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MINI-REVIEW

Biological nitrogen removal with nitrification and denitrification via nitrite pathway

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Abstract Presently, the wastewater treatment practices can be significantly improved through the introduction of new microbial treatment technologies. To meet increasingly stringent discharge standards, new applications and control strategies for the sustainable removal of ammonium from wastewater have to be implemented. Partial nitrification to nitrite was reported to be technically feasible and economically favorable, especially when wastewater with high ammonium concentrations or low C/N ratios is treated. For successful implementation of the technology, the critical point is how to maintain partial nitrification of ammonium to nitrite. Partial nitrification can be obtained by selectively inhibiting nitrite oxidizing bacteria through appropriate regulation of the system's DO concentration, microbial SRT, pH, temperature, substrate concentration and load, operational and aeration pattern, and inhibitor. The review addressed the microbiology, its consequences for their application, the current status regarding application, and the future developments.

Keywords Biological nitrogen removal · Denitrification · Partial nitrification · SHARON process · Shortcut nitrification–denitrification · Wastewater treatment

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Introduction

To protect lakes and other natural water from eutrophication, stringent nutrient level is set for the effluent from the wastewater treatment plants (WWTP). Because biological nitrogen removal is effective and inexpensive, it has been adopted widely in favor of the physical-chemical processes (EPA 1993). Various novel biological nitrogen removal processes such as short-cut nitrification and denitrification, anaerobic ammonium oxidation (Anammox), completely autotrophic nitrogen removal over nitrite (Canon) process and oxygen-limited autotrophic nitrification-denitrification (Oland) process, have been developed exclusively (Verstraete and Philips 1998). However, partial nitrification is a critical procedure for implementing these novel processes owing to nitrite is required as substrate or intermediary media (Philips et al. 2002). To date, nitrification and denitrification via nitrite technology have attracted more and more interests after successful application of SHARON (*S*ingle reactor system for *H*igh *A*mmonia *R*emoval *O*ver *N*itrite process) in practice. Partial nitrification process is based on the fact that nitrite is an intermediary compound in both nitrification and denitrification steps: a partial nitrification up to nitrite is performed followed by nitrite denitrification (Ferhan 1996; Fdz-Polanco et al. 1996), as shown in Fig. 1.

Compared to the traditional nitrification and denitrification via nitrate, the main advantages of partial nitrification

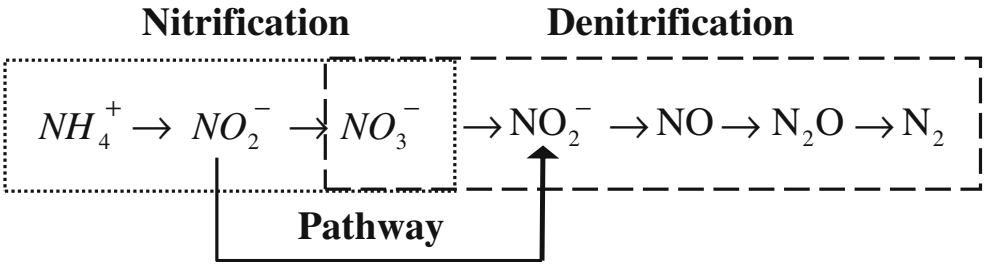

Fig. 1 Biological nitrification–denitrification via nitrite pathway

with respect to complete nitrification were reported as followed (Beccari et al. 1983; Turk and Mavinic 1987; van Kempen et al. 2001):

(1) 25% lower oxygen consumption in the aerobic stage implies 60% energy savings;
(2) in the anoxic stage the electron donor requirement is lower (up to 40%);
(3) nitrite denitrification rates are 1.5 to 2 times higher than with nitrate;
(4) reduces CO_2 emission by 20%;
(5) 33~35% lower sludge production in nitrification process and 55% in denitrification process.

Partial nitrification to nitrite and nitrite denitrification was reported to be technically feasible and economically favorable, especially when wastewater with high ammonium concentrations or low C/N ratios is treated (Surmacz-Gorska et al. 1997; Hellinga et al. 1998). SHARON process is the first successful process in which nitrification/denitrification with nitrite as an intermediate has been achieved under stable conditions and used in full-scale operation (Mulder et al. 2001). The full-scale application of SHARON process for nitrogen removal is shown in Table 1.

However, the operation conditions of SHARON, such as high temperature and high ammonium concentration, limit its development and application (STOWA 1995). Moreover, the application ranges mainly stage on rejection water of sludge digestion and leachate water of landfill site. How to achieve and maintain stable partial nitrification in other kinds of wastewater and in common conditions attracts more and more researchers' attention. Until now, successful partial nitrification processes recorded are obtained in sequencing operation process, and few are achieved in a continuous-flow process (Peng et al. 2003a; Khin and Annachhatre 2004). So in this review, various novel technologies for successful partial nitrification are discussed and critically reviewed. The current status regarding application and possible future research areas to further improve understanding of the process is also proposed.

Biological relationships of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria

Nitrification is a sequencing biological oxidation process, which involved two different groups of bacteria. The first step of nitrification is the oxidation of ammonia to nitrite over hydroxylamine (NH_2OH), involving the membrane-bound ammonia mono-oxygenase (AMO) and the hydroxylamine oxidoreductase (HAO), and is carried out by ammonia-oxidizing bacteria (AOB); the second group, nitrite-oxidizing bacteria (NOB), further oxidizes nitrite to nitrate. To date, any one group of bacteria that can directly oxidize ammonia to nitrate has not been found (Radajewski et al. 1994; Regan et al. 2002; Lipponen et al. 2004). Under normal conditions, the reaction of ammonia oxidation to nitrite is a velocity-limiting step; in contrast, nitrite is oxidized rapidly to nitrate, so nitrite is seldom accumulated in nitrifying reactors. In partial nitrification process, however, nitrite accumulation is required, and the second step must be restrained so as to accumulate AOB and washout NOB (Laanbrock and Gerards 1993).

