MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES

START-UP OF PILOT SCALE PARTIAL NITRIFICATION AND ANAMMOX REACTORS INSTALLED IN A SEWAGE TREATMENT PLANT

HİLAL UFLAZ

MASTER THESIS

Department of Environmental Engineering

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Hilal UFLAZ, a Master of Science student of Marmara University Institute for Graduate Studies in Pure and Applied Sciences, defended her thesis entitled "**Start-up of Pilot Scale Partial Nitrification and Anammox Reactors Installed in a Sewage Treatment Plant**", on 30 April 2018 and has been found to be satisfactory by the jury members.

Jury Members

Assoc. Prof. Bilge ALPASLAN KOCAMEMİ (Advisor)

Marmara University ....

Prof. Dr. Zehra CAN (Jury Member)

Marmara University ....

Prof. Dr. Ayşen ERDİNÇLER (Jury Member)

Boğaziçi University ....

APPROVAL

Marmara University Institute for Graduate Studies in Pure and Applied Sciences Executive Committee approves that Hilal UFLAZ be granted the degree of Master of Science in Department of Environmental Engineering Program on ………….., 2018. (Resolution no: …………….).

Director of the Institute

Prof. Dr. Bülent EKİCİ

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The completion of my dissertation of Master of Science has been a long journey for me. The scope of dissertation was very challenging and innovative as it includes not only desk study but also real implementation on the site. Therefore, during many phases of this research, I was supported by many people whom I am very grateful for.

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The biggest challenge of this research was establishment of the “Pilot plant” in a real Sewage Treatment Plant (STP) which is under operation. To achieve this, first we had to solve the following issues: funding sources, decision of location for the plant, technical support for the establishment of the plant, human resources support for the continuous operation of the plant in my absence.

As first, we searched for a water utility which is interested to help my project with financial support and can provide some space in its plant for pilot plant. Here, we gratefully acknowledge the funding received towards the pilot plant from Bursa Metropolitan Municipality Water and Sewage Administration (BUSKİ). Therefore, I would like to express my special thanks to Mr. İsmail Hakkı Çetinavcı- Former General Manager of BUSKİ and Mr. Talat Özen- Former Deputy General Manager of BUSKİ, Mr. Devrim İzgi-Former Head of Treatment Plants, Nurcan Aydoğan-Construction and Operation Branch Manager, who believed my project and allowed me to establish the pilot plant in Bursa Doğu STP and financially support the pilot plant. Also, I would like to thank Marmara University Scientific Research Projects Department (Fen C-YLP-080415-0123, Fen D project) for funding this study.

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ABSTRACT

Anaerobic Ammonium Oxidation (Anammox) process, which was discovered in 1995 is a lithoautotrophic process mediated by a group of Planctomycete bacteria which oxidize ammonium to N_2 gas using NO_2^- as electron acceptor under anaerobic conditions. The use of nitrite as an electron acceptor requires a preliminary partial nitritation process prior to Anammox applications. To date, partial nitritation followed by Anammox process has been successfully implemented and optimized for sidestream digester supernatant treatment in a cost-effective way both in laboratory-scale and full-scale studies. However, application of Anammox process for mainstrem sewage treatment is still limited in all over the world. The lack of local Anammox seed in most of the countries, extremely slow growth rate of Anammox bacteria and partial nitritation requirement of the process limit Anammox applications.

This study mainly focused on to evaluate the partial nitritation of sewage under real plant conditions and to assess enrichment of Anammox species locally under real sewage treatment conditions. For this purpose, two (2) pilot scale reactors, partial nitritation (PN) and Anammox (AN) reactors, were installed in Bursa Doğu Biological Nutrient Removal STP in Turkey and inocculated with mixed activated sludge of Bursa Doğu STP. The PN and AN reactors were operated with sewage for 360 days and 425 days, respectively.

Partial nitritation of sewage pilot study demonstrated the effect of dissolved oxygen, temperature, pH, FA and sludge age factors on PN efficiency. PN of sewage was not steady. The wide fluctuations occurred in effluent quality depending on the changes in plant influent characteristics. In the whole study, the NO_2^--N:NH_4^+-N effleunet ratio could be obtained maximum around 1.2 under DO: 1.1-1.2 mg/L, temperature: 20-24˚C, FA: 0.7-1.1 mg/L and SRT: 4-5 days. There were many periods resulted very low NO_2^--N:NH_4^+-N effleunet ratio which is not proper for Anammox. PN was identified as the major limiting factor for mainstream sewage Anammox applications. The use of two-stage partial Nitritation-Anammox systems for mainstream was found unfeasible for real case applications.

Anammox enrichment from local activated sludge was succcesfully achieved first time in Turkey under real plant conditions. Anammox population DNA copy number, which was

initially 10^3 ng/ml in mixed activated sludge reached to the levels of 10^7 ng/ml. The total N removal rate reached to the level of 89 mg N/d for total N loading of 96 mg N /d with about 93% removal efficiency at the end of 425 days enrichment period.

ÖZET

Anaerobik Amonyum Oksidasyon (Anammox) prosesi, 1995 yılında keşfedilen anaerobik ortamda Planctomycete bacteri grubunun aracılığı ile, NO_2^- elektron alıcısı olarak kullanıp amonyumu okside edip N_2 gazına dönüştüren bir litoototrofik prosestir. Nitrit'i elektron alıcısı olarak kullanımı, Anammox uygulamalarından önce bir ön-kısmi nitrasyon prosesi gerektirir. Bugüne kadar, Anammox öncesi kısmı nitritasyon prosesi laboratuvar ölçeğinde ve gerçek büyüklükteki çalışmalarda çürütücü süzüntü sularının ekonomik olarak arıtılmasında başarı ile uygulanmış ve optimize edilmiştir. Ancak, evsel atıksu arıtmasında, Anammox uygulanması tüm dünyada hala sınırlıdır. Pek çok ülkede yerel Anammox aşı çamurunun eksikliği, Anammox bakterilerinin aşırı yavaş büyüme oranına sahip olması ve Anammox prosesi öncesinde Kısmi nitrifikasyonun gerekli olması, Anammox uygulamalarını sınırlamaktadır.

Bu çalışma, esas olarak Türkiyede gerçek tesis işletme koşullarında atıksuyun kısmi nitrifikasyonunun incelenmesi ve Anammox türlerinin evsel nitelikli gerçek atıksudan kaynaklı aktif çamur kullanarak lokal olarak üretilmesi üzerine odaklanmıştır. Bu amaçla, iki (2) adet pilot ölçekli reaktörler, kısmı nitrifikasyon (KN) ve Anammox (AN) reaktörleri, Bursa Doğu Biyolojik Azot Giderim Atıksu Arıtma Tesisinde (AAT) kurulmuş ve Bursa Doğu AAT deki karışık aktif çamur ile aşılanmıştır. PN ve AN reaktörleri sırası ile 360 ve 425 gün boyunca işletilmiştir.

Atıksuyun kısmi nitritasyonu pilot çalışması, çözünmüş oksijen, sıcaklık, pH, FA ve çamur yaş faktörlerinin PN verimliliğine olan etkisini göstermiştir. Atıksuyun kismı nitritasyonu stabil değildir. Tesis giriş suyu karakteriksiğinde olan değişikliklere bağlı olarak, geniş aralıklarda çıkış suyu kalitesinde dalgalanmalar olmuştur. Tüm çalışmaya göre, max. 1.2 civarında NO_2^--N:NH_4^+-N oranı, 1.1-1.2 mg/L DO, 20-24˚C sıcaklık, 0.7-1.1 mg/L FA ve 4-5 gün SRT koşullarında elde edilebilir. Anammox için uygun olmayan, pek çok faz, düşük NO_2^--N:NH_4^+-N oranı ile sonuçlanmıştır. PN, evsel atıksuda Anammox uygulamaları için ana sınırlayıcı faktör olarak tanımlanmıştır. Evsel atıksuda iki aşamalı kısmi Nitritasyon-Anammox sistemlerinin kullanılması, gerçek uygulamaları için uygun bulunmamıştır.

Türkiye'de ilk kez, gerçek tesis işletme koşullarında yerel aktif çamurdan anammox üretimi, başarılı bir şekilde gerçekleştirilmiştir. Anammox populasyonu DNA kopya numarası, başlangıçta karışık aktif çamurda 10^3 ng/ml iken 10^7 ng/ml seviyesine çıktı. Toplam azot giderim oranı, 96 mg N /gün Azot yükünde 89 mg N/gün seviyesine ulaştı, 425 gün işletme sonunda, giderim oranı %93 oldu.

SYMBOLS

K_a : ionization constant of nitrous acid equilibrium equation

K_b : the ionization constant of ammonia

k_{dn} : endonegous decay coefficient for nitrifying bacteria, g VSS/g VSS.d

K_n : half-saturation coefficient for nitrifying bacteria, mg N/L

K_{NH} : half velocity coefficient for NH_4^+-N, mg /L

$K_{o,AOB}$: half velocity coefficient for DO for AerAOB, mg O_2/L

K_{AerAOB} : specific endonegous decay coefficient for AerAOB, g VSS/g VSS.d

K_{NO} : half velocity coefficient for NO_2^--N, mg /L

$K_{o,NOB}$: half velocity coefficient for DO for NOB, mg/L

K_{NOB} : specific endonegous decay coefficient for NOB, g VSS/g VSS.d

K_w : the ionization constant of water

S : nitrogen concentration, mg N/L

S_{NH} : NH_4^+ concentration, mg N/L

S_{NO} : NO_2^--N concentration, mg/L

T : Temperature (°C)

μ_n : specific growth rate of nitrifying bacteria, g new cells/g cell.d

μ_{mn} : maksimum specific growth rate of nitrifying bacteria, g new cells/g cell.d

ABBREVIATIONS

AOB : Ammonia Oxidizing Bacteria

AOA : Ammonia Oxidizing Archea

AerAOB : Aerobic Ammonium Oxidizing Bacteria

AnAOB : Anaerobic Ammonium Oxidizing Bacteria

AN : Anammox

ANAMMOX : **AN**aerobic **AMM**onium **Ox**idation

AMO : Ammonia Monooxygenase

STP : Sewage Treatment Plant

BOD : Biochemical Oxgen Damand

bCOD : Biodegradable Chemical Oxygen Demand

CANON : Completely Autotrophic Nitrogen Removal Over Nitrite

COD : Chemical Oxygen Demand

Deamonification: Combined Partial Nitritation and Anammox

DEMON : pH controlled “deammonification

DO : Dissolved Oxygen

FA : Free Ammonia

FNA : Free Nitrous Acid (HNO_2)

HAO : Hydroxylamine oxidoreductase

HRT : Hydraulic Retention Time

HRM : High Resolution Melting Analysis

MLSS : Mixed Liquor Suspended Solids

MLVSS : Mixed Liquor Volatile Suspended Solids

NH_3 : Ammonia

NH_4^+ : Ammonium

NO_3^- : Nitrate

NO_2^- : Nitrite

NOB : Nitrite Oxidizing Bacteria

OLAND : Oxygen Limited Autotrophic Nitrification and Denitrification

PN : Partial Nitritation

PLC : Programmable Logic Controller

PO_4 : Phosphate

qPCR : Quantitative Polymerase Chain Reaction

RAS : Return Activated Sludge

SBR : Sequencing Batch Reactor

SCADA : Supervisory Control and Data Acquisition

SHARON : Single Reactor System for High Activity Ammonia Removal Over Nitrite

SRT : Solid Retention Time

STP : Sewage Treatment Plant

SO_4 : Sulphate

TKN : Total Kjeldahl Nitrogen

TAN : Total Ammonia Nitrogen

TN : Total Nitrogen

VSS : Volatile Suspended Solids

WWTP : Wastewater Treatment Plant

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1. CHAPTER 1 - INTRODUCTION

1.1 Importance of Nitrogen Removal and Environmental Impacts

The nitrogen (N) is the one of the essential biological nutrient enhancing the growth and breeding of animals and plants. Nitrogen itself is not harmful to water bodies, however an excess amount of nitrogen compounds (e.g. ammonium nitrogen, nitrite and nitrate) could be toxic to aquatic life and negatively affects various plant life and organisms in receiving water bodies by causing oxygen depletion and eutrophication. It also causes a number of adverse health effects (Hu et al., 2013). There are lots of nitrogen sources such as; effluent from domestic and industrial wastewater treatment plant (WWTP), agricultural runoff (e.g. fertilizer), septic tanks and animal manure runoff. Because of the growth of the human population and development of the human activities, the huge amount of the nutrients especially nitrogen compounds transferred through natural environment from these sources. Namely, the effluent water coming from domestic/industrial WWTP generally discharged to the lakes, rivers and seas or agricultural run-off directly passes through environment. If total nitrogen amount in WWTP effluent water is higher than required discharge standards, it can cause serious problems in lakes, rivers and reservoirs such as; dissolved oxygen depletion, toxicity, eutrophication, deterioration of water quality and odors- occasionally result with decrease in animal and plant diversity, and affects daily use of the water for drinking, fishing, swimming, and boating (Gerardi M.H., 2003). Therefore, nitrogen removal from water and wastewater is one of the important strategies for the prevention of further eutrophication in receiving water bodies and to improve water quality.

1.2 The Complete Nitrogen Cycle

Nitrogen species are present in water in different forms including organic nitrogen (Org-N), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and free ammonia (FA – NH_3). Total Nitrogen (TN) is the sum of the un-oxidized Nitrogen (Total Kjeldahl Nitrogen (TKN); NH_3, NH_4^+, org-N) and oxidized Nitrogen (NO_3^-, NO_2^-). Total ammonia (NH_3) in aqueous solution consists of two main forms, the ammonium ion (NH_4^+) and the un-ionized ammonia (FA) (Epa Manuel, 1993).

The simplified version of the nitrogen cycle is shown in Figure 1.1. Fixation of nitrogen (conversion of nitrogen in the atmosphere into NH_3), nitrification (biological oxidation of NH_3/NH_4^+ to NO_2^- followed by the oxidation of the NO_2^- to NO_3^-), denitrification (reduction of NO_3^- to produce molecular nitrogen (N_2)), aerobic ammonium oxidation, Anaerobic Ammonium Oxidation (Anammox), and assimilation of nitrate (reduction of NO_3^- during photosynthesis and bacterial growth) are most of the important processes that contribute to the nitrogen cycle.

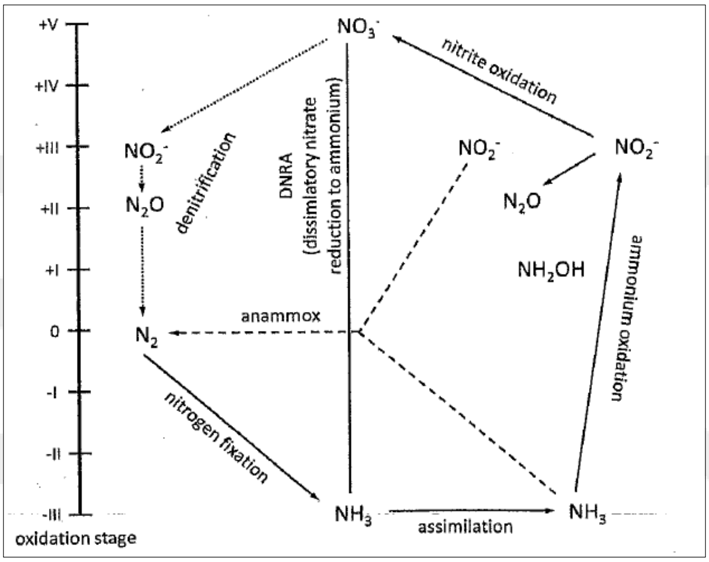


Figure 1-1. Complete Nitrogen Cycle (Morales et al., 2015a)

1.3 Biological Nitrogen Removal Processes

Biological nitrogen removal processes consist of conventional way of nitrogen removal process such as nitrification process followed by denitrification process and novel nitrogen removal processes.

1.3.1 Nitrification and denitrification process

Nitrification and denitrification are the conventional biological processes commonly used for nitrogen removal from wastewater. Nitrification is the conversion of NH_3 to first into NO_2^- (nitritation) by aerobic ammonium oxidizing bacteria (AerAOB) (Eqn.1.1, 1.2 and 1.3) then into NO_3^- (nitratation) by nitrite oxidizing bacteria (NOB) in the presence of oxygen (Metcalf & Eddy, 2014). These two groups of autotrophic nitrifying bacteria are predominantly chemolithoautotrophic, which means they derive energy from oxidation of inorganic nitrogen compounds and use carbon dioxide as a carbon source to growth (EPA Manuel, 2002).

$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$ [Eqn. 1.1]

$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$ [Eqn. 1.2]

$4CO_2 + HCO_3^- + NH_4^+ + H_2O \rightarrow C_5H_7O_2N + 5O_2$ [Eqn. 1.3]

While, *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus and Nitrosorobrio* are the most frequently identified AerAOB species which carry out oxidation of NH_3 to NO_2^-, *Nitrobacter, Nitrococcus, Nitrospira, Nitrospina and Nitroeystis* are the most frequently identified NOB species which carry out oxidation of NO_2^- to NO_3^- (Metcalf & Eddy, 2014).

The overall synthetic-oxidation reaction of nitrification is given in equation 1.4 (Lin et al., 2009);

$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7NO_2 + 1.041H_2O + 0.98NO_3^- + 1.88H_2CO_3$ [Eqn. 1.4]

Based on the equation 1.4, the oxygen required for complete oxidation of NH_3 is 4.33 g O_2/g N oxidized; 3.22 g O_2/g N used for NO_2^- production and 1.14 g O_2/g N used for NO_2^- oxidation and 7.07 g of alkalinity as $CaCO_3$ consumed per g of NH_3 oxidized. When synthesis is not considered, the amount of oxygen required is higher than 4.33 g O_2/g N which is 4.57 and alkalinity is higher than consumed 7.07 g of alkalinity as $CaCO_3$ which is 7.14 (Metcalf & Eddy, 2014).

The first step in nitrification is known to be rate limiting step due to AerAOB have lower specific growth rates than NOB at temperature less than 28°C (Morales et al., 2015a). There are researchers (Chandran and Smets, 2000a, Chandran and Smets, 2000b, Chandran and Smets, 2005) suggesting that both steps (reactions) are limiting at different stages of the process. For example, at high temperatures (25-40ºC) of wastewater and extremely low SRTs (1-2 days), AerAOB can grow faster than NOB. That is resulted with wash-out of NOB of the system and achieving nitrite accumulation, which is the basis of the single reactor system for high activity ammonia removal over nitrite (SHARON) process, as well.

The growth kinetics and effect of substrates on microbial growth of nitrifiers are described with Monod equation (Eqn. 1.5) (Epa Manuel, 1993; Rittmann and McCarty, 2001).

$$\mu_n = \mu_{mn}\left(\frac{S}{(K_n+S)}\right) - k_{dn} \qquad \text{[Eqn. 1.5]}$$

in which;

μ_n : specific growth rate of nitrifying bacteria, g new cells/g cell.d

μ_{mn} : maksimum specific growth rate of nitrifying bacteria, g new cells/g cell.d

S : nitrogen concentration, mg N/L

K_n : half-saturation coefficient for nitrifying bacteria, mg N/L

k_{dn} : endonegous decay coefficient for nitrifying bacteria, g VSS/g VSS.d

The biological growth rates of AerAOB and NOB are affected by the DO concentration during the reaction. The modified specific growth kinetic equation based on DO concentration as shown in Equations 1.6 and 1.7 (Metcalf & Eddy, 2014).

$$\mu_{AerAOB} = \mu_{m,AerAOB}\left(\frac{S_{NH}}{(K_{NH}+S_{NH})}\right)\left(\frac{DO}{(K_{o,AOB}+DO)}\right) - k_{AerAOB} \qquad \text{[Eqn. 1.6]}$$

in which;

S_{NH} : NH_4^+ concentration, mg N/L

K_{NH} : half velocity coefficient for NH_4^+-N, mg /L

$K_{o,AOB}$: half velocity coefficient for DO for AerAOB, mg O_2/L

DO : dissolved oxygen concentration, mg O_2/L

K_{AerAOB} : specific endonegous decay coefficient for AerAOB, g VSS/g VSS.d

and

$$\mu_{NOB} = \mu_{m,NOB}\left(\frac{S_{NO}}{(K_{NO}+S_{NO})}\right)\left(\frac{DO}{(K_{o,NOB}+DO)}\right) - k_{NOB} \qquad \text{[Eqn. 1.7]}$$

in which;

S_{NO} : NO_2^--N concentration, mg/L

K_{NO} : half velocity coefficient for NO_2^--N, mg /L

$K_{o,NOB}$: half velocity coefficient for DO for NOB, mg/L

K_{NOB} : specific endonegous decay coefficient for NOB, g VSS/g VSS.d

The key stoichiometric and kinetic parameters related to AerAOB and NOB are summarized in Tables 1.1 and 1.2, respectively.

Table 1-1. Basic and derived parameter values for AerAOB (Rittmann and McCarty, 2001)

Parameter	**5 °C**	**10 °C**	**15 °C**	**20 °C**	**25 °C**
Max. specific growth rate (μ_{nm}) (d^{-1})	0.32	0.42	0.58	0.76	1.02
Half-saturation coefficient (K_n) (mgNH_4^+-N/L)	0.18	0.32	0.57	1.00	1.50
Oxygen half-saturation coefficient (K_o) (mgO_2/L)	0.50	0.50	0.50	0.50	0.50
Endonegous decay coefficient (k_{dn}) (d^{-1})	0.045	0.06	0.082	0.11	0.15
Yield coefficient (Y) (mgVSS/mgNH_4^+-N)	0.33	0.33	0.33	0.33	0.33

Table 1-2. Basic and derived parameter values for NOB (Rittmann and McCarty, 2001)

Parameter	**5 °C**	**10 °C**	**15 °C**	**20 °C**	**25 °C**
Max. specific growth rate (μ_{nm}) (d^{-1})	0.34	0.45	0.61	0.81	1.10
Half-saturation coefficient (K_n) (mg NO_2^--N/L)	0.15	0.30	0.62	1.30	2.70
Oxygen half-saturation coefficient (K_o) (mg O_2/L)	0.68	0.68	0.68	0.68	0.68
Endonegous decay coefficient (k_{dn}) (d^{-1})	0.045	0.060	0.082	0.11	0.15
NO_2-N half-saturation coefficient (K_n) (mg NO_2^--N/L)	0.15	0.30	0.62	1.30	2.70
Yield coefficient (Y) (mgVSS/mg NO_2^--N)	0.083	0.083	0.083	0.083	0.083

As it is seen in the Tables 1.1 and 1.2, the half-saturation coefficient (K_o) for oxygen for AerAOB is lower than the K_o values of NOB at same temperatures, indicating that the NOB are not tolerant of low DO concentrations compared to the AerAOB. Hence, Rittmann and McCarty (2001) observed that operation at DO below oxygen half-saturation coefficient can lead to NOB out selection.

In addition to Rittmann and McCarty (2001) research, Vadivelu et al. (2006) reported 0.48 d^{-1} growth rate for Nitrobacter in mixed culture. Wiesmann (1994) reported oxygen half-saturation constant as 1.1 mg O_2/L for Nitrobacter.

Moreover, some researchers reported AerAOB kinetics in literature at 20°C. Brockmann et al. (2008), Pambrun et al. (2006) and Kaelin et al. (2009) investigated growth rates as 2.05, 1.96 and 1.1 d^{-1}, respectively while oxygen half-saturation constants as 0.6, 0.5 and 0.8 mg O_2/L, respectively. Similarly, Sin et al. (2008) reported oxygen half-saturation constants for AerAOB variation from 0.1 to 1.45 mg DO/L and for NOB variation from 0.3 to 1.1 mg DO/L. Wett et al. (2013), observed similar results in the study of full-scale pilot at the Strass WWTP, where K_o for AerAOB was 0.37 mg DO/L and for NOB was 0.16 mg DO/L.

Besides DO concentration, autotrophic nitrifying bacteria, both AerAOB and NOB are sensitive to environmental factors such as; temperature, pH, FA, free nitrous acid (FNA, HNO_2), the presence of synthetic organic chemicals and heavy metals, even inhibited by their own substrates (ammonia-N) and the intermediates (EPA Manuel, 2002).

Another key operation parameter is temperature has also large impact on both AerAOB and NOB. The maximum specific growth rates (μ_{mn}) of NOB is higher than AerAOB at temperature below than 25°C shown in Tables 1.1 and 1.2, that means NOB are can easily grow at a temperature in between 5-25°C than AerAOB. Regarding to the previous studies, it was investigated that the optimum temperature to wash-out NOB while surviving AerAOB in the system is recommended to be above 15°C (Hellinga et al., 1998).

In contrast to nitrification, denitrification (Eqn. 1.8, 1.9 and 1.10) is a heterotrophic process which means organic carbon is used for bacterial growth and NO_2^- or NO_3^- is reduced into N_2 by facultative heterotrophs (Metcalf&Eddy, 2014). This process occurs when DO levels are depleted and NO_3^- and NO_2^- replace with DO as primary oxygen source for microbial respiration. NO_3^- and NO_2^- are used as electron acceptors whereas organic carbon is used as electron donor (Epa Manuel, 1993; Metcalf&Eddy, 2014).

