Editorial Manager(tm) for Reviews in Environmental Science and Bio/technology
Manuscript Draft

Manuscript Number:

Title: Bio-removal of nitrogen from wastewaters—A review

Article Type: Reviews

Keywords: wastewaters, nitrogen removal, salinity, free ammonia, temperature, dissolved oxygen

Corresponding Author: Prof. Dr. rer.nat. Josef Winter, Doctor of natural sciences

Corresponding Author's Institution: University of Karlsruhe, Germany

First Author: Gaber Zayed, Professor

Order of Authors: Gaber Zayed, Professor; Josef Winter, Doctor of natural sciences

Abstract: If the present large volumes of nitrogen-containing wastewater of domestic and industrial origin are discharged into the environment without proper treatment, they lead to extensive soil and water pollution. Proper elimination of pollutants from these effluents is essential in industrialized countries and is becoming increasingly important from an environmental and human health point of view in developing and emerging countries. Beside the conventional nitrogen removal process (lithoautotrophic nitrification and denitrification), novel and cost-effective biological nitrogen elimination processes have been developed, including simultaneous nitrification and denitrification, anaerobic ammonium oxidation (Anammox), and its combined system (completely autotrophic nitrogen removal over nitrite, Canon). This review summarizes the recent studies dealing with agricultural, domestic and industrial wastewaters regarding their nitrogen content. Traditional and novel biological nitrogen elimination technologies are reviewed. Furthermore, recent studies dealing with temperature, dissolved oxygen, nitrate concentration, salinity, pH or the free ammonia concentration as factors affecting the nitrogen removal efficiency have also been incorporated.

Bio-removal of nitrogen from wastewaters—A review

Gaber Zayed

Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Egypt

Josef Winter*

Institut für Ingenieurbiologie und Biotechnologie des Abwasser, Universität Karlsruhe, Germany

Josef.Winter@iba.uka.de

Telefon: +49 (0) 721 608 2297: Telefax: +49 (0) 721 608 7704

***Corresponding authour**

Abstract

If the present large volumes of nitrogen-containing wastewater of domestic and industrial origin are discharged into the environment without proper treatment, they lead to extensive soil and water pollution. Proper elimination of pollutants from these effluents is essential in industrialized countries and is becoming increasingly important from an environmental and human health point of view in developing and emerging countries. Beside the conventional nitrogen removal process (lithoautotrophic nitrification and denitrification), novel and cost-effective biological nitrogen elimination processes have been developed, including simultaneous nitrification and denitrification, anaerobic ammonium oxidation (Anammox), and its combined system (completely autotrophic nitrogen removal over nitrite, Canon). This review summarizes the recent studies dealing with agricultural, domestic and industrial wastewaters regarding their nitrogen content. Traditional and novel biological nitrogen elimination technologies are reviewed. Furthermore, recent studies dealing with temperature, dissolved oxygen, nitrate concentration, salinity, pH or the free ammonia concentration as factors affecting the nitrogen removal efficiency have also been incorporated.

Keywords: wastewaters, nitrogen removal, salinity, free ammonia, temperature, dissolved oxygen.

1. Introduction

Clean, fresh and non-toxic drinking water is essential for humans and also for many animals. Water is vital both as a solvent in which many of the body's solutes dissolve and as an essential part of most metabolic processes within the body. Metabolism is the sum of anabolic and catabolic reactions. In anabolism, water is removed from molecules (through energy requiring enzymatic chemical reactions) in order to condensate small molecules to polymers (e.g. starch, triglycerides, poly β-hydroxybutyrate and proteins for storage of fuels and energy). In catabolism, water is used to break bonds in order to generate smaller molecules (e.g. glucose, fatty acids and amino acids from polymers to be used for energy generation or other purposes). Water is thus essential and central to these metabolic processes at the single reaction scale of life. Further, water plays an important role in ecology and its availability determines the world economy, as it functions as the basic solvent for a wide variety of chemical and industrial processes, e.g. it facilitates industrial cooling and transportation. Approximately 70 percent of freshwater is consumed for food and fed production by agriculture (Baroni et al. 2007). Although access to safe drinking water has improved steadily and substantially over the last decades in almost every part of the world (Lomborg 2001), in the developing countries 90% of all wastewater still goes untreated into local rivers and streams (UNEP 2002) and thus limits safe fresh water supply. Some 50 countries, with roughly a third of the world's population, also suffer from medium or high water stress, at least during the rain season and 17 of these extract more water annually than is recharged through their natural water cycles (Ravindranath et al. 2002**).** The increasing water demand not only affects surface freshwater bodies like rivers and lakes, but it also degrades groundwater resources. Due to more groundwater extraction than recharge it is expected that for instance the soil surface of Jakarta/Indonesia will settle 40-60 cm until 2020 (The Jakarta Post, 25. 08.2009) and periodic flooding of many city parts during the rain season will be the consequence. Eutrophication**,** associated with discharge of nitrogen compounds or nitrogen compounds-containing wastewater into freshwater has become a severe water pollution problem in many countries (Molinuevo et al. 2009). The water quality is deteriorated and potential hazards to human or animal health e.g. by toxic algal blooms are consequences. The presence of excess nitrogen in the environment has caused serious alterations of the natural nutrient cycle between the living world and the soil, water, and atmosphere **(**Obaja et al. 2003). Excess discharge of nitrate as a fertilizer but also as one the most common water and groundwater pollutants causes serious problems including cancer, blue-baby disease in new-born infants and methaemoglobinaemia **(**Höring and Chapman 2004). However there are many other pollutants in water such as e.g. antibiotics, X-ray contrasting agents, health care residues or sugar derivatives in industrial wastes or wastewater that are also potential toxicants. In recent years, a number of studies have focused on carbon, nitrogen and phosphate removal from domestic, agricultural and industrial wastewaters.

The objectives of this review are i) to identify nitrogen pollutants concentrations in domestic, agricultural and industrial wastewaters, ii) to compile the latest achievements of technologies developed for the removal of nitrogen from these wastewaters and iii) to clarify the effect of temperature, dissolved oxygen, nitrate concentration, salinity, pH and free ammonia concentrations as factors that influence the nitrogen removal efficiency.

2. Nitrogen -containing wastewaters

2.1. Nitrogen in agricultural wastewater

The growing demand for poultry and livestock production in both, developed and developing countries, has led to the accumulation of large quantities of nitrogen-containing animal wastes in limited areas. As a result too large quantities of animal and poultry manure are available as an organic fertilizer (Yetilmezsoy and Sapci-Zengin 2009). In recent years, several papers have addressed the recovery of nitrogen compounds from agricultural manures. The concentration of nitrogen compounds varies according to the origin of the respective manure. Poultry manure in a farm near Istanbul, Turkey, for instance, contained 1580 mg/l total Kjeldahl nitrogen and 1318 mg/l NH_4^+–N (Yetilmezsoy and Sapci-Zengin 2009). Livestock species, their type and age, the nature of their feed and how it is fed, whether or not the livestock are housed, weather and climate, all contribute to wastewater composition, volume and its rate of production (Knight et al. 2000). The variation of ammonium-N concentrations (mg N/l) in livestock wastewaters in south County Waterford, Ireland, was measured for almost a decade (Table 1). There were high annual variations of ammonia and even highr variations within single years (Harrington and McInnes, 2009).

Excessive amounts of chemical nitrogen fertilizers are applied in agriculture in many parts of the world under a broad spectrum of climatic conditions. Humid weather conditions may cause nitrate leaching, leading to pollution of surface and ground water resources. The consequences are eutrophication of surface waters and contamination of groundwater with nitrate. Such raw water sources should no longer be used as sources of potable water without treatment (Asadi et al. 2002; Jalali, 2005; Barton and Colmer 2006). The horizontal subsurface drainage system, in addition to controlling water table and leaching out harmful dissolved salts from the drained soil profile, may also cause losses of various forms of nitrogen through the drainage effluent (Singh et al. 2001). Such nitrogen losses, besides wasting a part of the applied fertilizer, are also likely to cause environmental degradation that will be detrimental to aquatic life, plants, and animals. Nitrogen leaching through subsurface drainage systems has been studied under different irrigation and fertilizer management regimes for semi-arid, arid and humid climates (Asadi et al. 2002; Tamini and Mermoud 2002; Darwish et al. 2003; Rajput and Patel, 2006). Gheysari et al. (2009 studied NO_3-N leaching from a soil depth of 30 cm under different nitrogen fertilizer levels and different irrigation systems. The estimated leached NO_3-N ranged from 3.1 kg /ha at no N application and deficit irrigation to 40.8 Kg/ha at fertilization level of 142 kg N/ha and full irrigation, which would be the minimum annual N-requirement for corn in European countries.

2.2. Nitrogen in domestic wastewater

2.2. Nitrogen in domestic wastewater

Effluent of domestic wastewater treatment plants contains high concentrations of inorganic nitrogen that may lead to eutrophication of the receiving water bodies (Mallick 2002; de-Bashan et al. 2004). In Rajasthan, India the raw sewage received at the activated sludge plant has a BOD of 600–800 mg/l and a NH_4^+-N concentration of 80–110 mg/l during summer when water shortage was acute (Gupta and Gupta 2001). Ammonia and eventually nitrate in the effluent caused eutrophication. The disposal of domestic wastewater in areas not served by sewer systems is almost exclusively by use of septic tanks and seepage fields. Effluents from septic tanks generally contain high concentrations of ammonia. Zeng et al. (2009) found that the ammonia concentration in real domestic wastewater from one septic tank in China was 54-74 mg/l. A similar finding was reported by Guo et al. (2009a). The effluent of septic tanks is usually discharged to aerobic seepage fields, where ammonia and organic nitrogen are transformed to nitrate, which may be trickling into the groundwater (Walker et al. 1992). Table (2) summarizes the values of total Kjeldahl nitrogen (TKN) and ammonia nitrogen (AN) found in domestic wastewaters in different locations during the recent years.

The traditional biological nitrogen removal processes applied in wastewater treatment plants involve the oxidation of ammonia (NH_4^+) to nitrate (NO_3^-) during nitrification and the reduction of nitrate with an organic carbon source (COD) to nitrogen gas in the denitrification process. Both, nitrification and denitrification, generate nitrite (NO_2^-) as an intermediate, which normally cannot be measured due to the fast following reaction. Hence, if nitrification would be stopped at nitrite (nitritation), then denitritation would require much less oxygen. However there may be situations where during denitritation on the way from nitrite to nitrogen some N_2O may be released, which is a climate-relevant gas emission that should be avoided.

2.3. Nitrogen in agroindustrial and industrial wastewaters

The concentration of nitrogen compounds in some industrial wastewaters is tremendously higher than what is found in agricultural and domestic wastewater. Ammonia and nitrate are the most problematic nitrogen compounds in this sort of wastewater. Ammonia in industrial wastewater is normally eliminated by nitrification which is achieved by the complete oxidation of ammonia (Sumino et al. 2006). Thus, nitrate removal from these types of industrial wastewater is an inevitable step in treatment. Different industrial and agro-industrial wastewaters are reported to contain more than 200 mg NO_3^--N/l (Zayed and Winter 1998; Peyton et al. 2001) and some contain even higher nitrate levels. For instance, the wastewater from glasshouses contained 325 mgNO_3^--N/l (Park et al. 2009). Several other industries generate wastewater with varying amounts of nitrate, being 81 mg/l in the wastewaters of the largest dairy factory in Israel (Arbeli et al. 2006), 222 mg/l in a tannery wastewater of Pisa. Italy, (Munz et al. 2008), 2320 mg/l in wastewater from the cochineal insects processing to produce natural carmine used principally as a colouring agent in cosmetics, beverages and products with low pH (Chimenos et al. 2003), 3600 mg/l generated from an initiating explosive factory in China (Shen et al. 2009) and 4000-6000 mg/l produced during the frosting process of bottles in a winery (Carrera et al. 2004). In Egypt the El-Nasr Pharmaceutical and Chemical Company, South-East of Cairo, discharges both industrial (6000 m^3/d) and municipal wastewater (128 m^3/d) into a nearby evaporation pond without any treatment. The generated raw wastewater is characterized by high values of COD (4100–13,023), TSS (20–330 mg/L), oil grease (17.4–600 mg/L) and ammonium (about 300 mg/l) (Badawy et al. 2009)

3. Processes for N-removal

3.1. Nitrification/denitrification

Biological autotrophic nitrification followed by heterotrophic denitrification has long been applied in municipal wastewater treatment. For application of nitrification-denitrification in industrial wastewater treatment, however, things become more complicated because the characteristics of wastewaters vary case by case and sometimes even day by day. Wastewater from antibiotics production, for instance, usually contains large amounts of fermentation products, some residual antibiotic activity, and a high concentration of ammonia. These wastewasters and some fermentation byproducts may not be easily utilized by denitrifiers as electron donors, and the residual antibiotics have a toxic effect on microorganisms (Watkinson 2007).

