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Research review paper

# Novel microbial nitrogen removal processes

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**Abstract**

The present-day wastewater treatment practices can be significantly improved through the introduction of new microbial treatment technologies. Recently, several new processes for nitrogen removal have been developed. These new nitrogen removal technologies provide practicable options for treating nitrogen-laden wastewaters. The new processes are based on partial nitrification of ammonium to nitrite combined with anaerobic ammonium oxidation. These processes include the single reactor system for high ammonia removal over nitrite (SHARON) process, which involves part conversion of ammonium to nitrite; the anaerobic ammonium oxidation (ANAMMOX) process, which involves anaerobic ammonium oxidation; and the completely autographic nitrogen removal over nitrite (CANON) process, which involves nitrogen removal within one reactor under oxygen-limited conditions. These new processes target the removal of nitrogen from wastewaters containing significant quantities of ammonium.


*Keywords:* Nitrification; Denitrification; Conventional nitrification and denitrification; SHARON process; ANAMMOX process; CANON process

## 1. Introduction

Deterioration of quality of inland and coastal waters is a serious environmental problem. Of particular concern is the wastewater containing organic nitrogen and phosphorus. This review is focused on processes for nitrogen removal from wastewaters. The removal of ammonium is of special interest because it can be toxic to aquatic species

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(Castens and Rozich, 1986). Ammonium can be removed from wastewaters by a variety of physicochemical and biological processes. Because biological nitrogen removal is effective and inexpensive, it has been adopted widely in favor of the physico-chemical processes (EPA, 1993).

Biological nitrogen removal proceeds slowly because the microorganisms responsible for the elimination reactions grow slowly. In addition, the operational control of aerobic and anaerobic conditions needed for nitrification and denitrification, respectively, can be difficult. To cope with these problems, various kinds of bioreactors have been studied for enhancing the efficiency of nitrogen removal. Examples of enhanced processes include the combined nitrification and denitrification (Kuenen and Robertson, 1994); immobilization of bacteria on polymeric gel beads in a moving bed biofilm reactor (Hem et al., 1994); and the formation of bacterial film on the surface of rotating disks or other packing in a moving bed biofilm reactor or aeration tank (Klees and Silverstein, 1992; Rusten et al., 1992). Unfortunately, these enhanced processes have generally not performed well when faced with wastewaters containing a high concentration of nitrogen. Variously, poor performance has been ascribed to the low nitrification and denitrification rates, low stability of immobilized bacteria and insufficient/unavailable carbon source for denitrification.

To overcome existing limitations, several novel nitrogen removal processes have been developed, including the SHARON process, the ANAMMOX process, the combined SHARON and ANAMMOX process and CANON process. This review is focused on these novel treatment technologies for nitrogen removal. For comparison, the conventional nitrification and denitrification technologies are also discussed briefly.

## 2. Conventional nitrification and denitrification

Conventional microbial nitrogen removal is based on autotrophic nitrification and heterotrophic denitrification. The removal involves aerobic nitrification (i.e., the conversion of $NH_4^+$ to $NO_2^-$ and further to $NO_3^-$) with molecular oxygen as the electron acceptor. The relevant reactions are as follows:

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

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2)$$

The anoxic denitrification (i.e., the conversion of $NO_3^-$ and $NO_2^-$ to gaseous nitrogen) is accomplished with a variety of electron donors, including methanol, acetate, ethanol, lactate and glucose (Grabinska-Loniewska, 1991; Tam et al., 1992; Akunna et al., 1993). The anoxic denitrification involves the following reactions:

$$2NO_3^- + 10H^+ + 10e^- \rightarrow N_2 + 2OH^- + 4H_2O \quad (3)$$

$$2NO_2^- + 6H^+ + 6e^- \rightarrow N_2 + 2OH^- + 2H_2O \quad (4)$$

As nitrification and denitrification are carried out under different conditions and by different microorganisms, experience shows that these processes have to be separated in