In this process, compared to nitrification process, a relatively broad range of bacteria can perform denitrification; such as Pseudomonas, Alcaligenes, Paracoccus, Thiobacillus, Bacillus and Halobacterium (Rittmann and McCarty, 2001; Wagner et al., 2002). These facultative anaerobe bacteria use nitrate instead of DO as an electron acceptor for respiration.

$$6\ NO_3^- + 2\ CH_3OH \rightarrow 6\ NO_2^- + 2\ CO_2 + 4\ H_2O \qquad \text{[Eqn. 1.8]}$$

$$6\ NO_2^- + 3\ CH_3OH \rightarrow 3\ N_2 + 3\ CO_2 + 3\ H_2O + 6\ OH^- \qquad \text{[Eqn. 1.9]}$$

The overall reaction of denitrification is;

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7NO_2 + 0.47N_2 + 1.68H_2O + HCO_3^- \qquad \text{[Eqn. 1.10]}$$

The hetetrophic denitrification process is an alkalinity producing process, which means bicarbonate alkalinity is produced and carbonic acid concentrations are reduced during denitrification. Theoretically, 3.57 g alkalinity as $CaCO_3$ is produced per mg of NO_3^--N reduced to N_2 and tendency to raise the pH of biological reaction (Metcalf&Eddy, 2014). This is the indication of pH has no significant effect on the denitrification rate unlike nitrification. Also, it has been reported that the denitrification is performed at pH between 7 and 8 without correction of pH and pH values outside these range can lead to accumulation of the denitrification intermediates which are NO_2^-, NO_2 and N_2O (Metcalf & Eddy, 2014; Rittmann and McCarty, 2001).

Although nitrification-denitrification processes are effective and economical processes among the alternative conventional physicochemical nitrogen removal processes (e.g., air stripping, breakpoint chlorination, and ion exchange), there are still several disadvantages of these processes compared to novel ammonia nitrogen removal processes developed in recent years (Epa Manuel, 1993; Fux et al., 2002; Metcalf & Eddy, 2014). One of the most important disadvantages is having high operational cost because of the high oxygen demand of the nitrification process (conversion of each gram of ammonia to nitrate takes

4.33 gram of oxygen), requirement of the exogenous carbon sources for denitrification process (2.72 mg biochemical oxygen demand (BOD_5) are required per mg of NO_3-N removed) and higher sludge production (Chandran et al., 2011; Chung et al., 2002; Jetten et al., 2005; Rittmann and McCarty, 2001; Sliekers et al., 2003; van Dongen et al., 2001; Vlaeminck et al., 2012). Siegrist et al., (2008) stated that, in order to remove organic matter in conventional STP, almost 50% of the energy is consumed in the aeration systems. Additionally, the denitrification process results with high emission of greenhouse gases (e.g., N_2O, NO and CO_2), which promote global warming (Glass and Silverstein, 1998; Metcalf & Eddy, 2014; Rittmann and McCarty, 2001).

1.3.2 Novel nitrogen removal processes

In order to overcome to the challenges in conventional biological nitrogen removal processes, several novel biological nitrogen removal processes have been developed in recent years. Novel nitrogen removal processes significantly reduce operational cost of wastewater treatment compared to conventional nitrification and denitrification processes (Jetten et al., 2005; Sliekers et al., 2003; van Dongen et al., 2001).

Among these novel technologies, the most popular ones are Partial Nitrification-Denitrification via nitrite (Eqn. 1.9 and 1.11), SHARON (Eqn. 1.12), ANAMMOX (Eqn. 1.13), Completely Autotrophic Nitrogen Removal Over Nitrite (CANON) (Eqn. 1.14 and 1.15) and Oxygen Limited Autotrophic Nitrification and Denitrification (OLAND) (Eqn. 1.16) (Sliekers et al., 2003; Strous et al., 1999a; Kuai and Verstraete, 1998; van-Loosdrecht and Salem, 2006).

Partial Nitrification- Denitrification via Nitrite Route:

It also is called Nitritation-denitritation process in which the oxidation of NH_3 to NO_2^- using oxygen as electron acceptor by eliminating formation of NO_3^- by means of NOB wash-out and then biological reduction of the accumulated NO_2^- to dinitrogen gas. (Yuan tao et l., 2010, Morales et al., 2015a).

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$ [Eqn. 1.11]

$$6NO_2^- + 3\ CH_3OH \rightarrow 3\ N_2 + 3\ CO_2 + 3\ H_2O + 6\ OH^- \qquad \text{[Eqn. 1.9]}$$

SHARON:

The term of SHARON is **S**ingle reactor system for **H**igh **A**ctivity **A**mmonia **R**emoval **O**ver **N**itrite that has been developed and operated at laboratory scale by group of researchers from Delft University (Hellinga et al., 1998).

The SHARON process is the first successful technique was achieved by which NH_3 removal over NO_2^-. This process is well-proven technology and it has been applied for more than 16 years for treating of sidestream (anaerobic digester supernatant) and performed without sludge retention (also referred to as chemostat) at high operation temperature (30-40°C) and pH (7-8) (Hellinga et al., 1998; Morales et al., 2015a; Mulder et al., 2001).

$$NH_4^+ + 0.75O_2 + HCO_3^- \rightarrow 0.5NO_2^- + 0.5NH_4^+ + CO_2 + 1.5H_2O \qquad \text{[Eqn. 1.12]}$$

ANAMMOX:

Anaerobic ammonium oxidation (Anammox) process is a lithoautotrophic (Chemolithoautotrophic) biological conversion process in which, bacteria oxidize NH_4^+ to N_2 using NO_2^- as electron acceptor under anaerobic conditions (Jetten et al., 1999, 2001; Strous et al., 1998).

The stoichiometric equation of Anammox process is (Strous et al., 1998);

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- +$$

$$0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \qquad \text{[Eqn. 1.13]}$$

CANON:

In the Completely Autotrophic Nitrogen Removal Over Nitrite (CANON) process, AerAOB and anaerobic ammonium oxidizing bacteria (AnAOB) perform two reactions at the same time to remove ammonia in single reactor under oxygen-limited conditions (Nielsen et al., 2005; Sliekers et al., 2003).

In the first step of reaction, AerAOB partially oxidize NH_3 to NO_2^- according to Eqn. 1.14.

$$NH_3 + 1.5\ O_2 \rightarrow NO_2^- + H_2O + H^+ \qquad \text{[Eqn. 1.14]}$$

In the second step of reaction, anaerobic ammonia-oxidizing bacteria convert ammonia to dinitrogen gas with nitrite according to equation 1.15.

$$NH_3 + 1.32\ NO_2^- + H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 2\ H_2O \qquad \text{[Eqn. 1.15]}$$

OLAND:

OLAND is oxygen-limited autotrophic nitrification and denitrification process (Eqn. 1.16) in which autotrophically oxidation of NH_4^+ to N_2 with NO_2^- under oxygen-limited conditions. It was first applied for a mixed culture of nitrifying bacteria (Kuai and Verstraete, 1998) and then examined in more detail in a lab-scale plants (Pynaert et al., 2003; Pynaert et al., 2004; Vlaeminck et al., 2012). In this process, the first step consists of the aerobic oxidation of about half of the NH_4^+ to NO_2^-, performed by AerAOB. The second step is anaerobic oxidation of the residual NH_4^+ with NO_2^- to produce N_2 and some NO_3^- (Vlaeminck et al., 2012).

$$NH_4^+ + 0.792O_2 + 0.08HCO_3^- \rightarrow 0.435N_2 + 0.111NO_3^- + 1.029H^+ + 0.052CH_{1.4}O_{0.4}N_{0.2} + 0.028CH_2O_{0.5}N_{0.15} + 1.46H_2O \qquad \text{[Eqn. 1.16]}$$

Among novel nitrogen removal technologies, the combination of partial nitritation (nitrite shunt) and Anammox processes is a promising technique for removing nitrogen from wastewater especially for wastewater with high ammonium concentration (e.g. digester supernatant, industrial wastewater) and low C/N ratio. Today, partial nitritation and Anammox processes are used efficiently for treatment of NH_4^+ in digester supernatants and a few industrial wastewaters (Lotti et al., 2015). Their applications for municipal wastewater treatment has received great attention in recent uyears and are still under investigation.

1.4 Partial Nitritation Process

1.4.1 General description

Partial nitritation (PN) process is the first step of conventional nitrification, in which NH_4^+ is partially converted to NO_2^- by AerAOB and it eliminates formation of NO_3^- via NOB out-selection (Morales et al., 2015a). This is the main goal of PN to provide out-selection of NOB while favouring the growth of AerAOB in the system.

Conventional nitrification/denitrification process is effective but expensive process due to high energy requirement (Daigger et al., 2011). Beccari et al. (1983), Turk and Mavinic, (1987) and van Kempen et al. (2001) reported 25% lower oxygen consumption, lower electron donor, 20% of reduction in CO_2 emission and lower sludge production (33∼35% in nitrification and 55% in denitrification) can be achieved in PN reaction compared to traditional nitrification and denitrification via nitrate process. In addition to this, in the study of Law and Stinson, (2012), it was reported nitrite shunt processes have 40% less sludge production and 20% lower CO_2 emission compared to conventional process.

Currently, PN process is mainly applied to treat rejection water of anaerobic digestion, leachate water and industrial waters with highly loaded nitrogen concentrations, low C:N ratios and high temperature (25-40ºC) which is technically feasible and economically favourable (Vlaeminck et al. 2012). Hence, wash-out of NOB of the system and nitrite accumulation in these types of water can be easily achieved when compared to mainstream application due to having ideal conditions of sidestream for PN such as; high operation temperatures (Lemaire et al., 2011). The out-selection of NOB to limit nitrate production in the mainstream is the main challenge in implementing shortcut nitrogen removal processes. The proper operation conditions for PN in mainstream are still being investigated; however, there have been some studies and their findings for this purpose (Morales et al., 2015a). In order to provide successful implementation of the PN to suppress NO_2^- oxidation at mainstream (low temperatures and low ammonium concentration), the reactor control strategies have to be assessed. The accumulation of NO_2^- in uncomplete nitrification shall be controlled by the production rate of NO_2^- and the inhibition of NOB. One parameter alone is not enough to control PN, for instance low level of DO to accumulate high rate of NO_2^- itself may not enough, since small amount of NO_3^- will always be formed. Because, there are other environmental and operating factors that impact the AerAOB and NOB community and PN reactions. Therefore, it is essential to monitor the biokinetics of PN in order to ensure the proper design and operation of bioreactors for nitrogen removal.

In the application of PN both in sidestream and mainstream, the main concern is how to maintain partial nitritation in the system and it attracts most of the researchers' attention. Biochemical experiments carried out over more than sixty years on different bacteria species and it was clearly indicated that kinetics is influenced by many physico-chemical and biological environmental factors. In previous studies, the effect of the most important factors includes DO, temperature, pH, FA, FNA, as well as sludge age on PN were monitored at the same time to control microbial community in relation to changing environmental variables (Alpaslan Kocamemi and Dityapak, 2016b; Bae et al., 2002; Dityapak, 2012; Park et al., 2010; S.W.H. Van Hulle, et al., 2007). These factors were discussed in the following subsections.

1.4.2 Factors influencing partial nitritation

1.4.2.1 Dissolved oxygen

Nitrification is an extremely oxygen intensive process and therefore DO concentration is the important parameter that significantly effects on the nitrification process and energy cost associated with air consumption in the plants. The most part of the energy consumption in wastewater treatment plants is caused from air consumption in biological process to remove nitrogen especially in highly nitrogen loaded systems (Fux et al., 2003). For this reason, it may be challenging to supply adequate oxygen for nitritation. For example, from the Eqn. 1.1, as the reaction is stopped in the first step which means nitritation, the oxygen consumption by the micro-organisms is reduced by 25%.

Both AerAOB and NOB utilize the DO as electron acceptor and they have different half saturation coefficients that affects the growth rates of these bacteria. Besides, there is no report in the previous studies that recommends exact DO concentrations for NOB out-selection for partial nitritation process (Morales et al., 2015a). As discussed in section 1.3.1 and as it is seen from Tables 1.1 and 1.2, the oxygen half-saturation coefficient (K_o) for AerAOB is lower than the K_o values of NOB at temperature of 15, 20 and 25 °C. Additionally, Grady (2011) was reported in literature that K_o for AerAOB was estimated to be 0.74 -0.99 mg/L and K_o for NOB was estimated to be 1.4-1.75 mg/L. Therefore, the growth of NOB is more restrictive to low DO than the growth of AerAOB. The previous studies reported that lowering the DO concentration resulted in reduction of the NOB activity, decreased the NH_4^+-N conversion and accumulated the NO_2^- concentration due to NOB are not tolerant of low DO concentrations compared to the AerAOB (Alpaslan Kocamemi et al., 2015; Alpaslan Kocamemi and Dityapak, 2013; 2014; Bae et al., 2002; Çiçek, 2008; Gilbert et al., 2014). In addition to this, NOB have a significantly higher maximum specific oxidation rate than the AerAOB at temperature range between 15-25 °C. When the DO operation concentration is reduced from 2.5 mg/L to 1.5 mg/L at these temperature in suspended cultures, NOB was affected more sensitively by the decrease of DO than AerAOB (Bae et al., 2002). Peng and Zhu, (2006) were reported the suppression of the growth of NOB while allowing AerAOB to grow at different DO concentrations with suspended system in sidestream. In another study, 95% of NH_4^+ oxidation and 75% of NO_2^- accumulation were observed at DO concentration of 1.4 mg/L in a suspended reactor that was fed with a synthetic wastewater (Ciudad et al., 2005). Alpaslan Kocamemi and Dityapak, 2016b; Dityapak, 2012 reported as DO concentration of 0.8-1 mg/L is the optimum working conditions to provide efficient partial nitritation in mainstream sequencing batch reactor (SBR) system. Besides, in the study of Alpaslan Kocamemi and Çiçek, 2015 and Çiçek, 2008 performed with synthetic wastewater simulating mainstream sewage ammonium level, NH_4^+ and NO_2^- accumulations were observed when DO concentration decreased from 1 to 0.54 mg/L in suspended-growth system, whereas NO_2^- accumulation was observed as DO concentration decreases from 3.64 mg/L to 1.21 mg/L in submerged packed bed biofilm system. Similary, Al-Omari et al. (2012) investigated that NOB suppression and NO_2^- accumulation can be achieved at low DO concentration in lab-scale SBRs operated at Blue Plains Advanced WWTP and full-scale pilot plant at Strass WWTP (Wett et al., 2013). In the pilot study conducted by Hampton Roads Sanitation District in mainstream, NOB out-selection via NO_2^- accumulation were demonstrated at high DO (> 1.5 mg/L) concentration and low solid retention time (SRT) (Morales et al., 2015a).

On the other hand, Gilbert et al. (2014) investigated that lowering of DO in moving bed biofilm reactor, did only affect AerAOB and not NOB, his findings also confirm other studies, NOB were not suppressed at a lower DO (Sliekers et al., 2005; Tokutomi et al., 2006).

Additionally, there have been many shortcut nitritation process demonstrated both in sidestream and mainstream with different DO operation concentrations, some of these studies are summarized in Table 1.3 (Park et al., 2010).

Table 1-3. DO Concentrations Causing Nitrite Accumulation (adapted from Park et al., 2010)

System	Wastewater	DO*	Effect**	Reference
Suspended growth	Lab scale	0.5	inhibition of NO_2^- accumulation	Hanaki et al.,1990
Activated sludge	industrial	0.7	96% as NO_2^-, 67% of NH_4^+	Ruiz et al., 2003
Biofilm air lift reactor	sidestream	1.0	100% as NO_2^-	Kim et al., 2003
Biofilm air lift reactor	sidestream	1.5	100% as NO_2^-, 50% of NH_4^+	Garrido et al., 1997
Biofilm reactor	synthetic	0.5	90% as NO_2^-, 100% of NH_4^+	Bernet et al., 2001
Actv. sludge with biofilm reac.	synthetic	<2	93% as NO_2^-, 88% of NH_4^+	Chung et al., 2007

* mg/L

**Accumulation rate of NO_2^- and removal rate of NH_4^+

As it is seen from Table 1.3, the optimum oxygen operation level for nitrite accumulation is in the range of 0.5-1.5 mg O_2/L for biofilm reactors, 0.5 mg O_2/L for suspended growth reactor (Blackbourne et al., 2008; Wang et al., 2011). Moreover, in the study of Wett et al. (2013) and De Clippeleir et al. (2013), it was observed that operation at a DO concentration of 1.5 mg O_2/L provides efficient NOB out-selection in mainstream deammonification.

For these reasons, DO control strategy becomes one of the most important parameter to achieve NO_2^- accumulation via inhibiting the growth of NOB while encourage the AerAOB in the system.

1.4.2.2 Temperature

Temperature is another important parameter for bacteria activity and biological reaction rates in nitrogen removal process. The basic idea behind the novel nitrogen removal process is to take advantage for the side stream which has already high temperature of wastewater. However, it cannot be said for the mainstream which has low wastewater temperature. From aspect of maximum specific growth rates (Tables 1.1 and 1.2), both AerAOB and NOB have higher growth rates at 25°C than the growth rates at 15°C and 20°C. In addition, the maximum specific growth rates of NOB are higher than the maximum specific growth rates of AerAOB at a temperature of 15°C, 20°C and 25°C.

At elevated temperature, biomass activity of nitrifiers increases with temperature up to 40°C as seen in Figure 1.2. At ambient temperatures, NOB grow faster than AerAOB resulting that NH_4^+ is completely oxidized to NO_3^- without achieving any NO_2^- accumulation. Except that the reverse reaction is true at elevated temperatures in which resulted with NO_2^- accumulation. This means that by carefully selecting the optimum residence time at ambient temperature, NO_2^- oxidizers can be washed out, while NH_4^+ oxidizers are survived in the reactor.

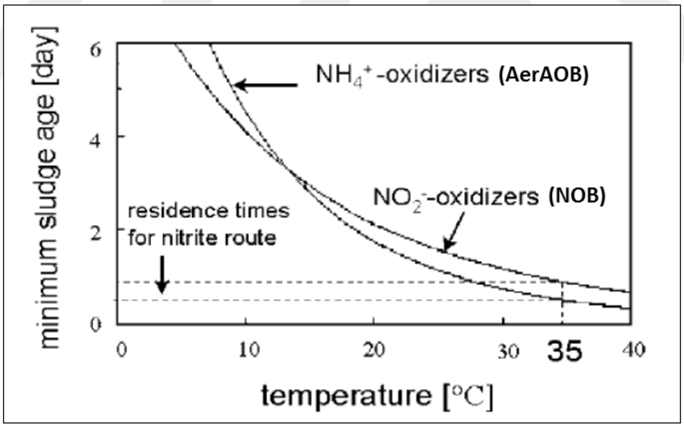


Figure 1-2. Min. SRT for AerAOB and NOB as function of the temperature (Hellinga et al., 1999; Notenboom et al., 2002)

The effect of temperature on nitritation was evaluated in different studies. In the study of Lackner et al. (2014) and Huang et al. (2010), it was observed that, temperature above 28°C, AerAOB become more active than NOB in mainstream and resulted in washout of NOB in the suspended system. Tonkovic et al., (1998) reported observation of NO_2^- accumulation in an activated sludge plant especially during the summer period.

All these previous studies showed that, why most of the studies for partial nitrification were performed at sidestream with high temperature so far (Padin et al., 2009; Hong-Fang et al., 2009; Vilar et al., 2010). However, there have been lots of studies reported, high rate of nitrogen removal can be possible at low water temperature even at 10 -12°C with low influent nitrogen concentration at mainstream (Hendrickx et al., 2012; Lotti et al., 2014c). De Clippelier et al. (2013) investigated that at temperature below 15°C or 20°C, wash-out of NOB was achieved and NO_2^- accumulation can be observed in mainstream. Moreover, depletion of the accumulated NO_2^- was also investigated as temperature lower than 10°C (Bae et al., 2002; Gilbert et al., 2014; Persson et al., 2014; Perez et al., 2014).

1.4.2.3 pH and effects FA and FNA

Nitrification is alkalinity consuming process and it reduces the pH during the reaction. Therefore, it is important to provide adequate alkalinity in the system to ensure adequate buffer for pH stability to be completed reaction (Grady et al., 2011).

The PN can be affected by pH in two ways (EPA Manuel, 2002);

(i) directly changing the reaction via reduction of total alkalinity (consumption of bicarbonate in the conversion of NH_4^+ to NO_2^-)

(ii) indirectly by means of inhibiting species of FA and FNA concentrations which change with pH and effect on their chemical equilibrium

As discussed in section 1.2, total ammonia occurs in aqueous solution in two forms as NH_3 (FA) and NH_4^+ (ionized ammonium). They are in equilibrium and that is affected by the pH of the solution (Eqn. 1.17). When the pH increases, the reaction shifts the right side that is resulted with increase in concentration of the NH_3.

Ammonia equilibrium;

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \qquad \text{[Eqn. 1.17]}$$

As is seen in Equation 1.18, when NO_2^- oxidation occurs, the pH of the solution decreases due to release of hydrogen ions. As the pH decreases, the concentration of FNA increases.

$$NH_4 + 1.5O_2 \rightarrow H_2O + H^+ + NO_2^- + H^+ \leftrightarrow HNO_2 \qquad \text{[Eqn. 1.18]}$$

The FA and FNA concentrations are directly correlated to pH, temperature, and the Total Ammonia Nitrogen (TAN) and NO_2^- concentrations, as shown in Eqn. 1.19 and 1.20, respectively (Anthonisen et al., 1976).

$$FA = \frac{17}{14} x \frac{[TAN] x 10^{pH}}{\frac{K_b}{K_w} + 10^{pH}} \qquad \text{[Eqn. 1.19]}$$

in which:

FA : Free ammonia as NH_3, mg/L

TAN : Total ammoniacal nitrogen (NH_4^++NH_3), mg/L

Kb : the ionization constant of ammonia

K_w : the ionization constant of water

where; $K_b/K_w = e^{\frac{6,344}{273+T\,(°C)}}$

and;

$$FNA = \frac{46}{14} x \frac{[NO_2 - N]}{K_a x\ 10^{pH}} \qquad \text{[Eqn. 1.20]}$$

in which:

FNA : Free nitrous acid as HNO_2, mg/L

K_a : ionization constant of nitrous acid equilibrium equation

$K_a = e^{\frac{-2,300}{273+T\,(°C)}}$

Boundary conditions for FA and FNA inhibition of nitrifiers were first described by Anthonisen (1976) as shown in Figure 1.3. Anthonisen et al. (1976) reported that at certain concentrations of FA, nitrification reaction is inhibited.

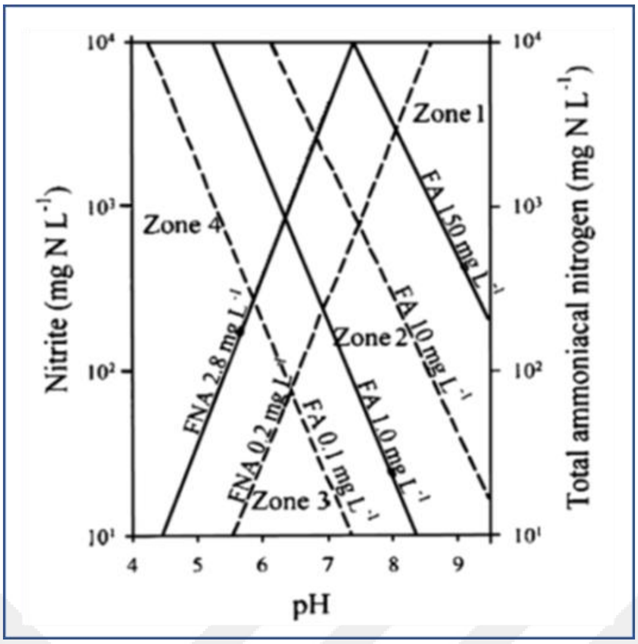


Figure 1-3. Relationships of FA and FNA and inhibition to nitrifiers (Anthonisen et al., 1976).

In Figure 1.3, the dashed lines represent lower limit, the solid lines represent the upper limit of each zone. There are four separate zones describing the different FA and FNA concentrations' inhibitory effect in terms of pH, TAN and NO_2^--N on nitrification. Zone 1=inhibition of nitritation and nitratation by FA; Zone 2=inhibition of nitratation by FA because due to lower concentrations of FA; Zone 3=complete nitrification, still lower FA concentration; Zone 4=inhibition of nitratation by FNA (Anthonisen et al., 1976; Peng et al., 2006).

Nitrifiers, both AerAOB and NOB, are very sensitive to pH and different nitrogen forms; FA and FNA. For instance; the optimal pH range for AerAOB (Nitrosomonas) is approximately 7.0 and 8.0, and the optimum pH range for NOB is approximately 7.5 to 8.0 (Epa manuel, 2002). In study of Bae et al. (2002), it was observed pH variations affect the growth rate of both AerAOB and NOB. Park and Bae (2009), investigated that initial FA and FNA concentrations affect NO_2^- oxidation process more seriously than ammonium oxidation processes which is resulted with accumulation of NO_2^-. In other words, lower FA concentrations have less inhibitory to AerAOB than to NOB. For this reason, FA concentrations play a role in NOB repression in partial nitritation because it is inhibitory to NOB even at low concentrations, while only high concentrations of FA are inhibitory to AerAOB as explained above. In addition to this, AerAOB use FA as actual substrate during the nitritation process and higher FA concentrations allow AerAOB to grow faster than NOB which means easily inhibition of NOB. Above the certain concentration of FA and FNA compounds has inhibitory effects on nitrification (EPA Manuel, 1993; Philips et al., 2002). In the study of Anthonisen et al. 1976, the inhibition concentration of nitrite oxidation began at 0.1–1.0 mg/l of FA for NOB, while that of ammonia oxidation was observed at 10 to 150 mg/l which inhibites AerAOB. Thus, a selective inhibition of nitrite oxidation would be achieved at a range of 1.0 to10 mg/L of FA concentration (Anthonisen et al., 1976). Moreover, Balmelle et al. (1992) reported Nitrobacter was completely inhibited at FA concentration of 3 mg/L. Similarly, Bae et al. (2002) observed NO_2^- accumulation occurred at an initial FA concentration of 4.7 mg/L. Vadivelu et al. (2007) stated inhibition of AerAOB species up to 16.0 mg NH_3-N/L FA levels. Alpaslan Kocamemi and Dityapak, (2013; 2014) reported the PN can be operated successfully at FA concentrations up to 3 mg/L in mainstream SBR system.

