ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

AMMONIUM REMOVAL FROM WASTEWATERS CONTAINING HIGH AMMONIUM CONCENTRATIONS USING PARTIAL NITRIFICATION-DENITRIFICATION PROCESS

M. Sc. Thesis by
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JUNE 2005

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

YÜKSEK AMONYAK İÇEREN ATIKSULARDAN KISMİ NİTRİFİKASYON-DENİTRİFİKASYON PROSESİ AMONYAK GİDERİMİ

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ABBREVIATIONS

BOD	: Biochemical Oxygen Demand (mg/L)
COD	: Chemical Oxygen Demand (mg/L)
EPA	: Environmental Protection Agency
SS	: Suspended Solids (mg/L)
VSS	: Volatile Suspended Solids (mg/L)
SRT	: Sludge Retention Time
HRT	: Hydraulic Retention Time
DO	: Dissolved Oxygen
T	: Temperature
NH_3	: Free ammonia
NH_4^+	: Ammonium ion
O_2	: Oxygen
NO_2^-	: Nitrite
NO_3^-	: Nitrate
CH_3OH	: Methanol

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ΔG_S : free energy required for the synthesis of an electron equivalent of biomass, (kcal/e^- eq.)

ΔG_r : free energy released by the oxidation of an electron equivalent of the substrate,(kcal/e^-eq.)

ΔG_P : the energy for the conversion of the carbon source into pyruvate, (kcal/e^-eq.)

ΔG_n : the energy that would be required to reduce nitrate or nitrite to ammonia, (kcal/e^-eq.)

ΔG_C : the energy required for the conversion of the ammonia to the cell material, (kcal/e^-eq.)

m : energy constant,

Y : the ratio of the e^- equivalent of substrate utilized for the energy to the e- equivalent of the biomass,

Y_A : autotrophic yield coefficient

μ_A : spesific growth rate of nitrifying bacteria, $time^{-1}$

μ_{HD} : spesific growth rate of denitrifying bacteria, $time^{-1}$

$\hat{\mu}_A$: maximum spesific growth rate of nitrifying bacteria, $time^{-1}$

$\hat{\mu}_{HD}$: maximum spesific growth rate of denitrifying bacteria, $time^{-1}$

K_S : saturation constant numerically equal to the growth-limiting nutrient concentration at which $\mu = \frac{\mu_{\max}}{2}$, mass $volume^{-1}$

S : residual growth limiting nutrient concentration, mass $volume^{-1}$; for nitrification this is considered to be the energy source

S_S : concentration of the electron donor of denitrification process (mg COD/L)

S_{NO} : concentration of the nitrate nitrogen (mg NO_3^--N/L)

K_S : half-saturation constant for the organic material (mg COD/L)

K_{NO} : half-saturation constant for the nitrate nitrogen (mg NO_3^--N /L)

q : substrate utilization rate

f_X : conversion factor (1.42 g VSS/g COD)

f_E : the fraction of inert biomass

C_{SO}, C_S : the concentration of biodegradable organic matter in the influent and effluent of the wastewater

Δt : time

b_{HD} : endogenous decay constant for the denitrifying bacteria (day^{-1})

$b, (k_d)$: endogenous decay rate coefficient (day^{-1})

X_A : autotrophic biomass concentrtion (mg/L)

K_{OA} : half saturation constant for So function [$M(O_2)/L^3$].

μ_M : maximum growth rate of Nitrosomonas,day^{-1}

μ_S : maximum growth rate of Nitrobacter, day^{-1}

K_{SH} : saturation constant for the unionized substrate

K_{IH} : inhibition coefficient for the unionized substrate

K_{O_2} : oxygen saturation coefficient

$[NH_4^+]$: ammonia concentration

$[NO_2^-]$: nitrite concentration

$[O_2]$: DO concentration

$e^{(A_E/T)}$: equilibrium constant for the dissociation of the substrates, where A_E is the activation energy and T the absolute temperature.

NH_3 : Free ammonia

NH_4^+ : Ammonium ion

O_2 : Oxygen

NO_2^- : Nitrite

NO_3^- : Nitrate

CH_3OH : Methanol

AMMONIUM REMOVAL FROM WASTEWATERS CONTAINING HIGH AMMONIUM CONCENTRATIONS USING PARTIAL NITRIFICATION-DENITRIFICATION PROCESS

SUMMARY

Biological nitrogen removal process consists principally of the two sub-processes, nitrification and denitrification. In nitrification process, ammonium is oxidized to nitrate via nitrite under aerobic conditions, whereas in denitrification process, produced nitrate is reduced to nitrogen gas under anoxic conditions in the presence of a carbon compound as an electron acceptor. However, conventional removal of ammonium usually requires large amounts of energy for aeration and supply of organic carbon sources for denitrification. Ammonium removal over nitrite is a low-cost alternative to conventional nitrification-denitrification process in which ammonium is oxidized to nitrite; called as the *partial nitrification*; and produced nitrite is denitrified with addition of a carbon compound, particularly methanol. Utilization of the partial nitrification-denitrification process results in theoretically 25% less oxygen and 40% less methanol requirements compared to conventional nitrification-denitrification process. This process is reported to be a suitable option for wastewaters with a low carbon and high ammonium content such as sludge digester effluents and some industrial wastewaters.

In this study, nitrogen removal from wastewaters containing high ammonium concentrations was investigated for the determination of optimum process conditions by using partial nitrification-denitrification process. For this purpose, a nitrifying-denitrifying culture was acclimatized to synthetic wastewater in an intermittently aerated chemostat system. In the first stage of the experimental study, a nitrifying culture was enriched, which oxidized ammonium only to nitrite. During this period, system was examined for ammonium conversion as well as nitrite and nitrate formation at varying temperature and sludge age values. Denitrification of produced

nitrite under anoxic conditions with addition of a carbon compound was investigated after a stable partial nitrification stage was achieved.

YÜKSEK AMONYAK İÇEREN ATIKSULARDAN KISMİ NİTRİFİKASYON-DENİTRİFİKASYON PROSESİ AMONYAK GİDERİMİ

ÖZET

Biyolojik azot giderimi, amonyağın aerobik koşullarda nitrit üzerinden nitrata oksitlenmesi ve anoksik koşullarda elektron vericisi olarak organik maddenin kullanılması ile nitratın nitrit üzerinden son ürün olan azot gazına indirgenmesidir. Aerobik koşullarda nitrifikasyonun gerçekleşmesi sırasında, atıksuda mevcut olan organik madde de büyük ölçüde oksitlenir. Bu durum özellikle organik maddenin kısıtlı olduğu atıksularda, denitrifikasyon için gerekli olan karbon kaynağının yetersiz kalmasına neden olur. Bu nedenle amonyağın nitrat yerine nitrite kadar oksidasyonu ve denitrifikasyonun da nitrit azotundan azot gazına kadar yapılması, hem nitrifikasyonda sisteme verilmesi gereken oksijenin hem de denitrifikasyonda gerekli olan organik madde ihtiyacının azaltılmasını sağlayacaktır. Amonyağın sadece nitrite kadar oksidasyonu *kısmi nitrifikasyon* olarak adlandırılmakta ve nitritin nitrata oksitlendiği ikinci adımın engellenmesi ile elektron alıcısı olarak oksijen ihtiyacında yaklaşık %25'lik bir tasarruf sağlanmaktadır. Ayrıca, oluşan nitritin denitrifikasyonu için nitratın denitrifikasyonundan daha az organik madde gerekmektedir. Organik maddenin metanol olması durumunda teorik olarak %40 tasarruf sağlanmaktadır. Bu yöntem daha çok yüksek amonyak ve düşük karbon içeren çamur çürütme sistemlerinin çıkış suları ve gene aynı nitelikteki endüstriyel atıksular için uygulanabilir.

Bu projede yüksek amonyak düşük karbon içeren atıksuların kısmi nitrifikasyon-denitrifikasyon prosesleri ile arıtılması için optimum işletme koşullarının belirlenmiştir. Bu amaç doğrultusunda sentetik atıksu ile beslenen aralıklı havalandırmalı kemostat bir sistem kullanılmıştır. Sistem farklı çamur yaşlarında ve farklı sıcaklıklarda çalıştırılarak öncelikle nitrit oluşumu gözlenmiştir. Kısmi nitrifikasyonun sağlanması için düşük çamur yaşında pH ve sıcaklık izlenmiştir.

Sistemde denitrifikasyondaki organik madde ihtiyacını karşılamak üzere kolay ayrışabilir nitelikte sentetik madde olan metanol kullanılmıştır.

1. INTRODUCTION

Domestic and industrial wastewaters are being treated almost for a century to overcome the harmful affects on the receiving water bodies and on human health. Conventionally, only the treatment of carbonaceous materials in wastewater was concerned, until it was understood that the nitrogen and phosphorous contents were also important. These two inorganic compounds are nutrients and therefore their presence in excess amounts is the major reason for eutrophication, low DO level and death of fish in the receiving water. Besides, if the pH of the medium becomes basic, ammonia nitrogen is converted to the free ammonia form which has a toxic effect on fish even at low conditions.

The current studies on treatment of high ammonium containing wastewaters are mainly directed to the improvement of efficiency of the existent technologies. Since nitrification requires high amounts of energy, options like energy saving and also development of new technologies are being investigated to achieve the conversion of ammonium into harmless forms of nitrogen.

In this study, partial nitrification and complete denitrification of wastewaters containing high ammonium concentrations were studied. For this purpose, nitrifying culture was enriched by inoculating a mixed-culture seed taken from domestic wastewater into a fill and draw type reactor. Then a partial nitrification system was set-up and aerobic ammonium oxidizers were enriched in a continuous flow reactor. Reactor was acclimatized to high ammonia loading rates and finally denitrification was performed.

2. FUNDAMENTALS OF NITRIFICATION AND DENITRIFICATION

2.1 Forms of Nitrogen

Nitrogen naturally exists in various compounds with a valence ranging from -3 to +5. Transformations of nitrogen forms resulting in valence changes are associated with metabolic activities of different types of organisms. The various forms of nitrogen present in nature, and pathways by which these forms are changed, are schematically depicted in Figure 2.1 (Orhon and Arhan, 1994).

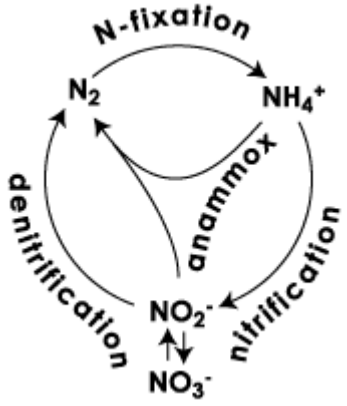

Figure 2.1 The Nitrogen Cycle

Ammonia nitrogen exists in aqueous solution as two different compounds - molecular or free ammonia (NH_3) and ammonium ion (NH_4^+). The ratio of the molar concentrations of these forms varies depending on the pH of the solution, in accordance with the following equilibrium reaction:

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (1)$$

As seen from Equation 1, the reaction balance will move leftward as pH increases, which results in enhancement of the free ammonia concentration and hence the reaction will move rightward as pH decreases (Peng et al., 2004).

Nitrite nitrogen is relatively unstable and easily oxidized to nitrate form. It is produced during the oxidation of ammonia nitrogen to nitrate nitrogen. Presence of nitrite in drinking water causes methahemoglobinemia (blue baby) disease (Tchobanoglous and Burton, 1991).

Organic nitrogen and ammonium are the main nitrogen forms in domestic wastewater.

The transformations of nitrogen in biological treatment processes are shown in Figure 2.1 (Tchobanoglous and Burton, 1991).

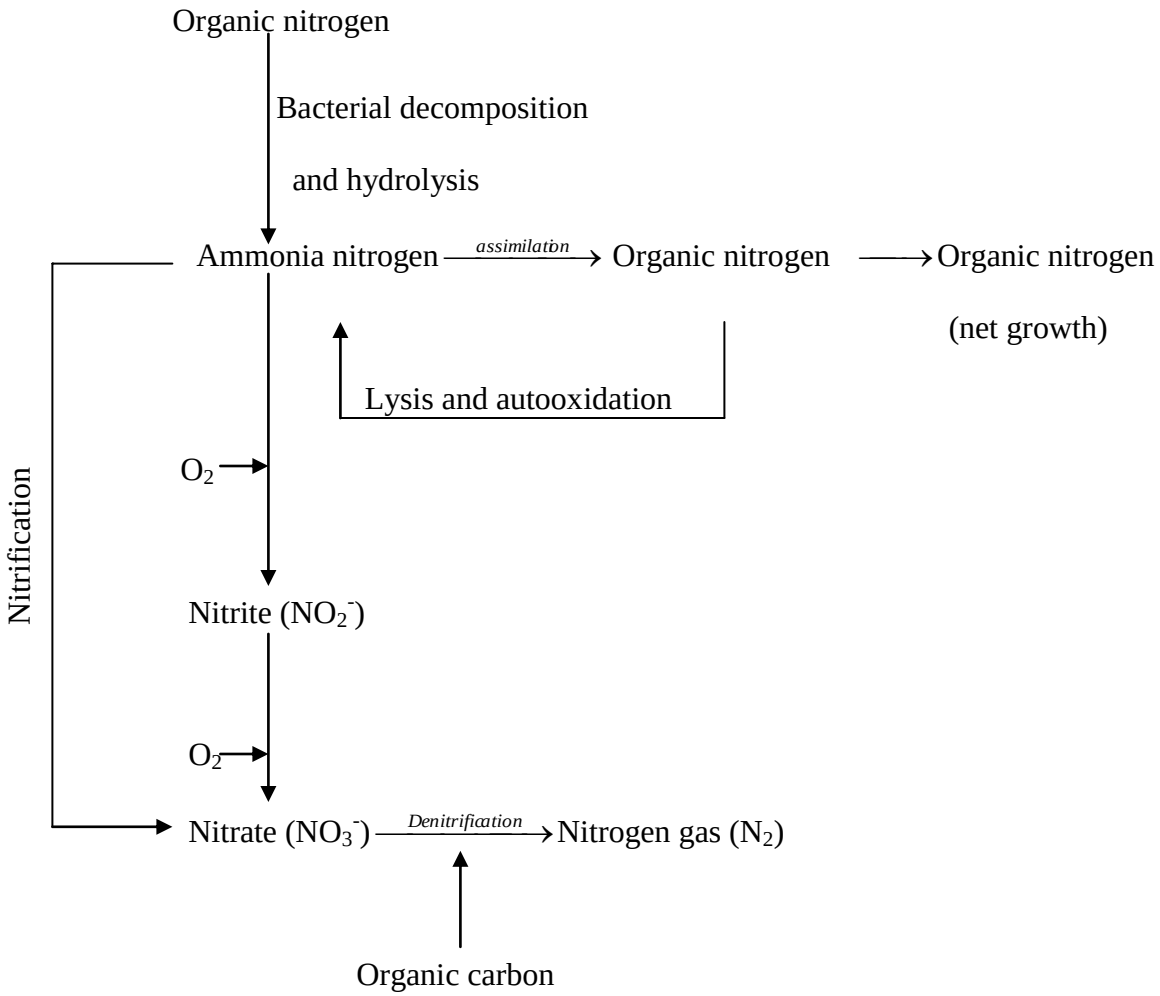

Figure 2.2 Nitrogen cycle in wastewater treatment

2.2 Biological Nitrification

In nitrification process, ammonium is oxidized to nitrate via nitrite under aerobic conditions by the two-step sequential reaction as follows:

$$2NH_4^+ + 3O_2 \xrightarrow{nitrosomonas} 2NO_2^- + 4H^+ + 2H_2O \qquad \text{(Nitritification)} \qquad (2)$$

$$2NO_2^- + O_2 \xrightarrow{Nitrobacter} 2NO_3^- \qquad \text{(Nitratification)} \qquad (3)$$

First step is called as Nitritification and second step is called as Nitratification. In the process of nitrification, 2 moles of oxygen are required to oxidize 1 mole of ammonia to nitrate which is a high oxygen requirement, and 2 moles of H^+ is produced per ammonia oxidized. Since nitrifiers are very sensitive to the environmental conditions, production of H^+ will decrease the pH of the media. Moreover, when pH of the media decrease below 6.5, then complete inhibition of nitrification takes place. Therefore alkalinity is required to buffer the pH of the reactor.

Under normal operational conditions of the treatment plants (5-20^oC), nitrifiers have relatively low growth rates and hence high sludge retention times are required for the nitrification process, which also necessitate large aeration volumes.

2.2.1 Microbiology of nitrification

Two physiological groups of microbes are largely responsible for nitrification, collectively called; the nitrifiers or the nitrifying bacteria. The nitrifying bacteria are autotrophs, chemolitotrophs and obligate aerobes. Inorganic carbon is used as the carbon source and inorganic nitrogen is used as the energy source. Together they mediate the two-step oxidation of ammonia to nitrite (autotrophic ammonia oxidizers) and nitrite to nitrate (autotrophic nitrite oxidizers). Autotrophic ammonia oxidizers have the prefix nitroso- and the autotrophic nitrite oxidizers have the prefix nitro- to distinguish between the two groups of microbes (Seviour and Blackal, 1998).

2.1.1.1 The Autotrophic Ammonia-Oxidizing or Nitroso Bacteria

The autotrophic ammonia-oxidizing bacteria are a group of obligatory chemoautotrophic Gram-negative bacteria that oxidize ammonia to nitrite to obtain energy (Seviour and Blackal, 1998).

Nitroso bacteria are all obligate aerobes, however some can apparently grow at reduced dissolved oxygen (DO) tensions (Seviour and Blackal, 1998). Nitrosomonas is the most recognized genus responsible for the oxidation of ammonia to nitrite, but Nitrosococcus, Nitrosospira, Nitrosovibrio and Nitrosolobus are also able to carry out this step (Seviour and Blackal,1998).

2.1.1.2 The Autotrophic Nitrite-Oxidizing or Nitro Bacteria

The autotrophic nitrite-oxidizing bacteria are not all obligate chemoautotrophs unlike the Nitroso bacteria (Watson et al., 1989). In fact, many strains of Nitrobacter can grow as heterotrophs, where both energy and carbon are obtained from organic carbon sources, or mixotrophically. These bacteria are collectively known as facultative chemoautotrophs, or lithoautotrophs (Seviour and Blackal, 1998).

Five genera, currently identified to be responsible for the oxidation of nitrite are Nitrobacter, Nitrospina, Nitrococcus, Nitrospira and Nitrocystis (Rittmann and McCarty, 2001).

