T.C.
MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES IN
PURE AND APPLIED SCIENCES

APPLICABILITY OF ANAMMOX PROCESS FOR THE NITROGEN REMOVAL FROM DOMESTIC WASTEWATER

Duygu DİTYAPAK

THESIS
FOR THE DEGREE OF MASTER OF SCIENCE
IN
ENVIRONMENTAL ENGINEERING

SUPERVISOR
Assist. Prof. Bilge ALPASLAN KOCAMEMİ

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ABSTRACT

APPLICABILITY OF ANAMMOX PROCESS FOR THE NITROGEN REMOVAL FROM DOMESTIC WASTEWATER

In the process engineering point of view, Anammox is an attractive alternate to conventional biological nitrogen removal, since it does not require any carbon and oxygen. This process uses NO_2-N as an e^- acceptor so a preliminary partial nitrification (PN) stage in which about half of the NH_4-N must be oxidized to NO_2-N, with a fraction of NH_4-N remaining unconverted is required prior to Anammox process.

In this study, treatment of domestic wastewater via Anammox process applied following to PN process was studied. PN and Anammox processes were studied in two different reactor systems, namely PN-SBR and Anammox-SBR systems, which were started-up with mixed activated sludge from a local STP. Both systems were started-up successfully and the effluent of PN-SBR, which was continuously fed with real domestic wastewater coming from a local STP, was continuosly fed to the Anammox-SBR for 240 days. The limiting step of the whole process was identified as partial nitrification due to low strength characteristic of domestic wastewater. In PN-SBR, the effluent NO_2-N:NH_4-N ratio proper for the Anammox feeding could not be obtained by controlling a single operational parameter. Increasing free ammonia (FA) concentration in combination with the limited DO and low SRT conditions was found to be the best suitable control strategy in order to obtain PN effluent proper for Anammox feeding. The optimum working conditions were identified as 0.8-1 mg/L DO, about 3 mg/L FA concentration and 2.5 days SRT. In Anammox-SBR, Anammox process was always proceded simultaneously with denitrification process. However, the dominance of denitrification activity was found to be dependent on the COD removal efficiency of PN-SBR. Feeding the Anammox-SBR system with well partially nitrified effluent with low organic carbon content and proper NO_2-N:NH_4-N ratio decreased the denitrification activity significantly and the Anammox process became the dominant process.

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ÖZET

EVSEL ATIKSULARDA AZOT GİDERİMİ İÇİN ANAMMOX PROSESİNİN UYGULANABİLİRLİĞİ

Anammox prosesi, konvensiyonel biyolojik azot giderim sistemleri karbon ve oksijen ihtiyacı mühendislik açısından kıyaslandığında daha iyi bir alternatiftir. Prosesin nitriti elektron alıcı olarak kullanmasından dolayı, Anammox prosesi öncesinde amonyağın %50'sinin nitrite dönüştüğü ve geri kalan kısmının amonyak olarak kaldığı bir kısmi nitrifikasyon prosesine ihtiyaç duyulmaktadır.

Bu çalışma kapsamında kısmi nitrifikasyon prosesini takiben Anammox prosesinin evsel atıksu giderimi için kullanılabilirliği incelenmiştir. Kısmi nitrifikasyon ve Anammox prosesleri, yerel bir atıksu arıtma tesisinden alınan karışık bakteri kültürü ile aşılanarak PN-SBR ve Anammox-SBR adlı iki adet reaktör sisteminde incelenmiştir. Başlangıç aşaması her iki sistemde de başarı ile gerçekleştirilmiş ve yerel atıksu arıtma tesisinden alınan gerçek evsel atıksu ile beslenen PN-SBR çıkışı ile beslenen Anammox SBR sistemi 240 gün işletilmiştir. Tüm prosesi sınırlayan basamak düşük kirlilik yüküne sahip olan evsel atıksuyun kısmi nitrifikasyonu olarak belirlenmiştir. PN-SBR sistem çıkışında Anammox girişi için uygun NO_2-N:NH_4-N oranının tek bir işletme parametresi kontrolü ile elde edilememiştir. Anammox sistemine uygun kısmi nitrifikasyon çıkışı sağlayabilmek için kısıtlı oksijen, düşük çamur yaşı ve artan serbest amonyak konsantrasyon parametrelerinin kombinasyonunun en uygun kontrol stratejisi olduğu bulunmuştur. Uygun çalışma koşulları 0,8-1 mg/L çözünmüş oksijen, 3 mg/L serbest amonyak konsantrasyonu ve 2,5 gün çamur bekletme yaşı olarak belirlenmiştir. Anammox-SBR sisteminde, Anammox prosesi denitrifikasyon prosesi ile simultane olarak gerçekleşmektedir. Fakat denitrifikasyon aktivitesinin baskınlığının PN-SBR sisteminin KOİ giderimin verimliliğine bağlı olduğu görülmüştür. Anammox-SBR sisteminin kısmi nitrifikasyon uygulanmış düşük organik karbon içeriğine ve uygun NO_2-N:NH_4-N oranınına sahip çıkış suyu ile beslenmesi ile denitrifikasyon aktivitesinin ciddi mertebede azaldığı ve Anammox'un baskın proses hale geldiği gözlemlenmiştir.

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SYMBOLS

f_s : Fraction of electron donor used for cell synthesis
K_a : The ionization constant of the nitrous acid equilibrium
K_b : The ionization constant of the ammonia equilibrium
k_{dn} : Endogenous decay coefficient for AOB (g VSS/ g VSS ·d)
K_n : Half-saturation coefficient for NH_4-N (mg/L)
K_o : Half-saturation coefficient for DO (mg/L)
$K_{o,\ AOB}$: DO half-saturation coefficient for AOB (mg O_2/L)
$K_{o,\ NOB}$: DO half-saturation coefficient for NOB (mg O_2/L)
K_w : The ionization constant of water
q_n : Specific NH_4^+-N oxidation rate (g NH_4^+-N oxidized/ g VSS/d)
q_{nm} : Maximum specific NH_4^+-N oxidation rate (g NH_4^+-N oxidized/ g VSS/d)
R : ideal gas coefficient (LbarK^{-1}mol^{-1})
$SOUR_{max}$: Maximum specific oxygen uptake rate (mg O_2/ mg VSS.d)
T : Temperature (°C)
V_G : Volume of headspace of the reactor (L)
VL : Volume of the liquid in the reactor (L)
Y : AOB yield coefficient (g AOB grown (VSS)/ g NH_4^+-N removed)
μ : Specific growth rate of microorganisms (d^{-1})
μ_n : Specific growth rate of AOB (g new cells/ g cells.d)
μ_{nm} : Maximum specific growth rate of AOB (g new cells/ g cells.d)
$\Delta_{NH4\text{-}N}$: consumed NH_4-N (g NH_4-N)
$\Delta_{NO2\text{-}N}$: consumed NO_2-N (g NO_2-N)
$\Delta_{NO3\text{-}N}$: produced NO_3-N (g NO_3-N)
θ : temperature coefficient

ABBREVIATIONS

AMO : Ammonium Monooxygenase
ANAMMOX : Anaerobic Ammonium Oxidation
AOB : Ammonium-oxidizing bacteria
BNR : Biological Nitrogen Removal
BOD : Biochemical Oxygen Demand
bsCOD : Biodegredable soluble Chemical Oxygen Demand
CANON : Completely Autotrophic Nitrogen removal Over Nitrite
COD : Chemical Oxygen Demand
CO_2 : Carbondioxide
DGGE : Denaturing Gradient Gel Electrophoresis
DO : Dissolved Oxygen concentration
ESEM : Environmental Scanning Electron Microscope
EPS : Extracellular Polymetric Matrix
FA : Free Ammonia
FISH : Fluorescence in-situ hybridization
FNA : Free Nitrous Acid
HAO : Hydroxylamine Oxidoreductase
HH : Hyrazine hydrolase
HNO_2 : Un-ionized nitrous acid
HRT : Hydraulic Retention Time
HZO : Hydrazine Oxidising Enzyme
MLVSS : Mixed Liquor Volatile Suspended Solids
N_2 : Nitrogen gas
N_2O : Nitrous Oxide
NCl_3 : Nitogen trichloride
NH_2Cl : Monochloramine
$NHCl_2$: Dicloramines
NH_3 : Un-ionized ammonia
NH_4^+-N : Ammonium nitrogen

NH_2OH : Hydroxylamine

NIR : Nitrite Reducing Enzyme

N_2H_4 : Hydrazine

NO : Nitric Oxide

NO_2^--N : Nitrite nitrogen

NO_3-N : Nitrate nitrogen

NOB : Nitrite-oxidizing bacteria

NOR : Nitrite Oxidoreductase

O_2 : Molecular oxygen

ORP : Oxidation Reduction Potential

OLAND : Oxygen Limited Autotrophic Nitrification Denitrification

pCOD : particulate Chemical Oxygen Demand

PLC : Programmable Logic Controller

PN : Partial Nitrification

Q-RT PCR : Quantitative- Real Time Polimerase Chain Reaction

SAA : Specific Anammox Activity

sbCOD : slowly biodegradable Chemical Oxygen Demand

SBR : Sequencing Batch Reactor

SCADA : Supervisory Control and Data Acquisition

SDA : Specific Denitrification Activity

SDAA : Specific Denitrification-Anammox Activity

SHARON : Single reaction system for High Ammonium Removal Over Nitrite

$SO4^{2-}$: Sulphate

SRB : Sulphate Reducing Bacteria

SRT : Sludge Retention Time

STP : Sewage Treatment Plant

TAN : Total Ammonical Nitrogen

TKN : Total Kjeldahl Nitrogen

TN : Total Nitrogen

TOC : Total Organic Carbon

TSS : Total Suspended Solids

VSS : Volatile Suspended Solids

WWTP : Wastewater Treatment Plant

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CHAPTER I

INTRODUCTION and AIM

Nitrogen compounds (ammonium (NH_4^+), organic N and nitrate (NO_3^-)) are major pollutants, which enter water bodies through aqueous wastes from several key industries (e.g. fertilizer, fish canning, refinery, tannery), agricultural run-off and domestic wastes (Mahne et al., 1996, Zhu et al., 2008). The traditional way for nitrogen removal mainly includes two biological steps: (i) aerobic nitrification (the transformation of NH_3 to nitrite (NO_2^-) or NO_3^-) by nitrifying bacteria, (ii) anoxic denitrification (the transformation of NO_2^- or NO_3^- to nitrogen gas (N_2)) by denitrifiers (Li et al., 2004). The treatment of wastewaters by a conventional autotrophic nitrification-hetetrophic denitrification process is expensive due to high aeration requirements and the need for an external carbon supply for wastewater containing low C/N ratio. So the development of processes based on anaerobic ammonium oxidation (Anammox) metabolism has resulted in a revolution in the field of nutrient removal. Mulder discovered Anammox in a laboratory-scale anaerobic fluidized denitrifying bed used in treating effluent from a methanogenic reactor (Mulder et al., 1995).

Anammox is a lithoautotrophic biological conversion process, mediated by a group of *Planctomycete* bacteria [van de Graaf et al., 1996]. In this process, NH_4^+ is oxidized autotrophically to nitrogen gas (N_2) gas while NO_2^- is used as an electron acceptor under anaerobic conditions. The main product of the Anammox process is N_2 gas, but about 10% of the nitrogen feed is converted to NO_3^- (Ahn et al., 2006).

Since anaerobic-autotrophic microorganisms are used to provide Anammox activity, oxygen requirement and organic carbon are not necessary for the process. This autotrophic growth mode leads to an overall stoichiometry showing a relatively low biomass yield which causes low sludge production (Jung et al., 2007). Minimal sludge production is an important design criteria because the traditional biological treatment leads to a sizeable amount of produced sludge that must be treated in a proper manner (Fux et al., 2002). Up to 90 % less nitrous oxide (N_2O), nitric oxide (NO) and carbondioxide (CO_2) emissions which are greenhouse gases make the process favorable. For these reasons, Anammox process seems a cost-effective solution against the conventional nitrification-denitrification processes.

As discussed above, NO_2^- is used as electron acceptor in the Anammox process. Therefore, this process requires a preliminary partial nitrification stage in which about half of the NH_4^+ must be oxidized to NO_2^-, with a fraction of NH_4^+ remaining unconverted. The key step for controlling partial nitrification is to obtain a nitrification reactor with a stable NO_2^- accumulation. In previous studies reported in literature, different strategies and approaches have been used. Those are mainly based on the control of dissolved oxygen (DO) (Bernat et al., 2011, Jubany et al., 2009, Guo et al., 2009, Bagchi et al., 2009, Okabe et al., 2011, Li et al., 2011, Zafarzadeh et al., 2011, Tian et al., 2008, Aslan et al., 2009, Blackburne et al., 2008, Zhang et al., 2010) temperature (Kim et al., 2008, Guo et al., 2010, Padin et al., 2010, Xue et al., 2009), sludge age (Gee et al., 2004, Hellinga et al., 1998, Munz et al., 2011, Ahn et al., 2008), pH as well as the presence of free ammonia (FA) and free nitrous acid (FNA) (Munz et al., 2011, Vadivelu et al., 2007, Jimenez et al., 2011, Zhou et al., 2011, Wu et al., 2011, Ma et al., 2011, Ye et al., 2009, Ciudad et al., 2007). In the economic point of view, among those strategies, partial nitrification based on DO control seems a very attractive solution.

The fully autotrophic combined partial nitrification – Anammox process is still under development. The previous studies reported in literature were mainly investigated the applicability of Anammox process for the treatment of digestor supernatant, piggery manure, fish canning and leachate which have high ammonium content. There is almost no study for evaluating the applicability of Anammox process for nitrogen removal from domestic wastewater. The main reason of this is the difficulty of preliminary partial nitrification of domestic wastewater containing low ammonia.

The main aim of this study is to evaluate the applicability of Anammox for the nitrogen removal from domestic wastewater. For this pupose, initially a partial nitrification (nitritation) system and an Anammox system were started up using a mixed activated sludge seed obtained from a local domestic wastewater treatment plant (WWTP). Partial nitritation of domestic wastewater was deeply studied under different operating conditions while the mixed activated sludge was being enriched for Anammox bacteria. Following to the optimization of partial nitritation process and enrichment of Anammox bacteria, treatability of domestic wastewater was studied by applying partial nitritation combined with Anammox by operating aforementioned reactors.

CHAPTER II

GENERAL BACKGROUND

II.1 NITROGEN CYCLE

Nitrogen exists in many forms in the enviroment. Transformation of nitrogen compounds is characterized by the nitrogen cycle and occurs through several mechanisms as shown in Figure II.1. Those of importance include fixation, ammonification, nitrification, denitrification and Anammox (Epa Manuel, 1993).

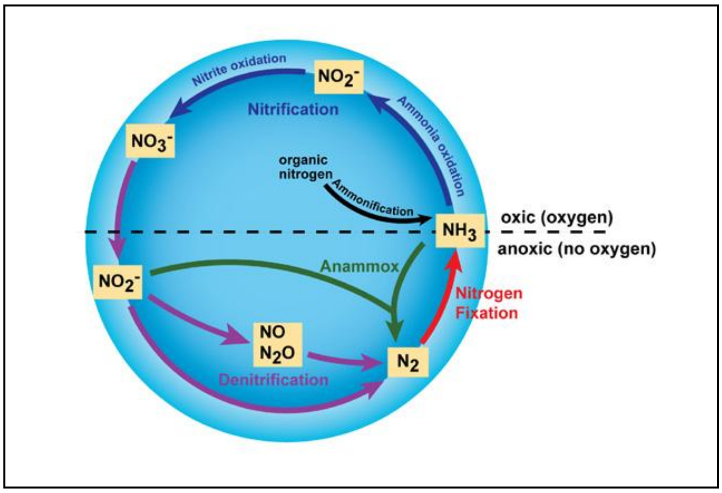


Figure II. 1 Major transformations in the nitrogen cycle

Nitrogen fixation means the incorporation of inert gaseous nitrogen into a chemical compound such that it can be used by plants and animals. Fixation of nitrogen from N_2 gas to organic nitrogen is predominantly accomplished biologically by specialized microorganisms and the associations between such microorganisms and plants (Eqn. II.1). Atmospheric fixation (Eqn. II.2) by lightning and industrial fixation (Eqn. II.3) processes plays smaller, but significant, role as a fixation method (Epa Manuel, 1993).

	Fixation process		**Product**	
↗	Biological	→	organic nitrogen compounds	(II.1)
N_2 gas →	lightening	→	nitrate	(II.2)
↘	industrial	→	ammonium, nitrate	(II.3)

Ammonification (Eqn. II.4) is the change from organic nitrogen to the ammonium form. In general, it occurs during decomposition of animal and plant tissue and animal fecal matter (Epa Manuel, 1993).

$$\text{Organic nitrogen (protein, amino acids, etc.)} + \text{microorganisms} \rightarrow NH_3/NH_4^+ \tag{II.4}$$

Nitrification (Eqn. II.5) is the microbiological oxidation of NH_4^+ to NO_2^- and NO_3^-. Process is done in two steps, first to NO_2^- form, then to NO_3^- form. Two specific chemoautotrophic bacterial genera are involved, using inorganic carbon as their source of energy (Epa Manuel, 1993).

$$NH_4^+ + O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- \tag{II.5}$$

Denitrification (Eqn. II.6) is biological reduction of NO_3^- to N_2. It can proceed through several steps in the biochemical pathway. A fairly broad range of hetetrophic bacteria are involved in the process, requiring an organic carbon source for energy (Epa Manuel, 1993).

$$NO_3^- + \text{organic carbon} \rightarrow NO_2^- + \text{organic carbon} \rightarrow N_2 + CO_2 + H_2O \tag{II.6}$$

Anammox (Eqn. II.7) process is for oxidation of NH_4^+ to N_2 gas while NO_2^- is used as an electron acceptor under anaerobic conditions. This process is mediated by a group of *Planctomycete* bacteria (Liang et al., 2008).

$$NH_4^+ + 1.3\ NO_2^- \rightarrow N_2 + 0.26\ NO_3^- + 2\ H_2O \tag{II.7}$$

II.2 ENVIRONMENTAL SIGNIFICANCE OF NITROGEN AND REMOVAL TECHNOLOGIES

Discharge of wastewater containing nitrogen in the form of NH_3/NH_4^+ can be toxic to aquatic life because of oxygen depletion, eutrophication and negative effect of chlorine disinfection efficiency. Eutrophication generally promotes excessive plant growth and decay, and causes a severe reduction in water quality (Metcalf&Eddy, 2003). Consumption of contaminated ground water with NO_3^- can possibly cause methemoglobinemia (blue baby syndrome) and formation of carcionogenic nitrosamines and nitrosamides. Microbial activities such as denitrification contribute to the production of greenhouse gases (NO and N_2O) in the atmosphere (Ye et al., 2001). For these reasons, there is a great interest in developing new technologies for nitrogen management to prevent environmental and health problems (Jung et al., 2007).

For the removal of nitrogen compounds from wastewater, different physicochemical and biological technologies can be used. Ammonia stripping, breakpoint chlorination and ion exchange are the most common physicochemical processes that are used to remove nitrogen compounds from wastewater.

Ammonia stripping is a simple desorption process used to lower the ammonia content of a wastewater stream. Ammonia (a weak base) reacts with water (a weak acid) to form ammonium hydroxide. In ammonia stripping, lime or caustic is added to the wastewater until the pH reaches 10.8 to 11.5 standard units which converts ammonium hydroxide ions. Scale formation, not appropiate for freezing conditions and chemical requirements are some of the disadvantages for ammonia stripping (Epa Manuel, 1993).

Breakpoint chlorination is the addition of chlorine to the water or wastewater source until the chlorine demand has been satisfied. Chloramination process takes place in a number of different stages. Chlorine will first react with NH_3 to form monochloramines (NH_2Cl). Additional free chlorine then reacts with monochloramines to form dichloramines ($NHCl_2$) and then trichloramines or nitrogen trichloride (NCl_3) and NO_3^- before the breakpoint is achieved. Breakpoint chlorination is most commonly used to remove small amounts of NH_4-N remaining after treatment with other nitrogen removal options; it is seldom used to remove the relatively high concentration of ammonia nitrogen found in conventional activated sludge effluents, although this is technically feasible and has been demonstrated. Serious odor problems have developed during breakpoint chlorination operations because of the formation of nitrogen trichloride and related compounds (Fux et al., 2002).

Ion exchange is a unit process in which ions of a given species are displaced from an insoluble exchange material by ions of a different species in solution. It has been used in wastewater applications for the removal of nitrogen, heavy metals, and total dissolved solids. For nitrogen control, the ions typically removed from the waste stream are NH_4^+, and NO_3^-. It has had limited application because of the extensive pretreatment required, concerns about the life of the ion exchange resins, and the complex regeneration system required. High concentrations of influent total suspended solids (TSS) can plug the ion-exchange beds, causing high headlosses and inefficient operation (Metcalf&Eddy, 2003).

As discussed above, limitations and costs of physicochemical processes made the biological treatments more favorable for the nitrogen removal from wastewater.

II.3 BIOLOGICAL NITROGEN REMOVAL

Biological nitrogen removal (BNR) removes total nitrogen (TN) from wastewater through the use of microorganisms under different environmental conditions in the treatment process (Metcalf&Eddy, 2003).

II.3.1 Conventional Biological Nitrogen Removal Technologies

Most widely used method for nitrogen removal from wastewater is a conventional two-step process, involving nitrification followed by denitrification.

II.3.1.1 Nitrification

Nitrification is normally a two-step aerobic biological process for the oxidation of NH_4^+ to NO_3^- . In the first stage, NH_4^+ is oxidized to NO_2^- (nitritation) by one group of autotrophic bacteria *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus* and *Nitrosorobrio*. In the second stage, NO_2^- is oxidized to NO_3^-

(nitratation) by another group of autotrophic bacteria *Nitrobacter*, *Nitrococcus*, *Nitrospira*, *Nitrospina* and *Nitroeystis* (Metcalf&Eddy, 2003).

The first step of ammonia oxidation (**nitrititation**) is actually a net reaction of two reactions that occur within Ammonium Oxidizing Bacteria (AOB). The first reaction (Eqn. II.8) involves the enzyme ammonium monooxygenase (AMO), which catalyzes the oxidation of NH_4^+ to hydroxylamine (NH_2OH). The second reaction (Eqn. II.9) is catalyzed by hydroxylamine oxidoreductase (HAO) with water as the source of oxygen for the second oxygen atom in nitrite (Arp et al., 2003).

$$NH_4^+ + 0.5O_2 + 2\ e^- \rightarrow NH_2OH + H^+ \qquad (II.8)$$

$$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^- \qquad (II.9)$$

Overall synthetic-oxidation of yield and oxygen consumption of nitritation is given in Eqn. II.10.

$$5.5NH_4^+ + 7.6O_2 + 1.09HCO_3^- \rightarrow C_5H_7O_2N + 5.4NO_2^- + 5.7H_2O + 1.04H_2CO_3 \qquad (II.10)$$

The second step of ammonia oxidation (**nitratation**) reaction (Eqn. II.11) occurs within Nitite Oxidizing Bacteria (NOB). This reaction involves the enzyme nitrite oxidoreductase (NOR), which catalyzes the oxidation of NO_2^- to NO_3^- .

$$4NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 1.95O_2 \rightarrow C_5H_7O_2N + 3H_2O + 4NO_3^- \qquad (II.11)$$

Overall synthesis and oxidation reaction in nitrification can be shown in Eqn. II.12.

$$NH_4^+ +1.83O_2+1.98HCO_3^- \rightarrow 0.021C_5H_7O_2N+0.98NO_3^- +1.041H_2O+1.88H_2CO_3 \qquad (II.12)$$

According to Eqn. II.10, 3.16 mg O_2 is consumed for per mg NH_4-N oxidation while 1.11 mg O_2 is consumed for per mg NO_2-N oxidation in Eqn. II.11. The actual total oxygen consumption is 4.33 g O_2/g N (Eqn. II.12). 7.07 mg alkalinity as $CaCO_3$ is required for per mg NH_4-N oxidation (Eqn. II.12). Yield for AOB is 0.15 mg cells/mg NH_4-N oxidized (Eqn. II.10) while yield for NOB is 0.02 mg cells/mg NO_2-N oxidized (Eqn. II.11) (Ahn et al., 2006).

AOB growth is limited by concentration of NH_4^+-N while NOB growth is limited by the concentration of NO_2^--N. The kinetic equation proposed by Monod

[Eqn. (II.13)] is used to describe the kinetics of biological growth of either AOB or NOB (Epa Manuel, 1993).

$$\mu_n = \frac{(\mu_{nm} N)}{K_n + N} - k_{dn} \tag{II.13}$$

where;

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

N : nitrogen concentration, g/m^3

K_n : half velocity constant, g/m^3

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

Nitrification rates are affected by the liquid DO concentration. The rate increase up to DO concentration 3 to 4 mg/L. To account for the effects of DO, the expression for the specific growth rate is modified as shown in Eqn. II.14 (Metcalf&Eddy, 2003).

$$\mu_n = \mu_{nm} \frac{N}{K_n + N} \frac{DO}{K_o + DO} - k_{dn} \tag{II.14}$$

where;

DO : dissolved oxygen concentration, g/m^3

K_o : half-saturation coefficient for DO, g/m^3

The basic and derived parameters for AOB and NOB were summarized in Table II.1 and II.2, respectively. When comparing the K_o values of AOB and NOB, it was seen that the high values of K_o quantify that NOB are not tolerant of low DO concentrations (Rittman, 2001).

Another key operation parameter, temperature, has distinct effects on the competition between AOB and NOB. Raising temperature expand the differences of specific growth rates (μ_{nm}) between AOB and NOB. As sen from the Table II.1 and II.2, AOB had a higher μ_{nm} than NOB at 20 °C, while the μ_{nm} of AOB was lower than that of NOB at 15 °C. These findings results that NOB are much more active than AOB below 25°C. In general, the optimum temperature for expanding the differences of μ_{nm} between AOB and NOB is recommended to be above 25 °C.

AOB would out-compete NOB at the relatively higher temperatures, due to higher μ_{nm} for AOB. Therefore, most of the studies on partial nitrification to nitrite were achieved at relatively high water temperatures (Rittman, 2001).

On the other hand, f_s^0 is very low for AOB and NOB that is shown in Table II.1 and II.2. They conserve very few electrons in biomass and these low f_s^0 values translate directly to low Y values (Rittman, 2001).

Table II. 1 Basic and derived kinetic parameters for AOB (Rittman, 2001)

Parameter	Temperature		
	15°C	**20°C**	**25°C**
Fraction of electron donor used for cell synthesis (fs)	0.14	0.14	0.14
Yield coefficient (Y) ($mg\ VSS / mg\ NH_4^+\text{-}N$)	0.33	0.33	0.33
Maximum specific NH_4^+-N oxidation rate (q_{nm}) ($mg\ NH_4^+\text{-}N/ mg\ VSS.d$)	1.70	2.30	3.10
Maximum specific oxygen uptake rate ($SOUR_{max}$) ($mg\ O_2/ mg\ VSS.d$)	5.10	6.80	9.20
Maximum specific growth rate (μ_{nm}) (d^{-1})	0.58	0.76	1.02
Half-saturation coefficient for NH_4^+-N (K_n) ($mg\ NH_4^+\text{-}N/L$)	0.57	1.00	1.50
Half- saturation coefficient for DO (K_o) ($mg\ O_2/ L$)	0.50	0.50	0.50
Endogenous decay coefficient (k_{dn}) (d^{-1})	0.082	0.11	0.15

Nitrification is affected by a number of environmental factors (e.g., DO, pH, FA, FNA, alkalinity, temperature). Nitrifiers are strict aerobes, which mean they need free DO to oxidize NH_4^+. Nitrification requires minimum of 1 mg/L of free DO; thus, under less than 0.5 mg/L, the growth rate is minimal. The rate increase up to DO concentration 3 to 4 mg/L. The optimum pH for AOB and NOB is between 7.5 and 8.5. Nitrification stops at a pH below 6.0. Nitrifying bacteria can also be inhibited by FNA greater than 2.8 mg/L and FA greater than 10 mg/L. An alkalinity of no less than 50-100 mg/L is required to insure adequate buffering. Nitrification reaches a maximum rate at temperatures between 30 and 35 °C. At temperatures of 40°C and higher, nitrification rates fall to near zero. At temperatures below 20 °C, nitrification proceeds at a slower rate, but will continue at temperatures of 10 °C and less (Rittman, 3rd edition, 2001).

Table II. 2 Basic and derived kinetic parameters for NOB (Rittman, 2001)

Parameter	Temperature		
	15°C	**20°C**	**25°C**
Fraction of electron donor used for cell synthesis (fs)	0.10	0.10	0.10
Yield coefficient (Y) ($mg\ VSS / mg\ NO_2^- \text{-} N$)	0.083	0.083	0.083
Maximum specific NO_2^--N oxidation rate (q_{nm}) ($mg\ NO_2^- \text{-} N/ mg\ VSS.d$)	7.30	9.80	13.00
Maximum specific oxygen uptake rate ($SOUR_{max}$) ($mg\ O_2/ mg\ VSS.d$)	7.50	10.10	13.50
Maximum specific growth rate (μ_{nm}) (d^{-1})	0.61	0.81	1.10
Half-saturation coefficient for NO_2^--N (K_n) ($mg\ NO_2^- \text{-} N/L$)	0.62	1.30	2.70
Half- saturation coefficient for DO (K_o) ($mg\ O_2/ L$)	0.68	0.68	0.68
Endogenous decay coefficient (k_{dn}) (d^{-1})	0.082	0.11	0.15

II.3.1.2 Denitrification

Denitrification is the dissimilatory reduction of NO_3^- or NO_2^- to N_2 gas. NO_3^- and NO_2^- replace oxygen for microbial respiration in this reaction. As shown through Eqn. II.15-II.18 several enzymes are involved in the process of denitrification (Rittman, 2001).

$NO_3^- + 2e^- + 2H+ \rightarrow NO_2^- + H_2O$ Nitrate Reductase (II.15)

$NO_2^- + e^- + 2H+ \rightarrow NO\ (g) + H_2O$ Nitrite Reductase (II.16)

$2NO + 2e^- + 2H+ \rightarrow N_2O\ (g) + H_2O$ Nitric Oxide Reductase (II.17)

$N_2O + 2e^- + 2H+ \rightarrow N_2\ (g) + H_2O$ Nitrous Oxide Reductase (II.18)

Denitrifiers are common among the Gram-negative *Proteobacteria*, such as *Pseudomonas, Alcaligenes, Paracoccus* and *Thiobacillus*. Some Gram-positive bacteria including *Bacillus* can denitrify. Even a few halophilic Archea, such as *Halobacterium* are able to denitrify. All the denitrifiers are facultative aerobes, which means that they shift to NO_3^- or NO_2^- respiration when O_2 becomes limiting. Also they are chemotrophs that can use organic or inorganic electron donors (Rittman, 2001).