Additionally, while nitrifying organisms were affected at a concentration of 0.22 -2.8 mg/L FNA (Anthonisen et al., 1976), Nitrosomonas was reported to have a much higher level of tolerance to FNA compared to NOB (Vadivelu et al., 2007). Park and Bae, 2009 reported that FNA concentration in the process is important to achieve nitrite accumulation via NOB out-selection because FNA is more inhibitive to NOB than AerAOB. FNA inhibits the PN process as pH < 7.5 depending on the initial concentration of un-ionized ammonia in wastewater.

Even through, there is a complex relationship between pH and the growth of the AerAOB and NOB, stable nitrite shunt process operation can be achieved at pH 7 or higher (Hellinga et al., 1998). In the mainstream process, FA concentrations are too low to cause NOB inhibition (Anthonisen et al., 1976).

1.4.2.4 Free hydroxylamine

In addition to FA and FNA inhibitory effects, free hydroxylamine (NH_2OH-N) has been reported as toxic to nitrifiers. It is an intermediate product in nitrification process NH_3 is initially oxidized to hydroxylamine by ammonia monooxygenase (AMO), and then the hydroxylamine is further converted to nitrite by hydroxylamine oxidoreductase (HAO) and low concentrations (0.42 mg/L) of NH_2OH-N effectively inhibits nitrite oxidation

(Morales et al., 2015a). Noophan et at. (2004) reported that irreversible inhibition occurred at a free hydroxylamine concentration of 2-5 mg/L. In the study of Xu et al. (2012), it was stated that hydroxylamine has selectively inhibitory effect on growth of NOB over AerAOB in aerobic granule system. Moreover, despite hydroxylamine concentration below 5 mg/l had little effect on NO_2^- accumulation, hydroxylamine concentration at 10 mg/l, complete nitritation was achieved.

1.4.2.5 Solids retention time

Solid retention time (SRT) or sludge age is one of the important parameter for the design of WWTPs that is relating to growth rate of microorganisms and indicates the mean residence time of bacteria in the reactor (Clara et al., 2005).

Working at short SRTs combined with high temperature is commonly used mechanism in sidestream PN process for wash-out of NOB in the system. An increase in SRT causes increase of the NOB population and decrease of the nitrite accumulation (Ahn et al. 2008; Garrido et al., 1997; Ruiz et al., 2003). As it is shown in Figure 1.2, it is possible to retain AerAOB in the system, while washout of NOB by controlling SRT at higher temperatures that are typical for sidestream. For instance, at operation temperature of 35°C, NOB out selection of the system can be achievable by providing SRT almost 1 day.

Bock et al. (1983) reported that the minimum doubling times of AerAOB and NOB to be 7–8 h and 10–13 h, respectively. In addition, by providing appropriate sludge retention time in the system, NOB can be washed-out. van Kempen et al. (2001) investigated the optimum SRT for suppression of NOB and stable operation is between 1 and 2.5 days depending on the temperature. In the study of Alpaslan Kocamemi and Dityapak, (2013; 2014), the optimum sludge ager to achive appropriate NO_2^--N: NH_4^+-N ratio in the PN effluent was reported as 2 days in a real domestic wastewater Sequencing Batch Reactor **(**SBR) system. Similarly, it has been reported that NOB out-selection and 57% of NO_2^- accumulation ratio was achieved together with low effluent TN in Changi water reclamation plant at SRT 2.5 days and low operation temperature (Morales et al., 2015a). Similarly, efficient NOB out-selection was observed at operation conditions of short SRT (3.5 days), low DO (0.1 to 0.3 mg O_2/L) concentration and high wastewater temperature (Morales et al., 2015a).

1.5 Anammox Process

As briefly described in section 1.3.2, Anammox process is an alternative pathway for NH_3 removal from wastewater. It is an autotrophic ammonium oxidation process using CO_2 as only carbon source and NO_2^- as electron acceptor under strictly anaerobic conditions (Strous et al., 2002). Anammox process is mediated by Planctomycetes species.

The stoichiometric equation of Anammox process is shown in the Eqn. 1.13, in section 1.3.2 (Strous et al., 1998);

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- +$$

$$0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \qquad \text{[Eqn. 1.13]}$$

As it is seen from Eqn. 1.13, for every mole of NH_4^+-N, 1.32 moles of NO_2^--N are consumed and 0.26 moles of NO_3^--N are produced. The Anammox reaction also provides alkalinity since 0.13 moles of H^+ are consumed according to the stoichiometry (Ahn et al., 2006; Jetten et al., 1999; Strous et al., 1998). Apart from above reaction, CO_2 fixation generated separately and for each mol of NH_4^+, 0.2 mol NO_3^- and 20 mg biomass are produced (van de Graaf et al., 1996).

In Anammox process (Eqn. 1.21 and 1.22) catabolic reaction is carried out 15 times to fix one molecule of CO_2 with NO_2^- as electron donor leading to the anaerobic production of NO_3^- in anabolism (Jetten et al., 2009).

Catabolism $\quad NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad$ [Eqn. 1.20]

Anabolism $\quad CO_2 + 2NO_2^- + H_2O \rightarrow CH_2O + 2NO_3^- \quad$ [Eqn. 1.21]

The physiological parameters of anaerobic ammonium oxidation were given in Table 1.4.

Table 1-4. Important physiological parameters for anaerobic ammonium oxidation (Strous et al., 1999)

Parameter	Result	Unit
Max. specific NH_4^+ consumption rate	1.1	g of NH_4^+-N. g of protein^{-1}day^{-1}
Biomass yield	0.07	g of protein. g of NH_4^+-N^{-1}
Activation energy	70	kJ. mol^{-1}
Temperature range	20–43	°C
pH range	6.7–8.3	

1.5.1 The biochemical pathway

The Anammox reaction a high sensitivity to environmental conditions (temperature, DO, substrates, etc.) which make them extremely difficult to cultivate (Jin et al., 2012; Lotti et al., 2012). As seen from Figure 1.4, in the first step of Anammox process NO_2^- is reduced to NO and then in the second step the produced NO reacts with ammonium to form hydrazine (N_2H_4). In the last step N_2H_4 is oxidized to N_2 (Kartal et al., 2011). N_2H_4 is very reactive and toxic compound and it has adverse effect on most living organisms. It is normally used as a high-energy rocket fuel.

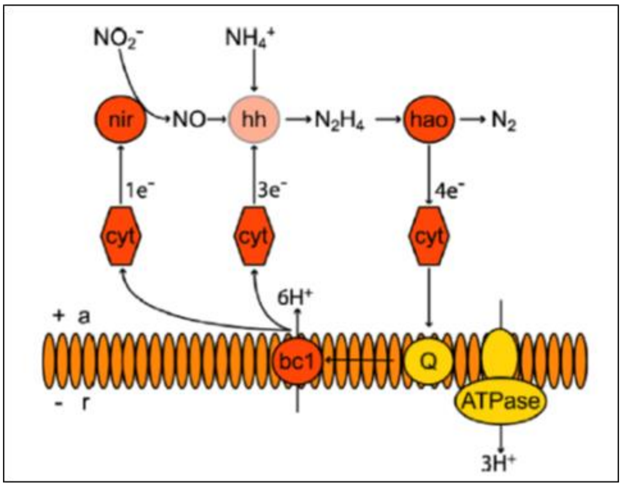


Figure 1-4. Biochemical pathway of Anammox process. Nir- nitrite reductase; hh-hydrazine hydrolase; hao-hydroxylamine oxidoreductase; cyt-cytochrome; bc1-cytochrome bc1 complex; Q-coenzyme Q; a-anammoxosome compartment; r-riboplasm compartment (Niftrik et al., 2008)

Hydroxylamine oxidoreductase (HAO) is a key enzyme used by AerAOB for conversion of hydroxylamine to NO_2^- in the oxidation of NH_4^+ (Hooper et al., 1997).

1.5.2 Characterization of Anammox bacteria

Anaerobic Ammonium Oxidizing Bacteria (AnAOR) are predominantly lithoautotrophic and Planctomycetes are the dominant species in the Anammox process (Isaka et al., 2008; M. Schmid, et al., 2003; Strous et al., 1998). To date, there are five Anammox species that were discovered, namely Brocadia, Kuenenia, Anammoxoglobus, Jettenia (fresh water). These four species were all enriched from activated sludge plants. The fifth species is Scalindua (marine) species were found in natural habitats (Jetten et al, 2001, 2009; Schmid, et al., 2003; Strous et al., 1999; Quan et al., 2008). Recent studies showed that Anammox bacteria significantly contribute to the release of fixed nitrogen as dinitrogen gas (N_2) to the atmosphere from the oceans (Arrigo et al., 2005; Devol et al., 2015; Kuypers et al., 2005, Lam and Kuypers, 2011).

Bacterial cells in terms of taxonomic purposes, can be subdivided into two classes: Gram-negative and Gram-positive. While, Gram-positive bacteria have cytoplasmic membrane and thick peptidoglycan layer (15–30 nm), the Gram-negative bacteria have cytoplasmic membrane, relatively thin peptidoglycan layer (1.5–15 nm), periplasmic space (filled peptidoglycan gel), and the outer membrane (Vollmer et al., 2008; van Teeseling et al., 2015).

Planctomycetes are the gram-negative bacteria and Anammox bacteria are unique in its biology due to existing of N_2H_4 as an intermediate product of catabolism, ladderane lipids and cytoplasm differentiation. The striking properties of these bacteria are; they have a membrane bound compartment are unique for each species inside the cytoplasm and proteinaceous cell wall without peptidoglycan (Figure 1.5). Compared with most other Planctomycetes, Anammox bacteria have three separate cytoplasmic compartments in their cytoplasm that are divided by unique intracellular membranes; outer membrane, cytoplasmic membrane and anammoxosome membrane. All process takes place in one compartment known as the anammoxosome which is the largest and innermost cytoplasmic compartment also contains unique 'ladderane' lipids and so far, unique in biology. There is cytoplasm between anammoxosome membrane and cytoplasmic membrane, which holds the nucleoid (DNA) as well as the ribosomes. The third and the outermost compartment is a unique cytoplasmic compartment (also called as the paryphoplasm) or a periplasm, which may be covered by S-layer protein (Boumann et al., 2009, Damste et al., 2002; Jetten et al., 2009; Kartal and Keltjens, 2016; Lindsay et al., 2001; Strous et al., 1999b; Van Niftrik et al., 2008a).

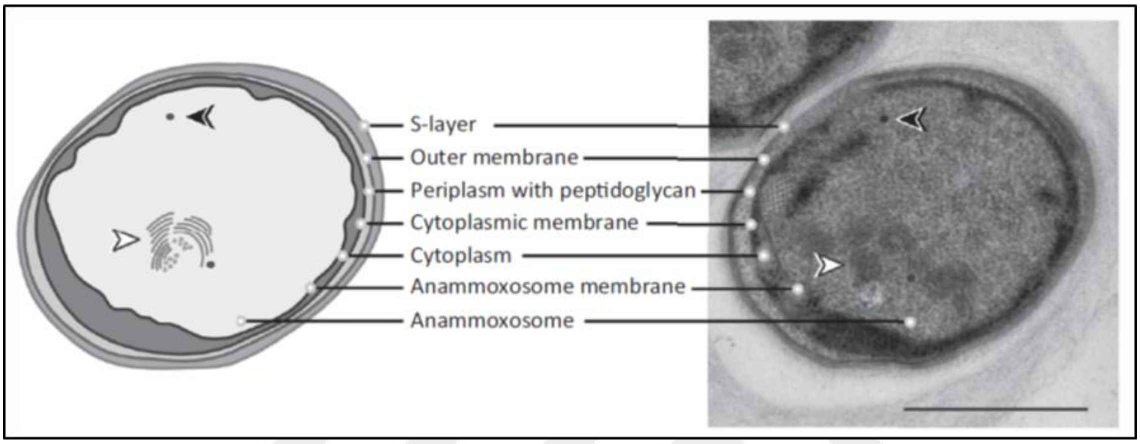


Figure 1-5. Cell plan and Lipid Structures of Anammox bacteria (Kartal and Keltjens, 2016)

Planctomycetes were divided by polar budding due to lack of peptidoglycan and FtsZ division ring gene (Fuerst, 1995; Jogler et al., 2012) and unlike other planctomycetes, Anammox bacteria were divided by binary fission (van Niftrik et al., 2009).

The AnAOR are the slowly growing bacteria and the grow rate is spectacularly low and reported as 0.05 to 0.2 day^{-1} (Strous et al., 1999; Tsushima et al., 2007; Van der star 2008). Doubling time and biomass yield of AnAOB range from 11 to 30 days and 0.11 to 0.13g VSS/g NH_4^--N oxidized, respectively (Fux et al., 2004; Jetten et al., 2009; Strous et al., 1998, 2002; Schmid et al., 2003; Van der Star et al., 2008; Wett et al., 2007a). AnAOR have specific activity more than seven times lower than when compared to AerAOB (Strous et al., 1998). Therefore, isolation and identification of Anammox species are very difficult and Anammox requires enrichment prior to start-up of the system. In North America, it took almost 2.5 years to accumulate sufficient biomass for the designing of the full scale Anammox plants due to slow growth rates of bacteria and requirement of a long start-up period (Wett, 2007b).

1.5.3 Factors influencing Anammox process

The Anammox reaction is very susceptible and irreversibly inhibited by many environmental factors, such as DO, temperature, pH, NH_4^+-N, NO_2^--N, organic matter, phosphate, sulphide, and salinity (Dapena-Mora et al., 2007; Fernández et al., 2012; Jin et al., 2012; Kartal et al., 2006; Lotti et al., 2012; Strous et al., 1999a; Van Hulle, et al., 2010).

1.5.3.1 Dissolved oxygen

The dissolved oxygen concentration is the critical operational parameter for Anammox bacteria activity. Because, Anammox bacteria are obligate anaerobes and they are active in the absence of O_2, which means their metabolism is reversibly inhibited at very low DO concentrations (Bagchi et al., 2010; Cema et al., 201; Jetten et al., 1999; Strous et al., 1997). Joss et al. (2009) reported that DO has to be less than 1 mg O_2L^{-1} for single-stage process since higher DO would also lead to the growth of NOB and hence could resulted with depletion of nitrite to be required for Anammox process as electron acceptor (Joss et al., 2009). At low oxygen concentrations (less than 1% air saturation), it was investigated that reversible inhibition of the Anammox process observed and at higher oxygen (higher than 18% air saturation), the Anammox reaction was inhibited irreversibly. In the study of Alpaslan Kocamemi and Dityapak, 2016b and Dityapak, 2012, it was observed that Anammox species' activities almost stopped at DO concentration above 0.6 mg/L. Therefore, DO level in the system should be continuously monitored.

1.5.3.2 Temperature

It was investigated in many studies that the optimal water temperature for Anammox biological activity was 30-40°C (Dosta et al., 2008; Isaka et al., 2008). Sudden or step-wise decrease in temperature impact on ammonia removal rates has been reported because low temperature reduces the activity of AnAOB which impacts process performance (Dosta et al.,2008; Gilbert et al., 2014; Isaka et al., 2007, 2008; Yang et al., 2007; Persson et al., 2014). Additionally, the temperature higher than 45 °C, irreversibly affects Anammox activity (Dosta et al., 2008).

There are studies (Gilbert et al.; 2014; Hendrickx 2012; Isaka, et al., 2007; Persson et al., 2014; Rysgaard et al., 2004) demonstrated Anammox activities at 10°C-22°C. In the study of Hu et al. (2013b), temperature of a combined partial nitritation/Anammox reactor operating at 25°C with high ammonium levels was decreased gradually to 12°C in 10 days and adaptation of Anammox species to low temperature levels were reported. Gilbert et al. (2014) also reported that single-stage reactor has been operated at 10°C with a relatively low range of Anammox activities.

Lotti et al. (2015) performed a study in order to evaluate short-term effect of temperature changes on activity of Anammox bacteria sampled from two full-scale reactors and two lab-scale reactors. The result showed that adaptation of Anammox bacteria after long term cultivation at 20°C and 10°C was observed and biomass specific activities decreased with temperature. Moreover, Plaza et al. (2011) and Hollowed et al. (2013) reported at operation temperature of 25 to 30°C, 70-85% of inorganic nitrogen removal was achieved.

In the study of Alpaslan Kocamemi, B. et al., 2016a, the optimum temperature was identified as 32.7 ˚C for sewage.

1.5.3.3 pH, FA and FNA

Strous et al. (1999a) and Egli et al. (2001) observed optimal pH range for Anammox activity is 6.7–8.3, 6.5-9.0, respectively. Besides, in the study of Jaroszynski et al. (2011), better performance was obtained in a biofilm reactor operating at pH 6.5 compared to operating at pH 7.8-8.

The low pH causes increase of FA while decreases of FNA and the pH is associated with low FA concentrations were investigated to be critical to stable Anammox activity in the MBBR (Jaroszynski et al., 2011). AnAOB are not as sensitive to FA as are NOB (Fernández et al., 2012). Stable operation of Anammox system was observed for less than 20–25 mg/L FA levels. In addition to this, Waki et al. (2007) also reported Anammox activity was affected with FA levels of 13–90 mg/L.

In the study of Fernández et al (2012), the inhibitory effect of FNA has been reported as 4.4 µg HNO_2-N/L.

1.5.3.4 NH_4^+-N and NO_2^--N

Strous et al. (1999a) reported that NH_4^+ or NO_3^- up to concentrations of 1 gN/L are not inhibitory to Anammox process although other researchers have reported that high levels of NH_4^+ caused suppression of Anammox activity (Fernández et al., 2012; Dapena-Mora et al., 2007). Dapena-Mora et al. (2007) observed that NH_4^+ concentration up to 770 mg/L caused 50% of activity loss of Anammox process.

Although NO_2^- is one of the key parameter of the Anammox process, excess amount of NO_2^- has inhibitory effect on Anammox activity. The negative effect of NO_2^- on Anammox activity has been studied widely and reported in previous studies. NO_2^- concentrations higher than 0.1 g N/L completely inhibited the Anammox process in which Anammox activity decreased with increasing NO_2^- concentration. Moreover, this inhibition was reversible and addition of Anammox process intermediates (hydrazine, hydroxylamine) were restored the activity rapidly (Strous et al., 1999a). In another study on NO_2^- inhibition, it was investigated that exposure to NO_2^- concentrations as high as 1 g N/L is inhibitory to Anammox process but it could be reversible for less than 24-hr exposure to NO_2^-. In addition, NO_2^- rather than nitrous acid is inhibiting compound exposures at three different pH-values (Lotti et al., 2012). NO_2^- concentrations of 5 mg N/L and 40 mg N/L have been reported as inhibitory by Wett et al. (2007a) and Fux et al. (2003), respectively. Strous et al. (1999) reported complete inhibition of Anammox activity for NO_2^- concentration of more than 100 mg/L. In contrast, Dapena-Mora et al. (2007) observed 50% inhibition of Anammox activity at NO_2^- concentration of 350 mg/L. Moreover, it was observed that influent NO_2^- concentrations higher than 280 mg/l inhibited Anammox activity and each Anammox species have different tolerance for NO_2^- (Isaka et al., 2007; Kimura et al., 2010; Wett et al., 2007a). The earlier studies on the inhibitory effect of NO_2^- on the Anammox activity showed that inhibitory effects of NO_2^- are based on exposure time as well as NO_2^- concentration in the system (Dapena-Mora et al., 2007; Egli et al., 2001; Strous et al., 1999a). Similarly, Dityapak, 2012 and Alpaslan Kocamemi et al., 2016a observed NO_2^--N concentration up to 50 mg/l has no significant effect on Anammox activity; however, at 71.5 mg NO_2^--N /l concentration, almost 50-60 % decrease of Anammox activity observed.

1.5.3.5 Organic and inorganic matter

Organic matter can be found in domestic wastewater at a low concentration which is favourable for Anammox process due it is autotrophic process. AnAOB and heterotrophic bacteria can coexist in mainstream and biodegradable organic carbon does not directly affect these bacteria in the system. However, an excess amount of organic carbon in the system causes heterotrophic bacteria would dominant, due to much lower growth rate of AnAOB compared to heterotrophs and resulted in uncomplete Anammox process (Chamchoi et al., 2008; Dapena-Mora et al., 2007; Jenni, 2015; Lackner et al., 2014). Chamchoi et al. (2008) observed that Anammox activity is suppressed when Chemical oxygen demand (COD) concentration is higher than 300 mg/L. Moreover, if the influent COD is high, rapid uptake of nitrite can be observed due to growth of heterotrophic bacteria (Morales et al., 2015a).

Sulphate (SO_4^{2-}) under anaerobic conditions, is converted into Sulphide (S^{2-}) which has inhibitory effect to Anammox bacteria (Dapena Mora et al., 2007). In the study of Dityapak, 2012; Alpaslan Kocamemi and Dityapak, 2016b, the short-term inhibitory effect of various organic (acetate, glucose, propionate) and inorganic compounds (NO_2^-, SO_4^{2-}, potassium (K^+), phosphate (PO_4^{3-})) were studied in mainstream Anammox SBR system. As a result of this study, 50% inhibition of Anammox activity was observed at concentrations as 5,000, 2,500, 1,500 mg/L COD for glucose, propionate and acetate, respectively. In addition to this, at concentrations of 3,500 mg PO_4^{3-}-P /l, 2,400 mg SO_4^{2-} /l and 1,384 mg K^+/l, 50% decrease observed in Anammox activity.

1.5.4 The history and applications Anammox process

Anammox has been defined as a “deammonification” process which involves partial nitritation of NH_4^+ and subsequently the anaerobic oxidation of NH_4^+ and NO_2^- to N_2 (Metcalff and Eddy, 2014).

Many different aspects of the conventional wastewater treatment technologies for nitrogen removal have been studied and applied thoroughly. At the end of these studies, it was investigated that application of conventional processes is not cost-effective or /and environment-friendly. On the other hand, the applications of Anammox process in combination with partial nitrification offer a quite attractive alternative solution compared to conventional processes and Anammox-based processes are becoming increasingly widespread all over the world (Ahn, 2006; Jetten et al., 2001; 2002, Op den Camp et al., 2006; Schmidt et al., 2003b). The potential plant operational saving was investigated as 40% saving for nitritation and denitritation process and 84% saving for partial nitritation and Anammox process compared to the conventional nitrification/denitrification process. (De Clippeleir et al., 2012).

Anammox process was first discovered in a pilot scale denitrifying fluidized bed reactor in Delft, The Netherlands (Mulder et al., 1995) and currently it is successfully implemented in the treatment of sidestream which have high concentration of ammonia and high-water temperature (>30 °C) such as; anaerobic digester reject waters and anaerobic industrial treatment effluents (food-processing, fermentation, winery industries, etc) (Lackner et al., 2014; Vlaeminck et al., 2012).

The first full-scale Anammox reactor at industrial scale (75 m^3), was implemented for the treatment of sidestream in 2002 in Sluisjesdijk, Rotterdam, Netherlands (Abma et al., 2007; van der Star et al., 2007). Later on, the first full scale one-stage Anammox plant (CANON) was successfully installed at Strass WWTP, Austria and it has been operated since 2004 (Wett et al., 2007a). In this plant, partial nitritation and Anammox processes happen in the same reactor under oxygen limitation (Third et al., 2001). To date, over 100 full scale Anammox-based plants for the treatment of wastewaters with high nitrogen concentrations at elevated temperature have been installed worldwide, primarily in Europe and Asia (Lackner et al., 2014; Morales et al., 2015a). It has been investigated that 75% of total inorganic nitrogen elimination and 80% of ammonia oxidation can be achieved in the sidestream process. There are also more than 50 plants are operational, under construction or under design, mainly in Europe and the United States.

Although, Anammox process is the most important promosing novel technology, its application for nitrogen removal was not straightforward especially in mainstream due to some challenges during the operation. The main challenge for application of Anammox in the mainstream is to obtain low effluent nitrogen concentration even under low wastewater temperatures (Lotti et al., 2015).

The Anammox application in the mainstream which have low water temperature (10-15°C), and relatively low ammonium concentration, is more recently studies in pilot-scale and full-scale. To the best of our knowledge, the number of reactors installed for the investigation of Anammox process in mainstream sewage treatment has been increasing (Cao et al., 2013; Dosta et al., 2008; Gilbert et al., 2014; Gilbert et al., 2015; Hendrickx et al., 2012; Hu et al., 2013b; Isaka et al., 2008; Laureni et al., 2015; Lotti et al., 2015; Lotti et al., 2014a, 2014b, 2014c; Morales et al., 2015b; Regmi et al., 2014; Vàzquez-Padìn et al., 2011; Wett et al., 2013; Winkler et al., 2011).

1.5.5 System configuration for Anammox application

Adequate NO_2^- concentrations should be supplied in Anammox process, for this purpose two methods can be used to accumulate NO_2^- in the system. NO_2^- can be either produced in a separate reactor and then subsequently be fed into Anammox reactor (two-stage) or produced in a single-stage (combined) system with an oxygen limited conditions (van Dongen et al., 2001; Sliekers et al. 2003; Third et al., 2001). The aim of the both systems is to convert part of available NH_4^+-N into NO_2^--N by AerAOB. Following to partial nitritation, the remaining NH_4^+-N and the formed NO_2^--N are converted to N_2 by AnAOB. Compared to two-stage processes, the capital cost of one-stage processes is generally lower. However, two-stage systems can be more flexible in operation since nitritation and Anammox processes can be controlled and optimized separately.

Nowadays, combined Anammox process followed by partial nitrification process applications in a mainstream are mainly preferred operational process. To date, three dominant reactor approaches were determined and applied in both sidestream and mainstream Anammox systems in which: SBRs, suspended growth (granular and flocculant) reactors, and attached growth reactors (Morales et al., 2015a).

SHARON, CANON, DEMON (pH controlled “deammonification”), and OLAND systems have been developed exclusively for this purpose (Kuai and Verstraete, 1998; Pynaert et al., 2004; Third et al., 2001; 2005; Vlaeminck et al., 2007; Wett, 2007b).