2.2.2 Stoichiometry of nitrification

The stoichiometry of nitrification basically defines a chemoautotrophic process where NH_4^+ serves as the electron donor and oxidized to nitrate, O_2 as the electron acceptor and CO_2 as the carbon source. The half-reaction for the complete oxidation of NH_4^+ to NO_3^- may be shown as:

$$NH_4^+ + 3H_2O \rightarrow NO_3^- + 10H^+ + 8e^- \quad (4)$$

It shows that 1/8 mole or 1.75 g of NH_4^+-N is available in the process for 1 mole of electron equivalent. The oxidation of NH_4^+ involves both the energy and biosynthesis reactions. A portion of the electrons released by the oxidation of ammonia is transferred

to O_2 in the energy reaction, while the remaining is used as the reducing power to convert CO_2 to the oxidation level of cellular constituents. Assuming that $C_5H_7O_2N$ describes the composition of the autotrophic biomass, the following reactions may be developed to define the growth process in nitrification (Sözen, 1992)

First step:

Energy reaction:

	ΔG^o (kcal/e$^-$.e)	
$\frac{1}{6}NH_4^+ + \frac{1}{3}H_2O \rightarrow \frac{1}{6}NO_2^- + \frac{4}{3}H^+ + e^-$	7.852	(5)
$\frac{1}{4}O_2 + H^+ + e^- \rightarrow \frac{1}{2}H_2O$	-18.675	(6)
$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + 2H^+ + H_2O$	ΔG_r = -10.823	(7)

Biosynthesis reaction:

$$\frac{1}{6}NH_4^+ + \frac{1}{3}H_2O \rightarrow \frac{1}{6}NO_2^- + \frac{4}{3}H^+ + e^- \quad (5)$$

$$\frac{1}{4}CO_2 + \frac{1}{20}NH_3 + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{8}{20}H_2O \quad (8)$$

$$\frac{1}{6}NH_4^+ + \frac{1}{4}CO_2 + \frac{1}{20}NH_3 \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{1}{6}NO_2^- + \frac{1}{3}H^+ + \frac{1}{15}H_2O \quad (9)$$

Second step:

Energy reaction:

	ΔG^o (kcal/e$^-$.e)	
$\frac{1}{2}NO_2^- + \frac{1}{2}H_2O \rightarrow \frac{1}{2}NO_3^- + H^+ + e^-$	9.425	(10)
$\frac{1}{4}O_2 + H^+ + e^- \rightarrow \frac{1}{2}H_2O$	-18.675	(6)
$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^-$	ΔG_r = -9.250	(11)

Biosynthesis reaction:

$\frac{1}{2}NO_2^- + \frac{1}{2}H_2O \rightarrow \frac{1}{2}NO_3^- + H^+ + e^-$	(10)
$\frac{1}{4}CO_2 + \frac{1}{20}NH_3 + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{8}{20}H_2O$	(8)
$\frac{1}{2}NO_2^- + \frac{1}{4}CO_2 + \frac{1}{20}NH_3 + \frac{1}{10}H_2O \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{1}{2}NO_3^-$	(12)

By combining the half reactions above, the overall reactions for energy and synthesis for nitrification are obtained.

Overall energy reaction:

	ΔG^o (kcal/e$^-$.e)	
$\frac{1}{8}NH_4^+ + \frac{3}{8}H_2O \rightarrow \frac{1}{8}NO_3^- + \frac{10}{8}H^+ + e^-$	8.245	(13)
$\frac{1}{4}O_2 + H^+ + e \rightarrow \frac{1}{2}H_2O$	-18.675	(6)
$\frac{1}{8}NH_4^+ + \frac{1}{4}O_2 \rightarrow \frac{1}{8}NO_3^- + \frac{2}{8}H^+ + \frac{1}{8}H_2O$	ΔG_r = -10.43	(14)

Overall biosynthesis reaction:

$\frac{1}{8}NH_4^+ + \frac{3}{8}H_2O \rightarrow \frac{1}{8}NO_3^- + \frac{10}{8}H^+ + e^-$	(13)
$\frac{1}{4}CO_2 + \frac{1}{20}NH_3 + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{8}{20}H_2O$	(8)
$\frac{1}{8}NH_4^+ + \frac{1}{4}CO_2 + \frac{1}{20}NH_3 \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{1}{6}NO_3^- + \frac{2}{8}H^+ + \frac{1}{40}H_2O$	(15)

As it is seen from the above reactions, at each step, a portion of the oxidized nitrogen is used for the energy reactions, and the rest is used for the synthesis reactions.

The distribution of electrons transferred from electron donor to the electron acceptor and synthesized biomass is shown (percentage of specific conversion rate) as;

$$Y = \frac{Transferable\ electrons\ in\ the\ synthesized\ biomass}{Transferable\ electrons\ in\ the\ energy\ source} \quad (16)$$

Y_A is the autotrophic yield coefficient which represents the amount of biomass production per unit amount of substrate utilized. The theoretical calculation of Y_A can be demonstrated by the use of following expressions and chemical reactions:

$$\Delta G_S = \frac{\Delta G_P}{k^m} + \frac{\Delta G_n}{k} + \Delta G_C \qquad (17)$$

where,

ΔG_S = free energy required for the synthesis of an electron equivalent of biomass, (kcal/e$^-$eq.)

ΔG_r = free energy released by the oxidation of an electron equivalent of the substrate,(kcal/e$^-$eq.)

ΔG_P = the energy for the conversion of the carbon source into pyruvate, (kcal/e$^-$eq.)

ΔG_n = the energy that would be required to reduce nitrate or nitrite to ammonia, (kcal/e$^-$ eq.)

ΔG_C = the energy required for the conversion of the ammonia to the cell material, (kcal/e$^-$eq.)

m = energy constant, (if G_P>0, m = 1; if G_P<0, m = -1)

k = energy transfer efficiency (0.4-0.8)

As the oxidation of electron donor is achieved through pyruvate, a significant part of the energy is consumed during the conversion of the CO_2 to pyruvate. The half reactions between the electron acceptor and the pyruvate are;

	ΔG^o(kcal/e$^-$eq)	
$\frac{1}{4}O_2 + H^+ + e^- \rightarrow \frac{1}{2}H_2O$	18.675	(6)
$\frac{1}{5}CO_2 + \frac{1}{10}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{10}C_3H_3O_3^- + \frac{2}{5}H_2O$	8.545	(18)

From the equations 6 and 18, it is found that ΔG_P = 27.22 kcal/e$^-$eq. When the ammonia is used as the nitrogen source in the synthesis reaction, ΔGn becomes equal to 0. The values for ΔGr and ΔG_C are obtained from the energy reactions as -10.43 kcal/e$^-$eq. and

7.5 kcal/e$^-$eq., respectively. Since $\Delta G_P > 0$, m = +1 and k value is suggested to be taken as 0.6, are put in the eqution 17 (Orhon and Arhan, 1994).

$$\Delta G_S = \frac{27,22}{0,6^{+1}} + 0 + 7,5 = 52,87 kcal / e^- eq.$$

Y_A is then calculated via the following equation:

$$Y_A = \frac{1}{1+A}$$

where;

A = the ratio of the e$^-$ equivalent of substrate utilized for the energy to the e- equivalent of the biomass, and;

$$A = -\frac{\Delta G_S}{k \Delta Gr}$$

Rearranging the expressions above yields;

$$A = -\frac{\Delta G_S}{k \Delta Gr} = \frac{52.87}{0.6 * 10.43} = 8.45$$

$$Y_A = \frac{1}{1+A} = \frac{1}{1+8,45} = 0,106 e^- eq.cell / e^- eq.N$$

2.2.2.1 Oxygen requirement

From the overall reactions, it can be seen that, oxygen and alkalinity are required for the ammonium conversion process. As the energy reaction (Eq.14) implies, for each mole of ammonia that will be oxidized, 2 moles of oxygen is required. Therefore, 1 mg NH_4^+-N needs 4.57 mg O_2 to be oxidized. On the other side, according to the experimental studies, it was observed that, for each mole of nitrate produced, 4.33 g of oxygen is consumed (Sözen, 1992).

The oxygen requirement due to the growth of nitrifiers may be expressed as:

Net O_2 requirement = (Theoric O_2 required to oxidize NH_4^+-N) – (experimental O_2 required to oxidize ammonium)

$$4.57 - 4.33 = 0.24 gO_2 / gN$$

$0.24 * 1.75 / 8 = 0.0525 e^-$ equivalent cell COD / e^- equivalent N

$$Y_A = 0.24 * 5.65 / 8 = 0.17 gVSS / gN \quad (19)$$

The above expression yields the stoichiometric coefficient that relates O_2 to the growth of nitrifiers in the kinetic description of nitrification (Sözen, 1992).

2.2.2.2 Alkalinity consumption

The energy equation shows that, for each mole of ammonia (NH_4^+) oxidation, 2 moles of H^+ is produced.

$$2 moles H^+ / mole NH_4^+ - N = 100 gCaCO_3 / 14 gN$$

$$= 7.14 gCaCO_3 / 14 gN$$

The overall equation predicts that 2 moles of HCO_3^- are consumed per mole of NH_4^+-N. This is equivalent to a reduction of 7.14 g $CaCO_3$ per g of NH_4^+-N. Alkalinity requirement is an important factor in the design of nitrifying activated sludge systems because nitrifiers can grow efficiently over a relatively narrow pH range. Consequently, the process will consume alkalinity equivalent to the amount of protons released. Furthermore there will be an additional reduction in the alkalinity corresponding to the ammonia conversion, which is used as the nitrogen source for biosynthesis and converted into organic nitrogen in cellular constituents (Sözen, 1992):

$$H^+ + HCO_3^- \rightarrow CO_2 + H_2O \quad (20)$$

$$NH_4^+ + HCO_3^- \rightarrow organic N + CO_2 + H_2O \quad (21)$$

2.2.3 Kinetics of nitrification

The main processes in nitrification kinetics are; growth of autotrophs, decay of autotrophs, hydrolysis of particular organic nitrogen and ammonification of the soluble organic nitrogen.

2.2.3.1 Growth of autotrophs

During nitrification process, the rate of ammonia oxidation depends on the growth of microorganisms. Monod equation is often used to define the relationship between active biomass and primary substrate:

$$\mu = \hat{\mu}\frac{S}{K_s + S} \tag{22}$$

where,

μ = spesific growth rate of nitrifying bacteria, time^{-1}

$\hat{\mu}$ = maximum spesific growth rate of nitrifying bacteria, time^{-1}

K_s = saturation constant numerically equal to the growth-limiting nutrient concentration at which $\mu = \frac{\mu_{max}}{2}$, mass volume^{-1}

S = residual growth limiting nutrient concentration, mass volume^{-1}; for nitrification this is considered to be the energy source

b = endogenous decay rate coefficient (day^{-1})

Rate limiting substrate for Nitrosomonas is the ammonia nitrogen and likewise the nitrite nitrogen for Nitrobacter (Orhon and Arhan, 1994). Nitrobacter can grow faster than Nitrosomonas at 20 ^{o}C (Rittman and McCarty, 2001). Therefore, within the whole nitrification process, the oxidation of the ammonia nitrogen to nitrite nitrogen is the rate limiting step.

The net specific growth rate expression can be shown as the following equation, which also includes the endogenous decay rate (Orhon and Arhan, 1994):

$$\mu = (Yxq) - k_d \quad (23)$$

where,

Y = growth yield

q = substrate utilization rate

k_d = endogenous decay rate coefficient (day^{-1})

X_A = autotrophic biomass concentration (mg/L)

Many organisms need energy for cell maintenance. The measured growth yield, Y must be corrected by considering the amount of cell decay during the declining phase of growth. This will give the true growth yield coefficient, which is lower than the measured yield (Tchobanoglous and Burton, 1991).

According to energy and biosynthesis reactions, the oxidation of ammonia to nitrate require high amount of energy, which is compensated by the energy produced during the reduction of oxygen. Besides this, CO_2 needs energy to be reduced to pyruvate. Thus, the energy, which can be used for cellular synthesis is very low compared to aerobic heterotrophs. Due to this lower yield, the maximum specific growth rates are also low. This value is less than 1 d^{-1} at 20^oC for both groups (Rittmann and McCarty, 2001).

The concentration of nitrifiers (X_A) in a reactor is defined as the following expression;

$$\frac{dX_A}{dt} = \mu_A X_A - b_A X_A = \mu_A \frac{S_{NH}}{K_{NH} + S_{NH}} X_A - b_A X_A \quad (24)$$

As seen from the above expression, the change in the nitrifier biomass is described with the growth and the endogenous decay.

Specific growth rate of nitrifying bacteria is also affected by: (1) concentration of the electron acceptor (i.e., molecular oxygen), (2) operating temperature, and (3) operating pH.

2.2.3.2 Effect of dissolved oxygen (DO)

Dissolved oxygen is an essential nutrient for nitrification. Downing and Scragg (1961) reported a S_O of 0.2 mg/l as the level at which nitrification ceased. The effect of DO level on nitrification is best evaluated in terms of its impact on the growth kinetics. It is generally accepted that as S_O decreases, it becomes the growth limiting substrate. This is expressed by a double saturation function relating the rate of autotrophic biomass growth to S_{NH} and So:

$$\mu_A = \mu_A \left(\frac{S_{NH}}{K_{NH} + S_{NH}} \right) \left(\frac{S_O}{K_{OA} + S_O} \right) \tag{25}$$

where, K_{OA} is the half saturation constant for So function [$M(O_2)/L^3$].

The two major steps of nitrification are reported to exhibit different responses to dissolved oxygen variations as shown in Table 2.1, which shows Nitrobacter is somewhat more affected by low DO concentration than Nitrosomonas. In support of this observation, Shoberl and Engel found that pure cultures of Nitrosomonas europea were unaffected by DO concentrations of more than 1 mg/L at 30°C, while pure cultures of Nitrobacter winogradskii showed reduced activity below an S_O of 2 mg/L.

Similarly, it was found that maximum nitrification rate was achieved at the DO concentrations of 2.5 mg/L (Yoo et al., 1998). Likewise, Carera et. al. (2002), observed that, at 3mg O_2/L dissolved oxygen concentration, it is possible to remove high concentrations of ammonia nitrogen at a very high nitrification rate. Besides, Uygur et al.(2004) reported that 31% of ammonia removal efficiency was achieved in an SBR reactor for wastewaters with high concentrations of ammonia, at 2 mg/L DO concentration. However, Antileo et al. (2002), studied at 6 mg O_2/L DO value with high strength ammonia and chloride contained wastewaters and observed that nitrification could be achieved with the selected microflora. Campos et al. (2001), found that over 2 mg/L DO values nitrification of wastewaters containing high strength ammonia and salinity was achieved.

Table 2.1 Relationship between Dissolved Oxygen Concentrations and Growth Constants of Nitrosomonas and Nitrobacter at 18,8^{o}C (Antileo et al., 2002)

Average Dissolved oxygen concentration (mg/L)	Max. growth rate day $^{-1}$		Half saturation constant (mg/L)	
	μ_M (Nitrosomonas)	μ_S (Nitrobacter)	K_M (Nitrosomonas)	K_S (Nitrobacter)
8.4	0.7	0.9	0.6	1.5
4.7	0.7	1.1	0.6	1.7
3.4	0.7	0.8	0.6	1.7
2.0	0.7	0.9	0.6	2.0
1.4	0.6	0.67	0.6	1.9
0.6	0.5	0.6	3.0	2.5

2.2.3.3 Effect of pH

The rate of nitrification is extremely sensitive to the pH of the growth medium for two main reasons. First, there is a significant inhibitory action of both the hydrogen [H^+] and hydroxyl, [OH^-] ions on the growth rate of nitrifiers. Second, nitrification consumes alkalinity in the medium, with a potential drop in pH. Experimental results show that in mixed cultures, such as activated sludge, there is an optimum pH range of 7.5 to 8.5 for the growth of nitrifiers. Downing and Knowles (1964) suggested the following equation to calculate the effect of pH on the maximum growth rate of Nitrosomonas, for pH values below 7.2:

$$\mu_{ApH} = \mu_A[1 - 0{,}833(7{,}2 - pH)] \quad (26)$$

Orhon and Artan have assumed μ_A to be constant for a pH range between 7.2 and 8.5 and did not consider the values above 8.5.

Different pH ranges are reported for nitrification in the literature as 6.0 to 9.0 (Koops and Moller, 1992), 5.8 to 8.5 (Watson et. al., 1989) and 7.8 to 8.3 (Antileo et. al., 2002). The optimal pH is reported to be around 7.5 (Carera et. al., 2002, 2003). Yoo et al., (1998) recommended the optimal pH value of 7.

Growth and activity of nitrifying bacteria decrease dramatically below the neutral pH. However, the pH value is a parameter indicating a potential limitation, but is not a limiting factor itself (Wett et. al., 2003). The pH has no effect on nitrification in the range 8.65 to 6.35, but at a pH lower than 6.35 or over than 9.05, complete inhibition of nitrification takes place (Ruiz et al., 2003).

2.2.3.4 Effect of temperature

As in most biochemical reactions, temperature significantly effects the nitrification kinetics. Experimental observations also suggest that the effect of temperature on μ_A may be expressed by an Arrhenius-type equation, in the range of 7-30°C:

$$\mu_{AT} = \mu_{A20}\theta^{T-20} \tag{27}$$

where, θ = temperature constant and lies between 1.08 and 1.123 (Orhon and Arhan, 1994).

A similar temperature function is also suggested for the decay coefficient, where b_A is expressed as;

$$b_{AT} = b_{A20}(1{,}029)^{T-20} \tag{28}$$

However, it is very difficult to experimentally substantiate this temperature dependency for b_A, as evidenced by the widely varying observations in the literature (Orhon and Arhan, 1994).

Extensive characterization of nitrifiers reveals that their optimal growth temperature lies between 25-30°C (Bock et al., 1986; Watson et al., 1989; Koops and Moller, 1992) and

30-35°C (Blackburn, 1983) but a much wider temperature range of 5-30°C will support growth (Watson et al, 1989). Antileo et al. (2002) found that at 30^{o}C, high ammonia conversion rates were achieved. Besides, Campos et. al.(2001) found that high ammonia conversion to nitrate was achieved at a constant temperature of 20 ^{o}C. Carera et. al. (2002), observed very high ammonia loading rate values at 15^{o}C, 20^{o}C and 25^{o}C which were 0.10, 0.21 and 0.37 g NH_4^+-N/gVSSd, respectively. The maximum nitratation activity was achieved when the temperature was below 15 ^{o}C (Yoo et al, 1998).

2.2.3.4 Effect of inhibitors

Aside from environmental factors exemplified by dissolved oxygen, pH and temperature; nitrifiers are affected by a number of organic and inorganic compounds which inhibit nitrification. Campos et al.(2001), found that over 525 mM salt concentration, ammonia accumulation started and nitrification efficiency decreased sharply. Oslislo et al. (1983), reported that methanol, formalin and acetone can inhibit the nitrification process with the inhibition constants (Ki) of; 116.0 mg/L, 61.5 mg/L and 804.2 mg/L, respectively. However, glucose when applied with concentrations up to 11.325 mg/L, had no effect on the nitrification process. Beg et al, (1987) found that trivalent arsenic, hexavalent chromium and flouride can inhibit the nitrification process.

2.2.3.5 Effect of C/N ratio

High organic loadings decrease the growth of autorotrophic bacteria. This results in excess oxygen requirement. Thus, any increase in C/N ratio, results in a decrease in the fraction of nitrifiers in the activated sludge system. Table 2.2 summarizes the relationship between the fraction of nitrifying organisms and BOD_5/TKN ratio.

Table 2.2 The relationship between the fraction of nitrifying organisms and the BOD_5/TKN ratio. (Tchobanoglous and Burton, 1991)

BOD_5/TKN ratio	Nitrifier fraction	BOD_5/TKN ratio	Nitrifier fraction
0.5	0.35	5	0.054
1	0.21	6	0.043
2	0.12	7	0.037
3	0.083	8	0.033
4	0.064	9	0.029

2.3 Biological Denitrification

Denitrification, is the biological reduction of nitrate (and nitrite) to gaseous products, namely N_2, NO, and N_2O by anaerobically respiring chemoheterotrophs. The process is achieved in the presence of organic carbon source under anoxic conditions. In this system, the nitrate, nitrite and other nitrogen oxides, are used as electron acceptors instead of oxygen (Seviour and Blackal, 1998):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (29)$$

2.3.1 Microbiology of denitrification

A wide taxonomic range of bacteria can denitrify and all are aerobes, which have an alternative method for carrying out electron transport phosphorylation by reducing nitrogen oxides if O_2 becomes limiting. A diverse group of Gram-negative and Gram-positive heterotrophic and autotrophic bacteria are capable of denitrifying, and most will use nitrate if available, in preference to nitrite as an electron acceptor. They are therefore facultative anaerobes. The autotrophic denitrifiers will use either bicarbonate or CO_2 as

their carbon source, while the heterotrophic denitrifiers depend upon an organic carbon source, and their overall growth during denitrification will therefore depend upon both the nature and concentration of the carbon source (Seviour and Blackal, 1998).

