Bio-removal of nitrogen from wastewaters—A review

Gaber Z Breisha*

Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Minia, Egypt
gaberbresha@yahoo.com
Telefon: 002 03 5776848 : Fax: 002 086 2362182
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

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.

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1. Introduction

Although access to safe drinking water has improved steadily and substantially over the last decades in almost every part of the world [1, 2], in the developing countries 90% of all wastewater still goes untreated into local rivers and streams [3] 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 [4]**.** 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 0.4-0.6 m 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 [5]. 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 [6]. 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 [7]. 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 a) to identify nitrogen pollutants concentrations in domestic, agricultural and industrial wastewaters, b) to compile the latest achievements of technologies developed for the removal of nitrogen from these wastewaters and c) 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

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^{-1} total Kjeldahl nitrogen and 1318 mg l^{-1} NH_4^+-N [8]. 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 [9]. The variation of ammonium-N concentrations (mg l^{-1}) 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 higher variations within single years [10].

Table (1): Ammonium concentration (mg l^{-1}) in livestock wastewater includes discharges from eight cattle/dairy farmyard and one sheep yard [10].

Year	Mean	SD[1]	N	SEM	%CV	Min	Max
2001	41.30	74.29	46	10.95	179.89	179.89	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

[1]SD = standard deviation; N= sample size (l); SEM = standard error of the mean; %CV = coefficient of variation; Min. = minimum; Max. = maximum.

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 [11-13]. 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 [14]. 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 [15-17]. Gheysari et al. [18] 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^{-1} at no N application and deficit irrigation to 40.8 Kg ha^{-1} at fertilization level of 142 kg N ha^{-1} and full irrigation, which would be the minimum annual N-requirement for corn in European countries.

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 [19, 20]. In Rajasthan, India the raw sewage received at the activated sludge plant has a BOD of 600–800 mg l^{-1} and a NH_4^+-N concentration of 80–110 mg l^{-1} during summer when water shortage was acute [21]. 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. [22] found that the ammonia concentration in real domestic wastewater from one septic tank in China was 54-74 mg l^{-1}. A similar finding was reported by Guo et al. [23]. 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 [24]. 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.

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

Location	Description	TKN ($mg\ l^{-1}$)	AN ($mg\ l^{-1}$)	Reference
Belgium	Collected domestic wastewater samples for a period of 450 days	40	24 ±11	[183]
Australia	Weekly collected domestic wastewater samples after on-site primary edimentation and predenitrification treating.	43	-	[184]
China	Domestic wastewater derived mainly from restaurants and dormitories.	70	40	[185]
Nigeria	Samples collected from a septic tank	17	13	[186]
China	Samples collected from a septic tank	85	79	[187]

TKN = total Kjeldahl nitrogen
AN = ammonia nitrogen

2.3. Nitrogen in agro-industrial 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. 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 l^{-1} NO_3^--N [25, 26] and some contain even higher nitrate levels. For instance, the wastewater from glasshouses contained 325 mg l^{-1} NO_3^--N [27]. Several other industries generate wastewater with varying amounts of nitrate, being 222 mg l^{-1} in a tannery wastewater of Pisa. Italy, [28], 2320 mg l^{-1} in wastewater from the cochineal insects processing to produce natural carmine used principally as a coloring agent in cosmetics, beverages and products with low pH [29], 3600 mg l^{-1} generated from an initiating explosive factory in China [30] and 4000-6000 mg l^{-1} produced during the frosting process of bottles in a winery [31]. In Egypt the El-Nasr Pharmaceutical and Chemical Company, South-East of Cairo, discharges both industrial (6000 m^3 d^{-1}) and municipal wastewater (128 m^3 d^{-1}) into a nearby evaporation pond without any treatment. The generated raw wastewater is characterized by high values of ammonium (about 300 mg l^{-1}) [32].

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 wastewaters and some fermentation byproducts may not be easily utilized by denitrifiers as electron donors, and the residual antibiotics have a toxic effect on microorganisms [33].

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

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (1)$$

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

$$NO_2^- + 0.5\ O_2 \rightarrow NO3^- \quad (2)$$

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* [34]. The complete nitrification, as seen during wastewater treatment can be expressed as follow:

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + H_2O + 2H^+ \quad (3)$$

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 [35, 36]. Unlike some contaminants which are in need for a certain microbe to be treated, denitrifying bacteria are ubiquitous in nature [37] 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 [38]. 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 [39, 40] and acetic acid which have been used for wastewater denitrification as well as in full-scale plants of drinking water treatment [41]. 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 [42]. Park et al. [43] 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. The theoretical methanol requirement for nitrate is 2.47 mg CH_3OH per mg NO_3-N as indicated in equation (4) [44].

