T.C.

MARMARA UNIVERSITY

INSTITUTE FOR GRADUATE STUDIES IN

PURE AND APPLIED SCIENCES

ASSESMENT OF PARTIAL NITRIFICATION/NITRITIFICATION PROCESS UNDER OXYGEN-LIMITED CONDITIONS

Esra ÇİÇEK

THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

IN

ENVIRONMENTAL ENGINEERING

SUPERVISOR

Assist. Prof. Bilge ALPASLAN KOCAMEMİ

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"Our true mentor in life is science"

Mustafa Kemal ATATURK

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ABSTRACT

ASSESMENT OF PARTIAL NITRIFICATION/ NITRITIFICATION PROCESS UNDER OXYGEN-LIMITED CONDITIONS

In the scope of this study, partial nitrification/nitritification processes based on dissolved oxygen (DO) concentration control will be investigated through 365-days continuous flow experiments performed in an enriched nitrifying suspended-growth sequential batch reactor (SBR) and a submerged packed bed biofilm system under gradually decreasing DO concentrations. The batch specific ammonium utilization rate and oxygen uptake rate experiments performed in the continuous-flow SBR system at certain time intervals were provided detailed kinetic analyses of the nitrifier culture which were exposed to limited oxygen conditions for prolonged time.

The experimental results showed that in both systems limited oxygen conditions caused ammonium and nitrite accumulation. In the SBR system, ammonium and nitrite accumulations were initially observed upon decrease of DO from 1 to 0.54 mg/L, whereas in the biofilm system they were initially observed upon decrease of DO from 3.64 mg/L to 1.21 mg/L. The findings from both systems indicated that ammonium oxidizing (AOB) and nitrite oxidizing (NOB) cultures, which were exposed to the limited oxygen conditions for prolonged time were physiologically adapted to low DO in some extent. In both systems, nitrite accumulation and ammonium accumulation occurred in parallel with each other, indicating that free ammonia concentration partially effective on nitrite accumulation. In the biofilm system limited oxygen conditions caused higher nitrite accumulation than in the SBR system and higher nitrite accumulation/ammonium accumulation ratio was observed with respect to the SBR system. In the biofilm system, it was observed that ammonium and nitrite accumulations showing a stable trend under limited oxygen conditions might change in the long term due to increasing amount of bacteria in time.

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ÖZET

KISMİ OKSİJEN ŞARTLARINDA NİTRİFİKASYON/ NİTRİTİFİKASYON PROSESLERİNİN İNCELENMESİ

Bu çalışma kapsamında çözünmüş oksijen (ÇO) konsantrasyonu kontrolüne dayalı kısmi nitrifikasyon/nitritifikasyon prosesleri zenginleştirilmiş nitrifikasyon kültürü içeren askıda çoğalan ardışık kesikli reaktör (SBR) ve dolgu yataklı batık biyofilm sistemlerinde aynı şartlar altında 365 gün süresince kademeli olarak azalan ÇO konsantrasyonlarında gerçekleştirilen sürekli akış deneyleri ile incelenmiştir. Ayrıca, sürekli akış altında işletilen SBR sisteminde belirli zaman aralıklarında gerçekleştirilen kesikli spesifik amonyum tüketim hızı ve oksijen tüketim hızı deneyleri ile uzun süreli limitli ÇO konsantrasyonuna maruz kalan nitrifikasyon bakterilerinin detaylı kinetik analizi gerçekleştirilmiştir.

Deneylerden elde edilen sonuçlar her iki sistemde de kısıtlı oksijen şartlarının amonyak ve nitrit birikimine yol açtığını göstermiştir. SBR sisteminde 1 mg/L'den 0.54 mg/L ÇO'ya geçildiğinde görülmeye başlanan amonyak ve nitrit birikimi biyofilm sisteminde 3.64 mg/L'den 1.21 mg/L'ye geçildiği süreçte görülmeye başlanmıştır. Her iki sistemde de uzun süreli kısıtlı oksijene maruz kalan amonyum oksitleyen AOB ve nitrit oksitleyen NOB kültürlerinin düşük ÇO'ya fizyolojik olarak bir miktar adapte olduklarına dair bulgular elde edilmiştir. Nitrit birikiminin her iki sistemde amonyak birikimine paralel olarak seyretmesi, serbest amonyak miktarının kısmen de olsa nitrit birikimini etkilediğini göstermiştir. Kısıtlı oksijen şartlarının biyofilm sisteminde SBR sistemine göre daha ciddi nitrit birikimine sebep olduğu ve nitrit birikim/amonyak birikim oranının daha yüksek seyrettiği bulunmuştur. Biyofilm sisteminde kısıtlı oksijen şartlarında stabil olarak seyreden amonyak ve nitrit birikimlerinde zamanla artış gösterecek bakteri miktarına bağlı olarak uzun vadede değişim olabileceği gözlemlenmiştir

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SYMBOLS

DO : Dissolved Oxygen concentration (mg/L)

CO_2 : Carbondioxide (mg/L)

f_s : Fraction of electron donor used for cell synthesis

f_e : Fraction of electron donor used for energy

HNO_2 : Un-ionized nitrous acid (mg/L)

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 $\cdot$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_s : Half-saturation or half-velocity coefficient (mg/L)

$K_{s,NH4\text{-}N}$: NH_4^+-N half-saturation coefficient for AOB (mg NH_4^+-N/L)

$K_{s,NO2\text{-}N}$: NO_2^--N half-saturation coefficient for NOB (mg NO_2^--N/L)

K_w : The ionization constant of water

N_2 : Nitrogen gas

NH_3 : Un-ionized ammonia (mg/L)

NH_4^+-N : Ammonium nitrogen concentration (mg/L)

NH_2OH : Hydroxylamine concentration (mg/L)

NO_2^--N : Nitrite nitrogen concentration (mg/L)

NO_3^--N : Nitrate nitrogen concentration (mg/L)

O_2 : Molecular oxygen (mg/L)

q_n : Specific NH_4^+-N oxidation rate (g NH_4^+-N oxidized/ g VSS/d)

$q_{NH4\text{-}N}$: Specific NH_4^+-N oxidation rate (g NH_4^+-N oxidized/ g VSS/hr)

$q_{max,\ NH4\text{-}N}$: Maximum specific NH_4^+-N oxidation rate (g NH_4^+-N oxidized/ g VSS/hr)

q_{nm} : Maximum specific NH_4^+-N oxidation rate (g NH_4^+-N oxidized/ g VSS/d)

S : Growth- limiting substrate concentration (mg/L)

$SOUR_{max}$: Maximum specific oxygen uptake rate (mg O_2/ mg VSS.d)

$SOUR_{max,AOB}$: Maximum specific oxygen uptake rate for AOB (mg O_2/ mg VSS.d)

$SOUR_{max,NOB}$: Maximum specific oxygen uptake rate for NOB (mg O_2/ mg VSS.d)

Y_n : AOB yield coefficient (g AOB grown (VSS)/ g NH_4^+-N removed)

μ : Specific growth rate of microorganisms (d^{-1})

μ_m : Maximum 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)

ABBREVIATIONS

ANAMMOX : Anaerobic ammonium oxidation
AOB : Ammonium-oxidizing bacteria
BF : Biofilm reactor
BOD : Biochemical oxygen demand
CANON : Completely autotrophic nitrogen removal over nitrite
CFSTR : Continuous-flow stirred-tank reactor
FA : Free ammonia
FBR : Fluidized bed reactor
FISH : Fluorescence in-situ hybridization
FNA : Free nitrous acid
HRT : Hydraulic retention time
MLVSS : Mixed liquor volatile suspended solids
MLSS : Mixed liquor suspended solids
NOB : Nitrite-oxidizing bacteria
OLAND : Oxygen limited autotrophic nitrification denitrification
OUR : Oxygen uptake rate
RBC : Rotating biological contactor
RPM : Rotation per minute
SBR : Sequencing batch reactor
SHARON : Single reaction system for high ammonium removal over nitrite
SRT : Sludge retention time
SS : Suspended solids
TF : Trickling filter
VSS : Volatile suspended solids

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CHAPTER I

INTRODUCTION and AIM

Nowadays, the preventation of the receiving water bodies has gained great importance and hence there are very stringent discharge limits for nitrogen, which causes serious environmental problems especially eutrophication (EPA, 1993). Nitrogen in form of ammonium is of special interest because it can be toxic to aquatic species (Khin and Annachhatre, 2004) and causes excessive oxygen demand in the receiving waters (Bernet et al., 2005). Therefore, in recent years, the nitrogen removal from domestic and industrial wastewater has received great attention. In the treatment of ammonium from wastewater, biological processes, which are more efficient and cheaper with respect to physico-chemical processes are preferred (EPA, 1993). Nitrogen compounds are generally eliminated from wastewater by a combination of two conventional processes, nitrification and denitrification (Jianlong and Ning, 2004). Conventional nitrification-denitrification process is based on autotrophic nitrification and heterotrophic denitrification (Khin and Annachhatre, 2004).

In the application of conventional nitrification-denitrification process for domestic and industrial wastewater treatment, high oxygen requirement of nitrification process and the necessity of external carbon addition for denitrification of low C/N containing industrial wastewaters increase the operational cost of treatment plants. However, recently developed novel biological nitrogen removal processes (e.g., Anammox, Canon, Oland) seem attractive alternatives to the conventional nitrification-denitrification process considering their potential for minimization of oxygen and chemical (external carbon) consumption in nitrogen removal. These processes of which basis depend on partial nitrification/nitritification were mainly developed for the high ammonium (NH_4-N) containing wastewaters having low C/N ratio. The applicability of these processes for the removal of nitrogen from domestic wastewater necessitates the detailed investigation of the maintaining stable partial nitrification or partial nitritification via inhibiting nitrite

(NO_2^--N) oxidation to nitrate (NO_3^--N) in nitrification process. The possibility of achieving partial nitrification/nitritification by modifying the dissolved oxygen (DO) concentration (i.e., aeration patterns) instead of other operational parameters seems to be an attractive solution considering energy consumption in air supply accounts for a high percentage of the total energy consumption in wastewater treatment systems.

In the present study, the applicability of partial nitrification/nitritification processes based on dissolved oxygen concentration control will be investigated for domestic wastewater. For this purpose, the effect of the DO concentration on the ammonium oxidation and nitrite accumulation were investigated in the long term through continuous-flow experiments performed in an enriched nitrifying suspended-growth sequential batch reactor (SBR) and a submerged packed bed biofilm system under gradually decreasing DO concentrations. The batch specific ammonium utilization rate and oxygen uptake rate experiments performed in the continuous-flow SBR system at certain time intervals were provided detailed kinetic analyses of the nitrifier culture which were exposed to limited oxygen conditions for prolonged time. Additionally, the molecular microbiological analyses of the sludge samples taken both from the SBR and biofilm systems were provided to monitor microbial ecology, community shifts and quantity changes of the nitrifying bacteria during continuous-flow experiments.

CHAPTER II

GENERAL BACKGROUND

Since waste water discharges containing nitrogen can cause oxygen depletion and eutrophication in receiving water, stimulate aquatic growth, exhibit toxicity toward aquatic life and present health hazard, reducing nitrogen levels from discharges is necessary (EPA, 1993; Ahn, 2006).

Therefore, in recent years, nitrogen removal from waste waters has received great attention. Nitrogen, in the form of ammonium, can be removed from wastewaters by a variety of physicochemical and biological processes. Because biological nitrogen removal is effective and inexpensive, it has been adopted widely in favor of physicochemical processes (EPA, 1993).

Nowadays, the most common way to remove nitrogen from wastewater is the combination of two sequential biological processes: autotrophic nitrification and heterotrophic denitrification (Cuidad et al., 2007). However, great research effort has been spent to develop several novel and cost-effective biological nitrogen removal processes, of which basis depend on partial nitrification/nitritification process.

II.1. NITRIFICATION

Nitrification is the term used to describe two-step biological process in which ammonium (NH_4^+-N) is oxidized to nitrite (NO_2^--N), and nitrite is oxidized to nitrate (NO_3^--N) (Metcalf and Eddy, 2004).

II.1.1. Physiology of Nitrifying Bacteria

Nitrification, as noted above, is a two-step process involving two groups of bacteria, which are autotrophs, chemolithotrophs, and obligate aerobes. NH_4^+-N is converted to NO_2^--N by autotrophic *Nitroso*-bacteria which are also called ammonium-oxidizing bacteria (AOB) and then to NO_3^--N by *Nitro*-bacteria which

are also called nitrite-oxidizing bacteria (NOB) under aerobic conditions via using inorganic carbon as carbon source (Rittmann and McCarty, 2001).

The most commonly recognized genus of bacteria that carries out the first step is *Nitrosomonas*; however *Nitrosococcus, Nitrosopira, Nitrosovibro* and *Nitrosolobus* are also able to oxidize NH_4^+ to NO_2^-. The ammonium-oxidizing nitrifiers, which all have the genus prefix *Nitroso*, are genetically diverse, but related to each other in the beta subdivision of the proteobacteria (Rittmann and McCarty, 2001).

Although *Nitrospira*, *Nitrospina, Nitrosococcus* and *Nitrocystis* are known to sustain themselves from the second-stage reaction, *Nitrobacter* is the most famous genus of the NO_2^- oxidizers. Within the *Nitrobacter* genus, several subspecies are distinct, but closely related genetically within the alpha subdivision of the proteobacteria. Recent findings using oligonucleotide probes targeted to the 16S rRNA of *Nitrobacter* indicate that *Nitrobacter* is not the most important nitrite oxidizing genus in most wastewater treatment processes. *Nitrospira* more often is identified as the dominant nitrite oxidizer (Rittmann and McCarty, 2001).

II.1.2. Stoichiometry of Nitrification

Conversion of NH_4^+-N to NO_2^--N and NO_3^--N involves a complex series of reactions that control the overall conversion process (Jianlong and Ning, 2004; Metcalf and Eddy, 2004).

II.1.2.1. Energy Reactions

The energy yielding two-step oxidation of NH_4^+-N to NO_3^--N is as follows (Metcalf and Eddy, 2004):

Nitroso- Bacteria:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \qquad \text{(II.1a)}$$

Nitro- Bacteria:

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad \text{(II.1b)}$$

Total Oxidation Reaction:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad \text{(II.1c)}$$

Based on the above total oxidation reaction [Eqn. (II.1c)], the oxygen required for complete oxidation of NH_4^+ is 4.57 g O_2/g N oxidized with 3.43 g O_2/ g used for NO_2^- production and 1.14 g O_2/ g NO_2 oxidized. When the synthesis is considered, the amount of O_2 required is less than 4.57 g O_2/g N. In addition to oxidation, O_2 is obtained from fixation of carbon dioxide and nitrogen into cell mass (Metcalf and Eddy, 2004).

Neglecting cell tissue, the amount of alkalinity required to carry out of the reaction is given in Eqn. (II.2)

$$NH_4^+ + 2HCO_3^- + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_2O \qquad \text{(II.2)}$$

In the above equation, for each g of NH_4^+-N converted, 7.14 g of alkalinity as $CaCO_3$ will be required (Metcalf and Eddy, 2004).

II.1.2.2. Cell Synthesis Reactions

Along with obtaining energy a portion of the NH_4^+ ion is assimilated into cell tissue. The biomass synthesis reaction can be represented as follows (Metcalf and Eddy, 2004).

$$4CO_2 + HCO_3^- + NH_4^+ + H_2O \rightarrow C_5H_7O_2N + 5O_2 \qquad \text{(II.3)}$$

The chemical formula $C_5H_7O_2N$ is used to represent the synthesized bacterial cells (Metcalf and Eddy, 2004).

II.1.2.3. Overall Energy and Cell Synthesis Reactions

The overall synthesis and energy reaction for nitrification is given in Eqn. (II.4). In Eqn. (II.4) fraction of electron donor used for cell synthesis (f_s) and used for energy (f_e) were assumed as 0.05 and 0.95, respectively.

$$NH_4^+ + 1.863O_2 + 0.098CO_2 \rightarrow 0.0196C_5H_7O_2N + 0.98NO_3^- + 0.0941H_2O + 1.98H^+ \quad \text{(II.4)}$$

From Eqn. (II.4) it will be noted that for each g of NH_4^+-N converted, 4.24 g of O_2 are utilized, 0.16 g of new cell are formed, 7.07 g of alkalinity as $CaCO_3$ are removed, and 0.08 g of inorganic carbon are utilized in the formation of the new cells. The oxygen required to oxidize 1 g of NH_4^+-N to NO_3^--N (4.25 g) is less than the theoretical value of 4.57 g computed using Eqn. (II.1c), because the ammonia for cell synthesis is not considered in Eqn. (II.1c). Similarly the alkalinity required for nitrification in Eqn. (II.4) (7.07 g as $CaCO_3$/g NH_4^+-N) is less than the value of 7.14 g calculated without considering the conversion of some of the NH_4^+ to cellular nitrogen [Eqn. (II.2)] (Metcalf and Eddy, 2004).

II.1.2.4. Growth Kinetics of Nitrifying Bacteria

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.5)] is used to describe the kinetics of biological growth of either *Nitrosomonas* or *Nitrobacter* (EPA, 1993). According to the Monod kinetics, the observed effect of growth-limiting substrate concentration on specific growth rate of microorganisms is shown in Figure II.1.

$$\mu = \mu_m \frac{S}{K_s + S} \quad \text{(II.5)}$$

where:

μ : specific growth rate of microorganisms, d^{-1}

μ_m : maximum specific growth rate of microorganisms, d^{-1}

S : growth- limiting substrate concentration, mg/L

K_s : half-saturation or half-velocity coefficient (equivalent to the growth-limiting substrate concentration at half the maximum specific growth rate), mg/L

For nitrification systems operated at temperatures below 28°C ammonia-oxidation kinetics versus nitrite-oxidation kinetics are rate-limiting, so that designs

are based on saturation kinetics for NH_4^+-N oxidation as given in Eqn. (II.6), assuming excess dissolved oxygen (DO) is available (Metcalf and Eddy, 2004).

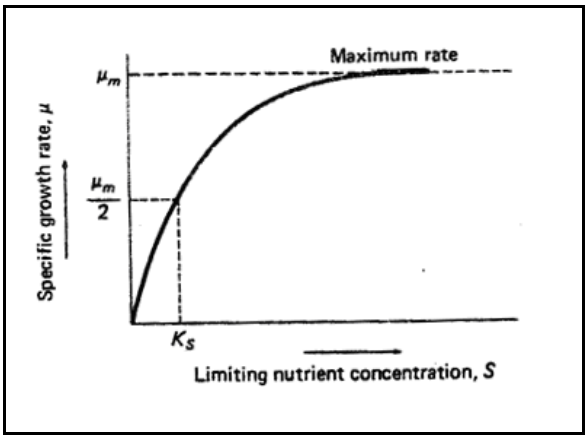


Figure II. 1 Effect of growth limiting substrate concentration on specific growth rate of bacteria based upon Monod kinetics (Metcalf and Eddy, 1991)

$$\mu_n = \frac{(\mu_{nm} N)}{K_n + N} - k_{dn} \qquad \text{(II.6)}$$

where:

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

N : NH_4^+-N concentration, mg/L

K_n : half-saturation coefficient for NH_4^+-N, mg/L

k_{dn} : endogenous decay coefficient for AOB, g VSS/ g VSS.d

The rate of NH_4^+-N oxidation is controlled by the growth of AOB and is related to this growth by the *Nitrosomonas* yield coefficient. The relationship between the oxidation rate and growth rate of AOB can be expressed as follows (EPA, 1993):

$$q_n = \frac{\mu_n}{Y_n} = q_{nm} \frac{N}{K_n + N} \qquad \text{(II.7)}$$

where:

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

Y_n : AOB yield coefficient, g AOB grown (VSS)*/ g* NH_4^+-N removed

Other terms are as defined previously.

In Eqn. (II.6) and Eqn. (II.7), it is apparent that when K_n is low with respect to N, the growth rate and the NH_4^+-N oxidation rate are independent of the concentration of NH_4^+-N and the AOB organisms are growing at their maximum rate. In complete-mix activated sludge systems, however, N can be lower than K_n, in which case kinetics approach first order (i.e., growth rate is dependent on substrate concentration) (EPA, 1993).

Table II.1 and Table II.2 summarize the basic and derived parameters that describe the stoichiometry and kinetics of AOB and NOB, respectively (Rittmann and McCarty, 2001).

Table II. 1 Basic and derived parameter kinetics for AOB (Rittmann and McCarty, 2001)

Parameter	Parameter Values at T =				
	5°C	10°C	15°C	20°C	25°C
Fraction of electron donor used for cell synthesis *(fs)*	0.14	0.14	0.14	0.14	0.14
Yield coefficient (*Y*) (*mg VSS / mg* NH_4^+*-N*)	0.33	0.33	0.33	0.33	0.33
Maximum specific NH_4^+-N oxidation rate (q_{nm}) (*mg* NH_4^+*-N/ mg VSS.d*)	0.96	1.30	1.70	2.30	3.10
Maximum specific oxygen uptake rate ($SOUR_{max}$) (*mg* O_2*/ mg VSS.d*)	2.90	3.80	5.10	6.80	9.20
Maximum specific growth rate (μ_{nm}) (d^{-1})	0.32	0.42	0.58	0.76	1.02
Half-saturation coefficient for NH_4^+-N (K_n) (*mg* NH_4^+*-N/L*)	0.18	0.32	0.57	1.00	1.50
Half- saturation coefficient for DO (K_o) (mg O_2/ L)	0.50	0.50	0.50	0.50	0.50
Endogenous decay coefficient (k_{dn}) (d^{-1})	0.045	0.060	0.082	0.11	0.15

Table II. 2 Basic and derived parameter kinetics for NOB at (Rittmann and McCarty, 2001)

Parameter	Parameter Values at T =				
	5°C	10°C	15°C	20°C	25°C
Fraction of electron donor used for cell synthesis *(fs)*	0.10	0.10	0.10	0.10	0.10
Yield coefficient (*Y*) (*mg VSS / mg* NO_2^-*-N*)	0.083	0.083	0.083	0.083	0.083
Maximum specific NO_2^--N oxidation rate (q_{nm}) (*mg* NO_2^-*-N/ mg VSS.d)*	4.10	5.50	7.30	9.80	13.00
Maximum specific oxygen uptake rate ($SOUR_{max}$) (*mg* O_2*/ mg VSS.d*)	4.20	5.60	7.50	10.10	13.50
Maximum specific growth rate (μ_{nm}) (d^{-1})	0.34	0.45	0.61	0.81	1.10
Half-saturation coefficient for NO_2^--N (K_n) (*mg* NO_2^-*-N/L*)	0.15	0.30	0.62	1.30	2.70
Half- saturation coefficient for DO (K_o) (mg O_2/ L)	0.68	0.68	0.68	0.68	0.68
Endogenous decay coefficient (k_{dn}) (d^{-1})	0.045	0.060	0.082	0.11	0.15

Nitrification rates are affected by the liquid DO concentration in activated sludge. In contrast to what has been observed for aerobic heterotrophic bacteria degradation of organic compounds, nitrification rates increase up to DO concentrations of 3 to 4 mg/L. To account for the effects of DO, the expression for the specific growth rate of AOB [Eqn. (II.6)] is modified as follows in Eqn. (II.8) (Metcalf and Eddy, 2004):

$$\mu_n = \mu_{nm} \frac{N}{K_n + N} \frac{DO}{K_o + DO} - k_{dn} \qquad \text{(II.8)}$$

where:

DO : dissolved oxygen concentration, mg/L

K_o : half- saturation coefficient for DO, mg/L

Other terms are as defined previously.

II.1.3. Factors Affecting Nitrification

II.1.3.1. Temperature

The nitrification process occurs over a range of temperature (approximately 4-45°C) with about 35°C optimum for *Nitrosomonas* and 35-42°C optimum for *Nitrobacter* (EPA, 1993). Nitrification rates have been found to double for every 10°C increase in temperature between 10°C and 30°C (EPA, 2003). In the study of Philips et al. (2002), it was determined that the NH_4^+-N and NO_2^--N oxidation rates increase 2.6 and 1.8 times per 10°C in a physiologically relevant temperature range. Nitrification slows down dramatically or may stop together at around 5^oC (EPA, 2003).