During the nitrification process, AOB and NOB co-exist and benefit from the close physical association. On one hand, the close physical association is useful for energetic reasons: NOB are able to efficiently intercept the nitrite

Table 1 Full-scale application of SHARON process for nitrogen removal

Full-scale plants	Capacity (pe)	Influent	N removal (%)	N load (kg N/d)	Operational year
Utrecht	400,000	Rejection water	>95	900	1997
Rotterdam	470,000	Rejection water	>95	850	1999
Zwolle	200,000	Rejection water	>95	410	2003
Beverwijk	320,000	Rejection water and condensate	>95	1.200	2003
Garmerwolde	300,000	Filtrate and condensate	>95	2,500	2005
Hague	930,000	Centrate	>95	1,200	2005

(i.e., their substrate) produced by the AOB, helping to cope with the poor energy yield of nitrite oxidation. On the other hand, AOB like the presence of the NOB, as the latter relieve them from the toxic nitrite. It therefore aids in the defense against the toxicity of nitrite by preventing its accumulation or formation of toxic by-products such as NO that can interact with bacterial enzymes (Stein and Arp 1998). But recent studies in which comparative 16S rRNA analyses of ammonia- and nitrite-oxidizing bacteria have clarified the phylogenetic relationships of these bacteria have demonstrated that they belong to two separate lineages within the *Proteobacteria* (Head et al. 1993; Teske et al. 1994).

The bacterial genera responsible for the oxidation of ammonia and nitrite are presumed to be predominantly the genera *Nitrosomonas* and *Nitrobacter*, both of which are chemolithoautotrophic members of the class *Proteobacteria* (Wheaton et al. 1994). The autotrophic ammonia-oxidizing bacteria that have been characterized belong exclusively to the β subdivision of the *Proteobacteria* and are typified by *Nitrosomonas europaea* (Fig. 2). The SHARON process is carried out largely by *Nitrosomonas eutropha* (Logemann et al. 1998). These bacteria form a distinct group within the β subdivision and are affiliated with an iron-oxidizing bacterium and the photosynthetic bacterium *Rhodocyclus purpureus*, along with methylotrophic bacteria. The only exception is *Nitrosococcus oceanus*, which is a marine species that belongs to the γ-proteobacterial lineage. The nitrite-oxidizing bacteria are more widespread in the *Proteobacteria*. The most commonly studied autotrophic nitrite-oxidizing bacteria belong to the á subdivision of the *Proteobacteria*, of which *Nitrobacter winogradskyi* is a representative species. Other chemolithoautotrophic nitrite-oxidizing bacteria that have been characterized are phylogenetically widespread in the class *Proteobacteria*, occurring in the α, δ, and γ subdivisions (Fig. 2).

Methods and strategies for achieving and maintaining partial nitrification

To date, researchers have developed many control methods and strategies to achieve partial nitrification. The main objective of these methods and approaches was to accumulate AOB and washout NOB through different activation energies, different sludge ages, different dissolved oxygen half-saturation coefficients, and different anti-toxic capacities of AOB and NOB. These methods mainly include appropriate regulation of the reactor system's temperature, pH, dissolved oxygen (DO) concentration, sludge retention time (SRT), substrate concentration and load, operational and aeration pattern, inhibitor and so on (Surmacz-Gorska