In biological denitrification process, the electron donor can be one of three sources: (1) the bsCOD in the influent wastewater, (2) the bsCOD produced during endogenous decay and (3) an exogenous source such as methanol or acetate. Reaction stoichiometry for different electron donors is shown in Eqn. II.19-II.21 (Metcalf&Eddy, 2003).

Wastewater : $C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^-$ (II.19)

Methanol : $5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$ (II.20)

Acetate : $5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$ (II.21)

In all above hetetrophic denitrification reactions, one equivalent of alkalinity is produced per equivalent NO_3-N reduced, which equates to 3.57 g alkalinity (as $CaCO_3$) production for each g NO_3-N consumed (Metcalf&Eddy, 2003). Heterotrophic biomass produced can be estimated as 0.4 g volatile suspended solids (VSS) produced / g COD consumed (Imajo et al., 2004).

Table II.3 summarizes representative stoichiometric and kinetic parameters for hetetrophs using methanol, other organic material, H_2, and elemental sulfur (S) as the electron donor for denitrification.

As discussed before, alkalinity is produced during denitrification reactions and the pH is generally elevated, instead of being depressed as in nitrification reactions. No significant effect on the denitrification rate has been reported for pH between 7 and 8 (Metcalf&Eddy, 2003). Very low concentrations of the electron donor or too high concentrations of DO concentration can lead to accumulation of the denitrification intermediates: NO_2^-, NO_2 and N_2O. A low concentration of donor limits the supply of electrons to drive the reductive half reactions. A high DO concentration tends to repress the nitrite and nitrous oxide reductases before nitrate reductase is repressed. This means denitrification can occur when DO concentrations are well above zero (Rittman, 2001).

Table II. 3 Representiative stoichiometric and kinetic parameters for denitrifiers at temperature 20°C (Rittman, 2001)

Electron donor	**Methanol**	**BOD**	**H_2**	**S^0**
Carbon source	**Methanol**	**BOD**	**CO_2**	**CO_2**
Fraction of electron donor used for cell synthesis (f_s)	0.36	0.52	0.21	0.13
Yield coefficient (Y) (*mg VSS / mg donor*)	0.27	0.26	0.85	0.1
Maximum specific oxidation rate (q) (*mg donor/ mg VSS.d*)	6.9	12	1.6	8.1
Maximum specific growth rate (μ) (d^{-1})	0.05	0.05	0.05	0.05
Half- saturation coefficient (K) ($mg\ O_2/ L$)	9.1	1	1	-

II.3.2 Novel Nitrogen Removal Technologies

The treatment of wastewaters by a conventional autotrophic nitrification-hetetrophic denitrification process is expensive due to high aeration requirements and the need for an external carbon supply for wastewater containing low C/N ratio. Moreover, the traditional biological treatment leads to a sizeable amount of produced sludge that must be treated in a proper manner. Detectable quantities of the greenhouse gases, N_2O and CO_2, are produced during nitrification and denitrification processes (Mahne et al., 1996). Therefore, in recent years, researchers have been developed new technologies to minimize energy and chemical consumption. These novel systems are denitrification via nitrite, ANAMMOX (Anaerobic Ammonium Oxidation), CANON (Completely Autotrophic Nitrogen removal Over Nitrite), and OLAND (Oxygen Limited Autotrophic Nitrification Denitrification).

In denitrification via nitrite route process, nitrification is stopped at the nitrite stage (nitritation), followed directly by reduction to N_2 in anoxic conditions with carbon addition (denitritation). AOB and denitrifying bacteria are capable of this process. Compared to conventional nitrification-denitrification, this process saves 25% oxygen consumption and 40% carbon resource (Yongtao et al., 2009).

Anammox is a lithoautotrophic biological conversion process, mediated by a group of *Planctomycete* bacteria (van de Graaf et al., 1996). In this process, NH_4^+ is oxidized autotrophically to N_2 gas while NO_2^- is used as an electron acceptor under anaerobic conditions. The main product of the Anammox process is N_2 gas, but about 10% of the nitrogen feed is converted to NO_3^-. Compared to conventional nitrification and denitrification, this process saves 100% of a required organic carbon source (e.g. methanol) and 60% of the required oxygen. In addition, the biomass yield is very low so that little sludge is produced (Jetten et al., 2003).

In the **CANON** process, Nitrosomonas-like aerobic ammonium-oxidizing bacteria (AOB) and Planctomycete like Anammox bacteria perform two sequential reactions simultaneously under oxygen limited conditions. The nitrifiers oxidize NH_4^+ to NO_2^-, consume oxygen and so create anoxic conditions for the Anammox bacteria need. The produced NO_2^- is utilized with the remainder of the NH_4^+ by Anammox bacteria and converted into dinitrogen (Nielsen et al., 2005). Compared to conventional nitrification and denitrification, this process saves 100% of a required organic carbon source and 63% of the required oxygen. This leads to a decrease in CO_2 emissions of more than 100% and a decrease in energy demand (Third et al., 2005).

OLAND is one-step process in which ammonium is oxidized directly to N_2. This autotrophic conversion of NH_4^+ to gaseous N compounds involved two steps: (i) aerobic nitrification of NH_4^+ to NO_2^- or NO_3^- with O_2 as the electron acceptor and (ii) anoxic denitrification of NO_2^- or NO_3^- to gaseous N with NH_4^+ as the electron donor. The AOB and anaerobic ammonium-oxidizing bacteria are two major groups of bacteria are responsible for autotrophic nitrogen removal. This process consumes 63% less oxygen (Kuai et al., 1998, Vlaminck et al. 2007).

The above-summarized novel nitrogen removal technologies, which are based on partial nitrification/nitritification processes, were basically developed for the high ammonium containing wastewaters having low C/N ratio (e.g., the digester supernatant). The applicability of these processes for the removal of nitrogen from domestic wastewater necessitates the detailed investigation of the maintaining stable partial nitrification/nitritification via inhibiting nitrite oxidation to nitrate in nitrification process.

II.4 PARTIAL NITRIFICATION/NITRIFICATION

As discussed above, in recent years several novel nitrogen removal technologies have been developed. For these technologies, around 55-60% of the influent NH_4-N in wastewater needs to be converted to NO_2-N by nitrifying aerobic microorganisms. The key point is to favour the nitritation process and at the same time to inhibit or suppress the nitratation process in order to have biomass enriched in AOB and poor in NOB. A number of studies were performed to identify the optimal operational conditions to obtain PN. The main factors affecting AOB and NOB activities in a different degree and useful to achieve PN are temperature, SRT, pH, DO and alkalinity (Choi et al., 2011). These operational parameters were discussed briefly in the following sections.

II.4.1 Effect of temperature on partial nitrification

Due to the potential benefits on energy/cost savings from PN, different studies focused on the influences of temperature on PN (Zeng et al., 2010).

The relation between the growth rates of the nitrifiers executing the two constituting steps of nitrification, the AOB and NOB, changes with temperature. The μNOB is significantly higher than μAOB at temperatures between 10 and 20 °C (Figure II.2). Increase in temperature causes the decrease of μNOB and increase of μAOB. In general, the optimal temperature for expanding the differences of specific growth rates between AOB and NOB is recommended to be above 25 °C. AOB would out-compete NOB at the relatively higher temperatures, due to higher specific growth rate for AOB. Therefore, most of the studies on partial nitrification to nitrite were achieved at relatively high water temperatures (Hwang et al., 2005, Padin et al., 2009, Hong-Fang et al., 2009, Vilar et al., 2010). This characteristic is used in the

SHARON process in which a chemostat is operated at a HRT, equal to SRT, of 1 day and 35°C with very low biomass concentration (Ye et al., 2001). These conditions favour the growth of AOB and to washout the NOB (Pollice et al., 2002).

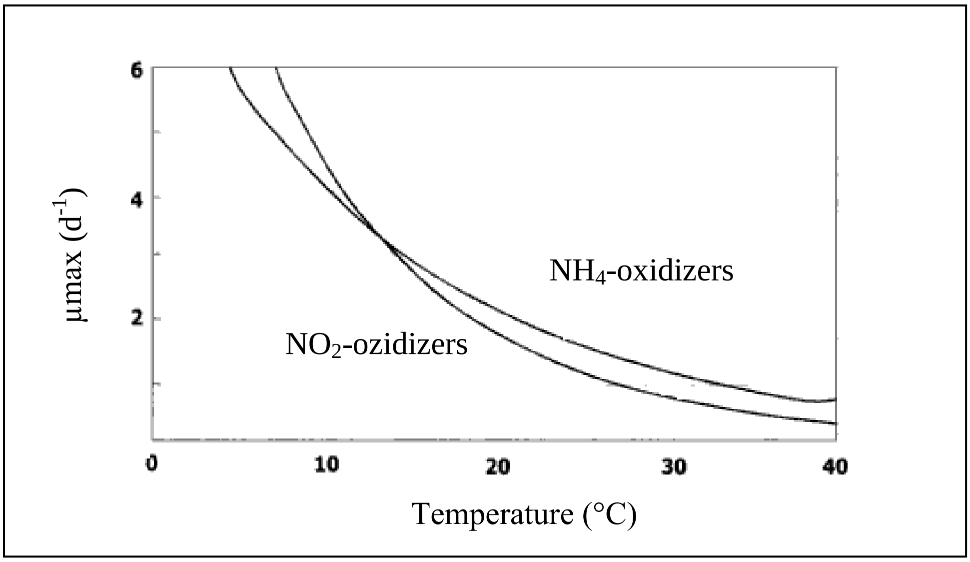


Figure II. 2 The effect of temperature on the maximal growth rate of NH_4 and NO_2 oxidizers (Padin et al., 2009)

Also, an increase in temperature causes an increase in FA concentration, according to FA equation that is described in Section II.4.3, briefly. Fdz-Polanco showed that rising temperatures differentially affect AOB and NOB via the formation of NH_3 that is more inhibitory to NOB.

On the other hand, the fact that the activation energy of the NH_4-N oxidation step is higher than that of the NO_2-N oxidation step provokes that an increase of temperature involves an increase on the NH_4-N oxidation rate larger than that experienced by the NO_2-N oxidation rate (Sinha et al., 2007, Hu et al., 1990). The ammonia oxidation rate depends more sensitively on lower temperatures; correspondingly the temperature coefficient (θ). At low temperature (5-20 °C), θ is higher than the temperature between 20-35 °C so the activation energy which is in correlation with θ is higher between 5-20 °C compared to 20-35 °C. An exponential increase of the maximum specific ammonia oxidation rate was observed for temperatures up to 35 °C. When the temperature decreased from 25 to 15 °C, the specific ammonia oxidation rate decreased by 1.5 times (Stüven et al., 1992).

II.4.2 Effect of SRT on partial nitrification

PN to NO_2^- can be accomplished by controlling sludge age. At stable temperature and pH, the sludge age is the critical parameter for PN when the oxygen supply is not limiting. Longer SRTs tend to favour NOB when oxygen is not limiting. Under limited oxygen supply, complete and stable conversion of NH_4-N into NO_2-N was obtained, independent of the sludge age (Jubany et al., 2009). AOB and washout NOB can be selectively accumulated by appropriate regulation sludge retention time in suspended-growth system because of different minimum required sludge ages which is in higher values for NOB than AOB. Bock et al., have reported the minimum doubling times of AOB and NOB to be 7–8 h and 10–13 h, respectively. If the nitratation process is inhibited in an extent that the minimum SRT required for NOB growth is higher than the current SRT of the system, they will be washed out and the effluent will not contain nitrate (Munz et al., 2011, Yan et al., 2009). Based on experiences from full-scale operation, van Kempen et al. suggest to maintain SRT between 1 day and 2.5 days.

II.4.3 Effect of pH on partial nitrification

The pH influences FA, FNA and free hydroxylamine concentration and the effects on PN were described briefly in three sections (Imajo et al., 2004, Anthonisen et al., 1976).

II.4.3.1 Effect of FA

Most of the literature related to NO_2-N accumulation using pH as a decisive factor states that pH in the range of 7.5–8.5 is most suited to inhibit the NOB. The NO_2-N accumulation is likely caused by the selective inhibition of NOB by FA which increased also exponentially above pH 7.5.

Ammonia is present in solution as NH_4^+ and un-ionized ammonia (NH_3). These are in an equilibrium that is affected by the pH of the solution (Eqn II.22).

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \qquad (II.22)$$

As seen from Eqn. II.23, FA is directly correlated to pH, temperature, and the concentration of ammonia (Anthonisen et al., 1976).

$$\text{FA as NH}_3\ (\text{mg/L}) = \frac{17}{14}\,x\,\frac{\text{total ammonia as N (mg/L)}\ x10^{\text{pH}}}{K_b/K_w + 10^{\text{pH}}} \qquad \text{(II.23)}$$

where;

K_b : The ionization constant of the ammonia equilibrium equation

K_w : The ionization constant of water

K_b/K_w : $e^{(6.344/273+T)}$

T : Temperature in ^{o}C

When the pH increases, the concentration of the NH_3 increases. It was postulated that, at certain concentrations, FA is completely inhibitory to both AOB and NOB. At lower concentrations FA will be less inhibitory to AOB than to NOB. The situation causes NO_2-N to accumulate (Anthonisen et al., 1976). Based upon FA and FNA effects, boundary conditions of zones of nitrification inhibition were determined as shown in Figure II.3.

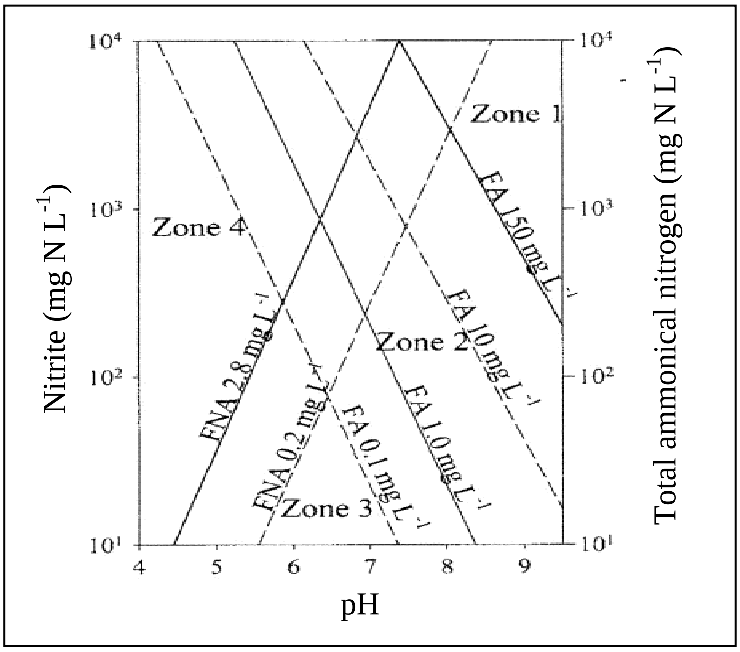


Figure II. 3 Relationship between concentrations of FA and FNA and inhibition to nitrifiers (Sinha et al., 2007)

As seen from Figure II.3, zone 1 (FA > 10–150 mg L^{-1}) represents the condition when the FA concentration is high enough to inhibit both AOB and NOB. No nitrification will occur and ammonia will accumulate in the system. At lower concentrations of FA, only NOB may be inhibited and NO_2^- accumulation will occur.

This condition is represented by Zone 2 (0.1–1.0 mg L^{-1}< FA < 10–150 mg L^{-1}). At still lower FA concentrations, neither NOB nor AOB are inhibited and complete nitrification will occur (Zone 3 (FA < 0.1–1.0 mg L^{-1})) (Anthonisen et al., 1976, Sinha et al., 2007).

II.4.3.2 Effect of FNA

As NO_2^- oxidation occurs, there is a release of hydrogen ions that decreases the pH to an extent related to the buffering capacity of the system. The pH regulates the equilibrium between nitrite and nitrous acid (Eqn II.24)

$$NH_4^+ + 1.5O_2 \rightarrow H_2O + H^+ + \boxed{H^+ + NO_2^- \leftrightarrow HNO_2^-} \qquad (II.24)$$

As seen from Eqn. II.25, FNA is directly correlated to pH, temperature, and the concentration of nitrite (Imajo et al., 2004, Anthonisen et al., 1976).

$$\text{FNA as } HNO_2 \text{ (mg/L)} = \frac{46}{14} x \frac{NO_2^- \text{ - N (mg/L)}}{K_a x 10^{pH}} \qquad (II.25)$$

where;

K_a : The ionization constant of the nitrous acid equilibrium equation

K_a : $e^{(-2.300/273+T)}$

Based upon FNA effects (Figure II.3), complete nitrification is possible in Zone 3 (FNA < 0.2-2.8 mg L-1) while in zone 4 NOB are inhibited by FNA (FNA > 0.2–2.8 mg L^{-1}) (Anthonisen et al., 1976, Sinha et al., 2007).

II.4.3.3 Influence on free hyroxylamine concentration

The presence of free hydroxylamine, a toxic intermediate in nitrification by AOB, appeared to have a consistent correlation with low nitrification activity (Guo et al., 2010). According to Hu, hydroxylamine exhibited acute toxicity to NOB, and this may also cause nitrite build-up in a nitrifying system. Moreover, this inhibitory effect of hydroxylamine on NOB was found to be irreversible. Hydroxylamine appeared likely to accumulate in a nitrifying system with high NH_3/NH_4^+ concentration, deficient oxygen, and high pH (Botruos et al., 2004).

A possible mechanism for the inhibition of nitrite oxidation by a low oxygen concentration is based on the accumulation of hydroxylamine, which is an intermediate product of the ammonium oxidation. It is based on the differences between the oxygen saturation coefficients of the Monod kinetics for ammonium oxidation and nitrite oxidation that are known to be 0.3 and 1.1 mg/L, respectively.

II.4.4 Effect of DO on partial nitrification

The operational costs of the conventional nitrification-denitrification nitrogen removal process are to a great extent related to the oxygen requirements. The amount of air supplied to nitrification process is an important design factor because energy consumption in air supply accounts for a high percentage of the total energy consumption in a wastewater treatment system. From the economic point of view, DO concentration and aeration duration are economically feasible control parameters (Ciudad et al., 2007, Yamamoto et al., 2008)ç

Oxygen limitation was selected as the main control parameter for inhibiting the growth of NOB and thus achieving only nitritation. As seen from Tables II.1 and II.2, low DO concentrations can affect the specific growth rate of both AOB and NOB, depending on its K_o constant; however, its influence on the NOB, (0.68 mg O_2/L) is greater than on the AOB (0.5 mg O_2/L). Low DO did not affect AOB, the growth yield of AOB was doubled at low DO, and this positive effect of low DO compensated the reduced ammonia oxidation rate per unit mass of cell. Nitrite oxidation was strongly inhibited by low DO and the growth yield of NOB was unchanged by low DO. In past studies, it was observed that oxygen level for NO_2-N accumulation is in the range of 0.5-1.5 mg O_2/l for suspended cultures (Blackbourne et al., 2008, Wang et al., 2011).

On the other hand, the floc size distribution has been suggested to largely affect oxygen mass transfer rates even in floccular biomass aggregates. Due to the oxygen mass transfer resistance, the DO concentration would not be uniform for bacteria inside different sized floccular aggregates. Hence, different specific oxygen uptake rates would be attained for different floc sizes at the same bulk DO concentration. As a consequence, the apparent K_o value will be affected by the oxygen mass transfer kinetics (Mueller, 1993, Bakti et al., 1992).

II.4.5 Effect of alkalinity on partial nitrification

Alkalinity is also an important factor for nitrification. Depending on the alkalinity of the wastewater, the SHARON reactor can convert a fraction or even the whole load of NH_4-N into NO_2-N. The proper ratio of NO_2-N:NH_4-N for the Anammox process can be easily achieved by controlling the alkalinity in the wastewater. Because the oxidation of 1 mole of NH_4-N to NO_2-N consumes 2 moles of bicarbonate, and the reaction stops when the pH is lower than 6.5, a molar ratio 1:1 between NH_4-N and bicarbonate produces a conversion of approximately 50 % of NH_4-N to NO_2-N and the rest remains as NH_4-N (Gali et al., 2007).

II.5 ANAEROBIC AMMONIUM OXIDATION (ANAMMOX)

Anammox process is a lithoautotrophic biological conversion in which NH_4^+ is oxidised to N_2 gas. This process is mediated by a group of *Planctomycete* bacteria under anaerobic conditions using NO_2^- as an electron acceptor. The catabolic reaction of fixing nitrite with one molecule of CO_2 leads to the anaerobic production of NO_3^- in the anabolism. Stoichiometry of Anammox is shown in Eqn. II.26.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad (II.26)$$

The main product of the Anammox process is N_2, but about 10% of the nitrogen feed is converted to NO_3^-. As seen from Eqn. II.26, the overall nitrogen balance gave a ratio of NH_4^+ to NO_2 conversion of 1:1.32 and ratio of NO_2 conversion to NO_3^- production 1:0.26 (Ahn et al., 2006). In theory, the molar ratio of NH_4^+ and NO_2 in Anammox catabolism is 1:1.3. The excess 0.3 mol of nitrite is anaerobically oxidized to nitrate and yields electrons for CO_2 fixation or reduction to the level of oxidation of cell material for cell growth (van de Graaf et al., 1997, Strous et al., 1999a, van Niftrick et al., 2004, Schmidt et al., 2003). The important physicological parameters for Anammox were summarized in Table II.4 (Strous et al., 1999b).

Metabolic pathway of ANAMMOX process is shown in Figure II.4. As seen from figure, initially (step 1) NH_4^+ is oxidized through NH_2OH to hydrazine (N_2H_4) by hydrazine hydrolase, the N_2H_4 forming enzyme. For the step 4, NO_2^- is oxidized to NH_2OH by hydroxylamine oxidoreductase. Hydrazine oxidizing enzyme, which has some similarity to hydroxylamine oxidoreductase, is the responsible enzyme for hydrazine oxidation to N_2 as seen from step 2 and 3. For step 5, NO_3^- formation could generate the reducing equivalents for biomass growth (Jetten et al., 1999, Jetten et al., 2001, Kuenen et al., 2008).

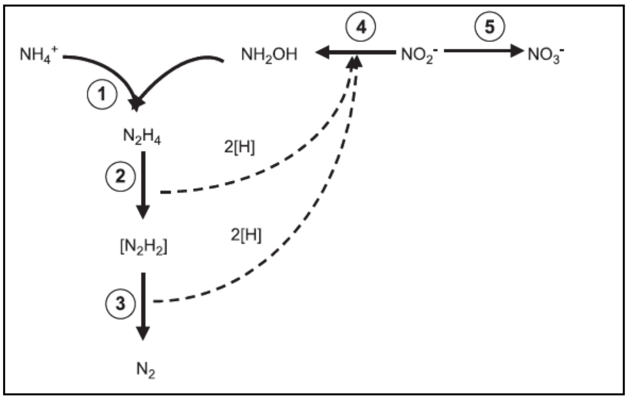


Figure II. 4 Possible metabolic pathway for Anaerobic Ammonium Oxidation (Jetten et al., 1999)

Table II. 4 Important physicological parameters for Anammox (Strous et al., 1999b)

Parameter	
Yield coefficient (Y) ($g\ VSS\ /\ g\ NH_4\text{-}N$)	0.07
Maximum specific NH_4-N oxidation rate (q) ($g\ NH_4\text{-}N/\ g\ VSS.d$)	1.1
Maximum specific growth rate (μ) (h^{-1})	0.003
Activation energy (*kJ/mol*)	70
K_s (NH_4-N) (μM)	5
K_s (NO_2-N) (μM)	< 5
Doubling times (*days*)	10.6

II.5.1 History of Anammox

Richards et al., have reported unexplainable loss of NH_4-N under anoxic conditions in studies of the nitrogen balance in anoxic fjords in 1965. Following to this observation, the Austrian biochemist Broda (1997) predicted the existence of microorganisms capable of NH_4-N oxidation with NO_2-N or NO_3-N as the electron acceptor based on thermodynamical calculations. He postulated that such a microbial conversion process could exist based on the fact that the conversion of NH_4-N and NO_2-N is a reaction with a large negative free energy.

Twenty years later, Mulder et al., discovered the Anammox in a laboratory-scale anaerobic fluidized denitrifying bed used in treating effluent from a methanogenic reactor. He made the first experimental observation while he was studying an autotrophic denitrification process with sulphide as electron donor. Initially it was assumed that NO_3-N was the preferred electron acceptor. Only when it was realised that NO_2-N is the actual electron acceptor it became possible to culture Anammox cultures reproducible in lab-scale reactors.

The first description of an Anammox bacterium dates from 1999 when Strous was able to physically purify anammox cells from a laboratory enrichment culture. Presently the micro-organism involved is reasonably well characterised and also the catabolic route has been established (Woebhen et al., 2008, Kartal et al., 2008). Since this first description, anammox based processes for wastewater treatments have been developed and in recent years the first applications have occurred. In 2002, the first full-scale anammox reactor has been taken into operation at the sludge treatment plant Sluisjesdijk, Rotterdam, NL. The reactor was fed with partially nitritated sludge liquor from an adjusted nitritation process. The reactor is now fully operational (van der Star et al., 2007).

Ten years later there are 11 full-scale Anammox references operational. Some of them were summarized in Table II.5 (Paques).

Table II. 5 Full-scale Anammox process used in industry around the world

Wastewater	NH_4-N (mg/L)	Location
Food	600	China
Yeast	300 - 800	China
Tannery	-	Lichtenvoorde, NL
Digestor liquor	-	Rotterdam, NL
Potato proccessing	-	Steenderen, NL

II.5.2 Biodiversity of Anammox

The anammox bacteria form a distinct, deep branching phylogenetic group in the order *Planctomycetales* as shown in Figure II.5 (Jetten et al., 2005).

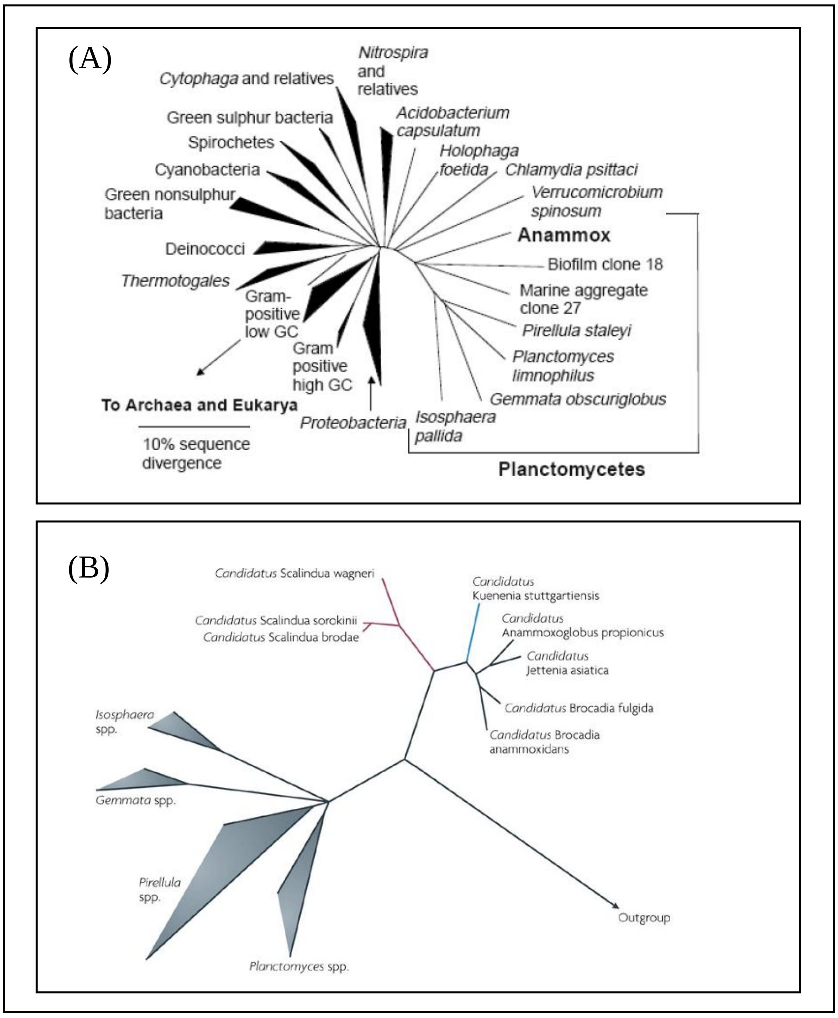


Figure II. 5 Phylogenetic tree of Anammox bacteria (A) within the domain bacteria (Kartal et al., 2008) (B) among the Planctomycetes (Jetten et al., 2003)

The group of anammox bacteria is currently associated with at least five genera, *Candidatus* Brocadia, *Candidatus* Kuenenia, *Candidatus* Anammoxoglobus , *Candidatus* Jettenia, and *Candidatus* Scalindua (Quan et al., 2008, van de Vossenberg et al., 2008, Kartal et al., 2006, Dalgaard et al., 2005, Trimmer et al., 2003, Ward, 2003, Risgaard-Peterson et al., 2004).

Bacteria able to carry out the Anammox process are being found in natural water sources and wastewater treatment plants spread all over the world. *Candidatus* Scalindua seem to appear both in marine environment and in freshwater ecesystems. Until now in nature saline ecosystems only Anammox species belonging to *Candidatus* Jettenia and *Candidatus* Scalindua genus have been dedected (Rysgaard et al., 2004). Studies indicate that anammox bacteria play a very important role in the oceanic nitrogen cycle because the original anammox habitats are the anoxic and microaerophilic zones of the oceans (Ward, 2003). The marine species *Candidatus* Scalindua sorokinii could be responsible for as much as 50% of the nitrogen conversion in the world’s oceans (Dalsgaard et al., 2002, Rysgaard et al., 2004). Many water treatment systems and fresh water ecosystems also appeared to contain significant populations of anammox bacteria that have been named *Candidatus* Kuenenia stuttgartiensis (Schmidt et al., 2005). *Candidatu*s Brocadia anammoxidans and *Candidatus* Kuenenia stuttgartiensis play an important role in the sustainable removal of nitrogen from wastewater (Jetten et al., 2002).