time or space to function effectively. The conventional nitrification/denitrification reactions have been known for a long time (Winogradsky, 1890; Beijerinck and Minkman, 1910; Kluyver and Donker, 1926). The nitrification reaction consumes a large amount of oxygen, requiring 4.2 g of oxygen for each gram of ammonium nitrogen nitrified (Gujer and Jenkins, 1974; EPA, 1975). During denitrification, the requirement of organic carbon is significant. For example, 2.47 g of methanol is required per gram of nitrate nitrogen for complete denitrification (McCarty et al., 1969). The requirement of added electron donors such as methanol makes full-scale denitrification quite expensive.

A relatively low-cost electron donor methane is commonly used for denitrification in the presence of oxygen (Davies, 1973; Sollo and Mueller, 1976; Werner and Kayser 1991; Thalasso et al., 1997; Costa et al., 2000; Lee et al., 2001). Methane is generally readily available in large amounts in wastewater treatment facilities through the anaerobic digestion of sludge. Denitrification with methane is brought about by the methanotrophic/methylotrophic association. Methanotrophs are strict aerobes and are capable of growth only on methane. An association of methanotrophs oxidizes methane to carbon dioxide and water (Mechsner and Hamer, 1985). This process does not denitrify per se but produces organic intermediate compounds under suitable environmental conditions (Megraw and Knowles, 1989; Roy and Knowles, 1994; Amaral and Knowles, 1995). It is these organic intermediates that serve as the carbon source for aerobic or anoxic denitrifying bacteria (Rhee and Fuhs, 1978; Mechsner and Hamer, 1985; Werner and Kayser, 1991; Thalasso et al., 1997; Costa et al., 2000; Lee et al., 2001). Methanol, formaldehyde and formate are the major known intermediate metabolism substrates of methane oxidation by methanotrophs (Hanson and Hanson, 1996). Other intermediate carbon compounds are also produced and excreted by the methanotrophs. These compounds include citrate (Rhee and Fuhs, 1978), methanol (Mechsner and Hamer, 1985), polysaccharides and proteins (Linton and Buckee, 1977; Ivanova and Nesterov, 1988; Mshenskii et al., 1988; Nesterov et al., 1988) and acetate (Costa et al., 2000). Unfortunately, although denitrification with methane is possible, it is quite slow (Werner and Kayser, 1991).

Because the organic carbon present naturally in the wastewater is quite limited, the complete removal of nitrogen from wastewaters that contain a high nitrogen concentration requires a large amount of an added carbon source for denitrification (van Dongen et al., 2001). Furthermore, most existing wastewater treatment facilities were not designed for nitrogen removal, and meeting the demands of the nitrification/denitrification steps in these facilities can be difficult. Thus, many wastewater treatment plants do not meet the current discharge standard of 10 mg N/l (Jetten et al., 2002). This was what drove the development of the new low-cost biotreatments for nitrogen-rich wastewaters.

## 3. Novel biological technologies for nitrogen removal

### *3.1. SHARON process*

The SHARON process (single reactor system for high ammonia removal over nitrite process) is a new process for biological nitrification. This process is operated without any biomass retention in a single aerated reactor at a relatively high temperature (35 °C) and

pH (above 7) (Brouwer et al., 1996; Hellinga et al., 1997). The process involves partial nitrification of ammonium to nitrite, and this greatly reduces the expense of aeration. The SHARON process can be carried out in a simple continuous stirred tank reactor (Hellinga et al., 1998) and is ideally suited to removing nitrogen from waste stream with a high ammonium concentration (>0.5 g N/l) (Jetten et al., 1997; van Dongen et al., 2001). This process was developed at the Technical University Delft, the Netherlands (Hellinga et al., 1998), and full-scale experience has recently been gained in its operation (Mulder et al., 2001; van Kempen et al., 2001). SHARON is the first successful process in which nitrification/denitrification with nitrite as an intermediate has been achieved under stable conditions (van Kempen et al., 2001). The possible metabolic pathways for nitrification and denitrification are shown in Fig. 1.