As it is evident that phosphorus uptake by poly-P organisms occur in anoxic zones of nutrient removal systems, poly- P organisms are capable of denitrification. Some of the poly P organisms can use nitrate as an electron acceptor some of them can use it as an electron acceptor. However, indications are that not all poly-P organisms have this ability (Seviour and Blackal, 1998).

2.3.2 Stoichiometry of denitrification

It is necessary to know the electron donor of the denitrification process to define the stoichiometry of the denitrification process. Three different sources as carbonaceous electron donors may be envisaged for denitrification of wastewaters:

1. An external carbonaceous energy source, added at the denitrification stage of the process
2. An internal carbonaceous energy source, organic matter present in wastewater
3. An endogenous energy source generated through death and lysis of biomass in the endogenous decay phase.

Using methanol as the carbon source, the stoichiometry of separate-stage denitrification can be described through the energy reactions presented in the following equations:

Overall energy reaction for the methanol as an electron donor:

	ΔG^o (kcal/e$^-$eq.)	
$\frac{1}{6}CH_3OH + \frac{1}{6}H_2O \rightarrow \frac{1}{6}CO_2 + H^+ + e^-$	-8,965	(30)
$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- \rightarrow \frac{1}{10}N_2 + \frac{3}{5}H_2O$	-17,1128	(31)
$\frac{1}{6}CH_3OH + \frac{1}{5}NO_3^- + \frac{1}{5}H^+ \rightarrow \frac{1}{6}CO_2 + \frac{1}{10}N_2 + \frac{13}{30}H_2O$		(32)

Biosynthesis Reaction:

$$\frac{1}{6}CH_3OH + \frac{1}{6}H_2O \rightarrow \frac{1}{6}CO_2 + H^+ + e^- \quad (30)$$

$$\frac{1}{4}CO_2 + \frac{1}{20}NH_3 + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7NO_2 + \frac{8}{20}H_2O \quad (33)$$

$$\frac{1}{6}CH_3OH + \frac{1}{4}CO_2 + \frac{1}{20}NH_3 \rightarrow \frac{1}{20}C_5H_7NO_2 + \frac{7}{30}H_2O \quad (34)$$

As the oxidation of the organic matter occurs via pyruvate, half reactions between pyruvate and electron donor can be presented as;

$$\frac{1}{6}CH_3OH + \frac{1}{6}H_2O \rightarrow \frac{1}{6}CO_2 + H^+ + e^- \quad (30)$$

$$\frac{1}{5}CO_2 + \frac{1}{10}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{10}C_3H_3O_3^- + \frac{2}{5}H_2O \quad (18)$$

From these reactions ΔG_P is calculated as -0.42 kcal/e^-eq. When ammonia is used as the nitrogen source in the synthesis reaction, $\Delta G_n = 0$, $\Delta Gc = 7.5$ kcal/e^-eq., k = 0.6 and m = -1 (Sözen,1992).

$$\Delta G_S = \frac{-0.42}{0.6^{-1}} + 0 + 7.5 = 7.248 kca/e^- eq$$

$$A = \frac{\Delta G_S}{k\Delta G_r} = \frac{7.248}{0.6 * 26.093} = 0.463$$

$$Y_{HD} = \frac{1}{1+A} = \frac{1}{1+0.463} = 0.683 e^- eq.cell/e^- eq.methanol$$

2.3.3 Kinetics of denitrification

2.3.3.1 Growth of denitrifiers

Growth kinetics of the denitrifiers is defined with the Monod type equation. As the rate limiting substrate can be both organic material and the nitrate nitrogen, the growth equation is expressed as (Orhon and Arhan 1994);

$$\mu_{HD} = \hat{\mu}_{HD} \frac{S_S}{K_S + S_S} \frac{S_{NO}}{K_{NO} + S_{NO}} \tag{35}$$

where,

μ_{HD} = specific growth rate of the denitrifying bacteria (1/d)

$\hat{\mu}_{HD}$ = maximum specific growth rate of the denitrifying bacteria(1/d)

S_S = concentration of the electron donor (mg COD/L)

S_{NO} = concentration of the nitrate nitrogen (mg NO_3^--N/L)

K_S = half-saturation constant for the organic material (mg COD/L)

K_{NO} = half-saturation constant for the nitrate nitrogen (mg NO_3^--N /L)

b_{HD} = endogenous decay constant for the denitrifying bacteria

The variation (alteration) of denitrifiers is expressed as;

$$\frac{dX_{HD}}{dt} = \mu_{HD} X_{HD} - b_{HD} X_{HD} \tag{36}$$

Kinetic coefficients for the denitrification process are demonsrated in Table 2.3.

Table 2.3 Typical kinetic coefficients for the denitrification process (Metcalf & Eddy, 1991)

		Value[a]	
Coefficient	Basis	Range	Typical
μ_{max}	d^{-1}	0.3 – 0.9	0.3
Ks	NO_3^- -N, mg/L	0.06 -0 .20	0.1
Y	NO_3^- -N,	0.4 – 0.9	0.8
k_d	d^{-1}	0.04 – 0.08	0.04

[a]Values reported are for 20'C.

Methanol, commonly used as a substrate for anaerobic respiration by these denitrifiers, is metabolized as follows (Eckenfelder and Argaman, 1991):

$$NO_3^- + 1{,}08CH_3OH + 0{,}24H_2CO_3 \rightarrow 0{,}056C_5H_7O_2N + 0{,}47N_2 + 1{,}68H_2O + HCO_3^- \quad (37)$$

$$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \quad (38)$$

This means that for each 2.47 g of methanol consumed, 0.45 g of new cell biomass is produced and 3.27 g of alkalinity is formed. Thus, some of the alkalinity lost during nitrification is recovered during denitrification. Nitrate can also replace O_2 as an electron acceptor during endogenous respiration, although it is reduced at very low rates. It is known that the rate of denitrification is affected by several parameters including temperature, DO levels and the concentration and biodegradability of carbon sources available to these cells (Eckenfelder and Argaman, 1991).

Electron acceptor is consumed by the mechanisms of growth and endogenous decay as shown below.

$$\frac{The\ amount\ of\ e^- acceptor}{\Delta t} = -(1 - f_X Y)\left(\frac{C_{SO} - C_S}{\Delta t}\right) - (1 - f_E) b f_X X \quad (39)$$

where;

f_X = conversion factor (1.42 g VSS/g COD)

f_E = the fraction of inert biomass

C_{SO}, C_S = the concentration of biodegradable organic matter in the influent and effluent of the wastewater

In aerobic conditions, oxygen consumption is determined as demonstrated in below;

$$\frac{\Delta S_O}{\Delta t} = -\frac{(1 - f_X Y_H)}{2{,}86 Y_H}\frac{(C_{SO} - C_S)}{\Delta t} - (1 - f_E) b_H f_X X_H \; f_E) \tag{40}$$

In anoxic conditions, the change in the amount of nitrate nitrogen is determined as demonstrated below;

$$\frac{\Delta S_{NO}}{\Delta t} = \frac{-(1 - f_X Y_{HD})(C_{SO} - C_S)}{2{,}86 \Delta t} - \frac{(1 - f_E)}{2{,}86} b_{HD} f_X X_{HD} \tag{41}$$

The amount of oxygen consumed per g of nitrogen is calculated as 2.86 g.

$$NO_3^- + 6H^+ + 5e^- \rightarrow \frac{1}{2} N_2 + \frac{3}{5} H_2O \tag{42}$$

5 moles of e^- is used for the half reaction above, of which the 2 moles are used for the conversion of NO_3^--N to NO_2^--N as shown in the reaction below;

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \tag{43}$$

The remaining 3 moles of electrons are used for the conversion from NO_2^--N to N_2 as given in the reaction below;

$$NO_2^- + 4H^+ + 3e^- \rightarrow \frac{1}{2} N_2 + 2H_2O \tag{44}$$

The oxygen consumed per g of nitrogen for the two stages of the reaction may be calculated as below:

In the first step;

$$\frac{8grO_2/e^-eq.}{(14/2)grN/e^-eq.} = 1{,}14grO_2/grN \quad (45)$$

In the second step;

$$\frac{8grO_2/e^-eq.}{(14/3)grN/e^-eq.} = 1{,}72grO_2/grN \quad (46)$$

$$1{,}14 + 1{,}71 = 2{,}86gr$$

Thus, yielding to a total of 2.86 g of O_2/N_2 if all the electrons are reduced sequentially:

According to growth expression, 1/Y part of the substrate is converted to biomass. Hence, in aerobic and anoxic conditions, oxygen consumption rates are calculated as demonstrated below;

$$\frac{dS_{NO}}{dt} = -\frac{(1-f_X Y_{HD})}{2{,}86Y_{HD}}\mu_{HD}X_{HD} - \frac{(1-f_E)b_{HD}f_X X_{HD}}{2{,}86} \quad (47)$$

$$\frac{dS_O}{dt} = -\frac{(1-f_X Y_H)}{Y_H}\mu_H X_H - (1-f_E)b_H f_X X_H \quad \textbf{(48)}$$

2.3.3.2 Effect of oxygen

During denitrification process, half reaction of the electron donor is;

$$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- \rightarrow \frac{1}{10}N_2 + \frac{3}{5}H_2O \quad (49)$$

An energy amounting to 17.128 kcal/e$^-$eq. is obtained from this reaction. If oxygen is available in the medium, microorganisms prefer the aerobic respiration to produce more energy and therefore, the amount of electron donor required for the denitrification process is decreased (Sözen, 1992). Accordingly, when the oxygen is used as electron donor (6), energy obtained becomes 18.675 kcal/e$^-$eq.

Normally, denitrification is considered an anoxic process, but under certain conditions, it appears that it can occur in the presence of oxygen. Generally, denitrification is thought

to be inhibited if the DO rises above 0.5 mg/L (Bryan 1993). (Seviour and Blackal, 1998). Similarly it was reported that, active denitrification took place when the DO concentration was below 1 mg/L (Yoo et al, 1998).

2.3.3.3 Effect of pH

The change in pH was shown to be a clear indicator of the denitrification reaction progress, including the ability to discriminate between the negligible pH effects of nitrate reduction to nitrite and the pH increase associated with the reduction of nitrite to non-ionic nitrogen products.

Rapid denitrification of wastewater with a relatively high nitrate concentration at pH values of 9-9.5 has been reported (Cook et al, 1993, Glass and Silvestein, 1998).

2.3.3.4 Effect of temperature

It was reported that denitrification rate is constant at temperatures over 20 ^{o}C (Sözen, 1992), and decreases below 5 ^{o}C. Optimum temperature is reported as 40 ^{o}C and maximum denitrification rate is achieved at 50 ^{o}C (Christensen and Harremoes, 1977).

2.3.3.5 Effect of inhibitors

Nitrate has an inhibitory effect on the enzyme activity of the denitrifying reductases. As a result of this, the mole fractions of nitrite, which is more toxic to the denitrification than nitrate (Kornaros et al., 1996), become increased.

Instead of nitrite ion concentrations, Abeling and Seyfried (1992) suggested that undissociated nitrous acid, is the form that inhibits bacterial denitrification. A HNO_2 concentration of 0.04 mg/L is proposed as the toxicity threshold for nitrite.

Denitrifying cultures appear to be inhibited by nitrate concentrations of 6000 mgNO_3^--N/L. In a study by Glass et al. (1998), shock loading of unacclimated activated sludge with 5400 mgNO_3^--N/L, resulted in complete inhibition of the denitrification process. Denitrification of the high nitrate containing wastewater can be obtained at pH 9, after the gradual acclimation of the activated sludge with a stepwise increase in nitrate loading from 2700 to 5400 to 8200 mgNO_3^--N/L.(Glass et al, 1998). Glass et al. (1997), found that high concentrations of nitrate, such as 2700 mg NO_3-N/L, can be denitrified

by activated sludge with relatively low inhibition if the pH of the mixed liquor is maintained at more than 8. Significant inhibition of denitrification was observed at nitrite concentrations less than 250 mgNO_2-N/L at a pH of 7. Amount of nitrite as low as 30 mgNO_2-N/L was found to be inhibitory to denitrification at pH 6. The increase of inhibition with decreasing pH can be explained by the accumulation of HNO_2, however, even at the relatively high pH of 8, high concentrations of nitrite can inhibit denitrification.

2.4 Partial Nitrification

Nitrification is carried out in two steps; first, ammonia is converted to nitrite by ammonia oxidizing bacteria (nitritation) and at the second step nitrite oxidizing bacteria convert nitrite to nitrate (nitratation) as mentioned previously. For 1 mole of ammonia, ammonia oxidizing bacteria use 1.5 moles of oxygen and nitrite oxidizing bacteria use 0.5 mole of oxygen. Complete nitrification requires 2 moles of oxygen per mole of nitrogen to be nitrified. This means that partial Nitrification to nitrite will only require 1.5 moles of oxygen per mole of nitrogen, implying a 25 % less oxygen demand for partial nitrification than complete nitrification (Verstraete et al., 1998,Ruiz et al., 2000).

$$NH_4^+ + 1{,}5O_2 \xrightarrow{NITRIFICATION} NO_2^- + H_2O + 2H^+ \quad (50)$$

$$NH_4^+ + 2O_2 \xrightarrow{NITRIFICATION} NO_3^- + H_2O + 2H^+ \quad (51)$$

↑ 25%O_2 saved

$$6NO_2^- + 3CH_3OH + 3CO_2 \xrightarrow{Denitrification} 3N_2 + 6HCO_3^- + 3H_2O \quad (52)$$

$$6NO_3^- + 5CH_3OH + CO_2 \xrightarrow{Denitrificaton} 3N_2 + 6HCO_3^- + 7H_2O \quad (53)$$

↑ 40% CH_3OH saved

In denitrification process, nitrate is converted to nitrite and then to nitrous oxide and nitric oxide and finally to nitrogen gas, each step consuming a certain COD. If post denitrification is considered, partial nitrification to nitrite, i.e., a shortcut of the nitrate

would mean a reduction in the total COD required for denitrification, because no COD is needed for the conversion of nitrate to nitrite (Ruiz et al., 2000).

To achieve partial nitrification, it is necessary to reduce the activity of nitrite oxidizing bacteria while maintaining the activity of ammonia oxidizing bacteria. This would be done by both selective inhibition of nitrite oxidizing microbes and reduction of the population of the nitrite oxidizing bacteria in the reactor (Yun et al., 2003). The effects of inhibitory factors on the growth of Nitrosomonas are different from those on Nitrobacteria due to different growth characteristics of these two kinds of bacteria. In comparison to Nitrosomonas, Nitrobacteria are more sensitive to the environmental changes. Therefore, Nitrobacteria are more likely to be inhibited from the initial stage under the presence of toxic materials. As a result, nitrite can accumulate due to the lacking Nitrobacteria and upon the prevention of the nitrite from further oxidizing to nitrate (Peng et al., 2004). Table 2.4 presents the kinetic expressions usually accepted for nitrifying biomass. It can be seen that, substrate concentration, temperature, pH, and DO affect each activity in different terms since the value of each constant is different. Additionally, pH will affect the substrate concentration for each step, because of the modification of the acid- base equilibrium.

Substrate concentration is not an operational parameter because it is the objective variable in terms of wastewater treatment.

Table 2.4 Kinetic expressions for ammonia oxidizing and nitrite oxidizing bacteria (Ruiz et. al.,2003)

Bacterial group	Kinetic expressions
Ammonia oxidizing bacteria	$\mu = \mu_{\max} \frac{\left[NH_4^+\right]}{K_{SH} e^{(A_E/T)} 10^{-pH} + \left[NH_4^+\right] \frac{\left[NH_4^+\right]^2}{K_{IH} e^{(A_E/T)} 10^{-pH}}} \frac{\left[O_2\right]}{K_{O_2} + \left[O_2\right]}$
Nitrite oxidizing bacteria	$\mu = \mu_{\max} \frac{\left[NO_2^-\right]}{K_{SH} e^{(A_E/T)} 10^{-pH} + \left[NO_2^-\right] \frac{\left[NO_2^-\right]^2}{K_{IH} e^{(A_E/T)} 10^{-pH}}} \frac{\left[O_2\right]}{K_{O_2} + \left[O_2\right]}$

where,

K_{SH} = saturation constant for the unionized substrate

K_{IH} = inhibition coefficient for the unionized substrate

K_{O_2} = oxygen saturation coefficient

$\left[NH_4^+\right]$ = ammonia concentration

$\left[NO_2^-\right]$ = nitrite concentration

$\left[O_2\right]$ = DO concentration

$e^{(A_E/T)}$ = equilibrium constant for the dissociation of the substrates, where A_E is the activation energy and T the absolute temperature.

Both organism groups involved in nitrification are aerobic and need CO_2 as carbon source. They stand out for low growth rates, high sensibility to pH value and temperature deviations as well as toxic matters. In practice, the product inhibition of Nitrosomonas is very important. It seems that this inhibition of Nitrosomonas by nitric acid is related to the nitrite concentrations depending on the pH and temperature (Figure 4). Nitric acid then causes inhibition of Nitrosomonas, so that concentration of ammonium, and respectively ammonia increases. On the one hand, ammonia can release a substrate inhibition of the Nitrosomonas; on the other hand, it can start a non-competitive inhibition of Nitrobacter. Under unfavorable conditions, there may occur a complete breakdown of nitrification (Abeling and Seyfried, 1992).

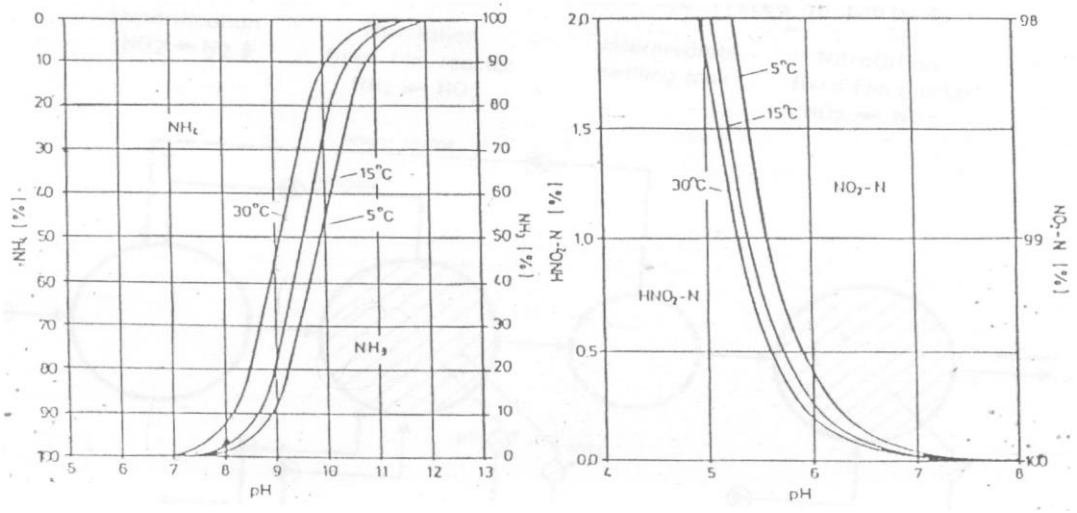

Figure 2.3 Dissociation Balances NH_4^+ / NH_3 and NO_2^- / HNO_2

In several tests with continuously operated experimental set-ups, Nyhuis (1985) could prove that Nitrosomonas ($NH_4^+ \rightarrow NO_2^-$) and Nitrobacter ($NO_2^- \rightarrow NO_3^-$) show different reactions on ammonia and nitric acid (Abeling and Seyfried, 1992).