Under strict aerobic conditions, complete nitrification is carried out in two sequential oxidative stages: ammonia is first converted to nitrite by ammonia-oxidizing bacteria:

$$\mathrm{NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + H_2O + 2\,H^+} \qquad (1)$$

Then the nitrite is further converted to nitrate by nitrite-oxidizing bacteria:

$$\mathrm{NO_2^- + 0.5\,O_2 \rightarrow NO_3^-} \qquad (2)$$

Each oxidative stage is performed by different bacterial genera which use ammonia or nitrite as an energy sources and molecular oxygen as electron acceptor, while carbon dioxide is used as a carbon source. The most commonly recognized genus of bacteria that carries out ammonia oxidation is *Nitrosomonas*. However, *Nitrosococcus*, *Nitrosopira*, *Nitrosovibrio* and *Nitrosolobus* are also able to oxidize ammonium to nitrite. These ammonia oxidizers are genetically diverse, but related to each other, and can be found in the beta subdivision of the *Proteobacteria*. For nitrite oxidation several genera such as *Nitrospira*, *Nitrospina*, *Nitrococcus*, and *Nitrocystis* are known to be involved. However, the most famous nitrite oxidizing genus is *Nitrobacter*, which genetically is closely related within the alpha subdivision of the *Proteobacteria* (Rittmann and McCarty 2001). The complete nitrification, as seen during wastewater treatment can be expressed as follow:

$$\mathrm{NH_4^+ + 2\,O_2 \rightarrow NO_3^- + H_2O + 2\,H^+} \qquad (3)$$

In a subsequent process denitrification is generally performed by heterotrophic denitrifyers under anoxic conditions. The oxidized nitrogen compounds (NO_2^- and NO_3^-) are reduced to gaseous nitrogen by heterotrophic microorganisms that use nitrite and/or nitrate instead of oxygen as electron acceptors and organic matter as a carbon and energy source. Denitrifiers are common among the Gram-negative bacteria such as *Pseudomonas*, *Alcaligenes*, *Paracoccus*, and *Thiobacillus*. Some Gram-positive bacteria (such as *Bacillus*) and a few halophilic archaeal microorganisms (e.g. *Haloferax denitrificans*) are able to denitrify (Kim et al. 2005; Cyplik et al. 2007b). Unlike some contaminants which are in need for a certain microbe to be treated, denitrifying bacteria are ubiquitous in nature (Szekeres et al. 2001) and numerous researchers cultivated them using mixed cultures taken from wastewater treatment plants as seeds. There has been a huge interest towards microbial removal of nitrate as the most environmentally friendly and cost-effective method, although biological denitrification may be slow, particularly for industrial wastewaters that contain high concentrations of nitrate (Foglar et al. 2005). The process of heterotrophic denitrification in environmental biotechnology is accomplished with a variety of electron donors and carbon sources. Both liquid and solid forms of organic carbon sources are conventionally used although the aqueous type is more common for treatment of water and wastewater. Among liquid carbon sources, the most common ones are methanol, ethanol (Gomez et al. 2003; Osaka et al. 2008) and acetic acid which have been used for wastewater denitrification as well as in full-scale plants of drinking water treatment (Feleke and Sakakibara 2002). A combined carbon source using methanol and acetic acid was found to be superior in nitrogen removal and additional benefits of this mixed carbon source included the excellent sludge settling properties compared to the use of methanol or acetic acid alone (Cho Molof 2004). Park et al. (2008) treated waste plant material either physically or biologically to produce several organic carbon rich liquors for use in denitrification experiments. The choice of substrate depends on a number of considerations such as costs, capacity and configuration of reactors and on the post-treatment process of the denitrified water (Szekeres et al. 2002). The theoretical methanol requirement for nitrate is 2.47 mg CH_3OH per mg NO_3-N as indicated in equation (4). Neglecting bacterial growth and the residual oxygen content of the wastewater from nitririfcation, the requirement is decreased to 1.9 (Young-Ho Ahn 2006).

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7O_2N + 0.47N_2 + 1.68H_2O + HCO_3^- \qquad (4)$$

Later studies tried to speed up biological denitrification by applying different process strategies through which a better contact of the nitrate in the water with microorganisms was maintained, such as packed beds (Daniel et al. 2009), rotating biological contactors (Teixeira Oliveira 2001) and fiber-based biofilm reactor (Wang et al. 2009). Efforts are still ongoing and some novelties in combination of biological and other methods, such as membrane biofilm reactors (MBR), were manifested (Terada et al. 2003).

3.2. Simultaneous nitrification and denitrification (SND)

Among many innovative technologies for nitrogen removal, nitrogen removal via nitrite was reported to be technically feasible and economically favorable. The process starts with a partial nitrification of NH_4^+ to nitrite and subsequently continues with a direct reduction of nitrite to N_2 gas (Jenicek et al. 2004; Laiet al. 2004). In SND nitrification and denitrification occur concurrently in the same reactor vessel under identical operating condition. If successful, this process could reduce the relatively large reactor volumes and energy costs for recirculation that are required for a separated aerobic and anoxic system. Several types of treatment units have been proposed in which SND can be realized (Guo et al. 2005; Zhang et al. 2007). Zhang et al. (2007) introduced a flexible biofilm reactor having adjustable aerobic, buffer and anoxic zones with liquid circulation being dependent on the aeration flow rate. Both studies were successful in proving the possibility of nitrification and denitrification in one reactor. Successful SND experiments were also carried out by Walters et al. (2009) who used a biofilm airlift suspension reactor with biodegradable carrier material. Investigation of Fux et al. (2006) of the shortened nitrogen removal pathway via nitrite revealed a high reduction of the COD demand for denitrification, a high rate of denitrification, low biomass yield during anaerobic growth and no apparent nitrite toxicity effects for the microorganisms in the reactor. SND is also effective in maintaining a neutral pH level in the reactor, without the addition of an acid or base. (Chang and Tseng 1999). This is important since a narrow optimal pH range between 7.5–8.6 is known to exist for the nitrifying bacteria. During nitrification alkalinity is consumed, but alkalinity is produced during denitrification. The optimal pH lies between 7 and 8 for denitrification with different optima for different bacterial populations. Further, Ma et al. (2009) constructed a bench-scale continuous flow system, consisting successively of an anaerobic hydrolysis column (column A), an anoxic column (column D) and two aerobic columns (columns B and F) to remove nitrogen and carbon simultaneously from terramycin crystallization mother solution (TCMS). Columns A and D were sludge-bed reactors, and columns B and F were biofilm reactors packed with polyethylene balls and soft fibers hung on plastic rings, respectively. Approximately 82% of the chemical oxygen demand (COD) and 81% total nitrogen were removed by the system when tap water diluted TCMS was continuously fed (dilution ratio, 1:4). Sulfide which was produced during anaerobic hydrolysis was used as part of electron donors for denitrification in column D.

Polymeric beads, in which a nitrifier and a denitrifier were co-immobilized, were used to remove nitrogen in a single step. Uemoto and Saiki (2000) investigated a novel immobilized-cell bioreactor containing packed gel envelopes capable of simultaneous nitrification and denitrification. The packed gel envelopes consisted of two polymeric gel plates with an internal space between them for injecting the electron donor for denitrification. An ammonia oxidizer, namely, *Nitrosomonas europaea*, and a denitrifier, namely, *Paracoccus denitrificans*, were coimmobilized in the plate gel. The immobilized *N. europaea* oxidized ammonia to nitrite on the outer surface of the plate that was in aerobic contact with the wastewater containing ammonia; the immobilized *P. denitrificans* reduced nitrite to nitrogen gas on the inside of the plate that was in anaerobic contact with the electron donor. This system did not require an additional aerobic step because the electron donors were not supplied to the wastewater directly but to the internal space of the gel plate. This resulted in an increase of the utilization efficiency of the electron donor for the denitrification process and a decrease in the quantity of surplus sludge. In another attempt, a bioreactor system with 30 packed gel envelopes was installed in a thermal power plant for the removal of nitrogen from ammonia-containing desulfurization wastewater. Each envelope consisted of doublesided plate gels containing *Nitrosomonas europaea* and *Paracoccus denitrificans* cells with an internal space in between for injecting an electron donor. The envelope could remove ammonia from wastewater in a single step. During continuous wastewater treatment with the bioreactor system 95.0% removal of the total nitrogen was obtained. The total nitrogen concentration in the outlet was below 9.0 mg L^{-1}. Since the bioreactor system could use the electron donor effectively, it was not necessary to use an additional aerobic tank to remove the electron donor and a settling tank to segregate the surplus sludge containing bacteria from wastewater (Morita et al 2008).

3.3. Autotrophic denitrification

The heterotrophic denitrification rate was strongly dependent on the type of carbon source, the concentration of the carbon source (Galvez et al. 2003; Gomez et al. 2003), and the C/N ratio (Rijn et al. 2006). This could vary for different microorganisms, water streams, and environmental conditions (Chiu and Chung 2003). In contrast, autotrophic denitrifiers utilized inorganic carbon substrates (carbon dioxide or bicarbonate) as a sole source of carbon. Some advantages of autotrophic over heterotrophic denitrification are; evasion of the poisoning effect of some organic carbon, low biomass build-up and less sludge production which results in reduction of reactor clogging and easier post-treatment (Rijn et al. 2006). Since some wastewaters have a very low concentration of biodegradable organic materials, autotrophic denitrification, which utilizes CO_2 from water as carbon source requires addition of an electron donor substrate. Elemental sulfur, (Broers 2004; Moon et al. 2004; Zeng and Zhang 2005; Sierra-Alvarez et al. 2007; Wan et al., 2009) and H_2 (Mansell and Schroeder 2002; Biswas and Bose 2005; Rezania et al. 2007) as electron donors for autotrophic denitrification systems have been studied extensively. Under typical aquifer conditions, iron sulphide (pyrite) is typically expected to be the electron donor (Pauwels et al. 2002) for denitrification assisted by *Thiobacillus denitrificans*:

$$5\ FeS_2 + 14\ NO_3^{-} + 4\ H^{+} \rightarrow 7\ N_2 + 10\ SO_4^{2-} + 5\ Fe^{2+} + 2\ H_2O \qquad (5)$$

In the sulfur-limestone autotrophic denitrification (SLAD) process element sulfur is used as electron donor and limestone is used to adjust the pH, but an increase of the sulfate concentration and hardness limits its application. Hydrogen gas would be an ideal electron donor for biological autotrophic denitrification. It has, however, a poor solubility in water (Wan et al. 2009**).** A biofilm-electrode reactor as a combined electrochemical and biological reactor was developed by Sakakibara et al. (1993) and improved by Prosnansky et al. (2002) to solve these problems. In this system, autotrophic denitrifying microorganisms are immobilized on the surface of the cathode and hydrogen gas as an electron donor is produced by electrolysis of water. Combining this bioelectrochemical and sulfur autotrophic denitrification system for water denitrification was proposed by Wang and Qu (2003) and applied at large scale by Wan et al. (2009). In such a process, sulfur and hydrogen autotrophic bacteria were integrated for the following reasons: the H^+ generated during denitrification with sulfur could be consumed by the bioelectrochemical denitrification with hydrogen to achieve neutralization, thus the limestone added into the SLAD system could be left away and the hardness increase could be avoided; the sulfate concentration of the effluent could be controlled by the nitrogen load of the autotrophic sulfur denitrification process, and would be lower than in the SLAD process. In general *Thiobacillus denitrificans* and *Thiomicrospira denitrificans* are the two most commonly reported autotrophic denitrifiers (Brettar et al. 2006). Because in nature these bacteria are likely to encounter autotrophic and heterotrophic conditions, it is of considerable interest that their nitrate removal characteristics under mixotrophic conditions is determined. However, no detailed information is available on the effect of organic compounds on sulfur-based autotrophic denitrification.