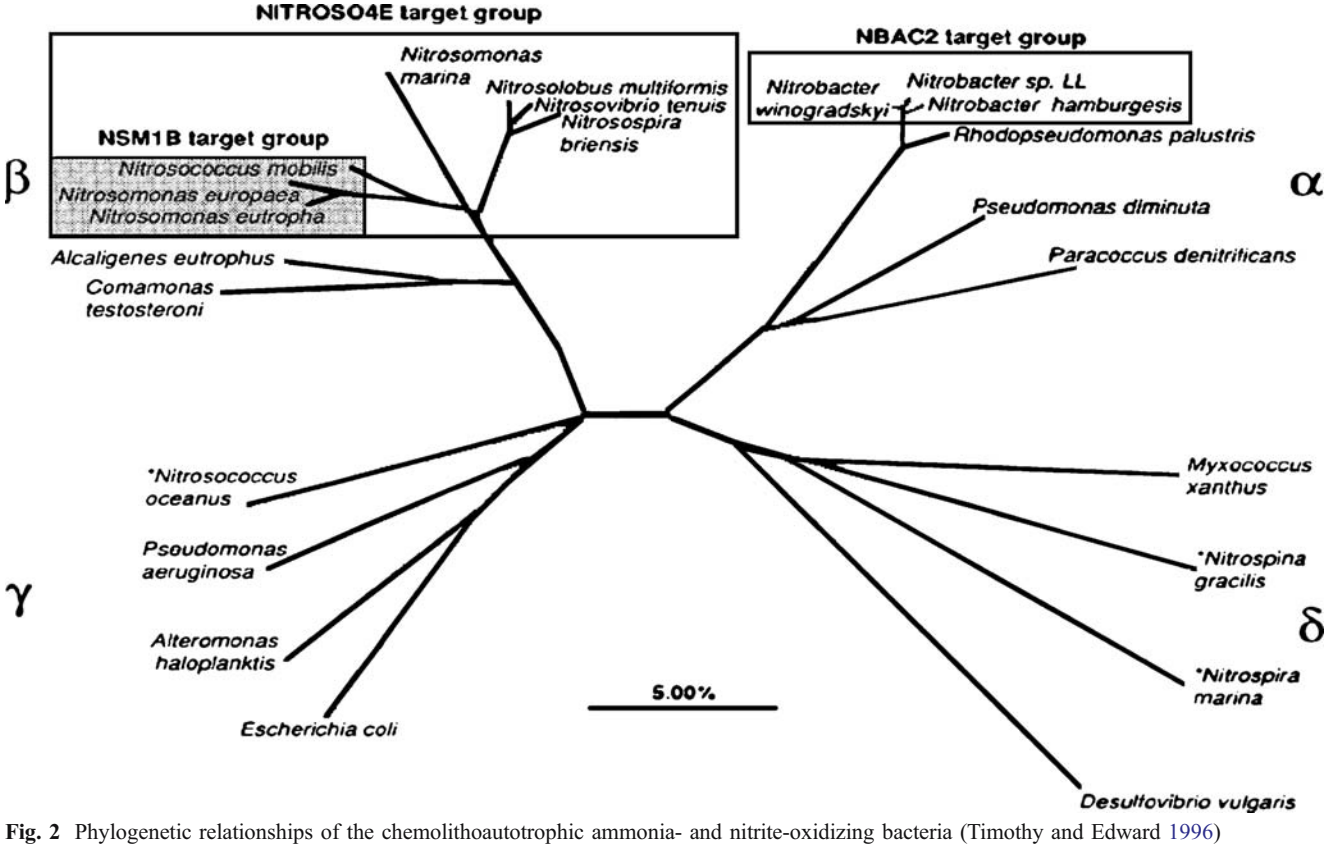

Fig. 2 Phylogenetic relationships of the chemolithoautotrophic ammonia- and nitrite-oxidizing bacteria (Timothy and Edward 1996)

et al. 1997; van Dongen et al. 2001; López-Fiuzaa et al. 2002). Details about these approaches are discussed in the following:

Reactor system's temperature

The activation energy of two groups of bacteria and their sensitivities to temperature changing are distinctly different (Hellinga et al. 1998). So raising temperature cannot only promote the growth rates of AOB, but can also expand the differences of specific growth rates between AOB and NOB, which was verified by some researchers (Yoo et al. 1999). Tonkovic (1998) noted that nitrite accumulated in an activated sludge plant especially over the summer period. There are various standpoints, however, about the optimal temperatures. Using pure cultures, optimum temperature was 35 °C for AOB and 38 °C for NOB (Camilla and Gunnel 2001). The SHARON process pre-mentioned was operated at 35 °C (Mulder et al. 2001). From the aspect of specific growth rate, only at temperatures above 25 °C is it possible for the ammonium oxidizers to effectively outcompete the nitrite oxidizers (Brouwer et al. 1996). But the opposite was the case at temperature below 15 °C.

Hellinga et al. (1998) have put forward the typical growth rate curves of AOB and NOB with temperature, as shown in Fig. 3. In Fig. 3, it can also be observed that the minimal residence time of AOB and NOB at temperature >25 °C is minimal. In other words, it is not necessary to excessively elevate temperature to achieve steady partial nitrification, which is also useful to explain why optimum temperatures in the literatures were different. It is not feasible to raise temperature of wastewater for the reason of higher specific heat of water (4.183 kJ kg^{-1} K^{-1}, 20 °C). The author suggests it is enough to keep the temperature at 25 °C when reactor system's temperature is the sole parameter. The experimental results of Yoo et al. (1999) also held out this suggestion.

Sludge age

Bock et al. (1986) have reported the minimum doubling times of AOB and NOB to be 7–8 h and 10–13 h, respectively. AOB and washout NOB can be selectively accumulated by appropriate regulation sludge retention time in suspended-growth system because of different minimum required sludge ages. Based on experiences from full-scale operation, van Kempen et al. (2001) suggest to maintain SRT between 1 day and 2.5 days.

More and more literatures about successful partial nitrification were reported under long sludge age. Nitrification tests were performed by Pollice et al. (2002a,b) in two lab-scale reactors operated under continuous and intermittent aeration, respectively. The results showed that partial nitrification to nitrite were steadily obtained under oxygen limitation, independent of the sludge age of 10, 14, and 40 days. Moreover, in our laboratory successful and stable nitrite accumulations were also fulfilled to treat domestic wastewater in pilot scale (54 m^3) and laboratory scale (38 l) under normal or even low temperature (<13 °C) with long sludge age (30 days) (Fig. 4). FISH (fluorescence in-situ hybridization) analysis results showed that AOB was the main nitrifying bacteria; no NOB was identified. The main AOB belonged to *Nitrosomonas*-like bacteria with PCR-DGGE. In pilot and laboratory scale experiments, the percent of AOB to total biomass is 3 and 12%, respectively (Zeng et al. 2006). Contrastingly, biomass retention is not required in the SHARON process. The dilution rate is the only adjustable parameter in the SHARON process that washes out the low-growth NOB and accumulates the AOB under high temperature so as to satisfy the biomass requirement. But this approach cannot be applied successfully into continuous flow processes.

Dissolved oxygen concentration

The dissolved oxygen half-saturation coefficients of AOB and NOB are 0.2–0.4 mg/l and 1.2–1.5 mg/l, respectively (Picioreanu et al. 1997). Therefore, low DO concentration is more restrictive for the growth of NOB than AOB, which will result in nitrite accumulation (Peng et al. 2004a).