It was found that, partial nitrification to nitrite is technically feasible and economically favorable, especially when wastewaters contained high ammonium concentrations or low C/N ratios (Jianlong et al., 2003). And also to achieve partial nitrification it is necessary to reduce the activity of nitrite oxidizing bacteria. This would be done by assuring favorable conditions for ammonia oxidizing bacteria.

Yoo et al. (1998), claims that for effective simultaneous nitrification and denitrification via nitrite, careful monitoring of the control parameters such as the DO level, pH, free ammonia, and free hydroxylamine concentration, temperature and duration of aeration is necessary.

Denitrification rates with nitrite are 1.5-2 times greater than with nitrate (Abeling et al., 1992). By changing some operation conditions, nitrite oxidizing bacteria becomes inactive, so that the partial nitrification occurs.

2.4.1 The Effect of pH and free ammonia

Nitrite accumulation by partial nitrification is controlled by free ammonia concentrations at alkaline pH values. Jianglong et al. (2003), studied a series of ammonia oxidation experiments at various pH values and free ammonia concentrations. It was found that, when the pH was too low, the free ammonia concentration limited the ammonia oxidation rate. Under such circumstances, high DO concentrations could enhance the ammonia oxidation rate significantly. When the pH was increased, the free ammonia concentration also increased and inhibited the ammonia oxidizing bacteria, so the ammonia oxidation rate decreased dramatically. The optimal pH was found as 7.5 for the nitritation process at T = 30 ^{o}C.

Additionally, pH will effect the substrate concentration for each step because of the modification of the acid-base equilibrium. It was reported that nitrite accumulation took place between the pH values of 8.65 and 8.95 (Ruiz et al. 2003). In contrast, optimal pH value was given by Pollice et al., (2002), as 7.2 for partial nitrification also in agreement with Jianglong et. al. (2003).

According to the research by Anthonisen (1976), the non-ionized forms of the ammonia (NH_3) and nitrous acid (HNO_2) have an inhibitory effect on both Nitrosomonas and Nitrobacter. Nitrobacter react more sensitively so that concentration of NH_3 in relatively low range is sufficient. Likewise, Abeling and Seyfried (1992) reported that concentration of 1 - 5mg NH_3/L inhibited nitratation but not the nitritation. In order to attain the highest nitritation rate, it was decisive to prevent the inhibition of the Nitrosomonas caused by free ammonia. The free ammonia concentration necessary for the inhibition of the Nitrobacter must be kept low enough to ensure that the inhibition of nitritation does not take place as well. At pH 8.5 and T=20 ^{o}C the optimal free ammonia concentration for maximum nitritation and minimum nitratation was found to be around 5 mg NH_3/L. In contrast, Ruiz et al. (2003), found that, at acidic pH, not only nitrite oxidizers but also ammonia oxidizers were inhibited by the HNO_2. On the contrary, Chung et al. (2004) found that, optimal free ammonia concentration for both stable and maximum ammonium removal was approximately 10 mg/L. When a lower ammonium concentration in the effluent is required, free ammonia concentration of 5 mg/L might be

applicable. Similarly, Peng et al. (2004), obtained that when the free ammonia concentration was above 5 mg/L, at temperatures between 25-30 ^{o}C, high and stable ammonia oxidation rate is achieved with more than 95% NO_2^--N/NO_X^--N ratio, where the NOx-N is the concentration of nitrate and nitrite concentrations. When free ammonia concentration is below 0.5 mg/L, the ammonia oxidation rate will decrease quickly with a NO_2^--N/NO_X^--N ratio less than 10% and therefore the process is shifted to the traditional nitrate type nitrification.

The free ammonia concentration can be controlled by; adjusting pH, wastewater feeding strategy and reactor type (Yun et al, 2003).

2.4.2 Effect of dissolved oxygen (DO) concentration

Different impacts of DO on nitrite and nitrate formation mechanisms may have serious implications on the balance of these two compounds, and may lead to nitrite accumulation at low dissolved oxygen concentrations.

DO is a co-substrate in the nitrification reaction in a dual-limitation manner. Yoo et al., found that the minimum DO level was generally below 0.4 mg/L. Ideal maximum DO concentration should be 2.0-2.5 mg/L. Ideal median DO level was 1.3 mg/L. However, Pollice et al., (2002), achieved the partial nitrification under oxygen limitation independent of the SRT. Jianglong et al. (2003) suggested that the optimal DO value of 1-1.5 mg/L is enough to achieve ammonia oxidation. Ruiz et al. (2003) found that, at the DO concentration of 1.7 mg/L there was a temporal accumulation of nitrite and when the DO concentration was between 1.4 and 0.7 mg/L nitrite accumulation increased while ammonia consumption stayed the same.

Yun et al. (2003), found that, DO was not the limiting factor for nitrification in the biofilm reactor. Nitrate concentration was low and the nitrite was the major oxidized nitrogen compound in the effluent of the biofilm reactor.

2.4.3 Effect of temperature

Temperature has a strong effect on the growth rate of both types of bacteria in different ways: At normal temperatures, in the wastewater treatment plants (5-20^{o}C), nitrite oxidizers grow more rapidly than ammonia oxidizers, and ammonia is oxidized to nitrate

in these systems. However, at high temperatures, this reaction changes its direction and ammonia oxidizers should have superior growth rates than nitrite oxidizers. As a result, nitrite will accumulate in the reactor. This is indeed the idea behind the SHARON process. However in most cases the temperature is not susceptible to be modified and controlled in full-scale reactors, mainly for economic considerations. Hence, pH and dissolved oxygen may be the main manipulated variables to control the system (Ruiz et. al. 2003). Jianglong et al. (2003) found that the optimal temperature for the nitritation process was 30 ^{o}C. Yoo et al. (1998) found that when the temperature was in the range of 22-27^{o}C during the entire operation cycle, the maximum nitritation activity was induced. However, Pollice et al., (2002), recommended the temperature of 32 ^{o}C to obtain partial nitrification. Similarly, Peng et al. (2004) found that the optimum pH for the partial nitrification ranges between 25-30 ^{o}C.

2.4.4 Effect of sludge retention time (SRT)

Pollice et. al. (2002) found that at constant temperature and pH value of 32 ^{o}C and 7.2, respectively, the sludge age becomes the critical parameter for partial nitrification when oxygen supply is not limited. Ammonium oxidation to nitrite was successfully obtained for SRTs around 10 days. Longer sludge retention times tend to favor nitrite oxidizers. When the oxygen supply is limited, complete and stable conversion of ammonia to nitrite was obtained independent of the SRT.

Yun et. al. (2003) found that selective free ammonia inhibition and reduced population of nitrite oxidizers made a contribution to accumulate nitrite in the biofilm reactor. Similarly, Tseng et al. (1997) found that partial nitrification was achieved at the sludge retention time of 10.7 days. Conversely, Hellinga et. al. found that the nitrite route can be maintained at 35 ^{o}C using a 1-day aerobic, 0.5-day anoxic residence times.

Chung et al. (2004) found that for the ammonia rich wastewaters, at 3-4 days of hydraulic retention time (HRT), nitrite accumulation was observed.

2.4.5 Effect of inhibitors

Carrera et al (2004) studied partial nitrification by using synthetic wastewaters with an initial ammonium concentration of 1000 mg NH_4^+-N/dm^3, at 23 ^{o}C, and at a pH of 7.5. It

was observed that only 30 % of the nitrite formed was oxidized to nitrate while ammonium concentration in the reactor was greater than 50 mg NH_4^+-N/dm^3. When the ammonium concentration decreased below this value, all of the nitrite formed was oxidized to nitrate.

2.5. SHARON Process

Since nitrite was found as a half-product in the nitrification denitrification processes, this idea proceeded to develop some partial nitrification techniques like the SHARON process that is used for the treatment of ammonia rich wastewaters (Helliga et al., 1998). SHARON Process was developed for the treatment of recycle waters of the sludge digesting unit of Rotterdam Dockhavens treatment plant together with Dutch Foundation for Applied Water Research, (STOWA) and Technical University of Delft (Hellinga et. al.,1998; STOWA 1996).

The nitrogen rich wastewaters in the treatment plants are commonly generated from the sludge digestion processes. These types of wastewaters (concentrate, filtrate, sludge water, sludge reject water) have high concentrations of ammonia and low concentrations of organic carbon. Instead of recycling sludge digestion wastewaters to the beginning of the treatment plant, these wastewaters can be treated in a different treatment unit in order to prevent the main treatment plant conditions from getting worse.

According to the SHARON Process, ammonium is first oxidized to nitrite and then nitrite is denitrified by saving 25 % of the oxygen requirement and 40 % of the energy requirement during the entire process.

$$NH_4^+ + 1{,}5O_2 \xrightarrow{Partial\ \ nitrification} NO_2^- + H_2O + 2H^+ \tag{50}$$

$$6NO_2^- + 3CH_3OH + 3CO_2 \xrightarrow{Denitrification} 3N_2 + 6HCO_3^- + 3H_2O \tag{52}$$

The SHARON reactor is operated at oxic and anoxic cycles. During the anoxic period, organic carbon source is added into the reactor because of the low COD/N ratios of the digesting sludge. Methanol is used as the carbon source as it is more economic than other organic carbon sources. Minimum residence time for ammonium and nitrite

oxidizers as a function of the temperature is given in Figure 5. It is observed from the figure that, the growth of ammonium oxidizers started (35 ^{o}C) at SRT of 0.5 days. However, SRT must be kept below 1.5 days to prevent the growth of the nitrite oxidizers in the reactor. In order to prevent nitrate formation, SRT for the nitritification process is to be selected as 1 day.

The SHARON reactor has intermittent aeration, continuous flow and completely stirred features and operated as a chemostat. Process operates at a short retention time (approximately 1 day) and high temperature (30-40^{o}C). This process is performed without sludge retention. This enables the prevention of nitrite oxidation and leads to lower operational costs (Hellinga et. al. 1998). As Nitrobacter has a distinctly lower growth rate than Nitrosomonas at high temperatures (Figure 5), by implementing a completely mixed reactor at short residence time (e.g. one day) and high temperatures, one can achieve wash out of Nitrobacter. In the reactor, when sludge retention time is equal to the hydraulic retention time, it helps to keep the amount of growth and the amount of washout of sludge in equilibrium in the reactor.

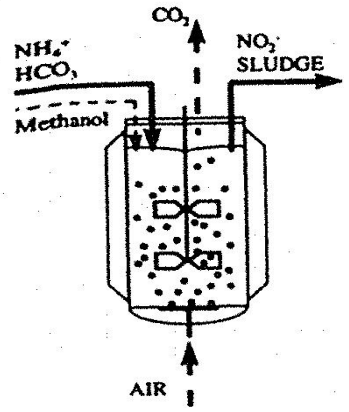

Figure 2.4 The SHARON process in a well mixed continuous flow reactor

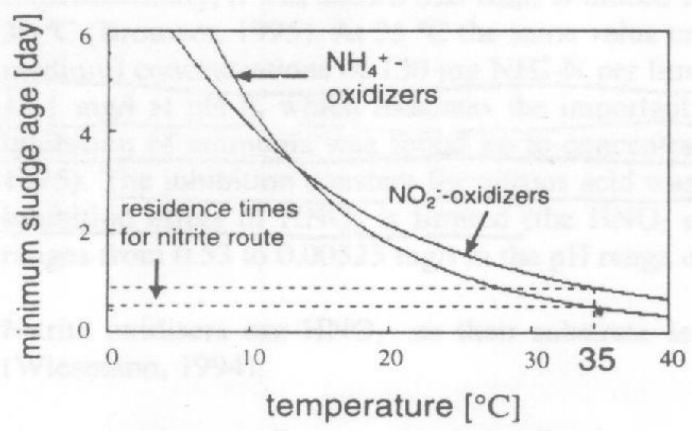

Figure 2.5 Minimum HRT for NH_4 and NO_2^- oxidisers as function of the temperature (Hunik 1998)

Previously, before the SHARON process was developed, a broad range of process factors such as, pH, DO, temperature, NH_4^+, HNO_2 and other inhibitors have been tested to achieve nitrification/denitrification via nitrite. Most of them initiated the formation of nitrite but could not prevent the formation of nitrate after longer periods. It was thought that, all these process factors were not successful for full-scale applications (STOWA, 1995). The SHARON process is the first successful technique by which nitrification/denitrification via nitrite under stable process conditions was achieved.

2.5.1 Description of the SHARON process

The SHARON process has been described in detail by Hellinga et al. (1998). This process (patented) distinguishes itself from other biological wastewater treatment processes by a complete absence of sludge retention.

Microorganisms show high activity, however the Ks value is rather high. As a result effluent concentrations at an aerobic retention time of 1 day may become several tens of milligrams. The effluent concentration is independent of the influent concentration, and the removal efficiency increases with higher inlet concentrations.

The pH control is very important due to high concentrations and high reaction rates. The bicarbonate in the anaerobic effluent and the denitrification process compensate the acidifying effect of the nitrification. Both constitute 50 % of the alkalinity requirement.

In the process, however, CO_2 stripping must be sufficient to allow complete use of the bicarbonate (van Kempen R. et al., 2001).

During the study of van Kempen R. et al. 2001, nitrification process started immediately after 3 weeks with the nitrification efficiency of over 95 %. After that the dosing of methanol was started in order to initiate the denitrification process. It was reported that this study proved it is possible to achieve nitrification without sludge retention.

Hellinga et. al. found that the SHARON process is most suitable for the substantial ammonium reduction in wastewater with a relatively high ammonium content and with an elevated temperature. Investment costs were found low because a simple well mixed tank reactor of modest dimensions without a sludge retention system was sufficient. It was recommended that, the reactor height to diameter ratio should be kept low in order to ensure high mass transfer rates for carbon dioxide thus reducing costs for pH regulation. Laboratory experiments revealed that the nitrite route could be maintained at 35^oC using 1-day aerobic, 0.5-day anoxic residence times. Achieving more than 5000 $mgNH_4^+/L$ conversion or lower effluent concentrations could only be obtained if the hydraulic residence time is increased. However, it is not clear that at which point complete oxidation to nitrate will occur.

3. MATERIAL METHOD

3.1 Experimental Studies

Experimental studies were composed of the successive steps of acclimation, achievement of continuous partial nitrification, and finally denitrification.

3.1.1 Acclimation studies

A 3 L glass reactor was used for the whole stages of the study. The reactor was run without sludge recycle and at the room temperature, where the sludge age and hydraulic retention times were both equal to 1 day. A glass cover was put at the top of the rector. The wastewater in the reactor was mixed with a magnetic stirrer, and aeration was achieved with diffusers. In order to buffer the pH, CO_2 was passed through the reactor. Figure 3.1 demonstrates the reactor shape.

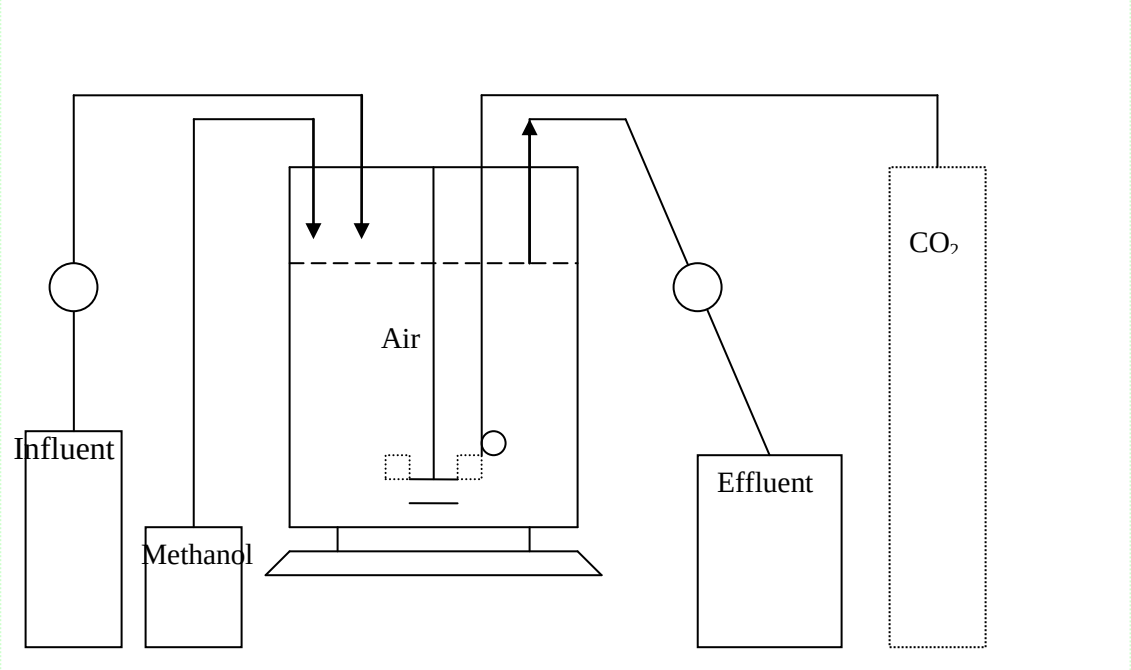

Figure 3.1 Demonstration of the experimental set-up

Synthetic wastewater was used for feeding the reactor. Since the experiments were carried out with synthetic wastewater the feeding solution did not contain organic carbon source, but only NH_4Cl and $NaHCO_3$ and also the trace elements (mineral supplements). The buffer solutions required for the microbiological reactions, were added to the reactor as described at the Table 3.1 (J.T.O. Connor, 1972).

3.1.1.1 Acclimation step

A 3 L glass reactor was used for acclimation step, which was operated in fill and draw mode with a hydraulic retention time of 1 day. The seed microorganisms were taken from the mixed culture of a lab-scale reactor fed with domestic wastewater, to obtain an enriched culture of nitrifiers. As the start-up period was in winter term, the nitrification could not be achieved within 6 days at room temperature. Therefore reactor was put into an incubator at 25±2 ^oC.

Table 3.1 Formulations of Solution A and Solution B

Solution A	K_2HPO_4	320 g/L
	KH_2PO_4	160 g/L
Solution B	$MgSO_4\ 7H_2O$	15 g/L
	$FeSO_4\ 7H_2O$	0.5 g/L
	$ZnSO_4\ 7H_2O$	0.5 g/L
	$MnSO_4\ 3H_2O$	0.3 g/L
	$CaCl_2$	2.0 g/L
NH_4Cl solution	NH_4Cl	46 g/L
$NaHCO_3$ solution	$NaHCO_3$	84 g/L

The initial ammonia concentration was gradually increased until the effluent concentration of NH_4^+-N was entirely removed. 200 ml of activated sludge was used as seed. Reactor was fed with an influent containing 60 mg NH_4^+-N/L, and 550 mg HCO_3/L with mineral supplements. In the beginning of the 1-week acclimation period, ammonia conversion efficiency was observed to be almost zero because of the winter term conditions. In order to speed up the acclimation period, reactor was started to be fed twice in a day after 4^{th} day of the acclimation period. At the end of 6 days, reactor volume was decreased to 2 L and put into the incubator at a constant temperature of 30 ± 2^oC. Nitrification could be observed at 30 days after when more or less stable conditions for nitrification was obtained.