3.4. Anaerobic ammonium oxidation (Anammox) process

Anaerobic ammonium oxidation (anammox) has received special attention since its discovery, because it is an efficient biological alternative to conventional nitrogen removal from wastewaters. Under anaerobic conditions, ammonium is oxidized to nitrogen gas with nitrite as the electron acceptor (Eq. 6) and carbon dioxide is used for growth of the anammox microorganisms involved. In comparison to traditional nitrification–denitrification process, this autotrophic process consumes 100% less biodegradable organic carbon and at least 50% less oxygen (Tal et al. 2006) and has, therefore, lower operating cost.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O \qquad (6)$$

Anammox needs ammonium and nitrite in a ratio of roughly one to one. For sludge digestor effluents, this ratio can be achieved without control, because these effluents contain bicarbonate as the counter ion for ammonium. When half of the ammonium is converted, the alkalinity of the water is depleted leading to a drop in pH and preventing further nitrification (Equation Eq. (7) (Jetten et al. 2001):

$$NH_4^+ + HCO_3^- + 0.75\ O_2 \rightarrow 0.5\ NH_4^+ + 0.5\ NO_2^- + CO_2 + 1.5\ H_2O \qquad (7)$$

If the Anammox process is combined with a preceding nitrification step, only part of the ammonium needs to be nitrified to nitrite, while the Anammox process combines the remaining ammonium with the nitrite to yield dinitrogen gas. This will reduce the oxygen demand in the nitrification reactor and reduce costs. The biomass yield is very low, and consequently, little sludge is produced. This is another factor that contributes to substantially lower operation costs of Anammox compared to the conventional denitrification process. However, the low biomass yield also necessitates an efficient system for sludge retention, and long start-up times are required to obtain a sufficient biomass concentration (Jetten et al. 1999).

The possible metabolic pathways for anaerobic ammonium oxidation are shown in Fig. 1 (van de Graaf et al. 1997). Using ^{15}N-labelling the experiments showed that the electron acceptor nitrite is reduced to hydroxylamine and that hydroxylamine somehow reacts with the electron donor ammonium, leading to the ultimate production of dinitrogen gas (Jetten et al. 1999). In batch experiments with excess hydroxylamine and ammonium, a transient accumulation of hydrazine was observed, indicating that hydrazine is the intermediate of this final step. Jetten et al. (1999) postulated that the oxidation of hydrazine to dinitrogen gas generates the electrons for the initial reduction of nitrite to hydroxylamine. It is well known that the occurrence of free hydrazine in microbial nitrogen metabolism is rare, if not unique (Schalk 2000) .

The anaerobic ammonium oxidizing bacteria (AnAOB) are autotrophic members of the *Brocadiales*, belonging to the phylum *Planctomycetes*, which is one of the major distinct divisions of the bacteria. Currently, five genera of AnAOB have been reported: *Candidatus* Brocadia, *Candidatus* Kuenenia, *Candidatus* Scalindua, *Candidatus* Anammoxoglobus, and *Candidatus* Jettenia. However the most common AnAOB are “*Brocadia anammoxidans*” (Strous et al. 1999a) and “*Kuenenia stuttgartiensis*” (Schmid et al. 2000). These two bacteria are very similar. They have the same overall structure and also produce hydrazine from exogenously supplied hydroxylamine. The high Anammox activity is detectable for both bacteria in a pH range between 6.4 and 8.3 and a temperature between 20 °C and 43 °C (Egli et al. 2001). The optimum pH and temperature of the two organisms are very similar. These bacteria have a highly unusual physiology, in that they live by consuming ammonia in the absence of oxygen. Furthermore, these metabolically versatile bacteria are, for example, capable of oxidizing short chain fatty acids with nitrate (Güven et al. 2005), co-oxidizing propionate and ammonium in the presence of nitrite and nitrate (Kartal et al. 2007a), and performing dissimilatory nitrate reduction to ammonium (Kartal et al. 2007b). Anammox is highly exergonic and linked to the energy metabolism of the organisms involved. In addition, Anammox bacteria were recently shown to be able to tolerate higher O_2 concentrations than originally established by Strous et al. (1997b) being metabolically active at oxygen concentrations of up to ~13 μmol O_2 L^{-1} (Jensen et al. 2008). Altogether, these results have important ecological and biogeochemical implications, since they extend the metabolic and environmental spectra of these bacteria.

The Anammox process is suitable for wastewater with low C:N ratios. At C:N ratios above 1, the Anammox bacteria are no longer able to compete with heterotrophic denitrifying bacteria (Güven et al. 2005). The organic loading rate was found to affect the Anammox process performance, but the exact inhibitory levels still remain unclear (Sabumon, 2007 and Wang and Kang 2005). An organic matter concentration above 300 mg COD/ l was found to inactivate Anammox communities in a UASB reactor fed with fat milk as organic matter source (Chamchoi et al. 2008). Concentrations of 50 mM of acetate resulted in 70% inhibition of the Anammox process (Dapena-Mora et al. 2007). Therefore it is necessary to clearly establish the COD levels inhibiting the Anammox process.

The Anammox process has also been maintained easily in a gas lift reactor achieving nitrogen removal rates of up to 8.9 kg $N/m^3 \cdot day$. This removal rate was 20 times higher compared to the removal rates previously achieved in the laboratory (Sliekers et al. 2003). Ammonia removal via Anammox has been developed for the treatment of many different wastewaters with low organic matter content (below 1700 mg COD L^{-1}), such as water from the secondary clarifier of a municipal wastewater treatment plant in a down flow biofilter (Li et al. 2005), nitrous organic wastewater in ASBR reactors (Jing-Ping et al. 2006) and landfill leachate in a continuous reactor (Liang and Liu 2008). Only a few studies have investigated the possibility of using the Anammox process for ammonia removal from animal waste treatment water, which is indeed a residue with high organic matter and nitrogen content (Waki et al. 2007). However, there is still a big gap regarding the effect of different pre-treatments (reducing organic and ammonia loads) of the wastewater streams on Anammox process performance. Up to 98.5 ± 0.8% of ammonia was removed from a diluted partially oxidized pig manure effluent (121 mg COD L^{-1}) using the Anammox process under different organic loadings in a semi-continuous UASB reactor. Mass balance clearly showed that an increase in organic loading (from 121 mg COD L^{-1} to 290 mg COD L^{-1} negatively affected the Anammox process and facilitated heterotrophic denitrification (Molinuevo et al. 2009).

3.5. Partial nitrification/Anammox

The increases in the operating costs of wastewater treatment systems is challenged by a novel microbial process, combining the Anammox reaction with partial nitrification in one reactor, entitled CANON (completely autotrophic nitrogen removal over nitrite) (Third et al. 2001). This combination of the preceding partial nitrification and the subsequent anaerobic ammonium oxidation is regarded as a promising new method of removing nitrogen from wastewater with a low C/N ratio and a large quantity of ammonium (Ciudad et al.

2005). Compared to the conventional nitrification and denitrification process, more than 50% (Fux et al. 2002) or 62.5% (Feng et al. (2007) less oxygen demand and the non-requirement of organic carbon addition, in the combined partial nitrification/Anammox process offer considerable cost savings. The combination of partial nitrification and Anammox is based on the fact that nitrite is an intermediary compound in both. Therefore, it will be convenient and economical to achieve 50% partial nitrification up to a condition wherein one-half of ammonia is converted to nitrite and the other half is not, followed by the anammox to ensure total nitrogen removal throughout an autotrophic process. (Khin and Annachhatre 2004; Yamamoto et al. 2008). In the CANON systems, *Nitrosomonas*-like aerobic ammonium-oxidizing bacteria and *Planctomycete*-like Anammox bacteria perform two sequential reactions simultaneously under oxygen limited conditions (Hao et al. 2002 and Sliekers et al. 2002). The nitrifiers oxidize ammonium to nitrite, consume oxygen and so create anoxic conditions needed by the Anammox bacteria. The produced nitrite is utilized with the remainder of the ammonium by Anammox bacteria and converted into dinitrogen gas (Nielsen et al. 2005). Equations number (8), (9) and (10) represent these reactions:

$$2\ NO_3^- + 12\ H^+ + 10\ e^- = N_2 + 6\ H_2O \qquad (8)$$

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 0.066\ CH_2O_{0.5}N_{0.15} + 1.02\ N_2 + 0.26\ NO_3^- + 2.03\ H_2O \qquad (9)$$

$$NH_4^+ + 0.85\ O_2 \rightarrow 0.44\ N_2 + 0.11\ NO_3^- + 1.43\ H_2O + 0.14\ H^+ \qquad (10)$$

Typically in the CANON process the *Nitrosomonas*-like ammonium-oxidizing bacteria are active in the outer aerobic region of both biofilm and aggregates, while Anammox bacteria are active in the inner anoxic region. This way the Anammox bacteria are protected from oxygen, which is consumed in the outer aerobic region. Oxygen would inhibit the Anammox activity (Strous et al. 1997b). The cooperation of these two groups of ammonium-oxidizing bacteria results in completely autotrophic nitrogen removal under oxygen limited conditions in one single reactor.

The CANON process has quite sensitive operational characteristics for dissolved oxygen, the nitrogen-surface load, biofilm thickness and temperature, etc. (Hao and van Loosdrecht 2004). The oxygen-mass transfer efficiency from gas to the liquid phase and effective biomass retention are considered two key rate-limited factors for the operation of a CANON system (Sliekers et al. 2003). Moreover, the growth rate of autotrophic ammonium oxidizing bacteria is lower than that of heterotrophic bacteria, with which they have to compete for oxygen. Without long retention times the suspended nitrifiers will be easily washed out of the reactor. The biomass concentration is increased by recirculation of the sludge after sedimentation, but limited by the efficiency of the sedimentation vessel. Besides, the ammonia oxidation rate is strongly influenced by the nature of nitrifying cultures and a variety of environmental factors, including substrate concentration, dissolved oxygen, temperature and pH. To overcome these problems and to promote the oxygen-mass transfer in a high biomass retention reactor configuration, immobilization techniques can be used. This is an important challenge in order to scale up CANON systems from laboratory to industrial application (Yan et al. 2009 and Gong et al. 2007). Immobilization is an efficient method to prevent biomass from being washed out and allows hyperconcentrated cultures. This can lead to relatively small reactors and provide some protection from adverse temperatures and toxic shocks, which would help in maintaining year-round treatment (Morita et al. 2007). Immobilized biomass can be divided into “naturally” attached biomass (biofilm) and “artificially” immobilized biomass. Biofilms have been widely applied in wastewater treatment. However, some particles can become anaerobic in the centre and settle to the reactor floor. Membrane-aerated biofilm reactor (MABR) represent a new technology for aerobic wastewater treatment, in which hydrophobic, gas-permeable membranes are used for bubbleless oxygen transfer (Casey et al. 1999b and Lapara et al. 2006). Membrane aeration is advantageous because gas transfer efficiencies are much higher than conventional bubble diffusers (Casey et al. 1999b). In an MABR, the microporous membranes play two roles: the oxygen gas supplemental material and the carrier for bacterial immobilization (Brindle and Stephenson 1996; Terada et al. 2006). The oxygen on the lumen side of the membrane is transported through the pores of the membrane wall without the formation of bubbles and utilized by microorganisms in the membrane attached biofilm. Extremely high oxygen-mass transfer efficiency can be achieved (Brindle et al. 1998 and Casey et al. 1999a). Recent research has demonstrated that thick membrane-aerated biofilms can simultaneously provide favorable conditions for both nitrification (near the membrane) and denitrification (near the biofilm-liquid boundary) within a single biofilm (Cole et al. 2004). Gong et al. (2007) developed a novel MABR, equipped with non-woven fabrics support around the microporous carbon tube membrane, and investigated its feasibility and process performance of the CANON-type single-stage autotrophic nitrogen removal to treat the synthetic ammonium-rich wastewater like anaerobic sludge liquids. This reactor allowed air to be supplied through the microporous carbon tube wall to the biofilm that was supported by non-woven fabrics. The partial nitrification and consumption of dissolved oxygen occurred in the inner layer and Anammox in the anoxic outer layer of the non-woven fabrics, thus realizing autotrophic nitrogen removal in a single reactor. This study demonstrated that MABR was a very suitable experimental set-up for the operation of the single-stage autotrophic nitrogen removal process

One of the most common techniques for artificially immobilization is gel entrapment. Both natural and synthetic polymers can be used as the immobilization support, but it must fulfill various requirements, such as photo-transparency, non-toxicity, retention of cellular viability, and stability in the culture medium (Mallick 2002). This immobilization technique is commonly used to immobilize a pure strain of bacteria because the mechanisms of pure strains are more easy to understand (Fierro et al. 2008; Hill et al. 2008). Nevertheless, the immobilization of activated sludge has also been reported (Rostron et al. 2001; Isaka et al., 2007). Compared to pure strains of bacteria, immobilization of activated sludge could remove multiple pollutants due to the biodiversity of the activated sludge. Yan et al. (2009) studied the characteristics of the partial nitrification and degradation of organics with an immobilized biomass in treating ammonium-rich organic wastewater. It serves as a first step in the Anammox process with partial denitrification via nitrite. They used four materials, i.e. sodium carboxymethylcellulose, sodium alginate, polyvinyl alcohol and sodium alginate, and chitosan for entrapping the biomass. Sodium alginate was selected as the best entrapment support after comparing partial nitrification rates and the adsorption efficiency.