II.5.3 Cell structure of Anammox

Anammox is carried out by an organism belonging to the order of the *Planctomycetales*. These are coccoid bacteria with a diameter of less than 1 µm and they reproduce by budding with a generation time of 10–30 days. All known anammox bacteria have a membrane-bound compartment in the cell, known as the anammoxosome (Figure II.6), in which the anammox process is believed to take place (van Niftrik et al., 2004, Keonig et al., 1984). Anammoxosome was considered to have three functions: (1) providing a place for catabolism; (2) generating energy for ATP synthesis through proton motive force across the anammoxsome membrane; (3) protecting the bacteria from the proton diffusion and intermediate toxicity due to their impermeable membranes. The membrane of the anammoxosome contains ladderane lipids which form an exceptionally tight barrier against diffusion and which appear to be unique to anammox bacteria

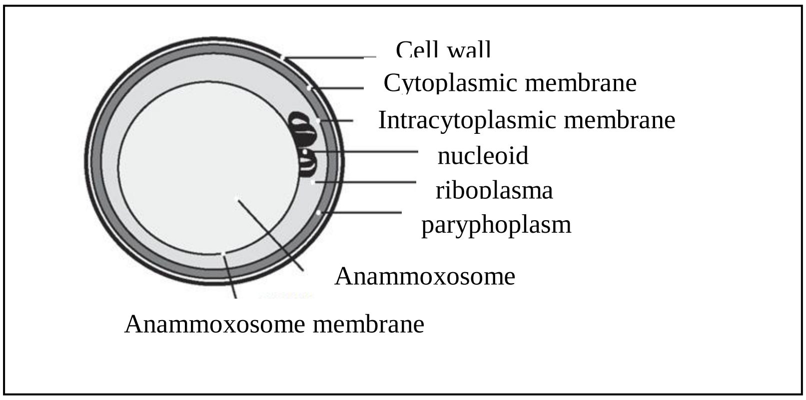


Figure II. 6 Cellular compartmentalization in anammox bacteria

Anammox bacteria lack peptidoglycan and have a proteinaceous cell wall instead (stackebrandt et al., 1986, Damste et al., 2002). Anammox lipids contain a combination of ester-linked and ether-linked fatty acids. Lipid membranes are essential to enable the existence of concentration gradients of ions and metabolites (Liesack et al., 1986). The lipids occur in a wide variety of types and derivatives. They contain one, two or both of two different ring-systems, X and Y (Figure II.7). Ring-system X is composed of three cyclobutane moieties and one cyclohexane moiety substituted with an octyl chain, which is ether-bound at its ultimate carbon atom to the glycerol unit. Ring-system Y is composed of five linearly concatenated cyclobutane rings substituted with a heptyl chain, which contains a methyl ester moiety at its ultimate carbon atom. All rings in ring-systems X and Y are fused by cis-ring junctions, resulting in a staircase-like arrangement of the fused rings, defined as ladderane (Damste et al., 2002). The structure of the ladderane membrane lipids is unique in nature. Ladderane membrane lipids have so far been found only in anammox bacteria.

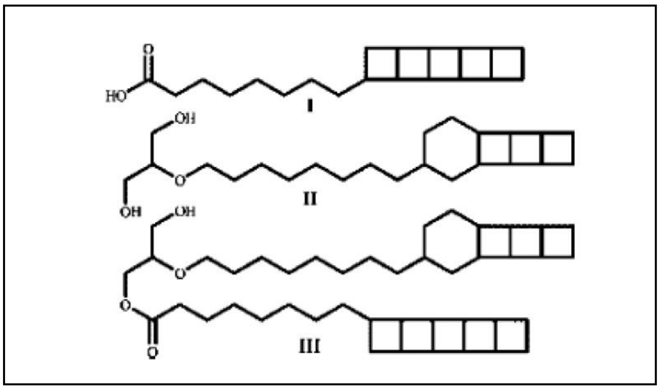


Figure II. 7 Structure of three characteristics ladderane lipids: (I) ladderane fatty acid-containing ring-system Y. (II) ladderane ether-containing ring-system X. (III) ladderane ether/ester containing both ring-systems, X and Y (Damste et al., 2002)

II.5.4 Biochemistry of Anammox

Anammox bacteria are anaerobic chemolithoautotrophs. They convert NH_4 with NO_2^- to N_2 (Eqn. II.27) with N_2H_4 and NH_2OH as intermediates. This 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 (Eqn. II.28) (Jetten et al., 2009).

Catabolism (15x) : $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ (II.27)

Anabolism : $CO_2 + 2NO_2^- + H_2O \rightarrow CH_2O + 2\,NO_3^-$ (II.28)

One of the key enzymes of the anammox reaction, the hydrazine-oxidizing enzyme, was purified and immunogold labeling showed that it was situated in the anammoxosome. This indicates that anammox catabolism takes place inside the anammoxosome.

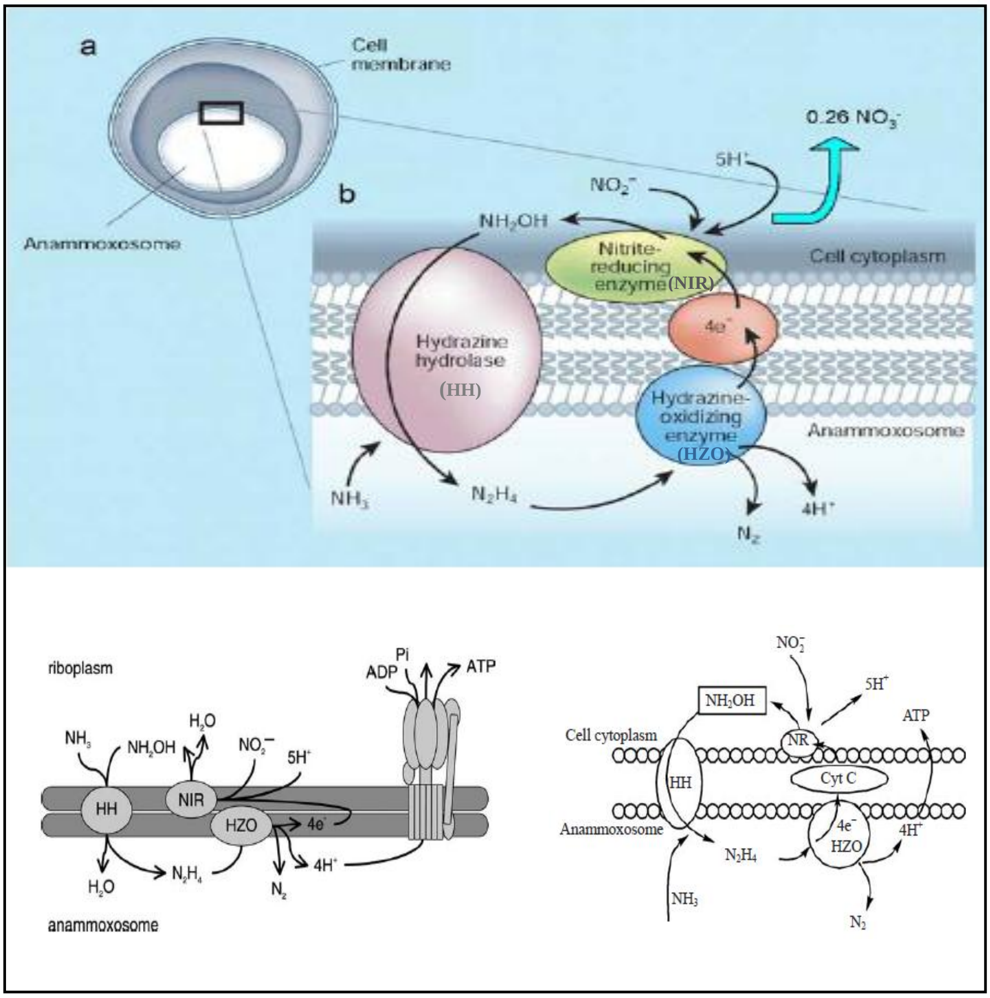


Figure II. 8 Biochemical pathway of Anammox reaction. (Cyt bc_1: cytochrome bc_1 complex, Q: co-enzyme Q) (Kuenen et al., 2008, Ahn et al., 2004)

A biochemical model (Figure II.8) has been proposed in which NH_4^+ and NH_2OH are combined to N_2H_4 by hydrazine hydrolase (HH), the hydrazineforming enzyme. Hydrazine is then oxidized by a hydrazine-oxidizing enzyme (HZO). The oxidation, taking place inside the anammoxosome, results in N_2, four protons and four electrons. These four electrons are then used together with five protons from the riboplasm by a nitrite-reducing enzyme (NIR) to reduce NO_2^- presumably to NH_2OH (Ahn et al., 2004).

II.5.5 Potentials of Anammox process compared to conventional nitrification/denitrification

Environmental and possibilities of economical advantages of Anammox process are important, and therefore give rise to large expectations in the future usage of naturally occurring Anammox bacteria in wastewater treatment technology (Gut et al., 2006). Anammox process can not be used without pretreatment of wastewater because of the electron donor which is NO_2^-. As compared conventional nitrification-denitrification process, less oxygen and no carbon requirements of PN-Anammox system make the process favorable (Figure II.9).

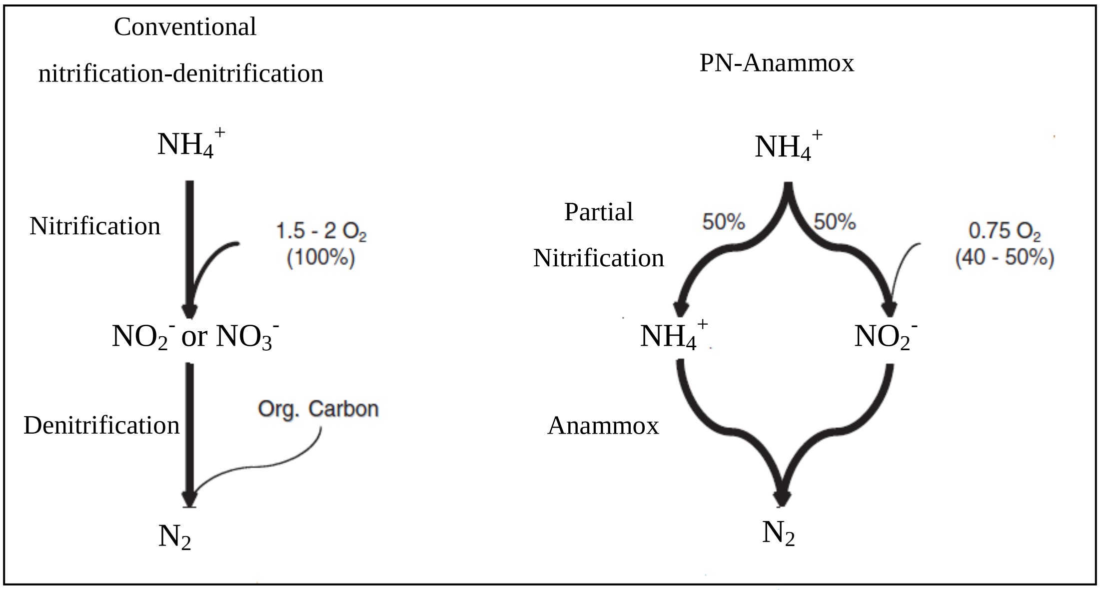


Figure II. 9 Comparison of oxygen and organic carbon consumption of conventional nitrification/denitrification and PN/anammox processes (Fux et al., 2004)

As seen from the Figure II.9, PN/Anammox allows over 50–60% of the oxygen to be saved and no organic carbon source is needed. The release of the gaseous intermediates is a great environmental concern (greenhouse gas, ozone depletion). Regarding the gases produced, autotrophic nitrogen elimination via PN/Anammox is definitely the preferred application from an environmental point of view.

Little practical experience is available for the anammox process, and this also leads to a certain caution and reluctance in constructing the full-scale applications (Abma et al., 2007). On the other hand, the biomass yield is very low (autotrophic organisms) that effects the amount of sludge disposed of the system. Minimal sludge production is an important design criteria because the conventional biological treatment leads to a sizeable amount of produced sludge that must be treated in a proper manner. Amount of produced sludge for conventional nitrification-denitrification and PN-Anammox processes were shown in Figure II.10.

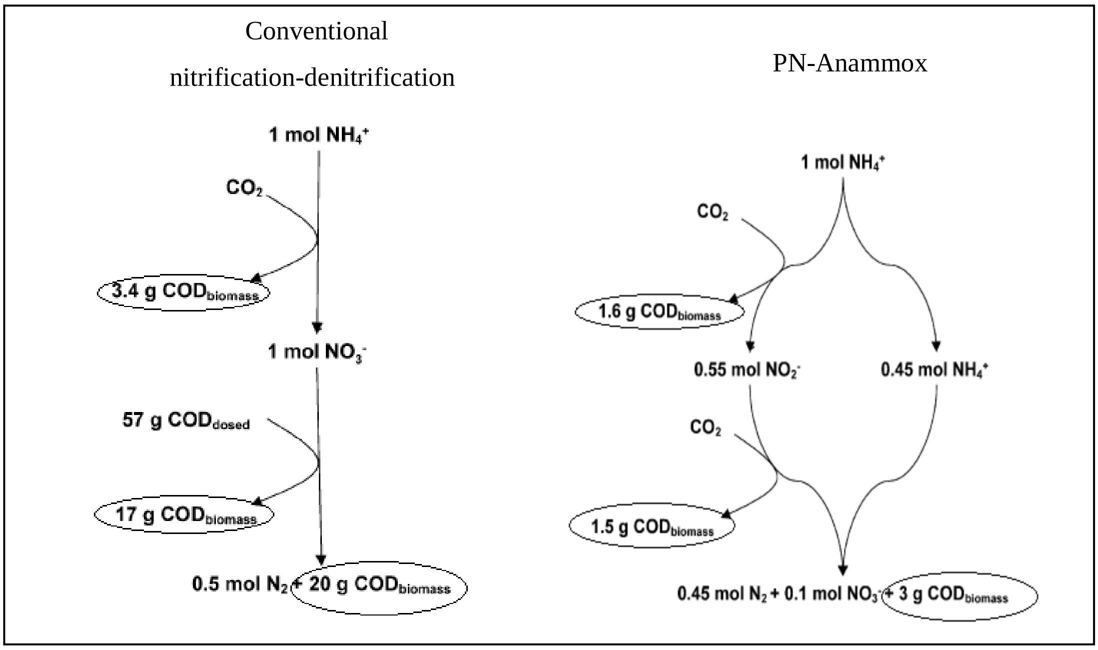


Figure II. 10 Comparison of sludge production for classical nitrification/ denitrification and PN/anammox (Fux et al., 2002)

II.5.6 Limitations of Anammox

The anammox process is a new and promising alternative to the conventional nitrogen removal processes. The application of Anammox to nitrogen removal would lead to a significant reduction of costs for aeration and exogenous electron donor as compared to the conventional nitrification–denitrification process (Fux et al., 2004). However, one of the main drawbacks common to application of the Anammox process is requirement of a long start-up period due to mainly slow growth rates (doubling time was reported to be approximately 11 days) of Anammox bacteria (Strous et al., 1998, van ge Graaf et al., 1996). Due to the long generation time of 11 days, it was so far not possible to isolate the anammox organisms in pure culture. Additionally, Anammox bacteria are strictly anaerobes and autotrophs so they are very difficult to be cultured. To promptly establish Anammox reactors, appropriate seeding sludge or starter cultures must be selected and used, and sufficient amounts of Anammox bacteria must be efficiently retained in the reactors (Bae et al., 2010).

As the Anammox is an anaerobic reaction, DO is not required and known to be inhibitor. As little as 1.1 μM O_2 was sufficient to completely inhibit Anammox activity in a bioreactor, reversebly (Strous et al., 1997). To date, different studies were conducted the inhibitory effect of various inorganic matter (PO_4^{3-}, SO_4^{2-}, NaCI, NO_2^-). SO_4^{2-} represents a problem, as under anaerobic conditions, it is converted into sulphide S^{2-} , which may inhibit Anammox bacteria, by sulphate reducing bacteria (SRB). Concentrations of S^{2-} between 1 and 2mM caused a decrease of 60% of activity (Dapena Mora et al., 2007) while wastewater containing salinity higher than 30 g/L NaCl inhibits Anammox activity, reversibly (Jin et al., 2011). In the case of PO_4^{3-}, concentration which is above 20 mM is inhibitory while the nitrite concentration higher than 100 mg/L inhibits the system irreversibly. It is suggested that the nitrite concentration in an Anammox reactor must be strictly controlled to avoid inhibition of the process (Jetten et al., 2003). On the other hand, organic carbon inhibition was evaluated with various compounds such as acetate, methanol, glucose and propionate. Methanol, a common organic form of many wastewaters, was found to the most inhibitory to Anammox bacterial activity and the inhibition was proven to be irreversible (Dapena Mora et al., 2007).

The Anammox activity occurs in a pH range between 6.4 and 8.3 and a temperature between 20 °C and 43 °C. So the pH and temperature is a problem in some cases.

As mentioned before, Anammox process requires NO_2-N as an electron acceptor, a pre-partial nitritation process should be initiated. In the first place, the NOB must be continuously suppressed, and secondly the NO_2-N/NH_4-N ratio produced must be about 1.3.

Other limitation of the process is the applicability to wastewaters characterized by low carbon to nitrogen ratio content and high ammonia concentrations. In last 10 years, Anammox is investigated for low ammonia containing wastewaters and results showed that it can be achieved successfully.

II.6. APPLICATIONS OF ANAMMOX

The combined PN-Anammox processes mentioned above with low operational costs have attracted much attention since their births. The previous studies reported in literature were mainly investigated the applicability of Anammox process for the treatment of digester supernatant, landfill leachate and also for the industrial wastewaters such as piggery wastewater, fish canning, tannery (Table II.6).

The most common application for the combined PN/Anammox system is for digester supernatant containing high ammonium nitrogen and low organic carbon content. Since anammox process was successfully applied to the treatment of sewage sludge digester liquor in the Netherlands, it opened doors for its application to many kinds of wastewater treatment such as industrial wastewater, livestock wastewater, and landfill leachate.

Table II. 6 Overview of the research and development of the Anammox process

Reactor configuration	Wastewater	Influent NH4-N	Organic matter	Location	References
UASB PN-Anammox	digester supernatant	717 mg/L	369 mg O_2/L	Hiimerfjarden WWTP	Gut et al., 2006
Biofilm PN-Anammox	digester supernatant	568 mg/L	210 mg COD/L	Laboratory scale	Szatkowska et al., 2007
Combined SBR Sharon-Anammox	digestor supernatant	800 mg/L	-	Barcelona WWTP	Gali et al., 2007
Combined SBR PN-Anammox	digestor supernatant	600 mg/L	-	Dokhaven WWTP, Rotterdam	van Dongen et al., 2001
Combined SBR PN-Anammox	digestor supernatant	657mg/L - 619 mg/L	4500 mg COD/L	Werdhoeizli, Zurich - Au, St.Gallen	Fux et al., 2002
Combined SBR PN-Anammox	digestor supernatant	-	-	-	Fux et al.,2004
Combined SBR PN-Anammox	digestor supernatant	1200 mg/L	-	Dokhaven WWTP, Rotterdam	van der Star et al., 2007
Combined SBR Sharon-Anammox	digestor supernatant	-	-	Beverwijk WWTP, NL	van Loodsrecht et al., 2006
Combined SBR Sharon-Anammox	digestor supernatant	600 - 900 mg/L	-	Utrecht WWTP, Rotterdam	Mulder et al., 2007
Combined SBR Sharon-Anammox	digestor supernatant	1000 - 1500 mg/L	-	Dokhaven WWTP, Rotterdam	Mulder et al., 2007
Combined SBR Sharon-Anammox	digestor supernatant	400 - 600 mg/L	-	Zwalle WWTP, Rotterdam	Mulder et al., 2007
Combined SBR Sharon-Anammox	digestor supernatant	700 - 900 mg/L	-	Beverwijk WWTP, Rotterdam	Mulder et al., 2007
Combined SBR Sharon-Anammox	digestor supernatant	900 - 1200 mg/L	-	The Hauge-Houtrust WWTP, Rotterdam	Mulder et al., 2007
Combined SBR Sharon-Anammox	digestor supernatant	700 - 800 mg/L	-	Groningen-Garmerwolde WWTP, Rotterdam	Mulder et al., 2007
Combined SBR PN-Anammox	digestor supernatant	-	-	-	Qaio et al., 2010
Combined SBR PN-Anammox	digestor supernatant	-	-	-	Zhang et al., 2009
Combined SBR PN-Anammox	digestor supernatant	-	-	-	Wanyuan et al., 2011
Combined SBR PN-Anammox	digestor supernatant	-	-	-	Ma et al., 2009
Combined SBR Sharon-Anammox	digestor supernatant	-	-	-	Padin et al., 2009
Combined SBR Sharon-Anammox	piggery waste	890 - 1070 mg/L	1220 - 4190 mg COD/L	Laboratory scale	Hwang et al., 2005
UASB PN-Anammox	piggery waste	4300 mg/L	47000 mg COD/L	Laboratory scale	Ahn et al., 2004
UASB PN-Anammox	piggery waste	3780 mg/L	4740 mg COD/L	Laboratory scale	Molinuevoet al., 2009
Biofilm PN-Anammox	landfill leachate	1400- 2800 mg/L	1100-2600 mg COD/L	Beijing, China	Liang et al., 2007
SBR PN-Anammox-Denitrification	landfill leachate	634 mg/L	554 mg COD/L	-	Wang et al., 2010
Combined SBR Sharon-Anammox	landfill leachate	1300 - 3100 mg/L	3000- 5000 mg O_2/L	CORSA landfill,Spain	Ganigue et al.,2008
Shortcut nitrification-Anammox	landfill leachate	1900-2400 mg/L	1900-2200 mg COD/L	Liulitun MSW, China	Liu et al., 2010
SBR PN-Anammox-Denitrification	landfill leachate	-	-	-	Lan et al., 2011
Combined SBR Sharon-Anammox	digested fish canning	260 - 800 mg/L	50 - 120 mg TOC/L	Dokhaven WWTP, Rotterdam	Dapena mora et al., 2006
Baffled PN- UASB Anammox	sewage	44,3 mg/L	44,4 mg COD/L	Gaobeidion WWTP, China	M0 et al.a, 2011

CHAPTER III

THE STUDY

In the scope of this study, nitrogen removal from domestic wastewater with Anammox process was evaluated. The reactor systems started-up and operated throughout the study were schematically summarized and chronologically illustrated in Figure III.1 and III.2, respectively. As seen from these figures, at the beginning of the study, two reactor systems namely PN-SBR and Stock-Anammox-SBR were started-up using a mixed activated sludge obtained from Istanbul Pasakoy STP recycle line.

In the PN-SBR system, partial nitritation of real domestic wastewater was deeply studied in order to obtain an effluent with NO_2-N/NH_4-N ratio (1.2-1.5) appropriate for the influent for Anammox in 350 days operational period. In this period both DO limitation, FA inhibition and operation at NOB growth-limiting SRT were used to obtain appropriate ratio in the effluent.

The Stock-Anammox-SBR was enriched for Anammox bacteria and operated for 515 days. In this operational period, the system was fed with synthetic wastewater and influent NH_4-N and NO_2-N concentrations were increased up according to the removal efficiencies. A new Anammox SBR reactor was started with this enriched Anammox culture and operated for 240 days. The system was initially fed with synthetic wastewater for 104 days to evaluate possible use of NO_3-N as electron acceptor. Following to the optimization of PN, treatability of domestic wastewater was studied by combining PN-SBR and Anammox-SBR systems. Prior of feeding PN effluent to Anammox SBR system, a serial batch experiments were performed to evaluate the possible organic carbon inhibition on Anammox bacteria. Sludge which was taken from a lab-scale Stock enriched Anammox SBR system was used in these experiments. This system was inoculated with seed sludge from a sludge treatment plant in Sluisjesdijk, Rotterdam and operated for 500 days by feeding synthetic wastewater [158, 159].

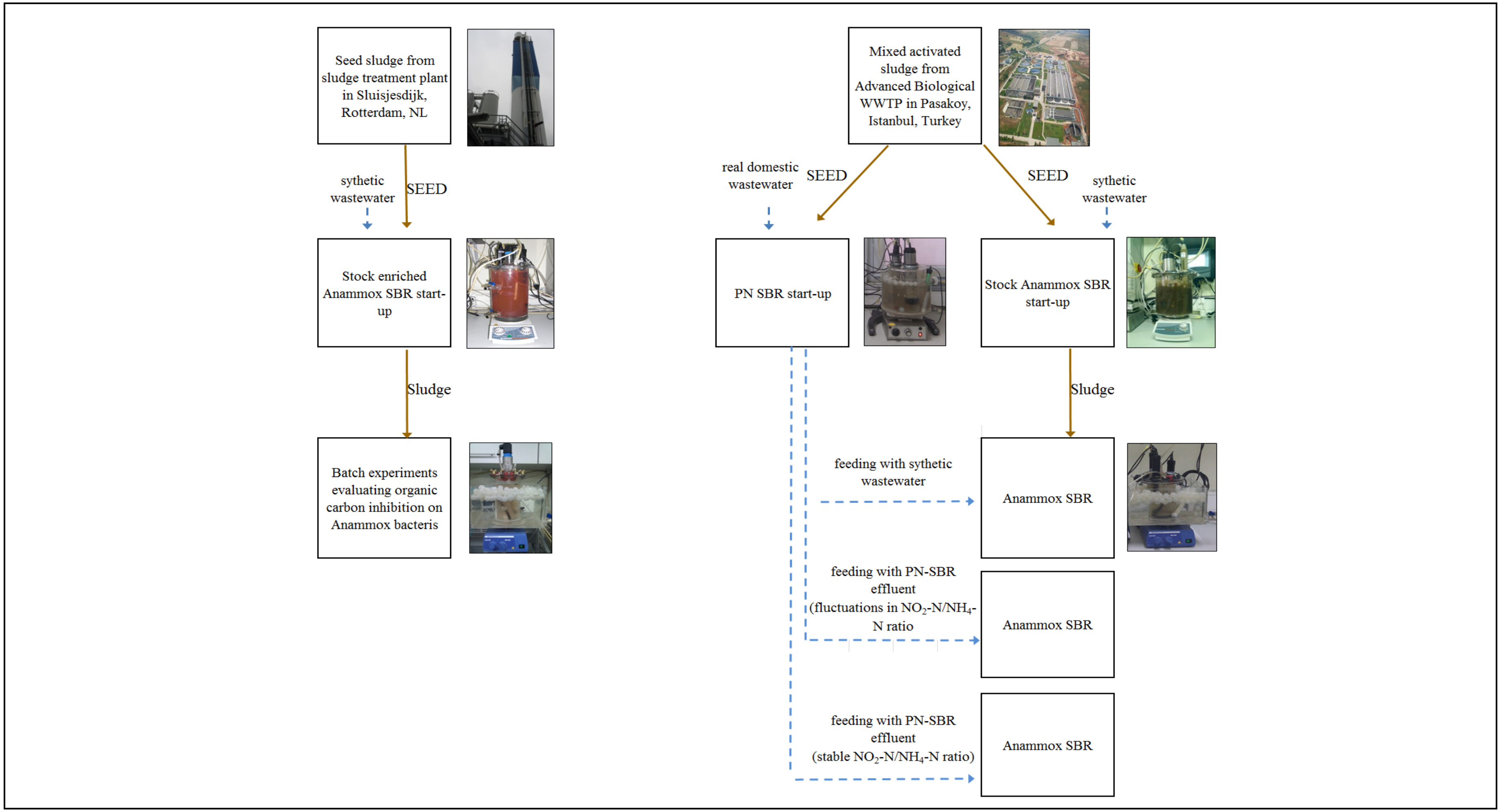


Figure III. 1 Operational chart of the study

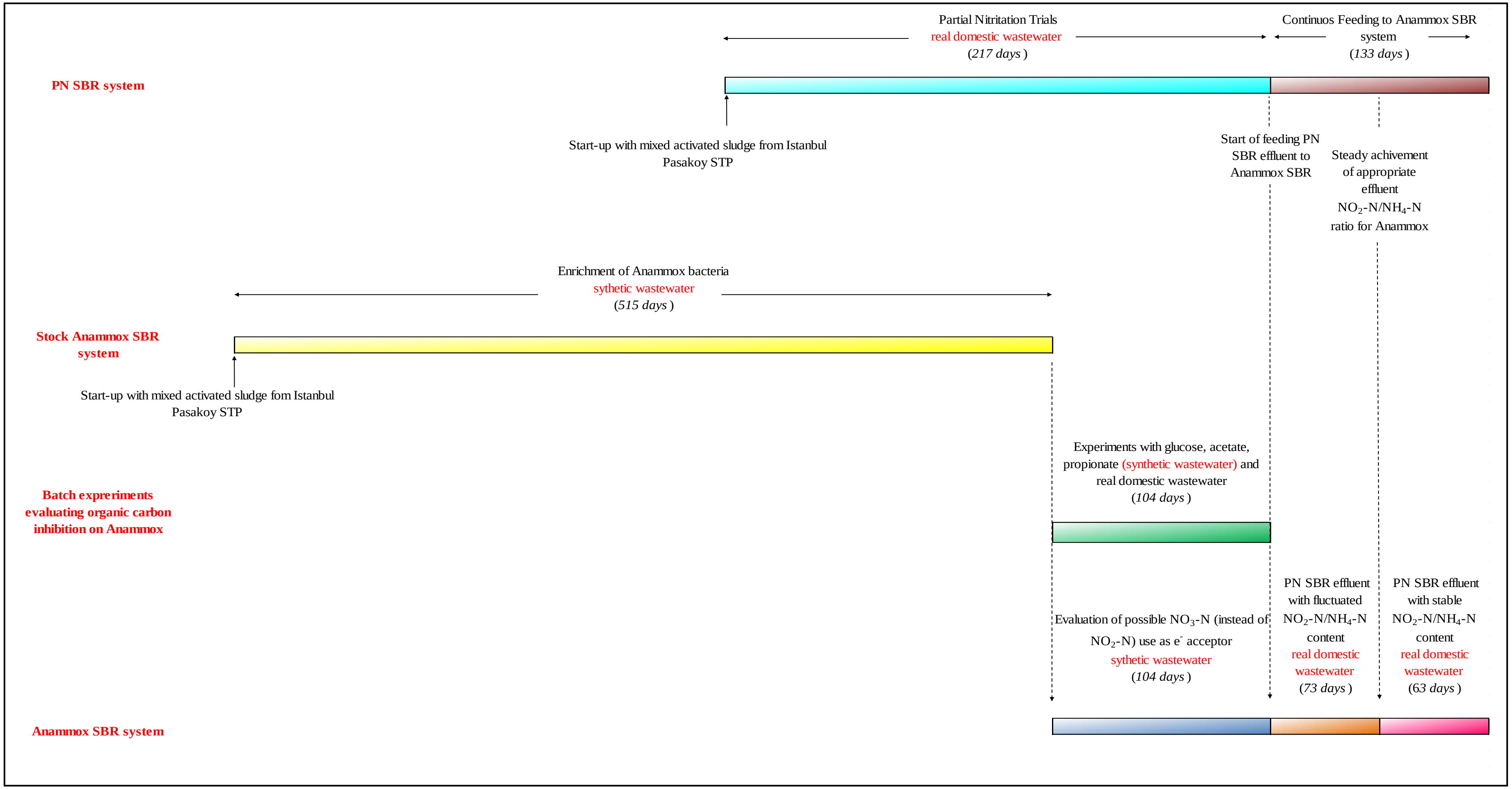


Figure III. 2 Chronological illustration of the study

III.1 PN-SBR SYSTEM

The reactor was initially inoculated with mixed activated sludge taken from Istanbul Pasakoy STP recycle line (Figure III.1, III.2). Initial MLVSS concentration of the reactor was 1066 mg/L. A lab-scale plexi-glass reactor (Figure III.3) having a 28 cm height and 15.5 cm diameter with a working volume of 2 L was operated as SBR.