To obtain the stable partial nitrification, the operating variables (temperature, pH, hydraulic retention time, substrate concentration, dissolved oxygen) are controlled in a chemostat operation (Beccari et al., 1979; Randall and Buth, 1984; Hellinga et al., 1998). Unfortunately, control of these process variables can be difficult in large-scale operations (STOWA, 1995).

Hunik (1993) reported that the ammonium oxidizers grow faster than the nitrite oxidizers at elevated temperatures (>15 °C). At the operational temperature of 35 °C, the maximum specific growth rate of nitrite oxidizers is approximately only half of that for the ammonium oxidizers (0.5 and 1 $day^{-1}$, respectively) (Hunik 1993). Only at temperatures above 25 °C is it possible for the ammonium oxidizers to effectively outcompete the nitrite oxidizers (Brouwer et al., 1996). The ammonium oxidizers have a shorter minimum required sludge age at temperatures of >20 °C. The sludge retention age of course can be controlled by the hydraulic retention time. When faced with a short hydraulic retention time, the nitrite oxidizers are selectively washed out (Hellinga et al., 1998).

The oxidation of ammonium is an acidifying process. Therefore, the control of pH is important for preventing process inhibition (van Kempen et al., 2001). The nitrite oxidizers

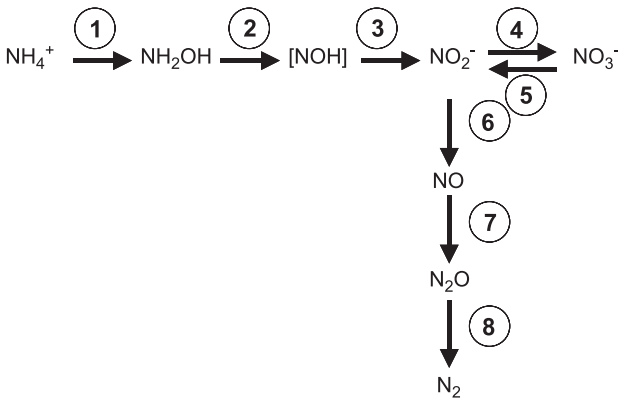


Fig. 1. Possible metabolic pathway for nitrification and denitrification. During nitrification, ammonium is oxidized to hydroxylamine (step 1). Hydroxylamine is oxidized to nitrite (steps 2 and 3). Nitrite is converted to nitrate (step 4). During denitrification, nitrate is reduced to nitrite (step 5) which is converted to gaseous NO, $N_2O$ and $N_2$ (steps 6, 7 and 8).

are particularly susceptible to a changing pH (Anthonisen et al., 1976; Truk and Mavinic, 1989; Abeling and Seyfried, 1992). When the pH drops below 6.5, the ammonium oxidation will no longer take place because of a pH-dependent equilibrium between the concentrations of $NH_3$ and $NH_4^+$. When pH drops too low, the free ammonium concentration becomes too low for sufficient growth of the ammonium oxidizers. Although the nitrite oxidizers do grow faster than the ammonium oxidizers at low pH values, the opposite is the case at high pH values. Therefore, a high pH is preferred for obtaining an effluent that is low in $NH_4^+$ concentration (Hellinga et al., 1998). Above pH 8, nitrification also declines. This is because too much $NH_3$ is apparently toxic for the nitrite oxidizers in this process (Anthonisen et al., 1976). The ammonium/nitrite ratio in the effluent of the SHARON process can be sensitively influenced by changing the reactor pH between 6.5 and 7.5 (van Dongen et al., 2001). Typically, for the sludge liquor the ratio of $HCO_3^-/NH_4^+$ is 1.1:1 (Hellinga et al., 1998), and consequently, about half of the ammonium in the liquor can be converted without any pH control, and this depletes the alkalinity of water. This leads to a pH drop and prevents further nitrification (Jetten et al., 2002).