The maximum specific growth rate of NOB is significantly higher than that of AOB at temperatures between 10°C to 20°C. At temperatures higher than 25°C however, the maximum specific growth rate of NOB is approximately in the same range as that of AOB, possible leading to nitrite accumulation. Researchers claim that at higher temperatures, the growth rate of NOB is lower than that of the AOB (Philips et al., 2002).

II.1.3.2. pH and Alkalinity

Alkalinity is a major chemical requirement needed for nitrification. The amount of the alkalinity required for nitrification, taking in to account cell growth, is about 7.07 g $CaCO_3$/g NH_4^+-N. In addition to the alkalinity required for nitrification, additional alkalinity must be available to maintain the pH in the range from 6.8 to 7.4. Typically the amount of residual alkalinity required to maintain pH near the neutral point is between 70 and 80 mg/L as $CaCO_3$ (Metcalf and Eddy, 2004).

Reactor pH conditions have a significant effect on the rate of nitrification. The pH moves to acidic range, the rate of ammonium oxidation declines. The nitrification rate may drop significantly as pH is lowered below the neutral range and that for performance stability it is best to maintain pH 6.5- 8.0 (EPA, 1993).

The oxidation of ammonium is an acidifying process. Therefore, the control of pH is important for preventing process inhibition. When the pH drops below 6.5 the ammonium oxidation will no longer take place because of a pH-dependent

equilibrium between the concentration of NH_3 and NH_4^+. The nitrite oxidizers grow faster than the ammonium oxidizers at low pH values (Khin and Annachhatre, 2004).

II.1.3.3. Dissolved Oxygen (DO)

The concentration of DO has a significant effect on the rates of nitrifier growth and nitrification in biological waste treatment systems. In contrast to what has been observed for aerobic heterotrophic bacteria degradation of organic compounds, nitrification rates increase up to DO concentration of 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.8) (Metcalf and Eddy, 2004). By modeling the growth of *Nitrosomonas* and *Nitrobacter* according to the Monod equation [Eqn. (II.8)], with DO as the growth-limiting substrate concentration, values for the half-saturation coefficient have been reported as 0.15-2.0 mg/L O_2 (EPA, 1993).

If mass transport or diffusional resistances are an inherent characteristic of nitrification reactor, as is the case with attached-growth reactors, the DO level achievable in designing the oxygen addition component should be relatively high. The value of DO at which nitrification is limited can be 0.5-2.5 mg/L in either suspended or attached-growth systems under steady-state conditions, depending on the degree of the mass-transport or diffusional resistance and the solid retention time (EPA, 1993). At low DO concentration (<0.5 mg/L) where nitrification rates are inhibited, the low DO inhibition effect has been shown to be greater for *Nitrobacter* than for *Nitrosomonas* (Metcalf and Eddy, 2004).

In a previous study it has been shown experimentally that the effect of DO on nitrification is affected by the activated-sludge floc size and density, and total oxygen demand of the mixed liquor. Nitrifying bacteria are distributed within a floc containing heterotrophic bacteria and other solids, with floc diameters ranging from 100 to 400 μm. Oxygen from bulk liquid diffuses into floc particles and bacteria deeper within the floc are exposed to lower DO concentrations (Metcalf and Eddy, 2004).

II.1.3.4. Free Ammonia (FA) and Free Nitrous Acid (FNA)

Nitrifying organisms are sensitive to certain forms of nitrogen. Un-ionized ammonia (NH_3), or free ammonia (FA), and un-ionized nitrous acid (HNO_2), or free

nitrous acid (FNA) are believed to be inhibitory to nitrifiers above certain concentrations (Anthonisen et al., 1976; EPA, 1993).

FA and FNA are present in accordance with the equilibrium reactions shown in Eqn. (II.9) and Eqn. (II.10), respectively (Anthonisen et al., 1976; EPA, 1993).

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \quad \text{(II.9)}$$

$$NH_4^+ + 1.5O_2 \rightarrow H_2O + H^+ + \boxed{H^+ + NO_2^- \leftrightarrow HNO_2^-} \quad \text{(II.10)}$$

FA and FNA concentration are directly correlated to pH, temperature, and the concentration, respectively, of ammonium plus ammonia ($NH_4^+ + NH_3$) and nitrite plus nitrous acid ($NO_2^- + HNO_2^-$) as shown in Eqn. (II.11) and Eqn. (II.12) (Anthonisen et al., 1976; EPA, 1993).

$$\text{FA as } NH_3 \text{ (mg/L)} = \frac{17}{14} x \frac{\text{total ammonia as N (mg/L)} \; x 10^{\text{pH}}}{K_b / K_w + 10^{\text{pH}}} \quad \text{(II.11)}$$

$$\text{FNA as } HNO_2 \text{ (mg/L)} = \frac{46}{14} x \frac{NO_2^- \text{-N (mg/L)}}{K_a x 10^{\text{pH}}} \quad \text{(II.12)}$$

where

K_a : The ionization constant of the nitrous acid equilibrium equation

K_b : The ionization constant of the ammonia equilibrium equation

K_w : The ionization constant of water

$K_b/K_w = e^{(6344/273+T)}$, T (Temperature) is in ^oC

$K_a = e^{(-2300/273+T)}$, T is in ^oC

Figure II.2 illustrates a relationship between pH and the concentration of the total NH_3 and NO_2^--N. In this figure, boundary conditions of zones of nitrification inhibition are shown. Zone 1 (FA> 10-150 mg/L) marks the inhibition of AOB and NOB by FA, while in zone 2 (0.1-1.0 mg/L< FA< 10-150 mg/L) FA inhibits only NOB. Complete nitrification is possible in zone 3 (FA<0.1-1 mg/L and FNA< 0.2-2.8 mg/L). In zone 4, NOB is inhibited by FNA (FNA> 0.2-2.8 mg/L). Because the concentrations of these two forms depend on the solution pH, FA is the main

inhibitor of nitrification at high pH (>8), for lower concentrations of FA than of FNA inhibit the nitrifiers, whereas FNA is the main inhibitor at low pH (<7.5) (Philips et al., 2002).

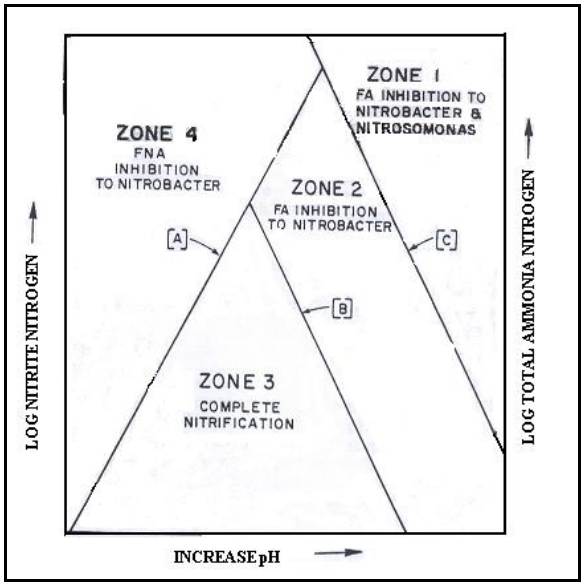


Figure II. 2 Postulated relationships of free ammonia (FA) and free nitrous acid (FNA) inhibition to nitrifying organisms (Anthonisen et al., 1976)

II.2. NITRIFICATION REACTOR SYSTEMS

The principle biological processes used for wastewater treatment can be divided in to main categories: suspended-growth and attached-growth (or biofilm) processes (Metcalf and Eddy, 2004).

II.2.1. Nitrification in Suspended-Growth Reactors

In suspended growth processes, the microorganisms responsible for treatment are maintained in the liquid suspension by appropriate mixing methods. The general types of suspended-growth reactor configurations incorporating nitrification process are plug flow, complete mix and sequencing batch reactors (SBR) (Metcalf and Eddy, 2004). Simplified schematic representations of these systems are presented in Figure II.3

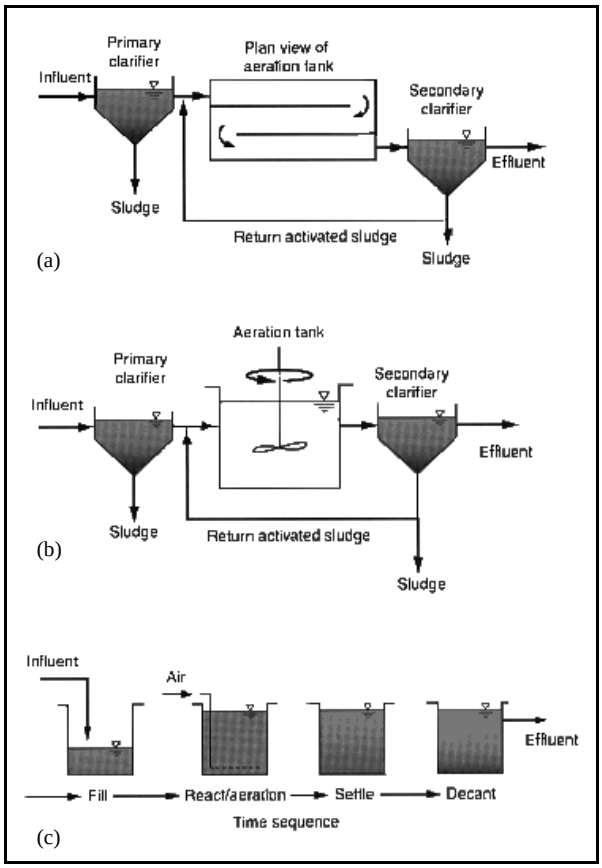


Figure II. 3 Schematic flow diagrams of suspended-growth reactor configurations incorporating nitrification process: (a) plug flow system (b) complete mix system, and (c) sequencing batch reactor (SBR) system (Metcalf and Eddy, 2004)

II.2.1.1. Plug Flow Systems

The plug flow systems (Figure II.3a) involve relatively long, narrow aeration basins, so that the concentration of soluble substances, colloidal and suspended solids (SS) varies along the reactor length. In the systems, fluid particles pass through the reactor with little or no longitudinal mixing and exit from the reactor in the same sequence in which they entered. The particles retain their identity and remain in the reactor for a time equal to the theoretical detention time. Although the process configurations employing long, narrow tanks are commonly referred to as plug flow processes, in reality, true plug flow does not exist. Depending on the type of aeration system, back mixing of the mixed liquor can occur and, depending on the layout of the reactor and the system reaction kinetics, nominal plug flow may be described more appropriately by the series of complete mix reactors shown (Metcalf and Eddy, 2004).

II.2.1.2. Complete Mix Systems

In complete mix systems, which are also known as the continuous-flow stirred-tank reactor (CFSTR) systems, complete mixing occurs instantaneously and uniformly throughout the reactor as fluid particles enter the reactor. Fluid particles leave the reactor in proportion to their statistical population (Metcalf and Eddy, 2004). The complete mix systems (Figure II.3b) provide uniformity of load to all points within the aeration tank, easing the problems of oxygen transfer that can be encountered at the head end of conventional plug-flow plants, and making this configuration attractive for handling organic shock loads. The contents of the complete mix reactor are homogenous and thus the mixed liquor feed to the clarifiers is identical to the mixed liquor through-out the reactor (EPA, 1993).

II.2.1.3. Sequencing Batch Reactor (SBR) Systems

SBR process (Figure II. 3c) utilizes a fill- and- draw reactor with complete mixing during the batch reaction step (after filling) and where the subsequent steps of aeration and clarification occur in the same tank (Metcalf and Eddy, 2004). It is a variation of the activated-sludge process which designed to operate under non-steady state conditions. It differs from activated-sludge plants because of the combination of the all treatment steps and processes in to a single basin or tank, whereas conventional facilities rely on multiple basins (Al-Rekabi et al., 2007). SBR performs biological treatment and secondary clarification in a single tank using a timed control sequence (EPA, 1999).

SBR system enables to tolerate peak flows or loads. In many conventional activated-sludge systems, separate equalization is needed to protect the biological system from peak flows, which may wash out the biomass or peak loads which may upset the treatment process (EPA, 1999). Additionally, SBR produces sludge with good settling properties providing the influent wastewater is admitted into the aeration in a controlled manner. The SBR system can be designed with the ability to treat a wide range of influent volumes whereas the continuous system is based upon a fixed influent flow rate. Thus there is a degree of flexibility associated with working in a time rather than in a space sequence (Al-Rekabi et al., 2007).

All SBR systems have five steps in common, which are carried out in sequence as follows: (1) fill, (2) react (aeration), (3) settle (sedimentation/clarification), (4)

decant (draw), and idle (waste) (Metcalf and Eddy, 2004). Each of these steps is illustrated on Figure II.4 and described in Table II.3.

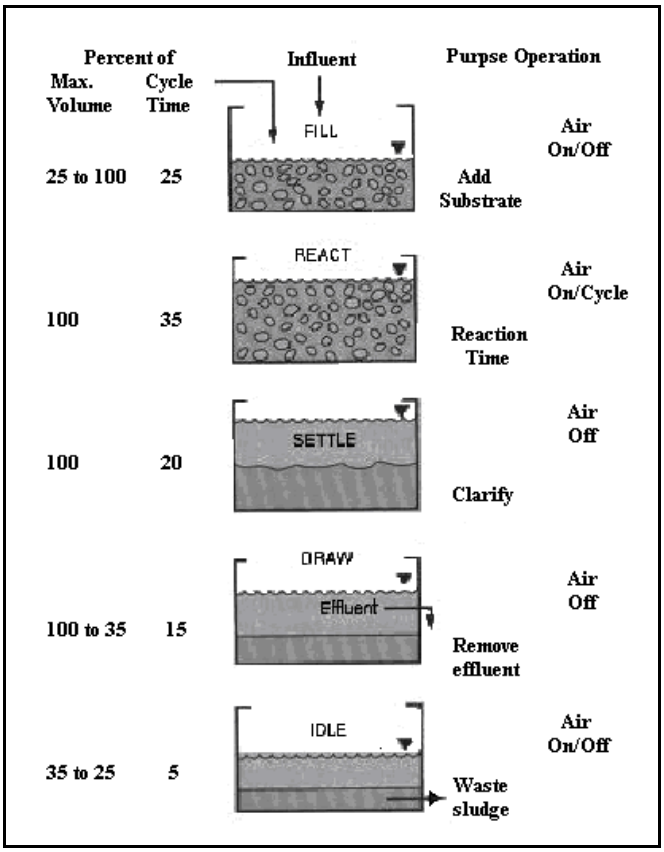


Figure II. 4 Schematic flow diagram of sequencing batch reactor (SBR) systems (Metcalf and Eddy, 2004)

Advantages of SBRs are listed below (EPA, 1999):

- Equalization, primary clarification, biological treatment and secondary clarification can be achieved in a single reactor vessel
- Operating flexibility and control
- Minimal footprint
- Potential capital cost savings by eliminating clarifiers and other equipment

Although SBR systems have many advantages, there are disadvantages such as difficult operating and maintenance because of complicated equipments and its automation (EPA, 1999).

Table II. 3 Description of operational steps for the sequencing batch reactor (SBR) (Metcalf and Eddy, 2004)

Steps	Description
Fill	During the fill operation, volume and substrate (raw wastewater or primary effluent) are added to the reactor. The fill process typically allows the liquid level in the reactor to rise from %75 of capacity (at the end of the waste period) to %100. During fill, the reactor may be mixed only or mixed only or mixed and aerated to promote biological reactions with the influent wastewater.
React	During the react period, biomass consumes the substrate under controlled environmental conditions. Sludge wasting usually occurs during the react phase so that a uniform discharge of solid occurs.
Settle	Solids are allowed to separate from liquid under quiescent conditions resulting in a clarified supernatant that can be discharged as effluent.
Decant	Clarified effluent is removed during decant period. Many types of decanting mechanisms can be used, with the most popular being floating or adjustable weirs.
Idle	The idle period is used in a multi tank system to provide time for one reactor to complete its fill phase before switching to another unit.

II.2.2. Nitrification in Attached-Growth Reactors

Reactors in which growth occurs on or within a solid medium are termed attached-growth, supported growth, or fixed film reactors (EPA, 1993). The reactor involves a solid media to which the bacteria fixed as a matrix called the biofilm, the surface of which is exposed to water passing through the reactor (Arvin and Harremoes, 1990).

A significant process feature of attached-growth process in contrast to activated-sludge treatment is the fact that the performance of biofilm processes is often diffusion-limited. As shown in Figure II.5, a stagnant liquid layer (diffusion layer) separates the biofilm from the bulk liquid that is flowing over the surface of the biofilm or is mixed outside of the fixed film. Substrates, oxygen, and nutrients diffuse across the stagnant liquid layer to the biofilm, and products of biodegradation

from the biofilm enter the bulk liquid after diffusion across the stagnant film (Metcalf and Eddy, 2004).

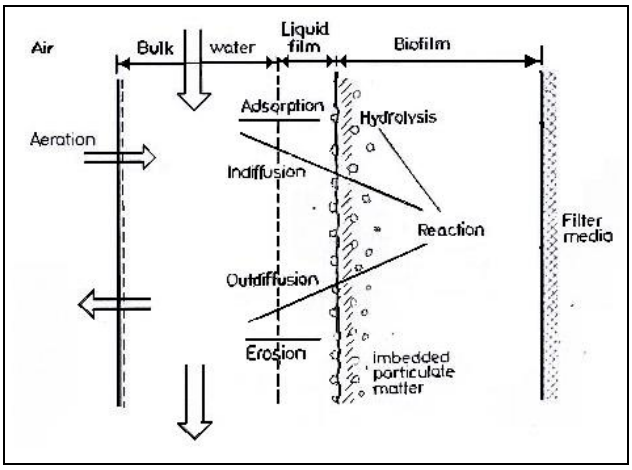


Figure II. 5 Mechanisms in attached-growth systems (Arvin and Harremoes, 1990)

The performance of biofilm reactors heavily depends on whether the substrate can penetrate the biofilm fully or partly. In order for the substrate to move into the film by diffusion there has to be a concentration gradient. If the rate of removal for unit volume is high and the diffusion is slow, the concentration may reach zero and the substrate penetrates the film only partly. Only part of the biofilm is active and the rate of reaction per unit surface is much slower than with a fully penetrated film. It is the bulk concentration that determines the transition from full to part penetration (Arvin and Harremoes, 1990).

The diffusion-limited concept is especially important when considering the measurable bulk liquid DO concentrations on attached-growth process biological reaction rates. Where a DO concentration of 2 to 3 mg/L is generally considered satisfactory for most suspended-growth aerobic process, such low DO concentrations can be limiting for attached-growth process. For uninhibited nitrification in the biofilm a much higher DO concentration may be required depending on the ammonia concentration (Metcalf and Eddy, 2004).

Main advantages of biofilm reactors over suspended growth reactors are as follows (Alpaslan Kocamemi, 2005):

- Simplicity of operation and maintenance
- Capability to remove slowly decomposable substrates
- Slower response and quicker recovery to sudden changes of influent

- Less sensitivity to toxic compounds
- less area requirement
- High effect on low concentration wastewater
- Operation at high sludge ages low cost

Since the autotrophic nitrifying organisms show relatively low growth rates and are more delicate towards environmental conditions than heterotrophic biomass, special attention has to be given to use of biofilm systems for nitrification process (Boller et al., 1994).

Attached-growth processes can be grouped into five classes: (1) Trickling filters (TF), (2) Rotating biological contactors (RBC), (3) Fluidized bed reactors (FBR), (4) Hybrid biofilm/suspended growth processes and, (5) Submerged fixed-film systems (Metcalf and Eddy, 2004).

II.2.2.1. Trickling Filters (TF)

Trickling filters (TF) are non-submerged fixed-film biological reactor using rock or plastic packing over which wastewater is distributed continuously. Treatment occurs as the liquid flows over the attached biofilm (Metcalf and Eddy, 2004). The wastewater is applied in a "trickling" or three-phase condition in which the water (first phase) moves downward along the surfaces of an unsaturated porous medium (second phase). Air moves upward or downward as a third phase for the purpose of supplying oxygen (Rittmann and McCarty, 2001). Rock filter designs have been classified as low- or standard-rate, intermediate-rate and high-rate. Plastic packing is used typically for high-rate designs; however, plastic packing has also been used at lower organic loadings, near the high end of those used for intermediate-rate rock filters. Both biochemical oxygen demand (BOD) removal and nitrification can be accomplished in rock or plastic packing trickling filters operated at low organic loadings. Heterotrophic bacteria, with higher yield coefficients and faster growth rates, are more competitive than nitrifying bacteria for space on the fixed-film packing. Thus, significant nitrification occurs only after BOD concentration is appreciably reduced. It was demonstrated that the effluent BOD had to be less than 30 mg/L to initiate nitrification and less than 15 mg/L for complete nitrification (Metcalf and Eddy, 2004).

II.2.2.2. Rotating Biological Contactors (RBC)

A rotating biological contactor (RBC) consists of a series of closely spaced circular disks of polystyrene or polyvinyl chloride that are submerged in wastewater and rotated through it. The cylindrical plastic disks are attached to a horizontal shaft (Metcalf and Eddy, 2004). The rotation causes turbulent mixing, circulation, and aeration of the liquid contents in the trough. Probably even more important is that the biofilm and its adhering water layer are exposed to oxygen when they are above the water level. The major of oxygen transfer to the biofilm is attributable to the above-water exposure (Rittmann and McCarty, 2001). Treatment systems employing RBCs have been used for BOD removal, pretreatment of industrial waste water, combined BOD removal and nitrification, tertiary nitrification, and denitrification. As for trickling filters, the organic loading affects BOD removal efficiency and the nitrogen loading after a minimal BOD concentration is reached affects the nitrification efficiency (Metcalf and Eddy, 2004).

II.2.2.3. Fluidized Bed Reactors (FBR)

In these systems, the biomass carrier material is suspended by the up flowing water and aerated by compressed air or pure oxygen (Boller et al., 1994). Fluidized beds work when the density of the carrier particles is greater than that of water. Carrier particles can range from sand or glass beads, which are much heavier than water, to coal or activated-carbon particles, which are only slightly heavier than water. Fluidized beds offer reduce volumes, due to their high specific surface area. Liquid detention times can be as low a few minutes. The size advantage of fluidized beds generally is limited by the ability to transfer oxygen to the water and the biofilm. Thus, surface loads for fluidized beds may be somewhat lower than for the other biofilm systems. As a consequence, volumetric loads probably cannot be increased in direct proportion to the increase in specific surface area. However, the short liquid detention times of fluidized beds are particularly advantageous for aerobic treatment of low concentrations of contaminants, for which the oxygen demand is relatively low (Rittmann and McCarty, 2001).

II.2.2.4. Hybrid Biofilm/Suspended Growth Processes

Activated-sludge processes can be enhanced in terms of capacity and reliability through the introduction of biofilm media that create hybrid biofilm/suspended-

growth systems. Existing activated-sludge processes can be upgraded by adding biofilm surface area inside the aeration basin. Immobile biofilms include plastic screen, ribbons, or lace strings that are held inside the aeration basin by fixed frames immersed in the mixed liquor. Mobile biofilm carriers are mixed into and travel with the mixed liquor. They include sponges, plastic mesh-cubes or cylinders, porous cellulosic pellets, or polyethylene glycol pellets. The latter can be imbedded with bacteria, although this is not necessary to achieve the mobile- biofilm effect. The mobile biofilm carriers must be easily separated from the mixed liquor and held in the aeration basin (Rittmann and McCarty, 2001). Whether immobile or mobile biofilm carriers are employed, the goal is to increase the total mass of active bacteria in the system and to increase the sludge retention times (SRT). The suspended and biofilm bacteria easily can have quite different SRTs. Normally, the biofilm SRT is longer, which makes it especially important for accumulating slow-growing species, such as, nitrifiers (Rittmann and McCarty, 2001).

II.2.2.5. Submerged Fixed-Film Systems

Aerobic submerged fixed-film processes consist of there phases: a packing, biofilm and liquid. The BOD and/or NH_4^+-N removed from the liquid flowing past the biofilm is oxidized. Oxygen is supplied by diffused aeration into the packing or by being pre-dissolved into the influent wastewater. Aerobic fixed-film processes include down-flow packed-bed reactors, up-flow packed-bed reactors, and up- flow fluidized-bed reactors. The type and size of packing is a major factor that affects the performance and operating characteristics of submerged attached growth processes. Designs differ by their packing configuration and inlet and outlet flow distribution and collection. No clarification is used with aerobic submerged attached-growth processes, and excess solids from biomass growth and influent SS are trapped in the system and must be periodically removed. The major advantages of submerged attached-growth processes are their relatively small space requirement, the ability to effectively treat dilute waste waters, no sludge settling issues as in activated- sludge process, and aesthetics (Metcalf and Eddy, 2004).