4. Factors affecting nitrogen removal efficiency

4.1. Effect of temperature

The temperature range of 22–37 °C gave best results in terms of maximum nitrogen and carbon removal from a shrimp aquaculture wastewater (Fontenot et al. 2007), but denitrification processes will normally occur in the range 2–50 °C (Brady and Weil 2002) and possibly beyond, where bacteria have evolved to cope with specific environmental conditions. Groundwater temperatures are typically around 10 °C (in northern Europe), with the exception of shallow groundwaters impacted by extreme surface temperatures. Reaction rates are typically assumed to double for every 10 °C increase in temperature (i.e. Arrhenius rate law). Elefsiniotis and Li (2006) investigated the role of temperature within the range of 10–30 °C on biological denitrification using synthetically produced volatile fatty acids as carbon sources. Their results confirmed that a temperature change from 10 to 20 °C exerted a greater effect on both the specific denitrification and carbon consumption rates than a further temperature increase from 20 to 30 °C, which was also evident in the corresponding temperature coefficient values. At a given temperature, the specific denitrification rate appeared to depend on the initial nitrogen concentration, while the specific carbon consumption rate was a function of the initial carbon content. Ilies and Mavinic (2001) investigated the nitrogen removal capabilities of the denitrification process, when treating sanitary landfill leachate containing an ammonia concentration of over 2200 mg N/L, at operating temperatures down to 10°C. When the operating temperature was decreased from 20 to 17°C, an approximate 15% decrease in denitrification was immediately experienced, with no noticeable effect on nitrification. With the temperature of 14°C, aerobic wasting was also stopped and methanol (carbon source for denitrification) loading was progressively decreased to match actual denitrification requirements. At 10°C, the system suffered major nitrification and denitrification inhibition. Changes in operating parameters, such as a decrease in influent ammonia and methanol loading, as well as an increase in ambient temperatures, from 10 to 15°C, did not significantly improve the overall system performance, within a reasonable time frame. Changes in the rate of denitrification with seasonal temperature variations may be masked by variations in the rate of organic carbon flux. For example, Cannavo et al. (2004) found that freeze–thaw cycles increase the flux of carbon to the unsaturated zone and can create anaerobic micro-environments in which denitrification can be established.

Present reports show that high temperature of 28–38 °C is favorable for nitrogen removal via nitrite due to the fact that the specific growth rate of AOB is higher than that of NOB (Brouwer et al. 1996). The reports about the effect of temperature on nitritation can be grouped into two classes: (1) achievement and maintenance of nitritation at high temperatures of 28–35 °C (Karakashev et al. 2008), and (2) start-up of nitritation at high temperatures and a gradually decline of the temperatures. Nitritation was maintained at room or low temperatures (Peng,et al. 2007). Some researches also proved that nitritation start-up could be promoted and accelerated at high temperatures (Zeng et al. 2008). However, the temperatures of real domestic wastewater (usually at 10–25 °C), especially in winter, cannot reach the optimal temperature of 30 °C for nitrogen removal via nitrite. In the temperature range of 10–20 °C, a high nitrite accumulation rate can hardly be maintained due to the fact that the specific growth rate of NOB is higher than that of AOB (van Dongen et al. 2001). Therefore, a relatively low wastewater temperature such as in domestic wastewater is the major obstacle for achievement and full-scale application of nitrogen removal via nitrite. However Zeng et al. (2009) achieved nitritation at a temperature of 19 ± 1 °C by controlling the dissolved oxygen (DO) concentration and pH. The dominance of ammonia oxidizing bacteria (AOB) was enhanced through the combination of a low DO concentrations (<1.0 mg/L) and a preset short-cycle control of the aeration time. Nitritation was successfully established with a NO_2^--N/NO_x^--N ratio over 95%.

Several authors (Strous et al. 1999b; Egli et al. 2001; Toh ei al. 2002) found that the optimum temperature for the operation of the Anammox process was around 30–40 °C. Perhaps for this reason, most of the works where this process was applied were carried out at temperature values higher than 30 °C (Van Dongen et al. 2001 and Imajo et al. 2004). Recently, Cema et al. (2007) proved that a rotating biological contactor (RBC) with an established Anammox process could be successfully operated at temperatures around 20 °C. Similar results were reported by Vázquez-Padín et al. 2009 and by Isaka et al. (2007b) who operated an anaerobic biological filtrater reactor (ABF) which treated 8.1 g N $(L\ d)^{-1}$. Moreover, several works done with marine Anammox samples reported measurable activities at low temperatures. Rysgaard et al. (2004) working with sediments of the east and west coasts of Greenland, observed Anammox activity between −2 and 30 °C, the optimum temperature being 12 °C. Similar results were found by Dalsgaard and Thamdrup (2002) working with marine sediments from the Skagerrak (Baltic-North Sea). These results indicate that the application of the Anammox process must not be restricted to effluents with temperatures around 30 °C. Therefore, Dosta et al. (2008) evaluated the effects of moderately low temperatures on the stability of this process. First, the short-term effects of temperature on the Anammox biomass were studied using batch tests and the maximum activity was found at 35–40 °C. Activity tests done at 45 °C showed an irreversible loss of the activity due to biomass lysis. Temperatures from 30 to 15 °C were used to determine long-term effects. The system was successfully operated at 18 °C but when the temperature was decreased to 15 °C, nitrite started to accumulate and the system lost its stability. On the other hand, some authors reported that the denitrification rate showed only a rather weak dependence on the temperature, the rate at 3°C being approximately 55% of that at 15°C. The maximum denitrification rate obtained at 15°C was 2.7 g NO_x^--N m^{-2} carrier d^{-1}. The maximum denitrification rate at 3°C during an 8-day period was found to be constant (Welander and Mattiasson 2003).

4.2. Effect of dissolved oxygen on denitrification

Denitrifiers are facultative bacteria that energetically prefer oxygen over nitrate as the terminal electron acceptor. Denitrifying bacteria use nitrogen oxides as terminal electron acceptors most rapidly in the absence of oxygen. Thus, the dissolved oxygen (DO) concentration has an important influence on the success of the nitrogen removal process. A high DO plays a crucial role in nitrification and has a negative influence on biological denitrification. DO can inhibit denitrification because oxygen functions as the electron acceptor for microorganisms over nitrate and aerobic conditions repress enzymes involved in denitrification (Zumft, 1997). Although high DO concentrations are necessary to enhance the activity of nitrifying bacteria in the biofilm reactor, denitrification is inhibited by oxygen. Lowering the aeration rate i.e. operating the wastewater treatment at low DO concentrations is a possible measure to control the inhibitory effect of DO on denitrification (Tan and Ng 2008). The negative effects of high DO concentrations on the denitrification process depended on the carbon source. Denitrification with alcohols such as ethanol and methanol was less affected by DO than with sucrose. The development of a biofilm was also influenced by the DO concentration as excess O_2 caused reduced biofilm growth. Biofilms that developed in presence of oxygen revealed a smaller bacterial density and a smaller atio of denitrifying versus nitrate reducing bacteria, which led to an unfavorable inorganic nitrogen removal and the presence of nitrite in the treated water. All these effects were more pronounced when sucrose was used as carbon source (Gómez et al. 2002).

Until now, a significant amount of research has focused on the partial nitrification and SND achieved by low DO (Aslan et al. 2009). Using low DO, Blackburne et al. (2008) achieved partial nitrification to nitrite in a lab-scale continuous-flow reactor treating synthetic wastewater containing ammonium as the sole energy source. Ma et al. (2006) showed a clear correlation between nitrite accumulation and low DO levels in a continuously run pilot plant. For nitrogen removal via nitrite with real wastewater the nitrite pathway in a continuous-flow system has not been fully demonstrated previously (Yuan et al. 2008). However, Ma et al. (2009) established the nitrite pathway in a pilot-scale continuous pre-denitrification plant ($V = 300$ L) treating domestic wastewater by controlling the DO concentration at 0.4–0.7 mg/L. It was demonstrated that the nitrite pathway could be repeatedly and reliably achieved, with over 95% of the oxidized nitrogen compounds at the end of the aerobic zone being nitrite. The nitrite pathway improved the total nitrogen removal by about 20% in comparison to the nitrate pathway, and also reduced aeration costs by 24%. Moreover, the short-term effect of DO on biological nitrogen removal has been discussed in many studies using batch test (Ciudad et al. 2005; Park and Noguera 2004). With the exception of the report of Guo et al. (2009b), limited reports are available on comparisons of partial nitrification performance under different DO for long-term operation. It is still doubtful whether high a DO level would destroy the stable and high nitrite accumulation ratio built by low DO or other operational factors. It is also not very clear whether high DO would cause the recovery of NOB after long time operation. Guo et al. (2009b) found that the average efficiencies of SND in a high DO (above 3 mg/l on average) and a low DO (0.4–0.8 mg) reactor were 7.7% and 44.9%, and the specific SND rates were 0.20 and 0.83 mg N/(mg MLSS h), respectively. Low DO did not produce sludge with poorer settling properties but attained lower turbidities of the effluent than high DO. AOB were the dominant nitrifying bacteria and NOB did not be recovered in spite of exposing nitrifying sludge to high DO.

4.3. Effect of nitrate concentration

Excess nitrate concentrations affect the denitrification process by inhibiting the formation of N_2 gas and causing the denitrification process to terminate with the formation of N_2O (Blackmer and Bremner 1978). A small number of research studies has been published to date on the denitrification of wastewater containing nitrate at concentrations higher than 600 mg NO_3-N/L (Austerman-Haun et al. 1999; Foglar et al. 2005; Cyplik et al. 2007a; Nair et al. 2007). Biological denitrification of high nitrate concentration in wastewater is a slow process. To increase the rate of denitrification, parameters such as pH, temperature, COD/NO_3^--N and biomass concentration of the process must be optimized. Acclimatization of sludge to nitrate wastewater is one of the methods used to develop the suitable consortium to treat high strength nitrate wastewater. Sludge, generally consists of different types of bacteria, broadly divided into two categories viz; nitrate tolerant and nitrate intolerant bacteria. Nitrate tolerant bacteria include nitrate respirators (capable of reducing nitrate to nitrite) and true denitrifiers (capable of reducing nitrate to nitrogen). The growth rate of nitrate tolerant and nitrate intolerant bacteria varies depending upon nitrate concentrations. At high nitrate concentration, the population of nitrate tolerant bacteria multiplies faster than that of nitrate intolerant bacteria. Thus, acclimatization is essentially a process of manipulating differential growth rates of two types of bacteria to obtain a desired population balance by subjecting them at controlled nitrate concentration. To acclimatize the sludge for treating high nitrate wastewater, it is subjected to high nitrate concentrations in which nitrate tolerant bacteria outgrow nitrate intolerant bacteria (Dhamole et al. 2007).

4.4. Effect of salinity

The effect of high salt concentration on nitrification and denitrification has been previously investigated (Campos et al. 2002; Moussa et al. 2006). Seawater has been used as an alternative water source for toilet flushing in some arid areas such as Hong Kong and some other coastal cities, resulting in a high salt content in the sewage (Wu et al. 2008; **Sudarno et al. 2009**). Salinity levels have a definite impact on the microbial community structure in the wastewater and may affect the nitrification and denitrification process (Colt and Tomasso 2001) and ultimately the performance of wastewater treatment systems. High salt concentrations in wastewater induce salt stress to the microbial flora, resulting in the inhibition of many enzymes, decreasing cell activity and eventually leading to plasmolysis (Uygur 2006). It was reported in these studies that nitrification and denitrification activities were sustained by gradual acclimatization of freshwater sludge to high salt conditions. Halophilic denitrifying bacteria were isolated from the long-term acclimated sludge, and higher denitrification performances were demonstrated when the long-term acclimated sludge was used as inoculums (Yoshie et al. 2006.). Furthermore, Furukawa et al. (1993) reported that nitrifying sludge taken from a night soil treatment plant employing a sea-water dilution in the summer season could adapt more smoothly to high salt condition than sludge from freshwater.

Rene et al. (2008) investigated the effect of different COD/N ratio (3–6) and salt concentrations (up to 3.2%) on organics and nitrogen removal efficiencies in fish market wastewater under different operating schedules. Different combinations of the COD/N ratio and salinity showed a negligible effect on organics removal, while they affected nitrification and denitrification efficiency to a larger extent. However, salt inhibition can be reduced significantly after long time acclimatization of the biomass. The treatment showed high COD (>80%) and nitrogen (>40%) removal efficiencies despite of high loading rates and COD/N fluctuations, which is due to the acclimatization of the biomass within the SBR. Using a sequencing batch reactor for the treatment of shrimp aquaculture wastewater, the results of Fontenot et al. (2007) indicated that the salinity of 28–40 parts per thousand (ppt), produced best results in terms of maximum nitrogen and carbon removal from the wastewater.