The nitrite oxidizers have a lower affinity for oxygen than the ammonium oxidizers (Hunik, 1993; Picioreanu et al., 1997); therefore, a low DO concentration is restrictive for the growth of nitrite oxidizers (Truk and Mavinic, 1989; Hanaki et al., 1990; Laanbroek and Gerards, 1993). Depending on the aerobic retention time, different concentrations of ammonium are achieved in the effluent (van Kempen et al., 2001). The ammonium oxidizers have a low affinity for ammonium (affinity constant 20–40 mg $NH_4^+$ N/l). In addition, $HNO_2$ inhibits the ammonium oxidizers, but they can tolerate high concentrations of nitrite (>0.5 g $NO_2^-$ N/l) at pH 7 (Jetten et al., 1997; van Dongen et al., 2001).

Of the various processes, the SHARON process appears to be the most practicable for substantially reducing the concentration of ammonium in wastewater that is relatively high in ammonium content. This can be achieved so long as operations are carried out at an elevated temperature and pH. A nitrogen removal efficiency of 90% can be achieved (van Kempen et al., 2001). The process requires relatively little initial investment because a simple well-mixed tank reactor of modest dimensions without sludge retention is sufficient (Hellinga et al., 1998). The process does not produce chemical sludge and has a relatively low production of biological sludge. It requires relatively little oxygen because the oxidation is sopped at the nitrite stage, and this saves on energy and the added carbon source. Compared to the traditional nitrification and denitrification via nitrate, the SHARON process demands 25% less aeration energy and 40% less added carbon.

### *3.2. ANAMMOX process*

In the past, oxidation of ammonium was known to be caused by ammonium oxidizers under aerobic and oxygen-limited conditions. Schmidt and Bock (1997) reported that ammonium was able to oxidized by ammonium oxidizers under anoxic conditions when gaseous nitrogen dioxide ($NO_2$) was present. Ammonium oxidizers, however, can denitrify with ammonium as the electron donor under oxygen-limited conditions (Goreau et al., 1980; Kuai and Verstraete, 1998). Denitrification under anoxic conditions occurs with hydrogen or organic compounds acting as electron donors (Bock et al., 1995; Mulder et al., 1995).

Mulder et al. (1995) discovered the anaerobic ammonium oxidation (ANAMMOX) in a laboratory-scale anaerobic fluidized denitrifying bed used in treating effluent from a methanogenic rector. Large quantities of ammonium were observed to disappear, while nitrate consumption and the dinitrogen gas production were simultaneously elevated (Eq. (5)) (Mulder et al., 1995). Later, van de Graaf et al. (1995) and Bock et al. (1995) observed that nitrite was the preferred electron acceptor for the process (Eq. (6)). Thus,

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+ \quad (5)$$

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

The main product of anaerobic ammonium oxidation was $N_2$, but about 10% of the N-feed (nitrite and ammonium) was converted to $NO_3^-$. The overall nitrogen balance (Eq. (7)) gave a ratio of $NH_4^+$ conversion to $NO_2^-$ conversion of 1:1.31 ± 0.06 (van de Graaf et al., 1996). The ratio of $NO_2^-$ conversion to $NO_3^-$ production was 1:0.22 ± 0.02 (van de Graaf et al., 1996); thus,

$$NH_4^+ + 1.31NO_2^- + 0.0425CO_2 \rightarrow 1.045N_2 + 0.22NO_3^- + 1.87H_2O + 0.09OH^- + 0.0425\underset{\text{biomass}}{CH_2O} \quad (7)$$

Strous et al. (1998) estimated the ANAMMOX stoichiometry based on mass balance over ANAMMOX enrichment cultures, as presented in Eq. (8):