The first SBRs for combined nitritation/Anammox processes were realized on two full-scale plants in Strass-Austria and in Glarnerland-Switzerland. They are currently operating Strass since 2004 and Glarnerland since 2007.

1.5.6 Challenges of Anammox process

Although, Anammox process is the most important promosing novel technology, there are some limitation of this process especially application in mainstream. The main challenges working in mainstreams are;

(i) Lack of locally mainstream Anammox seed (local enrichment- bacteria not available in pure culture) and Anammox seed is enable in Turkey – only in Europe and US,

Anammox process is strictly dependent on available local Anammox seed, but the absence of large volume of Anammox seed is the main obstacle to implement full scale plants. In addition to this, Anammox bacteria have slow growth rate and it requires long start-up period as well as a long period to accumulate sufficient biomass for the anammox plants (Wett, 2007b).

(ii) Low and variable operation temperatures and influent nitrogen concentrations and high effluent water quality requirement,

The ideal waste stream for Anammox process in terms of biodegradable chemical oxygen demand (bCOD) concentration and its ratio of TAN concentration is 2:1 or less, in which natural conditions of sidestream (Morales et al., 2015a). The lower operating temperatures and NH_4^+ concentrations, together with lack of unified strategies to provide NOB out-selection and high and stable nitrogen removal requirement (Lotti et al., 2015; Morales et al., 2015a) are another main challenge.

(iii) Partial nitirification of sewage required prior to Anammox process as preliminary step (NOB out-selection to avoid nitrite oxidation to nitrate),

At the beginning of Anammox process, ammonium and nitrite should be in the stoichiometric ratio of 1:1.3 - 1:1.67 which is the perfect influent for an Anammox reactor (Jetten et al., 1999; Mulder et al., 1995; Strous et al., 1998; Van de Graaf 1996; van Dongen et al., 2001). Therefore, partial nitrification to nitrite is a pre-requisite step for Anammox process in order to provide required stoichiometric ratio of nitrite to ammonia. The successful mainstream Anammox process depends on advance control strategies including online monitoring and flexibility of adjusting operational parameters. There are ongoing researches being investigated to optimize conditions for stable PN-AN process in mainstream (Morales et al., 2015a).

In Turkey, partial nitritation of sewage was studied both with synthetic wastewater and real sewage in various studies (Alpaslan Kocamemi et al, 2008; Alpaslan Kocamemi et al., 2015; Çiçek, 2008; Dityapak, 2012). In consistency with literature these studies showed that low strength characteristic of sewage makes it very difficult to partially nitrify. The effluent NO_2^--N/NH_4^+-N ratio proper for the Anammox feeding could be obtained by controlling various operational parameters (e.g. DO, FA, pH, SRT) simultaneously.

The first local Anammox enrichment in Turkey was obtained from Istanbul Paşaköy STP activated sludge under laboratory conditions using synthetic wastewater (Dityapak, 2012). The enrichment period was successfully followed through continuous monitoring of pH, ORP, and conductivity (Alpaslan Kocamemi et al., 2013; Alpaslan Kocamemi et al., 2014; Dityapak, 2012).

Dityapak, 2012; Alpaslan Kocamemi and Dityapak, 2016b investigated nitrogen removal from sewage with partial nitritation followed by Anammox process using Istanbul Paşaköy STP grit chamber effluent under laboratory conditions. The limiting step of the whole process was identified as Partial Nitritation due to low strength characteristic of sewage.

2. CHAPTER 2 - MATERIALS AND METHODS

2.1 General Description of the Pilot Plant

The study was performed in a pilot plant that was installed and still operation in Bursa Doğu Sewage Treatment Plant (STP) (Figure 2.1) in Turkey with the financial support of BUSKI (project number 3-030.99-19567).

Figure 2-1. General view of Bursa Doğu STP (http://www.tesisat.com.tr)

Bursa Doğu STP is the largest advanced wastewater treatment plant in Bursa which was designed and constructed in accordance with the extended aeration and five-stage Bardenpho process with a capacity of 320,000.0 m^3/day (third phase, 2030). It has been operated since 2006. The inlet wastewater coming through the plant is combined sewer including sewage and storm water. The Doğu STP also accepts effluent waters from textile industries.

The pilot plant (Figure 2.2) consists of two-reactor system with a working volume of 250 L; (i) Partial Nitritation (PN), (ii) Anammox (AN). These two reactors, PN and AN, were operated with the flow diagrams shown in Figure 2.3. PN (Fig. 2.2-b) was continuously fed with grit chamber effluent of Bursa Doğu STP. AN (Fig. 2.2-b) was continuously fed with secondary clarifier effluent of Bursa Doğu STP and spiked with NH_4^+-N and NO_2^--N. The overall system configuration (Figure 2.3) allows to divert effluent of PN to AN. This operation scheme was only applied after enrichment of Anammox bacteria in AN reactor in order to monitor partial nitritation followed by Anammox process (deammonification) for mainstream sewage treatment.

Figure 2-2. The Pilot Plant, (a) Cabinet, (b) PN and AN reactors, (c) PLC and SCADA System

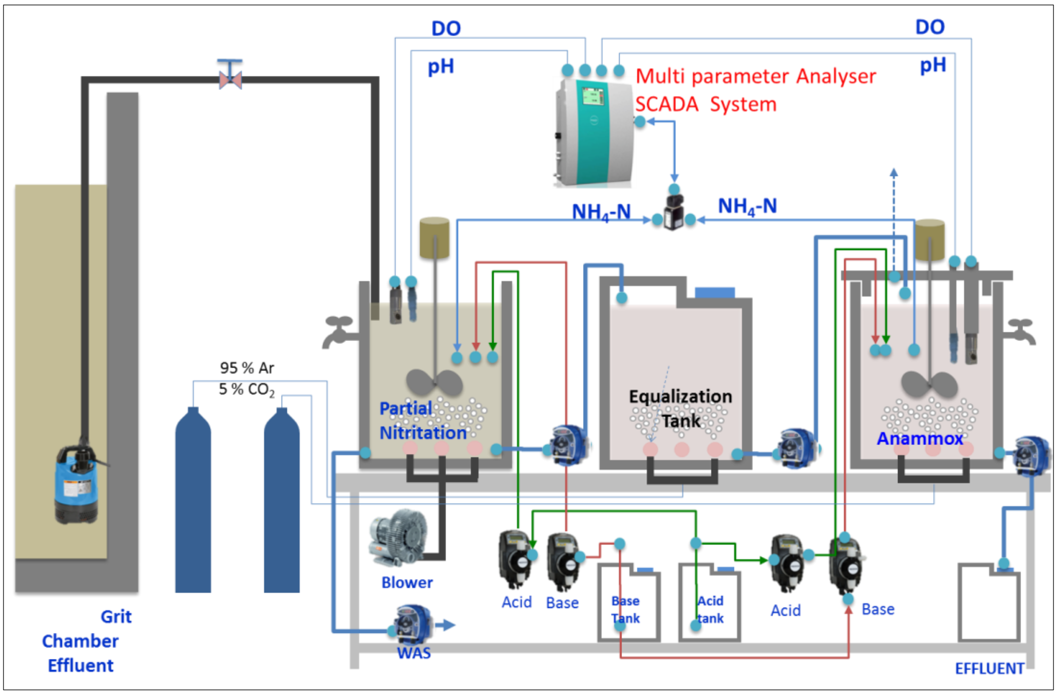


Figure 2-3. Pilot plant flow diagram

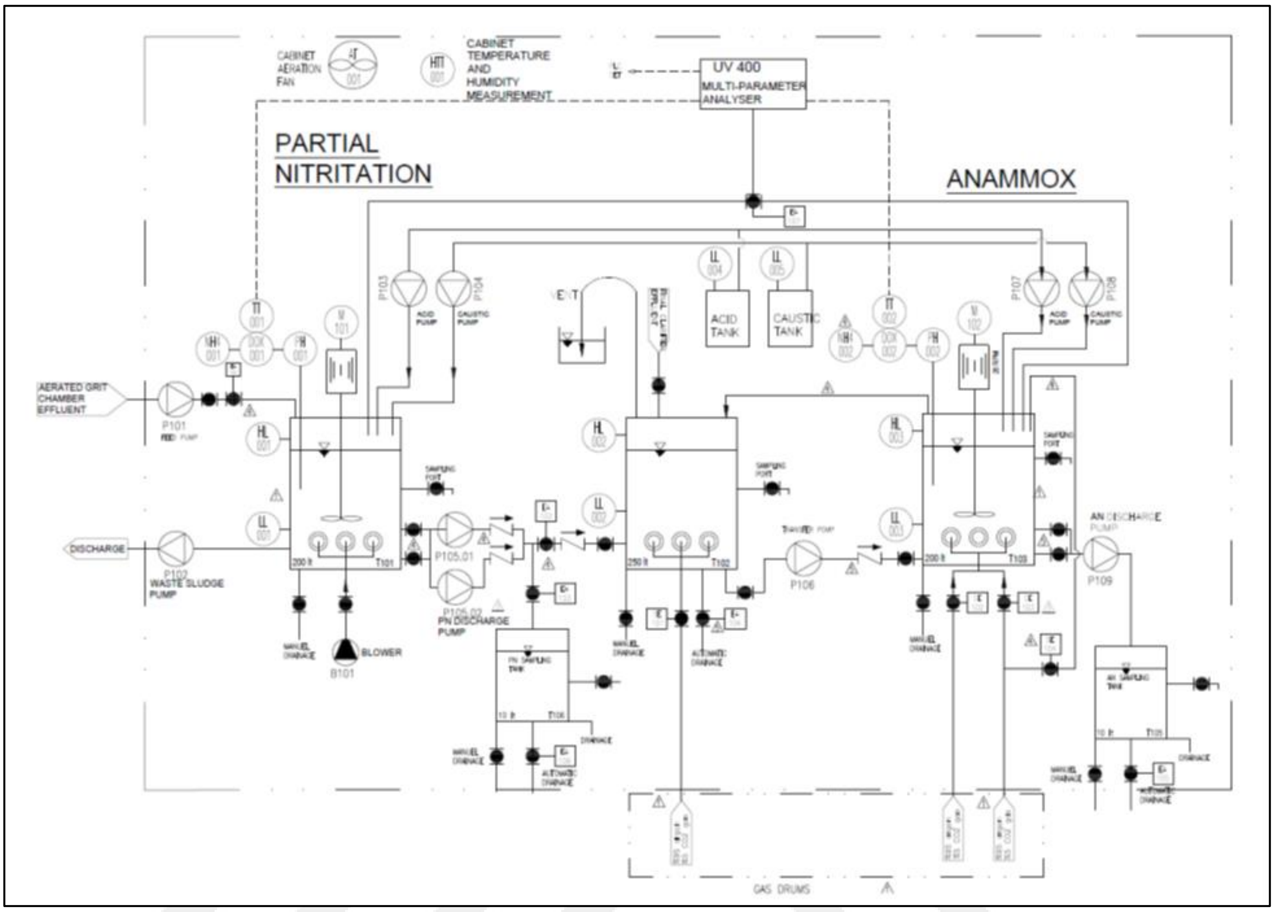


Figure 2-4. The overall system configuration (P&I)

2.2 Operation Strategy of the Pilot Plant Reactors

The pilot reactors (Figure 2.2-b) were operated in a cabinet (Figure 2.2-a) at water ambient conditions with a Programmable Logic Controller **(**PLC) and a SCADA (Figure 2.2-c) system. Both PN and AN reactors have been operated as sequencing batch reactors (SBR) via PLC system by following consecutive six basic phases; Fill, React, Waste, Settle, Idle, and Decant.

In order to obtain required operation conditions in reactors, crucial parameters effecting partial nitritation and Anammox such as; pH, temperature and DO were continuously monitored and recorded at 5-minute time intervals using Tethys probes connected to Tethys Online Water Analyzer-UV 400 (Fig. 2.2-b). On-line NH_4^+-N measurements were performed via Tethys Online Water Analyzer-UV 400. Daily influent and effluent samples from PN and AN reactors were analysed for TN, COD, NH_4^+-N, NO_3^--N and NO_2^--N parameters spectrophotometrically in accordance with methods described in

Section 2.5. Sludge samples were taken from PN and AN reactors regularly (usually monthly) for molecular analysis described in Section 2.6.

2.3 Partial Nitritation (PN) Reactor

The PN reactor (Figure 2.5) was constructed from Plexiglas material with an effective volume of 250 litres. The reactor was initially started-up by inoculating with the returned activated sludge (RAS) seed from Bursa Doğu STP. Initial biomass concentration (MLVSS) was around 2,710 mg/L. In order to monitor both partial nitritation and organic carbon removal efficiencies, the PN reactor has been operated for 360 days.

The system (Fig. 2.4) consists of a mechanical mixer, centrifugal blower with a blow-off valve, fine bubble diffusers, influent, effluent, sludge, acid/base pumps. During 360 days operation period, the system was continuously fed with effluent of Bursa Doğu STP grit chamber. Tubular fine bubble diffusers (Oxyflex) were connected to the blower (FPZ) to provide required air demand based on set DO level on Scada system in the reactor. Homogeneous mixing conditions was provided with a mechanical mixer (Aqua) with speed of 50 rpm.

The process control strategies of the PN reactor were as follows;

(i) DO control; set DO level was maintained by controlling on/off aeration of blower via on-line DO measurements,

(ii) pH control; set pH value was maintained by adjusting operation of proportional controlled acid/base pumps (Seko) via on-line pH measurements,

(iii) Sludge age control; the sludge age was adjusted by changing operation time of waste sludge pump,

(iv) NH_4^+-N control; discharge time of the reactor was controlled by comparing set NH_4^+-N value on SCADA with the NH_4^+-N value measured via an on-line ammonium analyser at certain time intervals.

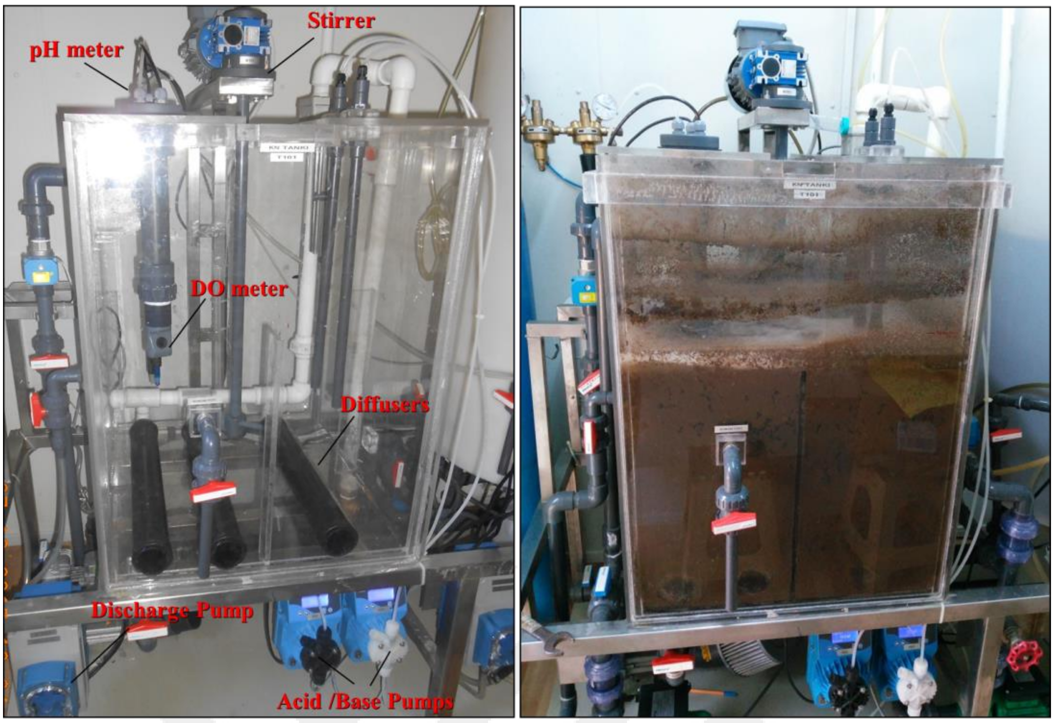


Figure 2-5. PN Reactor

Daily influent and effluent grab samples were analysed for NH_4^+-N, NO_2^--N, NO_3^--N, TN and COD in order to assess daily ammonium utilization, nitrite accumulation, nitrate production and COD removal rates. In addition to these daily analyses, hourly batch kinetic experiments were also performed through 1-year operation. Sludge samples for molecular analysis were taken monthly basis. In each week, MLSS and MLVSS measurements were performed in accordance with methods described in Section 2.5.

Depending on the duration of each batch cycle, hydraulic retention time (HRT) was ranged between 2-2.8 days.

The 1-year operation period of PN reactor was evaluated under ten (10) different phases depending on DO, pH, SRT and temperature changes in the reactor. These phases are summarized in Table 2.1.

Table 2-1. Operation conditions the PN reactor

Phases	Operation Days	DO (mg/l)*	pH*	SRT (day)	Temperature (°C)*
Phase I	0-19	0.6 ± 0.4	8.1 ± 0.4	4	19.9 ± 1.2
Phase II	20-53	1.2 ± 0.3	7.8 ± 0.2	5	20.3 ± 1.3
Phase III	54-107	0.7 ± 0.2	7.8 ± 0.1	5	22.7 ± 1.0
Phase IV	108-158	1.1± 0.1	7.8 ± 0.3	4	23.9 ± 1.3
Phase V	159-184	1.1± 0.2	7.7 ± 0.2	3	21.0 ± 1.5
Phase VI	185-231	1.2± 0.4	7.8 ± 0.1	4	18.6 ± 1.8
Phase VII	232-264	2.2± 0.2	7.8 ± 0.1	3	16.9 ± 1.5
Phase VIII	265-304	2.2± 0.6	7.9 ± 0.1	3	13.7 ± 2.4
Phase IX	305-335	1.5± 0.4	7.8 ± 0.2	3	15.7 ± 2.4
Phase X	335-360	1.3± 0.2	7.8 ± 0.0	2	16.0 ± 2.5

*Mean±S.D.

2.4 Anammox (AN) Reactor

The AN reactor was constructed from Plexiglas material with effective volume of 250 litres. The study was initiated with a temporary AN drum reactor (150 L) (Figure 2.5) and it was operated during construction period of pilot plant in order to start Anammox enrichment from mixed activated sludge. This temporary AN drum reactor was started-up via seeding with return activated sludge of Bursa Doğu STP. The initial MLVSS concentration was 2,100 mg/L. The drum reactor had been operated for 170 days. After pilot plant was commissioned, the sludge in drum reactor was transferred to the pilot scale AN reactor shown in Figure 2.6.

Figure 2-6. Temporary AN Drum Reactor

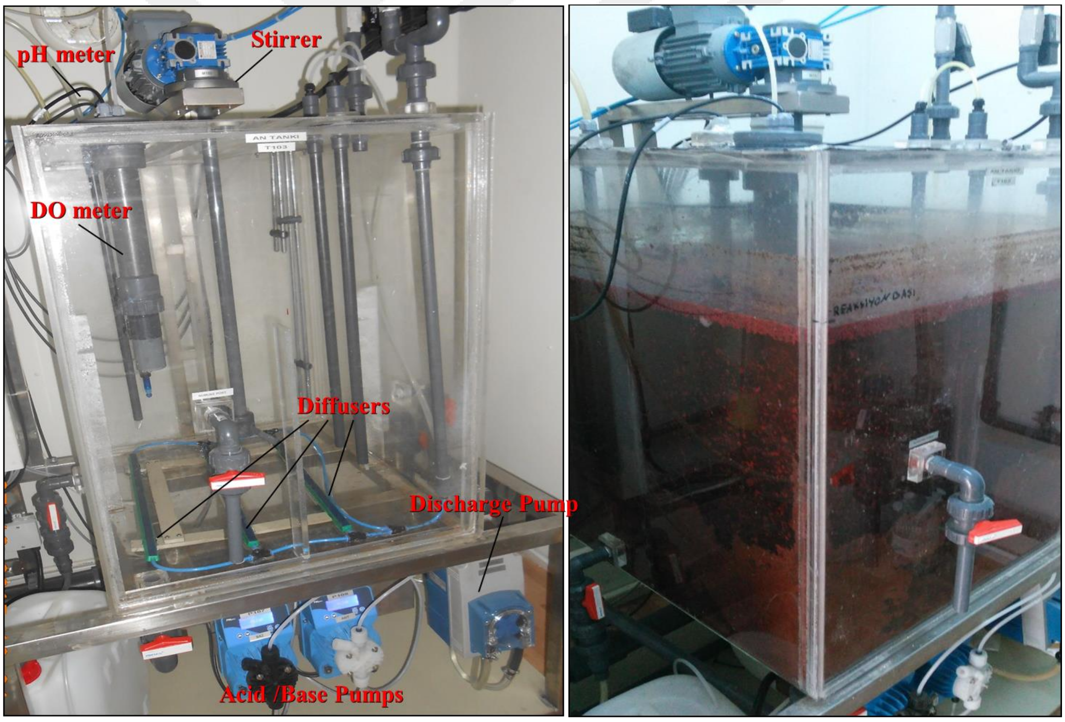


Figure 2-7. AN reactor

The pilot AN reactor (Fig. 2.7) consists of a mechanical mixer, diffusers influent, effluent, sludge, acid/base pumps. It was operated for 425 days as SBR with continuous flow of Bursa Doğu STP secondary clarifiers of effluent spiked with ammonium and nitrite.

The reactor was isolated completely to prevent air leakage into the reactor. However, the reactor was continuously flushed with 95% Ar + 5% CO_2 gas mixture to ensure strictly anaerobic conditions which is a must for Anammox bacteria. Homogeneous mixing in the reactor was provided with a mechanical mixer (Aqua) with operating a speed of 30 rpm. The process control strategies of the AN reactor were as follows;

(i) DO control; set DO level was maintained by controlling on/off gas mixture valves of reactor via on-line DO measurements,

(ii) pH control; set pH value was maintained by adjusting operation of proportional controlled acid/base pumps (Seko) via on-line pH measurements,

The AN reactor was operated as 24-hour batch cycles and each phase of the cycle was controlled by a PLC system.

The secondary clarifier effluent of Bursa Doğu STP was continuously spiked with NH_4^+-Cl, $NaNO_2$ and $NaHCO_3$ /$KHCO_3$ to simulate partially nitrified sewage and then fed to the AN reactor. During the first 30 days of operation, KNO_3 was also added to reactor in order to provide consumption of potential organic matter generated from endogenous decay activity. The spiking was gradually increased depending on Anammox activity of the reactor.

Daily influent and effluent grab samples were analysed for the presence of NH_4^+-N, NO_2^--N, NO_3^--N, TN and COD in order to assess ammonium, nitrite utilization, nitrate production rate and COD utilizations rates.

425 days operation period of the AN reactor was evaluated under six (6) different phases depending on treatment efficiencies. The major operation conditions are summarized in Table 2.2.

Table 2-2. Operation conditions of the AN reactor

Phases	Operation Days	DO (mg/l)*	pH*	Temperature
Phase I	0-22	0.05 ± 0.02	7.2 ± 0.2	29.6 ± 1.0
Phase II	22-95	0.09 ± 0.10	6.9 ± 0.3	21.0 ± 0.8
Phase III	96-129	0.56 ± 0.41	7.1 ± 0.5	15.4± 2.2
Phase IV	130-148	transfer from drum to pilot plant		
Phase V	149-235	0.06 ± 0.03	7.1 ± 0.4	20.2± 1.9
Phase VI	236-425	0.00	7.1 ± 0.8	22.2± 3.0

*Mean±S.D.

During 425 days operation periods NH_4^+-N and NO_2^--N loading rates to the reactor were increased gradually from 1.16 mg/d to 36.74 mg/d and 0.88 mg/L/d to 59.29 mg/d, respectively during the operation of the reactor. Table 2.3 summarizes the Anammox feed solution (spiked water).

Table 2-3. Spiked secondary clarifier effluent characteristics

Parameter	Min-max concentrations (mg/L)
TN	15-580
NH_4^+-N	1.3-220
NO_3^--N	2.5-224
NO_2^--N	0.1-355
COD	15-84

2.5 Analytical Methods

NH_4^+-N, NO_2^--N, NO_3^--N and TN concentrations of daily grab samples were taken from influent and effluent of both reactors, were analysed with WTW PhotoLab 6100 VIS spectrophotometer (Figure 2.7) by using Spectroquant Reactive Test Kit No: 14763, 14544, 14776 and 09713, respectively. COD concentrations were analysed in accordance with USEPA Approved method 5220 D with WTW PhotoLab 6100 VIS spectrophotometer. Weekly MLSS and MLVSS measurements were done in accordance with Standard Methods (1998).

2.6 Molecular Analysis of Sludge Samples

During operation of PN and AN reactors, monthly sludge samples were taken for the molecular analysis of bacteria species. Samples were stored at -20°C temperature for DNA extraction.

Whole microbial community composition and its dynamics in the reactors was monitored via high resolution melt (HRM) analysis based on melting peak molecular fingerprints. It has high-throughput capacity and short assay time, therefore large number of samples can be rapidly scanned for community structure (Hjelmsø et al., 2014). Different DNA sequences have different melting temperatures and they also have different melting peaks in HRM. HRM method was a robust, fast and reliable method for differentiation of methanogenic communities from different anaerobic digesters (Kim and Lee, 2014). Melting curve analyses were applied between 60°C-95°C at a florescence reading rate of 0.1°C/acquisition for HRM analysis. HRM profiles analysis was done as described by Reja et al. (2010). Microbial community profile dendrograms were obtained based on the Pearson distance using Minitab 17 software. The standard DNA's were prepared in the range of $1x10^5$- $1x10^9$ with cloned samples. Absolute quantitation was performed.