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

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 [45], rotating biological contactors [46] and fiber-based biofilm reactor [47]. Efforts are still ongoing and some novelties in combination of biological and other methods, such as membrane biofilm reactors (MBR), were manifested [48].

3.2. Simultaneous nitrification and denitrification (SND)

The SND process starts with a partial nitrification of NH_4^+ to nitrite and subsequently continues with a direct reduction of nitrite to N_2 gas [49, 50]. 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 [51]. Zhang et al. [52] 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. [53] who used a biofilm airlift suspension reactor with biodegradable carrier material. Investigation of Fux et al. [54] 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. This is important since the optimal pH for the nitrifying and denitrifying bacteria lies between 7 and 8.5 [55]. Further, Ma et al. [56] constructed a bench-scale continuous flow system, consisting to remove nitrogen and carbon simultaneously from terramycin crystallization mother solution (TCMS). 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 the anoxic reactor.

Polymeric beads, in which a nitrifier and a denitrifier were co-immobilized, were used to remove

nitrogen in a single step. Uemoto and Saiki [57] 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 double-sided 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 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 [58].

3.3. Autotrophic denitrification

The heterotrophic denitrification rate was strongly dependent on the type of carbon source, the concentration of the carbon source and the C/N ratio [59]. This could vary for different microorganisms, water streams, and environmental conditions [60]. 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 [61]. 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. Extensive studies have been carried out on elemental sulfur [62-66] and H_2 [67-69] as electron donors for autotrophic denitrification systems. Under typical aquifer conditions, iron sulphide (pyrite) is typically expected to be the electron donor [70] for denitrification assisted by *Thiobacillus denitrificans*:

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

In 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. A biofilm-electrode reactor as a combined electrochemical and biological reactor was developed by Sakakibara et al. [71] and improved by Prosnansky et al. [72] to solve these problems. In this system, autotrophic denitrifying microorganisms are immobilized on 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 [73] and applied at large scale by Wan et al. [66]. 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 http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6V73-4W45WFV-1&_user=2149863&_coverDate=07%2F31%2F2009&_alid=965208706&_rdoc=9&_fmt=full&_orig=search&_cdi=5831&_sort=r&_docanchor=&view=c&_ct=2424&_acct=C000056383&_version=1&_urlVersion=0&_userid=2149863&md5=c5def9212505431b485c79479846a42f - bib5[74]. 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 are determined.

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 [75] and has, therefore, lower operating cost.

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

Anammox needs ammonium and nitrite in a ratio of roughly one to one. For sludge digester 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) [76]:

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

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 [77].

The possible metabolic pathways for anaerobic ammonium oxidation are shown in Fig. 1 [78]. Using ^{15}N-labelling the experiments showed that the electron acceptor nitrite is reduced to hydroxylamine and that hydroxylamine somehow reacts with electron donor ammonium, leading to ultimate production of dinitrogen gas. 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. [77] postulated that 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 [79].

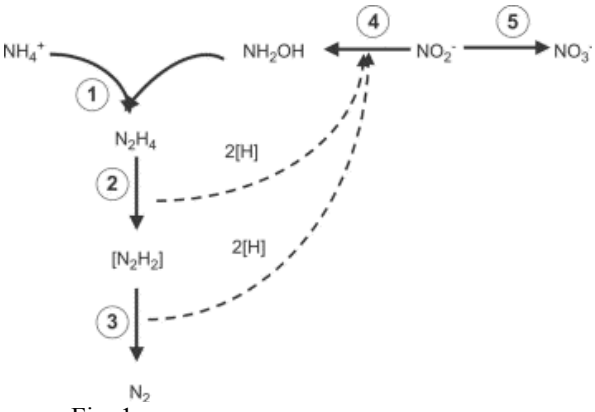

Fig. 1

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*" [80] and "*Kuenenia stuttgartiensis*" [81]. 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 [82]. 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 [83], co-oxidizing propionate and ammonium in the presence of nitrite and nitrate [84], and performing dissimilatory nitrate reduction to ammonium [85]. 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. [86] being metabolically active at oxygen concentrations of up to ~13 µmol $O_2\ l^{-1}$ [87]. 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 . The organic loading rate was found to affect the Anammox process performance, but the exact inhibitory levels still remain unclear [88, 89]. An organic matter concentration above 300 mg COD l^{-1} was found to inactivate Anammox communities in a UASB reactor fed with fat milk as organic matter source [90]. Concentrations of 50 mM of acetate resulted in 70% inhibition of the Anammox process [91]. 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·day. This removal rate was 20 times higher compared to the removal rates previously achieved in the laboratory [92]. 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 [93], nitrous organic wastewater in ASBR reactors and landfill leachate in a continuous reactor [94]. 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 [95]. 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 [5].