Since the partial nitritation–Anammox process was successfully applied to the treatment of sewage sludge digester liquor, it opened doors for application to many kinds of wastewater treatment such as industrial wastewater, livestock wastewater, and landfill leachate. However, these wastewaters contain high concentrations of salts which have been considered as an inhibition factor in the biological nitrogen removal process (van der Star et al. 2007). However, marine Anammox bacteria belonging to the genus *Scalindua* have been detected in natural surroundings (Schmid et al. 2007) and recently Nakajima et al. (2008) enriched them from an enclosed coastal sea in Japan using a continuous culture system. These results suggested that Anammox bacteria, inherently preferring high concentration of salts and living in the high salt habitats, would be enriched in the cultivations and would be available for industrial application. On the other hand, there is an inconsistent experiment. Kartal et al. (2006) adapted the Anammox sludge, which consisted of 50% of *Candidatus* Kuenenia stuttgartiensis and 50% of *Candidatus* Scalindua wagneri to a salt concentration of 30 g L^{-1}. Although this would be the culture conditions suitable for growth of *Candidatus* Scalindua, they reported that the major Anammox bacteria after the acclimation were *Candidatus* Kuenenia stuttgartiensis enriched from freshwater. Because Kartal et al. (2006) used the seed sludge containing marine Anammox bacteria besides a freshwater Anammox bacterium, the result that major Anammox bacteria at high salt conditions were freshwater Anammox species is an open question. In addition, Kartal et al. (2006) focused on only the population of Anammox bacteria species without the evaluation of the coexistent bacteria community. The effect of high salt concentration on the Anammox treatment was investigated by Liu et al. 2009 to establish an acclimation strategy under high salt concentration conditions. An Anammox fixed-bed reactor with non-woven biomass carrier was used and the salt concentration was gradually increased from 2.5 g L^{-1} to 33 g L^{-1}. The Anammox reactor revealed a stable nitrogen removal rate (NRR) of 1.7 kg-N m^{-3} d^{-1} for 65 days under a salt concentration of 30 g L^{-1}. However, the NRR sharply declined at a salt concentration of greater than 30 g L^{-1}.

4.5. Effect of pH

The pH range preferred by heterotrophic denitrifiers is between 5.95 and 7.9 (Salem et al. 2007), although the optimal pH level in an anoxic/oxic membrane bioreactor with over 99.9% of nitrate removal and without accumulation of nitrite was 7.5-8.5 (Shen et al. 2009). pH values outside this range may hinder the denitrification process, but the optimal pH is site-specific because of the effects of acclimation and adaptation to the microbial ecosystem. Strongly acidic environments ($pH < 5$) inhibit denitrification and tend to arrest the denitrification chain with the formation of nitrite or N_2O (Brady and Weil 2002). In well-buffered calcareous aquifers, such acidification is unlikely (Amirbahman et al. 1998). *Halomonas campisalis* (ATCC 700597) however was shown to completely reduce nitrate at 125 g/L NaCl and pH 9 in brine produced from regeneration of ion exchange resins with NaCl, containing a high concentration of nitrate that was difficult to remove using standard biological, physical, or chemical technologies (Peyton et al. 2001). On the other hand experiments of nitrate removal from high salinity wastewater are usually carried out without controlling the pH because denitrification from high salinity wastewater favors high pH levels (Hwang et al. 2006). Heterotrophic denitrification itself can increase the pH because it causes a release of hydroxyl ions and raises alkalinity. Each mg of nitrate-N reduced to N_2 causes an alkalinity increase of 3.57 mg $CaCO_3$ according to the following stoichiometry:

$2\ NO_3^- + 12\ H^+ + 10\ e^- = N_2 + 6\ H_2O$ (van Rijn et al. 2006).

Contrary to heterotrophic denitrification, autotrophic denitrification consumes alkalinity and, in addition, generates high concentrations of sulfate. High sulfate concentrations do not pose an undue problem in coastal areas, where treated wastewater can be discharged directly to the sea, which has a natural sulfate concentration of 2700 mg l^{-1}. 3.91 g alkalinity (as $CaCO_3$) will be consumed for reducing 1 g of NO_3^-–N to nitrogen gas. Previous research showed that the optimum pH for growth of *Thiobacillus denitrificans* (*T. denitrificans*) cultures was between 6.8 and 8.2, approaching zero at pH 5.5 (Koenig and Liu 2001) and with a maximum efficiency at 8.4. Increasing the pH above 8.6 caused a significant decrease in the nitrate removal rate and a dramatic increase in nitrite accumulation (Lee and Rittmann 2003). Therefore, alkalinity may have to be supplied to the autotrophic denitrification system to control the pH. The most effective and commonly used alkalinity source in research is $NaHCO_3$. For wastewater of low alkalinity, large amounts of $NaHCO_3$ are required to maintain the autotrophic denitrification process. An alternative and cheaper alkalinity source in conjunction with elemental sulfur particles is granular limestone. If the initial alkalinity of the wastewater is insufficient for complete denitrification, limestone can supply effective buffering capacity (Koenig and Liu 2002). Furthermore Jha and Bose (2005) evaluated the suitability of pyrite (FeS_2) as an in situ buffering agent for arresting pH increase during metallic iron assisted hydrogenotrophic denitrification by microorganisms that reduce nitrate to nitrogen gas by utilizing hydrogen as energy source. Pyrite is considered promising for this purpose because it is a mineral which is unstable under moderately reducing, i.e., anoxic conditions, where such denitrification takes place, and therefore is expected to consume hydroxide ions produced due to hydrogenotrophic denitrification reactions and get oxidized to ferrous hydroxide $Fe(OH)_2$. Experimental evaluation of the buffering efficiency of pyrite showed that it was effective in arresting a pH increase associated with denitrification in both, batch systems and during flow through reactive porous media. Furthermore, addition of pyrite had no demonstrable toxic effect on the denitrifying microorganisms, though elevated sulfate concentration was seen in the effluent after denitrification.

4.6. Effect of free ammonia concentration

Traditionally, accumulation of nitrite resulting from higher activities of AOB than NOB is considered undesirable in biological wastewater treatment systems. Factors such as pH, temperature, and the concentrations of DO, CO_2 and heavy metals were all found to influence the nitrite build-up (Lee and Rittmann 2003; Ma et al. 2006; Shen et al. 2009). However, one of the main causes is believed to be the inhibitory effects of free ammonia (FA) (Philips et al. 2002). Anthonisen et al. (1976) observed that both ammonium and nitrite oxidations are inhibited by FA; inhibition of nitrite oxidation by *Nitrobacter* began at a concentration of 0.1–1.0 mg FA/L, while ammonium oxidation by *Nitrosomonas* became inhibited at 10–150 mg FA/L, allowing selective inhibition of nitrite oxidation at a range of FA concentrations of 1.0–10 mg/L. Supporting this observation, Bae et al. (2001) reported that nitrite accumulation occurred at an initial FA concentration of around 4.7 mg/L, giving a high NO_2/NO_X ratio (up to 77%) in a batch reactor. Chung et al. (2007) accomplished a long-term accumulation of nitrite in a continuous-flow reactor by maintaining the FA concentration in the reactor around 20 mg/L. Chung et al. (2006), however, found that a FA concentration of 5–10 mg/L was most efficient in inhibiting nitrite oxidation without slowing down the rate of ammonium oxidation. To have appropriate kinetic expressions for both ammonium oxidation and nitrite oxidation under inhibition, Park and Bae (2009) studied inhibition of ammonium oxidation and nitrite oxidation by FA using three different sludges. An uncompetitive inhibition model fitted the experimental data well when the reactions were under FA inhibition. The estimates of the inhibition constant (K_I) were 46 μM for nitrite oxidation and 290–1600 μM for ammonium oxidation. The much smaller values of K_I for nitrite oxidation reflected the susceptibility of that reaction to inhibition by FA, which could lead to accumulation of nitrite during nitrification. Such studies revealed the impact of FA on the respiration of NOB. Little information was gained with regard to the impact of FA on the growth of NOB. However Vadivelu et al. (2007) indicated that FA has a limited inhibitory effect on the respiratory capability of *Nitrobacter*. While the real mechanisms remain to be identified, the study of Vadivelu et al. (2007) indicates that the FA inhibition of *Nitrobacter* is likely much more serious than suggested by previous studies where the presence of inorganic carbon (or the equivalent nitrite oxidation rate) was used as the sole measure of the inhibitory effects.

Acknowledgement

Gaber Zayed would like to thank Alexander von Humboldt Foundation, Bonn, Germany, for the financial support. He also thanks the stuff of Institut für Ingenieurbiologie und Biotechnologie des Abwasser, Universität Karlsruhe for supporting him during his stay in Karlruhe, Germany.

References

Aiyuk S, Amoako J, Raskin L, van Haandel A, Verstraete W (2004) Removal of carbon and nutrients from domestic wastewater using a low investment, integrated treatment concept. Water Res 38: 3031-3042

Almeida JS, Reis MAM, Carrondo MJT (1995) Competition between nitrate and nitrite reduction in denitrification by *Pseudomonas fluorescens*. Biotechnol Bioeng 46: 476–484.

Amirbahman A, Schönenberger R, Johnson CA, Sigg L (1998) Aqueous- and solid-phase biogeochemistry of a calcareous aquifer system downgradient from a municipal solid waste landfill (Winterthur, Switzerland). Environ Sci Technol 32: 1933–1940

Anthonisen AC, Loehr RC, Prakasam TBS, Srinath EG (1976) Inhibition of nitrication by ammonia and nitrous acid. J Water Pollut Control Fed 48: 835–852

Asadi ME, Clemente RS, Gupta AD, Loof R, Hansen GK (2002) Impacts of fertigation via sprinkler irrigation on nitrate leaching and corn yield in an acid–sulphate soil in Thailand. Agric Water Manage 52: 197–213

Aslan V, Miller L, Dahab M (2009) Ammonium oxidation via nitrite accumulation under limited oxygen concentration in sequencing batch reactors. Bioresour Technol 100: 659–664

Austerman-Haun U, Meyer H, Seyfried CF, Rosenwinkel KH (1999) Full scale experiences with anaerobic/aerobic treatment plants in the food and beverage industry. Water Sci Technol 40: 305–312

Badawy MI, Wahaab RA, El-Kalliny AS (2009) Fenton-biological treatment processes for the removal of some pharmaceuticals from industrial wastewater. J Haza Mater (In Press)

Bae W, Baek S, Chung J, Lee Y (2001) Optimal operational factors for nitrite accumulation in batch reactors. Biodegrad 12: 359–366

Baroni L, Cenci L. Tettamanti M, Berati M (2007) Evaluating the environmental impact of various dietary patterns combined with different food production systems. Eur J Clin Nutr 61: 279–286.

Barton L, Colmer TD (2006) Irrigation and fertilizer strategies for minimizing nitrogen leaching from turfgrass. Agric Water Manage 80: 160–175

Biswas S, Bose P (2005) Zero-valent iron-assisted autotrophic denitrification. J Environ Eng 131: 1212–1220

Blackburne R, Yuan Z, Keller J (2007) Demonstration of nitrogen removal via nitrite in a sequencing batch reactor treating domestic wastewater. Water Res 42: 2166-2176

Blackburne R, Yuan ZG, Keller J (2008) Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegrad 19: 303–312

Blackmer AM, Bremner, JM (1978) Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil micro-organisms. Soil Biol Biochem 10: 187–191

Brady NC, Weil RR (2002) The Nature and Properties of Soils, 13th edn, Prentice Hall, New Jersey

Brettar I, Labrenz M, Flavier S, Botel J, Kuosa H, Christen R, Hofle MG (2006) Identification of a *Thiomicrospira denitrificans*-like *Epsilonproteobacterium* as a catalyst for autotrophic denitrification in the Central Baltic Sea. Appl Environ Microbiol 72: 1364–1372

Brindle K, Stephenson T, (1996) The application of membrane biological reactors for the treatment of wastewaters. Biotechnol Bioeng 49: 601–610

Brindle K, Stephenson T, Semmens MJ (1998) Nitrification and oxygen utilisation in a membrane aeration bioreactor. J Membr Sci 144: 197–209

Broers HP (2004) Nitrate reduction and pyrite oxidation in the Netherlands. In: Razowska-Jaworek L, Sadurski A Editors, Nitrates in Groundwater, International Association of Hydrogeologists Selected Papers 5, Balkema, Leiden

Brouwer M, van Loosdrecht MCM, Heijnen JJ (1996) One reactor system for ammonium removal via nitrite. STOWA report 96-01, STOWA, Utrecht, The Netherlands