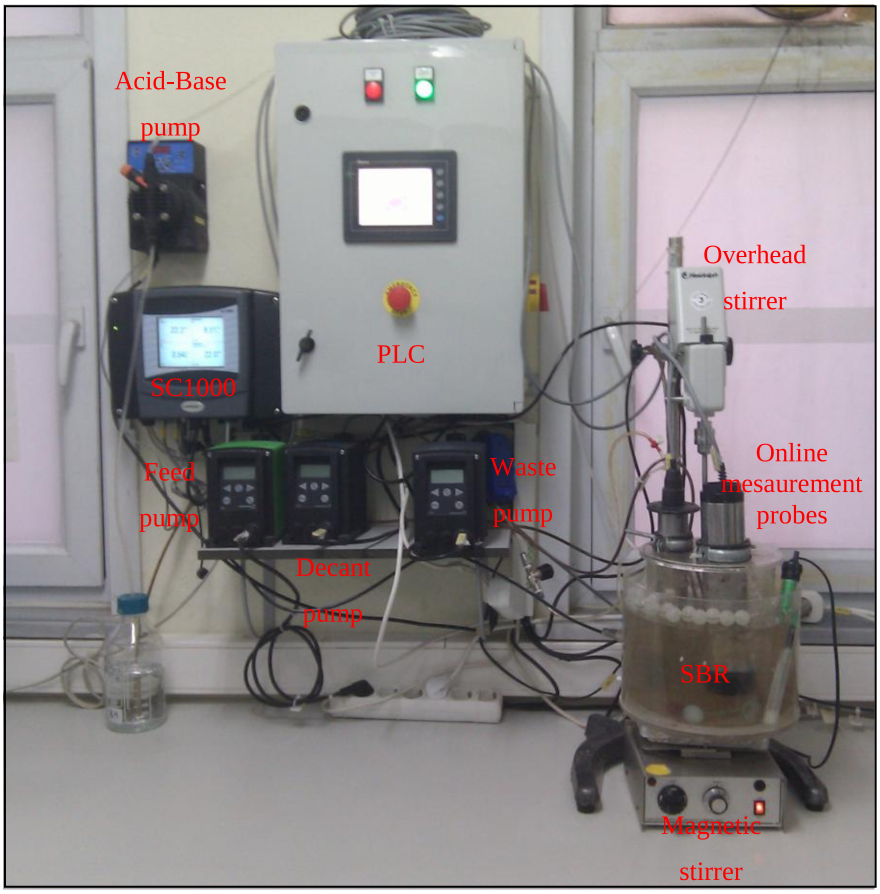


Figure III. 3 PN-SBR system

The schematic operation diagram of the system is shown in Figure III.4. The reactor was operated at 17 hours batch cycles and controlled with a Programmable Logic Controller (PLC) system. Each cycle comprised six phases including feed, react, waste, settle, decant and idle. The operational cycle of the reactor is detailed in Figure III.5.

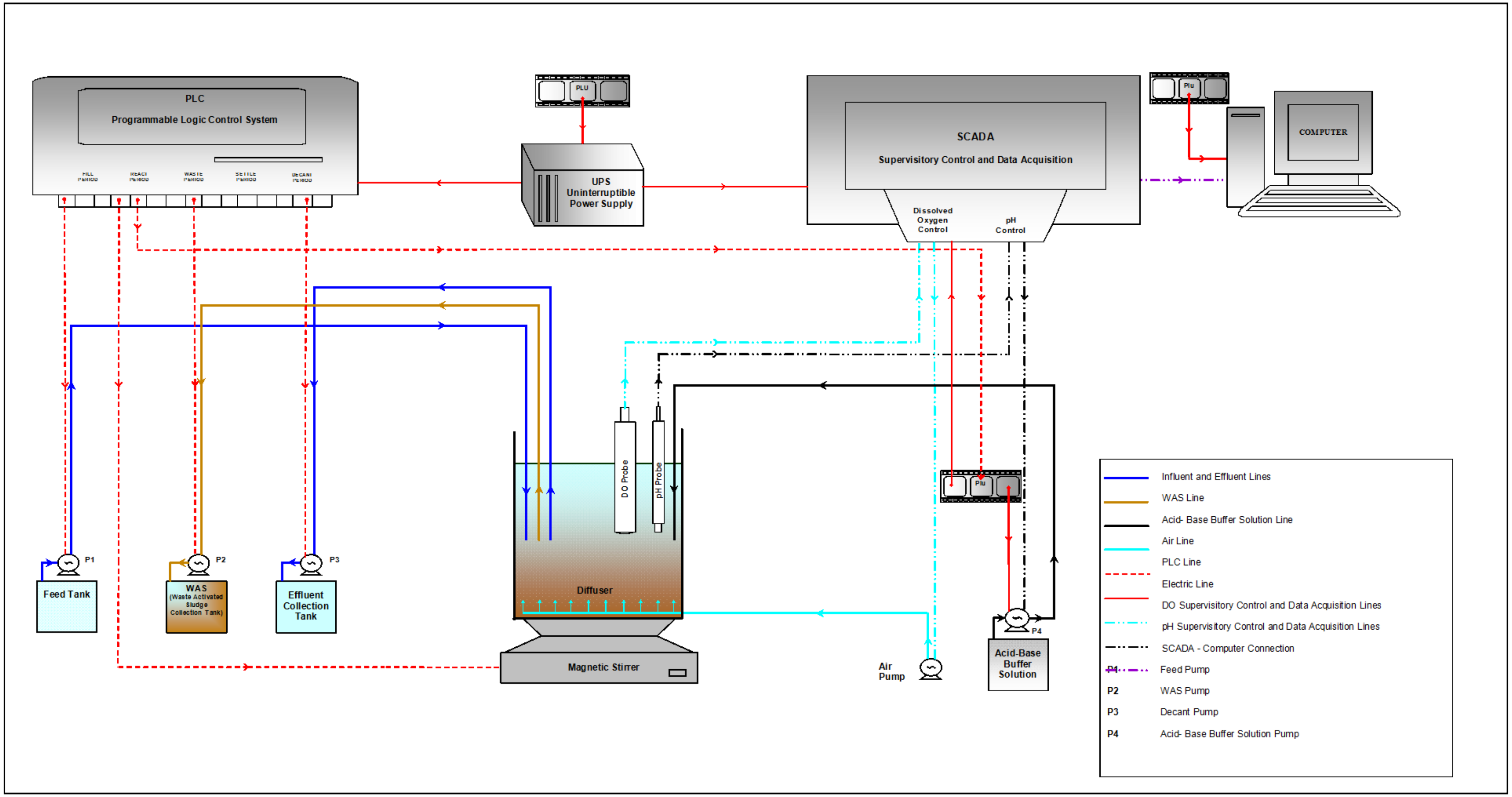


Figure III. 4 Schematic view of operation diagram for PN-SBR system

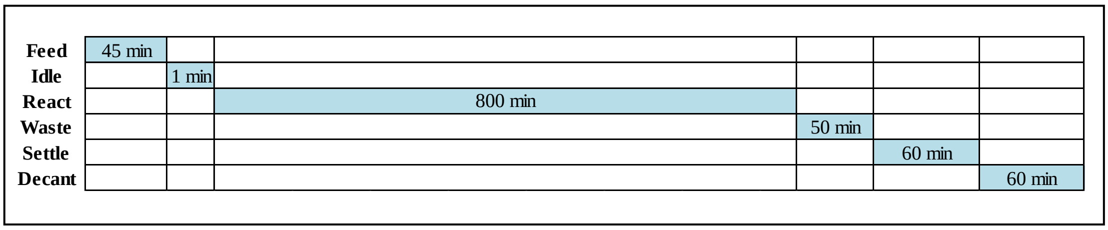


Figure III. 5 Operational cycle of the PN-SBR system

In each cycle, 1600 ml of working volume was discharged and 1600 ml real domestic wastewater was fed into reactor with diaphram dosing pumps (Grundfos DME 2-18). The reactor was constantly operated with a hydraulic retention time of 1.25 days as shown in Eqn. III.2.

$$\mathrm{HRT}=\frac{\text{Volume of the reactor (L)}}{\text{Influent flowrate (L/d)}}=\frac{2}{1.6}=1.25\ \mathrm{d} \tag{III.2}$$

In waste period, 800 ml mixed liquor was wasted with a diaphragm dosing pump (Grundfos DME 2-18). Sludge retention time (SRT) of the reactor was as 2.5 days as shown in Eqn. III.3.

$$\mathrm{SRT}=\frac{\mathrm{V.X}}{\mathrm{Qw.X}}=\frac{\text{Volume of the reactor (L)}}{\text{Influent flowrate (L/d)}}=\frac{2}{0.8}=2.5\ \mathrm{d} \tag{III.3}$$

Water temperature was set about 22-25°C by controlling the room temperature with an air conditioner. Complete mixing in the reactor was provided by a magnetic stirrer (Velp Are) and an overhead stirrer (Daihan Wisestir HS50A). Required DO concentration was supplied by air through diffusers placed into the bottom of the reactor and connected to an air pump. pH was fixed with an acid-base pump (ETATRON DLX MA/MB). These equipments were adjusted by a Supervisory Control and Data Acquisition system (SCADA-Hach Lange SC1000). pH (Hach-Lange pHD-S sc pH), DO (Hach-Lange LDO) and temperature were continuously measured in 5 minutes time arrivals by on-line probes connected to the SCADA system and the data were logged into this system.

The system was continously fed with real domestic wastewater for 350 days (Table III.1). The real domestic wastewater was obtained from the inlet of the grit chambers in Istanbul Pasakoy STP.

Table III. 1 Real domestic wastewater characteristics

Parameter	Concentration range (mg/L)
COD	45 - 415
TKN	26 - 95
NH_4-N	13 - 62
NO_2-N	0 - 2.5
NO_3-N	0 - 6
Alkalinity (as $CaCO_3$)	190 - 215

Throughout the study, NH_4-N, NO_2-N, NO_3-N, COD and TKN concentrations in the influent and effluent from the system were measured as described in Section III.5. The influent concentrations of the reactor was calculated by accounting the reactor content from a perivous cycle as described in Eqn. III.4.

$$\text{Influent N}=\frac{[\,1.6\ (\text{Feed solution N})+0.4\ (\text{Previous decant solution N})]}{2} \qquad \text{(III.4)}$$

III.2 STOCK ANAMMOX SBR SYSTEM

III.2.1 Start-up and enrichment

The reactor was initially inoculated with mixed activated sludge taken from Istanbul Pasakoy STP recycle line. The initial MLVSS concentration of the reactor was 3000 mg/L. Biomass was enriched in a lab-scale plexi-glass reactor (Figure III.6) having a 20 cm height and 19.4 cm diameter with a working volume of 4.5 L was operated as SBR.

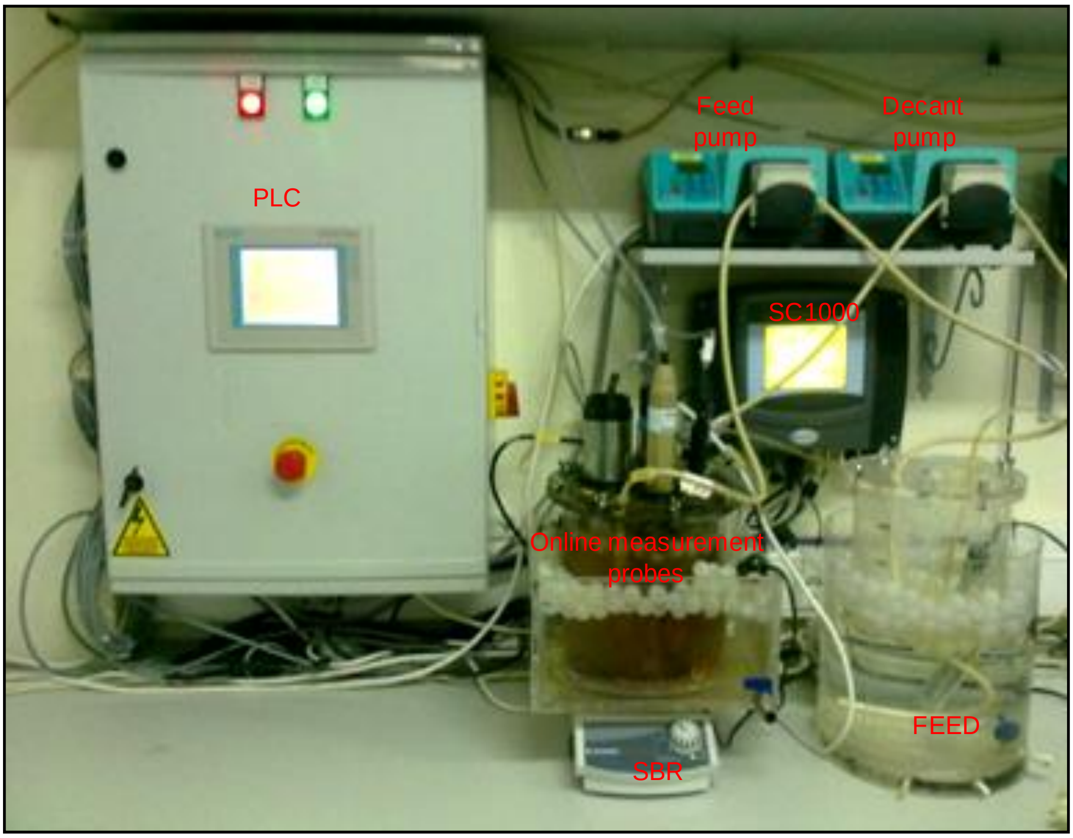


Figure III. 6 Stock Anammox SBR system

The schematic operation diagram of the system is shown in Figure III.7. The reactor was operated at 24 hours batch cycles and controlled with a PLC system. Each cycle comprised five phases including feed, react, settle, decant and idle. The operational cycle of the reactor is detailed in Figure III.8.

In each cycle, 1000 ml of working volume was discharged and 1000 ml synthetic wastewater was fed into reactor with peristaltic pumps (Watson Marlow 323 S/D). The reactor was constantly operated with a hydraulic retention time of 4.5 days as shown in Eqn.III.5.

$$\text{HRT} = \frac{\text{Volume of the reactor (L)}}{\text{Influent flowrate to the system (L/d)}} = \frac{4.5}{1} = 4.5\ \text{d} \tag{III.5}$$

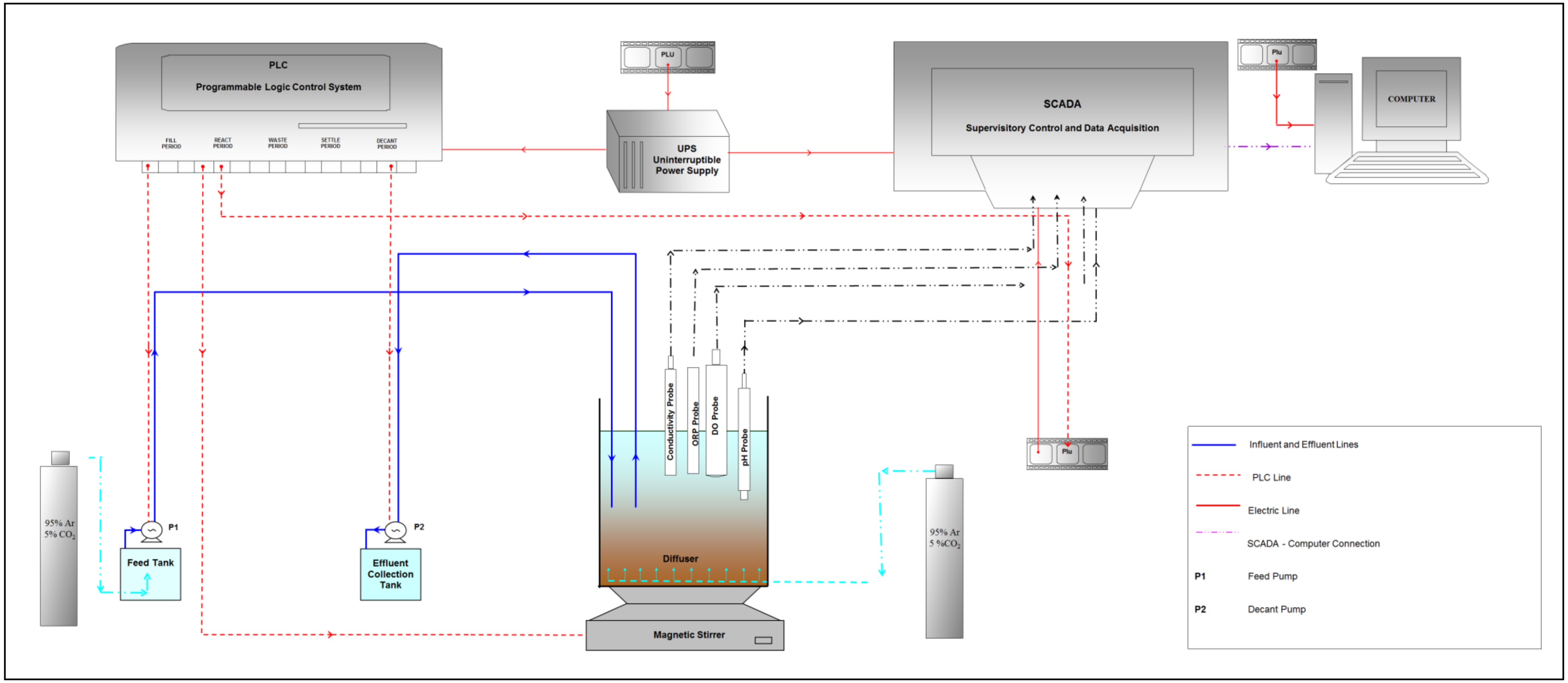
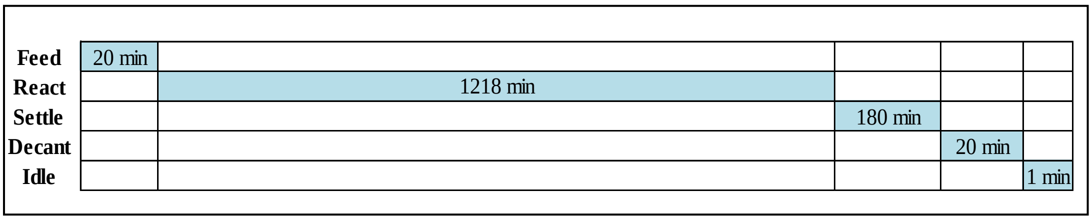


Figure III. 7 Schematic view of operation diagram for Stock-Anammox-SBR system



Feed	20 min				
React		1218 min			
Settle			180 min		
Decant				20 min	
Idle					1 min

Figure III. 8 Operational cycle of Anammox SBR reactor

Water temperature was set about 32-35°C by aquarium heater which was placed into water jacket surrounding the reactor. Complete mixing in the reactor was achieved by a magnetic stirrer (Heidolph MR Hei-Mix L). During react and feeding phases, reactor liquid was flushed with %95 Ar + %5 CO_2 to maintain anaerobic conditions and to supply inorganic carbon for Anammox bacteria. Feed solution and headspace of the reactor was also continuously purged with %95 Ar + %5 CO_2. The surface of the suspension level in the reactor and the surface of the feed liquid were covered with polypropylene balls (Cole-Parmer) to enhance the CO_2 solubility and to prevent oxygen enterance.

Conductivity (Hach-Lange 3700 sc), pH (Hach-Lange pHD-S sc pH), Oxidation Reduction Potential (ORP) (Hach-Lange pHD-S sc ORP), DO (Hach-Lange LDO) and temperature were continuously measured with 15 minutes time intervals by on-line probes connected to SCADA system (HACH Lange SC1000). The data were logged into the SCADA system.

Acclimatization was obtained by feeding the seed sludge with synthetic wastewater (Table III.2) for 515 days. The NH4-N and NO2-N loadings to the reactors were increased stepwise depending on the treatment efficiency.

Throughout the study, NH_4-N, NO_2-N and NO_3-N concentrations in the influent and effluent from the system were measured as described in Section III.5. The influent concentrations of the reactor was calculated by accounting the reactor content from a perivous cycle as described in Eqn. III.6.

$$\text{Influent N}= \frac{[\,1(\text{Feed solution N}) + 3.5\ (\text{Previous decant solution N})]}{4.5} \tag{III.6}$$

Table III. 2 Stock-Anammox-SBR system sythetic feed solution

Compound	Stock Concentration (g/L)	Concentration in the reactor (mg/L)	Volume added per liter (mL)
$(NH_4)_2.SO_4$	100 g/L as NH_4-N	4.5 - 209	0.2 - 9.4
$NaNO_2$	100 g/L as NO_2-N	9 - 225	0.4 - 10.1
KNO_3	100 g/L as NO_3-N	50	2
$KHCO_3$	250	1250	4
Mineral medium			
KH_2PO_4	27.2	27.2	1
$CaCl_2.2H_2O$	180	180	1
$MgSO_4.7H_2O$	300	300	1
Mineral Solution 1			
$Na_2EDTA \cdot 2H_2O$			
$FeSO_4.7H_2O$	5	5	1
Mineral Solution 2			
$Na_2EDTA \cdot 2H_2O$	15	15	1
$ZnSO_4.7H_2O$	0.43	0.43	
$CoCl_2$	0.12	0.12	
$MnCl_2.4H_2O$	0.99	0.99	
$CuSO_4.5H_2O$	0.25	0.25	
$NiCl_2.6H_2O$	0.19	0.19	
Na_2SeO_3	0.10	0.10	
H_3BO_3	0.011	0.011	

III.2.2 Microbial structure and morphological analysis of Stock-Anammox-SBR system

During the enrichment period, the microbial population dynamics were followed via molecular tools (Quantitative-Real Time Polimerase Chain Reaction (Q-RT PCR), Denaturing Gradient Gel Electrophoresis (DGGE), Fluorescense in Situ Hybridization (FISH)). These analyses were performed at Istanbul Technical University Molecular Biology and Genetics Department under the supervision of **Ass. Prof. Alper Tunga Akarsubaşı**. Morphological studies of the biomass were performed at Bogazici University Advanced Technologies Research and Development Centre with an environmental scanning electron microscope (Philips XL30 ESEM-FEG /EDAX) controlled with a computer system.

III.2.2.1 Sample Collection

Samples were stored at -20°C for DNA extraction. Biomass (2 ml) was harvested by centrifugation (10 min, 13000 RPM) and Fast DNA Spin Kit (MP Bio, USA) was used for extraction as described in manufacturer's instructions. DNA was resuspended in 75 µl elution buffer, and kept at -20 °C until further analysis. DNA occurance was observered by 1% (w/v) agarose gel electrophoresis, and concentration was measured with Qubit Fluorometer (Invitrogen, USA).

III.2.2.2 Eubacterial PCR

Eubacterial specific, 16S rRNA gene fragments were amplified using universal bacterial primers (VfGC-Vr). All PCR reactions contains 1 µM from each primer, 0.2 mM dNTP, 1U i-StarTaq^{TM} DNA polymerase (INTRON Biotechnology, Inc, USA). 1 µl of sample DNA (10 ng/µl) is used as template in a 25 µL of total PCR volume. Reactions were performed in C1000^{TM} Thermal Cycler (BIORAD, USA). Another primer set named pA-AGA GTT TGA TCC TGG CTC AG and pH-AGG GAG GTG ATC CAG CCG CA (Edwards et al., 1989) was also used for cloning. The PCR program was set as 5 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 55 °C, 40 s at 72 °C; and extension for 10 min at 72 °C for bacterial samples.

III.2.2.3 Q-RT PCR for Annamox bacteria

A combination of forward Pla46f-GGA TTA GGC ATG CAA GTC and reverse Amx667r-ACC AGA AGT TCC ACT CTC primers was used for quantifying anammox bacteria.

Q-RT PCR was performed with an iCycler iQ5 thermocycler and real-time detection system (Bio-Rad, Berkeley, CA). For the standard curves, 16S rRNA coding DNA specific PCR fragments from *Scalindua spp.* were cloned with the pGEM-T Easy cloning kit (Promega, Madison, WI) and used as positive control.

The thermal cycler's program was initiated with 5 min at 95°C denaturing step, followed by 45 cycles of 20 s at 95 °C denaturation, 20 s at 55 °C annealing, 60 s at

72 °C of extension steps. Melting curve analysis for SYBR green assay was done after amplification for the determination of nonspecific amplification if there were any.

The Anammox specific Q-RT PCR assay was very consistent, as shown by the strong inverse linear relationship between the threshold cycle numbers and the copy numbers of 16S rRNA gene ($R^2 = 0.998$). The amplification efficiencies were 103.3%, with slopes of -3.314.

III.2.2.4 DGGE

DGGE was performed by using the DCode universal mutation detection system (Bio-Rad Laboratories). 1-mm 10% (w/v) polyacrylamide (37:5:1 acrylamide: bisacrylamide) gels containing denaturant gradients of 35 to 65% were used to separate eubacterial PCR products. Gels were run at 60 °C and 180V for 330 minutes. The gels were stained with 0.5 µg/mL ethidium bromide (Sigma Aldrich, Inc., St. Louis, MO) in 1× TAE buffer for 20 min. Images were recorded using GelDoc imaging system (Bio Rad, Hercules, CA) after a 15min destaining processes in 1X TAE buffer.

DGGE band pattern analysed using Bionumerics Software Vers. 5.1 (Applied Maths, Belgium). Calculation of similarities of the DGGE profiles was based on the Pearson product moment correlation coefficient. Mean similarities and associated standard deviations were calculated from the similarity values of matrices compared pairwise within each samples.

III.2.2.5 Cloning, sequencing and phylogenetic analysis

A clone library of universal bacterial 16S rRNA gene was constructed using the PCR products and a TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, USA) as described in the manufacturer's instructions. Primers named pA and pH targeting a large fragment of the 16S rRNA gene was used for PCR prior to clone library analysis. PCR reaction was set up as described previously. One µL of genomic DNA was added as template in a total reaction volume of 50 µL. The clones were grown in LB medium plates supplemented with ampicillin (100 µg/ml) at overnight incubation. Clones were randomly selected for further analysis. QIAGEN MiniPrep Plasmid isolation kit (Qiagen, Inc., Valencia, CA) is used for plasmid extraction. The sequencing of the cloned nucleotides was performed with an automatic sequencer (ABI, USA). Affiliated phylotypes for the sequences of 16S rRNA gene were checked at the Ribosomal Database Project. Phylogenetic trees were constructed using the neighbour-joining method under the total gap remotion and Kimura's two-parameter substitution model and visualized using the MEGA 5 software package. The bootstrap analysis was conducted on 1000 resamplings. The nucleotide sequences were deposited in Genbank with accession numbers JN590920-JN590971.

III.3 BATCH EXPERIMENTS EVALUATING ORGANIC CARBON INHIBITION ON ANAMMOX

Organic carbon inhibition experiments were performed with glucose, acetate and propionate as synthetic carbon sources and domestic wastewater as natural carbon source. The experiments were performed before starting to feed of PN-SBR effluent to Anammox-SBR system to evaluate the possible inhibiton of Anammox bacteria by organic carbon in the effluent from PN-SBR.

The sludge used for experiments was taken from an enriched Anammox-SBR system which was started-up with the sludge obtained from the first full-scale Anammox reactor at the sludge treatment plant Sluisjesdijk, Rotterdam, NL. The system was operated for the enrichment of Anammox bacteria for 500 days [158, 159]. Schematic diagram of the batch experiment set-up is shown in Figure III.10. The methodology followed in batch experiments is as summarized below.

1. The empty reactor (Figure III.9) was flushed with 95%Ar + 5%CO_2 to get rid of O_2 and to maintain necessary anaerobic conditions for batch experiments.
2. The sludge taken from the enriched Anammox-SBR system was washed with three times with one-fifth diluted mineral solution (Table III.2) to remove the background media.
3. The reactor was filled with feed solution which was flushed by 95%Ar + 5%CO_2.
4. The headspace of the reactor was flushed with 95%Ar + 5%CO_2 to maintain anaerobic conditions.

5. Magnetic stirrer was turned on and samples were taken for MLVSS experiments.
6. N_2 gas was tracked by measuring the overpressure in the headspace with a pressure transducer (Endress Hauser PMC131).

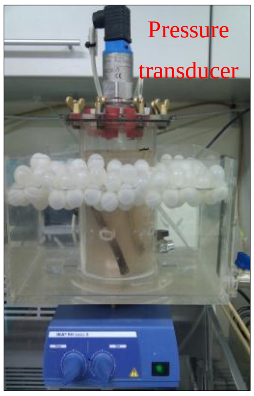


Figure III. 9 Batch experiment reactor

Batch experiments were performed as 4 sets as summarized in Table III.3. Each experiment included 2 different runs. In the first runs of each set, called blank test, the aim was to measure N_2 gas production resulting from denitrification. For this purpose, in these runs, the sludge was fed with only NO_2-N (175), NO_3-N (30), mineral solutions (Table III.2) and organic carbon source without addition of NH_4-N. In the second run, NH_4-N (140) was also included to the feed solution composition described for the first runs in order to measure N_2 gas production resulting from both Anammox and denitrification activities.

In experiment 3, propionic acid is used to observe possible organic carbon inhibiton with propionate. So the pH of the reactor had to be adjusted with NaOH. Proper amount of NaOH was added to the reactor with every dosage of propionate.

Table III. 3 Batch experiments performed for organic carbon inhibition

Experiment No	Organic matter	Concentration range (as COD/L)
1	Glucose	0 - 10000
2	Acetate	0 - 6000
3	Propionate	0 - 6500
4	Real domestic wastewater	215

In each run, pressure changes in the headspace due to N_2 gas production were monitored by a pressure transducer. The pressure changes were plotted against time and from the linearization the graph dP/dt (bar/min) values were calculated. By placing dP/dt values to the ideal gas law equation (Eqn. III.8), dN_2/dt vaues were calculated. From the linerization of these plottings, dN_2/dt values were found from the slopes (Eqn. III.7).

$$P.V = n.R.T$$

$$\frac{P}{t}.V = \frac{n}{T}.R.T$$

$$\frac{dP}{dt}.V = \frac{dN_2}{dT}.R.T$$

$$\frac{dN_2}{dt} = \alpha \frac{V_G}{RT} \qquad \text{(III.7)}$$

Where;

dP/dt : pressure change in headspace with respect to time (bar/min)

V_G : volume of the headspace (L)

R : ideal gas coefficient (0.00831447 L bar K^{-1} mol^{-1})

T : temperature (K)

In blank tests, Specific Denitrification Activity (SDA) was calculated from this values divided by the biomass concentration X (g VSS L^{-1}) as shown in Eqn. III.8.

$$\text{SDA (g N}_2\text{-N/mgVSS.d)} = \frac{\frac{dN_2}{dt}}{X\,V_L}\;\frac{28\text{ g N}_2\text{-N}}{\text{mol N}_2}\;\frac{1440\text{ min}}{\text{day}} \qquad \text{(III.8)}$$

where;

V_L : volume of the liquid (L)

In the second run of each test, the measured dP/dt values were due to both denitrifiers and Anammox activity. Specific Denitrification-Anamox Activity (SDAA) which was calculated with Eqn.III.9 and SDA.

$$\text{SDAA (g N}_2\text{-N/mgVSS.d)} = \frac{\frac{dN_2}{dt}}{X\,V_L}\;\frac{28\text{ g N}_2\text{-N}}{\text{mol N}_2}\;\frac{1440\text{ min}}{\text{day}} \tag{III.9}$$

The specific Anammox Activity was calculated as shown in Eqn. III.10.

$$\text{SAA (g N}_2\text{-N/mgVSS.d)} = \text{SDAA} - \text{SDA} \tag{III.10}$$

In each batch experiment set, the concentration of organic matter to be tested was gradulally increased till at least 70% inhibition of SAA inhibition was observed.