3.1.2 Continuous type feeding

After 107 days of operation, system was shifted to continuous type with an influent flow rate of 1081 ml/day and hydraulic retention time, equal to sludge retention time, of 1.85 days from the fill and draw reactor type. During continuous feeding, 10 ml HCl/L acid was started to be added to the feeding solution to buffer the pH. At the end of 152 days, the flow rate of the pump was increased from 1081 ml/day to 1735 ml/day, thus sludge retention time was decreased from 1.85 days to 1.15 days, and the temperature of the incubator was increased from 30 ± 2^oC to 35 ± 2^oC to achieve partial nitrification.

3.1.3 Denitrification step

After partial nitrification was achieved and the system reached the steady state. The reactor volume was increased to 2.6 L with the effluent of the same reactor to achieve oxic hydraulic retention time of 1 day and anoxic retention time of 0.5 day. The partial nitrification with denitrification application consisted of 80 minutes of oxic phase (partial nitrification) and 40 minutes of anoxic phase (denitrification) where these cycles were repeated 12 times in a day. Methanol feeding to the reactor was completed in the first minute. Aeration and methanol feeding was controlled by a timer. For the initial part of the denitrification step, a methanol dosage of 14 ml/L was used to obtain a C/N ratio of 1.2.

3.2 Methods of Analysis

All analyses were performed as stated in Standard Methods (APHA, 1989). Samples for the experiments were taken from the reactor and settled in the Imhoff Tank for 2 hr and then surface water (supernatant) was taken and filtered through the 0.45 μm pore sized durapore membrane filters.

Ammonia measurement was done in accordance with the 4500-NH_3 B. Preliminary Distillation Step Method 4500-NH_3 E. Titrimetric Method.

Nitrite measurement was done in accordance with the 4500-NO_2^- C. Ion chromatographic Method.

Nitrate was measured according to the 4500-NO_3^- C. Ion chromatographic Method.

Alkalinity was measured in the influent in according to the 2320 B. Titration method.

Suspended solids was measured in according to the 2540 B. Total solids dried at 103-105^oC.

Volatile suspended solids was measure in according to the 2540 E. Fixed and Volatile Solids Ignited at 550^oC.

4. EXPERIMENTAL RESULTS

Experimental studies to establish a system removing high ammonium loads via partial nitrification-denitrification process were performed mainly in three sequential steps. In the first part of the experimental work, autotrophic nitrifier culture was enriched from an activated sludge seed and adapted to high ammonium concentrations. As soon as a stable and reliable ammonium oxidation was achieved, a chemostat system was started to operate with the intention to achieve partial nitrification system, as the second step. Finally, denitrification process was examined subsequent to partial nitrification in the same chemostat system.

4.1 Fill and Draw Studies

A 2 liter glass reactor was used for acclimation and operated in fill and draw mode with a hydraulic retention time of 1 day. 200 ml of activated sludge was used as seed. In the beginning, reactor was fed with an influent containing 40 mg NH_4^+-N/L, and 450 mg HCO_3/L with mineral supplements. Until 7^{th} day of the acclimation period, no ammonium conversion was obtained mainly because of low temperature due to winter conditions. Thus, reactor was started to feed twice a day and placed into an incubator working at a constant temperature of 25±2^oC so as to accelerate the acclimation. System performance was slowly improved and at the day of 24, 89 % of ammonium removal was achieved where the influent ammonium concentration was 45 mg NH_4^+-N/L. At the day of 33, feeding conditions was changed again to once a day. After that time the influent ammonium concentration was gradually increased as soon as the effluent concentration of NH_4^+-N was entirely removed. Through out the acclimation period, pH, alkalinity, ammonium and biomass concentrations were measured and results were presented in Figures 4.1 to 4.24.

As can be seen from the Figure 4.1 and 4.2, 40 mg/L ammonium was completely removed within the 24 hour cycle. Maximum specific ammonium removal rate of 0.0085 mg NH_4^+-N/mgVSS.h was achieved in the first 6 hours. The pH fluctuated between 7.4 and 8.3 and remained in the safe range for nitrification.

At the 33rd day of the experiment, initial ammonium concentration was increased to 90 mg NH_4^+-N/L and almost 100 % ammonium removal was obtained. Thus, ammonium concentration was gradually increased in the next steps.

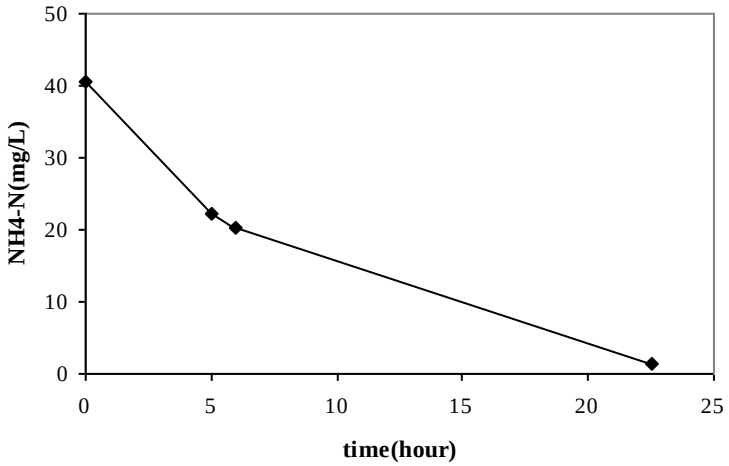

Figure 4.1 Ammonium removal time for the 30th day

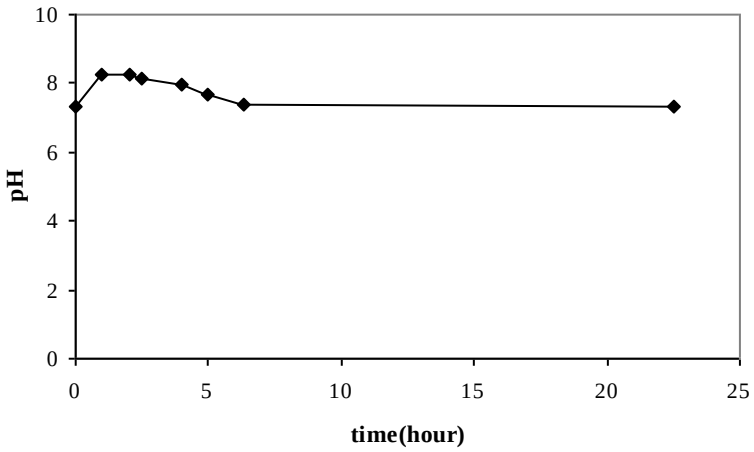

Figure 4.2 pH alteration versus time for the 30th day

At the 37th day of the experiment, same concentration of ammonium as 150 mg NH_4^+-N/L was supplied to the system. Maximum specific ammonium removal rate of 0.046 mg NH_4^+-N/mg VSS.h was obtained in the first 8 hours. Because it was not able to measure ammonium as soon as the sample was collected, system was controlled mainly by pH and bicarbonate measurements. Bicarbonate addition was increased to 1700mg HCO_3^-/L so as to provide adequate amount of alkalinity. Approximately 94 % of ammonium was oxidized within the first 9 hours of the experiment and there was still 650 mg HCO_3^-/L in the medium which was more than enough, thus pH remained in higher values around 8 and 9 during the whole day.

At 41st day of the experiment, influent ammonium concentration was increased to 200 mg NH_4^+-N/L, approximately 1.3 fold higher than the previous loading in order to acclimate the reactor to the high ammonium concentrations. Maximum specific ammonium removal rate of 0.048 mg NH_4^+-N/mg VSS.h was observed for the first 4 hours. Initial alkalinity was supplied as 1500 mg HCO_3^-/L provide a concentration ratio of HCO_3^-/NH_4^+-N = 7.7. Throughout the 24 hour cycle, 100 % of ammonium oxidation was obtained and almost all alkalinity was consumed. Although pH increased to 8.6 in the first 2 hours, it decreased to 7 at the end of the day, as a result of ammonium oxidation.

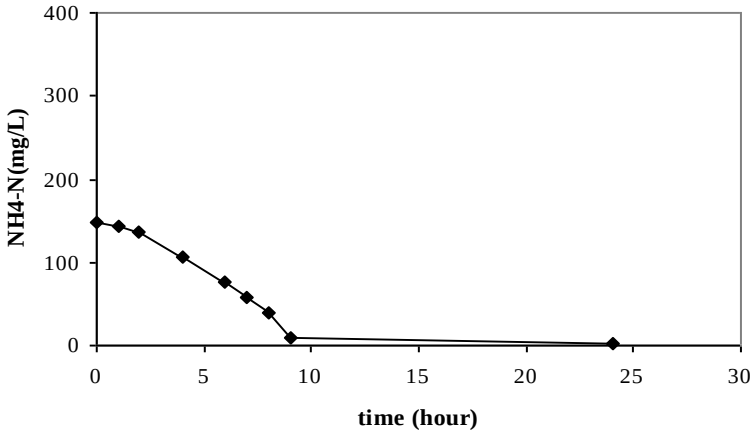

Figure 4.3 Ammonium removal versus time for the 37th day

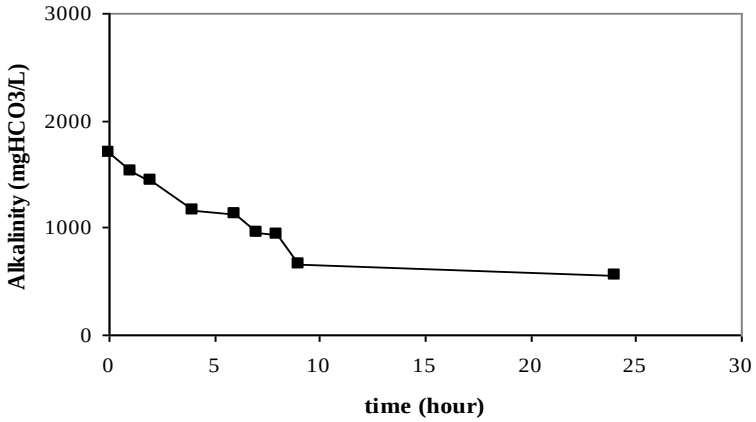

Figure 4.4 Alkalinity consumption versus time for the 37th day

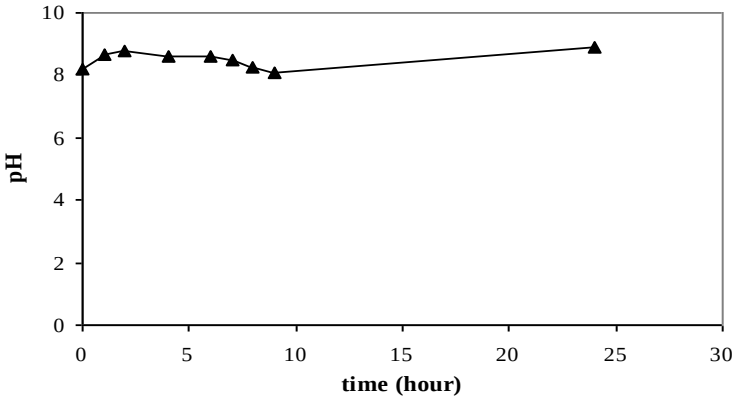

Figure 4.5 pH alteration versus time for the 37th day

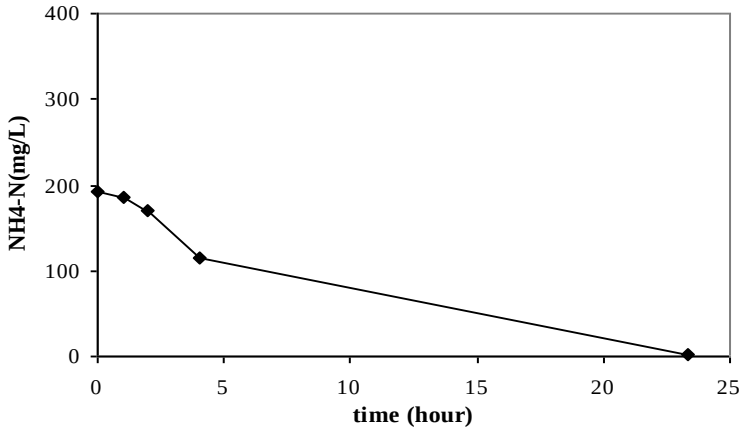

Figure 4.6 Ammonium removal versus time for the 41st day

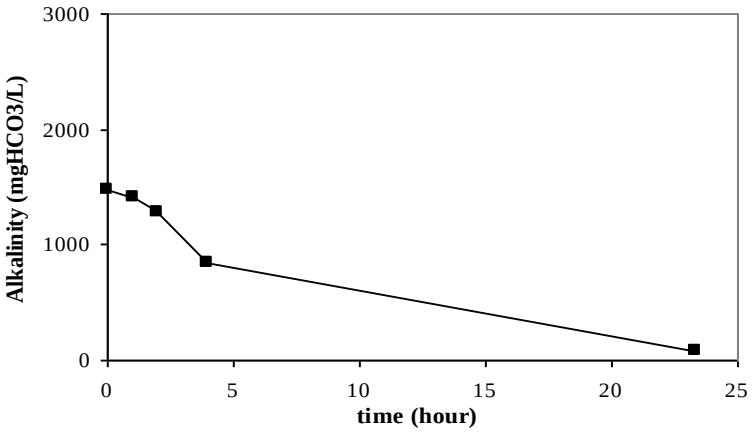

Figure 4.7 Alkalinity consumption versus time for the 41st day

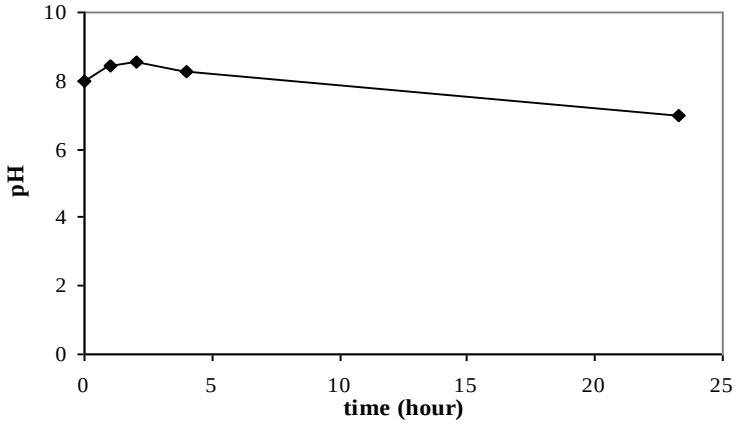

Figure 4.8 pH alteration versus time for the 41st day

Ammonium concentration of the influent was increased to 375 mg NH_4^+-N/L at 53rd day of the acclimation period, approximately 1.9 fold higher than the previous loading. Maximum specific ammonium removal rate of 0.042 mg NH_4^+-N/mgVSS.h was attained for the first 5.3 hours. As it can be seen from the Figure 4.13, alkalinity was fed into the reactor with a concentration ratio of HCO_3^-/ NH_4^+-N = 8.3 and 93 % of the alkalinity was consumed where 95 % of ammonium oxidation was obtained throughout the experiment. As a consequence pH decreased from 8.7 to 6.6.

At 54th day of the acclimation period, ammonium concentration of the influent was increased to 475 mg NH_4^+-N/L, approximately 1.3 fold higher than the previous loading. Maximum specific ammonium removal rate of 0.048 mg NH_4^+-N/mgVSS.h was observed for the first 5.5 hours. Approximately 5000 mg HCO_3^-/L alkalinity was fed into the reactor supplying a ratio of HCO_3^-/NH_4^+-N = 10.7. Because the alkalinity was more than enough, 1400mg HCO_3^-/L was remained unused in the system.

Figure 4.15, 4.16 and 4.17 shows the results of 69th day of the acclimation period. At this time, ammonium concentration of the influent was increased to 800 mg NH_4^+-N/L, approximately 1.7 fold higher than previous loading. Maximum specific ammonium removal rate of 0.06 mg NH_4^+-N/mg VSS.h was achieved for the first 4.3 hours, which is the highest rate that ever observed. This result confirms that the system was able to handle higher ammonium loads. Alkalinity was supplied to the system with a ratio of HCO_3^-/NH_4^+-N = 8.75 and 100 % ammonium oxidation where 88 % of alkalinity consumption was observed.

At 70th day, ammonium concentration of the influent was increased to 1400 mg NH_4^+-N/L. With this shock ammonium and bicarbonate load, pH increased from 8.1 to 9.1 which was inhibited nitrification since free ammonia concentration was increased in higher pH ranges. Due to over load of ammonium, system was inhibited and activity could not be completely recovered for the next 4 days.