$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (8)$$

The ANAMMOX process is a promising new way of removing nitrogen from wastewater (Jetten et al., 1997; Strous et al., 1997a,b). The possible metabolic pathways for anaerobic ammonium oxidation are shown in Fig. 2 (van de Graaf et al., 1997). The ANAMMOX process is based on energy conservation from anaerobic ammonium oxidation with nitrite as electron acceptor without addition of external carbon source (Jetten et al., 1999). Hydrazine and hydroxylamine are known to be some intermediates of the process (van de Graaf et al., 1997; Schalk et al., 1998; Jetten et al., 1999). Carbon dioxide is the main carbon source for the growth of ANAMMOX bacteria (van de Graaf et al., 1996).

Bacteria capable of anaerobically oxidizing ammonium had not been known earlier and were referred to as the "lithotrophs missing from nature." These missing lithotrophs were discovered and identified as the new autotrophic members of the order of *Planctomycete*, one of the major distinct divisions of bacteria (Strous et al., 1999a). The anaerobic ammonium oxidation reaction is carried out by two ANAMMOX bacteria that have been tentatively named as "*Brocadia anammoxidans*" (Strous et al., 1999a) and "*Kuenenia stuttgartiensis*" (Schmid et al., 2000). The former bacterium was observed in the Netherlands (Strous et al., 1999a), while the latter was found in several wastewater treatment facilities in Germany and Switzerland (Schmid et al., 2000; Egli et al., 2001). 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

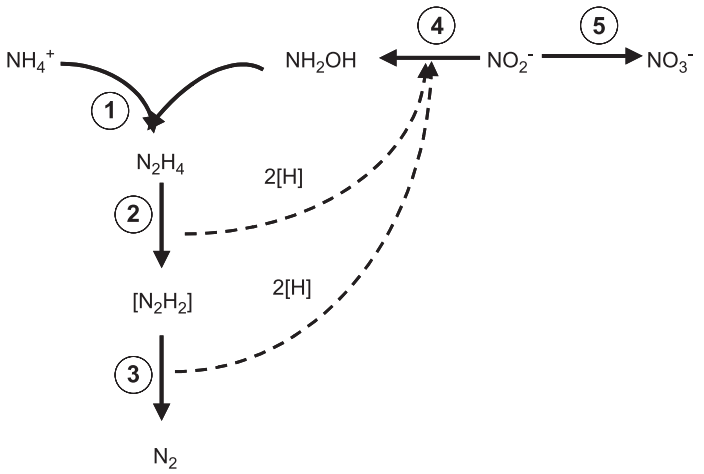


Fig. 2. Possible metabolic pathway for anaerobic ammonium oxidation. Consumption and production of $H_2O$ and $H^+$ are not shown. Ammonium is oxidized through hydroxylamine to hydrazine (step 1). Reducing equivalents derived from $N_2H_4$ then reduce nitrite to more hydroxylamine and nitrogen gas (steps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

for both bacteria in a pH range between 6.4 and 8.3 and a temperature between 20 °C and 43 °C (Strous et al., 1999b; Egli et al., 2001). The optimum pH and temperature of the two organisms are very similar. The highest ANAMMOX activity for *K. stuttgartiensis* was 26.5 nmol $N_2$/mg protein·min at pH 8 and 37 °C (Egli et al., 2001). This activity is lower than the maximum ANAMMOX activity of *B. anammoxidans* (55 nmol $N_2$/mg protein·min at pH 8 and 40 °C; Jetten et al., 1999). However, *K. stuttgartiensis* has a higher tolerance to nitrite, is more active in low cell density cultures and is less inhibited by phosphate compared to *B. anammoxidans* (Egli et al., 2001). The growth rates (doubling time 11 days) and growth yields (0.11 g VSS/g $NH_4N$) of both bacteria are extremely low.