III.4 ANAMMOX-SBR SYSTEM

The reactor was started with the sludge which was taken from the Stock-Anammox reactor (Section III.2) and diluted in a ratio of one-fifth. The initial MLVSS concentration of the reactor was 450 mg/L. A lab-scale plexi-glass reactor (Figure III.11) having a 16.5 cm height and 10 cm diameter with a working volume of 1 L was operated as SBR.

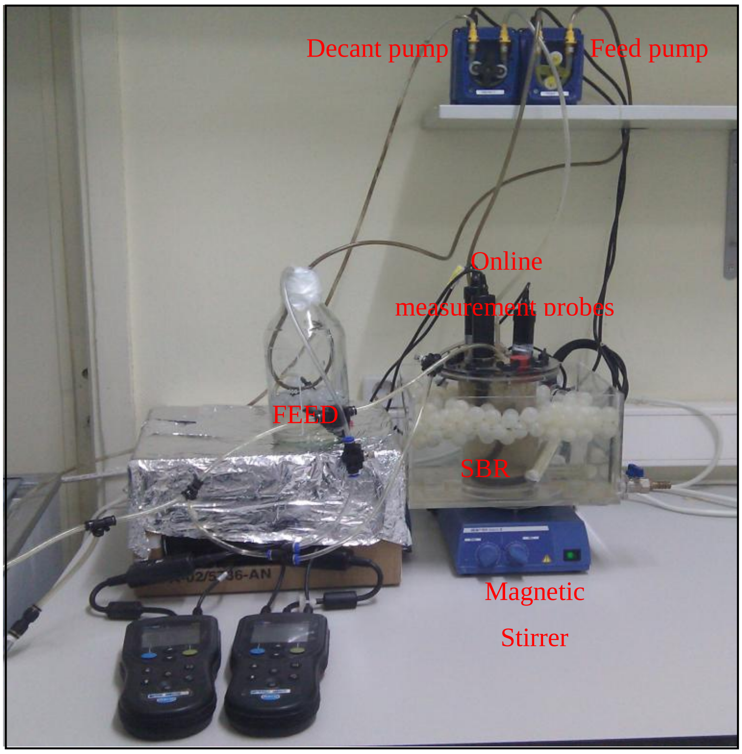


Figure III. 10 Anammox-SBR system

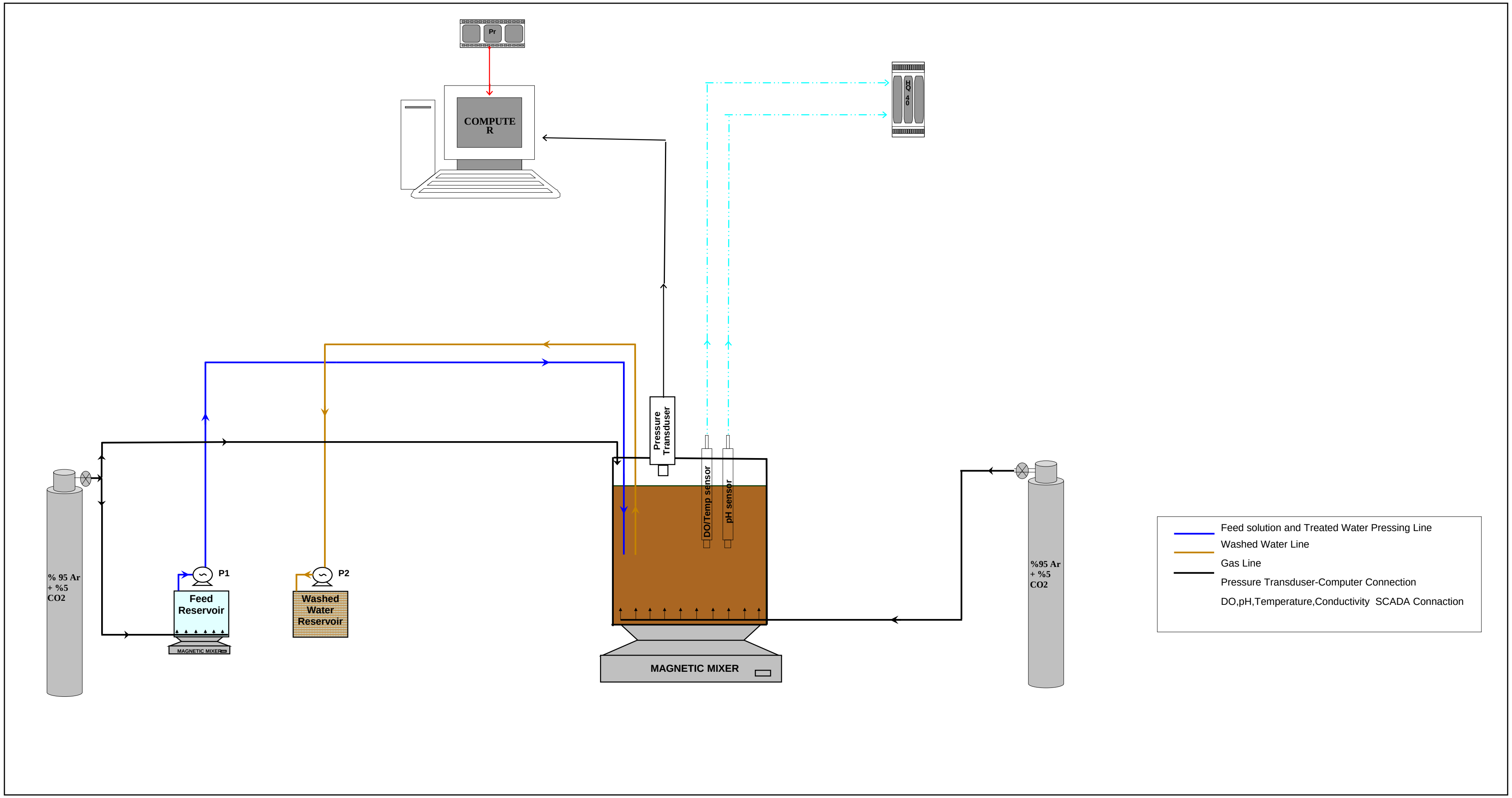
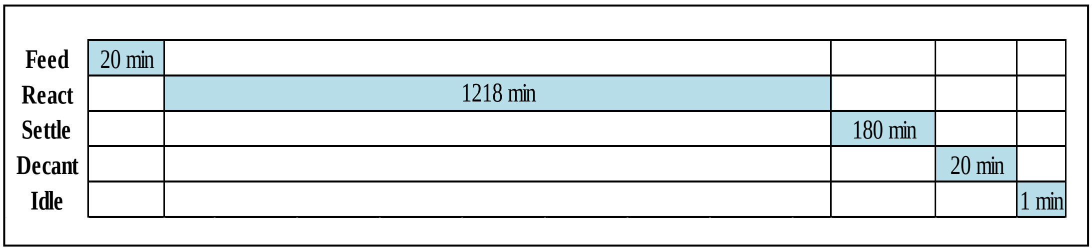


Figure III. 11 Schematic diagram of batch experiments set up

The schematic operation diagram of the system is shown in Figure III.13. Each cycle comprised five phases including feed, react, settle, decant and idle. The operational cycle of the reactor is detailed in Figure III.12.



Feed	20 min				
React		1218 min			
Settle			180 min		
Decant				20 min	
Idle					1 min

Figure III. 12 Operational cycle of Anammox SBR reactor

In each cycle, 900 ml of working volume was discharged and 900 ml wastewater was fed into reactor with peristaltic pumps (Seko). The reactor was constantly operated with a hydraulic retention time of 1.1 days as shown in Eqn.III.11.

$$\mathrm{HRT} = \frac{\text{Volume of the reactor (L)}}{\text{Influent flowrate to the system (L/d)}} = \frac{1}{0.9} = 1.1\ \mathrm{d} \qquad \text{(III.11)}$$

Water temperature was set about 32-35°C by aquarium heater which was placed into water jacket surrounding the reactor. During react phase, reactor liquid and headspace of the liquid were flushed with %95 Ar + %5 CO_2. The surface of the suspension level in the reactor and the surface of the feed liquid were covered with polypropylene balls (Cole-Parmer) to enhance the CO_2 solubility and to prevent oxygen enterance.

Initially, the system was continuosly fed with the synthetic wastewater (Table III.2 without $NaNO_2$) for 104 days. After partial nitritation process was performed successfully, PN-SBR effluent was fed to the system for a time period of 133 days. The concentrations in the reactor are summarized in Table III.4. pH, DO and temperature were continuously measured with 30 minutes time intervals by on-line probes (Hach Lange HQ40d).

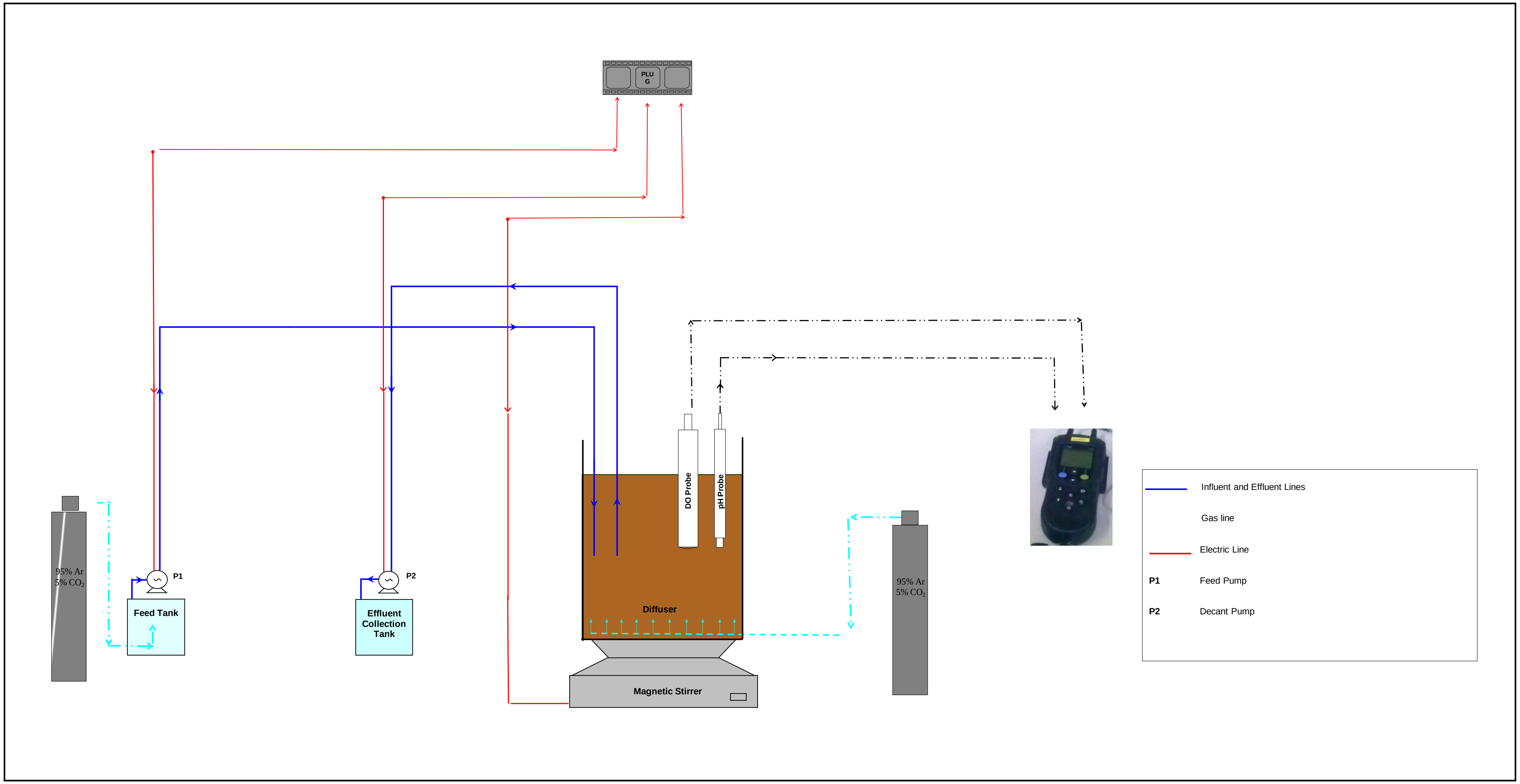


Figure III. 13 Schematic view of operation diagram for Anammox-SBR system

Table III. 4 Feed solution of Anammox-SBR system

	Sythetic Wastewater (*0-104 days*) (mg/L)	**PN SBR effluent** (real domestic wastewater) (*104-177 days*) (mg/L)	**PN SBR effluent** (real domestic wastewater) (*177-240 days*) (mg/L)
COD	-	22 - 115	11 - 78
NH_4-N	14 - 72	12 - 88.5	7 - 33
NO_2-N	-	0 - 50	5 - 26
NO_3-N	21 - 136	0 - 106	0 - 30.5
TKN	-	21 - 108	10 - 34
Mineral medium			
KH_2PO_4	27.2	-	-
$CaCl_2.2H_2O$	180	-	-
$MgSO_4.7H_2O$	300	-	-
Mineral Solution 1			
$Na_2EDTA\cdot 2H_2O$	5		
$FeSO_4.7H_2O$		-	-
Mineral Solution 2			
$Na_2EDTA.2H_2O$	15	-	-
$\text{ZnSO4.7H}_2\text{O}$	0.43	-	-
$CoCl_2$	0.12	-	-
$MnCl_2.4H_2O$	0.99	-	-
$CuSO_4.5H_2O$	0.25	-	-
$NiCl_2.6H_2O$	0.19	-	-
Na_2SeO_3	0.10	-	-
H_3BO_3	0.011	-	-

III.5 ANALYTICAL METHODS

NH_4-N, NO_2-N and NO_3-N concentration of samples were analyzed with IC Shimadzu 20A-Dual Injection shown in Figure III.14. Operational conditions of the ion chromotograph are summarized in Table III.5.

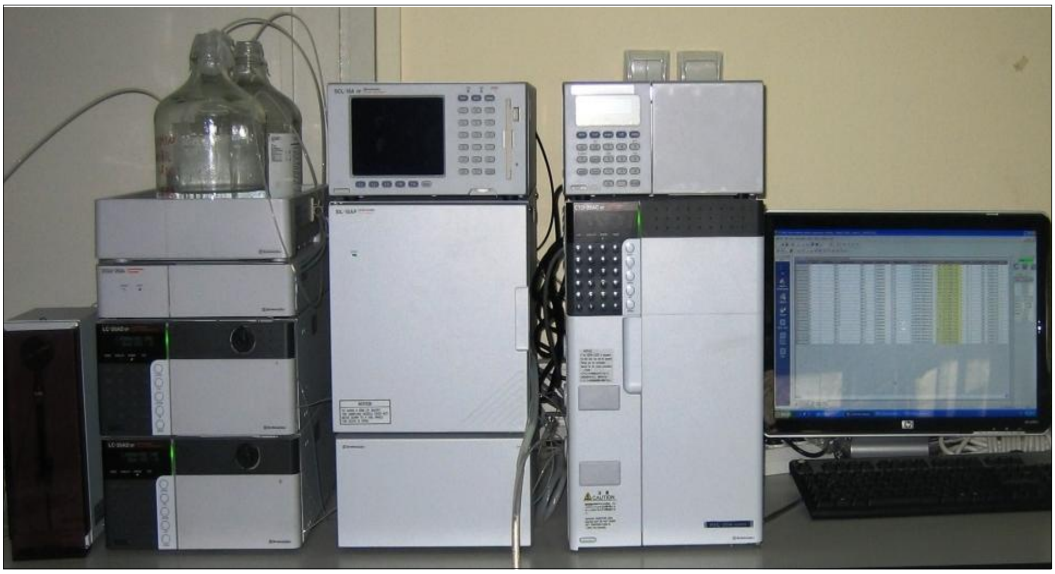


Figure III. 14 Ion chromotograph (IC Shimadzu 20A-Dual Injection)

Table III. 5 Operational parameters for ion chromotograph

Operational Parameter	**Cation**	**Anion**
Mobile phase	3.5 mM H_2SO_4	12 mM $NaHCO_3$ + 0.6 mM Na_2CO_3
Flow rate	0.8 ml/min	1 ml/min
Oven temperature	30^0C	30^0C
Column type	Shimpack IC-SC1 (150x4.6 mm)	Shimpack IC-SA2 (250x4 mm)
Injection time	20 min	20 min

A typical calibration chromotogram is shown in Figure III.15. The COD, MLSS and MLVSS measurements of the reactors were determined according to Standard Methods 5220C and 2540G, respectively.

TKN (Total Kjeldahl Nitrogen) analyses of the samples were performed with TKN analyzer (Gerhardt Vapodest 30S) by titration method.

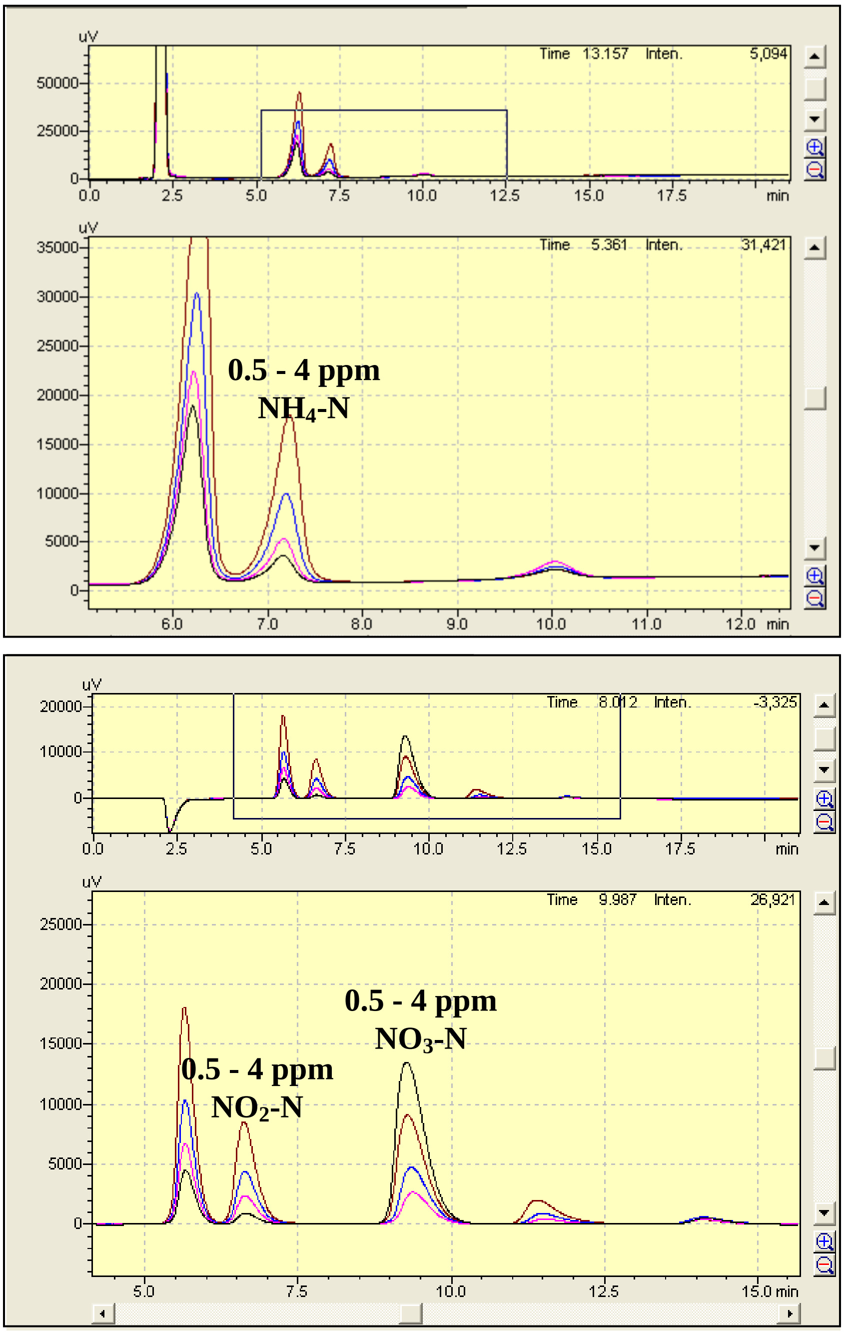


Figure III. 15 Calibration chromotograms of ion chromotograph

CHAPTER IV

RESULTS AND DISCUSSION

IV.1 PN SBR SYSTEM

IV.1.1 Start-up and enrichment

The PN-SBR reactor was inoculated with mixed activated sludge taken from the return activated sludge line of the Istanbul Pasakoy STP in Turkey. The system was continuously fed with the real domestic wastewater taken from the inlet of the grit chambers at Istanbul Pasakoy STP for 350 days as a preliminary treatment step for the Anammox-SBR reactor (see Section IV.4). The operational parameters (DO, pH, temperature) continuously measured and recorded with 5 minutes time arrivals in each day shown in Figure IV.1 as daily averages. The standart deviations from the averages in each cycle are also shown in this figure as error bars. Time course of influent and effluent changes for PN-SBR system are shown in Figure IV.2.

Throughout the study, the system was mainly operated under oxygen limited conditions (DO:0.4-1 mg/L) in order to obtain effluent having NO_2-N:NH_4:N ratio suitable for the Anammox-SBR system. This operational strategy was chosen in order to maximize the difference between AOB and NOB growth rates, which is the key point to achieve a fast and stable NOB washout. In literature, it was observed that the difference of K_o for AOB and NOB suggests that under limited DO conditions, AOB will out compete NOB because its influence on the NOB, is greater than on the AOB (Guo et al. 2009, Bagchi et al., 2009, Li et al., 2011). For this purpose, initially the system was started to operate with DO concentration of 0.2 mg/L. However, the DO concentration seemed to be too low as evidenced by the sudden increase of NH_4-N in the effluent (Figure IV.2). Hence, starting from day 28, the DO was adjusted in the range of 0.2-2 mg/L depending on the effluent accumulations observed. The aim was to obtain the desired NO_2-N:NH_4-N ratio in the effluent by adjusting DO concentrations. But, the set values of DO (Figure IV.1) could not be supplied successfully to the reactor due to probe malfunction which was realized later. Daily fluctuations in DO concentration through a batch cycle in each operation day are also apparent in error bars shown in Figure IV.1.

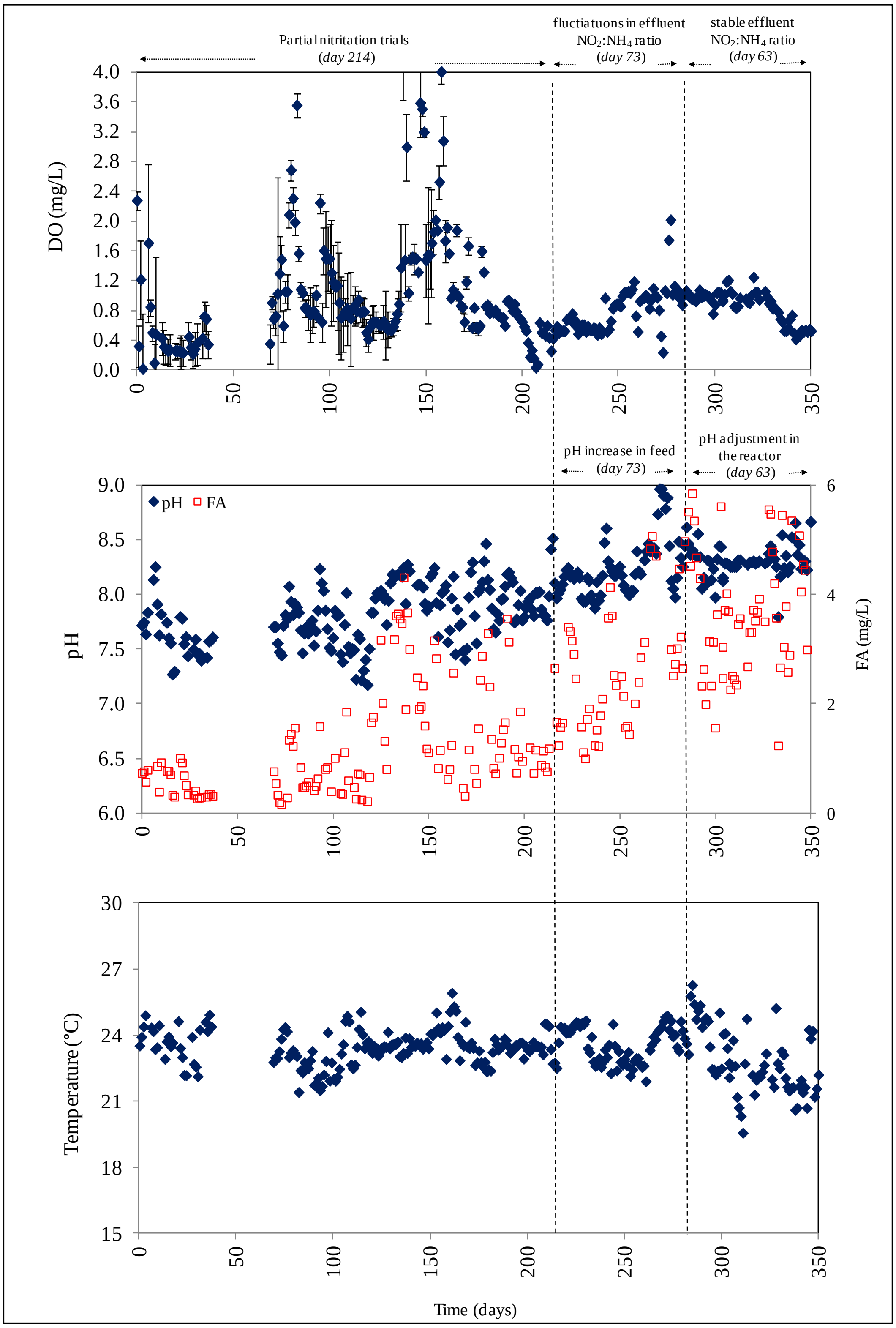


Figure IV. 1 Time course of daily DO, pH, FA, and temperature of the PN-SBR reactor

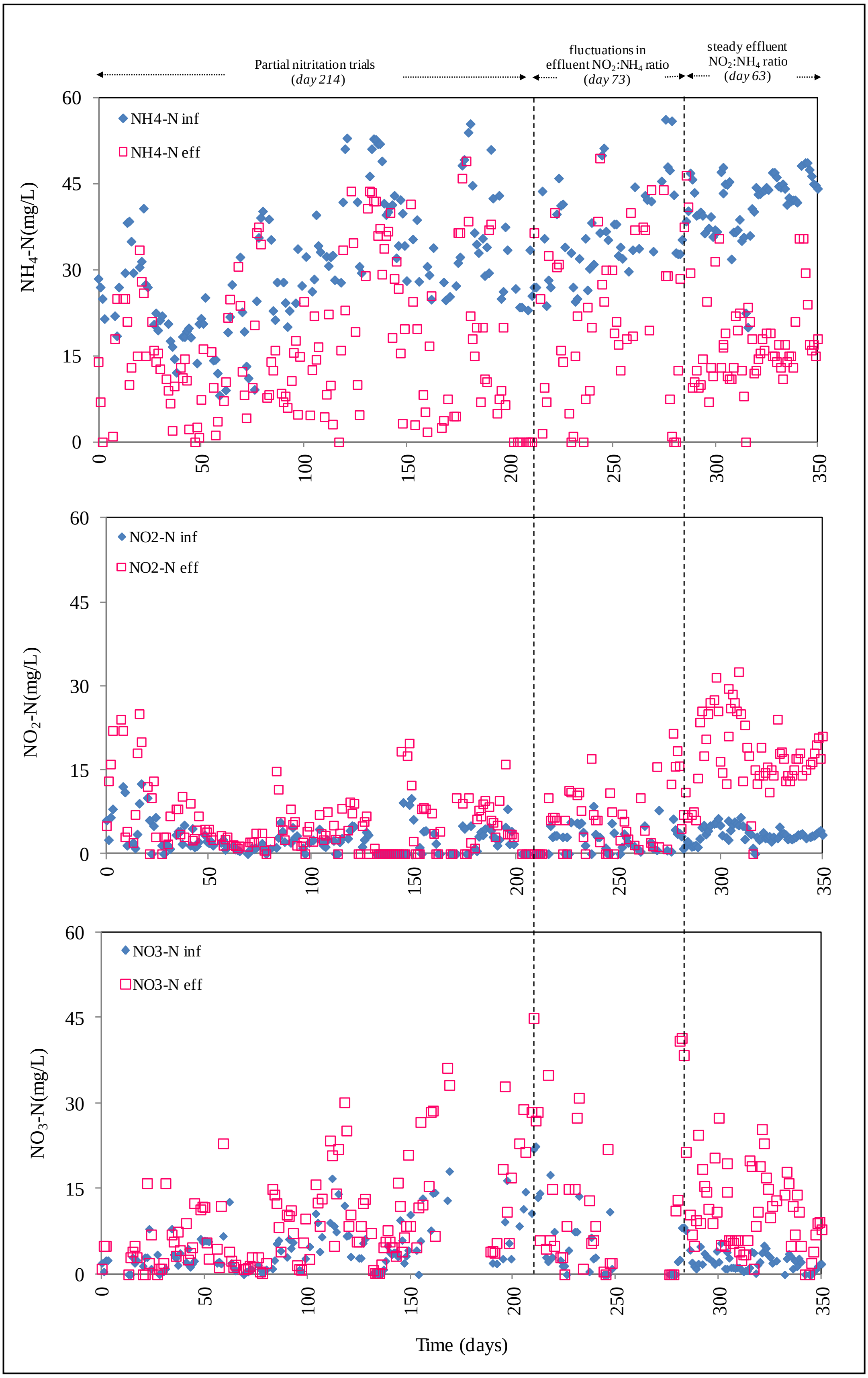


Figure IV. 2 Time course of influent and effluent nitrogen concentration changes of the PN-SBR system

In the same period (day 0-87), SRT was gradually decreased from 8 days to 5 days (at day 10) and to 2.5 days (at day 27). The aim was to wash-out NOB from the system. The MLVSS decrease from 1066 mg/L (at day 0) to 653 mg/L (at day 37) might be evaluated as an indication of NOB wash-out from the system due to SRT decrease. Similarly results were observed as in the past studies evaluating the PN under short SRT conditions (Munz et al., 2011, Ahn et al., 2008). SRT values ranging between 1-3 days resulted NO_2-N accumulation. On the other hand, the researchers found that NH_4-N was converted to NO_3-N when the SRT value of the PN system was increased from 3 days to 5 days. In that period (0-87 days), pH and FA were ranged between 7.27-8.25 and 0.16-1.55 mg/L, respectively (Figure IV.1). Therefore, there was no FA inhibition in that period. In the present study, decreasing the SRT value to the levels of 2.5 days in combination with the DO adjustment in the range of 0.2-2 mg/L resulted to obtain effluent containing NO_2-N:NH_4-N ratio of 0.03-5.5 which was not at the desired level for Anammox.