Quantitative analysis of AOB, NOB and comammox were performed to evaluate their population dynamic via Quantitative Polymerase Chain Reaction (qPCR). DNA of both sludge samples were extracted with Fastprep DNA extraction kit for soil (QBiogene, Carlsbad, CA) by following manufacturer's protocol. qPCR and HRM analysis were performed on SteponePlus (Applied Biosystems, USA) using evagreen Supermix (Biorad, USA). Primer sets of PCR amplification for Anammox reactor and KN reactor samples were given in the Table 2.4 and 2.5, respectively.

Table 2-4. Primer sets of PCR amplification- AN reactor

Primer	Sequence	Target	Annealing	References
341f	CCTACGGGAGGCAGCAG	bacterial 16S		
534r	TTACCGCGGCTGCTGGCAC		60	Koike et al.. 2007
la46f	GGATTAGGCATGCAAGTC	anammox 16S		
Amx368R	CCTTTCGGGCATTGCGAA		59	Schmid et al.,2003
amoA-1f	GGGGTTTCTACTGGTGGT	AOB amoA gene		
amoA-2r	CCCCTCKGSAAAGCCTTCTTC		55	Rotthauwe et al.,1997
ARC344F	ACGGGGYGCAGCAGGCGCGA	archaea 16S		
Arch806R	GGACTACVSGGGTATCTAAT		52	Raskin et al., 1994
amoA-AF	ATGGTCTGGCTWAGACG	AOA amoA gene		
amoA-AR	GCCATCCATCTGTATGTCCA		61	Francis et al., 2005

Table 2-5. Primer sets of PCR amplification- PN reactor

Primer	Sequence	Target	Annealin	References
341f	CCTACGGGAGGCAGCAG	bacterial 16S		
534r	TTACCGCGGCTGCTGGCAC		60	Koike et al., 2007
Pla46f	GGATTAGGCATGCAAGTC	anammox 16S		
Amx368R	CCTTTCGGGCATTGCGAA		59	Schmid et al.,2003
amoA-1f	GGGGTTTCTACTGGTGGT	AOB amoA gene		
amoA-2r	CCCCTCKGSAAAGCCTTCTTC		55	Rotthauwe et al.,1997
ARC344F	ACGGGGYGCAGCAGGCGCG	archaea 16S		
Arch806R	GGACTACVSGGGTATCTAAT		52	Raskin et al., 1994
amoA-AF	ATGGTCTGGCTWAGACG	AOA amoA gene		
amoA-AR	GCCATCCATCTGTATGTCCA		61	Francis et al., 2005
P338f	ACTCCTACGGGAGGCAGCAG	NOB 16S rRNA genes of Nitrobacter		
NIT3	CCTGTGCTCCATGCTCCG		65	Regan et al. (2002)
NSR1113f	CCTGCTTTCAGTTGCTACCG	NOB 16S rRNA genes of Nitrospira		
NSR1264r	GTTTGCAGCGCTTTGTACCG		60	Dionisi et al. (2002)

3. CHAPTER 3 - RESULT AND DISCUSSION

3.1 Partial Nitritation of Domestic Wastewater

PN reactor has been operated for 360 days under continuous-flow condition with grit chamber effluent of Bursa Doğu STP. The operation period of the pilot scale PN reactor was divided into 10 (ten) phases depending on temperature, DO concentration, pH and SRT changes in the reactor. Figure 3.1 shows daily average temperature, DO concentration, pH values calculated from online measured parameters in each batch cycle. FA concentrations shown in Fig. 3.2 were calculated in accordance with formula shown in Eqn. 1.9.

During one-year operation period, wastewater temperature, DO and pH ranged between 13-24°C, 0.6-2 mg/L and 7.7-8.1, respectively. SRT was adjusted between 2-5 days. Table 3.1. summarizes all operational conditions of the PN reactor. Based on the similarities in operating conditions, 10 operation phases were evaluated under 4 sub-groups: *Sub-group I*: Phases I-IV (April-September, 2016), *Sub-group II:* Phases V-VI (September-November, 2016), *Sub-group III:* Phases VII-VIII (November 2016-January 2017) and *Sub-group IV:* Phases IX-X (February-April, 2017).

TN, NH_4^+-N, NO_2^--N and NO_3^--N measurements of daily influent and effluent grab samples are given in Figure 3.3. Daily influent and effluent COD analysis results are given in Figure 3.4. In order to evaluate partial nitritation efficiency of the PN reactor during 1-year operation period, NH_4^+-N and NO_2^--N accumulation percentages and NO_3^--N consumption/production percentages were calculated based on daily measured influent and effluent TN, NH_4^+-N, NO_2^--N and NO_3^--N (Figure 3.3). These values are shown in Figure 3.5, 3.6, 3.7 and 3.8.

Phases I, II, III and IV, under *sub-group I* (April to September), were operated for 158 days. In this *Sub-group I (Table 3.1)*, wastewater temperature raised gradually from 20°C to 24°C. DO was adjusted in the range of 0.6 – 1.2 mg/L. pH was initially kept at a high level (8.1±0.4) to increase FA and then decreased to the levels of 7.8±0.3. SRT was adjusted between 4-5 days.

In Phase I, wastewater temperature was at 19.9 ±1.2°C. DO concentration and pH were kept around 0.6±0.4 mg/l and 8.1±0.4, respectively. SRT was 4 days. Total operation time

Table 3-1. Summary of Operation Phases of PN Reactor

Time Period	Phases	Sludge Samples	Temp. (°C)	DO (mg/L)	pH	FA (mg/L)	SRT (day)	Tot-N Inf. (mg/L)	Tot. NH_4^+-N Inf. (mg/L)	COD Inf. (mg/L)	sCOD Inf. (mg/L)
Apr-16	Phase I	KN S1	19.9 ± 1.2	0.6 ± 0.4	8.1 ± 0.4	1.12±0.6	4	15-58	10-30	447.2 ± 47	145.5 ± 15
May-16	Phase II	KN S2	20.3 ± 1.3	1.2 ± 0.3	7.8 ± 0.2	0.76±0.3	5	23-49	17-39	462 ± 76	150 ± 25
Jun-16 July-16	Phase III	KN S3 KN S4 KN S5	22.7 ± 1.0	0.7 ± 0.2	7.8 ± 0.1	0.77±0.1	5	28-48	21-37	526 ± 84	171 ± 27
Aug-16 Sep-16	Phase IV	KN S6	23.9 ± 1.3	1.1± 0.1	7.8 ± 0.3	1.1±0.6	4	27-51	18-53	525 ± 79	171 ± 26
Sep-16 Oct-16	Phase V	KN S7 KN S8	21.0 ± 1.5	1.1± 0.2	7.7 ± 0.2	0.62±0.3	3	34-49	20-39	559 ± 92	182 ± 30
Oct-16 Nov-16	Phase VI	KN S9 KN S10	18.6 ± 1.8	1.2± 0.4	7.8 ± 0.1	0.87±0.4	4	24-72	20-58	645 ± 113	210 ± 35
Nov-16 Dec-16	Phase VII	KN S11	16.9 ± 1.5	2.2± 0.2	7.8 ± 0.1	0.75±0.4	3	33-62	20-55	712 ± 126	232 ± 41
Jan-17	Phase VIII	KN S12	13.7 ± 2.4	2.2± 0.6	7.9 ± 0.1	0.76±0.3	3	28-150	20-58	566 ± 122	184 ± 40
Feb-17	Phase IX	KN S13 KN S14	15.7 ± 2.4	1.5± 0.4	7.8 ± 0.2	0.70±0.2	3	47-80	17-53	648 ± 68	204 ± 43
Mar-17 Apr-17	Phase X	KN S15 KN S16	16.0 ± 2.5	1.3± 0.2	7.8 ± 0.0	0.56±0.3	2	36-55	25-41	667 ± 93	209 ± 52

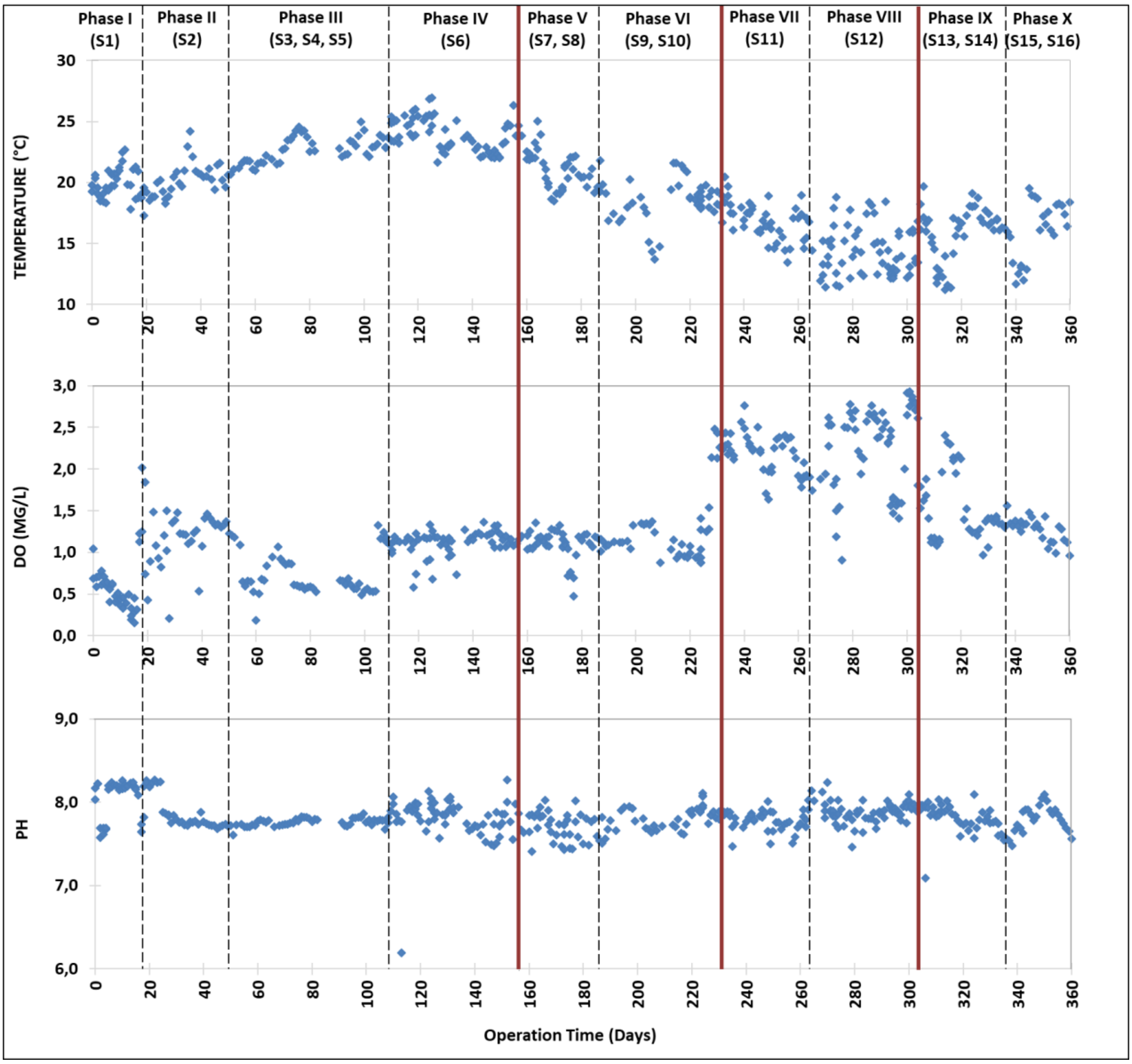


Figure 3-1. Operation conditions (real time Temperature, DO and pH) of the PN reactor

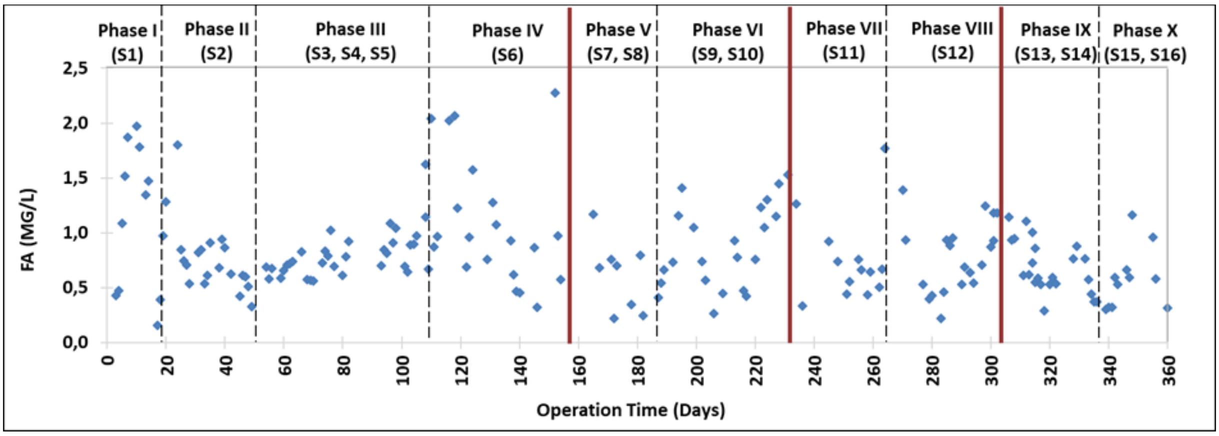


Figure 3-2. FA concentrations of the PN reactor

was 19 days. The observed differences between influent and effluent TN values indicated simultaneous nitrification and denitrification processes in the reactor. The average NH_4^+-N accumulation and NO_3^--N consumptions (Fig. 3.5) were around 57% and 7% respectively. NO_2^--N accumulation around 10 % was observed only in a few operational days. NH_4^+-N and NO_2^--N accumulations might be due to high FA levels (1.12 ±0.6 mg/l) in this period. During these days, DO fluctuations were high both in each cycle measurements and in daily basis measurements. The effluent NO_2^--N:NH_4^+-N ratio was monitored around 0.30±0.3 which is lower than the requirement of Anammox process. sCOD removal efficiency (Fig. 3.4) was 75%. qPCR analysis results (Fig. 3.5) demonstrated that population of the AerAOB in total bacterial species were very high (10^7). Nitrobacter (NOB) and Nitrospira (NOB) coexisted and Nitrobacter population (10^4) were dominant compared to Nitrospira population (10^2). The population of AnAOB were determined very low (10^2). Total Archea population was 10^4. But, no Ammonium-oxidizing Archea (AOA) were detected in the reactor.

In Phase II, wastewater temperature was at 20.3 ±1.3°C. DO level was increased to 1.2 mg/L to decrease NH_4^+-N accumulation and increase NO_2^--N accumulation. DO fluctuations were significantly less than Phase I. pH level decreased and was kept around 7.8. SRT was increased to 5 days. Total operation time was 34 days. Initially (during 7 days), NH_4^+-N accumulation was very high (around 90%), and no NO_2^--N and NO_3^--N productions were observed. This sharp increase in accumulation rate might be due to sudden change in FA level from 0.43 to 1.87 mg/L during these few days. Another reason of this sudden increase in NH_4^+-N accumulation might be the shock loads coming to the Dogu STP from textile industries. In the following days, while NH_4^+-N accumulation gradually decreased to the level of 23%, NO_2^--N accumulation and NO_3^--N production were observed around 42% and 22%, respectively. Increase in DO level minimized denitrification activity. FA levels around 0.76 mg/l favoured NOB inhibition. But, NO_3^--N production could not be prevented. The effluent NO_2^--N:NH_4^+-N ratio was fluctuated seriously and observed around 1.33 ±1.86 which is proper for Anammox process. sCOD removal efficiency (Fig. 3.4) was 75%. The qPCR results (Fig. 3.5) showed that while AerAOB and Nitrospira population increased one log, there were no significant change in Nitrobacter population. AnAOB and Archea population increased one log. AOA species were detected as well.

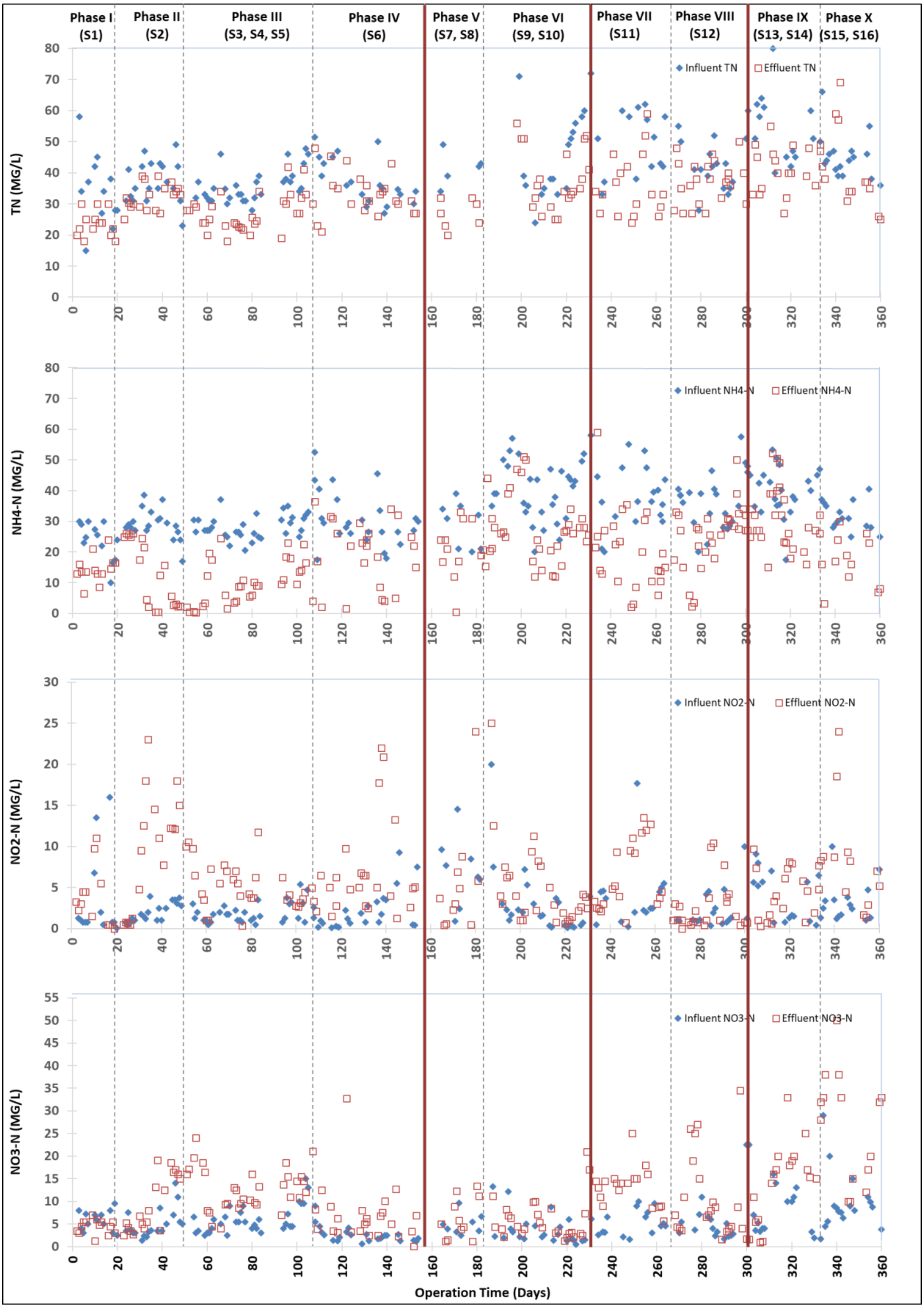


Figure 3-3. Influent and effluent N measurements of the PN reactor

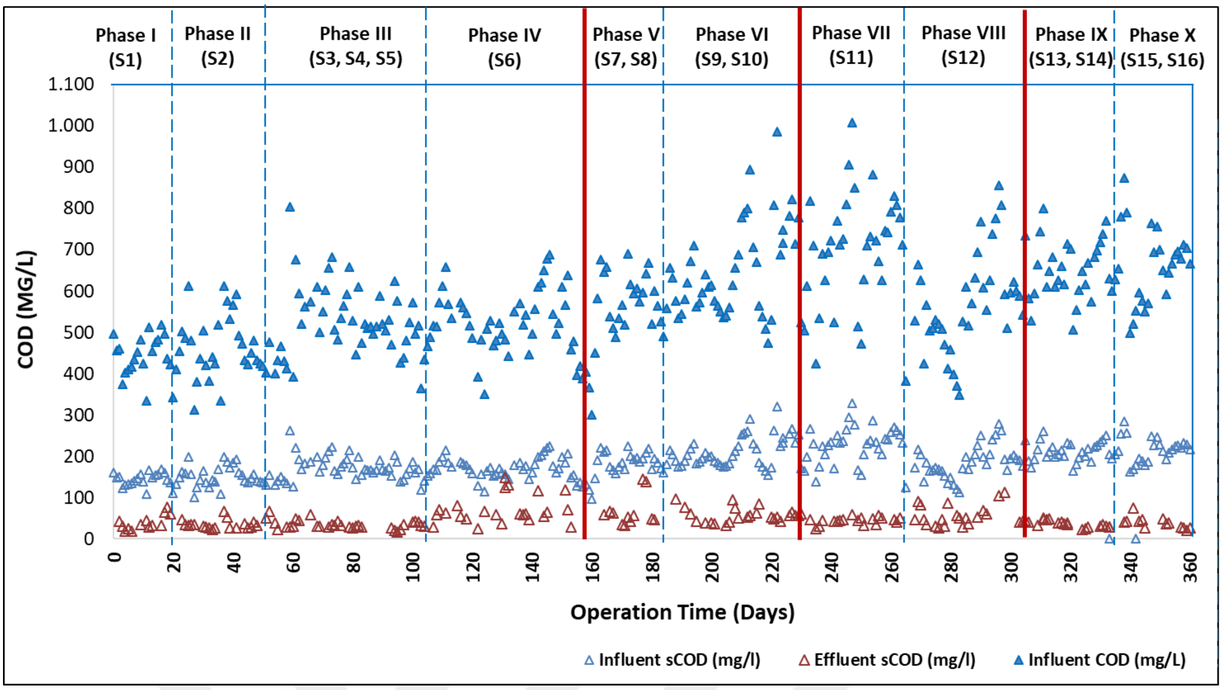


Figure 3-4. Influent and effluent COD measurement of PN reactor

In Phase III, wastewater temperature was at 22.7 ±1.0°C. DO level was decreased to 0.7 mg/L to adjust NH_4^+-N and NO_2^--N accumulations. pH level was kept around 7.8 ±0.1. SRT was around 5 days. Total operation time was 54 days. Initially, during a few days of operation, NH_4^+-N and NO_2^--N accumulations and NO_3^--N production were observed around 5%, 17% and 58%, respectively. That indicated almost full nitrification took place in the reactor mainly due to high DO level up to 1.2 mg/L. Besides, low level of NO_2^--N accumulation might be due to FA concentration around 0.77 mg/L. In the following 24 days of operation, decreasing DO levels due to increasing wastewater temperature resulted with NH_4^+-N accumulation (62%) and NO_2^--N accumulation (12 %) and minimized less NO_3^--N production (12%). However, after 68 days of operation, sharp decrease in NH_4^+-N accumulation (28%) occurred. In the same period, there was no significant changes in NO_2^--N accumulation (15%) and NO_3^--N production (21%). This might be due to decrease in AerAOB population during previous days of this period. The overall calculated effluent NO_2^--N: NH_4^+-N ratio was around 0.88 ±0.90 which is not very far away from Anammox process requirement. sCOD removal efficiency was 80%. qPCR results indicated 2-log decrease in copy number of AerAOB (from 10^8 to 10^6) and Nitrospira (from 10^3 to 10^1). There were no changes in copy numbers of Nitrobacter and

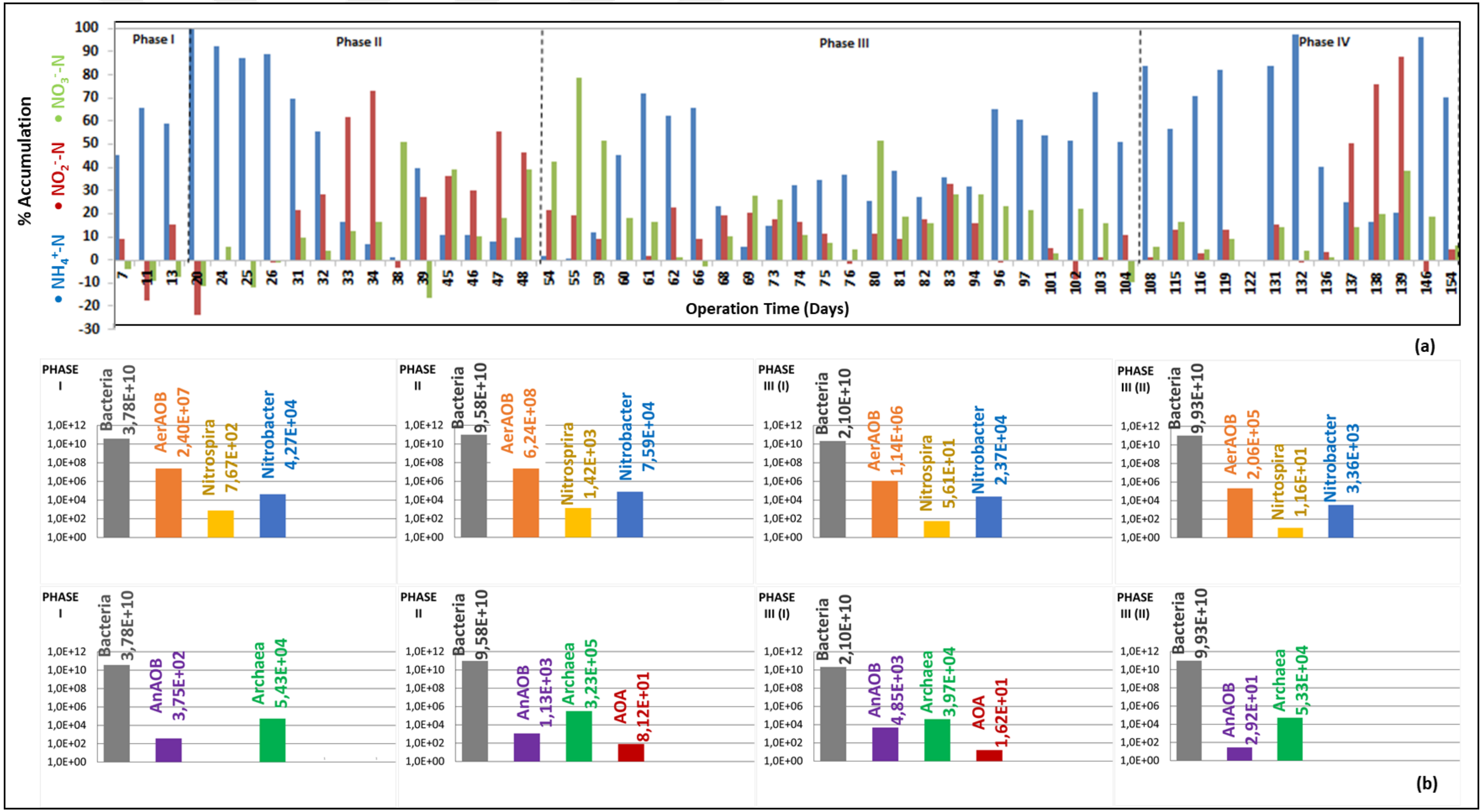


Figure 3-5. (a) Ammonia accumulation, nitrite accumulation and nitrate production of PN reactor (Phase I-IV), (b) Copy number of species determined for Phase I-IV

AnAOB species. Total Archea population decreased 1 log (from 10^5 to 10^4). But, there was no change in AOA population.