II.3. NOVEL NITROGEN REMOVAL TECHNOLOGIES

In recent years, many researches have been performed in order to develop novel biological treatment processes, which allow the minimization of energy and

chemical consumption in nitrogen removal. These novel biological processes are as follows (Jetten et al., 2002; Khin and Annachhatre, 2004; Schmidt et al., 2003; Verstraete, et al., 1998; Villaverde, et al., 2004): Partial nitrification-denitrification via nitrite, SHARON (**S**ingle reaction system for **H**igh **A**mmonium **R**emoval **O**ver **N**itrite), ANAMMOX (**AN**aerobic **AMM**onium **OX**idation), CANON (**C**ompletely **A**utotrophic **N**itrogen removal **O**ver **N**itrite), and OLAND (**O**xygen **L**imited **A**utotrophic **N**itrification **D**enitrification) processes.

In partial nitrification-denitrification process (Figure II.6), NH_4^+-N is oxidized to NO_2^--N but not to NO_3^--N and denitrification occurs via NO_2^--N route (Ciudad et al., 2005; Schmidt et al., 2003). This process allows not only 25% of O_2 saving with respect to conventional nitrification-denitrification process and but also approximately 40% reduces the external carbon addition necessary for the denitrification of wastewater containing low C/N ratio (Bae et al., 2002; Bernet et al., 2005; Villaverde, 2004; Volcke et al., 2002). Additionally, the sludge production in the partial nitrification-denitrification via nitrite process is about 30% less than that of conventional nitrification-denitrification process (van Kempen et al., 2005).

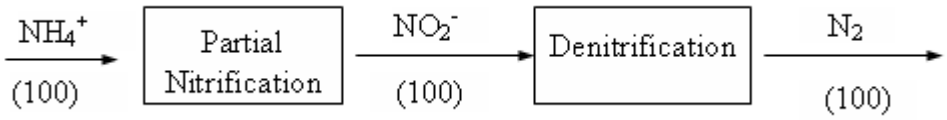


Figure II. 6 Schematic flow diagram of partial nitrification-denitrification process

SHARON process (Figure II.7) was developed for the treatment of sludge digester supernatant containing high ammonium. It is a partial nitrification technique which is applied with denitrification process in intermittently aerated chemostat systems at high temperature. It is based on the principle that at elevated temperatures (30-35^{0}C), NOB grows slower than AOB (Hellinga, 1998). For the waste streams with high NH_4^+-N concentration (> 500 mg/l) (e.g., sludge digester supernatant), this process can be operated as partial nitritification process without denitrification and allowing adequate 50% NO_2^--N formation necessary for the Anammox process (Ahn, 2006).

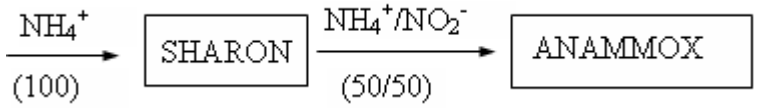


Figure II. 7 Schematic flow diagram of SHARON process

The ANAMMOX process (Figure II.8) is a lithoautotrophic biological conversion process, mediated by a group of *Planctomycete* bacteria, which reduces NH_4^+-N to N_2 gas by using NO_2^--N as an electron acceptor (Ahn, 2006; Jetten et al., 2002; Khin and Annachhatre, 2004; Villaverde, 2004). In the treatment of wastewaters with Anammox process, it is necessary to apply partial nitritification process prior to Anammox, which requires 50% NH_4^+-N-50% NO_2^--N in the influent.

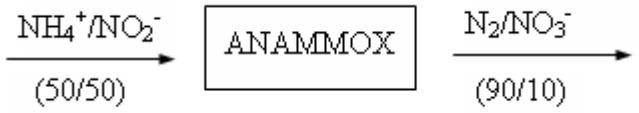


Figure II. 8 Schematic flow diagram of ANAMMOX process

CANON process (Figure II.9) is also the combination of partial nitritification and Anammox. However, this process performs two sequential reactions in a single reactor under oxygen-limited conditions, implying that the two groups of bacteria (*Nitrosomonas*-like aerobic microorganisms and *Planctomycete*-like anaerobic bacteria) cooperate in the whole process (Ahn, 2006).

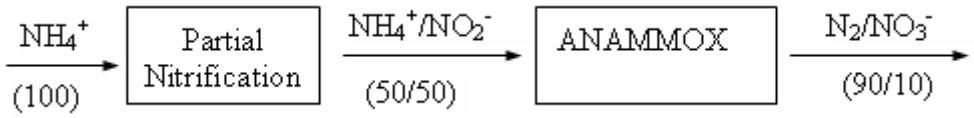


Figure II. 9 Schematic flow diagram of CANON process

OLAND process (Figure II.10) makes use of the denitrification activity of some conventional aerobic autotrophic nitrifiers under oxygen-limited conditions. In this process, ammonium oxidizers first oxidize 50% NH_4^+-N to NO_2^--N and then they reduce the remaining 50% NH_4^+-N to N_2 gas by using NO_2^--N as an electron acceptor (Ahn, 2006; Verstraete and Philips, 1998).

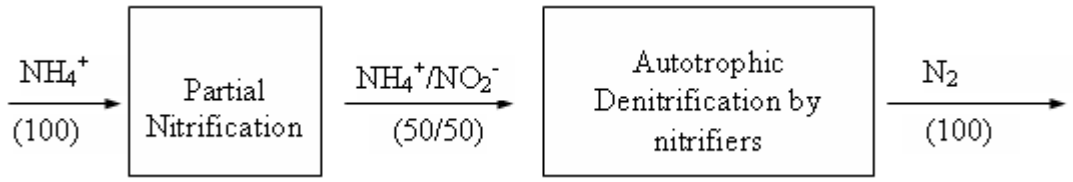


Figure II. 10 Schematic flow diagram of OLAND process

The above-summarized novel biological ammonium removal systems, 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, agro industry wastewater). The applicability of these processes for the removal of nitrogen from domestic wastewater necessitates the detailed investigation of the maintaining stable partial nitrification or partial nitritification via inhibiting nitrite oxidation to nitrate in nitrification process.

II.4. PARTIAL NITRIFICATION/NITRITIFICATION

As discussed in Section II.3, in recent years, several novel and cost-effective biological nitrogen removal processes have been developed. Those processes are mainly based on partial nitrification/nitritification process which entails the oxidation of NH_4^+ to NO_2^- (completely or partially), but not NO_3^-. However, NO_2^- normally does not accumulate in large amounts in biological treatment systems under steady-state conditions. Since the maximum growth rate of NOB is considerably higher than the maximum growth rate of AOB at temperatures below 20^oC (EPA, 1993). To achieve partial nitrification, it is necessary to selectively reduce the activity of the NOB without affecting the AOB (Ruiz et al., 2006). One powerful tool to achieve partial nitrification/nitritification is biochemical selection by inhibition of NO_2^--N oxidation. Some operational parameters, such as temperature, pH, FA, sludge age, and DO may produce partial nitrification/nitritification and inhibition of NO_2^--N oxidation to NO_3^--N. These operational parameters reported to allow NO_2^--N accumulation are discussed here after.

II.4.1. Influence of Temperature on Partial Nitrification/Nitritification

At normal temperatures in wastewater treatment plants (5-20^oC), NOB grow faster than AOB which means that NH_4^+ is completely oxidized to NO_2^- (Hellinga et al., 1998). Therefore, in wastewater treatment plants operated under optimum

conditions, NO_2^--N accumulation is not commonly observed and NH_4^+-N can be converted to NO_3^--N completely. At temperatures higher than 25°C however, the maximum specific growth rate of AOB is in the same range or higher than that of NOB, possibly leading to NO_2^--N accumulation. The Sharon process (Section II.3) makes use of this property to wash out the NOB. According to Eqn. (II.11), an increase in temperature causes an increase in FA, which is more inhibitory to NOB. For NOB the inhibition of the higher NH_3 concentrations at higher temperatures outweighs the activity stimulant allowing nitrite accumulations of 80% (Philips et al., 2002).

II.4.2. Influence of pH on Partial Nitrification/Nitritification

Different authors have reported that pH optimum for nitrification ranged between 7.0 and 8.0. For *Nitrosomonas*, the optimum pH varies from 7.9 to 8.2, whereas it ranges from 7.2 to 7.6 for *Nitrobacter* (Villaverde et al.,1997).

Regarding the optimum pH ranges for nitrification, three different effects of the pH on the nitrifying bacteria have been identified: activation-deactivation of nitrifying bacteria; nutritional effects, associated with the alkalinity and the species of inorganic carbon, and inhibition through FA and FNA. Activation-deactivation of nitrifying bacteria is linked to the binding of H^+ or OH^- ions to the weak basic groups of the enzymes, blocking the active sites in a reversible way. Nutritional effects are mainly associated with the availability of the mineral carbon, which is required as a carbon source for the nitrifying autotrophic microorganisms. At a low pH, the predominant carbon dioxide (CO_2) species can be easily removed from water by stripping. On the other hand, at high pH values, mineral carbon will mainly be present in the carbonate species, which can barely be assimilated. At higher pH values, FA increases. In an opposite way, nitrous acid concentration arises at a low pH (Parades et al., 2007). Although these three effects occur simultaneously FA inhibition is considered the most important (Villaverde et al., 1997).

Based on the results reported by Anthonisen et al. (1976), NOB are more sensitive than AOB to FA and FNA. As a consequence, if pH in the reactor is increased (higher FA) or lowered (higher FNA), NOB inhibition will occur and hence nitrite accumulation will be favored (Bernet et al., 2005).

II.4.3. Influence of Free Ammonia (FA)/ Free Nitrous Acid (FNA) on Partial Nitrification/Nitritification

Selective inhibition of FA on NOB usually occurs at 0.1 to 10 mg NH_3-N /L, while AOB are inhibited at 10-150 mg N/L. FNA is also known to inhibit NOB at 0.22-2.8 mg N/L (Kim et al., 2005). The inhibiting effect of FA on NOB may be the result of a combination of several factors such as initial NH_4^+-N concentration, pH and temperature. Notwithstanding the severe effects of high FA on NO_2^--N accumulation, in many researches continuous operation over long period of time under inhibitory FA concentrations, an acclimation of NOB to FA has been observed (Antileo et al., 2006; Anthonisen et al., 1976; Bae et al., 2002; Bernet et al., 2005; Jianlong and Ning, 2004; Kim et al., 2005; Parades et al., 2007; Philips et al., 2002). NOB has been found to be capable of acclimating to FA levels as high as 40 mg NH_3-N /L (Philips et al., 2002).

II.4.4. Influence of Sludge Age on Partial Nitrification/Nitritification

Partial nitrification to NO_2^- can be accomplished by controlling sludge age. In the study of Pollice et al. (2002), it was demonstrated that under non-limited oxygen conditions it is possible to obtain partial nitrification to NO_2^--N by operating at low sludge ages. NH_4^+ oxidation to NO_2^- was successfully obtained for SRTs around 10 day, although a certain instability was observed which turned into NO_3^- formation as soon as the sludge age slightly increased (Pollice et al., 2002). At the operational level, sludge age can not be easier to control. Additionally, very short sludge ages may result in biomass washout (Parades et al., 2007). Therefore, the possibility of converting NH_4^+-N in to NO_2^--N by modifying sludge age is not an attractive way to achieve NO_2^--N accumulation.

II.4.5. Influence of Dissolved Oxygen (DO) on Partial Nitrification/ Nitritification

DO concentration is an important factor for nitrification (Stenstrom and Poduska, 1980). Variation of the DO concentration is a possible way for enhancing the NO_2^- accumulation (Parades et al., 2007), which is due to higher oxygen half-saturation coefficients values of NOB (around 1.1 mg/L) than that of AOB (around 0.3 mg/L) (Jianlong and Ning, 2004; Kim et al. 2005). Based on oxygen half-saturation coefficients, NOB have a lower affinity for oxygen than the AOB;

therefore a low DO concentration is restrictive for the growth of NOB (Khin and Annachhatre, 2004).

A possible mechanism for the inhibition of NO_2^--N oxidation by a low DO concentration is based on the accumulation of hydroxylamine (NH_2OH), which is an intermediate product of the NH_4^+ oxidation. In general, obligatory AOB gain their energy by oxidizing NH_4^+ to NO_2^- in a two-step reaction with NH_2OH as an intermediate. The first step is the oxidation of NH_4^+, catalyzed by ammonia monooxygenase, whereas the second step is the oxidation of NH_2OH, catalyzed by hydroxylamine oxidoreductase (Parades et al., 2007). Under low DO concentration and high NH_4^+ concentration, NH_2OH can be accumulated. NH_2OH causes an inhibition, although NOB at values of around 250 μM, and values above 2000 μM even inhibit the AOB. However, in wastewater treatment, NH_2OH is typically ignored in nitrification processes due to an implicit assumption that it will be available in significant levels (Yang and Alleman, 1992).

Various investigations into controlling partial nitrification through DO have been carried out, including suspended and biofilm reactor systems (Parades et al., 2007). The results obtained from those studies are summarized in Table II.4. For suspended-growth biomass systems under limited DO supply, complete and stable conversion of NH_4^+ into NO_2^- was obtained, independent of the sludge age. However, when there was no oxygen limitation, the sludge age became the critical parameter for partial nitrification. In the case of biofilm systems, the results were similar to those obtained with suspended biomass, showing that low dissolved oxygen concentration led to NO_2^- accumulation, but the suggested mechanisms are different (Parades et al., 2007). With a biofilm airlift reactor, Kim et al. (2003) observed a stable nitrite accumulation (95%) for more than 200 days owning to a selective inhibition by FA (0.2 mg N/L) and oxygen limitation (1-2 mg/L). In addition, they noted that a stable 100 % NO_2^- accumulation could also be obtained at low DO concentrations. However, the NO_2^- oxidation activity was recovered as soon as the FA concentration was below the threshold level provided the DO concentration was not the limiting factor. Further Fluoresecence In Situ Hybridization (FISH) analysis showed that densely packed AOB were located outside the biofilm. In contrast, small clusters of NOB were surrounded by AOB and found in the deeper layer of the biofilm. The spatial distributions indicated that NOB

oxidizers are more exposed to oxygen limiting conditions than AOB (Parades et al., 2007).

Table II. 4 Effect of dissolved oxygen (DO) concentration on the nitrification processes (Parades et al., 2007)

System	DO (mg/L)	Effect
Suspended growth	0.5	Inhibition of NO_2^- oxidation and its accumulation
	6.0	Full nitrification
Activated sludge	<0.5	NH_4^+ and NO_2^- accumulation
	0.7	NO_2^- accumulation up to 67 % of the applied NH_4^+
	1.0	80 % oxidation of NH_4^+, 80 % oxidation of NO_2^-
	1.4	99 % oxidation of NH_4^+, 70 % oxidation of NO_2^-
	>1.7	Full nitrification
	2.4	99 % oxidation of applied NH_4^+, 10 % as NO_2^-
Biofilm airlift reactor	1.0	Stable and 100% NO_2^- accumulation
	<1.0	Low NH_4^+conversion and low NO_2^- and NO_3^- accumulation
	1.5	50% of NH_4^+ conversion to NO_2^-
	>2.5	Full nitrification, NH_4^+ oxidation depend on applied NH_4^+ load
Biological aerated filter	2.0-5.0	NO_2^- accumulation up to 60% of total NH_3 conversion
Completely stirred biofilm reactor	0.5	Stable NO_2^- accumulation (90%) and 100% NH_4^+ removal
	>0.5	Increasing dissolved oxygen increases NO_3^- concentration in the effluent. A further reduction produces NO_2^- accumulation again
Moving bed biofilm reactor	3.0	50% of NH_4^+ conversion to NO_2^-. After 11 months full nitrification took place

CHAPTER III

THE STUDY

III.1. SYNTHETIC WASTEWATER COMPOSITION

All experiments were performed with the synthetic wastewater simulating the characteristics of actual domestic wastewater without organic carbon. The composition of the synthetic wastewater including substrate, alkalinity, phosphate buffer and mineral solutions is given in Table III.1. All stock chemical solutions given in Table III.1 were prepared by dissolving chemicals supplied from Merck KGaA, Germany in deionised water.

Table III. 1 Composition of the synthetic wastewater

Compound	Stock concentration (g/L)	Volume added to	
		Final volume of 10L in continuous-flow experiments (ml)	Final volume of 300 ml in oxygen uptake rate experiments (ml)
Substrate			
$(NH_4)_2SO_4$	132.1	28	0.01-0.8([a])
$NaNO_2$	64	-	0.01-1.71 ([b])
Alkalinity and phosphate buffer			
$NaHCO_3$	84	112	0.9-3.21([c])
K_2HPO_4	136	175	5.25-6.38([c])
KH_2PO_4	174	90	2.7-4.05([c])
Mineral medium			
$MgSO_4.7H_2O$	4	25	0.75-1.3([c])
$CaCO_3$	0.2		
$MnSO_4.H_2O$	0.4		
K_2HPO_4	0.6		
$FeSO_4.7H_2O$	0.8		

([a]) 1 M stock $(NH_4)_2SO_4$ solution was diluted to the desired NH_4^+-N concentrations in the experiments
([b]) 1 M stock $NaNO_2$ solution was diluted to the desired NO_2^--N concentrations in the experiments
([c]) Adjusted proportional with the studied NH_4^+-N and NO_2^--N concentrations

III.2. SEQUENCING BATCH REACTOR (SBR) SET-UP AND OPERATION STRATEGY

The experimental set-up of SBR system is shown in Figure III.1. A cylindrical plexiglas reactor (15.5 cm diameter and 28 cm height) was used with a working volume of 3 L. The reactor was inoculated with the sludge which was taken from the sludge recycle line of Paşaköy Advanced Biological Wastewater Treatment Plant and was enriched for nitrifiers by feeding with only inorganic ammonium and necessary minerals since 2002 (Alpaslan Kocamemi, 2005).

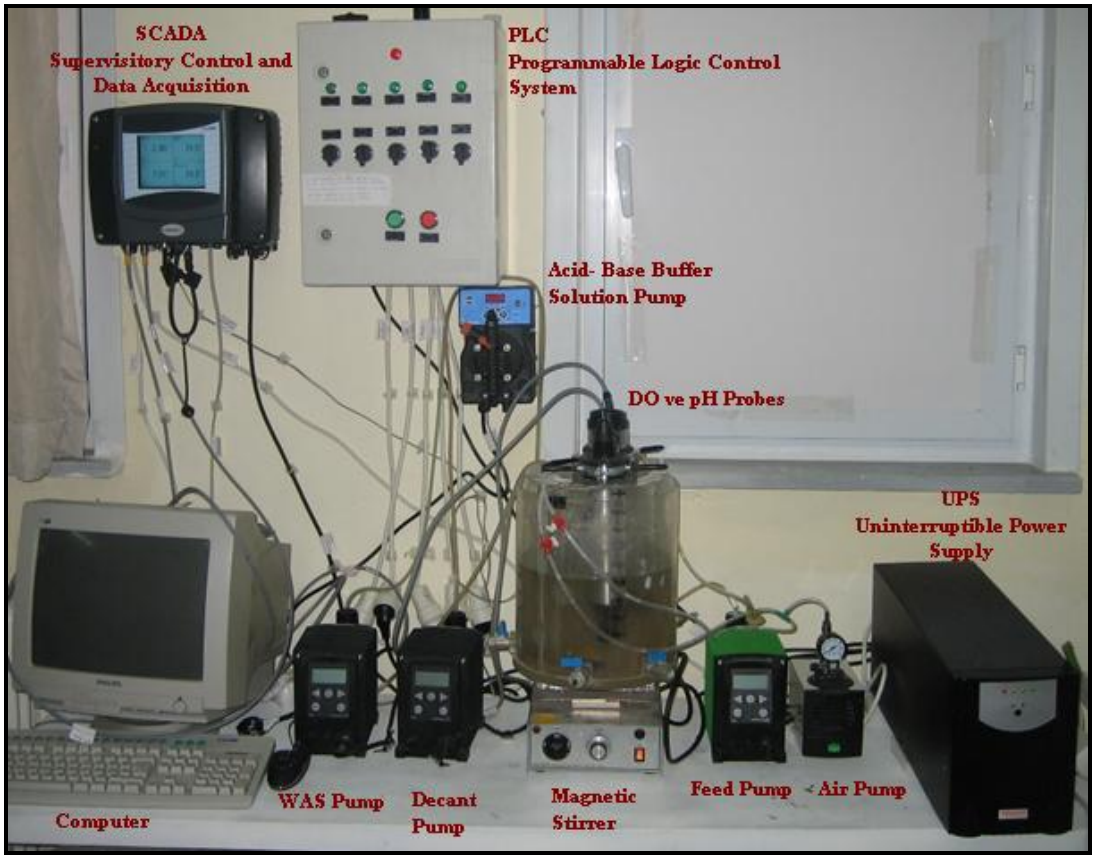


Figure III. 1 Experimental set-up of the SBR system

Complete mixing in the reactor was maintained with a magnetic stirrer (Velp Are). At low DO concentrations (≤2 mg/L) the mixing inside the reactor was achieved with a overhead mechanical stirrer (Daihan Wisestir® HS50A). The SBR system was operated at a fixed wastewater temperature of around 25°C by controlling the room temperature with an air-conditioner. Required DO concentration in the reactor was maintained by supplying air through diffusers placed to the bottom of reactor and connected to an air compressor (KNF N86 KN18).

Throughout the study, the SBR system was operated continuously at 24 hours batch cycles. The cycles were automated with a PLC (Programmable Logic Control) system (Siemens LOGO DM8230R). Each cycle comprised of five sequential phases: fill, react, waste, settle, and decant. Time schedule for all phases and the "on-line" and "off-line" equipment in each phase are summarized in Table III.2. All mechanical equipment listed in Table III.2 were connected to an uninterruptable power supply (UPS) (Pegasus 1200C) to maintain operation of the system during electricity cut off periods. Operation strategy of the system is also schematically shown in Figure III.2.

Table III. 2 Operational conditions of the SBR system

OPERATION PHASE	Fill	React	Waste	Settle	Decant
DURATION (min)	225	945	15	30	210
EQUIPMENT (On/Off- On-line/Off-line)					
Feed Pump	On	Off	Off	Off	Off
Waste Pump	Off	Off	On	Off	Off
Decant Pump	Off	Off	Off	Off	On
Acid-Base Dosing Pump	Off	Off	Off	Off	Off
Air Pump	Off	On	On	Off	Off
Magnetic Stirrer/ Over Head Stirrer	Off	On	On	Off	Off
SCADA System	Off-line	On-line	On-line	Off-line	Off-line

During the fill phase (225 min), the reactor was continuously fed with the synthetic wastewater described in Table III.1 by a diaphragm dosing pump (Grundfos DME 2-18) with a flow rate of 10 ml/min. In the react phase (945 min), DO concentration in the reactor was kept constant at the desired level which was predefined in the Supervisory Control and Data Acquisition (SCADA) system (Hach-Lange SC1000). The SCADA system continuously compared the on-line measured DO values in the reactor with the pre-defined DO level and sent on/off signal to the air compressor (KNF N86 KN18) for intermittent supply of air as required. A DO probe (Hach-Lange LDO) and a pH probe (Hach-Lange pHD-S sc) fitted inside the reactor were provided continuous monitoring of DO, pH,

Figure III. 2 Operation strategy of the SBR system

temperature values with 15 min. intervals during this period. The continuously measured data was logged into the SCADA system. During the waste phase (15 min), about 150 ml of the mixed reactor content was wasted by a diaphragm dosing pump (Grundfos DME 2-18) with a flow rate of 10 ml/min to keep the sludge retention time in the range of 13-15 days. Following to the sludge wasting, the biomass was allowed to settle for 30 min. Finally, in the decant phase (210 min) the supernatant liquid (about 70% of the working volume) was decanted from the reactor by a diaphragm dosing pump (Grundfos DME 2-18) with a flow rate of 10 ml/min.