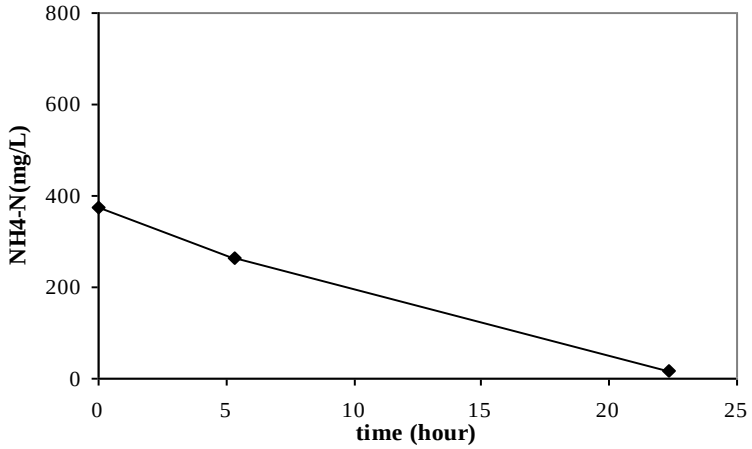

Figure 4.9 Ammonium removal versus time for the 53rd day

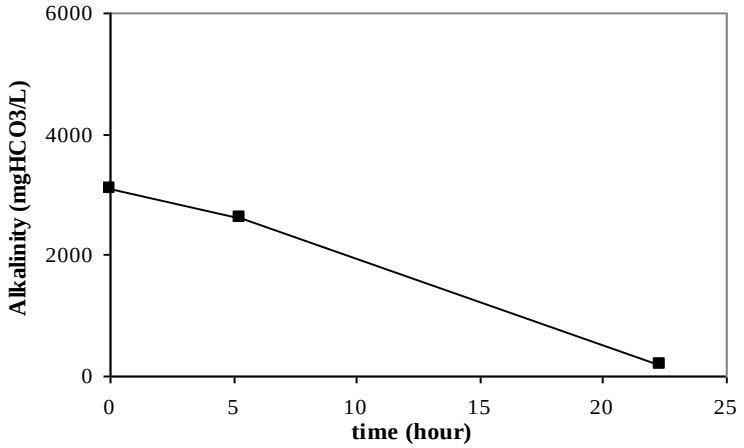

Figure 4.10 Alkalinity consumption versus time for the 53rd day

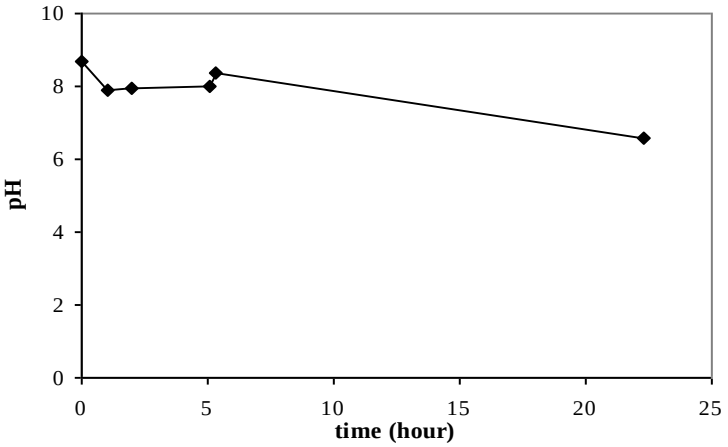

Figure 4.11 pH alteration versus time for the 53rd day

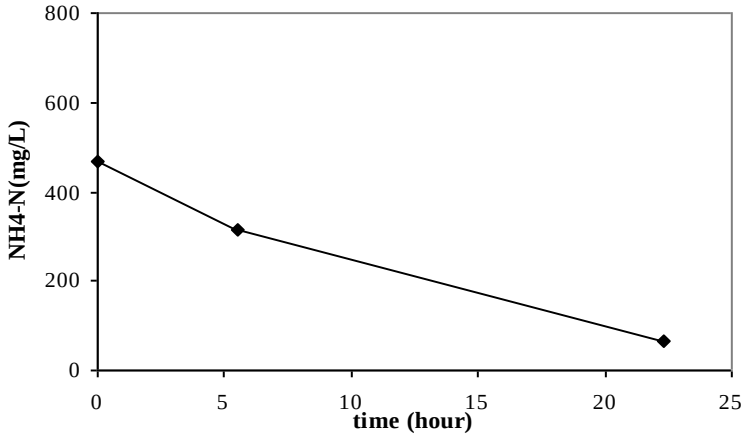

Figure 4.12 Ammonium removal versus time for the 54th day

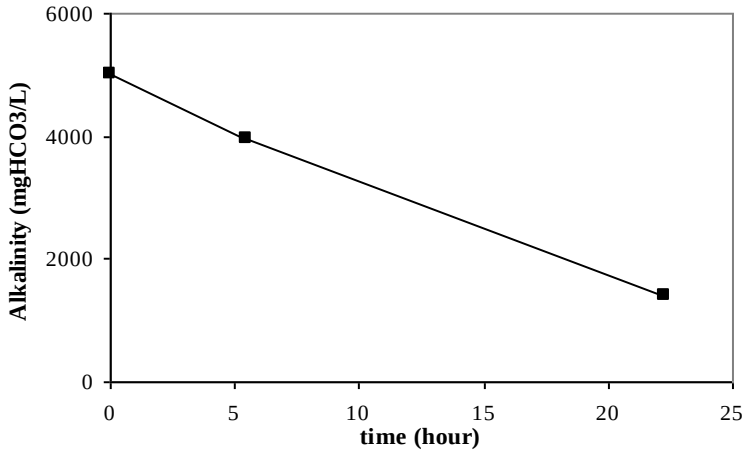

Figure 4.13 Alkalinity consumption versus time for the 54th day

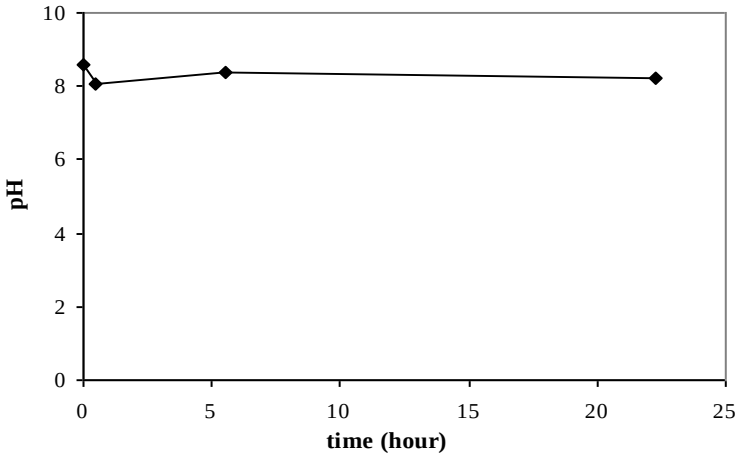

Figure 4.14 pH alteration versus time for the 54th day

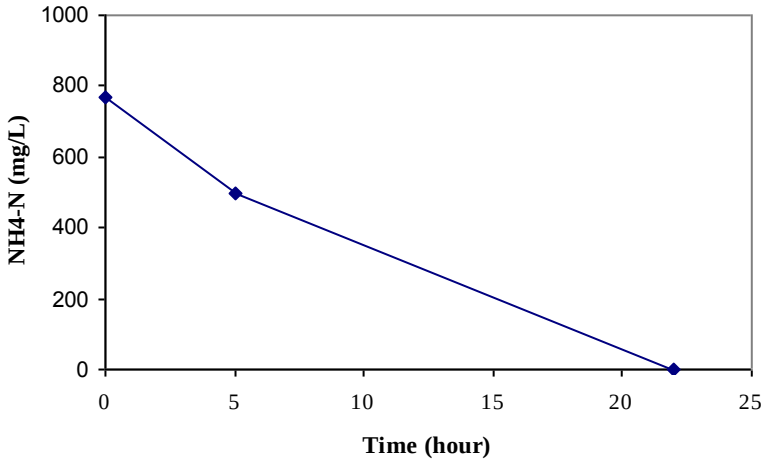

Figure 4.15 Ammonium removal versus time for the 69th day

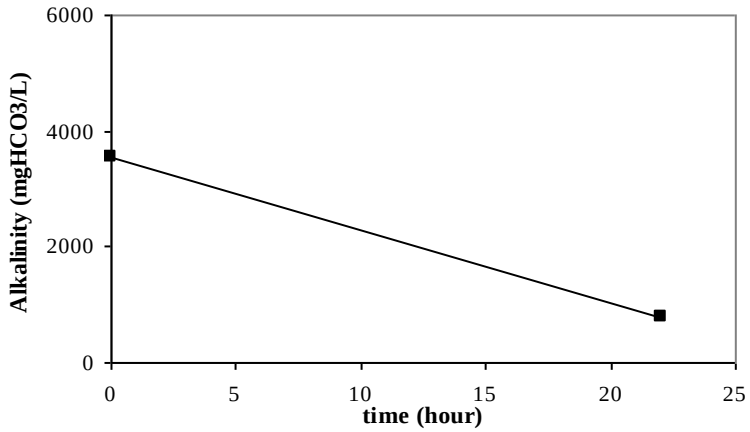

Figure 4.16 Alkalinity consumption versus time for the 69th day

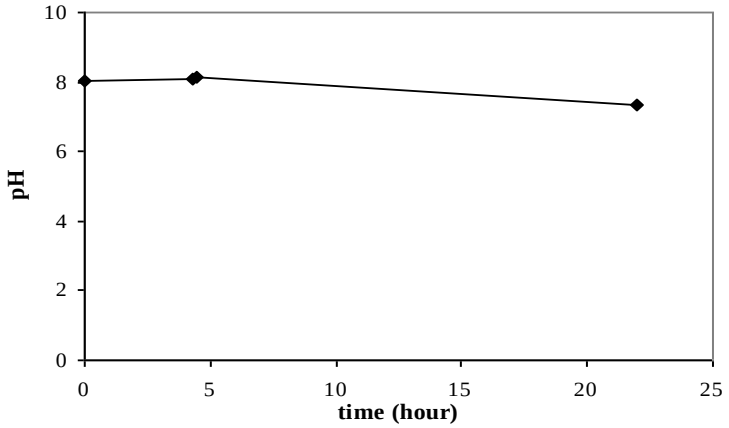

Figure 4.17 pH alteration versus time for the 69th day

Figure 4.18, 4.19 and 4.20 represents the 75th day of the experiments. At this time influent ammonium concentration was decreased to 475 mg NH_4^+-N/L so as to recover the ammonium oxidation activity. Throughout the cycle, 85 % of initial ammonium was oxidized indicating that, system could handle the load. However, maximum specific ammonium oxidation rate was obtained as 0.0334 mg NH_4^+-N/mgVSS.h which was considerably lower than the previous sets.

Ammonium concentration was increased stepwise up to 800 mg NH_4^+-N /L within the following 7 days. Results showed that the enriched culture of nitrifier was able to oxidize 800 mg NH_4^+-N /L per day.

Figure 4.21, 4.22 and 4.23 illustrates the results of the 82nd day of the acclimation period. At that time, maximum specific ammonium removal rate was obtained as 0.055 mg NH_4^+-N/mg VSS.h for the first 6 hours. pH was decreased from 8.3 to 7.6 during the experiments. As can be seen from the previous figures, when the alkalinity concentration was approximately 8.75 fold higher of the initial ammonium concentration, which is accurately close to the theoretical value of 8.7 for complete nitrification, enough alkalinity could be provided for the nitrification process. Ammonium removal efficiency was obtained as 98 % at day 82.

At 103rd day of the acclimation period, ammonium concentration of the influent was remained 800 mg NH_4^+-N/L. More than 96 % of the initial ammonium was oxidized throughout the 24 hour cycle.

For the next 4 days, system was fed with the same concentration of ammonium as 800 mg NH_4^+-N/L. During this period stable and more than 95 % of ammonium oxidation was obtained. Thus, fill and draw system was converted to a chemostat and started to operate in continuous flow mode with the intention of a partial nitrification system.

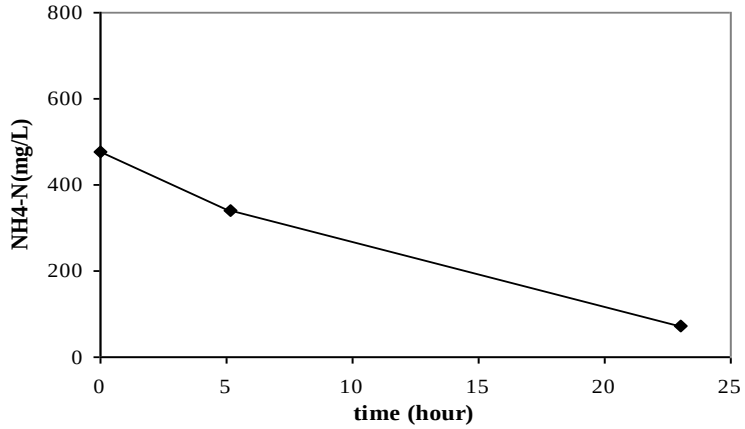

Figure 4.18 Ammonium removal versus time for the 75th day

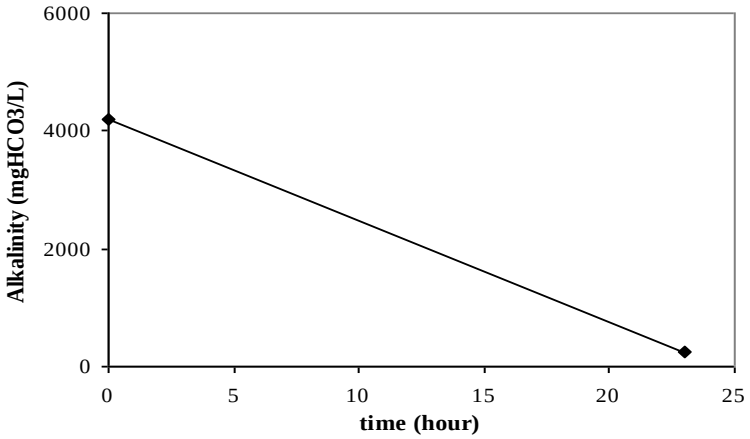

Figure 4.19 Alkalinity consumption versus time for the 75th day

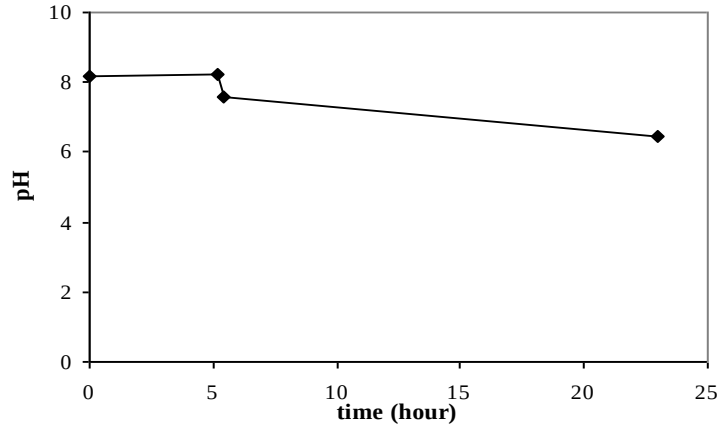

Figure 4.20 pH alteration versus time for the 75th day

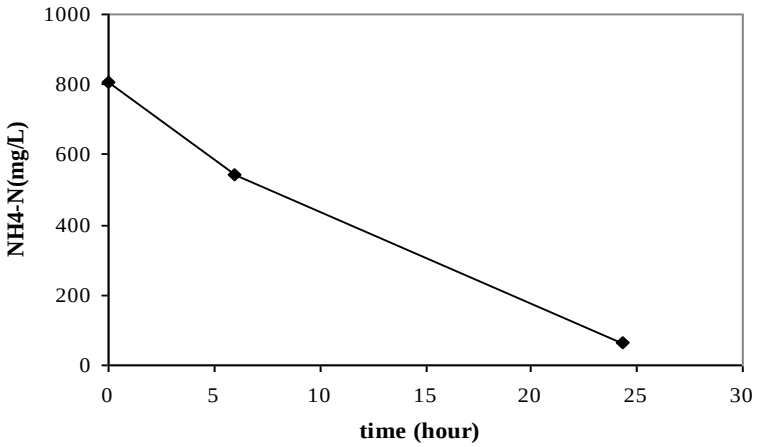

Figure 4.21 Ammonium removal versus time for the 82nd day

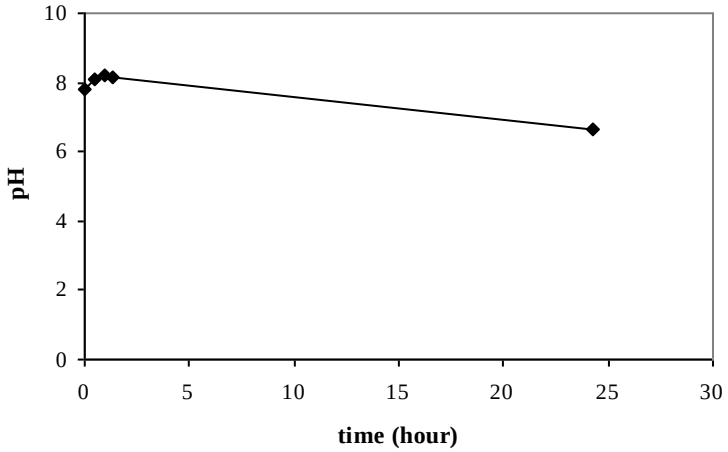

Figure 4.22 pH alteration versus time for the 82nd day of

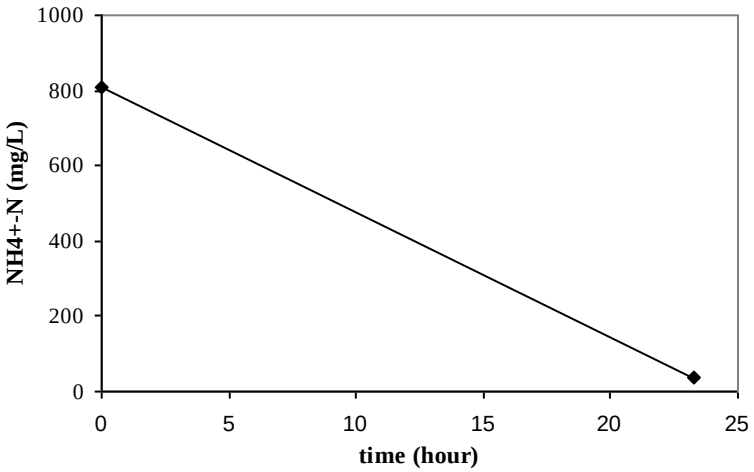

Figure 4.23 Ammonium removal versus time for the 103rd day

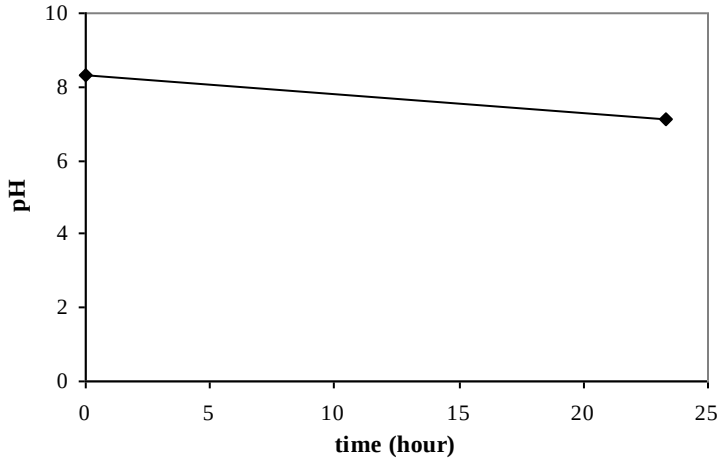

Figure 4.24 pH alteration versus time for the 103rd day

Table 4.1 represents the reactor performance interms of the maximum specific ammonium removal rates observed for a period of 82 days. As can be seen from the table 4.1, the maximum specific ammonium removal rates were varying within a range of 0.085 to 0.06 mg NH_4^+-N/mg VSS.h depending on the initial ammonium concentrations. For an initial ammonium concentration of 40 mg/L the maximum specific ammonium removal rate was observed as 0.085 mg NH_4^+-N/mg VSS.h as the lowest rate during the experiments, indicating that the growth was under substrate limiting conditions. The increase in ammonium concentrations from 150 mg/L to 475 mg/L did not reflect any major difference, in specific ammonium removal rates as a result of the same growth rate conditions. On the other hand, ammonium concentration to 800 mg/L caused much higher rate indicating the maximum specific

growth rateconditions. An average specific ammonium removal rate was calculated as 0.045 mg NH_4^+-N/mg VSS.h with omitting the extreme values in the table. pH was fluctuated in a wide range of 6.5 to 8.9 depending on the ammonium removal rate and bicarbonate concentration in the reactor.

Table 4.1 Maximum specific ammonium removal rates during the acclimation period

Days	Initial ammonium concentration (mg/L)	pH	average VSS (mg/L)	Maximum specific ammonium removal rate mg NH_4^+-N/mgVSS.h
0 – 30	40	7.3-8.2	400	0.0085
31 – 37	150	7.8-8.9	400	0.045
38 – 41	200	7.00-8.6	400	0.048
42 – 53	375	6.6-8.7	500	0.042
54	475	8.1-8.6	570	0.048
55 – 69	800	7.3-8.2	810	0.066
70 – 75	475	6.5-8.2	780	0.033
76 – 82	800	6.7-8.2	800	0.055
Average	385	7.2-8.5	530	0.045

4.2 Continuous Cultivation of Ammonium Oxidizers in a Chemostat Reactor

As mentioned in the previous section, an enriched culture of nitrifier was obtained in fill and draw reactors with an ammonium removal efficiency of 95 %. The culture was used as a seed for the continuous flow completely stirred tank reactor and the reactor was operated as a chemostat system that works in principally at the same dilution rate as the growth rate. Thus, it was possible to maintain a regular concentration of biomass in the reactor since the growth portion was washed out from the system. The reactor temperature was maintained between 30-35 ^oC to favor the growth conditions for ammonium oxidizers which have a faster growth rate than nitrite oxidizers at higher temperatures (30-40^oC) (Figure 2.5). Hence nitrite

oxidizers could be washed out by carefully selecting the HRT while ammonium oxidizers will be maintained in the reactor. In the system, SRT was set equal to HRT that means sludge residence time is controlled by the hydraulic retention time. Dilution rate was sustained between 0.87-1.02 d^{-1}.

4.2.1 Results of the partial nitrification

The results obtained from the chemostat system which was operated to achieve partial nitrification for a period of 129 days were presented in Figure 4.25 to 4.30.