In phase IV, wastewater temperature increased to 23.9 ±1.3°C. DO level was increased again to 1.1±0.1 mg/L to decrease NH_4^+-N accumulation. pH level was 7.8±0.3. SRT was decreased to 4 days in order to further washout NOB species from the system. Total operation time was 50 days. In the first 23 days (between 108-131) of operation, increase in influent NH_4^+-N (30-53 mg/L) to the plant, increase in wastewater temperature (25-26°C) resulted with increase in FA to the levels of 1.3 mg/L. The significant increase in FA caused very high NH_4^+-N accumulation (56-84%). Since the NH_4^+-N could not be removed, there were very low NO_2^--N accumulation (12%) and NO_3^--N production (10%) in this period. In the following 7 days (between 132-139), FA decreased to the range of 0.7-1 mg/L due to the changes in the influent NH_4^+-N (30 mg/ L) and wastewater temperature (22°C). Decrease of FA resulted to observe less NH_4^+-N accumulation (17-41%) and more NO_2^--N accumulation (50-88%). NO_3^--N production ranged between 2-15%. The changes observed in NH_4^+-N and NO_2^--N accumulation percentages regarding to changes in FA concentrations are in consistency with the past studies. As it was mentioned in section 1.4.2.3, while inhibition of NOB begins at 0.1–1.0 mg/l of FA, AerAOB inhibition occurs at 10 to 150 mg/l. In the last 20 days of Phase IV, even there was no increase in FA concentration significant NH_4^+-N accumulation (%70-%96) started again. There were no any other operational condition changes in the same period. Therefore, this sudden increase in NH_4^+-N accumulation might be due to shock loads coming to the Doğu STP from textile industries, similar to the Phase 2. The overall effluent NO_2^--N: NH_4^+-N ratio of Phase IV was around 1.2 ±1.9, which is proper for Anammox process. In contrast to Phases I-III, sCOD removal efficiency decreased to 60 %. Unexpectedly, no quantification of any species can be done in molecular samples most probably due to problems in DNA extraction.

Phases V and VI, under *sub-group II* (September to November), were operated for 94 days. In this *Sub-group II (Table 3.1)*, wastewater temperature decreased gradually from 21°C to 18.6°C. DO was kept at 1.1 mg/L. pH was kept at a high level 7.7 to 7.8. SRT was adjusted between 3-4 days to provide washout of NOB.

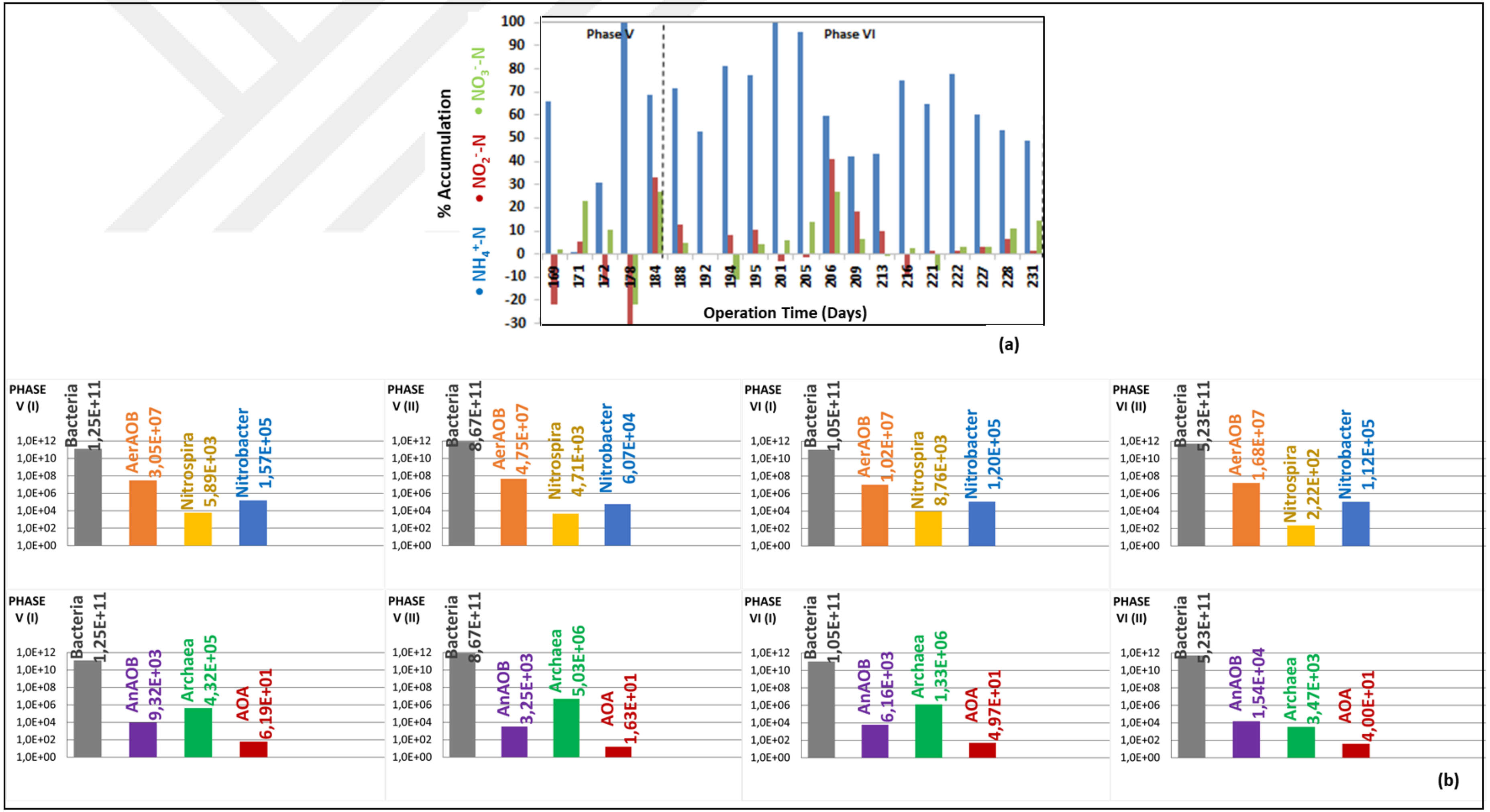


Figure 3-6. (a)Ammonia accumulation, nitrite accumulation and nitrate production of PN reactor (Phase V-VI), (b) Copy number of species determined for Phase V-VI

In phase V, wastewater temperature was at 21.0±1.5°C. DO level was kept around 1.1 mg/L. pH level was 7.7 and SRT was decreased to 3 days. Total operation time was 38 days. In this period, it was observed decreased in inlet NH_4^+-N concentrations (20-39 mg/L) resulted with decreased in FA concentration (0.62 mg/L). In addition, wastewater temperature decreased from almost 24 °C to 21°C due to seasonal changes compared to previous phase. As a result of these minor changes and minor fluctuations in FA resulted with very unsteady conditions in the reactor and AerAOB and NOB were affected adversely. As it was mentioned in section 1.4.3, AerAOB and NOB have different tolerance to FA concentrations and temperature changes due to having different growth rates. While FA concentration was higher than 1 mg/L, Ammonia Oxidizing Bacteria (AOB) inhibition was observed and resulted with NH_4^+-N accumulation, NOB inhibition was observed while $0.7<FA< 1$ mg/L and resulted with NO_2^--N accumulation. For example, the first 13 days of operation, the average FA concentration was 1.2 mg/L and NH_4^+-N accumulation (32.7%), low NO_2^--N consumption (9.6%) and low NO_3^--N production (11.9%) were observed. This is the indication of nitrification followed by denitrification took place in the reactor. In the following 6 days (between 172-178) of operation, DO values fluctuations were observed due to some mechanical problems. DO concentration in the reactor was around 0.5-1.1 mg/L. While NH_4^+-N accumulation ratio was increased to its peak value even through low FA concentration (0.6 mg/L), NO_2^--N consumption ratio was increased to 40% and NO_3^--N consumption was increased to 21.5%. It was obvious seen that fluctuations and decrease in DO level minimized nitrification activity and increase the denitrification activity. In the last 7 days of this period, DO fluctuations were minimized and significantly NO_2^--N accumulation (%33) was observed while NH_4^+-N accumulation was 69.1% and NO_3^--N production was 27.2%. It also might be due to low FA (0.52 mg/L) concentration in the reactor. The effluent NO_2^--N: NH_4^+-N ratio was calculated around 0.05 ±0.07 which is much lower than the requirement of Anammox process. sCOD removal efficiency (Fig. 3.4) was around 65-75%.

During this period, two sludge samples were taken in different time period. No significant change in copy number (Fig. 3.6) of AerAOB and NOB with respect to total bacteria population were observed

In phase VI, wastewater temperature was at 18.6±1.8°C. DO level was 1.1±0.2 mg/L. pH level was 7.7±0.2 and SRT was increased to 4 days. Total operation time was 47 days. During the operation of this phase, fluctuations in FA (0.26-1.54 mg/L) and DO concentrations were observed. As it was seen in figure 3.6, where FA concentrations is higher than 1 mg/L, NH_4^+-N accumulation was observed due to AerAOB inhibition. NO_2^--N accumulation was observed when FA concentration was in between 0.7-1.0 mg/L due to NOB inhibition. For example, in the days between 202 and 213, 60% NH_4^+-N accumulation, 23% NO_2^--N accumulation and 10% NO_3^--N production were observed when the average FA concentration was 0.7 mg/L in the reactor. On the other hand, in the last period of this phase, FA concentration higher than 1.0 mg/L caused to observe, 66% NH_4^+-N accumulation, 1% NO_2^--N accumulation and 4.5% NO_3^--N production. This observation indicates that, both AerAOB and NOB were affected adversely with high concentration of FA. The effluent NO_2^--N: NH_4^+-N ratio was calculated around 0.19 ±0.24 which is significantly lower than the requirement of Anammox process. sCOD removal efficiency (Fig. 3.4) was around 65-75%. The qPCR results (Fig. 3.6), indicated that many species increased in number but when compared with total bacteria populations which was around 10^{11}, no significant change was observed in each species with respect to total bacteria population.

Phases VII and VIII, under *sub-group III* (November to January), were operated for 73 days. In this *Sub-group III (Table 3.1)*, wastewater temperature decreased gradually from 17°C to 14°C. DO was kept at 2.2 mg/L. pH was kept at a high level 7.8 to 7.9. SRT was kept 3 days.

In phase VII, wastewater temperature was at 16.9 ±1.5°C. DO level was increased to 2.2 mg/L. pH level was 7.8 and SRT was decreased to 3 days. Total operation time was 33 days.

As it was mentioned in sections 1.4.1 and 1.4.2, AerAOB and NOB have different sensitivities against DO and temperature. At low temperature, NH_4^+-N tends to accumulate due to lower specific growth rate of AerAOB compared to NOB at temperature below 25°C. Additionally, the K_o for AerAOB is lower than the K_o values of NOB at temperature below than 25°C. Therefore, DO level was increased to 2.2 mg/L to decrease NH_4^+-N accumulation. During first few days of this phase (days of 231-240)

NH_4^+-N accumulation (90%), slightly NO_2^--N accumulation (6%) and NO_3^--N production (38%) were observed due to low temperature. In the last few days of this phase, NO_2^--N started to accumulate (21.3%) with NH_4^+-N accumulation of 46.7% and NO_3^--N production (19%) were observed despite of low temperature. This observation might be due to the inhibition of NOB with 0.74 mg/L FA in the reactor. The effluent NO_2^--N: NH_4^+-N ratio was around 0.82 ±1.29 which is not very far away from than the Anammox process requirement. sCOD removal efficiency was around 75-80%.

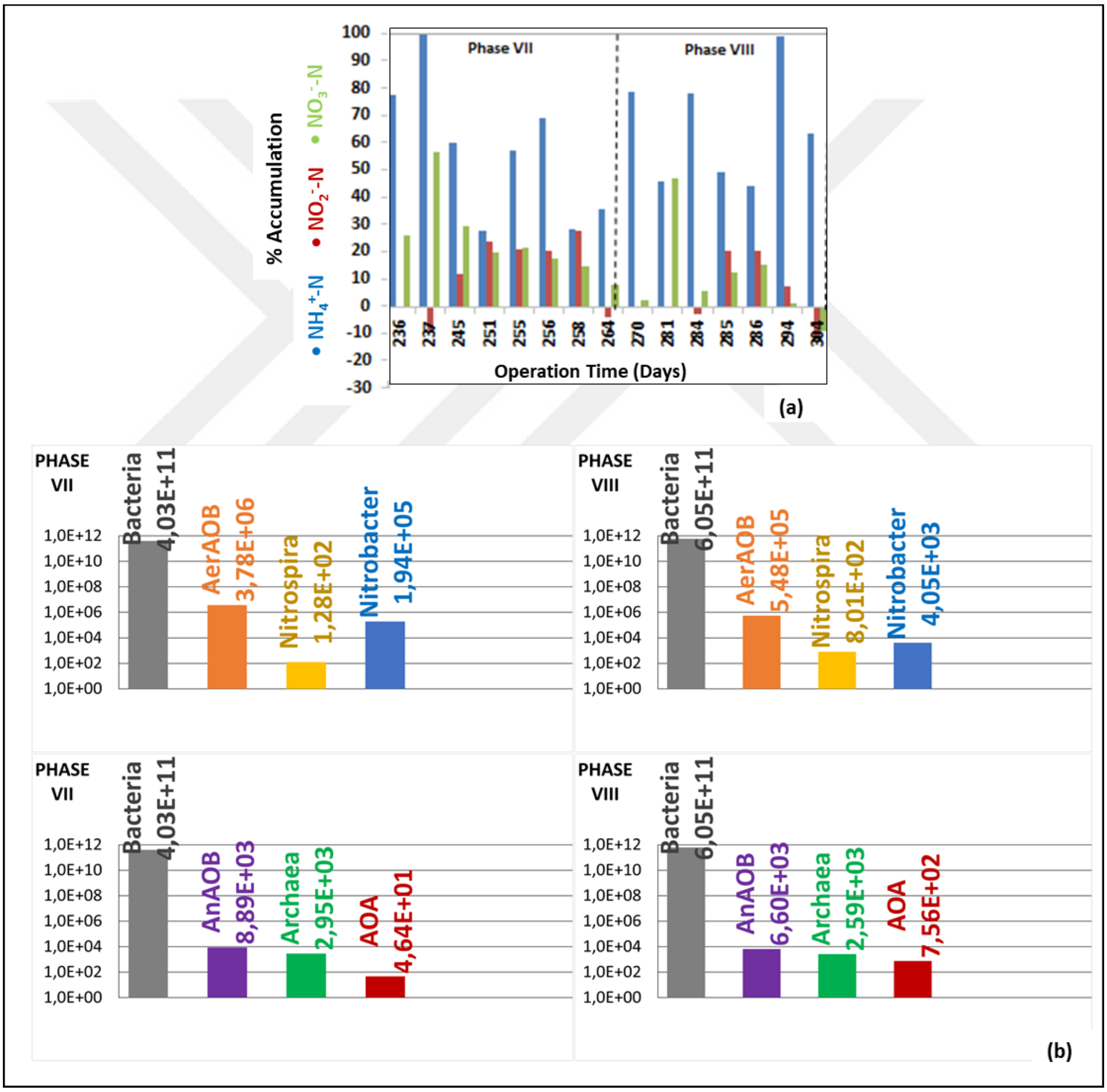


Figure 3-7. (a) Ammonia accumulation, nitrite accumulation and nitrate production of PN reactor (Phase VII-VIII), (b) Copy number of species determined for Phase VII-VIII

The qPCR analysis results (Fig. 3.7) showed that no significance change in the population of the almost all species.

In phase VIII, wastewater temperature decreased to 13.7±2.4°C. DO level was kept at 2.2 mg/L. pH level was 7.9 and SRT was 3 days. Total operation time was 40 days. Initially (days 265-284), significant NH_4^+-N accumulation (avg. 68%), almost no NO_2^--N accumulation and slight NO_3^--N production (avg. 18.6%) were observed. During last period of this phase (days 285-304), NO_2^--N started to accumulate (avg. 16.5%) NH_4^+-N accumulation percentage decreased to 64%, and NO_3^--N production percentage decreased to 5.5%. That showed the inhibition of NOB and AerAOB due to fluctuations in FA concentrations (0.45-0.1.18 mg/L) in the reactor. The effluent NO_2^--N: NH_4^+-N ratio was calculated around 0.18±0.22 which is significantly lower than Anammox process requirement. sCOD removal efficiency was 75-80 %. The qPCR results indicated 2-log decrease in copy number of Nitrobacter (from 10^5 to 10^3). No changes in the copy number of Nitrospira and AerAOB (Fig. 3.7). This shows washout of NOB in the system due to low SRT.

Phases IX and X, under *sub-group X* (February to April), were operated for 56 days. In this *Sub-group IV (Table 3.1)*, wastewater temperature raised gradually from 13.7°C to 16°C. DO was adjusted in the range of 1.3 – 1.5 mg/L. pH was kept at 7.8. SRT was adjusted between 2-3 days.

In Phase IX, wastewater temperature was at 15.7 ±2.4°C. DO concentration and pH were kept around 1.5±0.4 mg/l and 7.8±0.2, respectively. SRT was 4 days. Total operation time was 32 days. The observed differences between influent and effluent TN values indicated simultaneous nitrification and denitrification processes in the reactor as in Phase I. The average NH_4^+-N accumulation and NO_3^--N production (Fig. 3.8) were around 55% and 13% respectively. NO_2^--N accumulation around 28 % was observed. The effluent NO_2^--N:NH_4^+-N ratio was monitored around 0.32±0.63 which is lower than the requirement of Anammox process. sCOD removal efficiency (Fig. 3.4) was 80%. The qPCR results (Fig. 3.8) showed that there were almost no changes in all species population.

In Phase X, wastewater temperature was at 16.0 ±2.5°C. DO concentration and pH were kept around 1.3±0.2 mg/l and 7.8±0.0, respectively. SRT was decreased from 3 days to 2 days. Total operation time was 26 days. The average NH_4^+-N accumulation and NO_3^--N consumptions (Fig. 3.8) were around 61% and 18% respectively. NO_2^--N accumulation around 23 % was observed. The effluent NO_2^--N:NH_4^+-N ratio was monitored around 0.54±0.32 which is lower than the requirement of Anammox process. sCOD removal efficiency (Fig. 3.4) was 80%. In the first 10 days of this period, the AerAOB population increased 2 log (from 10^6 to 10^8), Nitrobacter population increased 1 log (from 10^4 to 10^5), and Nitrospira population decreased 1 log (from 10^4 to 10^3). But, there were no changes in copy numbers of AnAOB and Archea species. In the last 10 days of this period, the AerAOB population decreased 2 log (from 10^8 to 10^6). Nitrobacter population decreased 4 log (from 10^5 to 10^1). There were no changes in copy numbers of Nitrospira, AnAOB and Archea species. This shows the washout of the Nitrobacter in the reactor might be due to decrease in SRT.

During the operation of the reactors with different operation conditions, NO_2^--N accumulated most of the operational period. It was not the only limiting step, NO_3^--N production rate was also considered as limiting step as well as nitrite accumulation. The reduction of the reactor DO set value caused reduce in NO_2-N accumulation rate and NO_3^--N production rate whereas increase in NH_4-N consumption rate. That means, lowering operation DO concentration affected nitritation performance, but not so much. This finding confirms other studies, were a lower DO did also not suppress NOB.

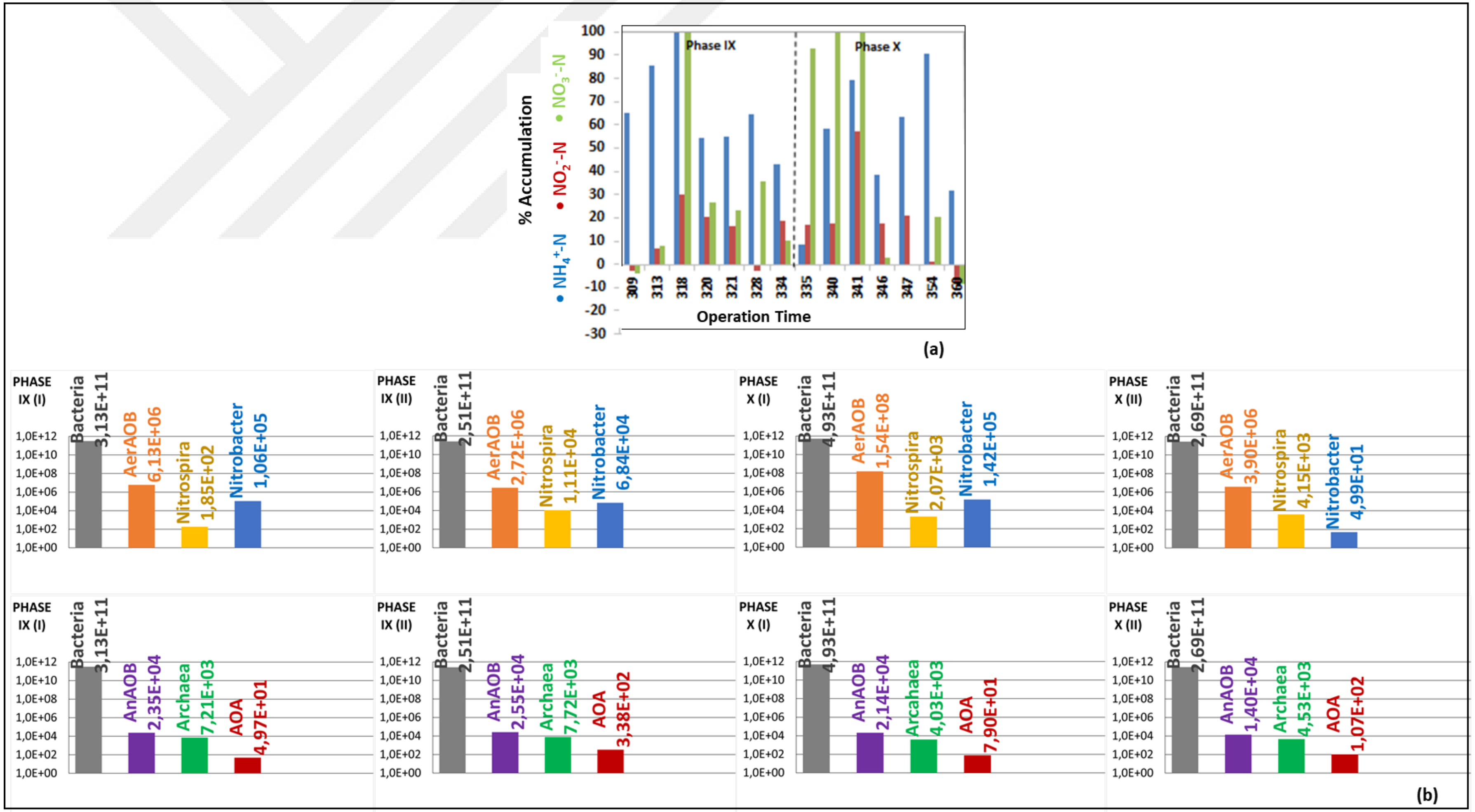


Figure 3-8. (a)Ammonia accumulation, nitrite accumulation and nitrate production of PN reactor (Phase IX-X), (b) Copy number of species determined for Phase IX-X.

Molecular Analysis of PN Samples

The evaluation of microbial structure in PN with HRM based on melting peak molecular fingerprints is shown in Figure 3.9 for total bacteria, AerAOB, AnAOB and Figure 3.10 for Nitrobacter, Nitrospira Total Archea and AOA.

Total bacteria HRM results (Figure 3.9) demonstrated that KNS4 (mid Phase III) is clustered separately and very dissimilar to other samples. KNS3 (early Phase III) is also clustered solely and its similarly to other samples was around 68%. Other samples were very similar to each other. AerAOB dendogram (Figure 3.9) showed two district clusters. KNS4 (mid Phase III), KNS5 (late Phase III), KNS11(Phase VII), KNS12 (Phase VIII) and KNS15 (Phase X) clustered together and their similarly to other clades was around 50%. Other samples clustered together. But, KNS3 was slightly dissimilar to other samples in the cluster. AnAOB species (Figure 3.9) demonstrated two separate clusters. These clusters were quite dissimilar to each other. The big cluster was also divided two clusters. Their similarity was around 70%.