III.2.1. Continuous-Flow Experiments in the Sequencing Batch Reactor (SBR) System

Continuous-flow experiments in the SBR system were performed to investigate the effects of various DO levels on NO_2^--N accumulation in a suspended-growth system with an enriched nitrifier culture. For this purpose, a SBR system, described Section III.2., was seeded with an enriched nitrifier culture and initially started-up at DO level of 7 mg/L. The initial mixed liquor volatile suspended solids (MLVSS) concentration of the reactor was 258 mg/L. The system was operated continuously at 24 hours batch cycles about 365 days for DO levels decreasing gradually from 7 mg/L to 0.22 mg/L. Table III.3 summarizes the operating conditions in all consecutive runs performed throughout the study.

As seen from the Table III.1, in all runs, the influent NH_4^+-N level was kept at a constant level of around 72 mg/L**.** Depending on the influent NH_4-N, temperature and pH values, at the start of each batch cycle FA was around 0.7-1.1 mg/L. As mentioned in Section III.2., the SBR system decanted 70% of the working volume at the end of each batch cycle. Therefore, at the beginning of each cycle the influent NH_4^+-N was diluted with the 30% of the reactor content that was remained in the reactor from the previous cycle. pH in the reactor was maintained around 7.4 with phosphate buffer in the influent. Wastewater temperature was around 25^oC. In each run, effluent decanted from the reactor at the end of each 24 hours batch cycle was collected in an effluent collection tank. Daily or 2-4 days composite effluent samples were taken from the effluent collection tank. Those samples were filtered by using 0.45 μm filters (Millipore Millex HV) in order to separate the liquid from the solids that might escape from the reactor during the decant phase. All samples were analyzed for NH_4^+-N, NO_2^--N, NO_3^--N as described in Section III.5.

Table III. 3 Operating conditions of continuous-flow SBR experiments

Run No.	Operation Period (days)	Working Volume (L)	Feed flowrate (L/d)	Feed NH_4^+-N concentration (mg/L)	DO level in the reactor (mg/L)
SBR Run No: 1	0- 96	3	2.1	72.6	7.06±0.04
SBR Run No: 2	97-140	3	2.1	72.7	4.19±0.09
SBR Run No: 3	141-168	3	2.1	73.2	3.18±0.14
SBR Run No: 4	169-243	3	2.1	72.8	2.20±0.12
SBR Run No: 5	244-299	3	2.1	72.9	1.27±0.17
SBR Run No: 6	300-323	3	2.1	71.9	1.00±0.20
SBR Run No: 7	324-355	3	2.1	71.0	0.54±0.04
SBR Run No: 8	356-365	3	2.1	73.8	0.22±0.01

III.2.2. Determination of Initial Substrate/Biomass (S_o/X_o) Ratio Reflecting Extant Kinetics

During the continuous-flow operation of the SBR system at various DO levels for a time period of 365 days, batch NH_4-N utilization rate and oxygen uptake rate experiments described in Sections III.2.3 and III.2.4 were periodically performed for the kinetic analyses of the enriched nitrifier culture in the SBR system The aim of those experiments were to find the kinetic parameters representative for the existing condition of the biomass in the SBR system (i.e. extant kinetic parameters rather than the intrinsic ones). Determination of extant or intrinsic kinetic parameters heavily depends on the initial substrate to biomass ratio (S_o/X_o) (Grady et al., 1996). Therefore, prior to the batch kinetic experiments, a set of preliminary initial NH_4^+-N utilization rate experiments were performed in order to determine the S_o/X_o ratio reflecting extant kinetic conditions. For this purpose, sludge samples from the continuous flow SBR system were either diluted or concentrated in order to maintain S_o/X_o ratio ranging between 0.07-0.45 for a constant initial NH_4^+-N (S_o) level of 40-50 mg/L. Initial rate experiments were then performed with those sludge samples at a constant temperature of 25°C. pH and DO levels were maintained around 7.4 and 8.5, respectively. During the experiments, NH_4^+-N measurements were periodically performed. MLVSS concentration was measured at the beginning of each

experiments for the calculation of specific NH_4^+-N utilization rates (q_{NH4-N}), measured NH_4^+-N values were plotted against time and the slope of those graphs obtained through linear regression analysis were divided to the present MLVSS values.

III.2.3. Batch Ammonium Utilization Rate Experiments

These experiments were performed in order to investigate the specific NH_4^+-N utilization rate (q_{NH4-N}) of the enriched nitrifier culture which were exposed to gradually decreasing DO levels during prolonged time continuous operation of the SBR system. For this purpose, during each continuous run of the SBR system (Section III.2.1), at certain days, initial rate experiments were performed inside the SBR system in Section III.2.1. The DO and pH probes fitted inside the SBR system provided continuous monitoring of those operational conditions in every 15 minutes intervals during these experiments. Each experiment was performed for S_o/X_o ratio less than 0.2. In each experiment, samples were taken from the SBR with intervals of 10-15 minutes at the beginning of the react period during which unlimited substrate (NH_4^+-N) conditions prevail. These samples were filtered by using 0.45 μm filters (Millipore Millex HV) and analyzed for NH_4^+-N. In all experiments, MLVSS concentration in the SBR was measured at the beginning of the experiments. It was considered that MLVSS changed insignificantly throughout the experiments because the experiments were performed over a short time period (about 1-2 hr) with too small initial NH_4^+-N/MLVSS (S_o/X_o) ratio. For the calculation of specific NH_4^+-N utilization rates (q_{NH4-N}), measured NH_4^+-N values were plotted against time and the slope of those graphs obtained through linear regression analysis were divided to the present MLVSS values.

III.2.4. Batch Oxygen Uptake Rate (OUR) Experiments

The aim of these experiments was to provide kinetic parameter estimates for AOB and NOB species which were exposed to gradually decreasing DO levels during prolonged time continuous operation of the SBR system. For this purpose, during the continuous-flow operation of the SBR system, OUR experiments were performed at certain time intervals to estimate maximum specific oxygen uptake rate and oxygen half saturation constants of AOB ($SOUR_{max,AOB,}$ $K_{o,AOB}$) and NOB ($SOUR_{max,NOB,}$ $K_{o,NOB}$) species in the SBR system .

All OUR experiments were performed in a 300 ml capped glass bottle without headspace. Complete mixing was provided by means of magnetic stirring. Wastewater temperature was kept around 25°C by controlling the room temperature with an air conditioner. pH was maintained around 7.3 with phosphate buffer. In each experiment, the sludge taken from the SBR was washed twice with deionized water to remove residual nitrogen species in the sample. The washed sludge sample was then either diluted or concentrated as required to maintain S_o/X_o ratio less than 0.2 in all experiments. In each experiment the sludge sample from the SBR was dosed with the synthetic wastewater (Section III.1) such that the initial substrate concentration (NH_4^+-N for the case of $K_{o,AOB}$ determination; NO_2^--N for the case of $K_{o,NOB}$ determination) was around 40-50 mg/L. The mixture was initially saturated to the DO level of around 8 mg/L and placed into the test bottle without headspace. During the experimental period, DO drops in the test bottle were recorded by Hach-Lange HQ40 DO meter with 15 sec. intervals. The test was ended when the DO in the bottle was fully exhausted (i.e. reached 0 mg/L). The MLVSS concentration in the test bottle was determined at the end of test period. The recorded DO values were plotted against time and the graph was divided into linear regions with respect to bulk DO concentrations. The slope of each linear region (OUR) obtained through linear regression analysis was divided to the present MLVSS value to obtain SOUR values. The SOUR values were then plotted against the corresponding bulk DO levels and this graph was analyzed by non-linear least square regression (NLSR) using EZ-FIT5 package program of Perella Scientific Inc.

III.3. BIOFILM REACTOR SET-UP AND OPERATION STRATEGY

The experimental set-up of the upflow aerated, submerged packed bed nitrifying biofilm system is shown in Figure III.3. The reactor was started up in 1994 using a mixed culture from a plant treating meat processing wastewaters and was used in different studies until 1998 (Çeçen, 1996; Çeçen and Orak, 1996; Çeçen and İpek, 1998; Orak, 1994). Between the years 1998-2004, it was fed with only synthetic ammonium solution and hence enriched for nitrifiers (Alpaslan Kocamemi, 2005). In year 2004, it was used in another study for 1 year (Alpaslan Kocamemi, 2005; Alpaslan Kocamemi and Çeçen, 2007).

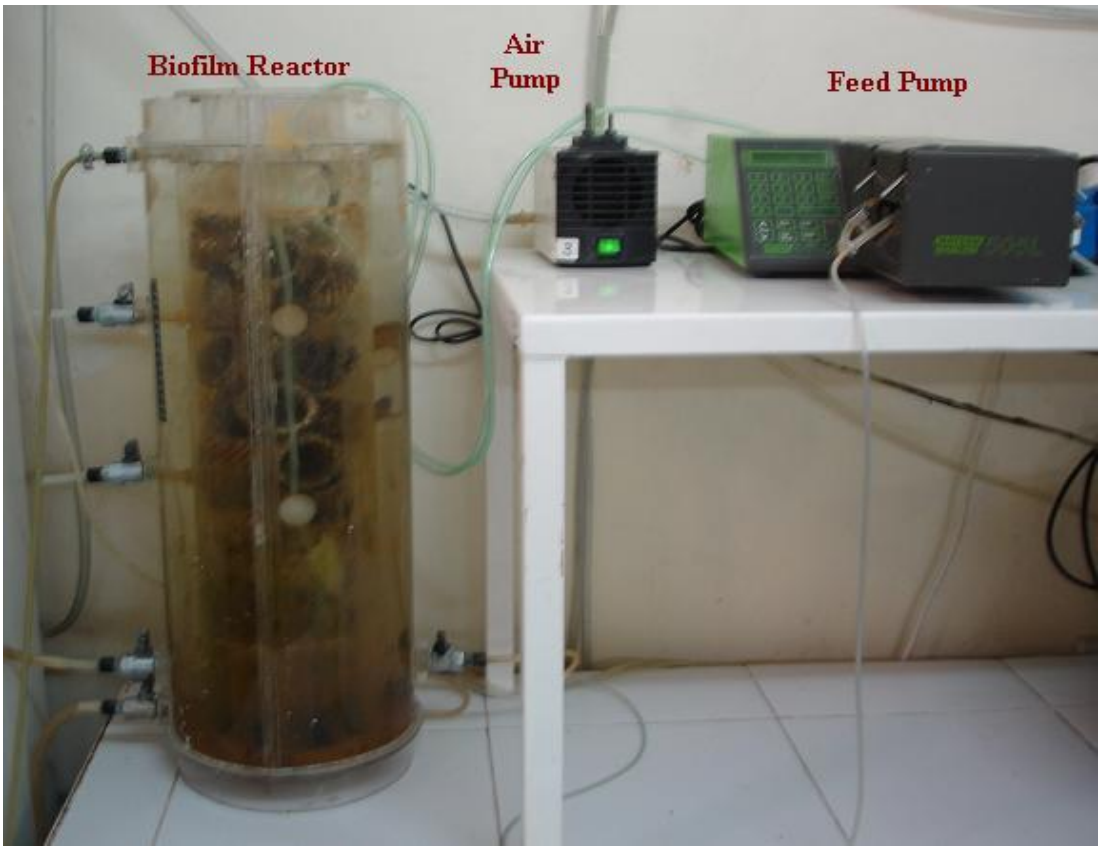


Figure III. 3 The experimental set-up of the nitrifying biofilm reactor

This cylindrical plexiglas reactor (11.65 cm diameter and 55 cm height) with a working volume of 4.25 L consists of randomly packed Bio-Pac15 polyethylene carrier material (Envicon Eng. GmbH) of which technical details are given in Table III.4. Since than, it has been fed with synthetic ammonium solution. Technical details of the carrier material for nitrifying biofilm reactor (Alpaslan Kocamemi, 2005; Alpaslan Kocamemi and Çeçen, 2007) are given in Table III.4.

Table III. 4 Technical details of the nitrifying biofilm reactor carrier materials (Çeçen, 1996; Çeçen and Orak, 1996; Orak, 1994)

Properties of Carrier Material	Values	Units
Number of carrier material	30	pieces
Length of carrier material	3	cm
Specific surface area of carrier material	150	m^2/m^3
Total surface area of the carrier materials in the reactor	0.192	m^2

Throughout the study, the nitrifying biofilm reactor (BF) was continuously fed with the synthetic wastewater described in Table III.1 by a peristaltic pump (Watson Marlow 505Di) with a flow rate of 4.1 L/day. The hydraulic retention time (HRT) was kept around 1 day based on 4.25 L working volume. The treated effluent was discharged by its own gravity.

The system was operated at a fixed wastewater temperature of around 25 ^{o}C by controlling the room temperature with an air-conditioner. Required DO concentration in the reactor was maintained by supplying air through diffusers placed to the bottom of reactor and connected to an air pump (Sonic 008). DO concentration and pH values inside the reactor were monitored at the middle and top ports of the reactor with daily measurements with Hach-Lange HQ40d DO and pH measurement device.

III.3.1. Continuous-Flow Experiments in the Biofilm Reactor System

Continuous-flow experiments in the nitrifying biofilm reactor were performed to investigate the effects of various DO levels on NO_2^--N accumulation in an attached-growth system with an enriched nitrifier culture. For this purpose, the nitrifying biofilm system described Section III.3 was initially started-up at DO level of 6.83 mg/L. The system was operated about 365 days for DO levels decreasing gradually from 6.83 mg/L to 0.93 mg/L. Table III.5 summarizes the operating conditions in all consecutive runs performed throughout the study.

Table III. 5 Operating conditions of continuous-flow experiments in the nitrifying biofilm reactor (BF)

Run No	Operation Period (days)	Feed Flow rate L/d	Feed NH_4^+-N Concentration (mg/L)	DO level at the top port (mg/L)
BF Run No: 1	0-78	4.02	72.6	6.83±0.55
BF Run No: 2	79-117	4.02	70.0	3.64±0.23
BF Run No: 3	118-230	4.02	73.3	1.21±0.23
BF Run No: 4	231-288	4.02	72.3	1.45±0.14
BF Run No: 5	289-365	4.02	72.5	0.93 ±0.25

In all runs, the system was fed with the synthetic wastewater described in Section III.1. As seen from the Table III.5, influent NH_4^+-N level was kept at a constant, level of around 72 mg/L. pH in the reactor was maintained around 7.37 with phosphate buffer. Wastewater temperature was around 24-25^oC. Depending on the pH, temperature, and NH_4^+-N concentrations in the reactor, FA ranged between 0.002-0.5 mg/L.

In each run, effluent discharged from the reactor by its gravity was collected in an effluent collection tank. Daily or composite effluent samples were taken from the effluent collection tank. These samples were filtered by using 0.45 μm filters (Millipore Millex HV) in order to separate the liquid from the solids that might escape from the reactor. All samples were analyzed for NH_4^+-N, NO_2^--N, and NO_3^--N as described in Section III.5.

III.4. CHARACTERIZATION OF THE MICROBIAL COMMUNITY IN THE SBR AND BIOFILM SYSTEMS

During the continuous-flow operation of both SBR and biofilm system for a time period of 365 days, sludge samples were taken from the reactor systems as the operation DO level was changed. Sludge samples from the biofilm system were always scraped from the surface of the carrier materials located between Port 1 and Port 2 of the reactor (Figure III.3). Those samples were analyzed with molecular tools, namely Fluorescence In-Situ Hybridization (FISH) and Slot-Blot Hybridization in order to investigate microbial ecology, community shifts and quantity changes in both systems during prolonged operation at various DO levels.

III.4.1. Sample Collection and Preparation

Sludge samples were concentrated by centrifuging for 5 min at 14000 rpm. Concentrated sludge samples were extracted and fixed in 4% paraformaldehyde-phosphate-buffered saline (PBS) for DNA extractions and FISH experiments immediately after centrifugation. The fixed cells were stored at - 20 °C

III.4.2. Fluoresecence In Situ Hybridization (FISH)

FISH analyses were performed in order to detect, identify and characterize the Bacteria domain and ammonia-and nitrite-oxidizing species). In situ hybridization of fixed cells was performed according to the protocol described by

Calli et al. (2006) using the following 16S rRNA-targeted oligonucleotide probes: BET42a for *β-proteobacteria* species, NSO190 for *Ammonium oxidizing β-proteobacteria* species, NIT3 for *Nitrobacter* species, NTSPA662 for *Nitrospira* genus. The sequences of these oligonucleotide probes were as reported by Mertoglu et al. (2005). All microorganisms were visualized by 4,6-diamidino-2-phenylindole (DAPI) staining. The slides were examined with the Leica DM-LB fluorescent microscope and the digital images of slides were captured with the Leica DC350F digital camera.

III.4.3. DNA Extraction

DNA extractions from the concentrated sludge samples were performed using FastDNA SPIN kit (Q-BIOgene) method according to the manufacturer's instructions as modified by Mertoglu et al. (2006).

III.4.4. Slot-Blot Hybridization

Slot-blot hybridization analyses were done to investigate the variations and activity of all microbial populations and also to quantify the relative abundance of microbial species. Slot-blot analyses of extracted DNA samples were performed with the DIG labeled oligonucleotide probes of EUB338 for Bacteria domain, NSO190 for *Ammonium oxidizing β-proteobacteria* species, NIT3 for *Nitrobacter* species, NTSPA662 for *Nitrospira* genus as described by Mertoglu et al. (2006).

III.5. ANALYTICAL METHODS

NH_4^+-N concentrations were measured by Nessler Method (Method 8038) with Hach DR 2000 spectrophotometer at 425 nm by using Hach mineral stabilizer, polyvinyl alcohol and Nessler reagent.

NO_2^--N concentrations were measured by Ferrous Sulfate Method (Method 8153) with Hach DR 2000 spectrophotometer at 585 nm by using Nitri Ver 2 nitrite reagent powder pillows.

NO_3^--N concentrations were measured by Cadmium Reduction Method (Method 8039) with Hach DR 2000 spectrophotometer at 500 nm by using Nitra Ver 5 nitrate reagent powder pillows. The samples including high NO_2^--N concentration (>10 mg/L) were pretreated with Hach bromine water and Hach phenol solution

before the addition of Nitra Ver 5 nitrate reagent powder pillows to compensate for nitrite interference.

SS and VSS concentrations were analyzed by Method 2540D and Method 2540E in Standard Methods (APHA, 1998), respectively. In SS analyses, well-mixed samples were filtered through 0.45 μm filter papers (Whatman GF52) and dried in an oven (Philip Harris INC95C) at 103°C-105°C for an hour. In the VSS analyses, the residues of SS analyses were ignited by a muffle furnace (Lenton 1200) at 550°C for 15 min (APHA, 1998).

CHAPTER IV

RESULTS and DISCUSSION

IV.1. CONTINUOUS-FLOW SBR EXPERIMENTS

The main operational parameters (temperature, DO, pH and MLVSS) and the influent-effluent NH_4^+-N, NO_2^--N, and NO_3^--N values measured in the SBR system which was operated continuously 365 days for DO levels decreasing gradually from 7.06 mg/L to 0.22 mg/L are chronologically shown in Figure IV.1 and Figure IV.2, respectively. As shown in Figure IV.1., during 365 days continuous operation the system was fed with a synthetic wastewater including 72.6 ± 0.8 mg/L NH_4^+-N. The each temperature, DO and pH value shown in Figure IV.1 is the average of continuously monitored values by the SCADA system with 15 min. intervals during the reaction period of 16 hr. As seen from Figure IV.1, wastewater temperature and pH were kept at a constant value of 24.7±0.6 ^oC and 7.4±0.1, respectively. Additionally, as seen from the DO versus time graph in Figure IV.1, no significant fluctuations were observed in daily measured DO concentrations. This indicates that SCADA system was successful to keep the pre-defined DO value at a constant level through the reaction period.

As seen from Figure IV.2, during initial three runs (168 days), in which DO levels were 7.06, 4.19 and 3.18 mg/L, the NH_4^+-N removal and NO_2^--N accumulation were observed around 99% and 1%, respectively and the NH_4^+-N removed was almost fully converted to NO_3^--N. On day 169, as a result of decreasing the DO level to around 2.20 mg/L, a decrease in the NH_4^+-N removal and an increase in the NO_2^--N accumulation were observed for 11 days. During this period, NH_4^+-N removal efficiency ranged between 80-90% and the NO_2^--N accumulation showed an increase up to 43%. However, in the next period of Run 4 (DO: 2.20 mg/L), NH_4^+-N removal efficiency increased to around 98%. NO_2^--N accumulation fluctuated between 1-15% from day 180 to day 243. In Run 5, which was started on day 244, the average DO in the reactor was reduced to the value of 1.27 mg/L. In this period, from day

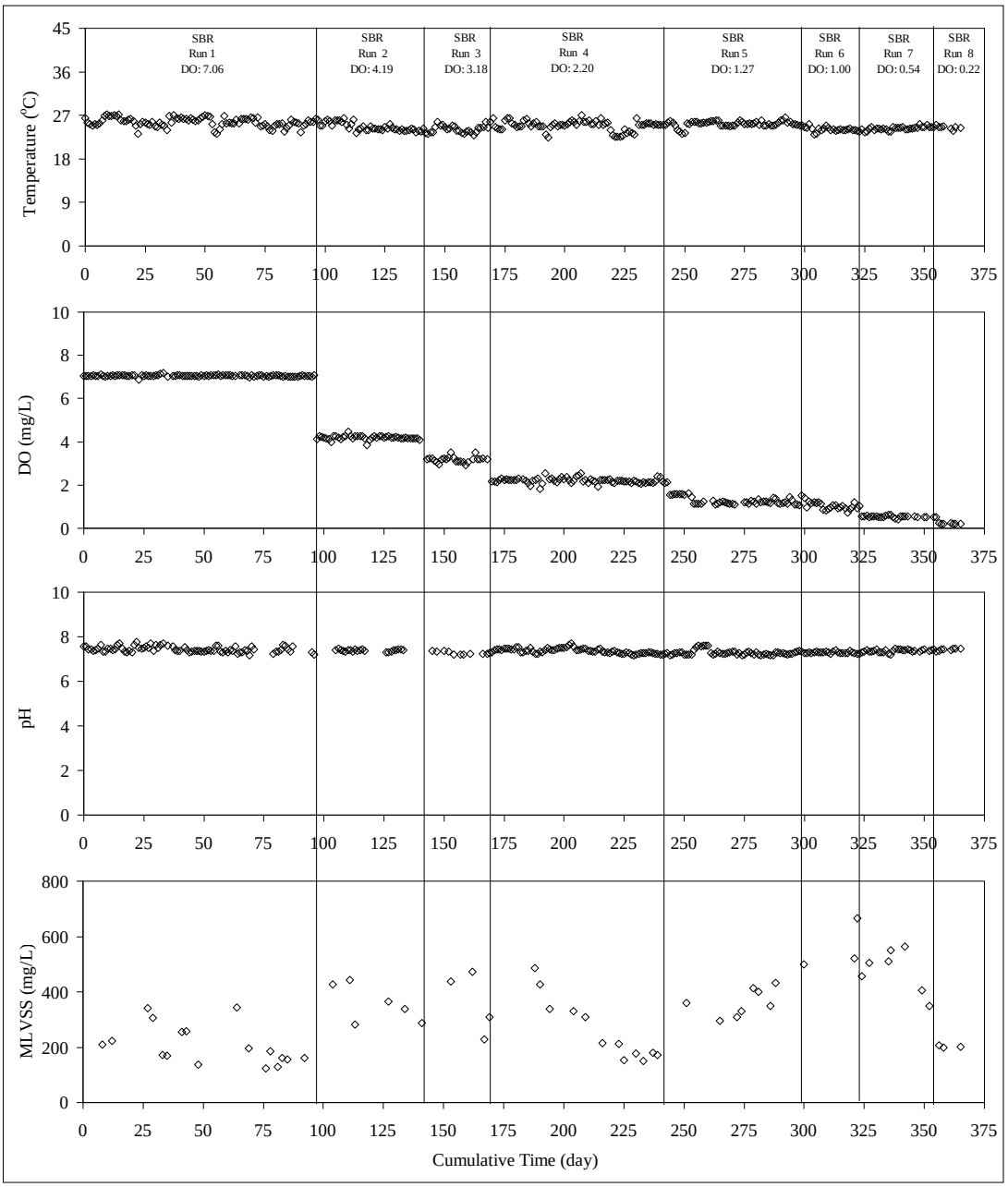


Figure IV. 1 Chronological illustration of temperature, DO, pH and MLVSS measurements during continuous-flow SBR experiments