In the first set, the system was operated at a SRT of 1.15 d which was equal to a dilution rate of 0.87 d^{-1} as seen in the figure. Due to operational problems, reliable results could only be achieved after 45 days. In the period which spans the days 45 to 104, partial nitrification could be achieved; however, the nitrite concentration in the effluent could not be kept stable. Figure 4.25 implies that there does not exist an average value for ammonium oxidation and nitrite production. The operational problems which were the interruption of aeration and failure of the effluent pump were also reflected on the figure on days 83 and 85, where the volumetric ammonium removal rates were decreased from 0.46 $kgN/m^3.d$ to 0.41 $kgN/m^3.d$ and then to 0.29 $kgN/m^3.d$, respectively while the maximum specific ammonium removal rate was decreased to 0.097mgN/mgVSS.h to 0.073 mgN/mgVSS.h. Accordingly, the nitrite production rate was also decreased. The decrease in the nitrite concentration in the reactor resulted also from the increase in the water level due to the failure of the discharge line.

In the second set, the sludge age was decreased to 0.99 d which was equal to a dilution rate of 1.02 d^{-1}. In that case, ammonium oxidation and nitrite production rates decreased dramatically which have led to washout of the ammonium oxidizers from the reactor. This result confirmed that the critical sludge age is 1 day as reported in the literature (Hellinga et al., 1998, van Kempen et al., 2001). As seen from the Figure 4.26 representing the biomass- and the dilution rate - time profiles, it is clear that the washout occurred between days 90 and 99 due to the higher dilution rates applied where dramatic decrease in nitrite production and ammonium removal was observed. After the 99^{th} day of operation, influent ammonium loading rate was decreased gradually, first to 0.591 $kgN/m^3.d$ and then to 0.153 $kgN/m^3.d$ with the maximum specific ammonium removal rate of 0.183 mgN/mgVSS.h to 0.029 mgN/mgVSS.h. During this time period, it was observed that the concentration of

VSS also decreased rapidly from 300 mg/L to 30 mg/L. In order to recover the system performance the microorganisms previously wasted from the reactor were added into the reactor and a HRT=SRT of 1.15 d was applied again. This have led to a gradual increase in ammonium loading rate to 0.305, 0.370, 0.414, 0.50 and finally to 0.521 kgN/m^3.day with maximum specific ammonium removal rate of 0.088, 0.085, 0.090, 0.125 and 0.147 mgN/mgVSS.h. From day 104 to day 113 between which the ammonium oxidation and nitrite production were achieved as 95 % and 100 %, respectively. This was another proof for the critical sludge age for partial nitrification at a temperature of 35^oC.

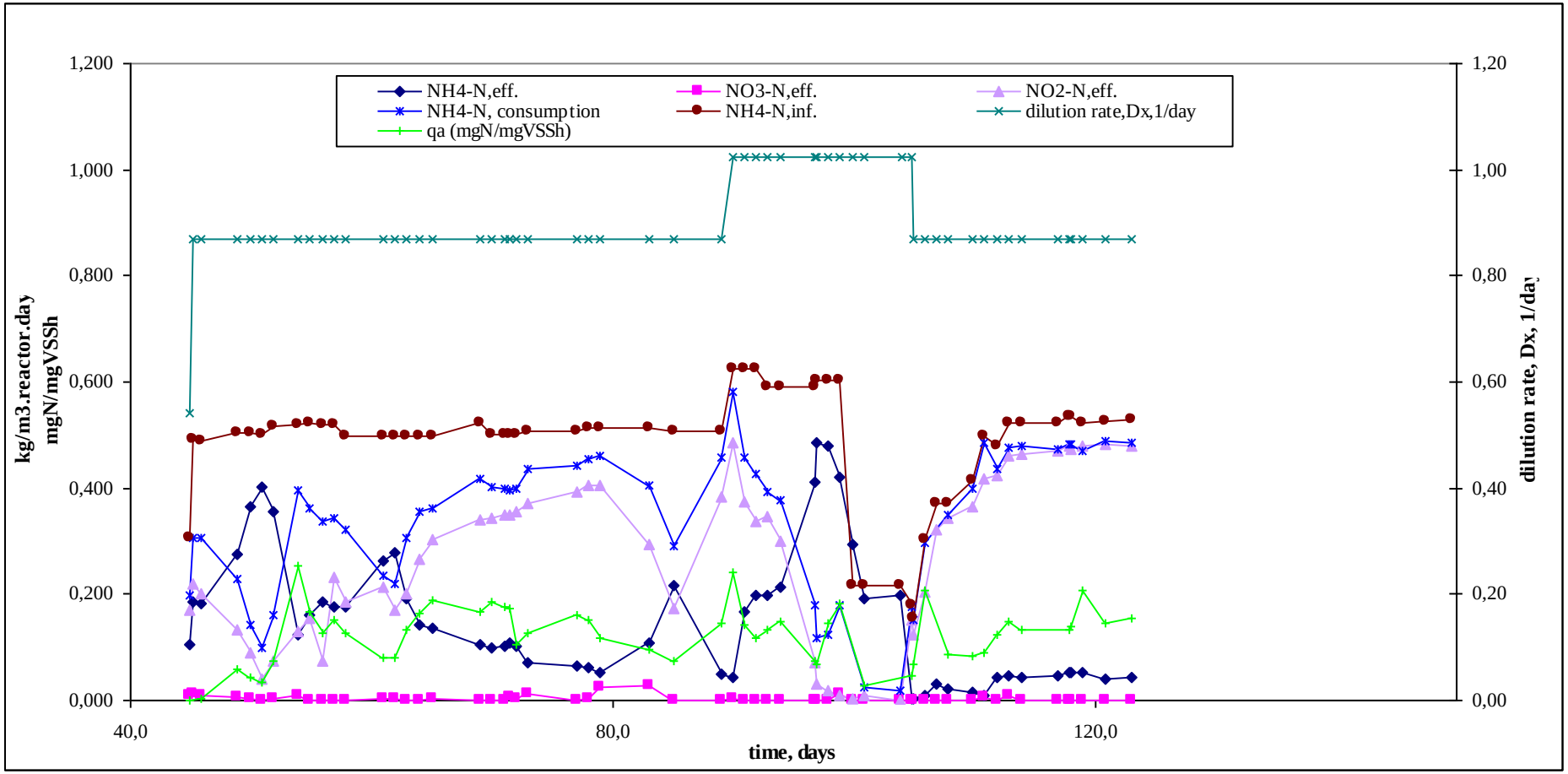

Figure 4.25 Volumetric ammonium loading, ammonium oxidation and effluent loading rates, nitrite and nitrate production rates and dilution rate versus time for continuous system

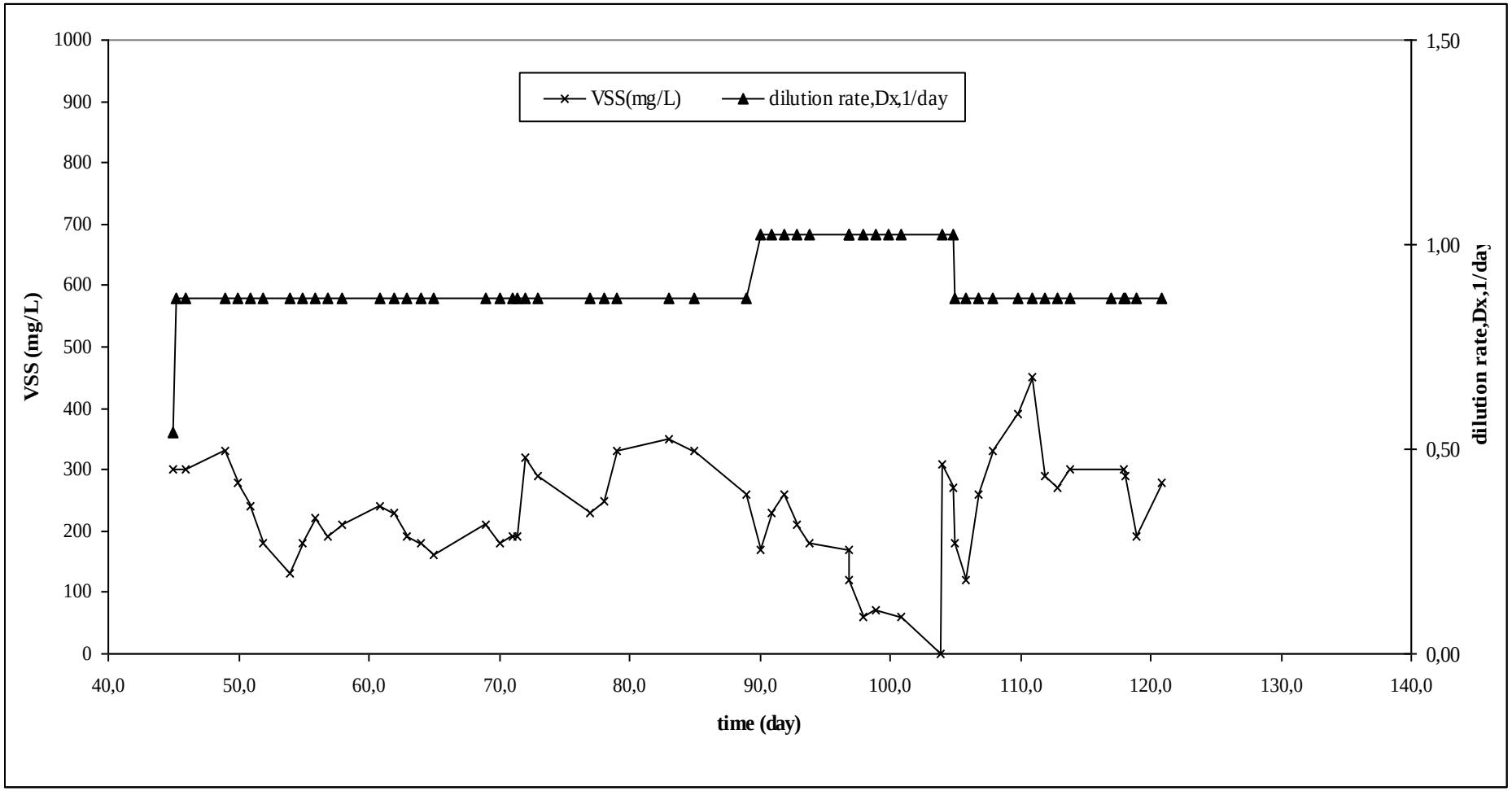

Figure 4.26 The interaction between VSS concentration and dilution rate versus time before methanol feeding

4.2.2 Results of the denitrification subsequent to partial nitrification

After a sustainable partial nitrification, a methanol feed of 100 mg COD/L was applied into the system on the 130th day. This was the start point of denitrification process for the following 16 days. For partial nitrification, aerobic retention time was set 1.15 days and anoxic period was set for 0.5 days for denitrification as reported in the literature (Hellinga et al., 1998). The Hydraulic Retention Time which was equal to Sludge Retention Time was changed to 1.65 days which was equal to a dilution rate of 0.67 d^{-1} by changing the reactor volume of 2.6 L. Figure 4.27 reflects the results of the methanol feeding period. Ammonium concentration in the influent was kept the same as before, volumetric loading and consequently removal rate was decreased due to increased volume of the reactor. Interestingly, volumetric ammonium removal rate was significantly decreased from 0.5 kg N/m^3.day to 0.2 kg N/m^3.day within one week. Similarly, nitrite production was sharply decreased, which reflects not only decreased ammonium removal rate but also denitrification of produced nitrite. Although 94 % of the inlet ammonium was oxidized, only 61 % of the oxidized ammonium was recovered as nitrite. This result reveals that approximately 33 % of the produced nitrite was denitrified with methanol addition. However, denitrification subsequent to partial nitrification of high ammonium containing wastewater could not be clearly established because both ammonium oxidizers and denitrifiers are very sensitive microorganisms to the environmental changes and more controlled systems are required to achieve denitrification subsequent to partial nitrification processes. As a result of operational problems only very few denitrification of nitrite was observed. Figure 4.28 represents the VSS concentrations depending on the dilution rate versus time after the methanol feeding and it is seen that VSS concentration was decreased from 280 mg/L to 200 mg/L after the dilution rate was decreased to 0.67 d^{-1}. During this time period more stable VSS concentrations were obtained in the system.

Both partial nitrification and denitrification subsequent to partial nitrification periods results are demonstrated in Figure 4.29.

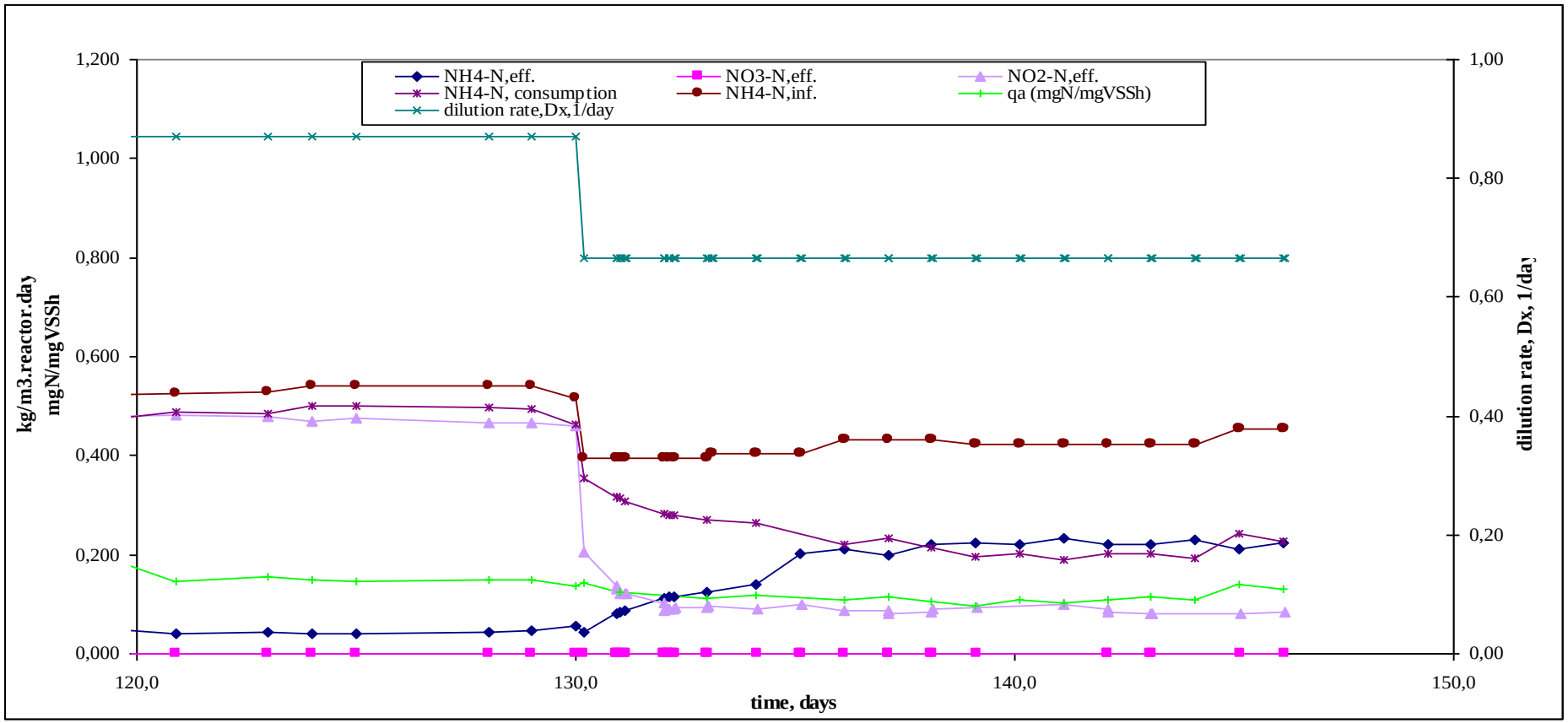

Figure 4.27 Ammonium loading, consumption and effluent loading rates, nitrte and nitrate production rates and dilution rate versus time graphic after methanol feeding in continuous system.

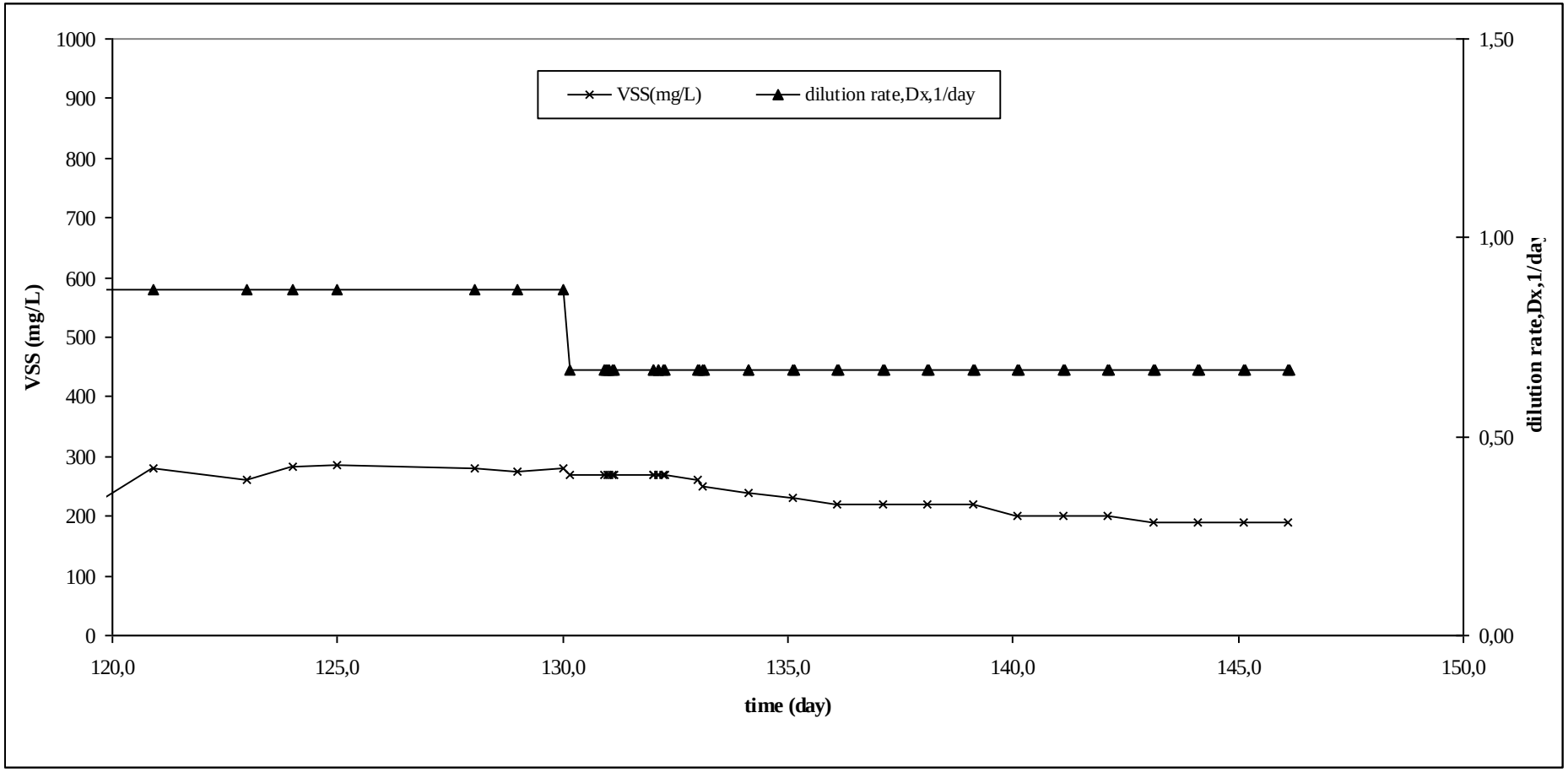

Figure 4.28 The interaction betwen VSS and dilution rate versus time after the methanol feeding in continuous system