Although many researchers reported that lower DO might inhibit the growth of NOB and cause AOB accumulation, the critical values of DO recorded in the literatures were different (Laanbrock and Gerards 1993;

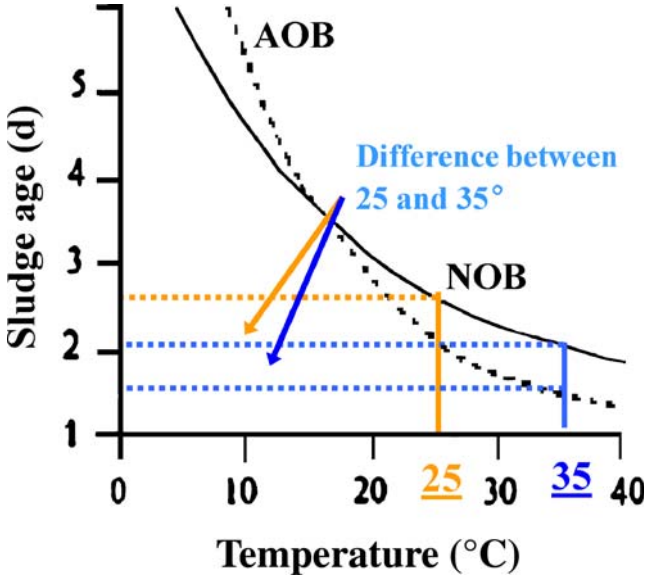

Fig. 3 Sludge age for AOB and NOB as a function of the temperature. There is little difference of minimal sludge age at temperature >25 °C. (After Hellinga et al. 1998)

Wyffels et al. 2004). In the report of Ciudad et al. (2005), an activated sludge reactor is operated under different DO levels, analyzing nitrite accumulation and ammonia removal. Results show that at 1.4 mg DO/l, 75% of nitrite accumulation takes place, with 95% of ammonia removal. Moreover, nitrite accumulation shows to be stable over more than 170 days of operation. Garrido et al. (1997) found that both ammonium oxidation rate and nitrite accumulation reached maximum when DO was 1.5 mg/l. Below 0.5 mg/l of DO ammonium was accumulated and over 1.7 mg/l complete nitrification to nitrate was achieved (Ruiz et al. 2003). On the other hand, it should be noticed that lower DO will lower nitrification rate and cause filamentous bulking sludge. Considering ammonia oxidation rate and nitrite accumulation, DO concentration should be maintained about 1.0–1.5 mg/l. It is a good idea to use real-time control methods to regulate DO concentration in the reactor. There is often appearance of "ammonia break point" in the DO profiles, and "ammonia valley" in the pH profiles at the end of nitrification for SBR process, shown in Fig. 5 (Wang et al. 2004). So the nitritation process can well be predominated by identifying "ammonia valley" and "ammonia break point" and avoiding from high DO and excess aeration.

Operational and aeration pattern

The aeration pattern is proposed as an alternative parameter to control ammonium oxidation to nitrite (Hidaka et al. 2002). Duration of aeration time was found to be inversely related to the degree of nitrite build-up (Turk and Mavinic 1987). Using of intermittent aeration was in favor of implementation of nitrite accumulation (Pollice et al. 2002a,b). Also, the continuous feeding of the influent wastewater from the bottom of the reactor was critical for effective nitrification and denitrification via nitrite (Yoo et al. 1999). As the induction of the different denitrification enzymes proceeds sequentially, the different intermediates of denitrification accumulate temporarily after the switch from aerobic to anoxic conditions. The length of the anoxic period is, therefore, important to achieve complete denitrification. According to Baumann et al. (1997), an anoxic period of 4 h is sufficient to synthesize all enzymes and have complete denitrification. Besides, Peng et al. (2004a) reported that using aeration control strategy partial nitrification had been achieved, although the temperature was decreased from 32 to 21 °C.

Many researchers have been working on SBR control based on common sensors such as pH measurements that should be easy to install with a little additional investment (Al-Ghusain and Hao 1995). The pH value decreases during the aeration period due to alkalinity consumption by nitrification. Theoretically, pH value in the course of converting nitrite to nitrate should not vary because there is no hydrogen ion produced, which is contrary to the process of ammonia conversion to nitrite. But when both nitritation and nitratation are completed, pH will increase because of CO_2 stripping. So there would be an inflexion of pH value in the liquid phase at the end of nitrification, as shown in Fig. 6. Stable partial nitrification can be achieved by online control technology, with variations of characteristic features and the differential coefficient as input variable parameters, thus preventing from excess aeration (Peng et al. 2003b). Long-time operation with online control strategy would result in AOB accumulating and NOB washout, which is a course of AOB community optimization.

pH

The value of pH influences partial nitrification in the following several aspects:

Influence on free ammonia (FA)

Free ammonia (FA) is a competitive inhibitor of nitrite oxidoreductase activity, which is located on the cell membrane of NOB (Yang and Alleman 1992). The inhibitions of free ammonia to two groups of bacteria are

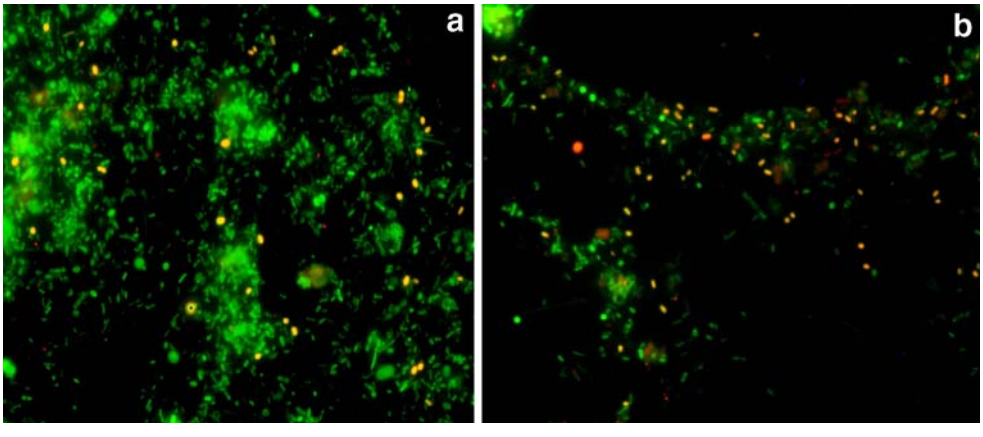

Fig. 4 In situ identification of nutrifying bacteria from pilot-scale SBR **a** and lab-scale SBR **b** a and b: *Green* for activated sludge (FITC labeled probe EUB_{mix}) and *red* for AOB β-*Proteobacteria* (CY3 labeled probe NSO 1225) (Zeng et al. 2006)