264 to day 267, a decrease in NH_4^+-N removal and an increase in NO_2^--N accumulation were observed. Those observations were most probably related with the operational problems of magnetic stirrer during the same period. The low DO level (1.27 mg/L) in this period resulted longer on/off intervals of the air pump than previous runs and hence air supply became insufficient to maintain complete mixing condition in the reactor during faulty operation of the magnetic stirrer. Except this period, between days 244-299 of Run 5 (DO: 1.27 mg/L), the NH_4^+-N removal was

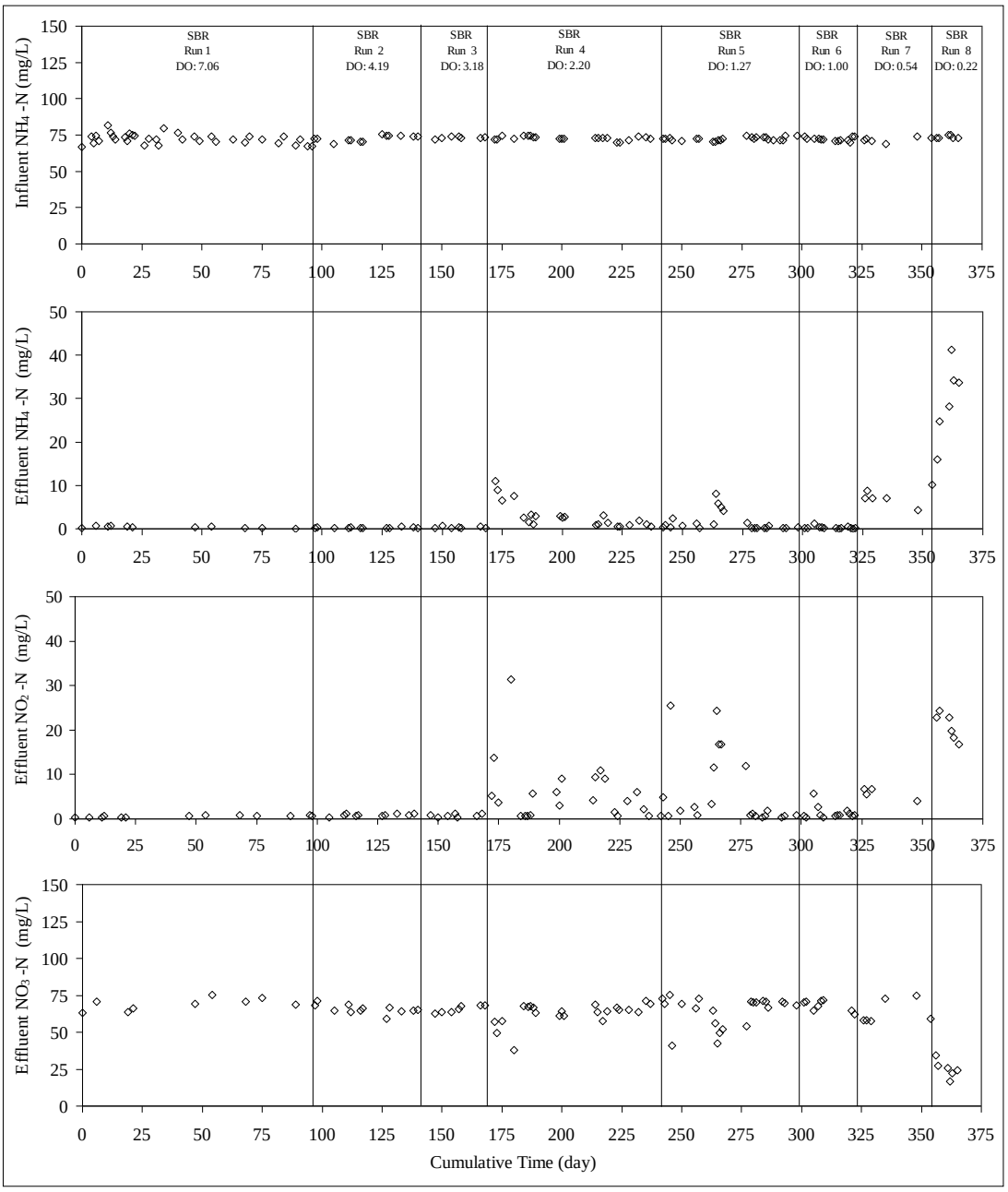


Figure IV. 2 Chronological illustration of daily influent and effluent of NH_4^+-N, NO_2^--N, and NO_3^--N measurements during continuous-flow SBR experiments (♦ influent values, ◊ effluent values)

about 99% and NH_4^+-N oxidized almost fully converted to NO_3^--N with negligible (1%) NO_2^--N accumulation. Comparison of the NH_4^+-N removals between Run 4 and Run 5, (DO concentrations were 2.20 and 1.27, respectively) shows that the NH_4^+-N removal efficiency of Run 5 was greater than Run 4 altough the DO level was lower. It is considered that this finding was most probably related with the higher MLVSS concentration of Run 5 with respect to Run 4 (Figure IV.1). In Run

6, between days 300- 323, DO level was reduced to 1 mg/L. The NH_4^+-N removal efficiency was observed about 99% and the NO_2^--N accumulation slightly increased and reached to 1.5 %. In Run 7, starting at day 324, as a result of decreasing DO level to 0.54 mg/L, NH_4^+-N removal efficieny decreased 10% and reached about 90% and the NO_2^--N accumulation increased to 9%. In this period, between days 340-342, the reactor was inadvertently fed with an influent including extremely high NH_4^+-N concentration (approximately 360 mg/L) and hence the system was inhibited about 1 week. During this inhibition period, very high NH_4^+-N and NO_2^--N concentrations were observed in the effluent. Those values are not shown in Figure IV.2. At day 348, the system recovered itself and the effluent values were turned back into the normal values. In Run 8, starting at day 356, the DO level was reduced to 0.22 mg/L and the system was operated at this DO level till the end of experiment at day 365. In this period, significant amount of NH_4^+-N and NO_2^--N accumulations were observed in the effluent. NH_4^+-N removal efficiency reduced to 56 % and NO_2^--N accumulation increased to the value of 28%.

The evaluation of the above summarized results as a whole clearly shows that from DO level of 7 mg/L to 0.54 mg/L no significant NH_4^+-N and NO_2^--N accumulations were observed in the SBR system. However, at DO level of 0.54 mg/L, with respect to the influent NH_4^+-N, 10% NH_4^+-N - 9% NO_2^--N accumulations were observed in the effluent. Those accumulations were increased to 44% NH_4^+-N-28% NO_2^--N at DO level of 0.22 mg/L. Those values correspond to 10% and 50% of the removed NH_4^+-N accumulated as NO_2-N at DO levels 0.54 and 0.22 mg/L, respectively. According to the those findings, it is obvious that at DO level of 0.54 mg/L NH_4^+-N accumulation and NO_2^--N accumulation started simultenously and decreasing DO level from 0.54 mg/L to 0.22 mg/L resulted in serious increase in both NH_4^+-N and NO_2^--N accumulations with similar ratios. These findings are in contrast with the results of previous studies in literature. For instance, in the study of Ruiz et al. (2006), in which a mixed culture system was operated at DO levels gradually decreasing from 5.5 mg/L to 0.5 mg/L for 49 days, it was reported that nitrification was not affected by DO concentrations in the range 5.7-1.7 mg/L At a DO concentration of 1.4 mg/L NO_2^--N accumulation took place, and increased as DO concentration decreased. The higher NO_2^--N accumulation, without affecting NH_4^+-N accumulation took place at 0.7 mg/L. At a DO of 0.5 mg/L NH_4^+-N conversion was affected. Similarly, in the study of Ciudad et al. (2005), in which a mixed

culture system was operated at DO levels 2.4, 1.4, 1 and 0.5 mg/L for seven days, it was reported that the serious NO_2^--N accumulation, without affecting ammonia removal, took place at 1.4 mg DO/L. At DO 1 mg/L, nitrite and NH_4^+-N accumulation took place simultenously. According to the findings in literature, accumulation of NO_2^--N started at higher DO concentrations than the present study. This is most probably due to the fact that the previous studies in literature were carried out by using mixed-cultures which include not only the nitrification bacteria but also heterotrophic bacteria whereas the present study was performed using enriched nitrifier culture. As mentioned by Stenstrom and Poduska (1980), compared to the enriched nitrifying cultures, which include only nitrification bacteria, in mixed cultures the nitrifying organisms are in large flocs and the DO concentration within the flocs may be considerably less than the bulk fluid concentration. Therefore, in mixed culture systems, it would be expected that diffusion of bulk DO into the bio-floc may proceed slowly and hence those cultures may be affected from low DO conditions more seriously than enriched nitrifying cultures. In the above mentioned previous studies given in literature, NO_2^--N accumulation started without NH_4^+-N accumulation. In these studies, NH_4^+-N accumulation started at significantly lower DO levels than those causing NO_2^--N accumulation. This may be related with the higher DO half saturation constant of NOB with respect to AOB as mentioned by many researchers (Blackburne et al., 2008; Hanaki et al., 1990; Parades, et al., 2007; Philips et al., 2002). However, in the present study, NO_2^--N accumulation and NH_4^+-N accumulation started simultenously and increased almost proportional with each other. This may be due to physiological adaptation of NOB bacteria in the enriched nitrifying culture by decreasing their DO half saturation constant as a result of prolonged exposure to the limited DO conditions. Similarly, in the study of Kowalchuk et al. (1998) it was reported that DO half-saturation coefficient of AOB cultures, which were exposed to limited DO concentrations for prolonged time, was decreased. Additionaly, in the study of Park et al. (2004), the stable and complete nitrification was observed in the mixed culture system operated continuously for a period of 300 days at DO levels 0.12-0.24 mg/L. In the same study, it was observed AOB cultures in the low-DO (0.12-0.24 mg/L) enrichment exhibited lower DO half saturation constant than the high-DO (8.5 mg/L) enrichment.

IV.2. KINETIC ANALYSES FOR ENRICHED NITRIFIER CULTURE DURING PROLONGED CONTINUOUS-FLOW OPERATION OF SBR SYSTEM

IV.2.1. Determination of Initial Substrate/Biomass (S_o/X_o) Ratio Reflecting Extant Kinetics

As it was mentioned in literature (Grady et al., 1996), the kinetics observed with a high S_o/X_o represent the maximum capability of the members of microbial community with the fastest growth kinetics. In other words, the measured kinetics with a high S_o/X_o ratio will reflect the characteristics of the fastest growing species rather than the characteristics of the original culture. Kinetic parameter estimates determined with these conditions are termed "*intrinsic*". Such kinetic measurements may provide insight into the immediate response (e.g., shock-load response) of the continuous culture from which the cells were obtained. Conversely, the kinetics observed with a low S_o/X_o represent the capabilities of the microbial culture as it existed in the parent bioreactor at the time the test specimen was removed. Kinetic parameter estimates determined with these conditions are termed "*extant*" or "*currently existing*". In literature, the S_o/X_o value required to give extant kinetics is uncertain for nitrifying organisms. Therefore, in the present study, prior to the batch kinetic experiments, a set of preliminary initial NH_4^+-N utilization rate experiments were performed in order to roughly estimate the S_o/X_o ratio reflecting extant kinetic conditions. Figure IV.3 shows the measured initial specific ammonium utilization rates at various S_o/X_o ratios ranging between 0.07-0.45 for a constant initial NH_4^+-N (S_o) level of 40-50 mg/L. As seen from the graph, up to S_o/X_o ratio of 0.2, the specific ammonium utilization rates were observed almost at the same level. But, the S_o/X_o ratios greater than 0.2 caused observation of higher specific ammonium utilization rates. This indicated that for the S_o/X_o ratios greater than 0.2 the measured ammonium utilization rates most probably reflected the characteristics of the fastest growing species in the culture rather than the characteristics of the whole culture. Therefore, in the later kinetic experiments (discussed in Sections IV.2.2 and IV.2.3) which were performed in order to estimate the extant kinetic parameters representative for the existing condition of the biomass in the SBR system, So/Xo ratio was always kept below 0.2.

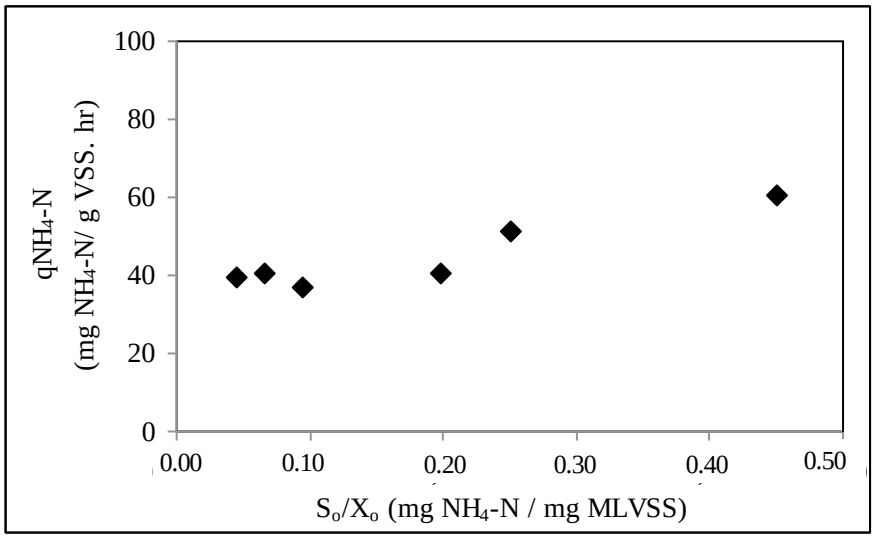


Figure IV. 3 Changes observed in qNH_4^+-N values depending on So/Xo ratio

IV.2.2. Changes in Specific Ammonium Utilization Rates

The results of the specific NH_4^+-N utilization rate (qNH_4^+-N) experiments performed during the continuous-flow operation of the SBR system at gradually reduced DO levels for a time period of 365 days were summarized in Table IV.1. These experiments were performed periodically due to DO level changes in continuous-flow SBR system as given in Tablo III.3. The qNH_4^+-N values measured during continuous-flow SBR experiments were chronologically illustrated in Figure IV.4.

As seen from Table IV.1 and Figure IV.4, during initial continuous-flow experiment of the SBR system performed at DO level of 7.06 mg/L for 97 days, the qNH_4^+-N value was around 51.8 mg/g.h. As a result of decreasing DO level to 4.19 mg/L at day 97, the qNH_4^+-N value decreased about 26% and reached a value of 39 mg/g.h. Until day 300, DO level in the SBR was gradually decreased from 4.19 mg/L to 1 mg/L. In this period, the observed qNH_4^+-N values showed slight fluctuations between 37.5 and 39 mg/g.h. However, in the period of day 300-323, as a result of decreasing DO level to 1 mg/L, the qNH_4^+-N value decreased 49% and reached a value of 19.23 mg/g.h. In the progressing days of the study, at day 324,

Table IV. 1 Specific NH_4^+-N utilization rates (qNH_4^+-N) in the SBR system

Phase	Experiment No	MLVSS (mg/L)	qNH_4^+-N $\frac{mg\ NH_4^+\text{-}N}{g\ VSS.\ hr}$
Seed sludge	-	201.33	47.68
SBR Run No.1	Exp. No: 1 (at 8th day)	210.00	51.43
DO=7.06 ± 0.04	Exp. No: 2 (at 12th day)	222.50	53.93
Operation day: 0-96	Exp. No: 3 (at 89th day)	160.00	50.18
	Average	***197.5±33.07***	***51.85±1.91***
SBR Run No.2	Exp. No: 1 (at 113th day)	282.22	38.27
DO=4.19 ± 0.09	Exp. No: 2 (at 141th day)	286.67	39.77
Operation day:97-140			
	Average	***284.44±3.16***	***39.02±1.06***
SBR Run No.3	Exp. No: 1 (at 153th day)	438.89	37.10
DO=3.18 ± 0.14	Exp. No: 2 (at 167th day)	228.89	39.32
Operation day:141-168			
	Average	***333.89±148.49***	***38.21±1.57***
SBR Run No.4	Exp. No: 1 (at 225th day)	153.33	39.13
DO=2.20 ± 0.12	Exp. No: 2 (at 237th day)	180.00	38.90
Operation day:169-243	Exp. No: 3 (at 239th day)	171.11	41.06
	Average	***168.15±13.58***	***39.70±1.19***
SBR Run No.5	Exp. No: 1 (at 272th day)	307.78	38.99
DO=1.27 ± 0.17	Exp. No: 2 (at 274th day)	330.00	37.24
Operation day:244-299	Exp. No: 3 (at 279th day)	413.33	36.29
	Average	***350.37±55.65***	***37.51±1.37***
SBR Run No.6	Exp. No: 1 (at 321th day)	520.00	19.23
DO=1.00 ± 0.20			
Operation day:300-323			
	Average	***520.00***	***19.23***
SBR Run No.7	Exp. No: 1 (at 352th day)	350	16.08
DO=0.54 ± 0.04			
Operation day:324-355			
	Average	***350***	***16.08***
SBR Run No.8	Exp. No: 1 (at 358th day)	296	13.70
DO=0.22± 0.01			
Operation day:356-365			
	Average	***296***	***13.70***

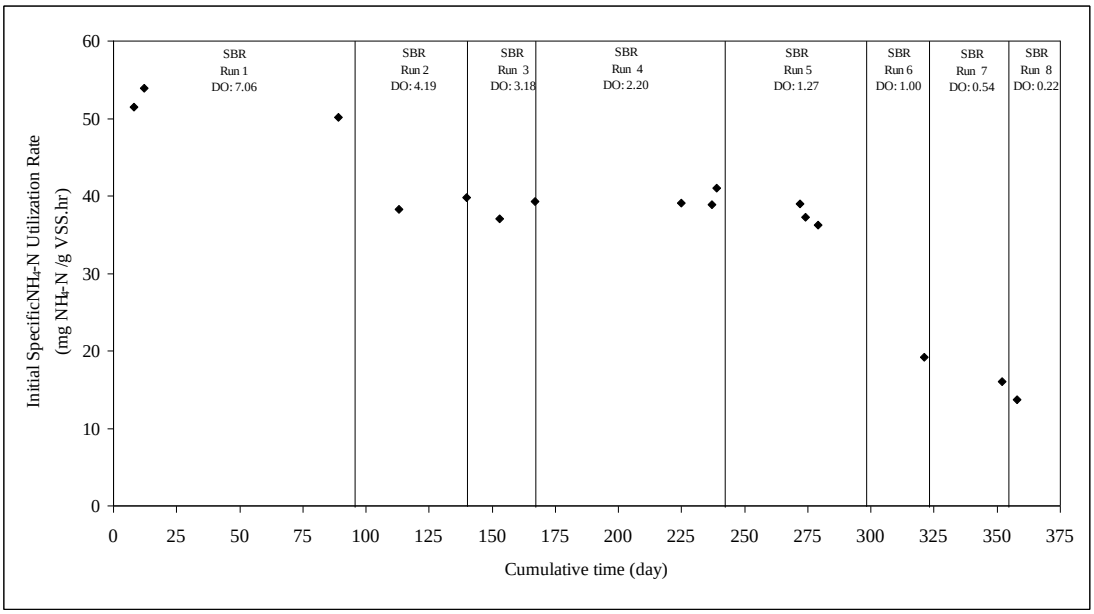


Figure IV. 4 Chronological illustration of initial specific NH_4^+-N utilization rates (qNH_4^+-N) changes in the continuous-flow SBR system

DO concentration was reduced to 0.54 mg/L in Run 7 and the qNH_4^+-N reduced 16% more and reached the value of 16.8 mg/g.h. And then, at day 355, DO concentration was reduced to 0.22 mg/L in Run 8 and an additional 15% reduction in $qNH4^+$-N was observed and it reached a value of 13.7 mg/g.h.

When qNH_4^+-N values measured at different DO concentrations during 365 days were evaluated according to the initial qNH_4^+-N value that was observed at 7.01 mg/L DO in Run 1; it is seen that the qNH_4^+-N value, which decreased 25 % due to the reduction of the DO from 7.01 to 4.19 mg/L, did not change significantly till 1 mg/L DO concentration; whereas at DO concentrations of 1 mg/l, 0.54 mg/L and 0.22 mg/L it decreased about 63%, 69% and 74%, respectively. However, the qNH_4^+-N values used in this evaluation were calculated based on the the total MLVSS concentration. Therefore, those values were not comparable unless the AOB ratio in the enriched nitrifying sludge was at the same level in all experiments.

The above summarized results of the batch qNH_4^+-N experiments demonstrate that the reason of not observing significant NH_4^+-N accumulation in the effluent of SBR system till DO level of 0.54 mg /L was heavily dependent on the too long reaction period in the SBR system (16 hr) during continuous-flow experiments.

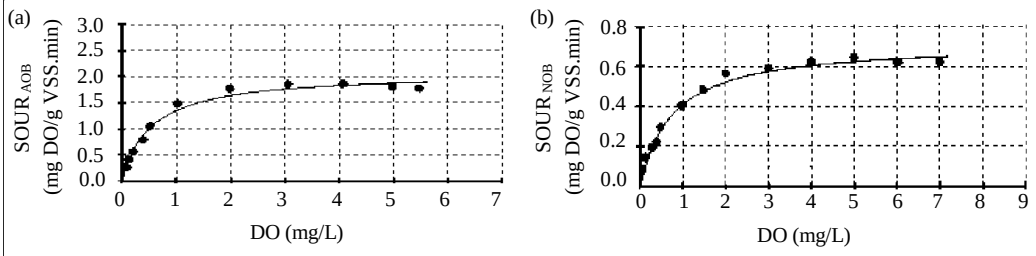
IV.2.3. Changes in Maximum Specific Oxygen Uptake Rates and Oxygen Half-Saturation Constants of AOB and NOB Species

The results of the oxygen uptake rate (OUR) experiments, which were performed during the last period of each experimental set of SBR system (Table III.3.), were given in Figure IV.5. The graphs shown in this figure were obtained using EZ-FIT5 package program of Perella Scientific Inc, which fits the experimental data to the Monod model via non-linear least square regression (NLSR) and provides to estimate maximum velocity and half saturation constant. Therefore, "K_m and V_{max}" values given below the graphs in Figure IV.5 (a) and Figure IV.5 (b) were automatically reported by the program and should be evaluated as "$K_{o,AOB}$, $SOUR_{max,AOB}$" and "$K_{o,NOB}$, $SOUR_{max,NOB}$", respectively. In view of the preliminary experiments results discussed in Section IV.2.1, the initial S_o/X_o value in each OUR experiment was kept under 0.2. Therefore, the kinetic constants obtained from these experiments reflect the actual kinetic capabilities of the biomass at a physiological state consistent with that in the continuous SBR system from which the biomass was obtained (i.e., extant kinetic parameters). $SOUR_{max}$ and K_o values obtained from the OUR experiments, which were carried out to investigate the kinetic constants of the AOB and NOB cultures exposed to gradually decreased DO levels for prolonged time were chronologically illustrated in Figure IV.6(a) and Figure IV.6(b), respectively.

As shown in Figure IV.6 (a), the $SOUR_{max,AOB}$ and $SOUR_{max,NOB}$ values of the sludge exposed to the 7.01 mg DO/L for 96 days were obtained 2.08 and 0.72 mgO_2/g MLVSS.min, respectively. $SOUR_{max,AOB}$ and $SOUR_{max,NOB}$ values exhibiting a decrease towards the end of SBR Run 2, which was performed at a DO level of 4.19 mg/L for 43 days, started to increase gradually in the later 100 days, during which the SBR system was operated at DO levels 3.18 and 2.2 mg/L consecutively, and reached their maximum values of 4.7 and 1.5 mgO_2/g MLVSS.min, respectively.