Nitrobacter and Nitrospira species (Figure 10) have single peak. Therefore, HRM analysis is not applicable for these species.

HRM of archaeal 16S rRNA genes showed two distinct clades. AOA HRM analysis showed similar dendogram to archeal dendogram.

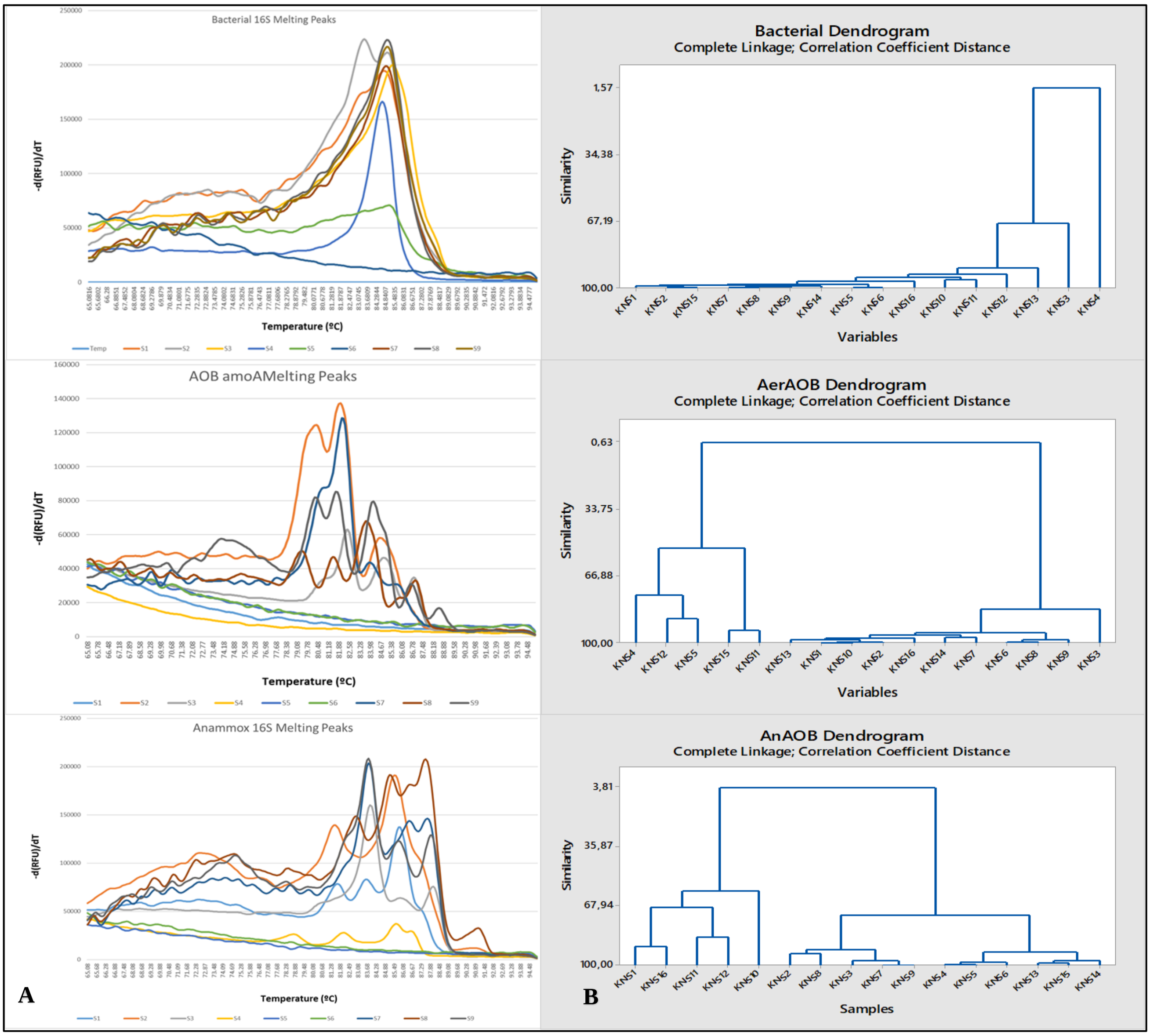


Figure 3-9. HRM analysis of Bacteria, Anammox, AerAOB and AnAOB genes from the samples Melting peak plots (a), and dendogram of samples diversity similarities based on HRM profiles (b)

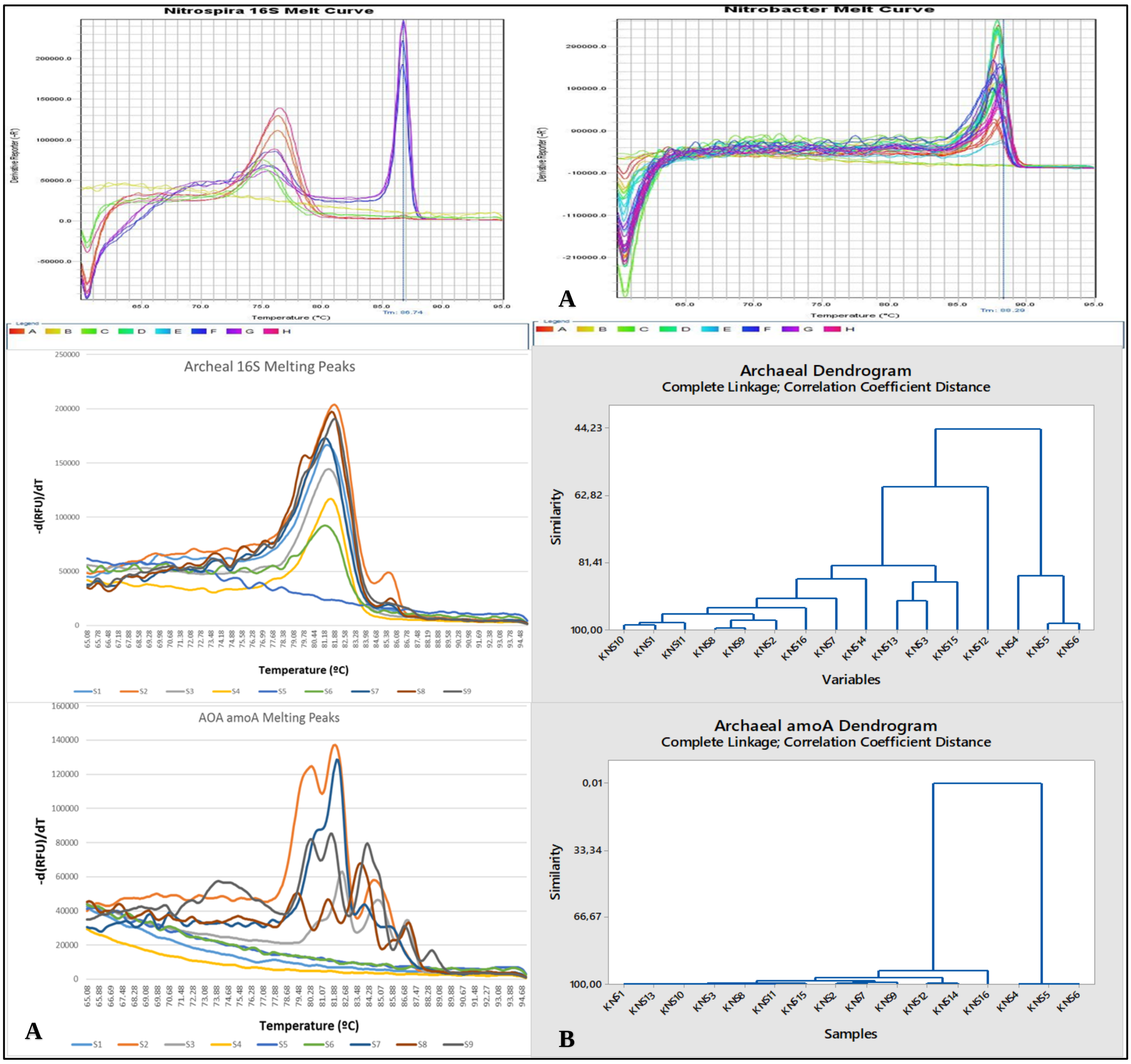


Figure 3-10. HRM analysis of Nitrospira genes, Nitrobacter genes, Archaeal and Archaeal amoA from the samples Melting peak plots (a), and dendogram of samples diversity similarities based on HRM profiles (b)

3.2 Enrichment of Anammox Bacteria Using Local Municipal Activated Sludge

AN reactor has been operated for 425 days under continuous-flow condition with secondary clarifier effluent of Bursa Doğu STP spiked with ammonium and nitrite. The operation period of the pilot scale AN reactor was divided into 6 (six) phases based on nitrogen constituent's changes in the reactor. Figure 3.12 shows daily average temperature, DO concentration, pH values calculated from on-line measured parameters in each batch cycle. Table 3.3. summarizes all operational conditions of the AN reactor. TN, NH_4^+-N, NO_2^--N and NO_3^--N measurements of daily influent and effluent grab samples are given in Figure 3.13. In order to evaluate Anammox activity of the reactor, consumed ΔNO_2^--N: consumed ΔNH_4^+-N and produced ΔNO_3^--N : consumed ΔNH_4^+-N were calculated based on daily measured influent and effluent NH_4^+-N, NO_2^--N, NO_3^--N values (Figure 3.13). These values are shown in Figure 3.14.

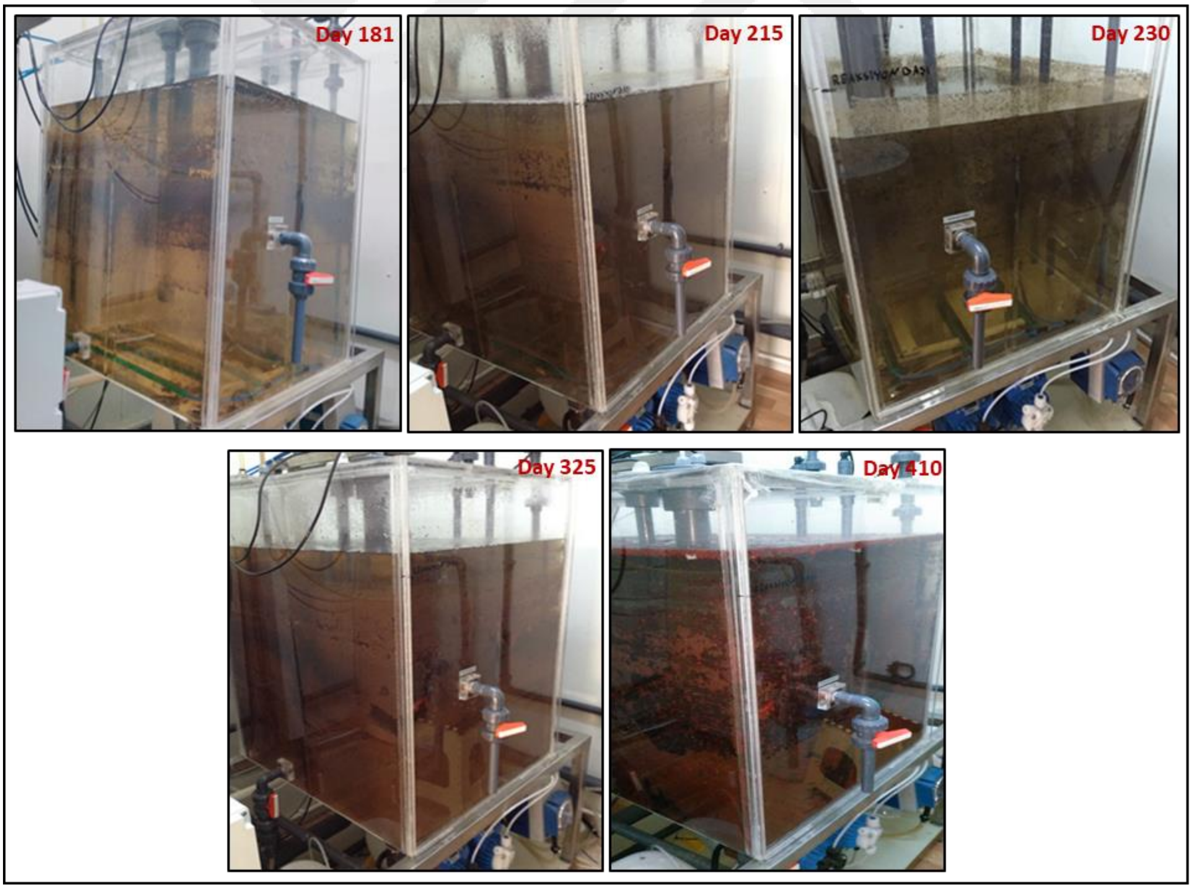


Figure 3-11. The photos of AN Reactor during the different operation periods

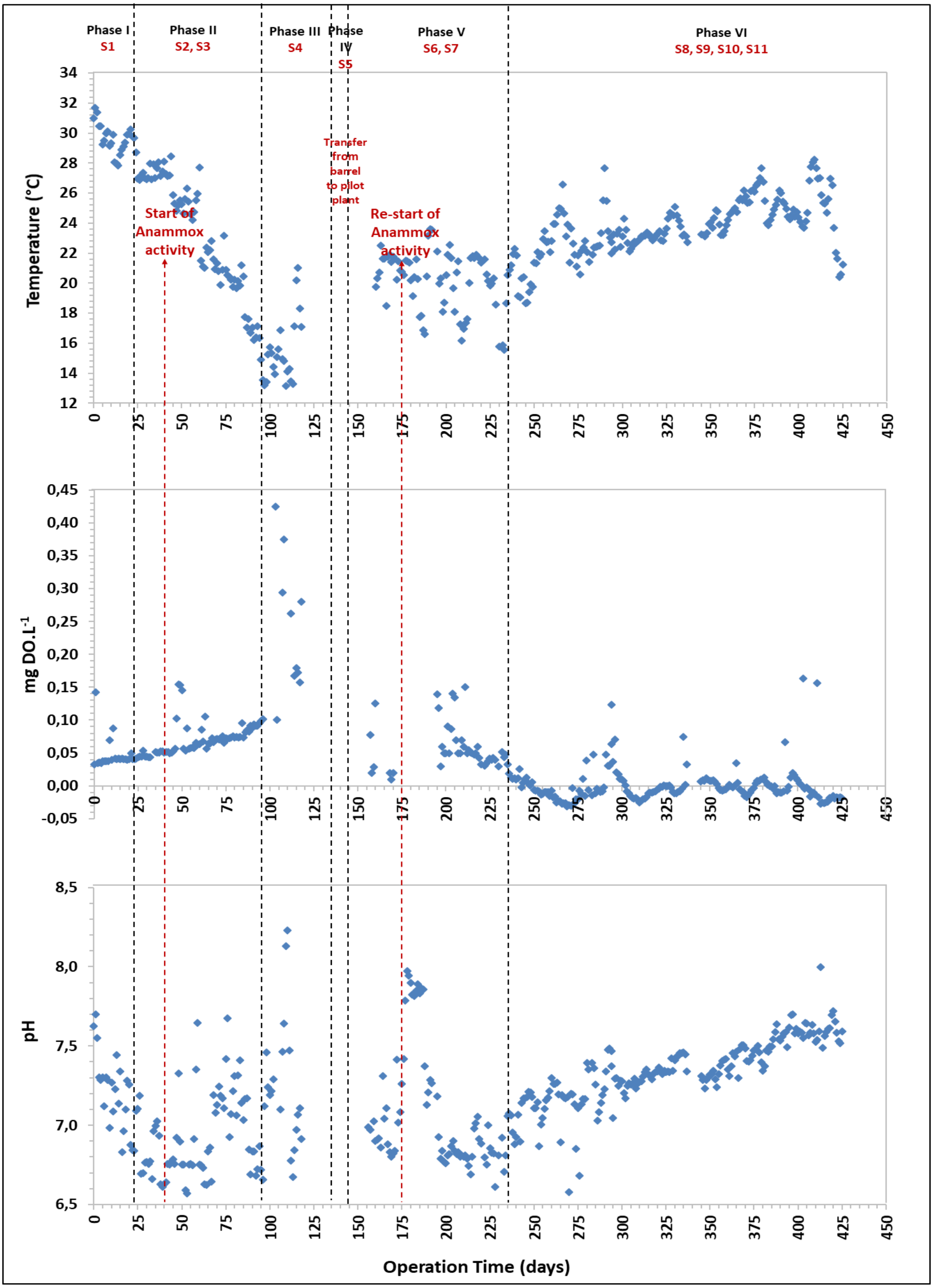


Figure 3-12. Operation conditions (real time Temperature, DO and pH) of the AN reactor

The system was started -up by seeding with only mixed activated sludge of Bursa Doğu STP without addition of any Anammox inoculum. In Phase I, influent average NH_4^+-N and NO_2^--N concentrations were 6.9±3.5 mg/L and 5.3±4.4 mg/L, respectively. Additionally, NO_3^--N were fed during the first month. In this period, wastewater temperature was at 29.6 ±1.0°C. DO concentration and pH were kept around 0.05±0.02 mg/L and 7.2 ±0.2, respectively. Increase in NH_4^--N levels was observed with a release rate of 0.20±0.22 mg/d most probably due to cell lysis. Slight decrease in NO_2^--N and significant decrease in NO_3^--N were observed with removal rates of 0.18±1.11 mg/d and 1.13±1.55 mg/d, respectively. These observations indicated that the dominant mechanism in Phase I was endogenous denitrification. No significant pH changes were observed. qPCR results (Fig. 3.14, S1) showed that population of Anammox species (10^3 copy number/ng DNA) in total bacterial species (10^{10} copy number/ng DNA) were low. Additionally, Archea (10^5 copy number/ng DNA) and AOA amoA (10^4 copy number/ng DNA) were detected.

In Phase II, wastewater temperature was at 21.0±0.8°C. DO concentration and pH were kept around 0.09±0.1 mg/l and 6.9±0.3, respectively. Total operation time was 73 days. The influent NH_4^+-N and NO_2^--N concentrations were 16.4±7.0 mg/L and 12.0±13.0 mg/L. NH_4^--N and NO_2^--N removal rate were observed 1.07±0.78 mg/L and 0.79±1.8 mg/L, respectively. NO_3^--N production was around 0.20±1.36 mg/d. Anammox activity was slightly observed after day-40 while endogenous denitrification was still the dominant process in the reactor. qPCR analysis results (Fig. 3.14, S2, S3) demonstrated that population of the Anammox increased 1-log and total bacterial species increased 3-log. On the other hand, no significant change was observed for Archea population. But, AOA amoA population decreased 2-log.

In Phase III, with the start of slight Anammox activity in Phase II, the influent NH_4^+-N and NO_2^--N concentrations were increased to the levels of 22.9±3.0 mg/L and 25.4±17.8 mg/L, respectively. The wastewater temperature was at 15.4±2.2°C. DO level increased to 0.56 mg/L due to sealing problem in temporary reactor. pH level was kept around 7.1 ±0.5. In this phase, decrease in both NH_4^--N and NO_2^--N removal rates were observed together with increase in NO_3^--N. NH_4^--N and NO_2^--N removal rates were around 0.11±0.62 mg/d and 0.26±1.67 mg/d, respectively. NO_3^--N production rate was around 0.83±1.87 mg/d. There were no significant changes in pH. Total bacterial population was almost same with Phase II (10^{11} copy number/ng DNA). However, the population of Anammox species increased 2-log (from 10^4 to 10^6 copy number / ng DNA) (Fig. 3.14, S4). This observation indicated that Anammox process became more dominant with respect to endogenous denitrification activity. Total Archea population and AOA amoA population increased 1-log with respect to Phase II.

At operation day of 130, the system was moved to a new pilot plant. This transfer period is called as Phase IV. The transfer of sludge from the drum to the new pilot plant resulted loss of Anammox bacteria. qPCR results (Fig. 3.14, S5) demonstrated 1-log decrease in total bacterial population and 3-log decrease in Anammox population. No change in Archea and AOA amoA population was observed.

In Phase V, the average influent NH_4^+-N and NO_2^--N concentrations were 10.8±3.0 mg /L and 13.4±8.8 mg /L for 87 days. The wastewater temperature was at 20.2 ±1.9°C. DO level was again back to 0.00 mg/L due to proper air sealing. There was no DO fluctuation observed in the reactor any more. pH level was around 7.1 ±0.4. During this phase, increase in the NH_4^--N and NO_2^--N removal rates were observed. They reached to 0.10±0.51 mg NH_4^--N/d and 0.37±0.76 mg NO_2^--N/d, respectively. NO_3^--N production rate was observed as 0.28±1.01 mg/d. These results indicated that Anammox activity re-started after operation day of 175 and increased gradually with time. Consistently, copy number of Anammox population (Fig. 3.14, S6, S7) significantly increased (4-log) in this period although total bacteria population was almost the same (10^{10}-10^{11} copy number/ng DNA). In addition to this, no changes in Archea and AOA amoA population were observed.

In Phase VI, after day 236, the loadings were gradually increased to enrich the Anammox population more. The average influent NH_4^+-N and NO_2^--N concentrations were 66.0±57.7 mg/L and 104.0±96.0 mg/L for 190 days. During this period, wastewater temperature was at 22.2 ±3.0°C. DO level and pH were 0.00 mg/L and 7.1±0.8, respectively. In this phase, gradually increase in the removal rate of NH_4^--N and NO_2^--N were observed, from 0.17 to 35.02 mg/d and from 0.04 to 54.28 mg/d, respectively. At the same time, NO_3^--N production and reduction was observed with slight denitrification activity. Almost same copy numbers were observed in total bacteria, Anammox, Archea and AOA amoA species (Fig. 3.14, S8, S9, S10, S11).