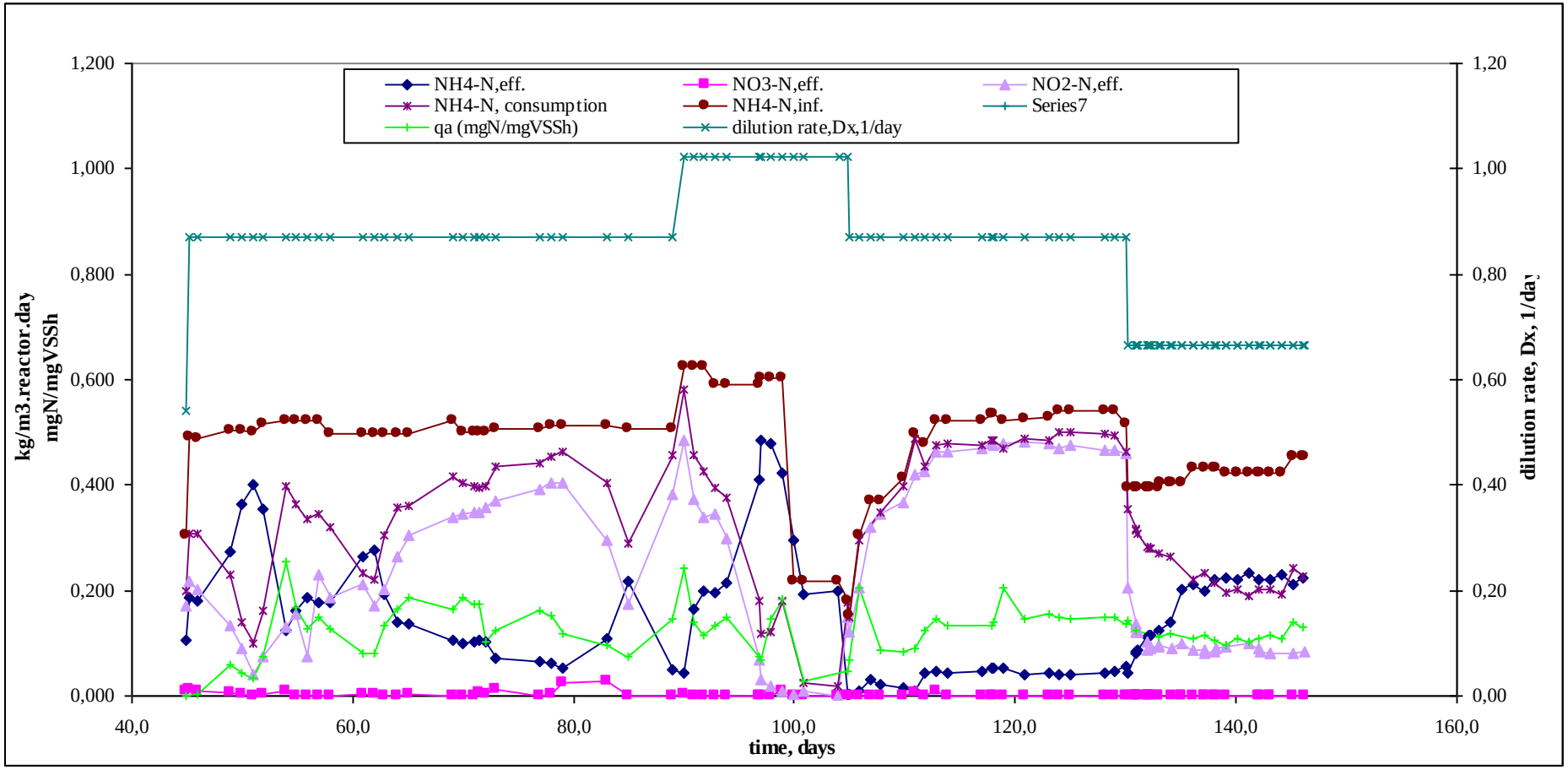

Figure 4.29 Volumetric ammonium loading, ammonium oxidation and effluent loading rates, nitrite and nitrate production rates and dilution rate versus time for continuous system before and after the methanol feeding

Throughout all experiments, pH varied in a wide range between 6.1 to 9.1 as can be seen from Figure 4.30, since it is effected from every single change in operational parameters. At 54th day, wastewater effluent and influents were interrupted and as a result, the lowest value of pH of the system was observed as 6.1. After problems were recovered, pH was increased 7.1 at 55th day. Another pH decrease was observed at 79th day because of influent wastewater interruption and pH value was measured as 6.2. As it can be seen from the Figure 4.25, although pH increased to 6.6 and then 7.3 at 83rd and 85th days, nitrite formation was decreased from 0.405 to 0.295 to 0.174 kgN/m^3 d values and nitrate formation was increased from 0.001 to 0.024 and then decreased to 0.001 kgN/m^{3}d values which was the highest nitrate formation rate in the system as a result of influent wastewater interruption at 79th, 83rd and 85th days. However during the whole experiments almost no nitrate formation was observed. At the day of 89, nitrite was increased to 0.383 and at 90th day to 0.486 by recovering system performance. At 89th day pH was measured as 6.8 as a result of aeration mixing and effluent wastewater interruptions at 85th day. At 90th day pH was measured as 7.03. After the methanol feeding, pH of the system fluctuated between 6.56 and 8.55. The lowest pH value was observed at 131st day depending on the influent pH value of 6.9.

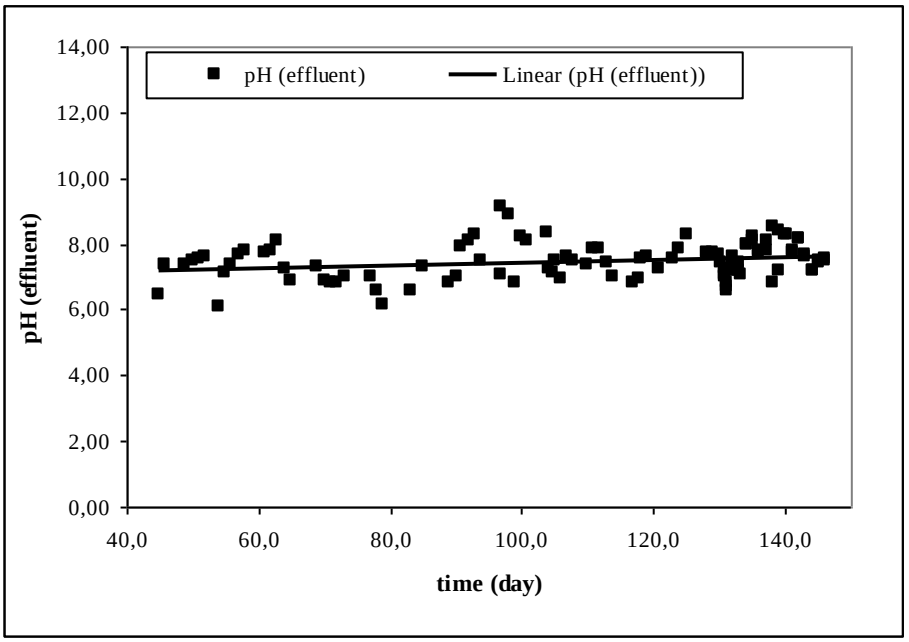

Figure 4.30 Effluent pH values versus time graphic for continuous system

Influent pH values were measured in the feeding solution stock. It was observed that the pH of the feed solution tended to increase with time (Figure 4.31) therefore it was decided to add HCl into the influent solution in order to prevent the pH from increasing which would cause free ammonia formation. However, it was observed that the pH changed between 6.9 and 9.3 at the influent stock solution.

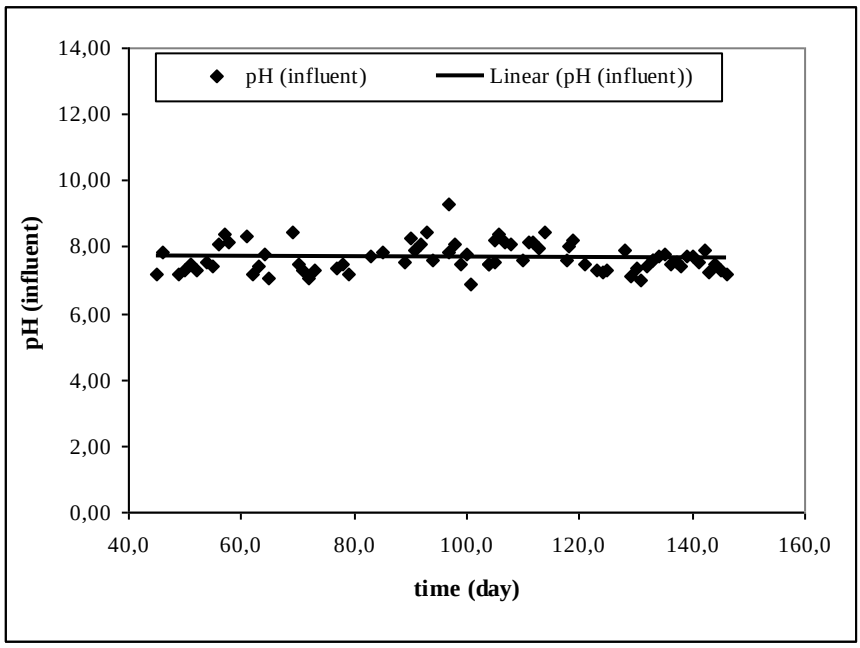

Figure 4.31 Influent pH values versus time graphic for continuous system

5. CONCLUSIONS

Partial nitrification-denitrification becomes the favourable choice of treatment for wastewaters with a high ammonium content particularly for the effluents from sludge digestion units since they mostly have elevated temperatures and low carbon contents.

In the scope of this study, the treatability of synthetic wastewaters having high ammonium contents by means of partial nitrification-denitrification was investigated. The experimental studies were conducted in two stages.

In the first stage, enrichment of the nitrifiers was obtained in a fill and draw reactor by using synthetic wastewater containing only ammonium, alkalinity and trace elements for the biomass growth. Since the feeding solution did not contain organic carbon, the enrichment of the nitrifiers were achieved successfully within 30 days. Then the acclimation of biomass to high ammonium concentrations in order to achieve partial nitrification was studied in fill and draw reactors keeping the sludge age at 25 days and a constant temperature at 30 ^{o}C. Except the results obtained with the ammonium loading ranging from 40 mg/L for 30 days to 800 mg/L for 69 days, the ammonium removal rates varied between 0.033 - 0.055 mgNH_4/mgVSS.h with an average value of 0.045 mgNH_4/mgVSS.h. The ammonium removal rates staying the same for the loadings of 150 to 800 mg/L indicated that the microorganisms were under the maximum growth conditions.

After the fill and draw mode of operation, experiments were carried out with the chemostat which revealed almost zero nitrate concentrations in the effluent hence full partial nitrititation was achieved. The results have shown that the system is very sensitive to the pH changes. It was also confirmed that the critical sludge age was 1 d. The rates for nitrite production and ammonium consumption were achieved as 0.405 kgN/m^{3}reactor.d and 0.46 kgN/m^{3}reactor.d, with maximum specific ammonium loading rate of 0.073 mgN/mgVSS.h. and 0.097 mgN/mgVSS.h. respectively for the sludge age of 1.15 d.

This study have shown that the conduction of partial nitrification was applicable but required substantial efforts. It was also proved that partial nitrification worked very well particularly for the treatment systems under organic limiting conditions.

At the last step of the experimental study, denitrification subsequent to partial nitrification was studied and system was operated for 12 cycles in a day with 80 minutes oxic and 40 minutes anoxic periods. The sludge retention time had been applied as the same as the hyraulic retention time of 1.65 days in the experimental period of 16 days. Methanol was used as an organic carbon source since it is economically more favourable than the others. For the anoxic cycle, methanol addition was completed in the first minute of the anoxic period. $HRT_{oxic} = SRT_{oxic}$ = 1.15 days applied again for the partial nitrification process and $HRT_{anoxic} = SRT_{anoxic}$ = 0.5 day was applied for denitrification process. Denitrification of high ammonium containing wastewaters was not clearly established since these processes requires more sensitive reactors. However, during the denitrification process 91 % ammonium removal was obtained with a 61 % nitrite production which means that approximately 33 % of the denitrification was obtained within a week of the denitrification process.

As a further step dynamic modelling can be applied on the collected data in order to determine the relevant kinetic coefficients more accurately.

REFERENCES

Abeling U., Seyfried C. F., 1992. Anaerobic- aerobic treatment of potatoe – starch wastewater. *Second International Symphosium on Waste Management Problems in Agro Industries* İstanbul Turkey

Almeida J.S., Reis M.A.M., Carrondo M.J.T., 1995 Competition between nitrate and nitrite reduction by denitri.cation by Pseudomonas fluorescens. *Biotechnol. Bio. Eng*; **46**: 476–84

Antileo C., Aspe E., Urrutia H., Zaror C. and Roeckel M., 2002. Nitrifying Biomass Acclimation to High Ammonia Concentration, *Journal of Environmental Engineering*, **128**:4(367)

APHA, 1989. *Standard Methods for the Examination of Water and Wastewater, 17th Edition, American Public Health Association*, Washington DC.

Beg S. A. and Mirza M. Hassan 1987. Effect of inhibitors on nitrifcation in a packed bed biological flow reactor. *Water Research* **21**(191-198).

Campos J. L., Mosquera-Corral A., Sanchez M., Mendez R., Lema J.M., 2002. Nitrification in saline wastewater with high ammonia concentration in an activated sludge unit. *Water Research* **36**(2555-2560)

Carera J., Baeza J. A., Vieent T., Lafuente J., 2003. Biological nitrogen removal of high strength ammonium industrial wastewater with two-sludge system. *Water Research* **47**(4211-4221)

Carera J., Jubany I., Carvallo L., Chamy R., Lafuente J., 2004. Kinetic models for nitrfication inhibition by ammonium and nitrite in a suspended and immobbilised biomass systems. *Process Biochemistry* **39**(1159-1165)

Chen G. H., Wong M. T., Okabe S., Watanabe Y., 2003. Dynamic response of nitrifying activated sludge batch culture to increased chloride concentration. *Water Research* **37**(3125-3135)

Downing, A. L., Painter H. A. and Knowles G., 1964. Nitrification in the Activted Sludge Process, *J. Inst. Sew. Purif.*, **3**(2):130.

Dinçer A. R., Kargı F., 2000. Kinetics of sequential nitrification and denitrification processes. *Enzyme and Microbial Technology,* **27** : 37–42

Gapes D., Pratt S., Yuan Z., Keller J., 2003. Online titrimetric and off-gas analysis for examining nitrification processes in wastewater treatment. *Water Research* **37** (2003) 2678–2690

Glass C. and Silverstein J., 1997. Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. *PII: S0043-1354*(97)00260-1

Glass C. and Silverstein J., 1997. Inhibition of denitrification in activated sludge by nitrite. *Water Environmental Research* **69**(1086).

Glass C. and Silverstein J., 1998. Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. *Water Research*, **32**(3), 831-839.

Glass C. and Silverstein J., 1998. Denitrification of high nitrate, high salinity wastewater. *PII: S0043-1354*(98)00177-8

Guo-min C., Qing-xiang Z., Xian-bo Sun, Tong Zhang., 2002. Characterization of nitrifying and denitrifying bacteria coimmobilized in PVA and kinetics model of biological nitrogen removal by coimmobilized cells. *Enzyme and Microbial Technology*, **30**: 49–55

Gupta S. K., Sharma R., 1996. Biological oxidation of high strength nitrogenous wastewater. *Water Research* **30**(593-600).

Hellinga C., Schellen A. A. J. C., Mulder J. W., van Loosdrecth M. C. M., Heijnen J. J., 1998. The SHARON process: An innovative method for nitrogen removal from ammonium rich wastewater. *Water Science and Technology* Vol **37** No 9 (135-142)

Jianlong W., Ning Y., 2004. Partial nitrification under limited dissolved oxygen conditions. *Process Biochemistry* **39** (1223-1229)

Kempen R. Van, Mulder J. W., Uijterlinde C. A., van Loosdrecth M. C. M., 2001. Overview: full scale experience of the SHARON process for

treatment of rejection water of digested sludge dewatering. *Water Science and Technology* Vol **44** No 1 (145-152)

Lee K.C., Rittmann B., 2003. Effects of pH and precipitation on autohydrogenotrophic denitrification using the hollow-.ber membrane-biofilm reactor. *Water Research* **37** (2003) 1551–1556

Oslislo A., Lewandowski Z., 1985. Inhibition of nitrification in the packed bed reactors by selected organic compounds. *Water Research* **19** (423-426)

Pollice A., Tandoi V., Lestingi C., 2002. Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. *Water Research* **36** (2541-2546)

Rittman, B.E. and McCarty, P.L., 2001. *Environmental Biotechnology*: *Principles and Applications, McGraw-Hill Companies*, Inc., NewYork

Ruiz G., 2000. Nitrification-denitrification via nitrite (Spanish) *Biochemical Engineering School, Universidad Catolica de Valparaiso*, Chile.

Ruiz G., Jeison D., Chamy R., 2003. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Water Research* **37**(1371-1377)

Sevior, R.J. and Blackall, L.L., 1998. *The microbiology of nitrogen removal in activated sludge systems*. London

Svenson A., Sanden B., Delhammar G., Remberger M., Kaj L., 2000. Toxicity identification and evaluation of nitrification inhibitors in wastewaters. John Wiley and Sons, Inc. *Environmental Toxicology*. **15**:527-532

Sözen, S., 1992. *Nitrifikasyon-Denitrifikasyon kinetiğinin deneysel Karakterizasyonu***,** İ.T.Ü. Fen Bilimleri Enstitüsü, İstanbul.

Tchobanoglous, G. And Burton, F.L., 1991. *Wastewater Engineering, treament, disposal and reuse/ 3rd Edition, Metcalf&Eddy Inc*., USA.

Thomas KL, Lloyd D, Boddy L., 1994. Effects of oxygen, pH and nitrate concentration on denitrification by Pseudomonas species. *FEMS Microbiol Lett*;**118**:181–6.

Tseng C. C., Potter T. G., Kopman B., 1997. Effect of influent chemical oxygen demand to nitrogen ratio on a partial nitrification/complete denitrification process. *Water Research* Vol:**32** (165-173)

Uygur A., Kargı F., 2004. Biological Nutrient removal from pre-treated landfill leachate in a sequencing batch reactor. *Journal of Environmental Management* **71**(9-14)

Verstraete W., Philips S., 1998. Nitrification-denitrification processes and Technologies in new contexts. *Environmental Pollution* **102** (717-726)

Wett B., Rauch W., 2003. The role of ınorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. *Water Research* **37**(1100-1110)

Yoo H., Ahn K. H., Lee H. J., Lee K. H., Kwak Y. J., Song K. G., 1999. Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification via nitrite in an intermitently aerated reactor.*Water Research* Vol. **33**, No. 1 (145-154)

Yun H. J., Kim D. J., 2003. Nitrite accumulation characteristics of high ammonia wastewater in an autotrophic nitrifying biofilm ractor. *Journal of Chemical Technology and Biotechnology* **78** (377-383)

Zhu S., Chen S., 2002. The impact of temperature on nitrification rate in fixed film biofilters. *Aquacultural Engineering* **26** : 221-237

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