Starting from day 244, the SBR reactor was operated under DO levels of 1.27 and 1 mg/ L consecutively for a time period of 78 days. At the end of this period, $SOUR_{max,AOB}$ and $SOUR_{max,NOB}$ values decreased and reached the values of 2 and 0.59 mgO_2/g MLVSS.min, respectively. These values did not significantly change while the SBR system were operated at DO level of 0.54 mg/L for 31 days. As a result of reduction of the DO level to 0.22 mg/L at day 356 and 9 days operation

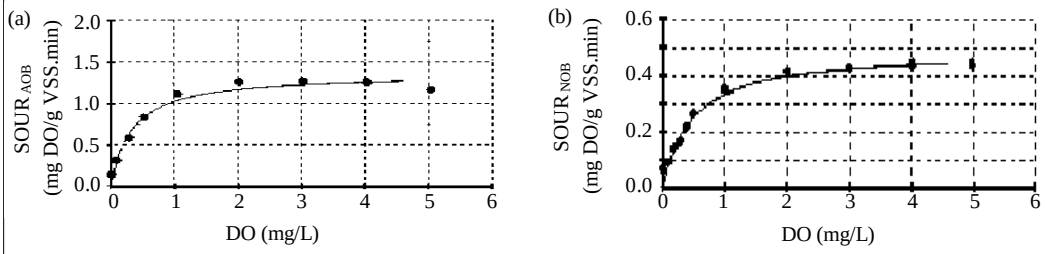
Exp. Phase: SBR Run No: 1



Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -1.389 Akaike Criterion= -25.219
RUNS test of Residuals= Pass p=0.05 1 - r^2= .1733
Km:= 0,5411 +/- 0,0646 (p=7,629E-06)
Vmax:= 2,0768 +/- 0,0646 (p=2,170E-09

Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -2.771 Akaike Criterion= -59.524
RUNS test of Residuals= Pass p=0.05 1 - r^2= .1726
Km:= 0.7473 +/- 0.0757 (p=4.189E-07)
Vmax:= 0.7191 +/- 0.0196 (p=3.218E-10)

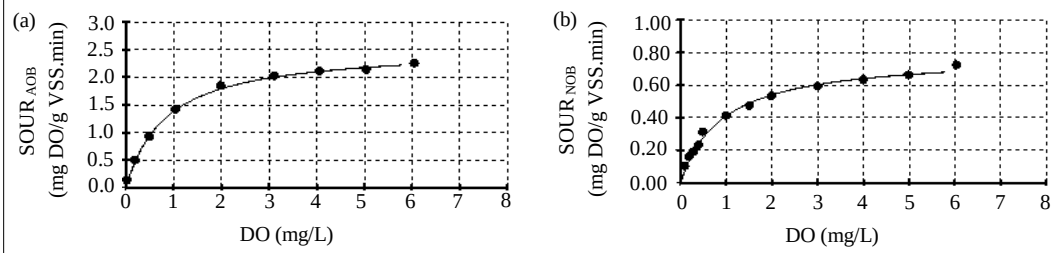
Exp. Phase: SBR Run No: 2



Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -1.243 Akaike Criterion= -26.17
RUNS test of Residuals= Pass p=0.05 1 - r^2= .1849
Km:= 0,3265 +/- 0,0559 (p=3,187E-04)
Vmax:= 1,3584 +/- 0,0506 (p=1,249E-08)

Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -1.181 Akaike Criterion= -57.283
RUNS test of Residuals= Pass p=0.05 1 - r^2= .2133
Km:= 0,4835 +/- 0,061 (p=1,193E-05)
Vmax:= 0,4526 +/- 0,0164 (p=-1,598E-09)

Exp. Phase: SBR Run No: 3

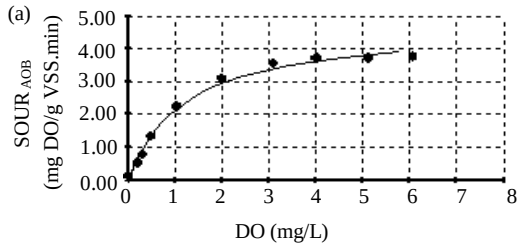


Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -3.881 Akaike Criterion= -39.267
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0132
Km:= 0.8343 +/- 0.0429 (p=1.211E-07)
Vmax:= 2.5396 +/- 0.034 (p=5.234E-10)

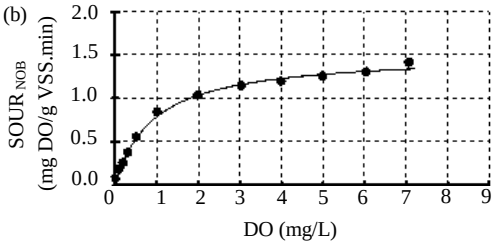
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -2.04 Akaike Criterion= -61.425
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0932
Km:= 0,9097 +/- 0,0726 (p=1,062E-07)
Vmax:= 0,7853 +/- 0,0196 (p=-7,521E-08)

Figure IV. 5 Determination of (a) $K_{o,AOB}$ and $SOUR_{max,AOB}$ (b) $K_{o,NOB}$ and $SOUR_{max,NOB}$ with batch OUR experiments during continuous flow SBR experiments

Exp. Phase: SBR Run No: 4

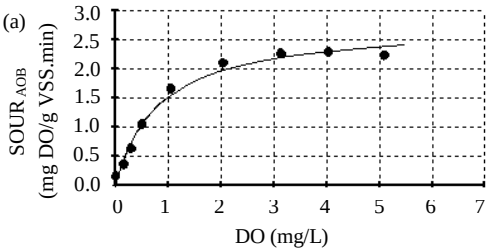


Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -2.183 Akaike Criterion= -14.241
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0756
Km:= 1.1873 +/- 0.1373 (p=1.239E-05)
Vmax:= 4.7043 +/- 0.17 (p=8.209E-09)

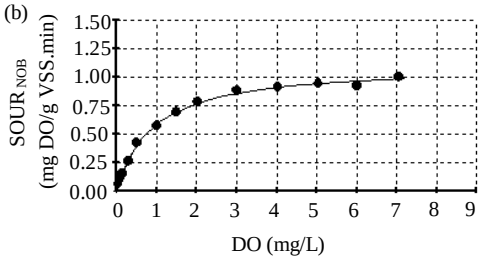


Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -2.507 Akaike Criterion= -47.329
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0584
Km:= 0,9136 +/- 0,0721 (p=3,210E-08)
Vmax:= 1,5093 +/- 0,0319 (p=4,752E-08)

Exp. Phase: SBR Run No: 5

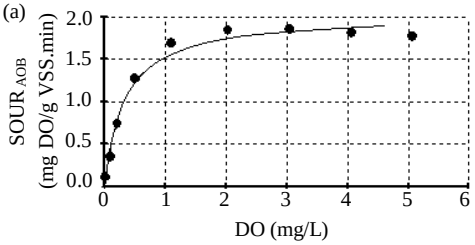


Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -1.716 Akaike Criterion= -17.832
RUNS test of Residuals= Pass p=0.05 1 - r^2= .1153
Km:= 0.8183 +/- 0.1242 (p=1.539E-04)
Vmax:= 2.7542 +/- 0.124 (p=5.042E-08)

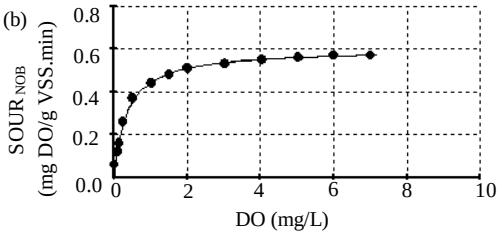


Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -3.054 Akaike Criterion= -66.949
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0347
Km:= 0,8964 +/- 0,0495 (p=1,836E-09)
Vmax:= 1,1074 +/- 0,0167 (p=-1,164E-09)

Exp. Phase: SBR Run No: 6



Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -1.475 Akaike Criterion= -19.856
RUNS test of Residuals= Pass p=0.05 1 - r^2= .1466
Km:= 0.3235 +/- 0.0526 (p=2.341E-04)
Vmax:= 2.0208 +/- 0.0707 (p=6.810E-09)



Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -2.172 Akaike Criterion= -71.975
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0838
Km:= 0.3524 +/- 0.0256 (p=1.638E-08)
Vmax:= 0.5984 +/- 0.0089 (p=2.387E-09)

Figure IV.5 Continue

at this DO level, $SOUR_{max,AOB}$ significantly reduced to the value of 0.51 mgO_2/g MLVSS.min. In the same period, however, $SOUR_{max,NOB}$ value slightly increased to the value of 0.83 mgO_2/g MLVSS.min.

There may be two possible reasons of observing significant fluctuations in $SOUR_{max,AOB}$ and $SOUR_{max,NOB}$ values during 365 days experimental period. First,

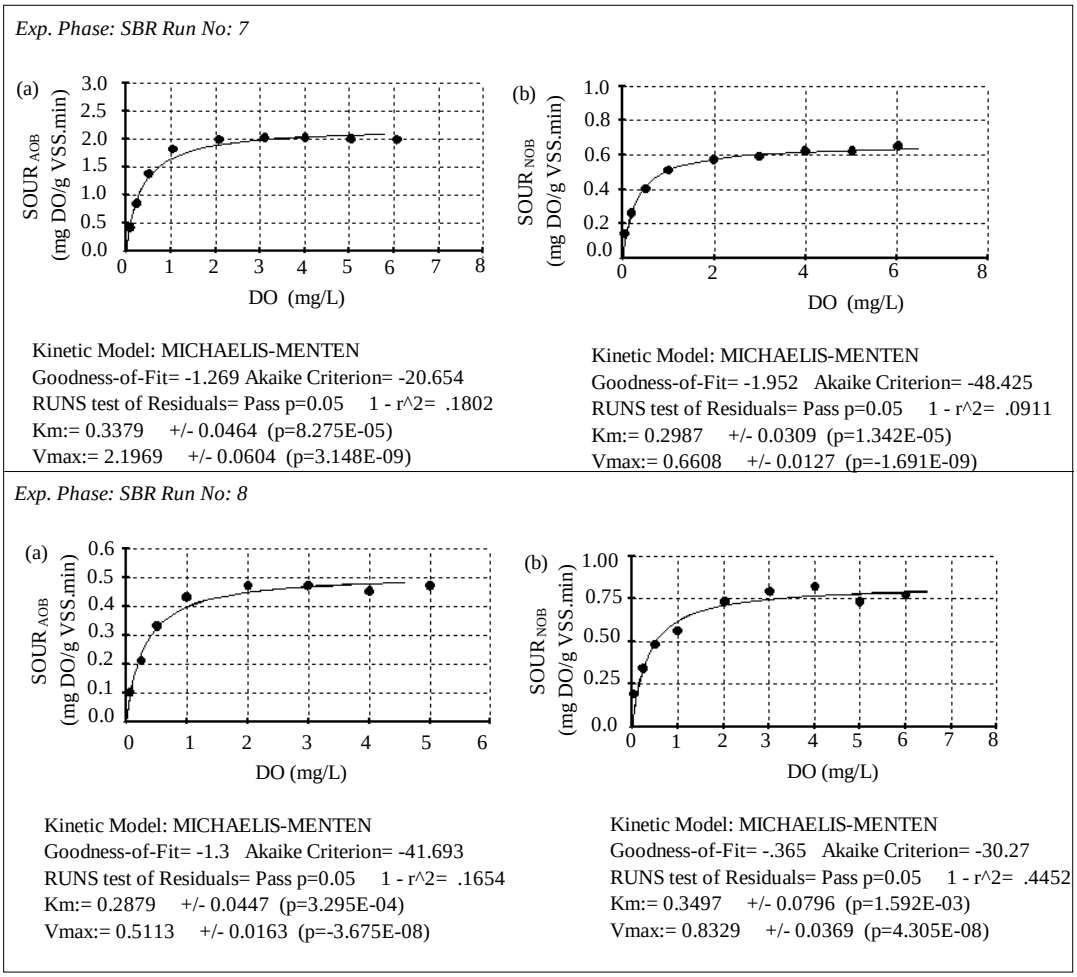


Figure IV.5 Continue

the reported $SOUR_{max,AOB}$ and $SOUR_{max,NOB}$ values were calculated according to total MLVSS concentration. In other words, the reported $SOUR_{max,AOB}$ and $SOUR_{max,NOB}$ values should not be considered as actual values unless the AOB/NOB ratio in the enriched nitrifying sludge was at the same level in all experiments. Due to the possible changes in the AOB/NOB ratio of the enriched nitrifying sludge during 365 days experimental period, $SOUR_{max,AOB}$ and $SOUR_{max,NOB}$ values might exhibit a fluctuating pattern with respect to time.

As seen from Figure IV.6(b), the $K_{o,AOB}$ and $K_{o,NOB}$ values of AOB and NOB cultures in the enriched nitrifying sludge,which exposed to the DO level of 7.01 mg/L for 96 days were obtained as 0.54 mg/L and 0.74 mg/L, respectively. As a result of exposure of the same culture to the DO level of 4.19 mg/L from day 97 to 134 for 43 days, the oxygen affinities of the AOB and NOB species increased and $K_{o,AOB}$ and $K_{o,NOB}$ values were observed as 0.33 mg/L and 0.48 mg/L, respectively.

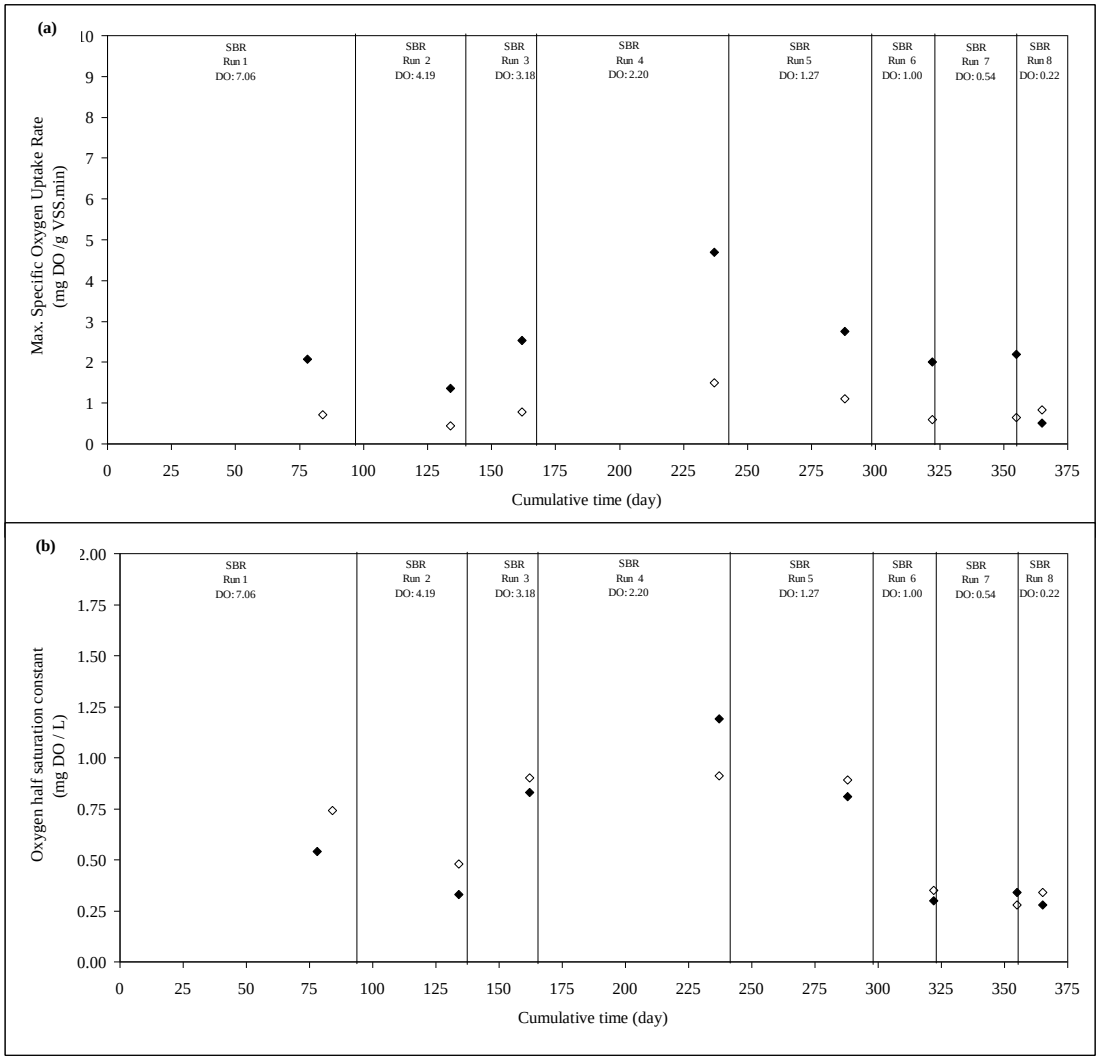


Figure IV. 6 Chronological illustration of changes observed for (a) maximum specific oxygen uptake rates (b) oxygen half saturation constants during continuous-flow operation of the SBR system (♦AOB species ◊ NOB species)

During this period (days 97-143), decreasing the DO level in the reactor to the level of 4.19 mg/L resulted in extension of on/off time intervals of the air pump and hence the speed of the magnetic stirrer was increased in order to maintain complete-mix conditions in the reactor. The increasing speed of the magnetic stirrer caused the reduction of the floc size, which could be seen with the naked eye. Therefore, the increasing oxygen affinities of AOB and NOB species observed in the same period is most probably related with the decreasing size of the flocs which resulted in increasing bulk DO diffusion into the bioflocs as mentioned by Stenstrom and Poduska (1980). After day 141, the DO level in the reactor was gradually decreased from 3.18 mg/L to 1.27 mg/L within 156 days. In this period, the reduction in the

oxygen affinities of both AOB and NOB species was observed. $K_{o,AOB}$ was observed in the range of 0.8 and 1.1 mg/L while $K_{o,NOB}$ was around 0.9 mg/L. In view of these observed half saturation constants of AOB and NOB species, in the period during which the SBR system was operated at 1.27 mg DO/L it may be expected to observe NH_4^+-N and NO_2^--N accumulations in the SBR effluent in some extent. However, as shown from the results given in Section IV.1, in contrast to the expectation, the NH_4^+-N and NO_2^--N accumulations were observed at the beginning of the period in which the reactor was operated at DO level of 2.2 mg/L, and towards the end of this period the NH_4^+-N accumulation completely disappeared while the NO_2^--N accumulation decreased. During the operation of the SBR system at 1.27 mg DO/L (except faulty operation period of the magnetic stirrer), neither NH_4^+-N nor NO_2^--N accumulation was observed. It is considered that this finding may be related with the possibly increasing subsrate affinities ($K_{s,NH4-N}$ ve $K_{s,NO2-N}$) of AOB and NOB species in the same period. Similarly, in the study of Park and Noguera (2007), it was reported that in low DO chemostats decreasing oxygen affinity of AOB species may be compensated by their increasing substrate affinity. As given in Section IV.2, in the same period, in spite of decresing DO level no change was observed in initial ammonium utilization rate of the culture. This finding also supports that substrate affinity of the culture probably increased. From day 300, the culture exposed to 1 mg DO/L for 23 days. In this period, a sudden increase in the oxygen affinities of both AOB and NOB species were observed and $K_{o,AOB}$ and $K_{o,NOB}$ values reduced to 0.3 mg/L and 0.35 mg/L, respectively. In consistent with these findings, in this period neither NH_4^+-N nor NO_2^--N accumulation was observed in the system effluent as mentioned in Section IV.1. In the period between the days 323-365, the DO level in the SBR system was consecutively reduced to 0.54 and 0.22 mg/L. In this period, no change was observed in the oxygen affinities of both AOB and NOB species. $K_{o,AOB}$ and $K_{o,NOB}$ values were around 0.3 mg/L. In consistent with these values, in the same period (days 323-365), contrary to the similar studies in literature (Cuidad et al., 2005; Ruiz et al., 2006) NH_4^+-N and NO_2^--N accumulation started simultenously at 0.54 mg DO /L and these accumulations increased almost proportional with each other as a result of decreasing DO level from 0.54 to 0.22 mg/L (Section IV.1). Although both $K_{o,AOB}$ and $K_{o,NOB}$ were around 0.3 mg/L, the reason of observing slightly higher NO_2^--N accumulation (50% in terms of removed

NH_4^+-N) with respect to the NH_4^+-N accumulation (44%) (Section IV.1) indicates the possible effect of FA concentration on NO_2-N accumulation.

IV.3. CONTINUOUS-FLOW BIOFILM EXPERIMENTS

The main operational parameters (temperature, DO, pH) and the influent-effluent NH_4^+-N, NO_2^--N, and NO_3^--N values measured in the biofilm system which was operated continuously 365 days for DO levels decreasing gradually from 6.83 mg/L to 0.93 mg/L are chronologically shown in Figure IV.7 and Figure IV.8. As shown in Figure IV.7, during 365 days continuous operation the system was fed with a synthetic wastewater including 72.1 ±1.24 mg/L NH_4^+-N. The wastewater temperature and pH were kept at a constant value of 24±0.4^{0}C and 7.37±0.07, respectively.

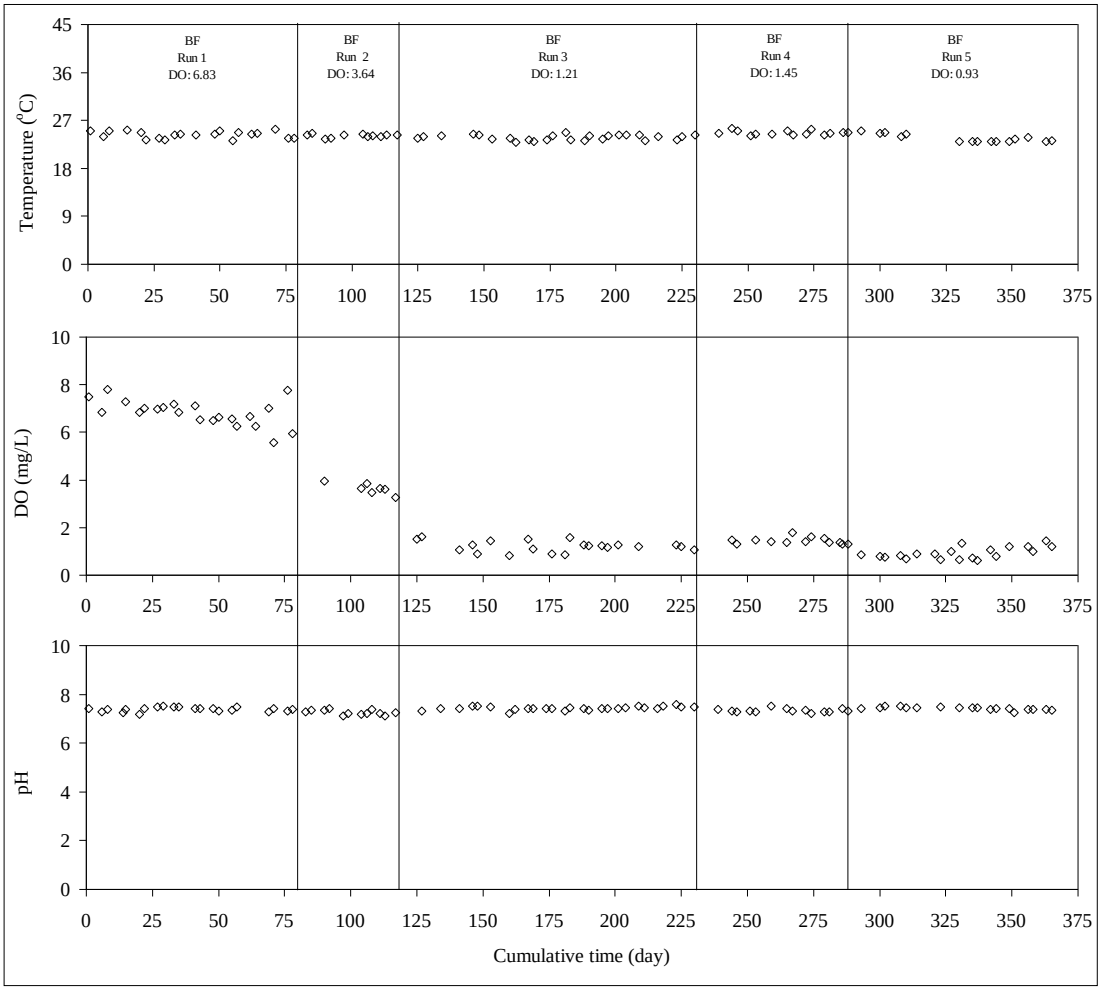


Figure IV.7 Chronological illustration of temperature, DO, pH during continuous-flow nitrifying biofilm reactor (BF) experiments

As seen from Figure IV.8, during initial two runs (117 days), in which DO levels were 6.83 and 3.64 mg/L, the NH_4^+-N removal and NO_2^--N accumulation were observed about 99% and 1%, respectively and the NH_4^+-N removed was almost fully converted to NO_3^--N. At day 118, Run 3 was started by decreasing the DO level to 1.21 mg/L. In the first 23 days of this run, the NH_4^+-N removal and NO_2^--N accumulation were observed around 80% and 8%, respectively. Between the days 141-148, NH_4^+-N removal started to decrease while NO_2^--N accumulation was increasing. Starting from day 153, 40% NH_4^+-N removal and 39% NO_2^--N accumulation were observed in the effluent for 30 days. At day 184, NH_4-N removal started to increase without significant change in NO_2^--N accumulation. Till day 197, NH_4^+-N removal and NO_2^--N accumulation was around 58% and 42%, respectively. Between the days 197- 230, the steady-state conditions were deteriorated due to failure of the feed pump frequently. Therefore, the effluent measurements performed within this period are not shown in Figure IV.8. In the period of Run 4 started at day 231, the DO concentration in the reactor was around 1.45 mg/L. In this period (58 days), the NH_4^+-N removal was around 94-95%. Hovewer, the NO_2^--N accumulation, which was around 31% during first 29 days of this period (till day 259),exhibited a sudden decrease at day 260 and later it was observed around 4% till the end of this period (day 288). At day 289, Run 5 was started by decreasing the DO level in the reactor to 0.93 mg/L. In the first 50 days of this period (till day 338), the NH_4^+-N removal and the NO_2^--N accumulation were observed around 55% and 29%, respectively. At day 340, the reactor was inadvertently fed with an influent including extremely high NH_4^+-N concentration (approximately 360 mg/L) and hence the steady-state conditions deteriorated due to short-term inhibition of the system. The effluent measurements in this period are not shown in Figure IV.8. After two weeks, the system recovered itself and the effluent values were turned back into the normal values. Between the days 355-365, the NH_4^+-N removal and the NO_2^--N accumulation were clearly observed around 73% and 55%, respectively. If these values are compared with those observed at a similar DO level prior to inhibition (Run 3), it is obvious that NH_4^+-N removal increased and the NO_2^--N removal decreased. There may be two possible reasons of this situation. First, NOB species might be affected from the inhibition more seriously than the AOB species. Second, AOB/NOB ratio in the enriched nitrifying sludge might change after inhibition of the system.