Campos JL, Mosquera-Corral A, Sanchez M, Mendez R, Lema JM (2002) Nitrification in saline wastewater with high ammonia concentration in an activated sludge unit. Water Res 36: 2555–2560

Cannavo P, Richaume A, Lafolie F (2004) Fate of nitrogen and carbon in the vadose zone: in situ and laboratory measurements of seasonal variations in aerobic respiratory and denitrifying activities. Soil Biol Biochem 36: 463–478

Carrera J, Vicent T, Lafuente J (2004) Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonium industrial wastewater. Process Biochem 39: 2035-2041

Casey E, Glennon B, Hamer G (1999a) Oxygen mass transfer characteristics in a membrane-aerated biofilm reactor. Biotechnol Bioeng 62: 183–192

Casey E, Glennon B, Hamer G (1999b) Review of membrane aerated biofilm reactors. Resour Conserv Recy 27: 203–215

Cast KL, Flora JRV (1998) An evolution of two cathode materials and the impact of copper on bio-electrochemical denitrification. Water Res 32: 63–70

Cema G, Wiszniowski J, Żabczyński S, Zabłocka-Godlewska E, Raszka A, Surmacz-Górska J (2007) Biological nitrogen removal from landfill leachate by deammonification assisted by heterotrophic denitrification in a rotating biological contactor (RBC). Water Sci Technol 55: 35–42

Chamchoi N, Nitisoravut S, Schmidt JE (2008) Inactivation of ANAMMOX communities under concurrent operation of anaerobic ammonium oxidation and denitrification. Biores Technol 99: 3331–3336

Chang YJ, Tseng SK, (1999) A novel double-membrane system for simultaneous nitrification and denitrification in a single tank. Lett Appl Microbiol 28: 453–456

Chimenos JM, Fernández AI, Villalba G, Segarra M, rruticoechea A, Artaza B, Espiell F, (2003) Removal of ammonium and phosphates from wastewater resulting from the process of cochineal extraction using MgO-containing by-product. Water Res 37: 1601-1607

Chiu YC, Chung MS (2003) Determination of optimal COD/nitrate ratio for biological denitrification. Int Biodeterior Biodegrad 51: 43–49

Cho E, Molof A, (2004) Effect of sequentially combining methanol and acetic acid on the performance of biological nitrogen and phosphorus removal. J Environ Manage 73:183-187

Chung J, Bae W, Lee YW, Rittmann BE (2007) Shortcut biological nitrogen removal in hybrid biofilm/suspended growth reactors. Process Biochem 42: 320–328

Chung J, Shim H, Park S, Kim SJ Bae W (2006) Optimization of free ammonia concentration for nitrite accumulation in shortcut biological nitrogen removal process. Bioprocess Biosyst Engin 28: 275–282

Ciudad G, Rubilar O, Muñoz P, Ruiz G, Chamy R, Vergara C, Jeison D (2005) Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. Process Biochem 40: 1715–1719

Ciudad G, Rubilar O. Munoz P, Ruiz G, Chamy R, Vergara C, Jeison D (2005) Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. Process Biochem 40: 1715–1719

Cole AC, Semmens MJ, LaPara TM (2004) Stratification of activity and bacterial community structure in biofilms grown on membranes transferring oxygen. Appl Environ Microb 70: 1982–1989

Colt J, Tomasso JR (2001) Hatchery water supply and treatment. In: Wedemeyer GA Editor, Fish Hatchery Management (second ed.), American Fisheries Society, Bethesda, Maryland: 91–186

Cyplik P, Grajek W, Marecik R, Króliczak P, Dembczyński R, (2007a) Application of a membrane bioreactor to denitrification of brine. Desalination 207: 134–143

Cyplik P, Grajek W, Marecik R, Kroliczak P (2007b) Effect of macro/micro nutrients and carbon source over the denitrification rate of *Haloferax denitrificans* archaeon. Enzyme Microbial Technol, 40: 212-220

Dalsgaard T, Thamdrup B (2002) Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. Appl Environ Microbiol 68: 3802–3808

Daniel LM, Pozzi E, Foresti E, Fabio Alexandre Chinalia A (2009) Removal of ammonium via simultaneous nitrification–denitrification nitrite-shortcut in a single packed-bed batch reactor. Bioresour Technol 100: 1100-1107

Dapena-Mora A, Fernández J, Campos JL, Mosquera-Corral A, Méndez R, Jetten MSM (2007) Evaluation of activity and inhibition effects on Anammox process and batch tests based on the nitrogen gas production. Enz Microb Technol 40: 859–865

Darwish T, Atallah T, Hajhasan S, Chranek A (2003) Management of nitrogen by fertigation of potato in Lebanon. Nutr Cycl Agroecosyst 67: 1–11

de-Bashan LE, Hernandez JP, Morey T Bashan Y (2004) Microalgae growth-promoting bacteria as “helpers” for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. Water Res 38: 466–474

Dhamole PB, Nair RR, D’Souza SF, Lele SS (2007) Denitrification of high strength nitrate waste. Bioresour Technol 98: 247-252

Dosta J, Fernández I, Vázquez-Padín JR, Mosquera-Corral A, Campos JL, Mata-Álvarez J, Méndez R (2008) Short- and long-term effects of temperature on the Anammox process. J of Hazard Mater 154: 688-693

Egli K, Fanger U. Alvarezz PJJ, Siegrist H, van der Meer JR, Zehnder AJB (2001) Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium rich leachate. Arch Microbiol 175: 198–207

Elefsiniotis P, Li. D (2006) The effect of temperature and carbon source on denitrification using volatile fatty acids. Biochem Engin J 28: 148-155

Feleke Z, Sakakibara Y (2002) A bio-electrochemical reactor coupled with adsorber for the removal of nitrate and inhibitory pesticide. Water Res 36: 3092–3102

Feng H, Hu L, Mahmood Q, Qiu C, Fang C, Shen D (2008) Anaerobic domestic wastewater treatment with bamboo carrier anaerobic baffled reactor. Intern Biodeterior Biodegrad 62: 232-238

Feng Y, Tseng S, Hsia T, Ho C Chou W (2007) Partial nitrification of ammonium-rich wastewater as pretreatment for anaerobic ammonium oxidation (Anammox) using membrane aeration bioreactor. J of Biosci Bioengin 104: 182-187

Fierro S, Sánchez-Saavedra MP, Copalcúa C (2008) Nitrate and phosphate removal by chitosan immobilized scenedesmus. Bioresour Technol 99: 1274–1279

Foglar L, Briski F, Sipos L Vukovic M (2005) High nitrate removal from synthetic wastewater with the mixed bacterial culture. Biores Technol 96: 879–888

Fontenot Q, Bonvillain C, Kilgen M, Boopathy R (2007) Effects of temperature, salinity, and carbon: nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. Bioresour Technol 98: 1700-1703

Francis CW Hatcher CW (1980) Biological denitrification of high-nitrate wastes generated in the nuclear industry. In: P.F. Cooper and B. Atkinson, Editors, Biological Fluidized Bed Treatment of Water and Wastewater, Ellis Horwood Ltd., Chichester

Furukawa K, Ike A, Fujita M (1993) Preparation of marine nitrifying sludge. J Ferment Bioeng 76: 134–139

Fux C, Boehler M, Huber P, Brunner I, Siegrist H (2002) Biological treatment of ammonium-rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (Anammox) in a pilot plant. J Biotechnol 9: 295–306

Fux C, Velten S, Carozzi V, Solley D Keller J (2006) Efficient and stable nitritation and denitritation of ammonium-rich sludge dewatering liquor using an SBR with continuous loading. Water Res 40: 2765–2775

Galvez JM, Gomez MA, Hontoria E, Gonzalez-Lopez J (2003) Influence of hydraulic loading and air flowrate on urban wastewater nitrogen removal with a submerged fixed-film reactor. J Hazard Mater 101: 219–229

Gheysari M, Mirlatifi SM, Homaee M, Esmaeil Asadi M, Gerrit Hoogenboom G (2009) Nitrate leaching in a silage maize field under different irrigation and nitrogen fertilizer rates. Agric Water Manag 96: 946-954

Gomez MA, Galvez JM, Hontoria E, Gonzalez-Lopez J (2003) Influence of concentration on biofilm bacterial composition from a denitrifying submerged filter used for contaminated groundwater. J Biosci Bioeng 95: 245–251.

Gómez MA, Hontoria E, González-López J (2002) Effect of dissolved oxygen concentration on nitrate removal from groundwater using a denitrifying submerged filter. J Hazard Mater 90: 267-278

Gong Z, Yang F, Liu S, Bao H, Hu S, Furukawa K (2007) Feasibility of a membrane-aerated biofilm reactor to achieve single-stage autotrophic nitrogen removal based on Anammox. Chemosphere (69): 776-784

Guo H, Zhou J, Su J, Zhang Z (2005) Integration of nitrification and denitrification in airlift bioreactor. Biochem Engin J 23: 57–62

Guo J, Peng Y, Wang S, Zheng Y, Huang H, Wang Z (2009b) Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. Bioresour Technol 100: 2796-2802

Guo JH, Peng YZ, Wang SY, Zheng YN, Huang HJ, Ge SJ (2009a) Effective and robust partial nitrification to nitrite by real-time aeration duration control in an SBR treating domestic wastewater. Process Biochem 44: 979-985

Gupta AB, Gupta SK (2001) Simultaneous carbon and nitrogen removal from high strength domestic wastewater in an aerobic RBC biofilm. Water Res 35: 1714-1722

Güven D, Dapena A, Kartal B, Schmid MC, Maas B, van de Pas-Schoonen K, Sozen S, Mendez R, Op de Camp HJM, Jetten MSM, Strous M, Schmidt I (2005) Propionate oxidation by and methanol inhibition of anaerobic ammonium oxidizing bacteria. Appl Environ Microbiol 71: 1066–1071

Hao XD, van Loosdrecht MCM (2004) Model-based evaluation of COD influence on a partial nitrification-Anammox biofilm (CANON) process. Water Sci Technol 49: 83–90

Hao XD, van Loosdrecht MCM, Heijnen JJ (2002) Model-based evaluation of kinetic, biofilm and process parameters in a one-reactor ammonium removal (CANON) process. Biotechnol Bioeng 77: 266–277

Harrington R, McInnes R (2009) Integrated Constructed Wetlands (ICW) for livestock wastewater management. Bioresource Technology (In press)

Hill BC, Khan E (2008) A comparative study of immobilized nitrifying and co-immobilized nitrifying and denitrifying bacteria for ammonia removal from sludge digester supernatant. Water Air Soil Poll 195: 23–33

Höring H, Chapman D (2004) Nitrates and Nitrites in Drinking Water. World Health Organization Drinking Water Series, IWA Publishing, London

Hwang C, Wu WM, Gentry TJ, Carley J, Carroll SL, Schadt C, Watson D, Jardine PM, Zhou J, Hickey RF, Criddle CS, Fields MW (2006) Change in bacterial community structure correlate with initial operating conditions of a field-scale denitrifying fluidized bed reactor. Appl Microbiol Biotechnol 71: 748–760

Imajo U, Tokutomi T, Furukawa K (2004) Granulation of Anammox microorganisms in up-flow reactors. Water Sci Technol 49: 155–163

Isaka K, Sumino T, Tsuneda S, (2007b) High nitrogen removal performance at moderately low temperature utilizing anaerobic ammonium oxidation reactions. J Biosci Bioeng 103: 486–490

Isaka K, Yoshie S, Sumino T, Inamori Y, Tsuneda S (2007) Nitrification of landfill leachate using immobilized nitrifying bacteria at low temperatures. Biochem Eng J 37: 49–55

Jalali M (2005) Nitrates leaching from agricultural land in Hamadan, western Iran. Agric Ecosyst Environ 110: 210–218

Jenicek P, Svehla P, Zabranska J, Dohanyos M (2004) Factors affecting nitrogen removal by nitritation/denitritation. Water Sci Technol 49: 73–79

Jensen, M.M., Kuypers, M.M.M, Lavik, G. and Thamdrup, B. 2008. Rates and regulation of anaerobic ammonium oxidation and denitrification in the Black Sea, *Limnology and Oceanography* 53 (2008), pp. 23–36

Jetten MSM, Strous M, van de Pas-Schoonen KT, Schalk J van Dongen L, van de Graaf, AA, Logemann S, Muyzer G, van Loosdrecht MCM, Kuenen JG (1999) The anaerobic oxidation of ammonium. FEMS Microbiol Rev 22: 421–437 A good overview on the history and microbiology of anaerobic ammonium oxidation.