After day 87, LDO cap of DO probe was changed so DO began to remain constant as set values. Between days 87-214, set DO concentration was being continuosly changed depending on the effluent accumulations observed. In that period, daily DO concentration ranged between 0.03-4.16 mg/L. The system was operated at SRT value of 2.5 days under average pH conditions of 7.17-8.46. There was still no significant amount of FA concentration in the reactor. Under these operating conditions, sufficient NO_2-N accumulation could not be achieved. NO_2-N:NH_4-N ratio in the effluent was observed at the levels of 0.02-3.77 which is not appropriate for Anammox. The observation of high NO_3-N production with insufficient amount of NO_2-N accumulation arised the question of Anammox bacteria can use both NO_3-N and NO_2-N as an electron acceptor. To find an answer to that question, The Anammox-SBR system was initially fed with synthetic wastewater containing NO_3-N but not NO_2-N as discussed in Section IV.4.

Starting from day 214, in some operational days, the feed pH was increased to the levels of 8.2 to evaluate the effect of FA concentration on NO_2-N accumulation. In that period, NO_2-N started to accumulate in the effluent more steadily. But, the effluent NO_2-N:NH_4-N ratio still exhibited fluctuations in the range of 0.08-7.7 till day 252. The observation of more steady NO_2-N accumulation was related to the three possible reasons: (1) Starting from day 214, DO values through a batch cycle started to fluctuate less. This is apparent in error bars shown in Figure IV.1. (2) Prolonged exposure of bacteria to the SRT level of 2.5 days (starting from day 27) might result to wash-out of significant amount of NOB from the system. (3) Feed pH increase to the levels of 8.2 resulted to observe high FA concentration (2.1-4.2). Increasing FA concentration might cause inhibition of NOB. At day 252, a malfunction of overhead stirrer occurred and the complete mixing in the reactor was provided only by means of magnetic stirrer for 10 days. This was resulted a floc size increase in the reactor and sudden NH_4-N accumulation in the effluent. As reported in literature, the floc size distribution affects oxygen mass transfer rates even in floccular biomass aggregates (Blackbourne et al., 2007, Hanaki et al., 1990, Piciorenau et al., 1997). Increasing floc size might cause of considerably less DO inside the floc with respect to bulk liquid DO. In the systems including high size flocs, even if the DO level was high, significant amount of accumulations might be observed. Therefore, a sudden NH_4-N accumulation that was observed at day 260 might heavily be related with sudden floc size increase explained above. To overcome this problem, DO concentration was sharply increased to the level of 1 mg/L as shown in Figure IV.1. Although a new overhead stirrer was placed to the system at the operation day of 262, the NH_4-N and NO_2-N accumulations were adversely effected from this operational problem till day 286.

In the operational days of 214-286, it is obvious that increasing FA concentration in the reactor resulted to obtain better effluent in terms of NO_2-N:NH_4-N ratio. Therefore, increasing FA concentration in combination with limited DO and SRT conditions were considered as a suitable control strategy for the proper PN of low strength domestic wastewater prior to Anammox process. Similarly, in the previous studies, FA was reported as a factor of inhibiting the growth of NOB. Some researchers metioned that PN could be achieved successfully when FA concentration was between 3-4 mg/L (Aslan et al., 2008, Gonzales et al., 2007, Antileo et al., 2006). To observe the combined effect of high FA, low DO and low SRT conditions on PN prior to Anammox, the system was operated by adjusting both DO and pH in the reactor between days 286-350. In that period DO concentration and pH ranged between 0.8-1 m/L and 7.79-8.66, respectively. FA concentration in the reactor was around 1.23-5.4 mg/L. Under these conditions, the effluent NO_2-N:NH_4-N ratio ranged 0.75-1.28.

Altough the obtained effluent NO_2-N:NH_4-N ratio was very close to the desired ratio of 1.2-1.5, the applied strategy (i.e high FA, low DO, low SRT conditions) could not prevent NO_3-N production in the system. Therefore, these results indicated that in case of Anammox application for the treatment of low strength domestic wastewater, the partially nitrified influent to the Anammox reactor will always contain not only NO_2-N but also a small portion of NO_3-N.

To obtain the optimum operational strategy for the PN systems to be placed prior to Anammox reactor treating a low strength domestic wastewater, the combined effect of DO and FA concentrations on the PN effluent NO_2-N:NH_4-N ratio was further evaluated in Figure IV.3. As seen from the figure, to maintain the effluent NO_2-N:NH_4-N ratio of 1.2-1.5 are DO concentration shall be in the range of 0.8-1 mg/L while the FA concentration shall be around 3 mg/L. These findings are in good aggrement with those reported in literature. In the past studies about PN under limited DO conditions (Zhang et al., 2010, Aslan et al., 2009, Peng et al., 2011). NO_2-N accumulation was mainly observed in DO concentration range of 0.6-1 mg/L. Similarly in the studies evaluating the effect of FA concentration increase on NO_2-N accumulation for low strength wastewater, the reported FA concentration ranges between 3-4 mg/L (Aslan et al., 2008, Gonzales et al., 2007, Antileo et al., 2006).

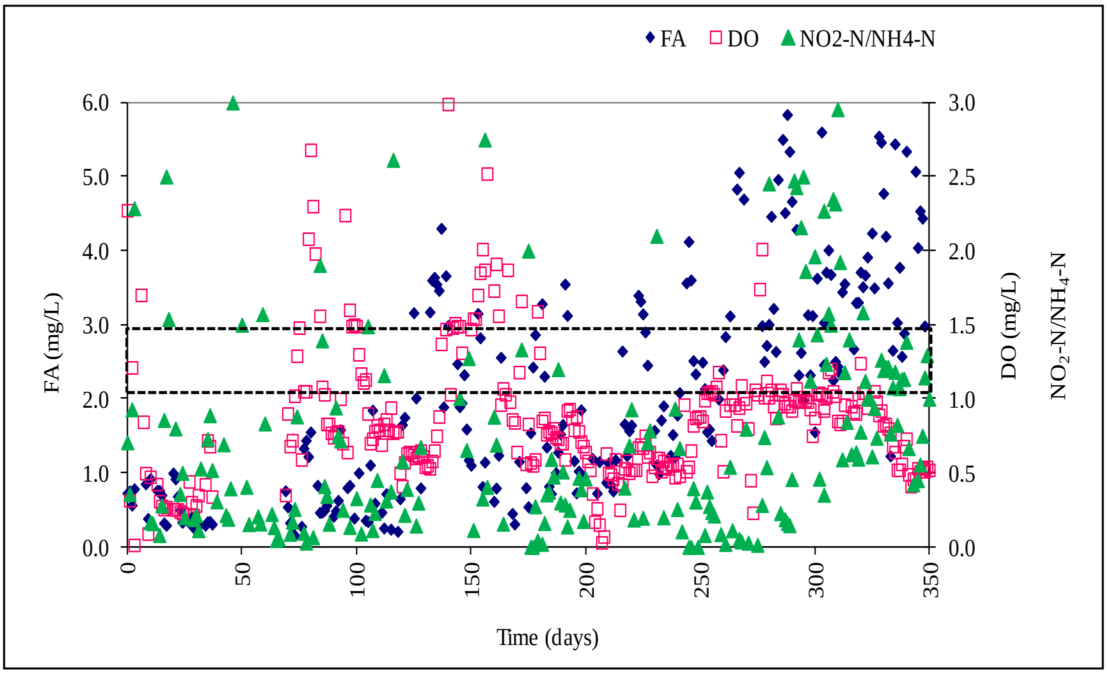


Figure IV. 3 Optimum working conditions (FA, DO) for PN

Time course of influent and effluent COD and TKN changes observed throughout 350 days operational periods are shown in Figure IV.4. Throughout the study, the COD removal of the reactor was around 76±11 and the effluent COD was observed around 50 mg/L.

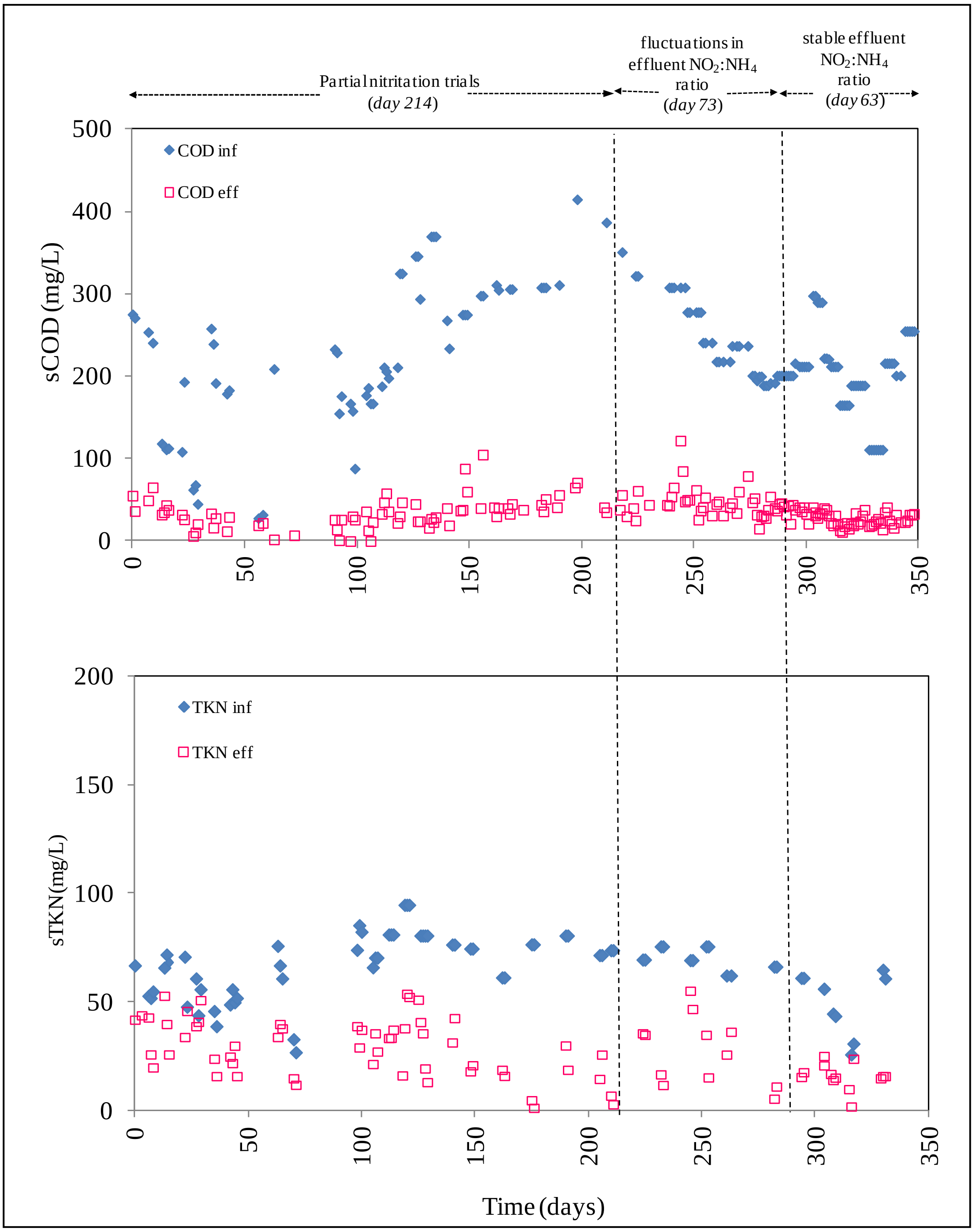


Figure IV. 4 Time course of influent and effluent sCOD and sTKN changes for PN-SBR system

These observations indicated that lowering DO concentration and increasing FA concentration did not negatively influence COD removal in the reactor. The soluble part of COD (mainly readily biodegradable portion (rbCOD)) was successfully degraded under limited DO conditions. The total COD measurements performed in few operational day (data not shown) indicated that the influent pCOD/COD ratio of 0.4 was decreased to around 0.12 at the end of each batch cycle. This indicated that under limited DO and high FA concentrations the great portion of pCOD could be first hydrolyzed and then be degraded. Hence, the partially nitrified effluent mainly consisted of nonbiodegredable and/or slowly biodegradable soluble COD which could not cause significant activation of denitrifiers in Anammox system. This can be the main advantage of Anammox process over CANON process in which partial nitrification and Anammox are performed simultaneously in the same reactor. In CANON process, the mixed culture faces with high concentration of rbCOD and results the activity of denitrifiers might increase significantly. So denitrifiers would be the dominant process on Anammox in the reactor.

IV.2 STOCK ANAMMOX-SBR SYSTEM

IV.2.1 Start-up and enrichment

The Stock Anammox reactor was inoculated with mixed activated sludge taken from the return activated sludge line of the Istanbul Pasakoy STP in Turkey. Initial MLVSS concentration of the reactor was 3000 mg/L. The system was fed with synthetic wastewater for 515 days by starting feeding with 30 mg/L NH_4-N, 17 mg/L NO_2-N and 20 mg/L NO_3-N. NO_3-N was fed to the reactor for the consumption of organic matter possible generated from endogenous decay activity during early stages of start-up. The time course of influent and effluent changes observed in the system are given in Figure IV.5. As seen from the figure, NH_4-N was either same as or higher from the influent concentrations in the first 7 days. It might be caused from the change in environment of seed sludge and the turnover of bacteria. The former dormant bacteria (such as nitrifying bacteria) might be died, causing cell lysis and breakdown of organic nitrogen to NH_4-N. In the same period, MLVSS sharply decreased from 3000 mg/L to 2320 mg/L.

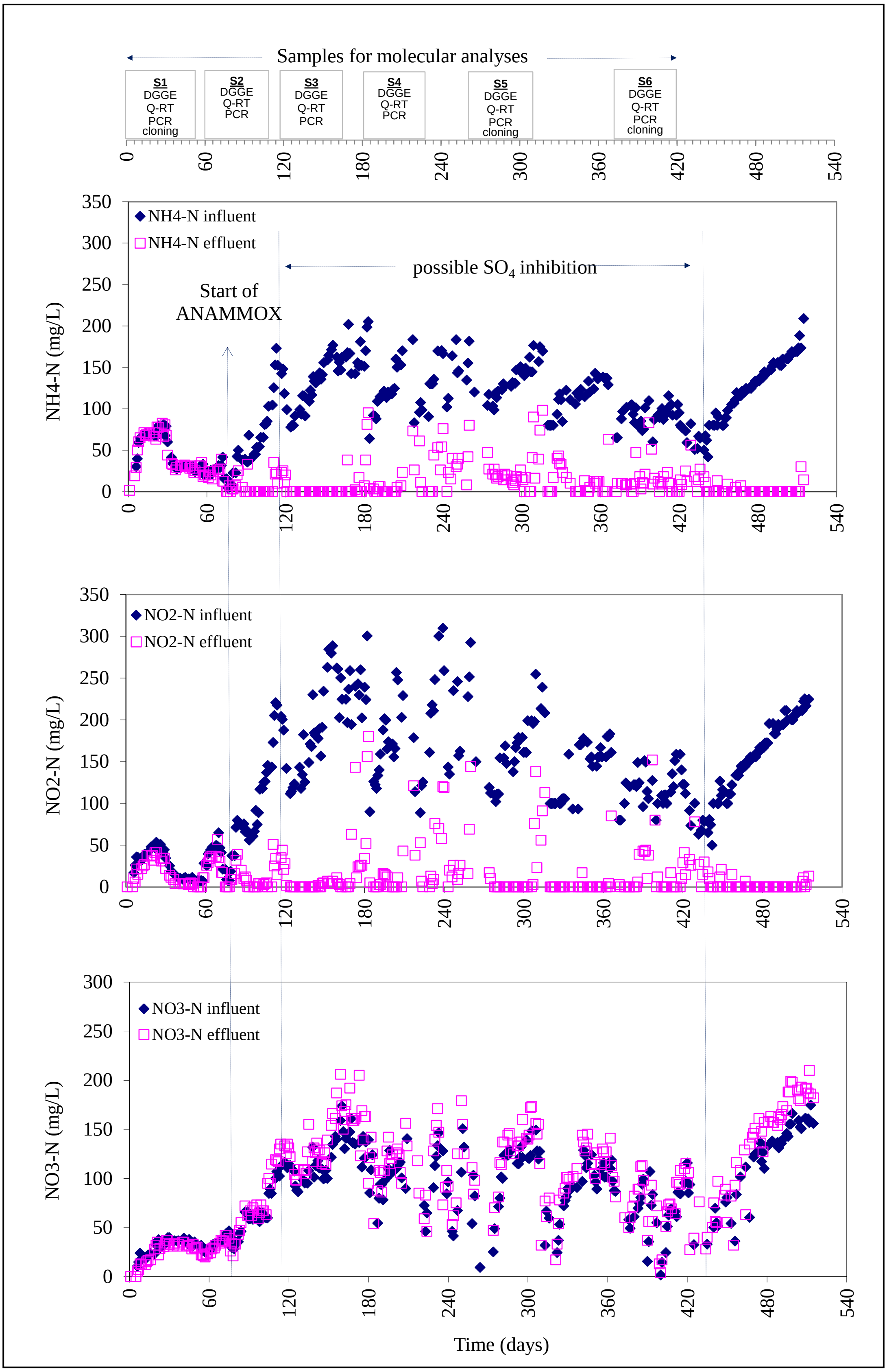


Figure IV. 5 Time course of influent and effluent changes of Stock Anammox-SBR system

Slight increase in NH_4-N and significantly decrease in NO_2-N and NO_3-N were observed in this period. These findings indicated possible endogeneous denitrification activity occurred via lysis of seed sludge. Between days 7-60, endogenous denitrification activity continued with decreasing rate.

After day 60, NH_4-N started to decrease very slightly. But NO_2-N and NO_3-N decreases were also apperent indicating that endogeneous denitrification was still prevalent in the system. Significant NH_4-N and NO_2-N removals were observed after day 75 but significant NO_3-N production was observed after day 92. Starting from day 75, NH_4-N and NO_2-N loadings to the reactor were gradually increased to enrich the Anammox culture in the system. At reaction day 510, the reactor NH_4-N and NO_2-N concentrations reached the maximum levels of 209 mg/l NH_4-N (0.0256 g NH_4-N / g VSS.day) and 225 mg/l NO_2-N (0.0276 g NO_2-N/g VSS.d). The treatment efficiencies were observed almost 100%. As seen from Figure IV.5, in some operational days (e.g day 119, 184, 260, 308, 366, 397, 429, 500), unexpected accumulations of NH_4-N and NO_2-N were observed in the system effluent. These sudden accumulations might be related to the increasing SO_4^{2-} loading due to $(NH_4)_2SO_4$ feeding to the system. Reduction of SO_4^{2-} to S^{2-} by sulphate reducing bacteria (SRB) might cause temporary inhibition of Anammox bacteria in those days.

IV.2.2 Stoichiometry

Stoichiometric analysis of Anammox process observed in Stock-Anammox-SBR system were done in terms of consumed NO_2-N(Δ_{NO2-N}) : consumed NH_4-N(Δ_{NH4-N}) and produced NO_3-N (Δ_{NO3-N}) : consumed NH_4-N (Δ_{NH4-N}) ratios which were calculated using Eqn. IV.1 and IV.2.

$$\frac{\Delta_{NO2-N}}{\Delta_{NH4-N}} = \frac{\text{Influent } NO_2\text{-N-Effluent } NO_2\text{-N}}{\text{Influent } NH_4\text{-N-Effluent } NH_4\text{-N}} \tag{IV.1}$$

$$\frac{\Delta_{NO3-N}}{\Delta_{NH4-N}} = \frac{\text{Effluent } NO_3\text{-N-Influent } NO_3\text{-N}}{\text{Influent } NH_4\text{-N-Effluent } NH_4\text{-N}} \tag{IV.2}$$

The time course changes of Δ_{NO2-N}: Δ_{NH4-N} and Δ_{NO3-N}: Δ_{NH4-N} are shown in Figure IV.6. As seen from the figure, serious fluctuations were observed prior to start of Anammox activity at day 64. The endogenous denitrification activity observed in the early stages of start-up period might cause to observe negative values in these ratios. After the start of Anammox activity, Δ_{NO2-N} : Δ_{NH4-N} and Δ_{NO3-N} : Δ_{NH4-N} ratios were steady. Δ_{NO2-N} : Δ_{NH4-N} and Δ_{NO3-N} : Δ_{NH4-N} ratios were determined as 1.42 and 0.17, respectively. The observed ratios are found consistent with the previously reported values in literature (van de Graaf, 1996).

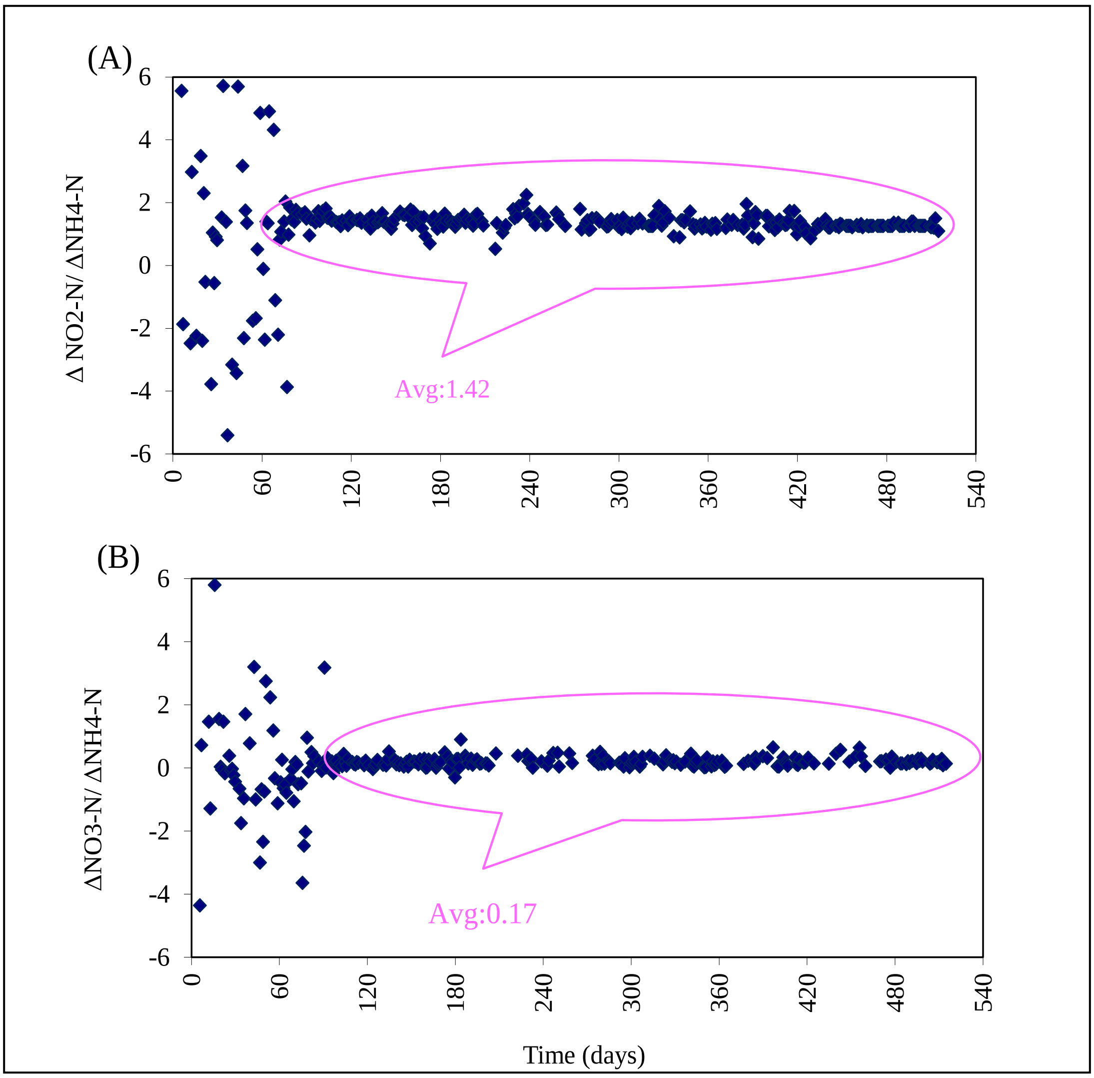


Figure IV. 6 Stoichiometric analysis of Stock Anammox-SBR system (A) time course changes of Δ_{NO2-N} : Δ_{NH4-N} (B) time course changes of Δ_{NO3-N} : Δ_{NH4-N}

IV.2.3 Monitoring of Anammox process via on-line measurements

On-line monitoring of Anammox process through real-time measurement of process constituents (NH_4^+, NO_2^-, NO_3^- and N_2) is costly and not practical. The process dynamics can easily and cheaply be monitored by real-time pH, ORP and conductivity measurements if the process is in a way correlated to profiles of pH, ORP and conductivity. During 515 days operational period of Stock-Anammox-SBR system, the operational parameters of pH, ORP, conductivity values were continuously monitored with 15 minutes time intervals at each batch cycle. pH, ORP and conductivity profiles developed from those measurements were then evaluated to explain the process condition in the reactor (Alpaslan et al., 2011). In view of findings discussed in Section IV.2.1, the 515 days operational period of Stock-Anammox-SBR system was divided into three main operational phases as follows: (1) endogeneous denitrification (*day 1-75*), (2) endogeneous denitrification with Anammox (*day 75-92*) and (3) Anammox (*day 92-515*).

Figure IV.7 demonstrates one example of pH, ORP and conductivity profile of a batch cyclebelonging to each of these phases. In phase I (Figure IV.7-A), through in which endogeneous denitrification was the dominant process in the reactor. pH profile exhibited slightly increasing values. Since the denitrification process produces alkalinity (see Eqn IV.3), increasing pH values exactly matches with the process condition.

$$NO_3^- + 5/6\ CH_3OH + 1/6\ CO_2 \rightarrow ½\ N_2 + 11/6\ H_2O + \boxed{HCO_3^-} \qquad (IV.3)$$

According to ORP profiles, it was seen that ORP value was as low as -80 mV at the beginning of batch cycle. This might be related with the absence of O_2 and presence of slight amounts of NO2-N and NO3-N at the beginning of the cycle. After feeding with low amount of NO_2-N and NO_3-N, ORP profile exhibited a tendency to increase. Through the cycle, gradual ORP decrease was observed with depletion of NO_2-N and NO_3-N by denitrification.

In phase II (Figure IV.7-B) through in which endogeneous denitrification and Anammox occurred simultaneously in the reactor. pH profile exhibited a slight increase with with respect to phase I. This might be heavily related with the decreasing endogeneous denitrification activity in the system. pH increase due to Anammox process is expected as shown in Eqn.IV.4. In this period Anammox

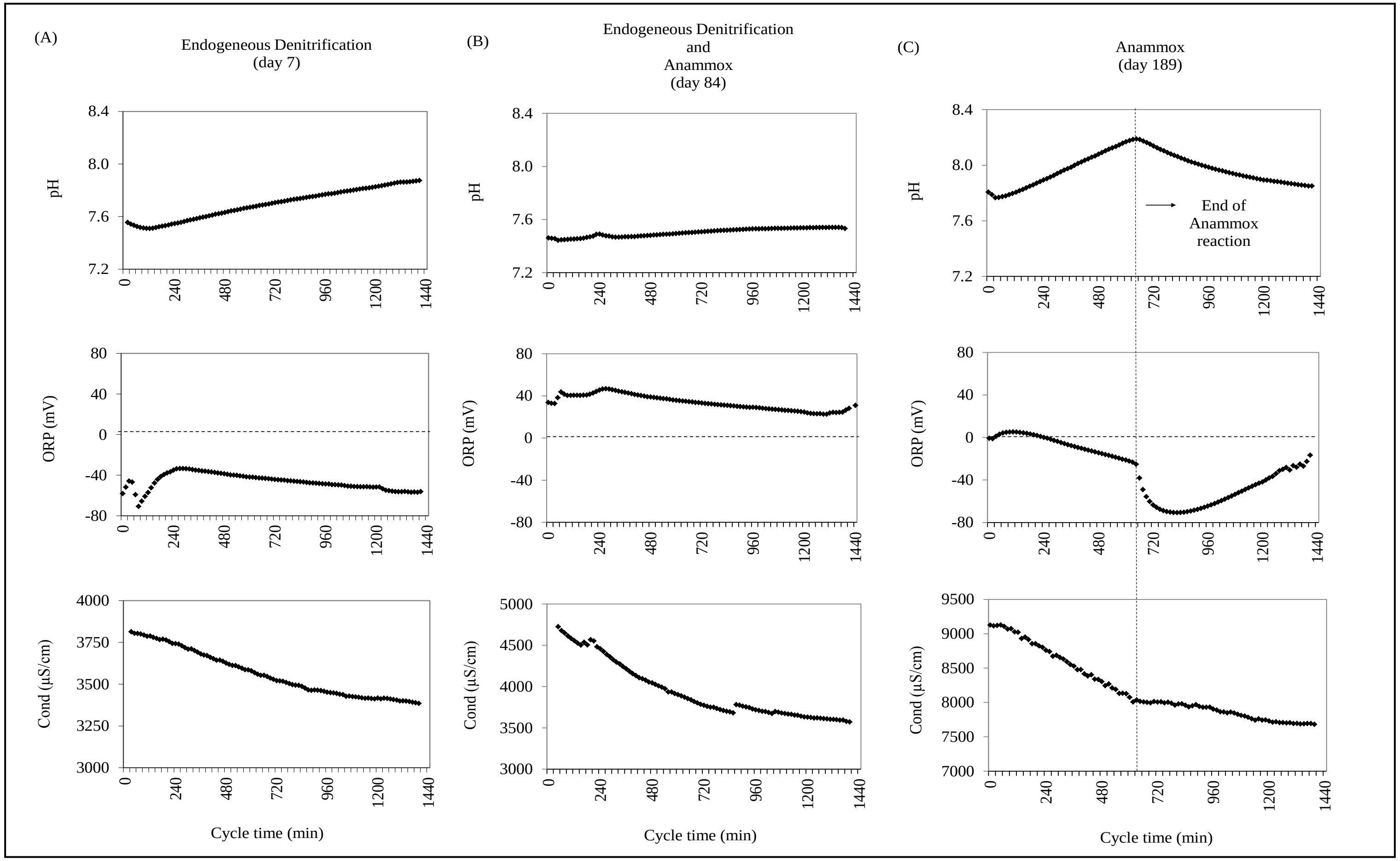


Figure IV. 7 pH, conductivity and ORP profiles of Stock-Anammox-SBR system observed in a batch cycle (A) at day 7, (B) at day 84, (C) at day 189 process was already started and hence generates very low H^+ due to feeding with low loadings of NH_4-N and NO_2-N.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- \boxed{+ 0.13H^+} \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad \text{(IV.4)}$$

According to ORP profiles, at the start of batch cycle, ORP value was around +40 mV possibly due to NO2-N and NO3-N remaining in the reactor from the perivous cycle. Through the cycle, gradual ORP decrease was observed with depletion of NO_2-N and NO_3-N by both Anammox and denitrification.