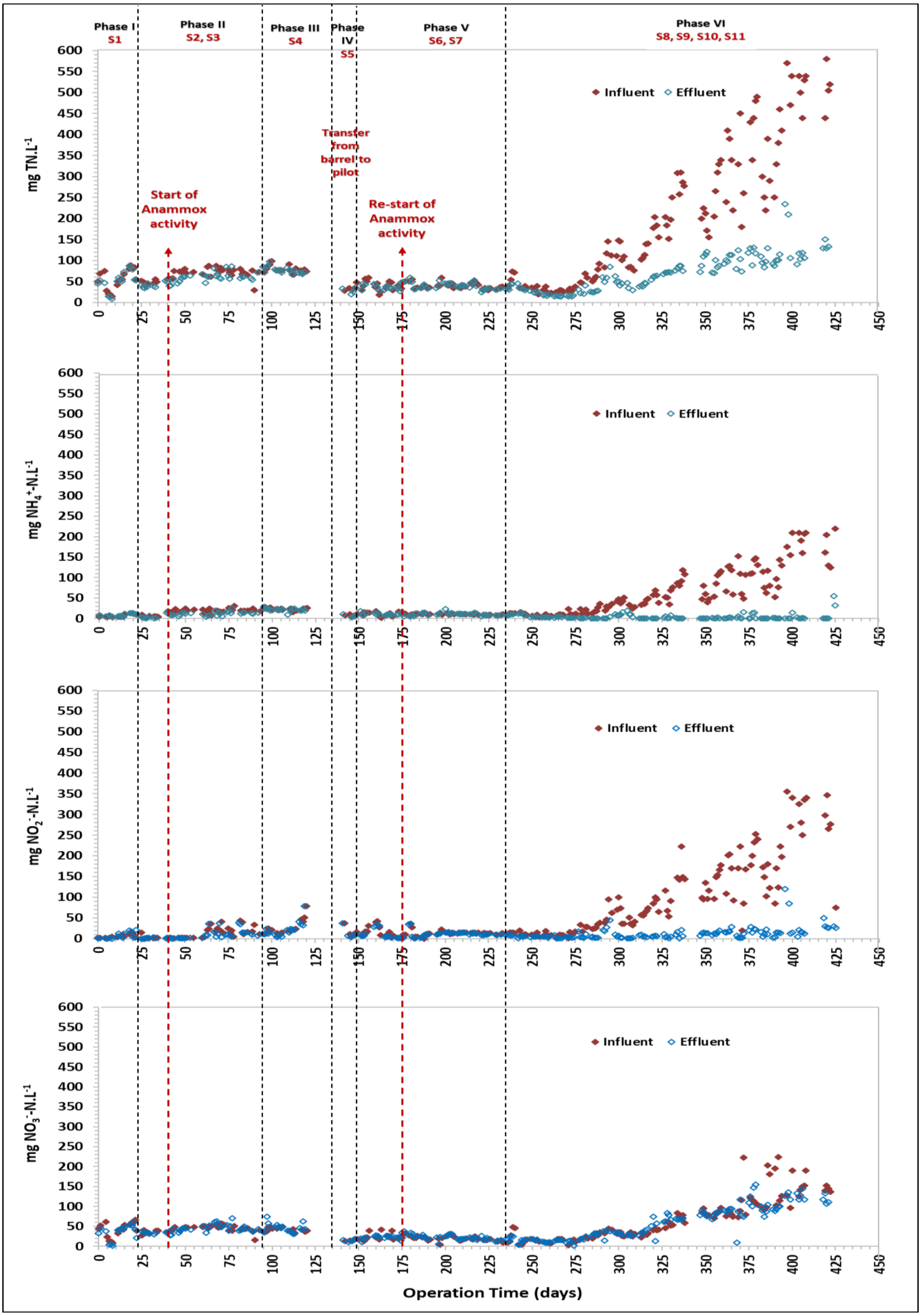


Figure 3-13. Influent and effluent N measurements of the AN reactor

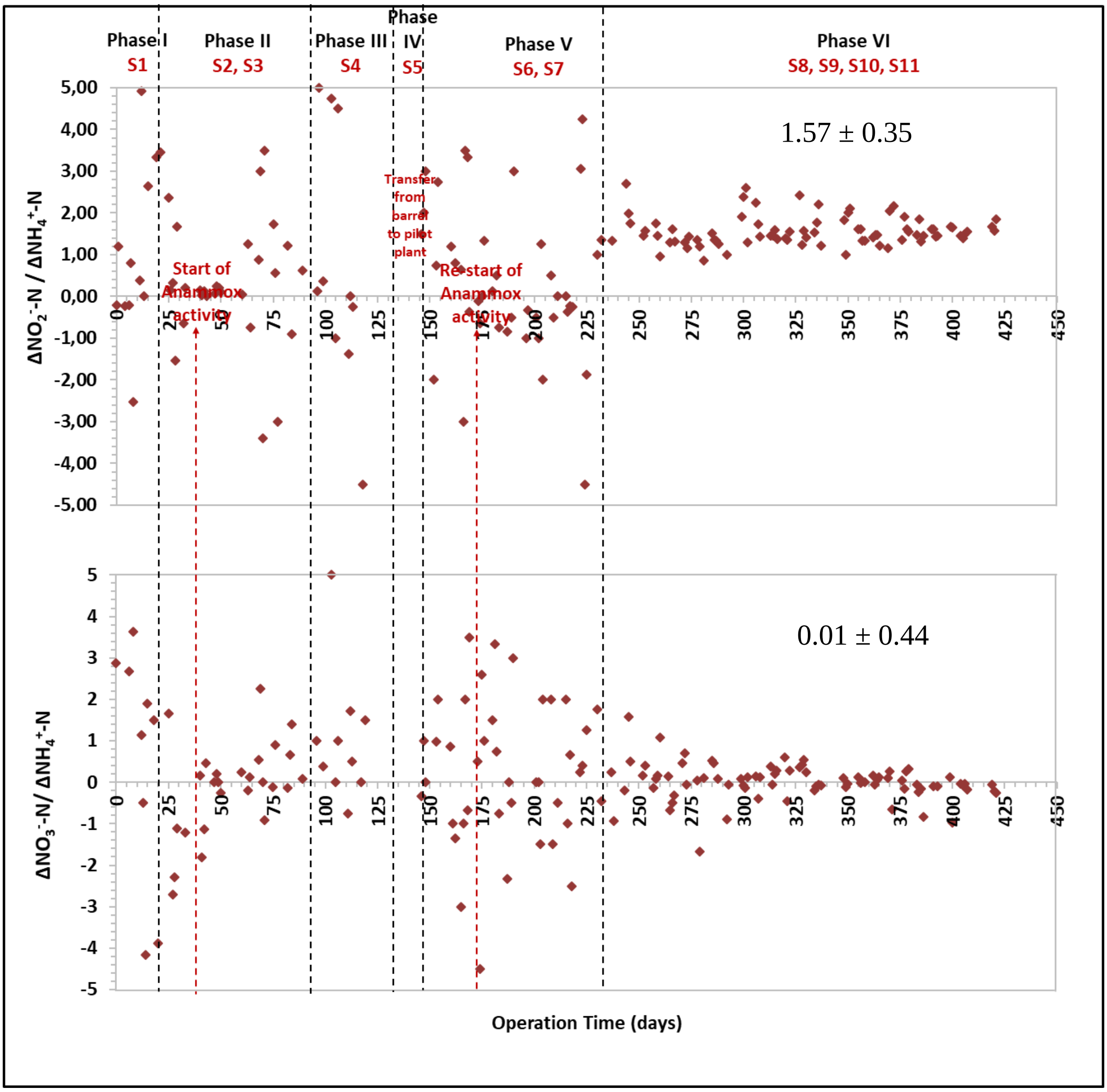


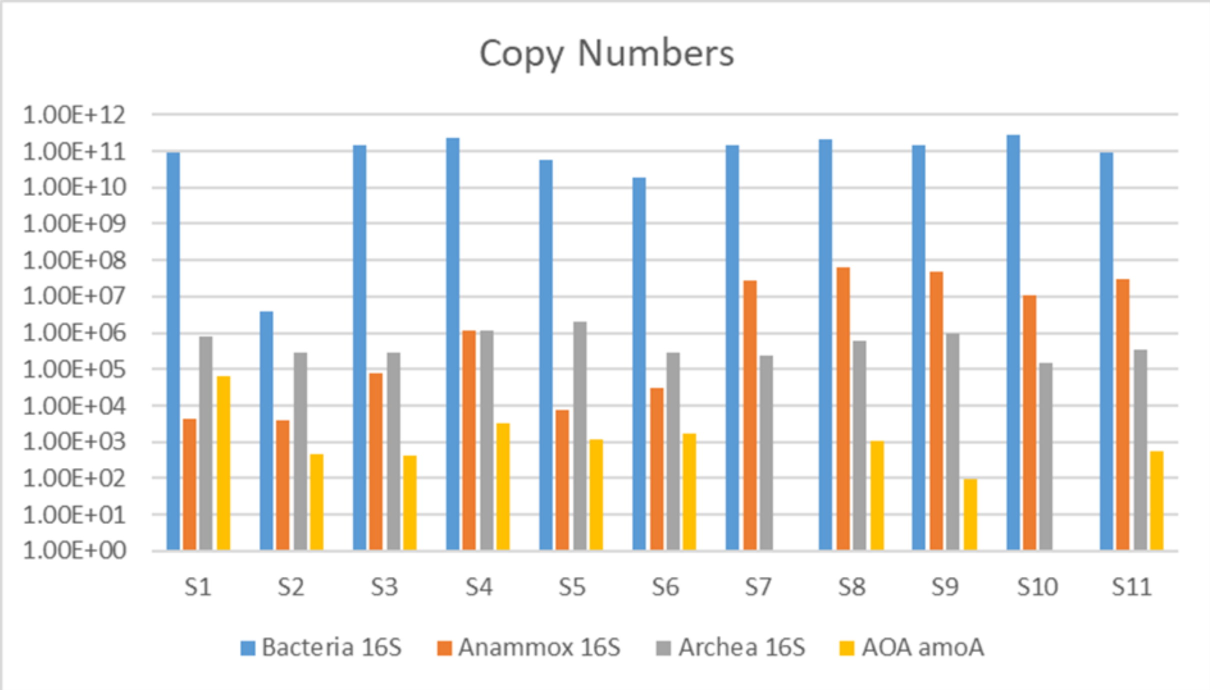
Figure 3-14 Influent and effluent N removal ratios of the AN reactor

The morphological changes in Anammox pilot reactor was observed visually with colour changes. As seen from Figure 3.11, as a result of wash-out of bacteria from the system between Phases I-V, the concentration of bacteria decreased significantly and light greenish colour appeared in the reactor. With the start of Anammox activity, this colour gradually turned to brownish and then reddish colour. In Phase VI, with the increase in Anammox population, granulation started in the reactor.

Molecular Analysis of AN Samples

The evaluation of microbial structure with HRM based on melting peak molecular fingerprints is shown in Fig. 3.16 and 3.17.

HRM analysis of bacterial 16S rRNA genes (Figure 3.16) showed two distinct clades. Bacterial community was changed drastically between AN-S5 (Phase IV) and AN-S6 (Phase V). AN-S1 (Phase I) to AN-S5 (Phase IV) except AN-S3 (Phase II) and AN-S6 (Phase V) to AN-S11 (Phase VI) clustered separate clades: Clade 1: AN-S3, AN S6; Clade 2: AN-S7 to AN-S11. AN-S1 gave four different melting peaks at around 78, 81, 85 and 88. Hence, AN-S1 had at least 4 different Anammox taxa. 78 and 81 peaks disappeared and new peak (83) appeared and at AN-S2. Intensity of Peak 83 and 88 increased for AN-S3 and stayed high for other samples, except AN-S6. Peak 85 appeared again for AN-S6 and disappeared again for AN-S7.



Samples	Bacteria 16S	Anammox 16S	Archea 16S	AOA amoA
S1	8.95E+10	4.34E+03	8.15E+05	6.19E+04
S2	3.86E+06	4.04E+03	2.88E+05	4.63E+02
S3	1.40E+11	7.50E+04	2.94E+05	4.30E+02
S4	2.26E+11	1.12E+06	1.17E+06	3.18E+03
S5	5.52E+10	7.62E+03	1.99E+06	1.15E+03
S6	1.83E+10	2.95E+04	2.79E+05	1.66E+03
S7	1.49E+11	2.86E+07	2.30E+05	NA
S8	2.11E+11	6.38E+07	6.08E+05	1.10E+03
S9	1.45E+11	4.70E+07	9.25E+05	9.15E+01
S10	2.78E+11	1.10E+07	1.54E+05	NA
S11	9.26E+10	3.03E+07	3.62E+05	5.84E+02

Figure 3-15. Absolute quantitation of Bacteria, Archea, Anammox 16S rRNA and AOA amoA gene copy number

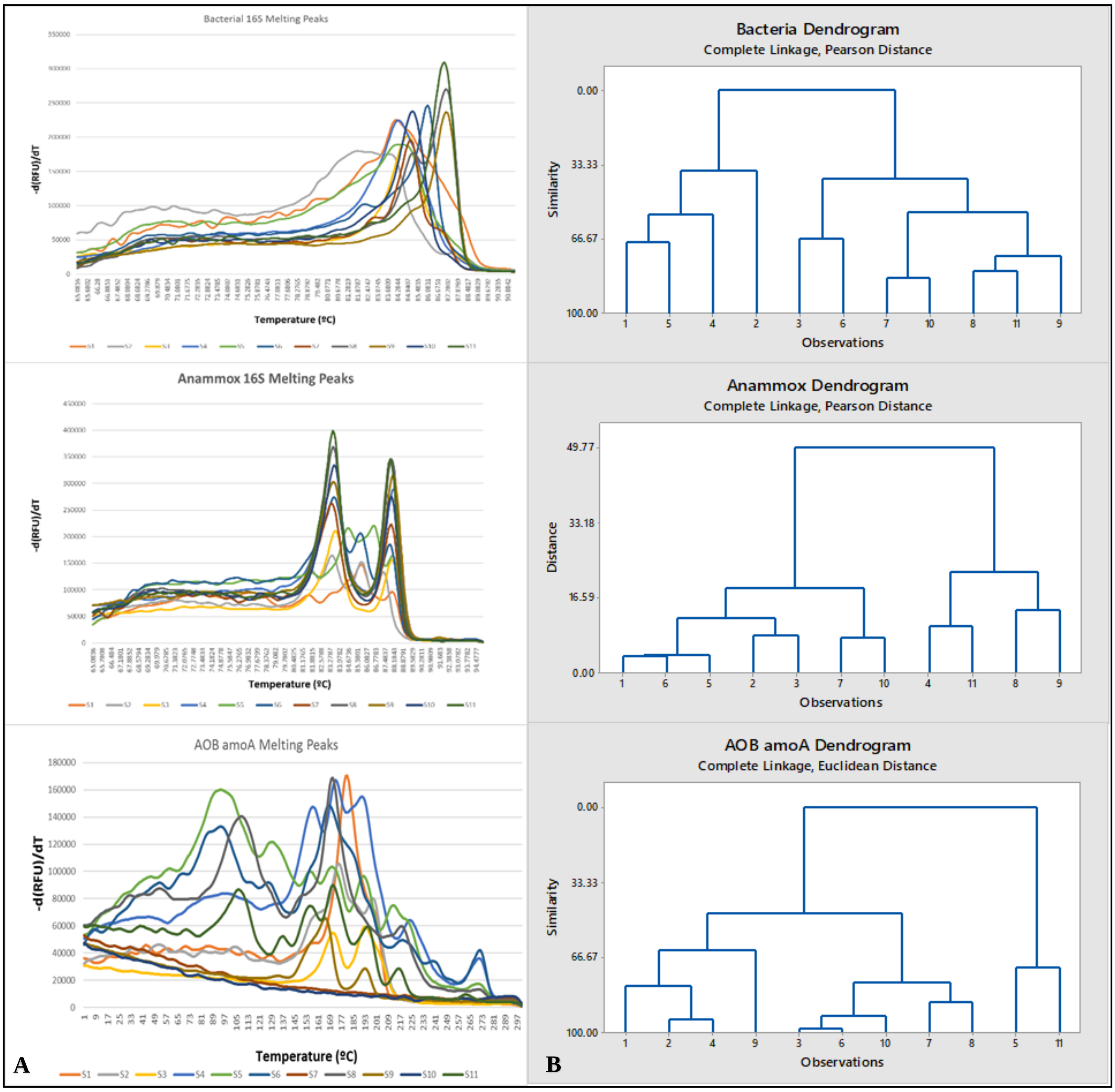


Figure 3-16. HRM analysis of Bacteria, Anammox, AOB amoaA genes from the samples Melting peak plots (a), and dendogram of samples diversity similarities based on HRM profile (b).

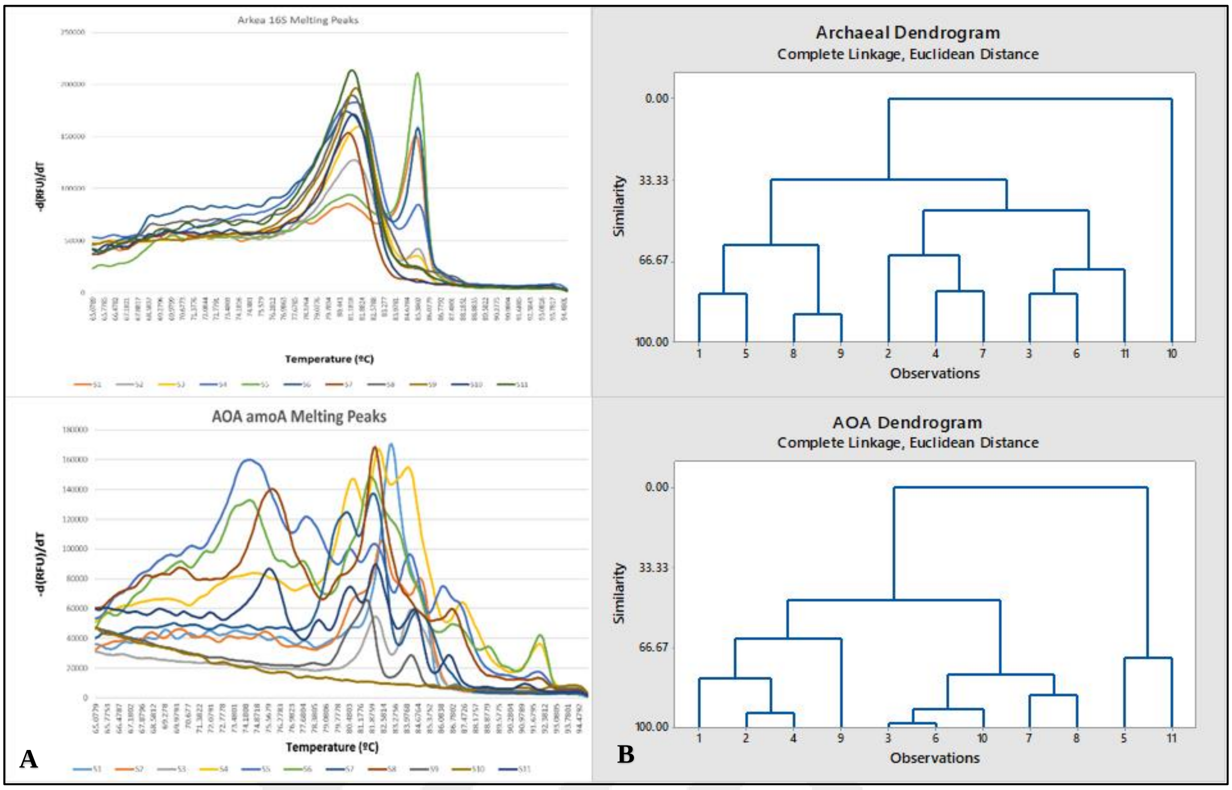


Figure 3-17. HRM analysis of Arheal and AOB genes from the samples Melting peak plots (A), and dendogram of samples diversity similarities based on HRM profile (B).

Table 3-2. Summary of Operation Phases of AN Reactor

	PHASE I	PHASE II	PHASE III	PHASE IV	PHASE V	PHASE VI
Sludge sample no	S1	S2, S3	S4	S5	S6, S7	S8, S9, S10, S11
Duration (operation day)	0-22	23-95	96-129	130-148	149-235	236-425
DO (mg/L)	0.05 ± 0.02	0.09 ± 0.10	0.56 ± 0.41		0.06 ± 0.03	0.00
pH	7.2 ± 0.2	6.9 ± 0.3	7.1 ± 0.5		7.1 ± 0.4	7.1 ± 0.8
Temperature (°C)	29.6 ± 1.0	21.0 ± 0.8	15.4± 2.2		20.2± 1.9	22.2± 3.0
NH_4^+-N loading rate, mg/d	1.16±0.58	2.74±1.17	3.82±0.50	Transfer from barrel to Pilot plant	1.81±0.50	0.42 ---> 36.74
NH_4^+-N release(-) / removal(+) rate[a], mg/d	-0.20±0.22	1.07±0.78	0.11±0.62		0.10±0.51	0.17 ---> 35.02
NO_2^--N loading rate, mg/d	0.88±0.73	2.00±2.19	4.24±2.97		2.23±1.46	0.08 ---> 59.29
NO_2^--N removal rate, mg/d	-0.18±1.11	0.79±1.81	0.26±1.67		0.37±0.76	0.04 ---> 54.28
NO_3^--N loading rate, mg/d	6.99±1.11	7.2±1.33	6.65±1.84		3.59±1.17	0.42 ---> 37.41
NO_3^--N removal (-) /production(+) rate[b], mg/d	-1.13±1.55	0.20±1.36	0.83±1.87		0.28±1.01	-24.72 ---> 5.75
Start of Anammox activity (operation day)	-	40	-		175 (re-start)	
ΔNO_2^--N/ΔNH_4^+-N	-	-	-		-	1.6±0.83
ΔNO_3^--N/ΔNH_4^+-N	-	-	-		-	-0.12±1.88
Quantity of Total bacteria, copies / ng DNA	S1: 8.95×10^{10}	S2: 3.86×10^{6} S3: 1.40×10^{11}	S4: 2.26×10^{11}	S5: 5.52×10^{10}	S6: 1.83×10^{6} S7: 1.49×10^{11}	S8: 2.11×10^{11}, S9: 1.45×10^{11} S10: 2.78×10^{11} , S11: 9.26×10^{10}
Quantity of Anammox bacteria, copies / ng DNA	S1: 4.34×10^{3}	S2: 4.04×10^{3} S3: 7.50×10^{4}	S4: 1.12×10^{6}	S5: 7.62×10^{3}	S6: 2.95×10^{4} S7: 2.86×10^{7}	S8: 6.38×10^{7}, S9: 4.70×10^{7} S10: 1.10×10^{7} , S11: 3.03×10^{7}

[a] Increase in effluent NH_4^+-N concentration with respect to influent is mentioned with (-) sign as release rate

[b] Decrease in effluent NO_3^--N concentration with respect to influent is mentioned with (-) sign as removal rate

4. CHAPTER 4 - CONCLUSIONS

In this study, which was performed at Bursa Doğu Biological Nutrient Removal Sewage Treatment Plant (STP) in Turkey, mainstream partial Nitritation-Anammox (Deammonification) process was evaluated with real sewage. For this purpose, two (2) pilot scale reactors, partial nitritation (PN) and Anammox (AN) reactors, were installed in Bursa Doğu STP in Turkey and inoculated with mixed activated sludge in Bursa Doğu STP and operated for 360 days and 425 days, respectively.

With this study, partial nitritation of sewage was studied first time in Turkey under real plant conditions using grit chamber effluent. Moreover, the enrichment of Anammox species from local activated sludge, without using any Anammox inoculum was achieved first time in Turkey under real plant conditions using mainstream effluent spiked with nitrogen. Optimum conditions to achieve partially nitrified sewage influent (proper NO_2^--N: NH_4^+-N ratio of 1.3-1.5) for Anammox reactor and enrichment of Anammox bacteria in Turkey using local activated sludge seed were deeply studied. Although, it is a challenging to operate Partial Nitritation and Anammox processes at lower and variable wastewater temperature and nitrogen concentrations, the following conclusions have been found during this study;

1. The evaluation of mainstream partial nitritation for one-year period covering all seasonal conditions showed that it is not easy to achieve *partial Nitritation of sewage* steadily under real plant conditions. The findings did not match with the previous lab-scale studies performed short-term and under controlled environmental conditions (Dityapak, 2012; Alpaslan Kocamemi and Dityapak, 2016b). The changing and fluctuating plant influent characteristics together with unexpected industrial shock loadings prevented to observe steady effluent quality from PN which is proper for Anammox process. These findings pointed out that it is better to focus on one-stage PN-AN systems instead of trying to obtain PN effluent with certain NO_2^-N:NH_4^+-N ratio in a two stage PN-AN system. It might be better to use online one-stage PN-AN systems. One stage PN-AN systems may easily use symbioting realtionship between nitrifying and Anammox species and hence may not be effceted seriously from unsteady infleuent characteristics. In such a system, the produced

NO_2^--N by AerAOB will simultenously be consumed by AnAOB using available NH_4^+-N in the system.

2. In this study, three (3) major operational parameters (DO, pH and SRT) were adjusted in order to obtain proper NO_2^--N:NH_4^+-N influent for Anammox. As expected, DO decreases, pH increases and SRT decrease resulted NO_2^--N accumulation. But, the degree of NO_2^--N accumulation fluctuated seriously not only dependent on these parameters but also dependent on influent characteristics (e.g., wastewater temperature, influent ammonium level) of which adjustments are not feasible. DO levels below 0.6 mg/L favored denitrification activity and hence prevented NO_2^--N accumulation in the system. It was found that the system must adjust DO level depending on other factors, especially temperature and FA. FA levels between 0.7 and 1 mg/L usually ended up with NO_2^--N accumulation. However, FA level greater than 1 mg/L caused inhibition of AerAOB and hence prevented NO_2^--N accumulation in the system. FA levels less than 0.5 mg/L usually resulted with serious NO_3^--N production. Through the study, SRT ranged usually between 3-5 days. The studied SRT values did not result enough washout of NOB species from the system. Overall data demonstrated that the proper NO_2^--N:NH_4^+-N effluent ratio (around 1.2) was obtained under DO:1.1-1.2 mg/L, temperature: 20-24˚C, FA: 0.7-1.1 mg/L and SRT: 4-5 days.

3. Molecular studies showed that at the start-up of PN, local activated sludge was containing high population of AerAOB, very low in AnAOB. The dominant NOB species were Nitrobacter. During one-year operation at various conditions, no significant change in the quantity of AerAOB and NOB were observed except last phase having SRT value 2 days. In these phase, significant decrease in Nitrobacter species (4-log) was observed. Non-observation of NO_2^--N accumulation even at low SRT values might be due to presence of Commamox species oxidizing ammonia directly to nitrate.

4. Partial Nitritation process was identified as the major limiting factor for mainstream sewage Anammox applications.

5. This study demonstrated the first successful enrichment of Anammox sludge in Turkey using local activated sludge under real STP conditions. The Anammox

activity started in 40 days as a result of continuous feeding with treatment plant effluent spiked with nitrite and ammonia. The expected initial endogenous denitrification activity upon seeding with activated sludge ended up in a very short time (around 20 days). The local activated sludge enrichment with Anammox species reached to the levels of 10^7 ng Anammox copy number/ml at the end of 236 days operational period under as is wastewater temperature conditions as low as 15˚C.

6. The Anammox enrichment period was not seriously affected from the organic carbon content in feed water (treatment plant effluent), which was very low and hence did not favour the growth of heterotrophic organisms. Through 425 days operational period, sCOD removal rate ranged between 5%-19%. Slight denitrification activity observed with nitrate reduction to nitrite was apparent time to time through 425 days.
7. The total N removal rate reached to the level of 89 mg N/d for total N loading of 96 mg N/d with about 93% removal efficiency at the end of 425 days enrichment period.
8. The stoichiometry of Anammox process under real plant conditions matched with the values observed in lab-scale studies. The removed nitrite nitrogen (ΔNO_2^--N): removed ammonium nitrogen (ΔNH_4^+-N) ratio was round 1.57±0.35. The nitrate nitrogen production (ΔNO_3^+-N): removed ammonium nitrogen (ΔNH_4^+-N) and the nitrate nitrogen production (ΔNO_3^+-N): removed total nitrogen (ΔNO_2^--N+Δ NH_4^+-N) was mostly around 0,30±0.30 and 013±0.14, respectively. The lower nitrate production stoichiometry indicated the presence of slight denitrification activity in the system.

The findings of overall study provided insight for the installation, start-up and operation of two-stage partial nitritation and Anammox systems in Turkey using local activated sludge under ambient temperature conditions with low to moderate nitrogen loadings. In view of findings of this study, focusing on one-stage partial Nitritation-Anammox systems, is strongly recommended to overcome unsteady characteristics of partial nitritation process under real plant conditions. In those studies, not only the on-line ammonium monitoring but also on-line nitrite and on-line nitrate monitoring are strongly advised in order to understand simultaneously occurring processes properly.

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CIRRICULUM VITAE

Personal Information

Name, Surname	Hilal Uflaz
Date of Birth	1982
2012-2018	M.S. Environmental Engineering, Marmara University Institute for Graduate Studies in Pure and Applied Sciences Istanbul, Turkey
1999-2004	B.S. Environmental Engineering, Marmara University Faculty of Engineering Istanbul, Turkey

Work Experiences

Sept 2015-present	MWH-Stantec Global Project Manager
Feb 2013 - Sept 2015	Kuzu Toplu Konut Inş. A.Ş. Project and Proposal Group Coordinator
Mar 2010 - Feb 2013	Kuzu Toplu Konut Inş. A.Ş. Senior Process and Project Engineer
Mar 2007-Mar 2010	Gezer Endüstri İnşaat San.ve Tic. A.Ş. Process and Project Engineer
Nov 2005 – Mar 2007	Eurosan Su Arıtım Sistemleri A.Ş. Sales / Proposal Engineer
Aug 2004 - Nov 2005	Gama Kimya Arıtma Sistemleri Ltd. Project Engineer