The ANAMMOX bacterial activity is 25-fold higher than aerobic nitrifying bacterial oxidation of ammonium under anoxic conditions when using nitrite as the electron acceptor (Jetten et al., 1999). Anaerobic ammonium oxidation is more than seven times slower than aerobic ammonia oxidation (Strous et al., 1998). ANAMMOX activity has been inactivated by gamma irradiation, heating of the pilot plant sludge and incubation with various inhibitors (Jetten et al., 1999). Acetylene, phosphate and oxygen strongly inhibit ANAMMOX activity (van de Graaf et al., 1996). ANAMMOX bacteria are very sensitive to oxygen and nitrite. Oxygen concentration as low as 2 μM and nitrite concentrations between 5 and 10 mM inhibit the ANAMMOX activity completely but reversibly (Jetten et al., 2001).

The process has a good potential for ammonium removal from sludge digestion effluent. Fixed bed and fluidized bed reactors are suitable reactor configurations for the ANAMMOX process (Strous et al., 1997a). The ANAMMOX process has also been maintained easily in a gas lift reactor. Nitrogen removal rates of up to 8.9 kg $N/m^3$·day were achieved. This removal rate was 20 times higher compared to the removal rates previously achieved in the laboratory (Sliekers et al., 2003). The ANAMMOX process

requires no COD (organic carbon chemical oxygen demand) addition to support denitrification (van de Graaf et al., 1996). Furthermore, 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 oxygen demand in the nitrification reactor and reduce cost. The biomass yield is very low, and consequently, little sludge is produced. This is another factor that contributes to a substantially lower operation cost of ANAMMOX compared to the conventional denitrification process. However, the low biomass yield also necessitates an efficient system for sludge retention, and long start-up times are required to obtain a sufficient biomass concentration (Jetten et al., 1997).

### 3.3. Combined SHARON and ANAMMOX process

The idea of coupling the SHARON process with ANAMMOX process has been successfully tested in the laboratory on sludge digester effluent (Jetten et al., 1997). The combined SHARON and ANAMMOX process can work stably over long periods, and full-scale implementation is being evaluated for treatment of sludge liquor (van Dongen et al., 2001).

The principle of the combined SHARON and ANAMMOX processes (Fig. 3) is that wastewater containing ammonium is oxidized in the SHARON reactor to nitrite using only 50% of the influent ammonium (Eq. (9)) (Jetten et al., 1997):

$$NH_4^+ + HCO_3 + 0.75O_2 \rightarrow 0.5NH_4^+ + 0.5NO_2^- + CO_2 + 1.5H_2O \quad (9)$$

The effluent from SHARON reactor containing a mixture of ammonium and nitrite is ideally suited as the influent for the ANAMMOX process where ammonium and nitrite are anaerobically converted to dinitrogen gas and water (Eq. (6)) (van Dongen et al., 2001).

The ratio of ammonia and nitrite needed for the ANAMMOX process is about 1. For sludge liquor, this ratio can be achieved without any pH control, because the sludge liquor contains bicarbonate as the counter ion for ammonium. When half of the ammonium in the liquor is converted, the alkalinity of the water is nearly depleted, leading to a pH drop and preventing further nitrification (Jetten et al., 2002).

The combined SHARON and ANAMMOX treatment is suitable for concentrated sludge reject waters (sludge liquor) and industrial wastewaters containing a high

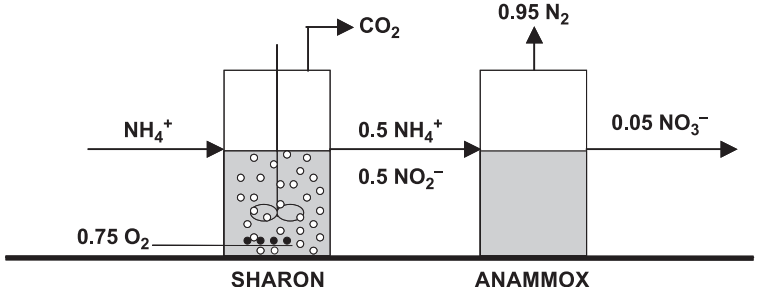


Fig. 3. Schematic presentation of the combined SHARON and ANAMMOX processes.