Jetten MSM, Wagner M, Fuerst J, van Loosdrecht M, Kuenen G, Marc Strous M (2001) Microbiology and application of the anaerobic ammonium oxidation (‘anammox’) process. Curr Opin Biotechnol 12: 283-288

Jha J, Bose P (2005) Use of pyrite for pH control during hydrogenotrophic denitrification using metallic iron as the ultimate electron donor. Chemosphere 61: 1020-1031

Jing-ping Z, Yong-you H, Hui-gian L (2006) Correlation of anaerobic ammonium oxidation and denitrification. J Environ Sci 18: 227–231

Karakashev D, Schmidt JE, Angelidaki I (2008) Innovative process scheme for removal of organic matter, phosphorus and nitrogen from pig manure. Water Res 42: 4083–4090

Kartal B, Kuypers MMM, Lavik G, Schalk J, Op den Camp HJM, Jetten MSM, Strous M (2007) Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. Environ Microbiol 9: 635–642

Kartal B, Koleva M, Arsov R, van der Star W, Jetten MSM, Strous M (2006) Adaption of a freshwater anammox population to high salinity wastewater. J Biotechnol 126: 546–553

Kartal B, Rattray J, van Niftrik L, van de Vossenberg J, Schmid M, Webb RI, Schouten S, Fuerst JA, Damste JS, Jetten MSM, Strous M (2007) Candidatus “Anammoxoglobus propionicus” gen. nov., sp. nov., a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. Syste Appl Microbiol 30: 39–49

Khin T, Annachhatre AP, (2004) Novel microbial nitrogen removal processes. Biotechnol. Adv 22: 519–532

Kim JK, Park KJ, Cho KS, Nam S, Park T, Bajpai R (2005) Aerobic nitrification–denitrification by heterotrophic *Bacillus* strains. Biores Technol 6: 1897-1906

Kleerebezem R, Mendezà, R (2002) Autotrophic dentrification for combined hydrogen sulfide removal from biogas and postdentrification. Water Sci Technol 45: 349–356

Knight R, Payne V, Borer RE, Clarke RA, Pries JH (2000) Constructed wetlands for livestock wastewater management. Ecol Eng 15: 41–55

Koenig A, Liu LH (2001) Microbial aspects of autotrophic denitrification of wastewaters. In: Matsuo T, Hanaki K, Takizawa S, Satoh H Editors, Advances in Water and Wastewater Treatment Technology: Molecular Technology, Nutrient Removal, Sludge Reduction and Environmental Health, Elsevier Science, Amsterdam pp. 217–226

Koenig A, Liu LH (2002) Use of limestone for pH control in autotrophic denitrification: continuous flow experiments in pilot-scale packed bed reactors. J Biotechnol 99: 161-171

Lai E, Senkpiel S, Solley D, Keller J (2004) Nitrogen removal of high strength wastewater via nitritation/denitritation using a sequencing batch reactor. Water Sci Technol 50:. 27–33

Lapara TM, Cole AC, Shanahan JW Semmens MJ (2006) The effects of organic carbon, ammoniacal-nitrogen, and oxygen partial pressure on the stratification of membrane-aerated biofilms. J Ind Microbiol Biotechnol 33: 315–323

Lee K, Rittmann B, (2003) Effects of pH and precipitation on autohydrogenotrophic denitrification using the hollow-fiber membrane-biofilm reactor. Water Res 37: 1551-1556

Li J, Xiong B, Zhang S, Yang H, Zhang J (2005) Anaerobic: ammonium oxidation for advanced municipal wastewater treatment is it feasible?. J Environ Sci 17: 1022–1024

Liang Z, Liu J (2008) Landfill leachate treatment with a novel process: Anaerobic ammonium oxidation (Anammox) combined with soil infiltration system. J Haz Mater 151: 202–212

Liu C, Yamamoto T, Nishiyama T, Fujii T, Furukawa K (2009) Effect of salt concentration in anammox treatment using non woven biomass carrier. J Biosci Bioeng 107: 519-523

Lomborg B (2001) The Skeptical Environmentalist. Cambridge University Press. p 22

Ma WL, Zhang Y, Qi R, Wang, V, Liang, C Z, Yang M (2009) Performance of a successive hydrolysis, denitrification and nitrification system for simultaneous removal of COD and nitrogen from terramycin production wastewater. Biochem Eng J 45: 30-34

Ma Y, Peng Y, Wang S, Yuan Z Wang X (2009) Achieving nitrogen removal via nitrite in a pilot-scale continuous pre-denitrification plant. Water Res 43: 563-572

Ma Y, Peng Y, Wang S (2006) Nitritation by real-time control in a pilot-scale pre-denitrification plant treating domestic wastewater. *Proceedings (DVD)* of the 4th International Conference on Marine Waste Water Disposal and Marine Environment *(MWWD 2006), Antalya, Turkey, November 06–10* (2006) ISBN 9944-5566-2-9

Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 15: 377–390

Mallick N, Rai LC (1994) Removal of inorganic ions from wastewaters by immobilized microalgae. Microbiol Biotechnol 10: 439–443

Mansell BO, Schroeder ED (2002) Hydrogenotrophic denitrification in a microporous membrane bioreactor. Water Res 36: 4683–4690

Molinuevo B, García MC, Karakashev D, Angelidaki I (2009) Anammox for ammonia removal from pig manure effluents: Effect of organic matter content on process performance. Bioresour Technol 100: 2171-2175

Moon HS, Ahn KH, Lee S, Nam K, Kim JY (2004) Use of autotrophic sulfur-oxidizers to remove nitrate from bank filtrate in a permeable reactive barrier system. Environ Pollut 129: 499–507

Morita M, Kudo N, Uemoto H, Watanabe A, Shinozaki H (2007) Protective effect of immobilized ammonia oxidizers and phenol-degrading bacteria on nitrification in ammonia- and phenol containing wastewater. Eng Life Sci 7: 587–592

Morita M, Uemoto H, Watanabe A (2008) Nitrogen-removal bioreactor capable of simultaneous nitrification and denitrification for application to industrial wastewater treatment. Biochem Eng J 41: 59-66

Moussa MS, Sumanasekera DU, Ibrahim SH, Lubberding HJ, Hooijmans CM, Gijzen HJ, van Loosdrecht MCM (2006) Long term effects of salt on activity, population structure and floc characteristics in enriched bacterial cultures of nitrifiers. Water Res 40: 1377–1388

Munz G, Gori R, Cammilli L, Lubello C (2008) Characterization of tannery wastewater and biomass in a membrane bioreactor using respirometric analysis. Bioresour Technol 99: 8612-8618

Nair RR, Dhamole PB, Lele SS, D'Souza SF (2007) Biological denitrification of high strength nitrate waste using preadapted denitrifying sludge. Chemosphere 67: 1612–1617.

Nakajima J, Sakka M, Kimura T, Furukawa K Sakka K (2008) Enrichment of anammox bacteria from marine environment for the construction of a bioremediation reactor, Appl Microbiol Biotechnol 77: 1159–1166

Nielsen M, Bollmann A, Sliekers O, Jetten M, Schmid M, Strous M, Schmidt I, Larsen LH, Nielsen LP, Revsbech NP (2005) Kinetics, diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor. FEMS Microbiol Ecol 51: 247–256

Obaja D, Macé S, Costa J, Sans C, Mata-Alvarez J (2003) Nitrification, denitrification and biological phosphorus removal in piggery wastewater using a sequencing batch reactor. Bioresour Technol 87: 103–111

Oladoja N, Ademoroti CMA (2006) The use of fortified soil-clay as on-site system for domestic wastewater purification. Water Res 40: 613-620

Osaka T, Shirotani K, Yoshie S, Tsuneda S (2008) Effects of carbon source on denitrification efficiency and microbial community structure in a saline wastewater treatment process. Water Res 42: 3709-3718

Park HD, Noguera DR (2004) Evaluating the effect of dissolved oxygen on ammonia-oxidizing bacterial communities in activated sludge. Water Res 38: 3275–3286

Park JBK, Craggs RJ, Sukias JPS (2008) Treatment of hydroponic wastewater by denitrification filters using plant prunings as the organic carbon source. Bioresour Technol 99: 2711-2716

Park JBK, Craggs RJ, Sukias JPS (2009) Removal of nitrate and phosphorus from hydroponic wastewater using a hybrid denitrification filter (HDF). Bioresour Technol 100: 3175-3179

Park S, Bae W (2009) Modeling kinetics of ammonium oxidation and nitrite oxidation under simultaneous inhibition by free ammonia and free nitrous acid. Process Biochem 44: 631-640

Pauwels H, Foucher J, Kloppmann W (2000) Denitrification and mixing in a schist aquifer: influence on water chemistry and isotopes. Chem Geol 168:. 307-324

Peng YZ, Yang Q, Liu XH, Zeng W, Mino T, Satoh H (2007) Achieve nitrogen removal via nitrite form municipal wastewater at low temperatures using real-time control to optimize nitrifying communities. Environ Sci Technol 41: 8159–8164

Peyton BM, Mormile MR, Petersen JN (2001) Nitrate Reduction with *Halomonas campisalis:* Kinetics of Denitrification at pH 9 and 12.5% NaCl. Water Res 35: 4237-4242

Philips S, Laanbroek HJ, Verstraete W (2002) Origin causes and effects of increased nitrite concentrations in aquatic environments. Environ Sci Biotechnol 1: 115–141

Prosnansky M, Sakakibara Y, Kuroda M (2002) High-rate denitrification and SS rejection by biofilm-electrode reactor (BER) combined with microfiltration. Water Res 36: 4801–4810

Rajput TBS, Patel N (2006) Water and nitrate movement in drip-irrigated onion under fertigation and irrigation treatments. Agric Water Manage 79: 293–311

Ravindranath N, Jayant H, Sathaye A (2002) Climate Change and Developing Countries. Springer.

Rene ER, Kim SJ, Park HS (2008) Effect of COD/N ratio and salinity on the performance of sequencing batch reactors. Bioresour Technol 99: 839-846

Rezania B, Oleszkiewicz JA, Cicek N (2007) Hydrogen-dependent denitrification of water in an anaerobic submerged membrane bioreactor coupled with a novel hydrogen delivery system. Water Res 41: 1074–1080

Rijn JV, Tal Y, Schreier HJ (2006) Denitrification in recirculating systems: theory and applications. Aquacul Eng 34: 364–376

Rittmann BE, McCarty PL (2001) Environmental biotechnology: principles and applications 10020, McGraw-Hill, New York, NY (2001).

Rostron WM, Stuckey DC, Young AA (2001) Nitrification of high strength ammonia wastewaters: comparative study of immobilisation media. Water Res 35: 1169–1178

Rysgaard S, Glud RN, Risgaard-Petersen N, Dalsgaard T (2004) Denitrification and Anammox activity in Arctic marine sediments. Limnol Oceanogr 49: 1493–1502

Sabumon PC (2007) Anaerobic ammonia removal in presence of organic matter: A novel route. J Haz Mater 149: 49–59

Sakakibara Y, Kuroda M (1993) Electric prompting and control of denitrification. Biotechnol Bioeng 42: 535–537

Salem Z, Lebik H, Cherafa WK, Allia K (2007) Valorisation of olive pits using biological denitrification. Desalination 204: 72-78

Schalk J (2000) A study of the metabolic pathway of anaerobic ammonium oxidation (PhD Thesis). Technology University, Delft

Schmid M, Twachtmann U, Klein M, Strous M, Juretschko S, Jetten MSM (2000) Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonia oxidation. Syst Appl Microbiol 23: 93–106

Schmid MC, Risgaard-Petersen N, van de Vossenberg J, Kuypers MMM, Lavik G, Petersen J, Hulth S, Thamdrup B, Canfield D, Dalsgaard T, Rysgaard S, Sejr MK, Strous M, den Camp HJMO, Jetten MSM (2007)

Anaerobic ammonium-oxidizing bacteria in marine environments: widespread occurrence but low diversity. Environ Microbiol 9: 1476–1484

Shen J, He R, Han W, Sun X, Li J, Wang L (2009) Biological denitrification of high-nitrate wastewater in a modified anoxic/oxic-membrane bioreactor (A/O-MBR). J Haz Mater (In Press).