In phase III (Figure IV-C) through in which the dominant process was Anammox, gradual increase in pH was obtained in a batch cycle due to consumption of H^+ ions in cell synthesis (Eqn.IV.4). Increasing loadings to the reactor resulted high amount of H+ consumption and hence sharp increase in pH values. At the end of the process, pH values decreased because the reactor was continously purged with CO_2 as carbon source. According to the ORP profile, at the beginning of the cycle, ORP was around 0 mV because of NO_2-N and NO_3-N remaining from the previous cycle. Through the cycle, gradual ORP decrease was observed with depletion of NO_2-N. After NO_3-N production ORP values started to increase.

The evaluation of the conductivity profiles (Figure IV.7-A,B,C) showed that in all operated phases, conductivity values exhibited a linear decrease till the process ended. The expected conductivity decrease in Anammox process can be calculated based on molar conductivity per concentration values of the ions involved in the Anammox. This calculation is summarized in Table IV.1.

Table IV. 1 The expected conductivity decrease in Anammox process based on the molar conductivity values of the ions involved

	Reaction	Change in ion content	mSm^2
Anammox Process	$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \longrightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$	$-1\ NH_4^+$	-7,34
		$-1.32\ NO_2^-$	-9,47
		$-0.066\ HCO_3^-$	-0,293
		$-0.13\ H^+$	-4,55
		$+0.26\ NO_3^-$	+1.85
			$\boxed{-19,803}$

NH_4^+= 7.34 mSm^2/mol, NO_2^- = 7.18 mSm^2/mol, HCO_3^-= 4.45 mSm^2/mol, H^+=35 mSm^2/mol, NO_3^-=7.14 mSm^2/mol

As seen from Table IV.1, according to the Anammox process stoichometry, 14 g/m^3 NH_4-N removal results in approxiamately -19803 μS/cm conductivity decrease in the system (Alpaslan et al., 2010). The conductivity decreases observed in profiles shown in Figure IV.7-C matches with this stoichometrically expected conductivity decrease. The further evaluation of phase III profiles indicated that end of Anammox reaction in a reactor system can easily be monitored through on-line pH, ORP and conductivity measurements. This condition is also apperent in Figure IV.7-C as shown with dotted line.

IV.2.4 Morphological analysis of microbial communities in Stock-Anammox-SBR system

The time course of morphological changes in Stock-Anammox-SBR system was followed by ESEM analyses as shown in Figure IV.8. The ESEM micrographs showed that in the seed sludge (Figure IV.8-A), the shape of bacterial clusters were not identificable since the bacteria in clusters were partly embedded in an extracellular polymeric matrix (EPS). Protozoa like organisms were also apperent.

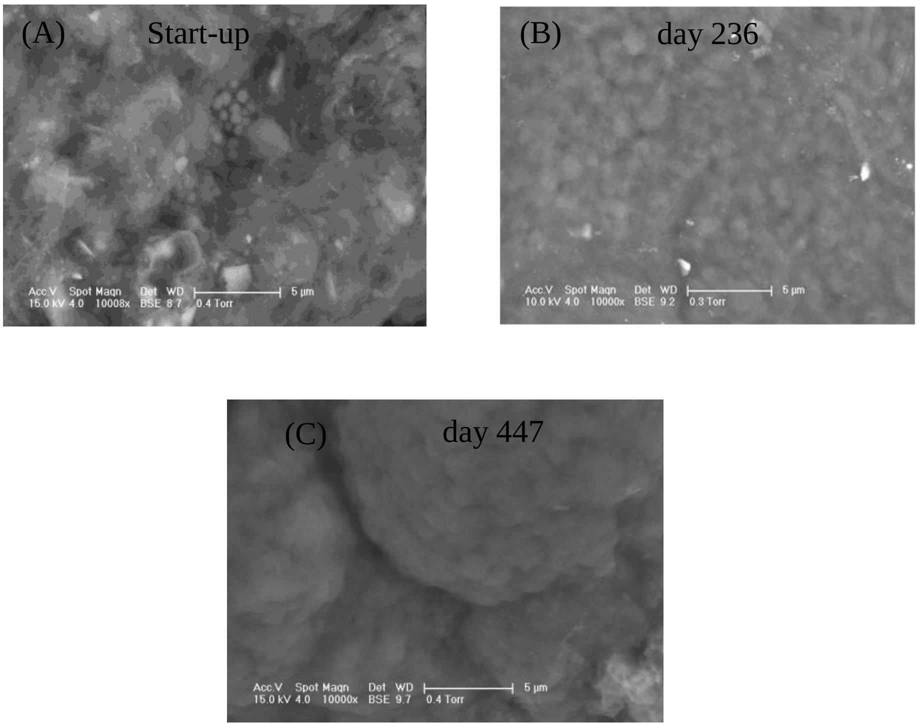
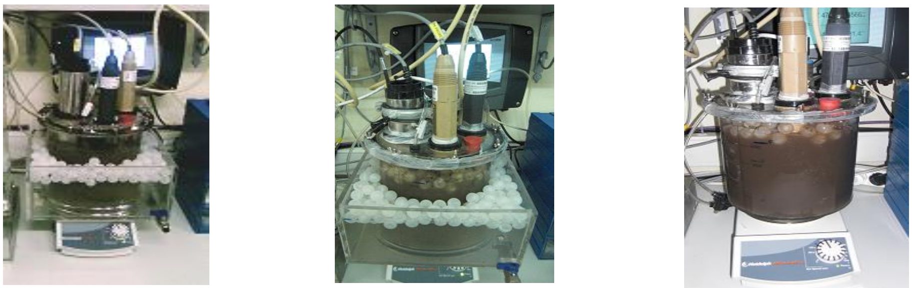


Figure IV. 8 ESEM micrographs of Stock Anammox-SBR system (A) at the start-up (B) at day 236 (C) at day 447

As the culture enriched for Anammox (Figure IV.8-B), spherical shape covered with thin EPS layer were apperent following to prolonged enrichment period. As seen from the Figure IV.8-B and C , the enriched biomass had a cauliflower like appearance, which was proved to be the characteristics of Anammox enrichment cultures in the past studies (Lindsay et al., 2001).

The morphological color changes of bacteria were also observed via photographs shown in Figure IV.9.



January 2010 (day 40)

February 2010 (day 54)

March 2010 (day 82)

April 2010 (day 117)

July 2010 (day 218)

October 2010 (day 299)

November 2010 (day 324)

February 2011 (day 439)

May 2011 (day 503)

Figure IV. 9 Chronological photos of the sludge in the Stock Anammox-SBR system

The initial brown color of seed sludge initially turned to light yellow after the decrease of endogeneous denitrification activity. After the start of Anammox activity the color of sludge gradually turned to red, which is the distinct feature of anammox bacteria accompanied by an increase in cytochrome content (van de Graaf et al., 1996).

IV.2.5 Microbial community structure analysis of Stock-Anammox-SBR system

In the start-up and enrichment period, the microbial population dynamics in the Stock-Anammox-SBR system were followed via molecular tools (FISH, qPCR, DGGE, cloning, sequencing and phlogenetic analyses).

As seen from Figure IV.10, at the start-up, no hybridization signals were observed for PLA46, AMX820, AMX368 and BS820 oliganucleotide probes indicating the low quantities of Anammox bacteria in the seed sludge. After the start-up of Anammox activity at day 75, positive signals detected with PLA46 and AMX820 probes (data not shown). As a result of prolonged enrichment of Anammox bacteria, at day 429, hybridization signals (Figure IV.11) with PLA46, AMX820, BS820 increased significantly. This indicated that in the enrichment Anammox culture *Ca. Brocadia Anammoxidans* and *Ca. Scalindua* were intensively present.

The DGGE analyses results of sludge taken from the Stock-Anammox-SBR reactor (Figure IV.12) clearly indicated the enrichment of microbial community for Anammox. Since the reactor started up using mixed activated sludge, diverse microbial community was observed at the start-up (S1). Following to the start of Anammox activity, microbial community drastically changed (S3 and S4). Later, microbial community profiles consisted of similar taxa with high similarity values (90-95%).

The success of enrichment for a time period of 515 days, was further evaluated by performing qPCR analysis. As shown from qPCR analyses results (Table IV.2), after the start of Anammox process, anammox cells in the reactor increased significantly.

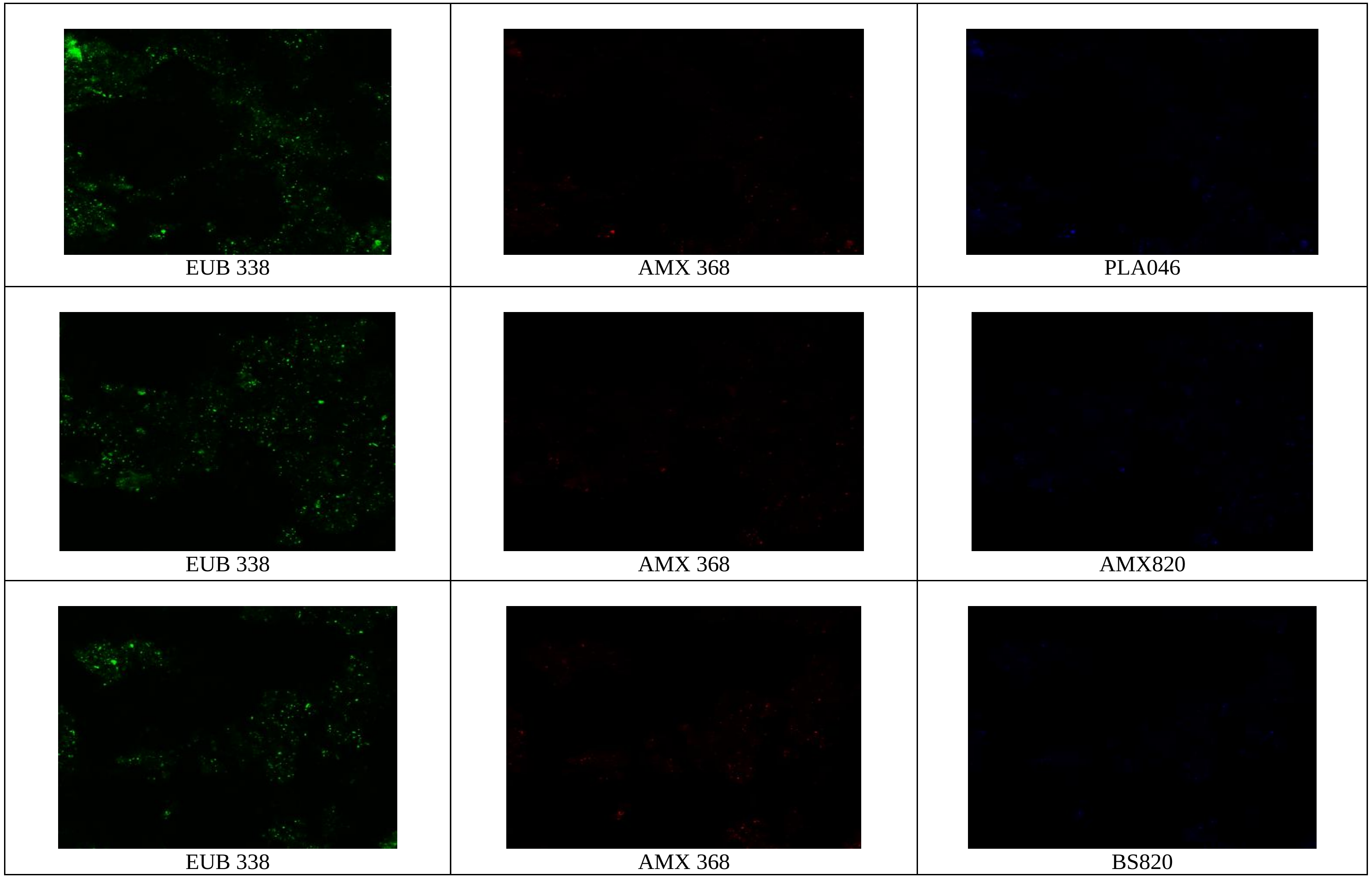


Figure IV. 10 Photomicrographs of FISH with oligonucleotideprobes EUB338, AMX368, BS820, PLA46 of Stock-Anammox-SBR system at the start-up

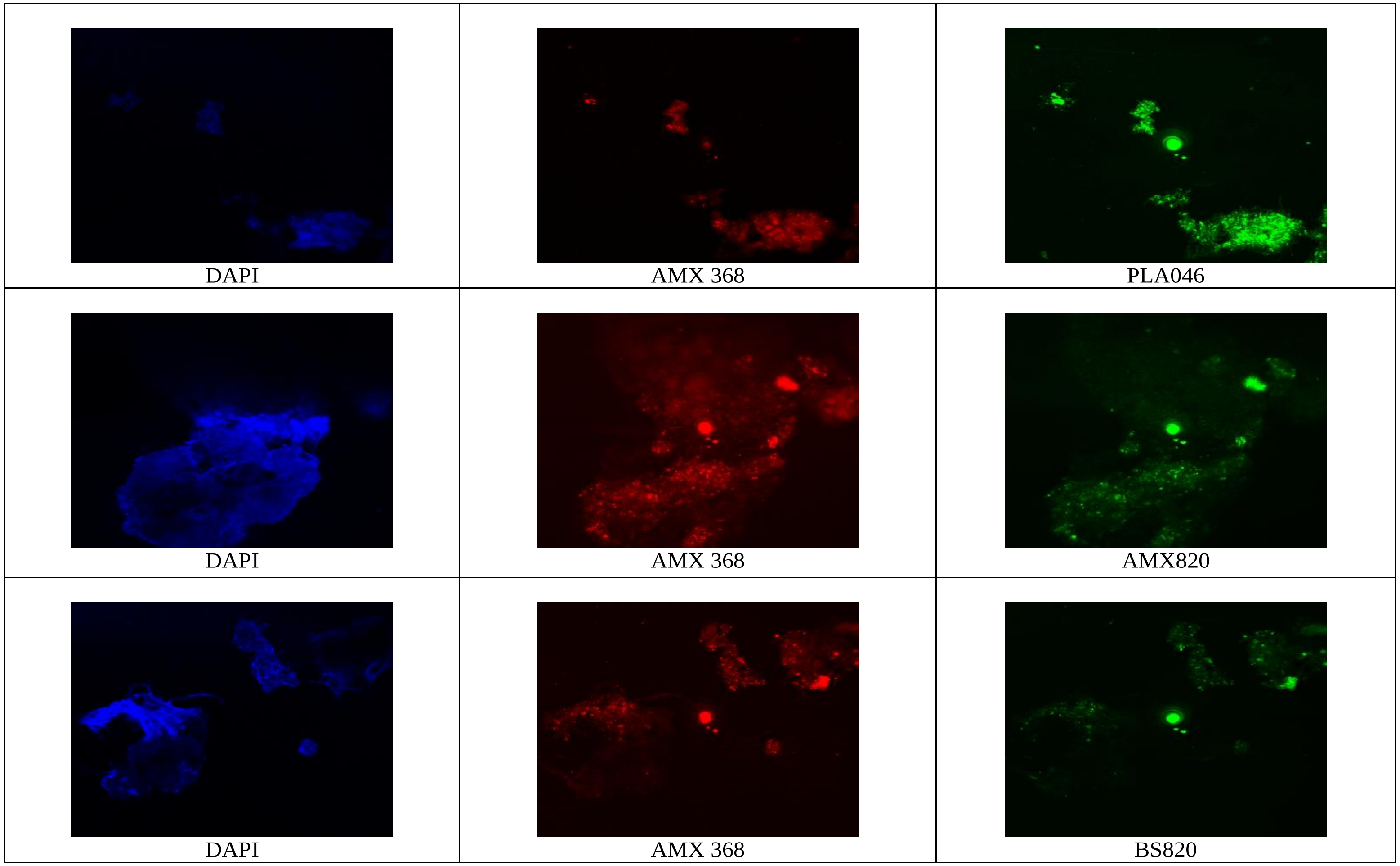


Figure IV. 11 Photomicrographs of FISH with oligonucleotideprobes AMX368, BS820, PLA46 of Stock-Anammox-SBR system at the operation day of 429 (All microorganisms were visualized by DAPI staining (blue))

Table IV. 2 QRT-PCR results of Stock Anammox SBR

Sample	Copy number (CN/ng DNA)
S1 (*day 1*)	2.14E+06
S2 (*day 72*)	1.05E+07
S3 (*day 122*)	5.87E+07
S4 (*day 222*)	5.34E+10
S5 (*day 299*)	1.11E+11
S6 (*day 429*)	3.11E+11

At the end of 429 days operation, the abundance of Anammox cells in reactor was in the order of 3.11×10^{11} CN/ng DNA which is significantly higher that the referential copy numbers reported in literature (Tsushima et al., 2007, Bae et al., 2010).

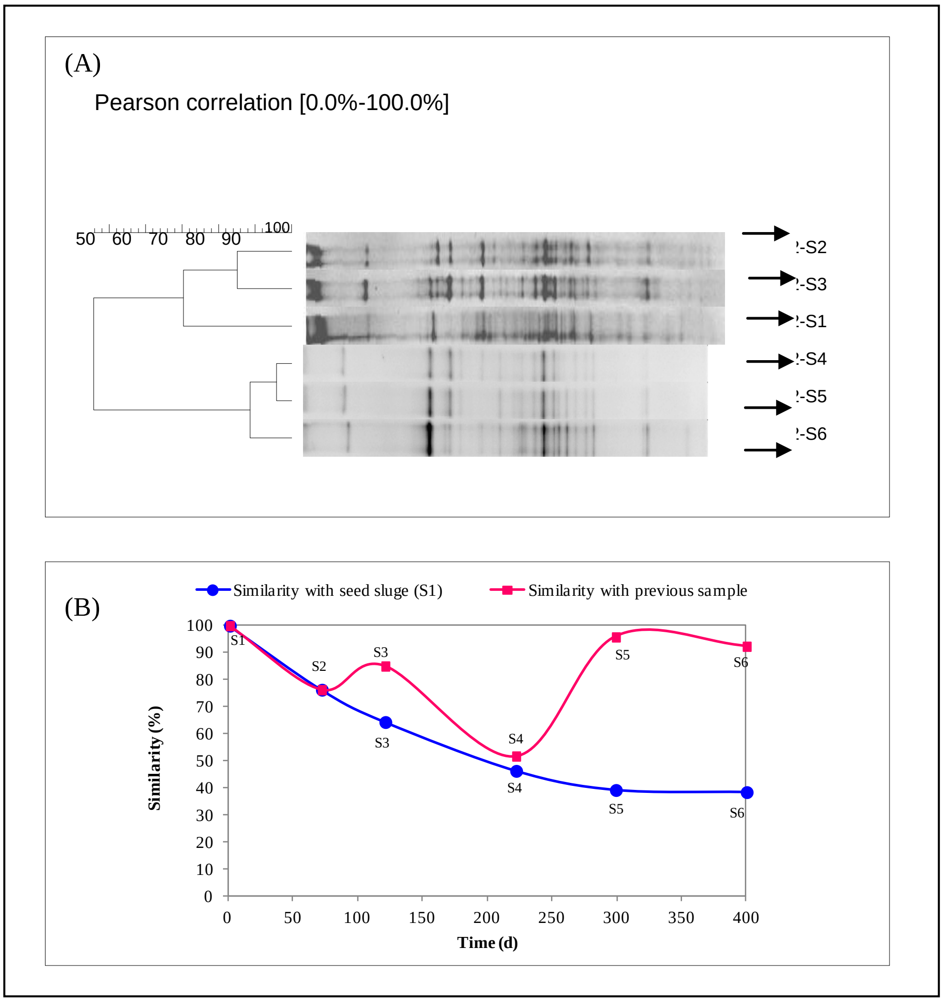


Figure IV. 12 DGGE results of Stock Anammox SBR

Cloning, sequencing and phlogenetic analyses results of seed sludge and the sludge sample taken from the reactor after enrichment are given in Figure IV.13 and IV.14, respectively

As seen from the figures, in the first sample the reactor 15% of the sequenced phylotypes for Stock Anammox SBR system was found to be related to bacteria that were previously found in anammox reactors. As the enrichment proceeds and anammox activity was maintained, agreeably clone library analysis demonstrates that the last sample was comprised of 80% of the phylotypes respectively related to microorganisms that are usually found in anammox reactors. Especially the abundance of phylotypes highly similar to Chloroflexi phylum with anammox activity agrees with previous studies where more than half of the clones detected in an anammox reactor was found as Chloroflexi-like bacteria. These bacteria are thought to be important role players for the granulation process in the reactor.

IV.3 BATCH EXPERIMENTS EVALUATING ORGANIC CARBON INHIBITION ON ANAMMOX

In order to apply the Anammox process for the treatment of domestic wastewater including organic carbon, it is necessary to know the effect of organic carbon on the Anammox bacteria. To date, several studies were performed to observe the inhibition effect of organic carbon. For this purpose, experiments with different organic carbon forms that may exist in wastewater (e.g. glucose, acetate, propionate) and real domestic wastewater were used (Alpaslan et al. 2008). In all experiments, the presence of organic carbon resulted to occur denitrification (data not shown).

The specific Anammox activity (SAA) values show in Figure IV.15 were calculated by accounting a pressure increase due to denitrification as explained in Section III.3 and hence only reflects the effect of organic carbon on Anammox process. As seen from Figure IV.15, the presence of organic carbon in all forms resulted inhibition of Anammox bacteria in some extent. 50% inhibition of SAA was observed at 5000 mg/L COD for glucose, 1500 mg/L COD for acetate and 2500 mg/L COD for propionate. These findings indicated that the inhibitory effect of organic carbon on Anammox process is significant at high COD levels.

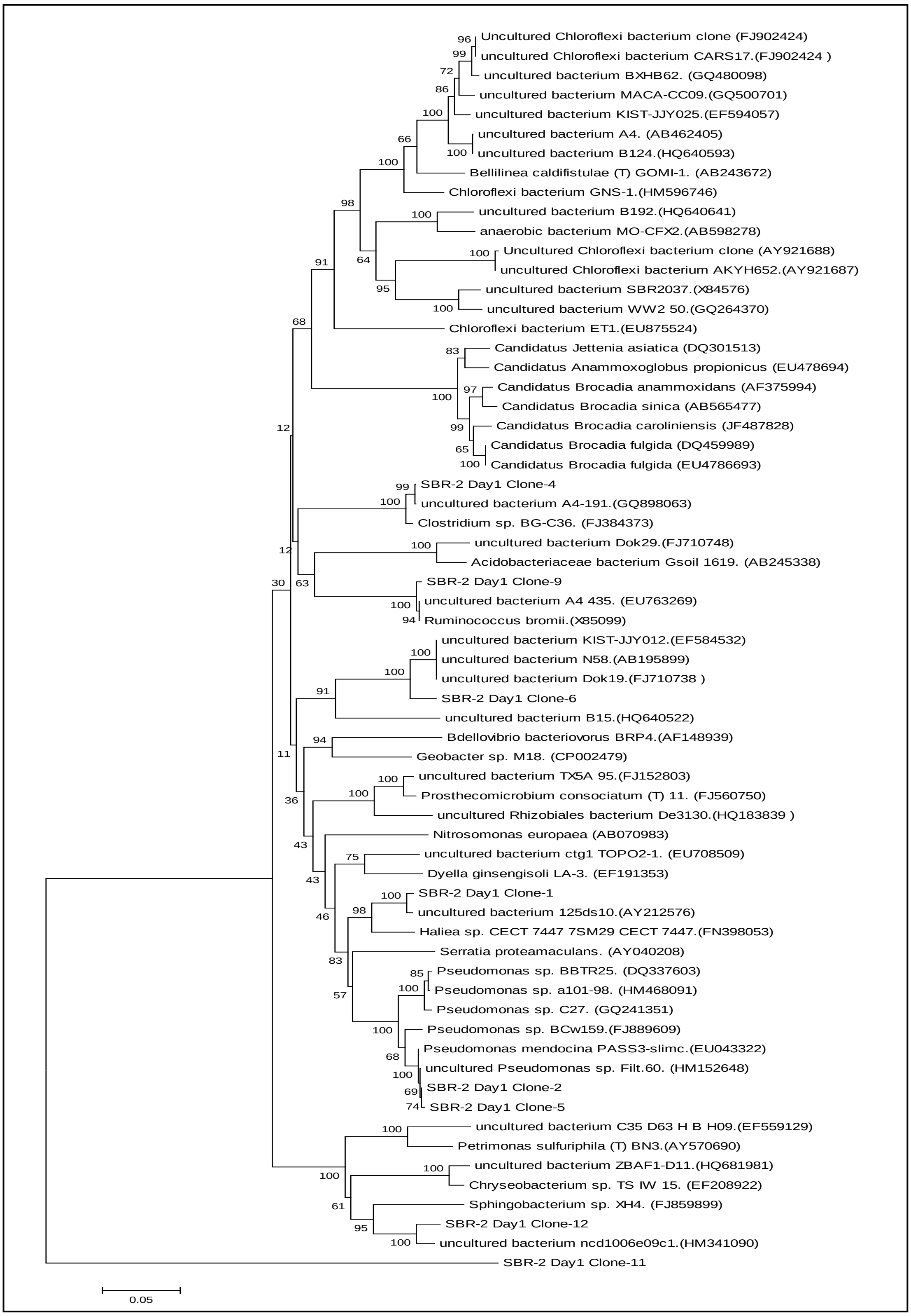


Figure IV. 13 Phylogenetic tree of 16s rRNA clone library sequences of start-up biomass sample constructed with the MEGA program using the neighbor-joining method with default settings. The values beside the branches represent the percentage of times that a node was supported in 1000 bootstrap pseudoreplications.

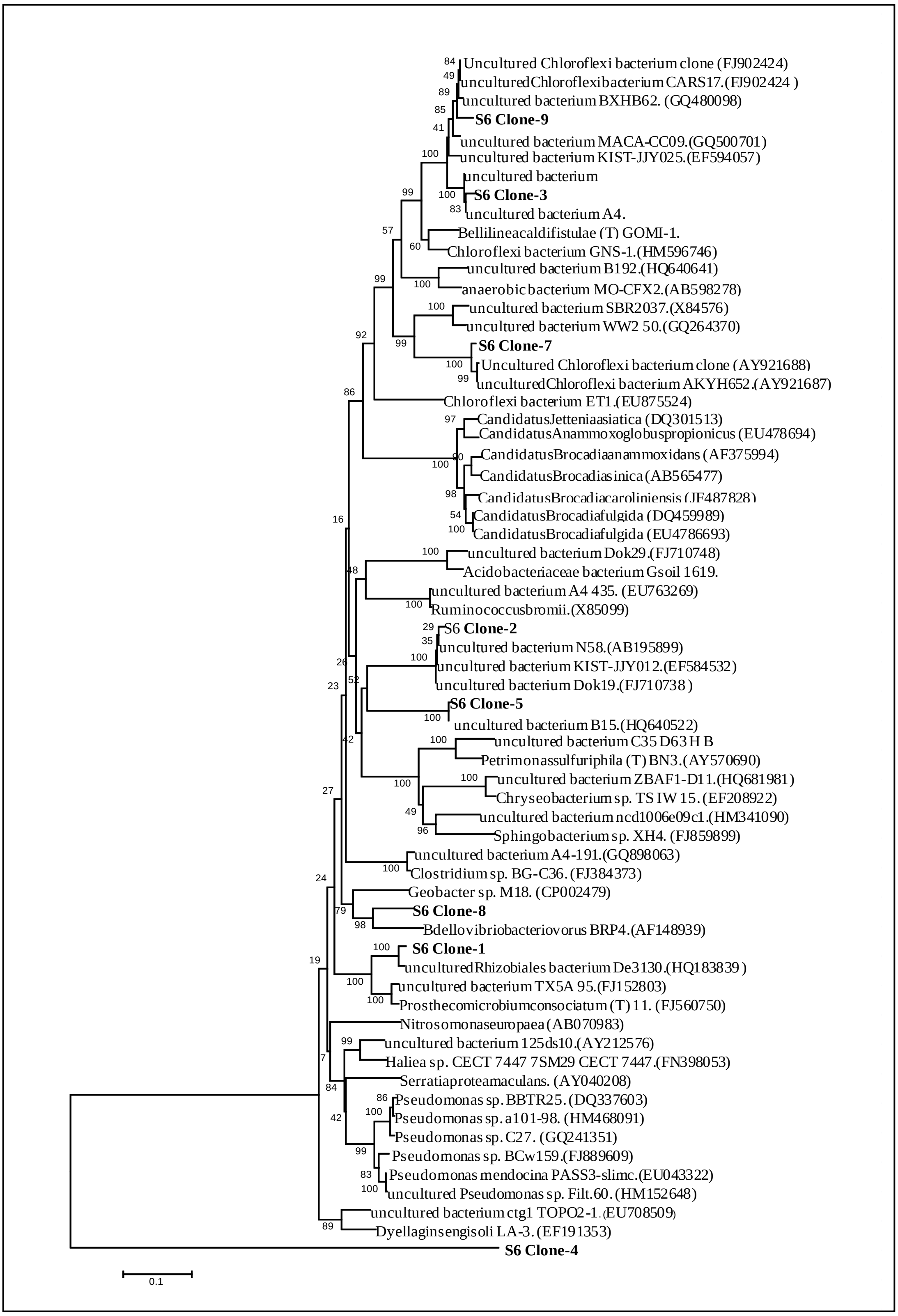


Figure IV. 14 Phylogenetic tree of 16s rRNA clone library sequences of day 400 biomass sample constructed with the MEGA program using the neighbor-joining method with default settings. The values beside the branches represent the percentage of times that a node was supported in 1000 bootstrap pseudoreplications.