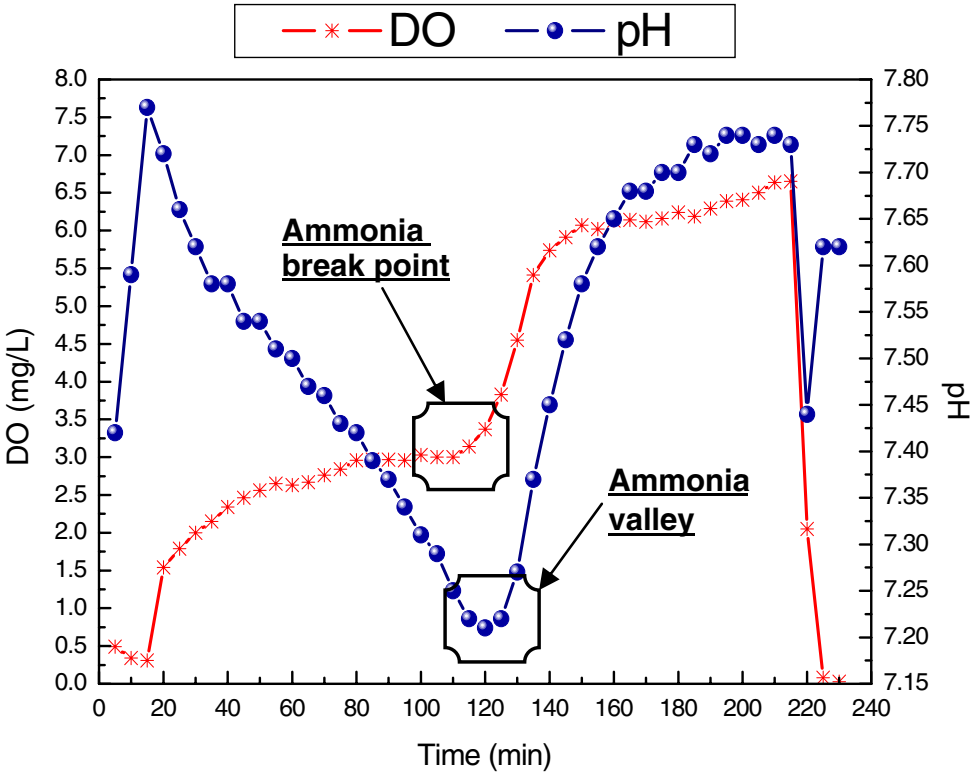

Fig. 5 The typical variations of DO and pH for organic substrate removal and nitrification process in SBR process

different, and the relationship between free-ammonia concentration and pH is as follows (Anthonisen 1976):

$$\mathrm{FA} = \frac{17}{14} \times \frac{[\mathrm{NH_4^+ - N}] \quad 10^{\mathrm{pH}}}{Ka/Kw \quad 10^{\mathrm{pH}}}$$

Here, FA is free-ammonia concentration, $[NH_4^+–N]$ is ammonium concentration, K_a and K_w are ionization constants of ammonia and water, respectively. Boundary conditions for FA inhibition of both AOB and NOB were first depicted in Anthonisen (1976) (Fig. 7).

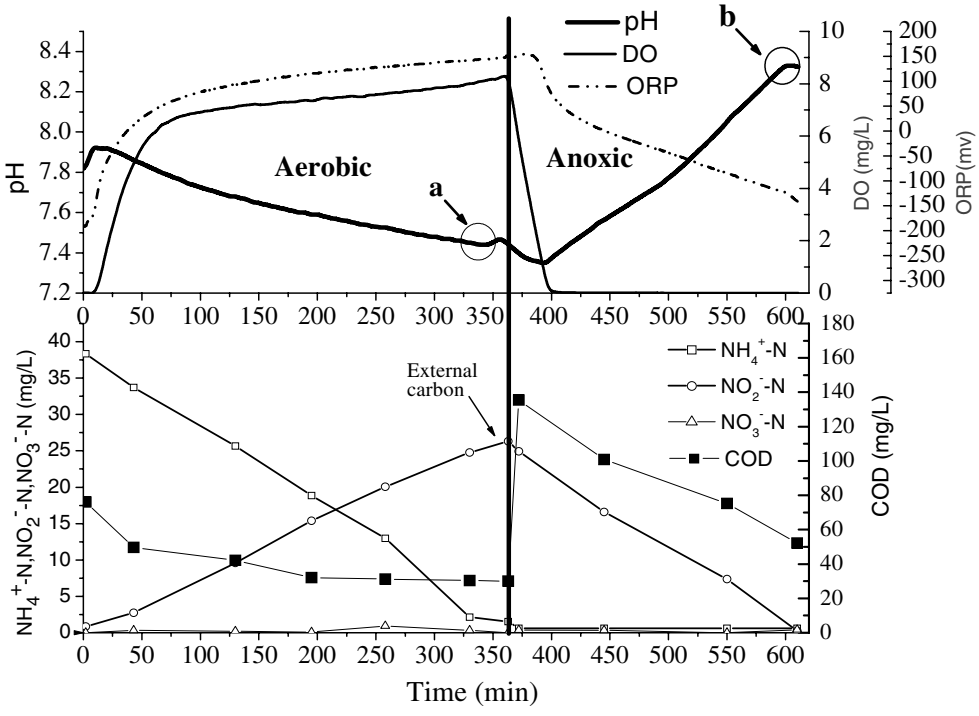

Fig. 6 Typical variations of DO, pH, and ORP value and concentrations of ammonia, nitrite, and nitrate during nitrification and denitrification process in SBR (Yang et al. 2006) Influent NH_4=56 mg/l; COD=254 mg/l; alkalinity=268 mg/l