Sierra-Alvarez R, Beristain-Cardoso R, Salazar M, Gómez J, Razo-Flores E, Field JA (2007) Chemolithotrophic denitrification with elemental sulfur for groundwater treatment. Water Res 41: 1253–1262

Singh M, Bhattacharya AK, Nair TVR, Singh AK (2001) Nitrogen loss through subsurface drainage effluent in coastal rice field from India. Agric Water Manage 52:.249-260

Sliekers AO, Derwort N, Gomez JLC, Strous M, Kuenen JG, Jetten MSM (2002) Completely autotrophic nitrogen removal over nitrite in one single reactor. Water Res 36: 2475–2482

Sliekers AO, Third KA, Abma W, Kuenen JG, Jetten MSM (2003) CANON and Anammox in a gas lift reactor. FEMS Microbiol Lett 218: 339–344

Strous M, Fuerst JA, Kramer EHM, Logemann S, Muyzer G, van de Pas-Schoonen KT (1999a) Missing lithotroph identified as new *Planctomycete*. Nature 400: 446–449

Strous M, Kuenen JG, Jetten MSM, (1999b) Key physiology of anaerobic ammonium oxidation. Appl Environ Microbiol 65: 3248–3250

Strous M, van Gerven E, Zheng P, Kuenen JG, Jetten, MSM, (1997b) Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (ANAMMOX) process in different reactor configurations. Water Res 31: 1955–1962

Strous, M, Van Gerven E, Kuenen JG, Jetten M (1997a) Effects of aerobic and micro-aerobic conditions on anaerobic ammonium-oxidizing (Anammox) sludge. Appl Environ Microbiol 63: 2446–2448

Sudarno S, Winter J, Gallert C (2009) Nitrification in fixed-bed reactors treating saline wasterwater. J Appl Microbiol Biotechnol

Sumino T, Isaka K, Ikuta H, Saiki Y, Yokot T (2006) Nitrogen removal from wastewater using simultaneous nitrate reduction and anaerobic ammonium oxidation in single reactor. J Biosci Bioeng 102: 346–351

Szekeres S, Kiss I, Bejerano TT, Soares MIM (2001). Hydrogen-dependent denitrification in a two-reactor bio-electrochemical system. Water Res 35: 715–719

Szekeres S, Kiss I, Kalman M Soares MIM (2002) Microbial population in a hydrogen-dependent denitrification reactor. Water Res 36: 4088–4094

Tal JEM, Watts J, Schreier HJ (2006) Anaerobic ammonium-oxidizing (Anammox) bacteria and associated activity in fixed-film biofilters of a marine recirculating aquaculture system. Appl Environ Microbiol 72: 2896–2904

Tamini T, Mermoud A (2002) Water and nitrogen dynamics under irrigation onion in a semi-arid area. Irrig Drain 51: 77–86

Tan TW, Ng HY (2008) Influence of mixed liquor recycle ratio and dissolved oxygen on performance of pre-denitrification submerged membrane bioreactors. Water Res 42: 1122-1132

Teixeira P, Oliveira R (2001) Denitrification in a closed rotating biological contactor: effect of disk submergence. Process Biochem 37: 345-349

Terada A, Hibiya K, Nagai J, Tsuneda S, Hirata A (2003) Nitrogen removal characteristics and biofilm analysis of a membrane-aerated biofilm reactor applicable to high-strength nitrogenous wastewater treatment. J Biosci Bioeng 95: 170–178

Terada A, Yamamoto T, Igarashi R, Tsuneda S, Hirata (2006) Feasibility of a membrane-aerated biofilm reactor to achieve controllable nitrification. Biochem Eng J 28: 123–130

Third KA, Olav Sliekers A, Kuenen JG, Jetten MSM (2001) The CANON system (completely autotrophic nitrogen-removal over nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. Syst Appl Microbiol 24: 588–596

Toh SK, Webb RI, Ashbolt NJ (2002) Enrichment of autotrophic anaerobic ammonium-oxidizing consortia from various wastewaters. Microb Ecol 43: 154–167

Uemoto H, Saiki H (2000) Nitrogen removal reactor using packed gel envelopes containing *Nitrosomonas europaea* and *Paracoccus denitrificans.* Biotechnol Bioeng 67: 80–86

UNEP International Environment (2002) Environmentally Sound Technology for Wastewater and Stormwater Management: An International Source Book. IWA Publishing.

Uygur A (2006) Specific nutrient removal rates in saline wastewater treatment using sequencing batch reactor, Process Biochem 41: 61–66

Vadivelu V, Keller J, Yuan Z (2007) Effect of free ammonia on the respiration and growth processes of an enriched *Nitrobacter* culture. Water Res 41: 826-834

van de Graaf AA, de Bruijn P, Robertson LA, Jetten MSM, Kuenen JG (1997) Metabolic pathway of anaerobic ammonium oxidation on the basis of N-15 studies in a fluidized bed reactor. Microbiol 143: 2415–2421

van der Star WRL, Abma WR, Blommers D, Mulder JW, Tokutomi T, Strous M, Picioreanu C, van Loosdrecht MCM (2007) Startup reactors for anoxic ammonium oxidation: experiences from the first full-scale anammox reactor in Rotterdam. Water Res 41: 4149–4163

van Dongen U, Jetten MSM, van Loosdrecht MCM (2001) The SHARON-ANAMMOX process for treatment of ammonium rich wastewater. Water Sci Technol 44: 153–160

van Rijn J, Yossi Tal Y, Schreier HJ (2006) Denitrification in recirculating systems: Theory and applications. Aquacultu Eng 34: 364-376

Vázquez-Padín J, Fernádez I, Figueroa M, Mosquera-Corral A, Campos J, Méndez R (2009) Applications of Anammox based processes to treat anaerobic digester supernatant at room temperature. Bioresour Technol 100: 2988-2994

Waki M, Tokutomi T, Yokoyama H, Tanaka Y (2007) Nitrogen removal from animal waste treatment water by anammox enrichment. Bioresour Technol 98: 2775–2780

Walker WG, Bouma J, Keeney DR, Olcott PG, (1992) Nitrogen transformation during subsurface disposal of septic tank effluent in sands II Groundwater quality. J Environ Qual 2: 521–525

Walters E, Hille A, He M, Ochmann C, Horn H (2009) Simultaneous nitrification/denitrification in a biofilm airlift suspension (BAS) reactor with biodegradable carrier material. Water Res (In Press),

Wan D, Liu H, Qu J, Lei P, Xiao S, Hou Y (2009) Using the combined bioelectrochemical and sulfur autotrophic denitrification system for groundwater denitrification. Bioresour Technol 100: 142-148

Wang HY, Qu JH (2003) Combined bioelectrochemical and sulfur autotrophic denitrification for drinking water treatment. Water Res 37: 3767–3775

Wang J, Kang J (2005) The characteristics of anaerobic ammonia oxidation (ANAEROBIC AMMONIA REMOVAL) by granular sludge from an EGSB reactor. Process Biochem 40: 1973–1978

Wang Q, Feng C, Zhao Y, Hao C (2009) Denitrification of nitrate contaminated groundwater with a fiber-based biofilm reactor. Bioresour Technol 100: 2223-2227

Watkinson AJ, Murby EJ, Costanzo SD (2007) Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. Water Res 41: 4164–4176

Welander U, Mattiasson B (2003) Denitrification at low temperatures using a suspended carrier biofilm process. Water Res 37: 2394-2398

Wu C, Chen Z, Liu X, Yongzhen Peng Y (2007) Nitrification–denitrification via nitrite in SBR using real-time control strategy when treating domestic wastewater. Biochem Eng J 36: 87-92

Wu Y, Tam NFY, Wong MH (2008) Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms Marine Pollu Bullet 57: 727-734

Yamamoto T, Takaki K, Koyama T, Furukawa K (2008) Long-term stability of partial nitration of swine wastewater digester liquor and its subsequent treatment by Anammox, Bioresour Technol 99: 6419–6425

Yan Y, Hu YY (2009) Partial nitrification to nitrite for treating ammonium-rich organic wastewater by immobilized biomass system. Bioresour Technol (100): 2341-2347

Yetilmezsoy K, Sapci-Zengin S (2009). Recovery of ammonium nitrogen from the effluent of UASB treating poultry manure wastewater by MAP precipitation as a slow release fertilizer. J Haza Mater 166: 260-269

Yoshie S, Ogawa T, Makino H, Hirosawa H, Tsuneda S, Hirata A (2006) Characteristics of bacteria showing high denitrification activity in saline wastewater Lett Appl Microbiol 42: 277–283

Young-Ho Ahn (2006). Sustainable nitrogen elimination biotechnologies: A review. Process Biochem 41: 1709-1721

Yuan Z, Oehmen A, Peng YZ, Ma Y, Keller J (2008) Sludge population optimisation in biological nutrient removal wastewater treatment systems through on-line process control: a review. Rev Environ Sci Biotechnol (sited from Ma et al 2009)

Zayed G, Winter J (1998) Removal of organic pollutants and of nitrate from wastewater from dairy industry by denitrification. Appl Microbiol Biotechnol 49: 469–474

Zeng H, Zhang TC (2005) Evaluation of kinetic parameters of a sulfur-limestone autotrophic denitrification biofilm process. Water Res 39: 4941–4952

Zeng W, Zhang Y, Li L, Peng Y, Wang S (2009) Control and optimization of nitrifying communities for nitritation from domestic wastewater at room temperatures. Enzym Microb Technol (In Press)

Zeng, W, Peng YZ, Wang SY, Peng CY (2008) Process control of an alternating aerobic-anoxic sequencing batch reactor for nitrogen removal via nitrite. Chem Eng Technol 31: 582–587

Zhang X, Zhou J, Guo H, Qu Y, Liu G, Zhao L (2007b) Nitrogen removal performance in a novel combined biofilm reactor. Process Biochem 42: 620–626

Ziv Arbeli Z, Brenner A, Aharon Abeliovich A (2006) Treatment of high-strength dairy wastewater in an anaerobic deep reservoir: Analysis of the methanogenic fermentation pathway and the rate-limiting step. Water Res 40: 3653-3659

Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 61: 533–616

List of Tables

Table (1): Ammonium concentration (mg/l) in livestock wastewater includes discharges from eight cattle/dairy farmyard and one sheep yard (Harrington and McInnes, 2009).

Year	Mean	SD[a]	N	SEM	%CV	Min.	Max.
2001	41.30	74.29	46	10.95	179.89	0.07	480.00
2002	85.95	193.28	6	78.91	224.87	5.88	470.00
2003	93.42	226.07	92	23.57	241.99	0.10	1900.00
2004	67.45	96.09	120	8.77	142.47	0.19	654.00
2005	51.80	64.64	193	4.65	124.78	0.03	613.90
2006	36.76	42.39	77	4.83	115.31	0.27	180.44
2007	32.50	35.38	80	3.96	108.85	0.07	185.70
2008	47.05	48.25	62	6.13	102.57	0.00	262.12
2009	43.86	43.38	14	11.59	98.91	4.85	152.27

[a] SD = standard deviation; *n* = sample size; SEM = standard error of the mean; %CV = coefficient of variation; Min. = minimum; Max. = maximum.

Table (2): Concentrations of TKN and AN in different domestic wastewaters.

Location	Description	TKN (mg/l)	AN (mg/l)	Reference
Ghent, Belgium	Collected domestic wastewater samples for a period of 450 days	39±9	24 ±11	Aiyuk et al. (2004).
Brisbane, Australia	Weekly collected domestic wastewater samples after on-site primary sedimentation and pre-denitrification treating.	43	-	Blackburne, et al. (2007).
Zhejiang, China	Domestic wastewater derived mainly from restaurants and dormitories.	70	40	Feng et al. (2008).
Beijing, China	Samples collected from a septic tank	85	79	Wu et al. (2007)
Auchi, Nigeria	Samples collected from a septic tank	17	13	Oladoja and Ademoroti (2006)

TKN = total Kjeldahl nitrogen

AN = ammonia nitrogen

List of Figure

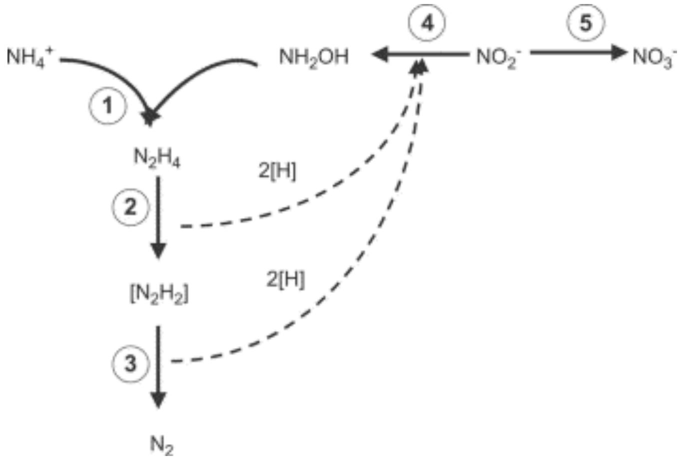

Fig. 1. Possible metabolic pathway for anaerobic ammonium oxidation. Consumption and production of H_2O and H^+ are not shown. Ammonium is oxidized through hydroxylamine to hydrazine (step 1). Reducing equivalents derived from N_2H_4 then reduce nitrite to more hydroxylamine and nitrogen gas (steps 2, 3 and 4). Nitrate formation could generate the reducing equivalents for biomass growth (step 5) (van de Graaf et al. 1997).