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) [96]. 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 [97]. Compared to the conventional nitrification and denitrification process, more than 50% [98] or 62.5% [99] 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. [100, 101]. In the CANON systems, *Nitrosomonas*-like aerobic ammonium-oxidizing bacteria and *Planctomycete*-like Anammox bacteria perform two sequential reactions simultaneously under oxygen limited conditions [102, 103]. 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 [104]. Equations number (8), (9) and (10) represent these reactions:

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

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

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

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 [86]. 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. [105]. 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 [92].

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 [106, 107]. 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 [108]. 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 [109, 110]. Membrane aeration is advantageous because gas transfer efficiencies are much higher than conventional bubble diffusers. In an MABR, the microporous membranes play two roles: the oxygen gas supplemental material and the carrier for bacterial immobilization [111]. 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 [112, 113]. 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 [114]. Gong et al. [107] 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. This immobilization technique is commonly used to immobilize a pure strain of bacteria because the mechanisms of pure strains are more easy to understand [115, 116]. Nevertheless, the immobilization of activated sludge has also been reported [117, 118]. 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. [106] 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 [119], but denitrification processes will normally occur in the range 2–50 °C [120] 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 [121] 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. The nitrogen removal capabilities of the denitrification process, when treating sanitary landfill leachate containing an ammonia concentration of over 2200 mg N/l^{-1}, were investigated 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, 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, freeze–thaw cycles increase the flux of carbon to the unsaturated zone and can create anaerobic micro-environments in which denitrification can be established [122].

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 [123]. 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 [124], and (2) start-up of nitritation at high temperatures and a gradually decline of the temperatures. Nitritation was maintained at room or low temperatures [125]. Some researches also proved that nitritation start-up could be promoted and accelerated at high temperatures [126]. 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 [127]. 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. [22] 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 [128,129] 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 [130]. Recently, Cema et al. [131] 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 other workers [132, 133] who operated an anaerobic biological filtrate 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. [134] 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 [135] 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. [136] 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 [137].

4.2. Effect of dissolved oxygen

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 [138]. 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 [139]. 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 [140].

Until now, a significant amount of research has focused on the partial nitrification and SND achieved by low DO [141]. Using low DO, Blackburne et al. [142] 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. [143] 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 [144]. However, Ma et al. [145] 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 [146, 147]. With the exception of the report of Guo et al. [148], 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. [148] 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/l) 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 [149]. 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^{-1} [150]. 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 [153].

4.4. Effect of salinity

The effect of high salt concentration on nitrification and denitrification has been previously investigated [154, 155]. 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 [156, 157]. Salinity levels have a definite impact on the microbial community structure in the wastewater and may affect the nitrification and denitrification process [158] 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 [159]. 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 [160]. Furthermore, Furukawa et al. [161] 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. [162] 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. [107] indicated that the salinity of 28–40 parts per thousand , 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 [163]. However, marine Anammox bacteria belonging to the genus *Scalindua* have been detected in natural surroundings [164] and recently Nakajima et al. [165] 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. [166] adapted *Candidatus Kuenenia stuttgartiensis* and *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. [166] 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. [166] 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. [167] 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 [168], 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 [30]. The 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 [120]. In well-buffered calcareous aquifers, such acidification is unlikely [169]. *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 [26]. 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 [170]. 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:

$$2NO_3^- + 12\ H^+ + 10\ e^- = N_2 + 6\ H_2O \text{ [171]}$$

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 2.7 g l^{-1}. Alkalinity of 3.91 g (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 [172] 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 [173]. 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 [174]. Furthermore Jha and Bose [175] 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 [30]. However, one of the main causes is believed to be the inhibitory effects of free ammonia (FA) [176]. Anthonisen et al. [177] 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^{-1}, allowing selective inhibition of nitrite oxidation at a range of FA concentrations of 1.0–10 mg/l. Supporting this observation, Bae et al. [178] 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. [179] 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. [180], 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 [181] 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. [182] 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. [182] 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.

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