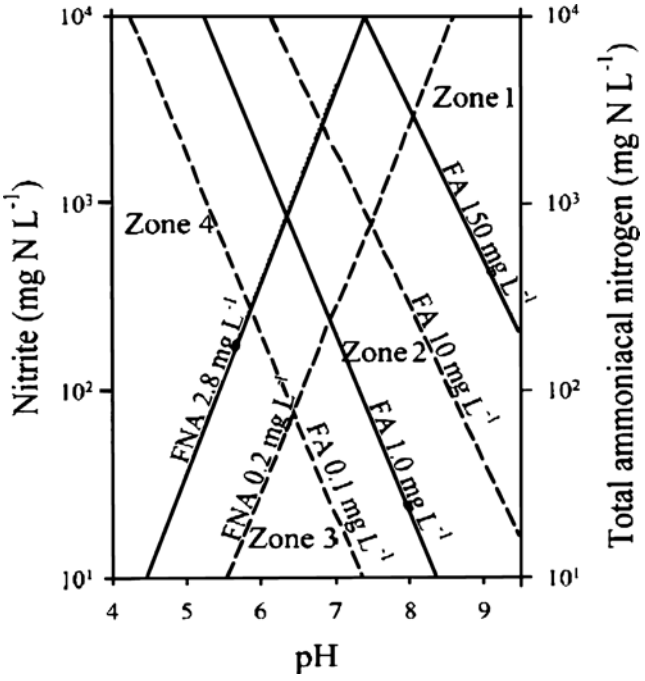

Fig. 7 Relationship between concentrations of free ammonia (*FA*) and free nitrous acid (*FNA*) and inhibition to nitrifiers. The *dashed lines* mark the lower limit and the *solid lines* mark the upper limit of the range of boundary conditions of zones of nitrification inhibition. *Zone 1*=inhibition of nitritation and nitratation by *FA*; *Zone 2*=inhibition of nitratation by *FA*; *Zone 3*=complete nitrification; *Zone 4*=inhibition of nitratation by FNA (Anthonisen et al. 1976)

Many researchers confirmed that nitrite accumulation could be achieved by regulating pH to control free-ammonia concentration (Ferhan 1996; Peng et al. 2004b). At FA ≥7 mg NH_3–N/l, ammonia oxidation reaction is inhibited, and ammonia oxidation reaction will almost cease at 20 mg NH_3–N/l (Abeling and Seyfried 1992). But the threshold inhibition concentrations of free ammonia found in the literature were different. It was reported that the system could stably operate at free-ammonia concentration of 50 mg NH_3–N/l, but nitrite oxidation activity would be inhibited in the presence of free-ammonia concentration higher than 3.5 mg NH_3–N/l (Wong-Chong and Loehr 1978). Free ammonia has only an inhibition effect on NOB, but does not kill them. After a period of cultivation, NOB will recover activity (Beccari et al. 1983; Han et al. 2003). It is necessary to synthetically consider other factors to achieve stable partial nitrification when only by regulating free-ammonia concentration (Fdz-Polanco et al. 1994; Surmacz-Gorska et al. 1997). Ford et al. (1980) reported that both ammonia and nitrite oxidation activities would be inhibited under the condition of free-ammonia concentration higher than 24 mg NH_3–N/l, but they would recover as soon as free-ammonia concentration was below the threshold concentration, and the system could operate in spite of free-ammonia concentration of 56 mg NH_3–N/l.

Influence on HNO_2

The research results suggested that an HNO_2 concentration of 0.13 mg/l was proposed as the toxicity threshold for nitrite to denitrifying bacteria (Abeling and Seyfried 1992). An inhibition mechanism that has been proposed for HNO_2 toxicity is that it acts as an uncoupler by donating a proton inside the cell. That intracellular proton directly interferes with the transmembrane pH gradient required for ATP synthesis (Glass et al. 1997).

The pH regulates the equilibrium between nitrite and nitrous acid. The relationship between HNO_2 distribute ratio and pH is as follows:

$$\mathrm{HNO}_2/\% = \frac{100}{1 + [K_\mathrm{b}] \big/ 10^{-\mathrm{pH}}}.$$

$[K_\mathrm{b}]$ is ionization constant of nitrous acid

Furthermore, pH values >7 have the double effect of limiting the conversion of nitrite into nitrous acid and ensuring concentrations of free ammonia that would selectively inhibit nitrite oxidizers, thus favoring the stability of partial nitrification.

In addition to higher organisms like human being, nitrite has also a known influence upon micro-organisms and their processes. It is a known bacteriostatic molecule due to its affinity for the metal ions in the center of enzymes (Wild et al. 1995). According to Sijbesma et al. (1996), nitrite acts as a protonophore that stimulates basal electron transport, inhibits ATP (adenosine triphosphate) synthesis, stimulates ATP hydrolysis, and inhibits various exchange reactions catalysed by the ATP-ase (Rottenberg 1990). The inhibition effects by nitrite of ammonia oxidation activity are shown in Fig. 8.