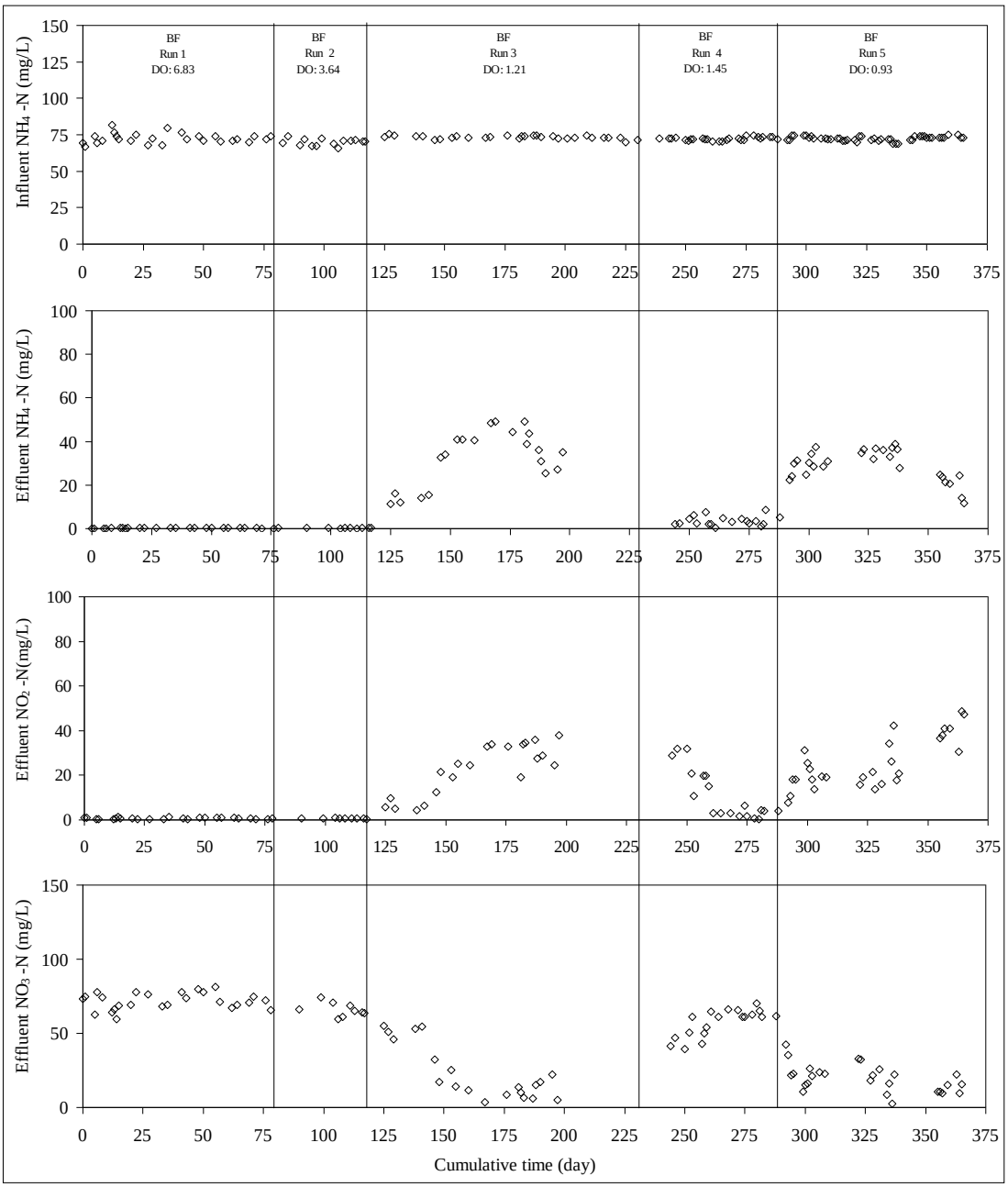


Figure IV. 8 Chronological illustration of daily influent and effluent of NH_4^+-N, NO_2^--N, and NO_3^--N measurements during continuous-flow nitrifying biofilm reactor (BF) experiments

The NH_4^+-N and NO_2^--N accumulation percentages observed during the 365 days operation of the enriched nitrifying biofilm system at gradually decreased DO levels were summarized in Table IV.2. As seen from Table IV.2, NO_2^--N accumulation and NH_4^+-N accumulation started simultaneously in the system at 1.21 mg DO /L. On the other hand, as discussed in Section IV.1, in the suspended-growth SBR system, which was also operated with gradually reduced DO levels for

365 days under same operational conditions with the biofilm system, NH_4^+-N and NO_2^--N accumulations were started simultenously at DO level of 0.54 mg /L. In the present study, the results obtained from continuous-flow SBR and biofim experiments were directly comparable with each other since the both systems include enriched nitrifying culture and were operated at gradually reduced DO levels for prolonged time. This comparison shows that in the attached-growth systems accumulation of NH_4^+-N and NO_2^--N starts at higher DO levels with respect to suspended-growth systems. This is mainly due to the fact that attached-growth systems are diffusion-limited systems as reported in the literature (Arvin and Harremoes, 1990; Metcalf and Eddy, 2003). In other words, in attached-growth systems the DO concentration in the biofilm is considerably less than the bulk DO concentration. Similar to the present study, in the previous studies performed to investigate the effect of DO on nitrite accumulation in attached-growth systems (Bernet et al., 2005; Park and Noguera, 2004; Kim et al., 2005; Garrido, et al., 1997), accumulation of NO_2^--N was observed at the DO concentrations in the range of 1-2 mg/L. As seen from Table IV.2, in the 66-days experimental period starting with decreasing DO level from 3.64 mg/L to 1.21 mg/L, NH_4^+-N accumulation initially showed a gradual increase and reached a stable value at the end of 23 days. The system kept this stable NH_4^+-N accumulation for 30 days. But, later NH_4^+-N accumulation decreased. Similarly, in the study of Bernet et al. (2001), as a result of transition from high DO to low DO levels, NH_4^+-N showed a very similar accumulation trend with the present study. Bernet et al. (2001) indicated that the reason of this observed trend might be related with the quantity changes in AOB species or a possible shift in dominant AOB species. Low growth rate of AOB species decrease more under oxygen limited conditions. Therefore, under limited DO conditions, it is logical to expect that doubling of AOB species proceed in the long term and accordingly a gradual increase in NH_4^+-N removal efficiency may occur. As seen from Table IV.2, as a result of increasing DO level from 1.21 mg/L to 1.45 mg/L, the NH_4^+-N accumulation significantly (about 87%) disappeared. But, in the later period in which the DO level was decreased to 0.93 mg/L, NH_4^+-N accumulation reappeared and similar to the trend observed at 1.21 mg DO/L steady-state NH_4^+-N accumulation observed 49 days decreased later. The NH_4^+-N accumulation decrease observed at DO level of 1.21 mg/L occurred in the similar time duration and almost in the same

Table IV.2. NH_4^+-N and NO_2^--N accumulation percentages observed in continuous-flow biofilm experiments

Exp. No.	Operation Period (day)	DO in the reactor (mg/L)	% NH_4^+-N Accumulation	% NO_2^--N Accumulation (in terms of influent NH_4^+-N)	% NO_2^--N Accumulation (in terms of removed NH_4^+-N)
BF Exp. No: 1	0-78	6.83±0.55	-	-	-
BF Exp. No: 2	79-117	3.64±0.23	-	-	-
BF Exp. No: 3	118-141	1.21±0.23	19	8	10
	153-183	1.21±0.23	60	39	96
	184-197	1.21±0.23	42	42	72
BF Exp. No: 4	231-259	1.45±0.14	5.5	31	32
	260- 288	1.45±0.14	5	4	4
BF Exp. No: 5	289- 338	0.93±0.25	45	29	52
	341	0.93±0.25	Inadvertent feeding of the system with an influent including extremely high NH_4^+-N concentration (approximately 360 mg/L)		
	342-355	0.93±0.25	Inhibition recovery period		
	355- 365	0.93±0.25	27	55	75

proportion with the NH_4^+-N accumulation decrease observed at DO level of 0.93 mg/L. This finding supports the above-mentioned hypothesis that relates the gradual decrase in NH_4^+-N accumulation with the lower groth rate of AOB species under limited DO conditions. The observation of lower NH_4^+-N accumulation in the period of 0.93 mg/L DO with respect to the previous period of 1.23 mg/L DO may be due to increasing substrate affinity of AOB species as a result of their long term exposure to limited DO conditions. Similarly, in the study of Park and Noguera (2007), it was reported that in low DO chemostats decreasing oxygen affinity of AOB species may be compensated by their increasing substrate affinity.

The NO_2^--N accumulation percentages (in terms of removed NH_4^+-N) given in Table IV.2 demonstrates that in the biofilm systems the effect of limited oxygen conditions on NOB species is different than the effect on AOB species. First of all, the NO_2^--N accumulation percentages were significantly higher than the NH_4^+-N accumulation percentages observed in the same periods. The main reason of this situation may be related with the localization of AOB and NOB species in the biofilm. As mentioned in many studies (Bernet et al., 2001; Garrido et al., 1997; Okabe and Watanabe, 2000), in attached-growth systems, AOB species usually located in the outer part of the biofilm while NOB species are located in the inner part of the biofilm. Accordingly, utilization of the bulk DO first by the AOB species located at the outer surface of the biofilm and the oxygen-diffusion limitation through biofilm thickness results in significantly lower DO levels in the inner part of the biofilm, where NOB species are located. Therefore, in attached-growth systems operating under oxygen limited conditions, NOB species activities are more severely affected from low DO levels than the AOB species activities. But, as seen from Table IV.2, at DO level of 1.21 mg/L, NO_2^--N accumulation occurred in parallel with the NH_4^+-N accumulation. This indirectly indicates that NO_2^--N accumulation that was mainly caused by the limited oxygen condition was also affected from FA concentration ranging between 0.4 - 0.5 mg/L depending on the NH_4^+-N accumulation in the system. Similarly, in the study of Kim et al. (2005) performed with an airlift biofilm reactor it was observed that NOB species significantly inhibited by FA concentration greater than 0.1 mg/L. As a result of increasing DO level from 1.21 mg/L from 1.45 mg/L, NH_4^+-N accumulation decreased suddenly in significant amount. In contrast to this, a gradual decrease in NO_2^--N accumulation was observed. Increasing bulk DO concentration in this period resulted in a decrease

in diffusion-limitation to the inner part of the biofilm, where NOB species presumably located. However, the quantity of NOB species in this period was most probably very low since their activity was seriously affected in the previous period (DO: 1.21 mg/L). Therefore, the gradual decrease observed in NO_2^--N accumulation at 1.45 mg DO/L may be related with the time period necessary for NOB species to increase in quantity. As a result of decreasing DO to the value of 0.93 mg/L, it was started to observe NO_2^--N accumulation in the system effluent. However, the observed NO_2^--N accumulation at 0.93 mg/L DO was slightly lower than at 1.23 mg/L DO.

In literature (Kim et al., 2005), oxygen half saturation constant of NOB species was reported around 1.1 mg/L. Therefore, the above finding indicate that either substrate or oxygen affinity of NOB species possibly increased due to their physiological adaptation upon exposure to low DO levels for prolonged time.

IV.4. CHARACTERIZATION OF THE MICROBIAL COMMUNITY IN THE SBR AND BIOFILM SYSTEMS

IV.4.1. Fluorescence In-Situ Hybridization (FISH) Results

In order to detect, identify and characterize the microbial community in the SBR and biofilm reactors, FISH technique was applied to the sludge samples taken from the reactors as the operation DO level was changed. The photomicrographs visualized by 4.6-diamidino-2-phenylindole (DAPI) staining for all microrganisms and by oligonucleotide probes of BET42a for *β-proteobacteria* species, NSO190 for *Ammonium oxidizing β-proteobacteria* species, NIT3 for *Nitrobacter* species, NTSPA662 for *Nitrospira* genus were shown in Figures A.1-A.4. In these figures, each photomicrograph couple represents the same field of the microscopic view and hence they are directly comparable with each other.

DAPI staining and hybridization results with BET42a (Figures A.1 and A.5) indicated that the dominating microbial populations both in the SBR and biofilm system belong to betasubclass of *Proteobacteria*. The comparison of the DAPI staining and hybridization signals of NSO 190 (Figures A.2 and A.5) revealed that *Nitrosomonas*-like ammonia oxidizing bacteria were the dominant beta *Proteobacteria* in both systems. This finding ensures that both the SBR and biofilm systems were enriched for nitrifiers. In the SBR system, hybridization signals

detected with NSO190 probe (Figures A.2) indicated that ammonia oxidizing bacteria formed spherically dense clusters. In contrast, in the biofilm system (Figure A.5), ammonia oxidizing bacteria formed small microcolonies in flocs.

In both systems, nitrite oxidizing species were examined with *Nitrobacter* specific probe NIT3 and *Nitrospira* specific probe NTSPA662. However, hybridization signals were only detected in the SBR system with NIT3 probe (Figure A.3). The signals detected in the SBR system showed that decreasing DO level with time resulted in a decrease in *Nitrobacter* population, which were observed as single scattered cells or small clusters.

IV.4.2. Slot Blot Hybridization Results

Slot-blot hybridization was performed with DNA isolates of SBR and biofilm sludge samples taken from the reactors as the operation DO level was changed. This was done to investigate the variations and activity of all microbial populations and also to quantify the relative abundance of microbial species. The results of slot-blot hybridization analyses of SBR and biofilm systems with oligonucleotide probes of EUB338 for Bacteria domain, NSO190 for *Nitrosomonas* species, NIT3 for *Nitrobacter* species, and NTSPA662 for *Nitrospira* species are shown in Figure IV.9. As seen from Figure IV.9, Nitrosomonas-like ammonia oxidizing bacteria (NSO 190) were predominant in both SBR and biofilm system. In contrast to FISH results, hybridization signals of *Nitrobacter* (NIT3) and *Nitrospira* (NTSPA662) species were observed for both systems. However, hybridization signals with NIT3 and NTSPA662 were considerably low. This indicates relatively low number of nitrite oxidizing species in both systems. Throughout the 365-days continuous operation of both SBR and biofilm system at gradually decreasing DO levels, no significant shift in the populations of ammonia and nitrite oxidizing species were observed.

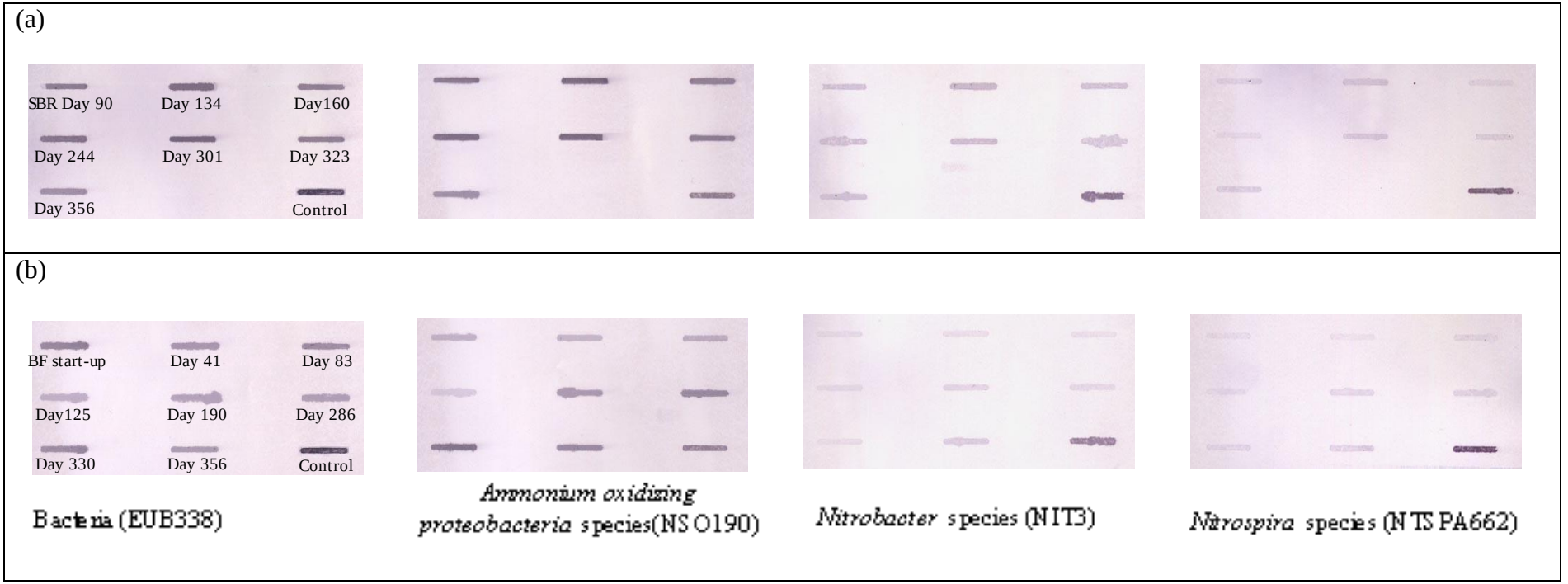


Figure IV. 9. Results of slot-blot analyses for (a) SBR system (b) biofilm system

CHAPTER V

CONCLUDING REMARKS and RECOMMENDATIONS

In the scope of this study, the partial nitritification/nitritification process based on dissolved oxygen concentration control was investigated in a suspended-growth SBR system and a submerged packed-bed biofilm system. Throughout the study, both systems including enriched nitrifying culture were operated under continuous-flow at similar temperature and pH conditions for 365 days. Additionally, the detailed kinetic analyses of the enriched nitrifying culture exposing the limited DO conditions for prolonged time was carried out via batch specific ammonium utilization rate and batch specific oxygen uptake rate experiments performed in the continuous-flow SBR system at certain time intervals. The major conclusions obtained from these experiments and the recommendations for future researches are summarized below:

1. In the SBR system, which was operated contiuously at DO levels gradually decreased from 7 mg/L to 0.22 mg/L, the accumulation of neither NH_4^+-N nor NO_2^--N were observed up to the DO level of 0.54 mg/L. However, at the DO levels of 0.54 mg/L and 0.22 mg/L, 10% NH_4^+-N - 9% NO_2^--N accumulations (10% in terms of removed NH_4^+-N) and 44% NH_4^+-N - 28% NO_2^--N accumulations (50% in terms of removed NH_4^+-N) were observed in the effluent, respectively.

In the present study, the SBR system was operated continuously with 24 hours batch cycles in which the reaction period was 16 hr. For the future studies, it is recommended that different batch cycles and/or reaction periods should be evaluated in order to maintain %50 NH_4^+-N -%50 NO_2^--N accumulation in the effluent, which is the required influent characteristic for the novel biological treatment processes such as Anammox, Canon and Oland.

2. The use of SCADA (Supervisory Control and Data Acquisition) system, which adjusts the on/off intervals of the air pump according to the continuously measured DO concentration in the system, was identified as a

successful method in order to keep the pre-defined DO value at a constant level through the reaction period in SBR sysytems.

3. The S_o/X_o value required to give extant kinetics of nitrifying culture in the SBR system was found less than or equal to 0.2.

4. During the 365-days experimental period, when the suspended-growth nitrifying culture were initially exposed to limited oxygen conditions the oxygen affinities of both AOB and NOB species decreased (i.e., their oxygen half saturation coefficients increased) but later as a result of their physiological adaptation to the limited DO conditions their oxygen affinities increased. At the beginning of the study, the oxygen half saturation coefficients of AOB ($K_{o,AOB}$) and NOB ($K_{o,NOB}$) species were observed as 0.54 mg/L and 0.72 mg/L, respectively. But, upon their exposure to the limited DO levels for prolonged time $K_{o,AOB}$ and $K_{o,NOB}$ almost equalized and both were observed around 0.3 mg/L.

5. In contrast to the previous studies in literature, the accumulation of NO_2^--N in the suspended-growth SBR system could not be related with the higher DO half saturation constant of NOB species with respect to AOB species. NO_2^--N accumulation started simultenously with the NH_4^+-N accumulation. Although the accumulation of NO_2^--N was mainly due to limited DO conditions, proportional increases observed in NH_4^+-N and NO_2^--N accumulations indicates the possible effect of FA concentration on NO_2^--N accumulation.

For the future studies in suspended-growth systems, it is recommended to investigate the short-term and long-term combined effect of limited DO and FA on nitrite accumulation.

6. When the nitrifying culture in the SBR system exposed to the limited DO condition (about 2.2 mg DO/L), initially NH_4^+-N and NO_2^--N accumulations were observed. But, in the later period, NH_4^+-N accumulation completely disappeared while the NO_2^--N accumulation decreased. In the same period, observation of the decreased oxygen affinities for both AOB and NOB species indicated that AOB and NOB species possibly increased their subsrate affinities ($K_{s,NH4-N}$ ve $K_{s,NO2-N}$) to compansate the decreasing oxygen affinities.

For the future studies, it is recommended to monitor changes in substrate half saturation constants of AOB and NOB species due to prolonged exposure to the limited DO conditions.

7. In the submerged packed bed biofilm system, which was operated contiuously at the DO levels gradually decreased from 6.83 mg/L to 0.93 mg/L accumulation of NH_4^+-N and NO_2^--N were observed at higher DO concentrations than the SBR sytem, indicating that diffusion-limited attached-growth systems are more sensitive to limited DO conditions than SBR systems.

In the present study, the NH_4^+-N and NO_2^--N accumulations started upon decreasing DO level from 3.64 mg/L to 1.21 mg /L. Therefore, this study could not allow monitoring the possible accumulations that would occur in the DO range of 3.64-1.21 mg/L. For the future studies, more gradual decrease from 3.64 to 1.21 mg DO/L and hence the monitoring of the process in this DO range is recommended.