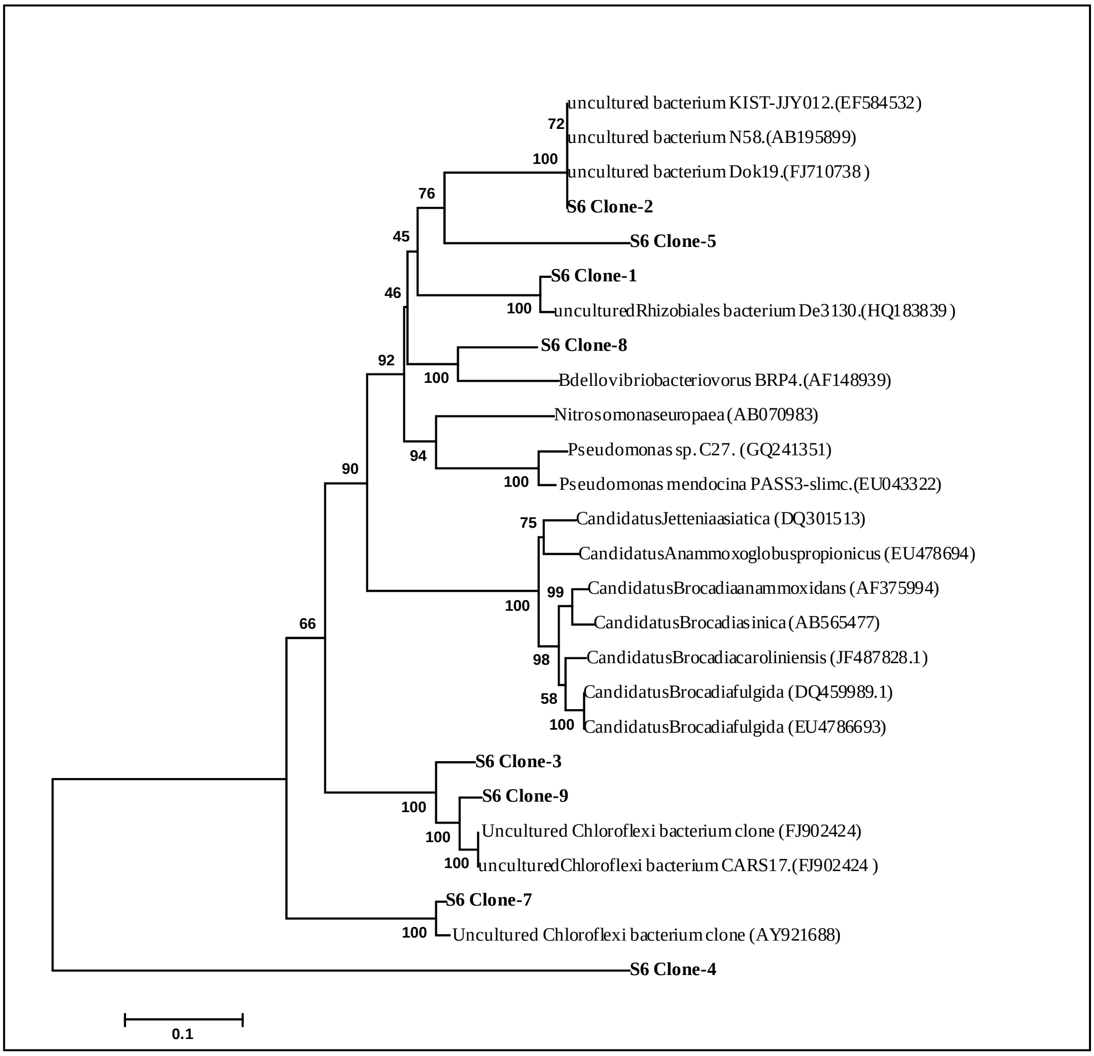
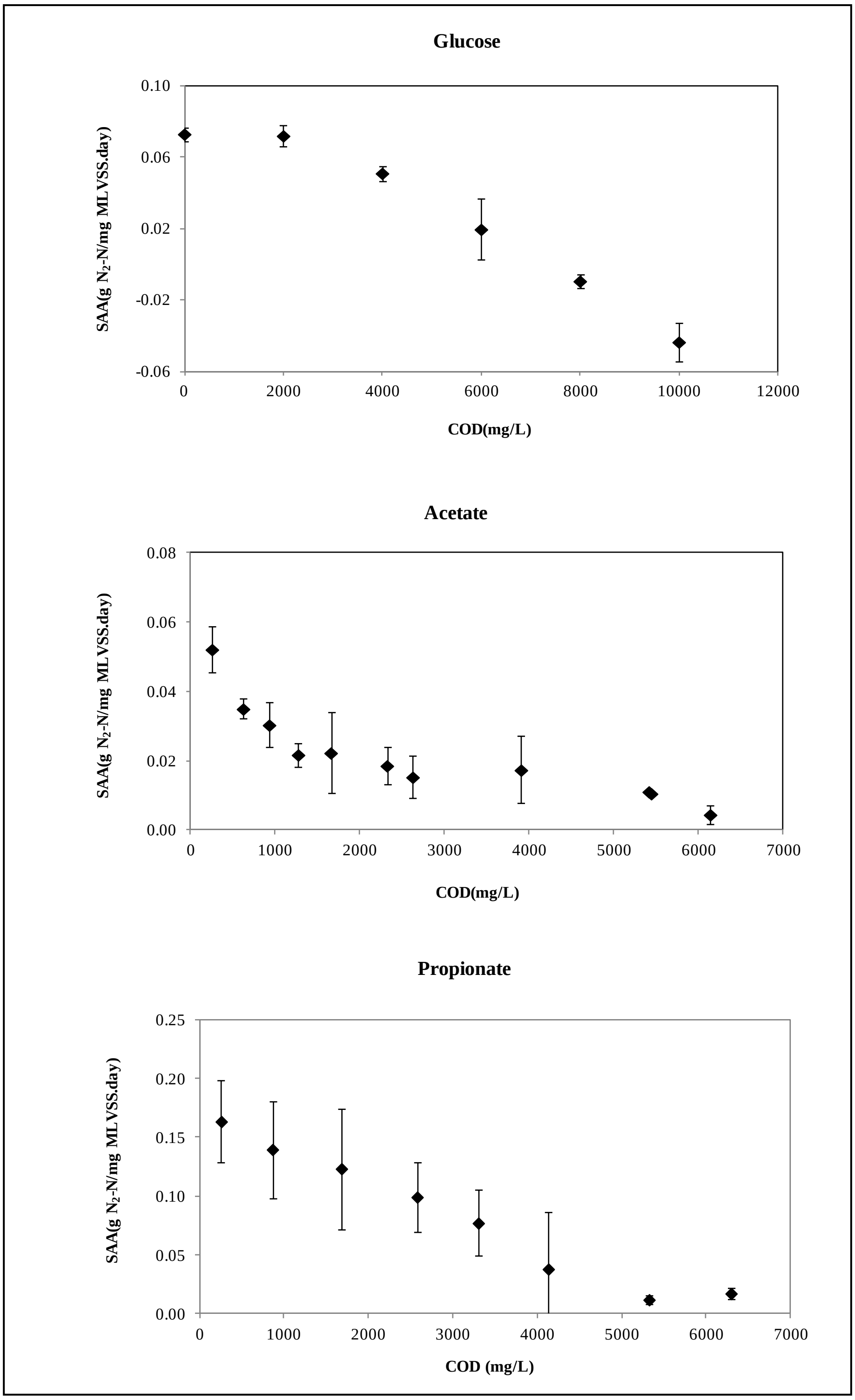


Figure IV.14 continue

Among the organic carbon forms studied, acetate has the highest inhibitory effect on Anammox. The batch experiment performed with real domestic wastewater containing 215 mg/L COD resulted no negative effect on Anammox process. The SAA value measured with real domestic wastewater exactly matched with the value measured for synthetic domestic wastewater without organic carbon. The NH_4-N removal rates calculated in these experiments proved that gas production in case of real domestic wastewater has resulted from only Anammox process.

In view of these findings, PN effluent was started to feed Anammox-SBR system as explained in Section IV.4.



Glucose

Acetate

Propionate

Figure IV. 15 Organic carbon effect of glucose, acetate and propionate on Anammox process

IV.4 ANAMMOX-SBR SYSTEM

The Anammox-SBR system was started-up using the sludge taken from the Stock-Anammox-SBR system which was enriched for 515 days as explained in Section IV.2. The system was operated in three phases to evaluate the followings: (I) Phase I: evaluation of possible use of NO_3-N as electron acceptor in Anammox process (104 days) (II) Phase II: evaluation of partially nitrified real domestic wastewater containing high organic carbon and improper NO_2-N:NH_4-N ratio for Anammox process (III) Phase III: evaluation of partially nitrified real domestic wastewater containing proper NO_2-N:NH_4-N ratio for Anammox process. The time course of influent and effluent changes observed in these phases are given in Figure IV.16. The stoichometric analysis of the process in these phases were done in terms of consumed NO_2-N+consumed NO_3-N ($\Delta_{NO2\text{-}N}+\Delta_{NO3\text{-}N}$): consumed NH_4-N ($\Delta_{NH4\text{-}N}$) as shown in Eqn IV.3. The results of these stoichometric analyses are shown in Figure IV.17.

$$\frac{\Delta_{NO2\text{-}N}+\Delta_{NO3\text{-}N}}{\Delta_{NH4\text{-}N}}=\frac{(\text{Influent } NO_2\text{-N-Effluent } NO_2\text{-N})+(\text{Influent } NO_3\text{-N-Effluent } NO_3\text{-N})}{\text{Influent } NH_4\text{-N-Effluent } NH_4\text{-N}} \tag{IV.3}$$

In the 1st phase of operation (day 0-104), the system was fed with sythetic wastewater containing only NH_4-N and NO_3-N without organic carbon (Table III.2). The aim was to evaluate the possible NO_3-N use by Anammox bacteria. As seen from Figure IV.16, both NH_4-N and NO_3-N concentrations decreased for the operational time period of 104 days. The specific removal rates for NH_4-N ($q_{NH4\text{-}N}$) and NO_3-N ($q_{NO3\text{-}N}$) were around 0.03 g NH_4-N/g VSS.d and 0.05 g NO_3-N/g VSS.d, respectively. This might indicate the use of NO_3-N by Anammox bacteria as an electron acceptor and this could be occurred due to two possible mechanisms. In the first possible mechanism, NO_3-N can be directly used by Anammox bacteria as shown stoichometrically in Eqn IV.3. In the second possible mechanism, NO_3-N can be first reduced to NO_2-N by denitrification and then the Anammox bacteria can use NO_2-N as electron acceptor for the degradation of NH_4-N. By considering the fact that the feed did not include any organic carbon, the occurance possibility of second mechanism is not expected. But, from stoichometric analyses of the process (Figure

IV.17), $\Delta_{NO3\text{-}N}/\Delta_{NH4\text{-}N}$ ratio was observed as 2.55 which is very far from the stoichiometrically expected ratio of 0.6 (Eqn IV.4). This indicated the possible use of NO_3-N in endogeneous denitrification.

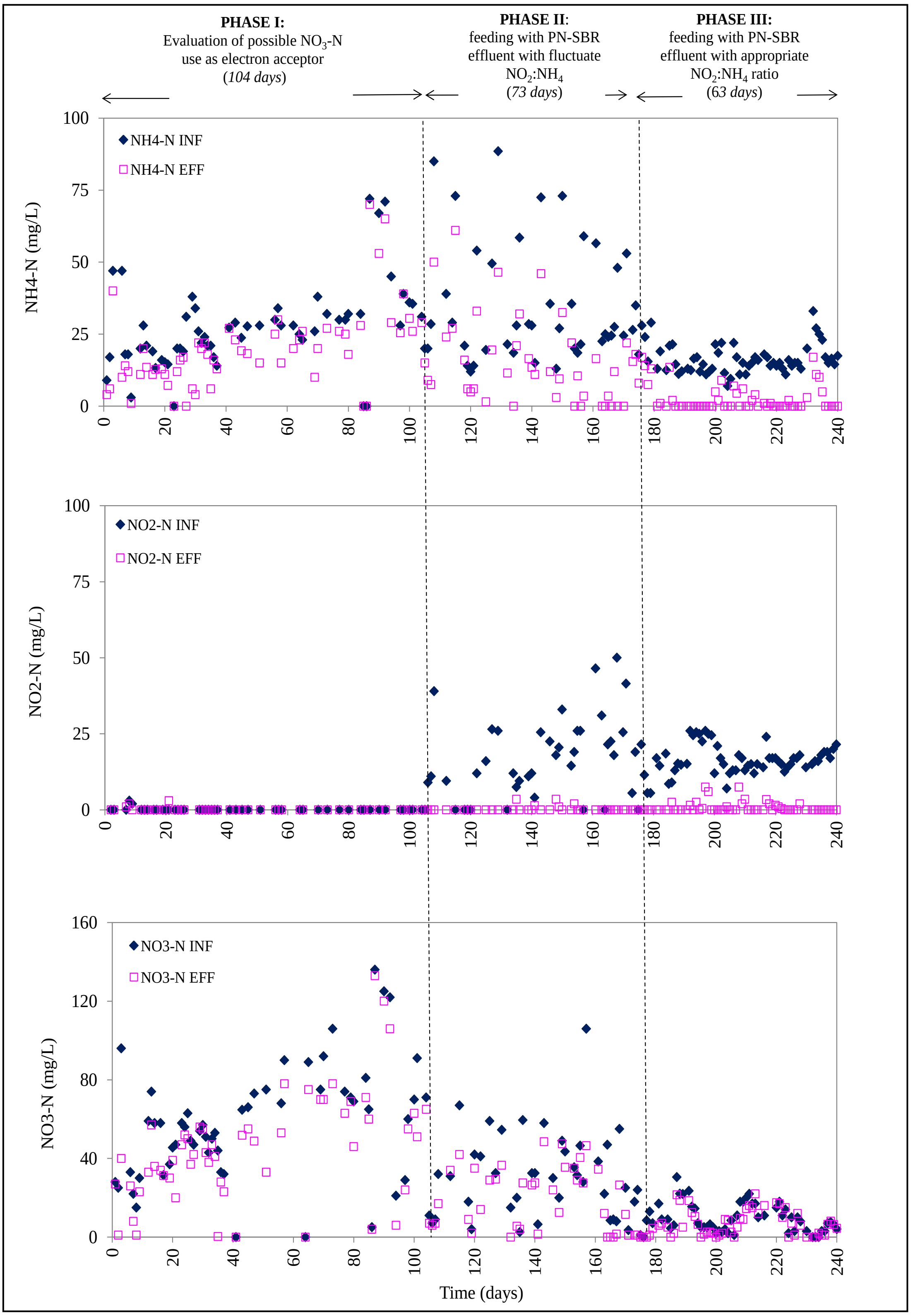


Figure IV. 16 Time course of influent and effluent changes for Anammox-SBR system

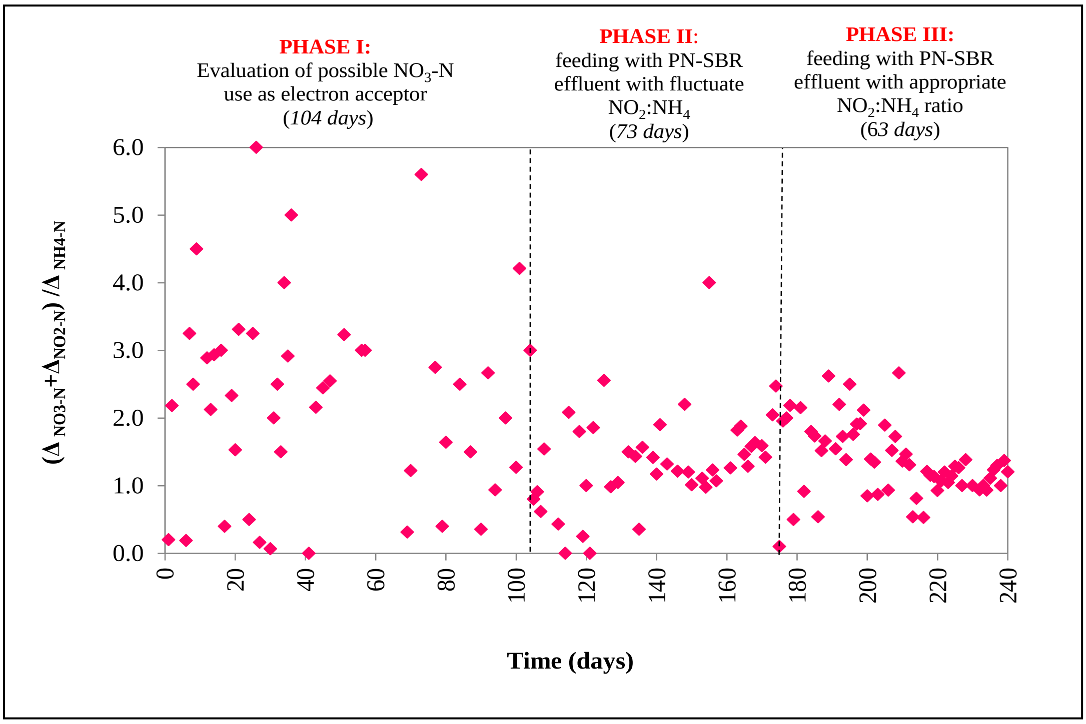


Figure IV. 17 Stoichiometric analysis for time course changes of $(\Delta_{NO2-N}+\Delta_{NO3-N})$ / (Δ_{NH4-N}) of Anammox-SBR system

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+ \qquad (IV.4)$$

In the 2nd phase of operation (days 107-177), as a result of feeding the system with partially nitrified domestic wastewater from the effluent of PN-SBR system (Section IV.1) NH_4-N removal rate suddenly increased to the levels of 0.06 g NH_4-N/g VSS.d. This indicated that NO_3-N use as electron acceptor, as tied in the 1st phase, results significantly lower NH_4-N removal rate. Similarly, in the study of Kumar, it was reported that longer reaction time period was necessary for the degradation of NH_4-N using NO_3-N as an electron acceptor instead of NO_2-N. NO_2-N removal was observed around 0.07 g NO_2-N/g VSS.d. Unexpectedly, instead of NO_3-N production, NO_3-N removal occurred with a rate of 0.04 g NO_3-N/g VSS.d. This observation might heavily be due to simultaneous Anammox and denitrification processes in the reactor. Organic carbon removals observed through COD analyses (Figure IV.18) prove this hypothesis. The stoichiometric analyses of the process indicated $(\Delta_{NO2-N}+\Delta_{NO3-N})/\Delta_{NH4-N}$ ratio was around 1.42, which is greater than the expected value of 1. This indicates that denitrification still prevailed in the system because of feeding the system with high organic carbon containing partially nitrified wastewater.

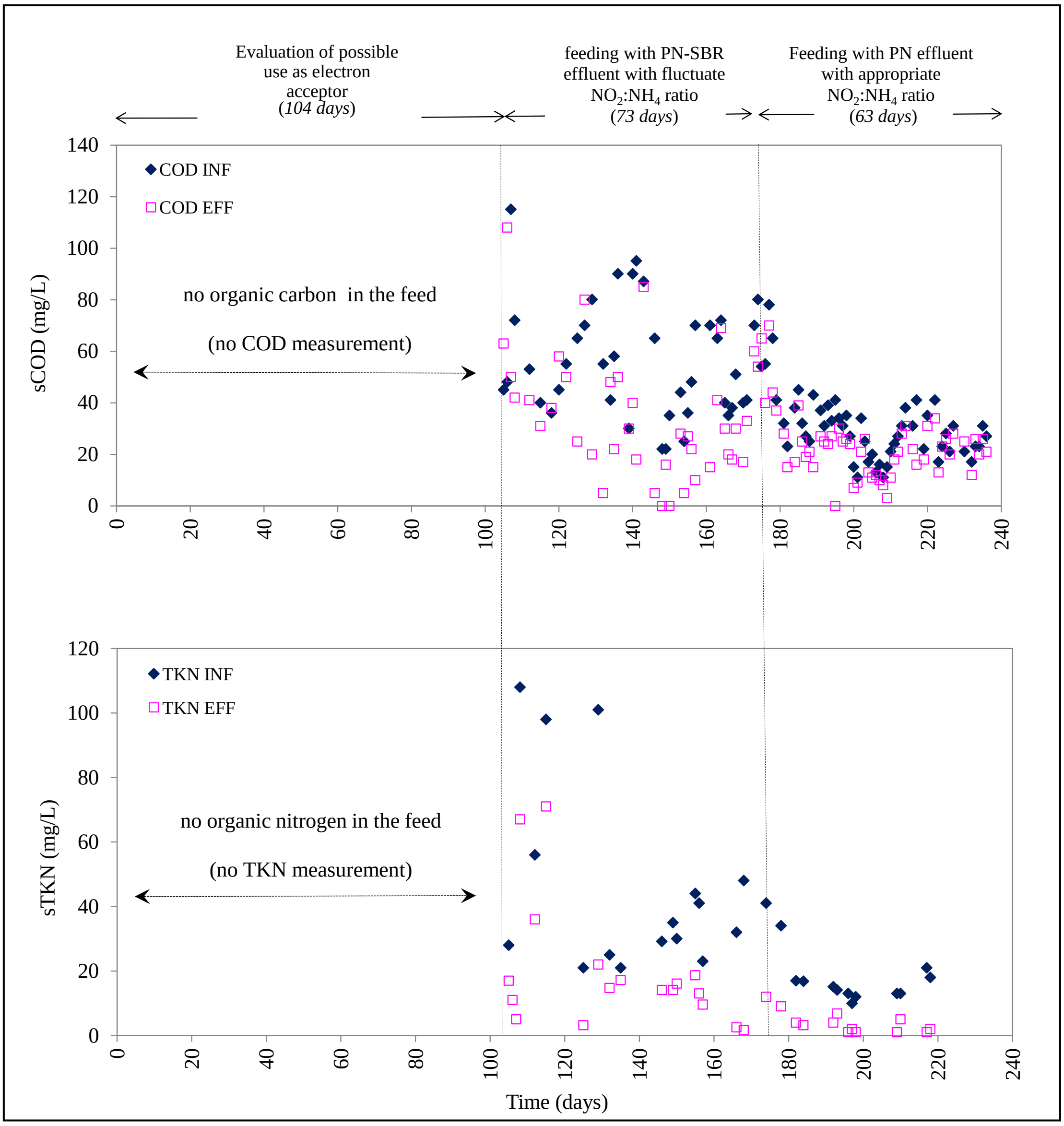


Figure IV. 18 Time course of sCOD and sTKN changes for Anammox SBR system

The sludge taken from Stock-Anammox-SBR system (Section IV.2) had been inoculated with mixed activated sludge. For this reason, the system had different cultures especially denitrification and Anammox. First and foremost, the coupling of denitrification and Anammox in a single reactor system could induce a competition between autotrophic anammox species and heterotrophic denitrifiers. When we compared Eqn. IV.5 and IV.6, ΔG° of denitrification reaction is higher than the Anammox reaction; therefore, the denitrification reaction is thermodynamically more feasible compared to Anammox reaction.

Anammox : $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$

$(\Delta G° = -357\ kJ/M)$ (IV.5)

Denitrification : $1.6NO_3^- + CH_3COOH \rightarrow 0.8N_2 + 2CO_2 + 1.2H_2O + 1.6OH^-$

$(\Delta G° = -843\ kJ/M)$ (IV.6)

In the 3rd phase of operation (178-240), the system was fed with a partially nitrified wastewater containing low amount of organic carbon and proper NO_2-N:NH_4-N ratio. The specific removal rates of NH_4-N, NO_2-N and NO_3-N were around 0.05, 0.03 and 0.02, respectively. As seen from those removal rates, the dominant process was Anammox but the denitrification activity could not be halted completely.

In the whole study, operational parameters were monitored online via measurement probes (pH, DO and temperature) in 30 minutes time arrivals. Throughout the operational period, temperature, DO and pH of the reactor were approxiametly 32.8±3.2 ^{0}C, 0.01±0.01, 7.81±0.51 respectively.

The combination PN-Anammox is a promising new and cost effective technology wastewater treatment, which should be extended into common municipal and domestic wastewater treatment.

CHAPTER V

CONCLUDING REMARKS AND RECOMMENDATIONS

In the scope of this study, the nitrogen removal from domestic wastewater with Anammox process was evaluated. For this purpose, two reactor systems, namely PN-SBR and Anammox-SBR systems were initially started-up to study partial nitritation and Anammox process, respectively. In the PN-SBR system, partial nitritation process, which is the preliminary treatment step required before Anammox process was studied for 350 days. The effluent of PN-SBR system was continuosly fed to the Anammox-SBR system for 240 days. Before the starting of feed the PN-SBR effluent to the Anammox-SBR system, a series of batch experiments were also performed to evaluate the possible organic carbon inhibition on Anammox process using different forms of organic carbon (synthetic wastewater containing glucose/acetate/propionate and real domestic wastewater). The major conclusions obtained from the experiments are summarized below:

1. The results obtained from the 350 days operation of PN-SBR system indicated that low strength characterictic of domestic wastewater makes it very difficult to partially nitrify. The effluent NO_2-N:NH_4-N ratio proper for the Anammox feeding could not be obtained by controlling a single operational parameter (e.g only DO limitation). The results indicated that increasing FA concentration in combination with the limited DO and low SRT conditions is the best suitable control strategy in order to obtain PN effluent proper for Anammox feeding. The optimum working conditions were identified as 0.8-1 mg/L DO, about 3 mg/L FA concentration and 2.5 days SRT. It must be noted that these conditions are changeable for different reactor configurations (e.g different MLVSS content, different AOB/NOB fraction)
2. The PN effluent observed at optimum operating conditions (see item1) showed that in case of low strength domestic wastewater, the partially nitrified influent to the Anammox system will always contain not only NO_2-N but also a small

portion of NO_3-N. NO_3-N production could not be prevented in the whole study because it is difficult to maintain long-term inhibition on NOB due to the low NH_4-N concentration and the reactor would suggest likely acclimatisation of the NOB to FA. The biomass might get acclimated to FA, and nitrite build-up may not be maintained for a long time.

3. The influent and effluent COD measurements performed in PN-SBR system indicated that lowering DO concentration and/or increasing FA concentration to achieve successful partial nitrification do not negatively influence COD removal in the PN reactor. The soluble biodegradable portion of COD is removed about 76% in all operating conditions. This indicates that almost all of the readily biodegradable portion of COD (rbCOD) is removed in PN-SBR. So, the influent COD to the Anammox-SBR system mainly consisted of nonbiodegradable and/or slowly biodegradable soluble COD can not result in activation of denitrifiers significantly in Anammox-SBR system.
4. In this study, the enrichment of Anammox bacteria from activated sludge of a local sewage treatment plant was successfully conducted as proven by molecular methods. These findings suggest that for the inoculation of newly constructed pilot or full-scale Anammox reactors seed sludge to be required in large volumes can be supplied from a local STP, instead of supplying from an already operational Anammox reactors mainly centered in Europe.
5. The pH, ORP, conductivity profiles observed through the 515 enrichment period of Anammox bacteria indicated that for an Anammox reactor newly started-up, *early start-up*, *start-up* and *enrichment* periods can easily be differentiated and monitored via online pH, ORP and conductivity measurements. Additionally, in a well operated Anammox reactor, the status of process can successfully be monitored and controlled through these online measurements.
6. Batch organic carbon inhibition experiments revealed that the inhibitory effect of acetate, glucose and propionate on Anammox process is significant at very high COD levels. The 50% inhibition concentrations were identified as 5000, 2500, 1500 mg/L COD for glucose, propionate and acetate, respectively. Among the organic carbon forms studied, acetate was identified as the most

inhibitory compound. The organic carbon content of typical domestic wastewater does not cause any inhibition on Anammox activity.

7. The operation of the Anammox-SBR system with only NH_4-N and NO_3-N feeding indicated that NO_3-N might possibly be used by Anammox process as an electron acceptor. But as compared to the use of NO_2-N as an electron acceptor, the degradation rate of NH_4-N will proceeded very slowly.
8. The operation of the Anammox-SBR system with the partially nitrified real domestic wastewater containing high organic carbon and improper NO_2-N:NH_4-N ratio showed that high organic carbon influences the denitrification activity in the system and Anammox and denitrification occurs simultaneously.
9. The operation of the Anammox-SBR system with partially nitrified effluent with low organic carbon content and proper NO_2-N/NH_4-N ratio indicated that in the treatment of domestic wastewater with Anammox process, denitrification activity can not be prevented completely. But if the partial nitrification process is done properly (i.e providing proper NO2-N:NH4-N ratio in the effluent) organic carbon load to Anammox-SBR system will decrease significantly. As a result, in the Anammox system denitrification activity will decrease significantly and the Anammox process will become dominant.

The findings of this study provided deep information for the applicability of Anammox process for the treatment of low strength domestic wastewater. In view of findings of this study, the followings are recommended for future studies:

1. In the partial nitrification studies, the microbial population must be quantified in terms of AOB and NOB using a qPCR technique to ensure NOB washout from the system by the applied operational conditions (e.g low SRT).
2. The findings of the study showed that partially nitrified effluent inevitably contain a portion of NO_3-N. The possible use of NO_3-N together with NO_2-N in the Anammox process must be studied deeply in the future studies.
3. In the present study, COD measurements were performed in terms of soluble COD. In the future studies, it is recommended to fractuate influent and effluent COD values (i.e as rbCOD, sbCOD, bCOD, nbCOD) for both PN-SBR and Anammox-SBR systems to evaluate the mass balance of COD in both systems.

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CIRRICULUM VITAE

Personal Information

Name, Surname	Duygu Dityapak
Date of Birth	04.05.1984

Education

2009 – 2012	M.S. Environmental Engineering, Marmara University Institute for Graduate Studies in Pure and Applied Sciences Istanbul, Turkey
2003 – 2007	B.S. Chemistry, Ege University Faculty of Science Izmir, Turkey
1999 – 2002	Kartal Koy Hizmetleri Anatolian High School, Istanbul, Turkey

Work Experience

2007 - 2008	Ant teknik Laboratuvar Cihazları Pazarlama Ltd. Şti. Istanbul/Turkey *Application and Service Engineer*
2009 – 2011	Scientific and Technological Research Council of Turkey (TÜBİTAK) – Project no: 108Y120 “Investigation of Anammox process for the Biological Nitrogen Removal from wastewaters” *Project Assistant*
2011 – …	Spektrotek Laboratuvar Cihazları Pazarlama Proje ve Danışmanlık Ltd.Şti. Istanbul/Turkey *Service Engineer*