Influence on free-hydroxylamine (FH) concentration

The presence of free hydroxylamine, a toxic intermediate in nitrification by AOB, appeared to have a consistent correlation with low nitrification activity (Castignetti and Gunner 1982; Stüven et al. 1992). According to Hu (1990), hydroxylamine exhibited acute toxicity to NOB, and this may also cause nitrite build-up in a nitrifying system. No nitrite oxidation occurred when 0.42 mgNH_2OH-N/l was present. Addition of 2.5–5 mgNH_2OH-N/l to a submerged filter system significantly enhanced nitrite accumulation during nitrification (Hao and Chen 1994). Moreover, this inhibitory effect of hydroxylamine on NOB was found to

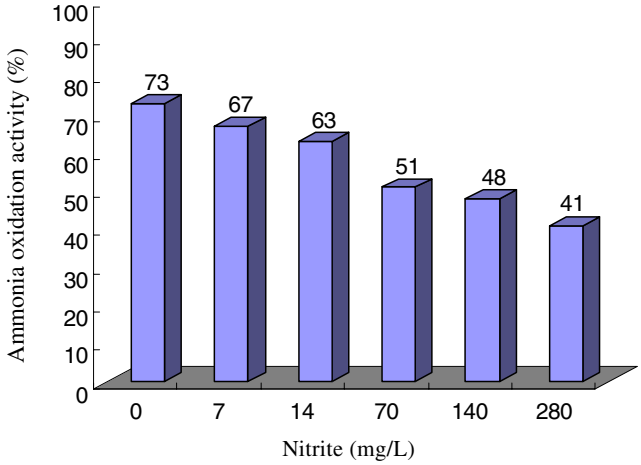

Fig. 8 Ammonia-oxidizing activity relative to the initial activity in *Nitrosomonas europaea* cells exposed to nitrite after a 24-h incubation (pH 8) in the absence of ammonium under aerobic conditions (after Stein and Arp 1998)

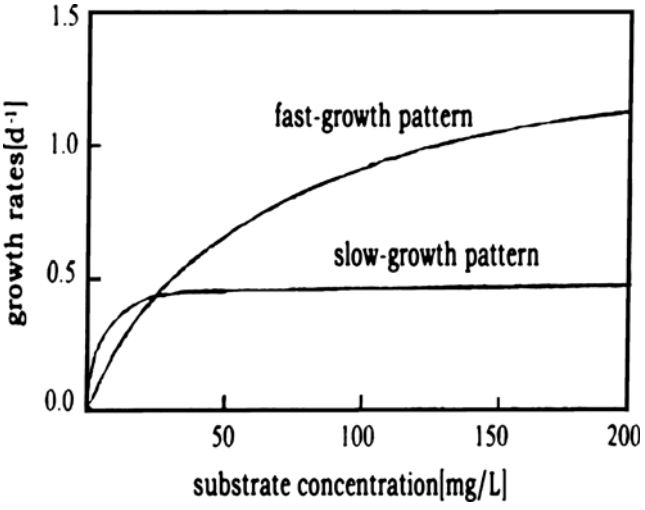

Fig. 9 The growth curve of two type ammonia oxidation bacteria. The substrate was the common limiting growth factors such as ammonia and DO. (Zheng et al. 2004)

be irreversible. Hydroxylamine appeared likely to accumulate in a nitrifying system with high NH_3/NH_4^+ concentration, deficient oxygen, and high pH (Stüven et al. 1992).

Control of substrate concentration and load

Research results showed that AOB could be divided into two different groups of bacteria: slow-growth and fast-growth pattern (Zheng et al. 2004). One group of ammonia oxidation bacteria (fast-growth pattern) has higher affinity for substrate ammonia (lower half-saturation coefficient for ammonia) and thus can grow under the condition of low-substrate concentration with the higher specific growth rate. Oppositely, another has lower affinity for substrate and can only grow under the condition of high substrate concentration. The curve growth rates of two types of AOB vs substrate concentration were shown in Fig. 9. In conventional wastewater treatment process, ammonia concentration generally would be controlled at the lower level to satisfy discharge standard. However, fast-growth-pattern AOB will accumulate in partial nitrification reactors because of high ammonia concentration (Surmacz-Gorska et al. 1997). These also demonstrate why it is easy to achieve partial nitrification to treat wastewater with high concentrations of ammonia, but difficult to implement and maintain steady partial nitrification with treatment of common domestic wastewater (Sliegrist et al. 1998; van Dongen et al. 2001).

Moreover, in the case of nitrification, according to Okabe et al. (1996), a higher C/N ratio retarded an accumulation of nitrifying bacteria, particularly NOB. Due to competition for common electron donors, nitrate in high concentrations has been found to inhibit the nitrite reduction (Almeida et al. 1995; van Rijn et al. 1996). In addition, Kornaros et al. (1996) found that nitrate has an inhibitory effect on the synthesis and activity of nitrite reductase. During dissimilatory nitrate reduction to ammonium, nitrite accumulates especially when high concentrations of nitrate resulted from inhibition of the nitrite reductase system by nitrate, 140 mg NO_3-N/l are present (Bonin 1996).

Experimental results of Nowak have shown that nitrite oxidation might be affected by phosphorus deficiency, the so-called phosphate block (Nowak et al. 1996). The phosphate half-saturation coefficient for NOB (0.2 mg P/l) is about one order of magnitude higher than for AOB (0.03 mg P/l) (Nowak et al. 1996). The nitrite oxidation was substantially reduced at phosphate levels below 0.2 mg PO_4^3-P/l. In this case, the presence of phosphate, rather than its absence, has also a profound effect on denitrification and causes nitrite accumulation. Nitrite reduction rate decreased from 4.9 to 2.7 mg NO_2-N/g protein min as phosphate concentration was increased from 0 to 15.5 mg P/l (Barak et al. 2000).

Inhibitor

The inhibitors for nitrification include heavy metal, toxicant, organic compounds, fulvic acids, oxidants, volatile fatty acids, and halide (Garrido 1998; Zhang et al. 2000; López-Fiuzaa et al. 2002). Heavy metals, chromium, nickel, copper, zinc, lead, and cadmium, might inhibit both steps of nitrification reaction, but the inhibition effects are different (Camilla et al. 1998). The ranges of metal concentrations of inhibition to nitrification under pure culture were shown in Table 2. No other metals were shown to be significantly inhibiting.