8. In the biofilm system, at a constant DO level, initially observed NH_4^+-N and NO_2^--N accumulations showed changes in the long term. At the DO level of 1.21 mg/L, the initially observed NH_4^+-N and NO_2^--N accumulations of 60% NH_4^+-N- 39% NO_2^--N (96% in terms of removed NH_4^+-N) were changed into 42% NH_4^+-N -42% NO_2^--N (72% in terms of removed NH_4^+-N) in course of time. At the DO level of 0.93 mg/L, the initially observed NH_4^+-N and NO_2^--N accumulations of 45% NH_4^+-N - 29% NO_2^--N (52% in terms of removed NH_4^+-N) changed into 27% NH_4^+-N -55% NO_2^--N (75% in terms of removed NH_4^+-N) in course of time, even tough the system was inhibited by the excess ammonium loading for a short time in the same period.

9. In biofilm systems, at a constant DO level, NH_4^+-N accumulation exhibiting a stable trend may abruptly decrease depending on the quantity changes of AOB species with time.

10. In the biofilm system, NH_4^+-N accumulation observed at a DO value of 1.21 mg/L DO showed a decrease in the next period even though the DO level was decreased to the level of 0.93 mg/L. This finding indicates the possible physiological adaptation of the AOB species to the low DO levels as a result of their long-term exposure to the limited DO conditions.

11. In the biofilm experiments, under limited DO conditions, the NO_2^--N accumulation was always higher than the NH_4^+-N accumulation, indicating that the NOB species were affected from limited DO conditions more seriously than the AOB species. It is thought that this situation was mainly due to the location of AOB and NOB species in the biofilm. As mentioned in previous studies, in attached-growth systems, AOB species usually located in the outer part of the biofilm while

NOB species are located in the inner part of the biofilm. Accordingly, utilization of the bulk DO first by the AOB species located at the outer surface of the biofilm and the oxygen-diffusion limitation through biofilm thickness results in significantly lower DO levels in the inner part of the biofilm, where NOB species are located. Therefore, in attached-growth systems operating under oxygen limited conditions, the activities of NOB species are more severely affected from low DO levels than of AOB species, as observed in the present study.

For future studies, it is recommended that in addition to the bulk DO, level DO levels through the biofilm thickness should be monitored via DO microelectrodes. Additionally, investigation of spatial distribution of AOB and NOB species through the biofilm thickness via microbological molecular tools is also recommended.

12. In the biofilm experiments, although the accumulation of NO_2^--N was mainly due to limited DO conditions, proportional increases observed in NH_4^+-N and NO_2^--N accumulations indicated the possible effect of FA concentration on NO_2^--N accumulation.

For the future studies in attached-growth systems, it is recommended to investigate short-term and long-term combined effect of limited DO and FA on nitrite accumulation.

13. In the biofilm system, NO_2^--N accumulation observed at DO level of 1.23 mg/L showed a decrease in the later period during which the DO was around 0.93 mg DO/L. By considering the commonly reported oxygen half saturation constant of NOB species (around 1.1 mg/L), this finding indicates that NOB species possibly adapted to low DO levels as a result of their long-term exposure to the limited DO.

14. FISH analyses revealed that *Nitrosomonas*-like ammonia oxidizing bacteria were the dominant beta *Proteobacteria* in both systems. In the SBR system, ammonia oxidizing bacteria formed spherically dense clusters. In contrast, in the biofilm system (Figure IV.13), ammonia oxidizing bacteria formed small microcolonies in flocs. In the biofilm system, neither *Nitrobacter* nor *Nitrospira* species could be detected by FISH analysis. In the SBR system, unlike *Nitrospira* species, hybridization signals could be detected for *Nitrobacter* species. The decreasing DO level with time resulted in a decrease in *Nitrobacter* population, which were observed as single scattered cells or small clusters.

15. In slot blot analyses, contrary to FISH analysis, hybridization signals of *Nitrobacter* and *Nitrospira* species were observed for both systems. *Nitrosomonas*-like ammonia oxidizing bacteria were found predominant in both systems. However, *Nitrobacter* and *Nitrospira* species comprised a small fraction in the total Bacteria domain. Throughout the 365-days continuous operation of both SBR and biofilm system at gradually decreasing DO levels, no significant shift in the populations of ammonia and nitrite oxidizing species were observed.

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APPENDIX

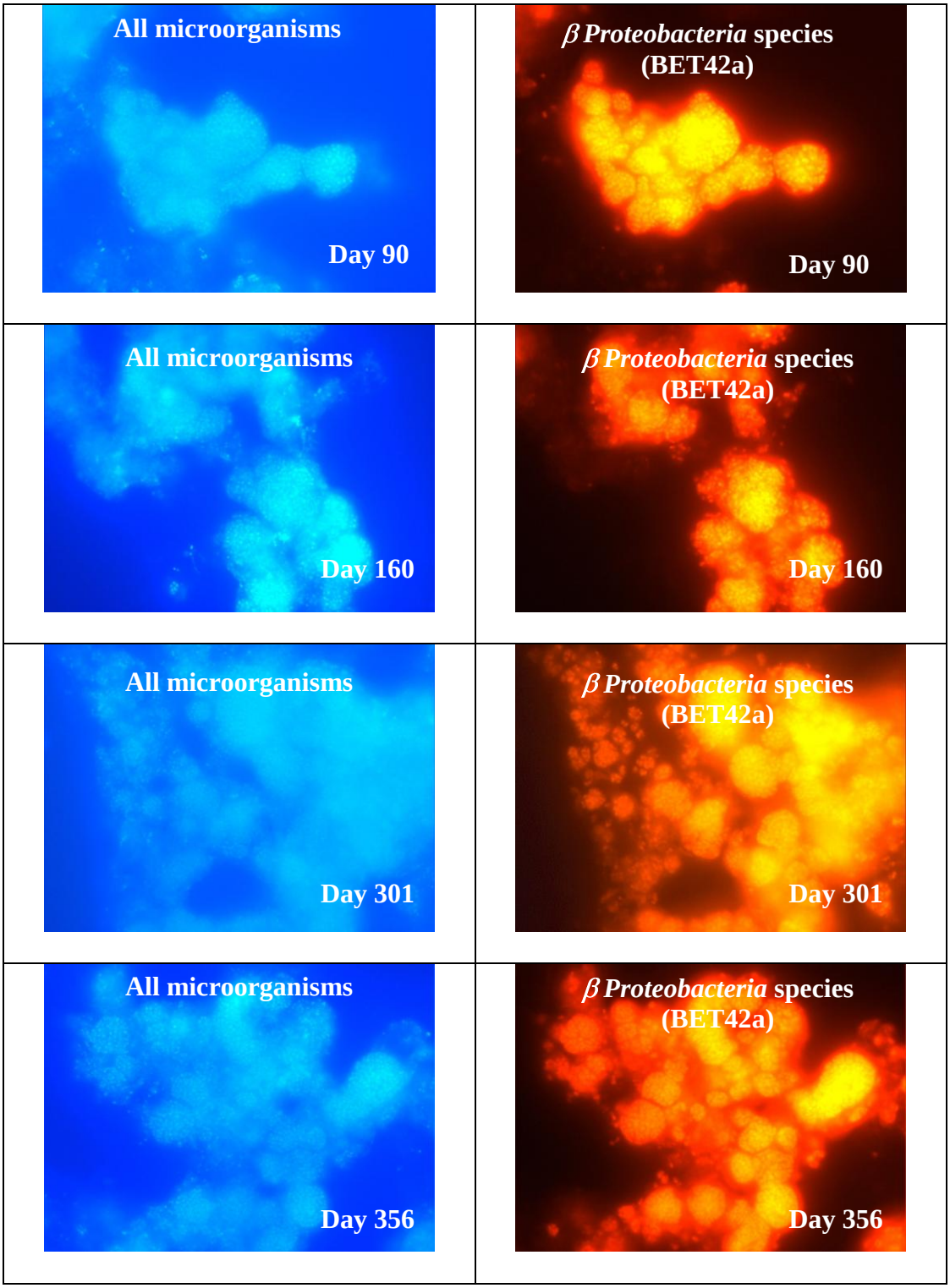

Figure A.1 All microorganisms and *β-proteobacteria* distribution in continuous-flow SBR system. DAPI and β-proteobacteria pictures were taken from the same microscopic field

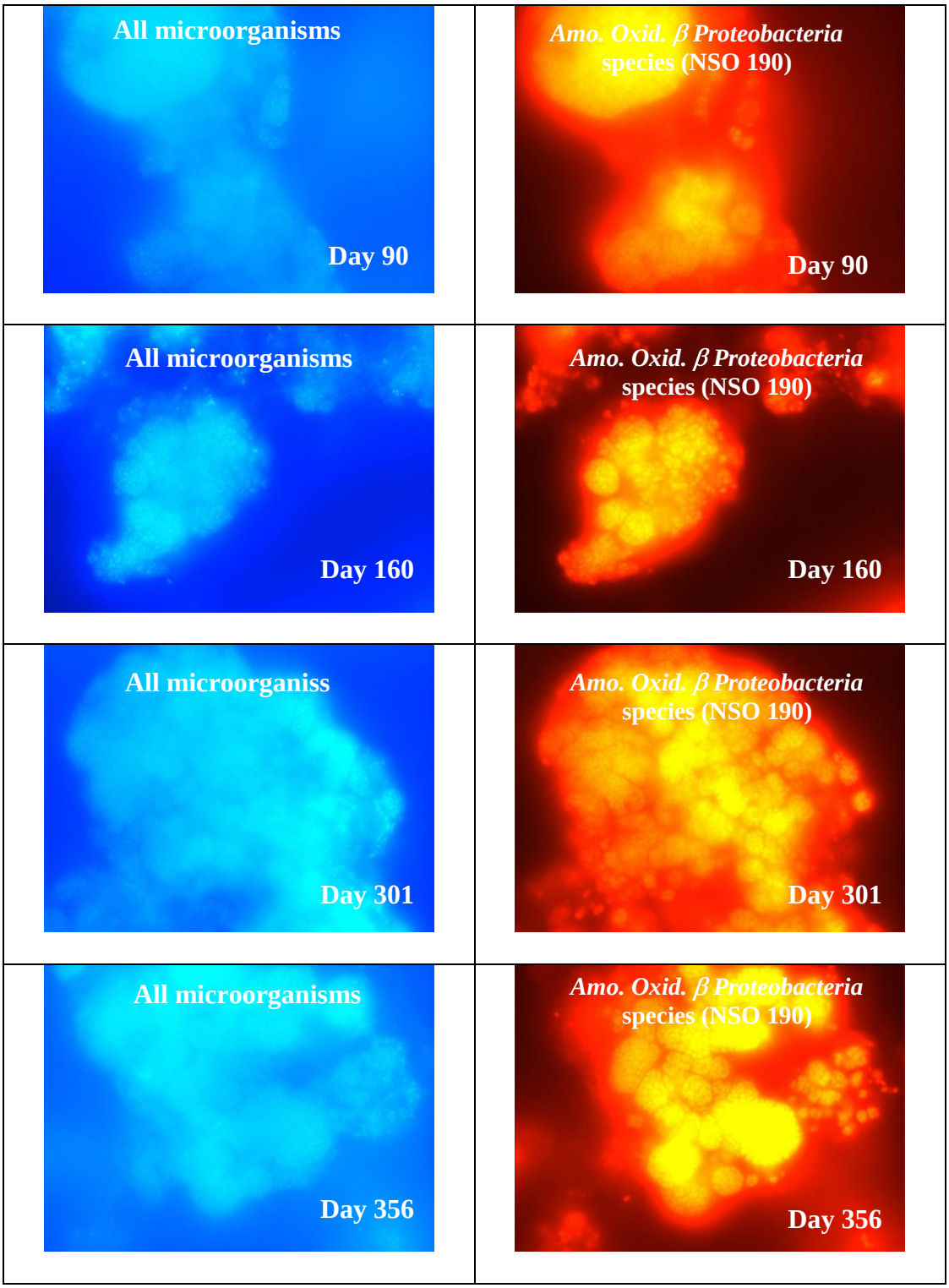

Figure A.2 All microorganisms and *Ammonium Oxidizing β-proteobacteria* distribution in continuous-flow SBR system. DAPI and β-proteobacteria pictures were taken from the same microscopic field

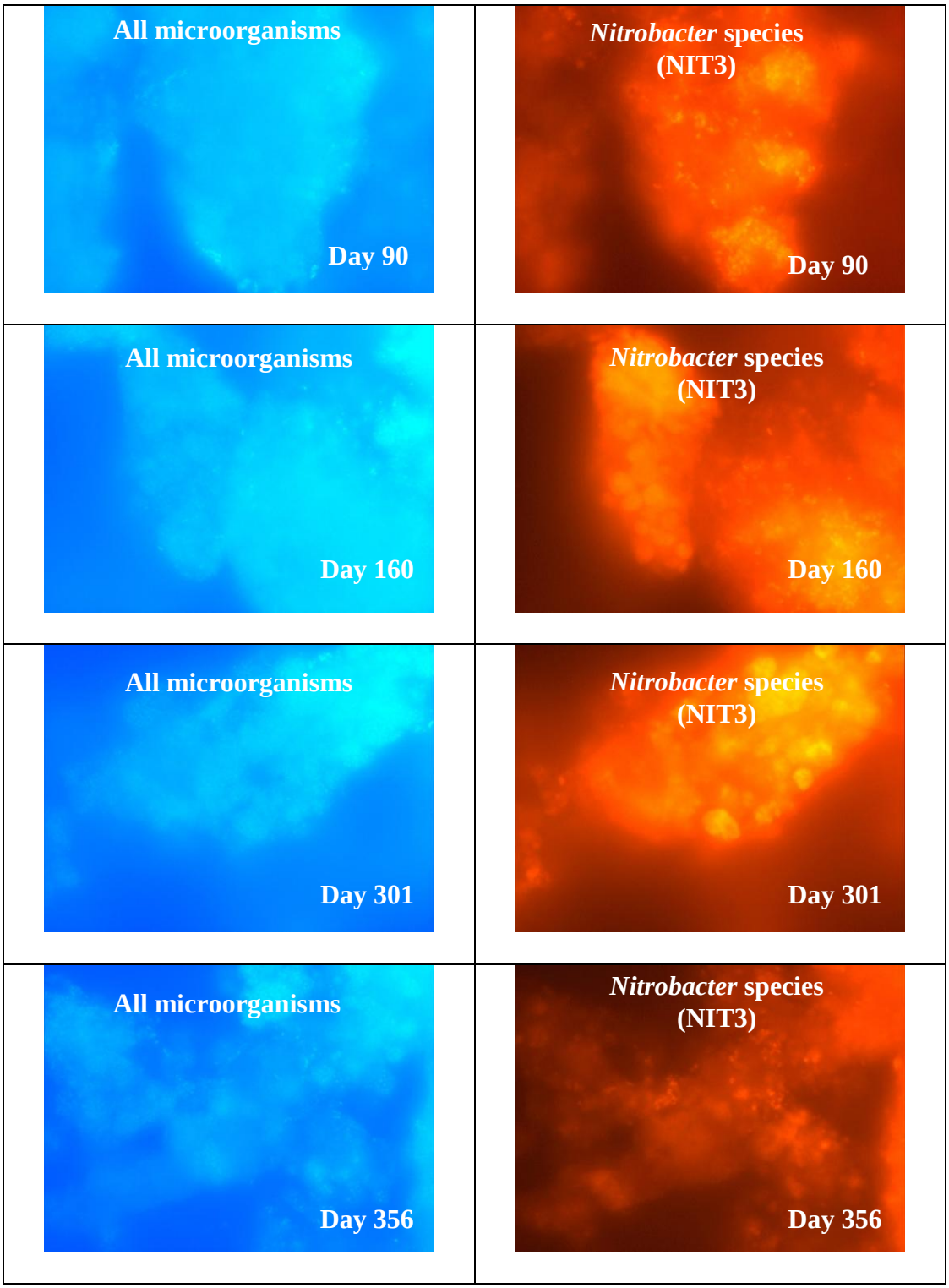

Figure A.3 All microorganisms and *Nitrobacter* distribution in continuous-flow SBR system. DAPI and β-proteobacteria pictures were taken from the same microscopic field

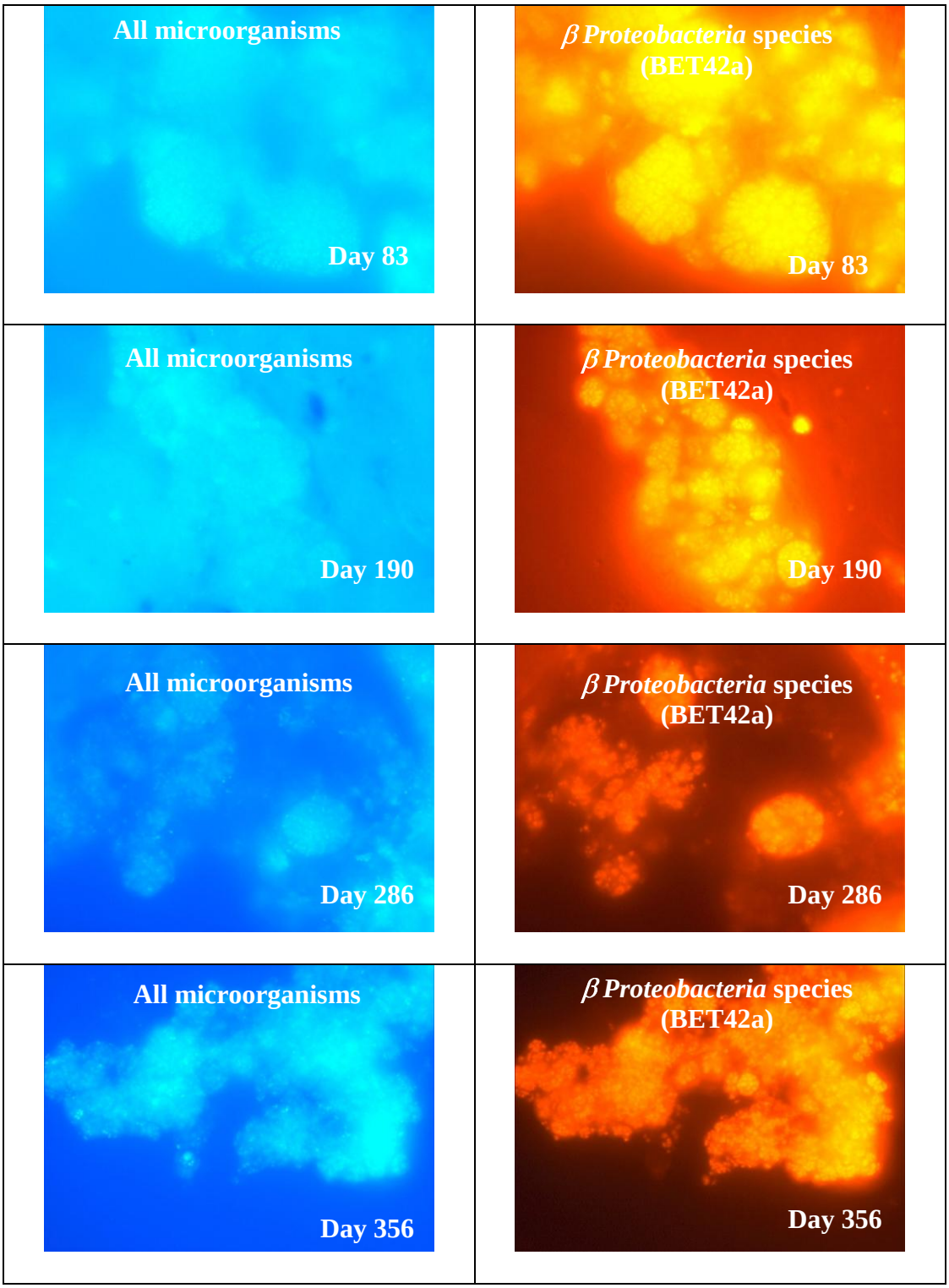

Figure A. 4 All microorganisms and *β-proteobacteria* distribution in continuous-flow biofilm system. DAPI and β-proteobacteria pictures were taken from the same microscopic field

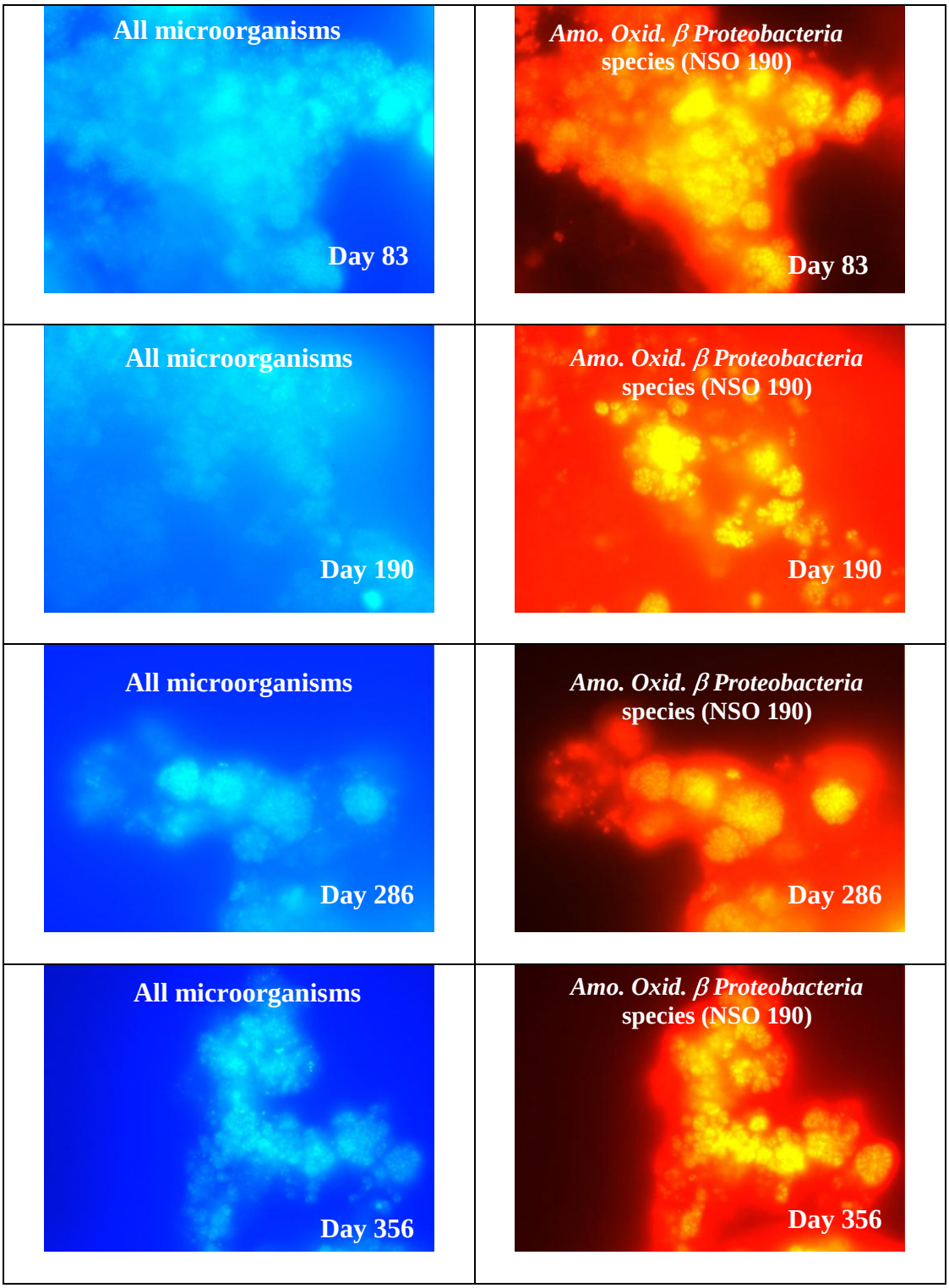

Figure A. 5 All microorganisms and *Ammonium Oxidizing β-proteobacteria* distribution in continuous-flow biofilm system. DAPI and β-proteobacteria pictures were taken from the same microscopic field.

AUTOBIOGRAPHY

I finished my primary, secondary and high school education in İstanbul. After the university degree in İstanbul University, Engineering Faculty, Environmental Engineering Department between 2000 -2004, I started to the master education in 2004 in Marmara University, Engineering Faculty, Environmental Engineering Department. I worked at "Gezer Endüstri İnşaat San. ve Ticaret A.Ş." as an environmental engineering between the years of 2004–2006. I am stil working at "ATS Arıtım Teknolojileri San. Tic. A.Ş." as an environmental engineering since 2007.