Nitrite accumulation in the biofilm process was related to fulvic acids loadings. When the fulvic acids loading was

Table 2 Ranges of metal concentrations of inhibition to nitrification (Camilla et al. 1998)

Metal	Concentration (μg/l)
Cr	0.7–785
Ni	3–860
Cu	3–5,730
Zn	3–1,000
Pb	0.09–1,680
Cd	0.01–20

less than 0.002 kg (TOC)/m^3 h, no nitrite build-up appeared, but when the loading was in the range of 0.002±0.02 kg (TOC)/m^3 h, nitrite built up and the concentration of nitrite could reach as high as 11.4 mg/l. When the loading was above 0.07 kg (TOC)/m^3 h, the nitrification process was completely inhibited (Zhang et al. 2000). Organic compounds such as aniline, ortho-cresol, and phenol have stronger inhibition influences on NOB than on AOB, so biological wastewater treatment with these organics might cause nitrite accumulation phenomenon (Neufeld et al. 1986) to occur.

Oxidants such as chlorite and chlorate have inhibition effect on NOB. The inhibition effect of chlorite and chlorate is selective, which is different with other inhibitors (Belser and Mays 1980). The growth of NOB would be inhibited when the concentration of potassium chlorate was during 0.001–0.01 mM. When the concentration of ClO_3 is 1–10 mM, the activity of NOB would be inhibited completely, and when the concentration of ClO_2 is 3 mM, it would also be the case. The real inhibitor to nitrite oxidation bacteria was verified to be ClO_2. The results proposed by Belser and Mays (1980) demonstrated that the concentration ClO_3 of 10 mM had no effects on the activity of AOB; in other words, ammonia oxidation rate would be fully identical with the rate without ClO_3 addition, but the activity of NOB was inhibited completely (Lees and Quastel 1945).

Volatile fatty acids, formic, acetic, propionic and *n*-butyric acid all inhibited nitrite oxidation, but exhibited no significant effect on ammonia oxidation (Eilersen et al. 1994). Eilersen et al. (1995) provided the critical concentrations of volatile fatty acids in Table 3, which is the concentration at which activity falls to 50% of the activity in the absence of the inhibiting compound.

Cl_2 and Br_2 as disinfectors used commonly in water treatment would also inhibit NOB (Cotteux and Duchene 2003; Peng et al. 2004c). The experimental results from biological treatment of wastewater containing seawater indicated that salinity had benefits to nitrite accumulation (Peng et al. 2005). When the ratio of seawater to wastewater was 30%, and the ammonia loading was below the critical value of 0.15 kgNH_4^+-N/(kgMLSS.d), the ammonia removal efficiency via nitrite pathway was above 90%.

Critical review on various control technologies

By comparing and evaluating steady nitrification and denitrification via nitrite pathway used in practical application, the most successful implementation was obtained in sequencing operation process. Few were achieved in continuous-flow process, also not in domestic wastewater with influent NH_4^+-N <50 mg/l. Moreover, how to accomplish partial nitrification in continuous flow systems has some problems to solve, as nitrite concentration in the effluent is equal to nitrite concentration recycled. How to implement stable partial nitrification process in the treatment of common domestic wastewater with continuous-flow system should be the future research direction. Besides, partial nitrification process can be achieved by inhibiting NOB growth with FA and chlorate. But chlorates only have irreversible inhibition on NOB, not AOB. Moreover, the effect mechanisms of FA and inhibitors are different, which FA only selectively inactivated, but inhibitors selectively deaden NOB. Consequently, this aspect should be researched in-depth. Stable partial nitrification can also be fulfilled under normal or long sludge age condition, which is contrary to the SHARON process. The on-line measurement and control technology with DO and pH and their differential coefficient as input variable parameters would be a feasible research direction. With

Table 3 Critical concentrations (mM) of some volatile fatty acids with respect to inhibition of nitrification and denitrification (Eilersen et al. 1994, 1995)

Volatile fatty acid	Ammonia oxidation	Nitrite oxidation	Nitrite reduction	Nitrate reduction
Formic acid	–	2	–	–
Acetic acid	–	115	–	–
Propionic acid	–	68	196	74
n-Butyric acid	–	33	–	–
Isobutyric acid	6	8	32	30
n-Valeric acid	37	75	57	36
Isovaleric acid	6	7	18	18
n-Caproic acid	36	81	110	105

this technology, stable partial nitrification can be fulfilled and applied in the treatment of common municipal wastewater other than high-ammonia wastewater, which is the most distinct advantage over other methods and approaches.

Conclusions

Biological nitrification and denitrification via nitrite pathway is technically feasible and economically favorable, especially when wastewater with high ammonium concentrations or low C/N ratios is treated. The paper reviewed the biological relationships of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria. Moreover, the review extensively discussed the factors and estimated all methods used to achieve steady partial nitrification. By discussing and analyzing impact factors, it is possible to implement steady nitrite accumulation only through regulating one of the factors. DO concentration is an economically feasible control parameter. Under the condition of lower DO concentration, the aeration will be saved, but it is possible to reduce COD biodegradation rate and cause filamentous bulking sludge. Furthermore, idiographic and practical conditions should be considered, too. For example, it is unpractical to raise temperature for treating common municipal wastewater, because of higher water-specific heat. It is necessary to consider economic feasibility when using temperature, pH, and inhibitor as regulation parameter. But based on the fact that the influence mechanisms of some parameters have not been confirmed and the interrelations among some other parameters possibly exist, such as pH and substrate concentration and FA concentration, it is necessary to detailedly and roundly consider these factors.

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