concentration of ammonia and a low amount of organic carbon. The combined process can usually be engineered in two separate reactors or a single vessel (Dijkman and Strous, 1999). The overall nitrogen removal in the combined process requires less oxygen (1.9 kg $O_2$/kg N instead of 4.6 kg $O_2$/kg N), no carbon source (instead of 2.6 kg BOD/kg N) and has a low sludge production (0.08 instead of approximately 1 kg VSS/kg N) (van Loosdrecht and Jetten, 1998).

The SHARON-ANAMMOX process could greatly contribute to improved wastewater management (van Loosdrecht and Jetten, 1998). Because the combined process does not require the input of COD, the COD and nitrogen removal operations can be optimized separately, eliminating the need for complex compromises between COD and N-removal as in the conventional N-removal process (Jetten et al., 1997; van Dongen et al., 2001). Compared to conventional nitrification/denitrification, the combined system saves 50% on required oxygen, 100% on the external carbon source and reduces $CO_2$ emission by more than 100% (the combined process actually consumes $CO_2$) (van Loosdrecht and Jetten, 1997). Overall, the combined process is 90% less expensive than the conventional processes (Dijkman and Strous, 1999).

### 3.4. CANON process

A high amount of nitrogen loss as elemental nitrogen has been observed from wastewaters that are highly loaded with ammonium and contain low concentrations of organic carbon (Hippen et al., 1997; Helmer and Kunst, 1998; Kuai and Verstraete, 1998; Siegrist et al., 1998; Helmer et al., 1999, 2001; Koch et al., 2000). The microorganisms responsible for this are autotrophic populations that denitrify under low dissolved oxygen conditions. Along similar observations, Dijkman and Strous (1999) described a new biological nitrogen removal process named the CANON process for completely autotrophic nitrogen removal over nitrite. This process removes ammonium from wastewaters containing low amounts of organic materials. The process can be carried out in a single reactor or biofilm under oxygen-limited conditions. This process is based on a partial nitrification and anoxic oxidation of ammonia.

Under oxygen-limited conditions (< 0.5% air saturation) a coculture of aerobic and anaerobic ammonium-oxidizing bacteria can be established (Strous, 2000), and this system is responsible for the CANON activity. The process relies on a stable interaction between the two groups of autotrophic microorganism populations: *Nitrosomonas*-like aerobic bacteria and *Planctomycete*-like anaerobic ammonium-oxidizing bacteria, under oxygen-limited conditions (Third et al., 2001). These autotrophic cultures convert ammonia directly to dinitrogen gas with nitrite as an intermediate. Application of this concept to wastewaters can potentially lead to complete ammonia removal in a single autotrophic reactor. The two groups of microorganisms interact and perform the two sequential reactions simultaneously.

Under oxygen-limited condition, ammonium is oxidized to nitrite by aerobic nitrifiers, such as *Nitrosomonas* and *Nitrososira* (Eq. (10)) (Hanaki et al., 1990):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (10)$$

Subsequently, anaerobic ammonium oxidizers *Planctomycete*-like ANAMMOX bacteria convert ammonium with the produced nitrite to dinitrogen gas and trace amounts of nitrate (Eq. (11)) (Strous, 2000):

$$NH_4^+ + 1.3NO_2^- \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \quad (11)$$

As the nitrite also serves as an electron donor for the formation of biomass from carbon dioxide, the formation of nitrate in the reaction is stoichiometrically coupled to growth. The combination of the above two reactions results in nitrogen removal as follows (Eq. (12)) (Strous, 2000):

$$NH_4^+ + 0.85O_2 \rightarrow 0.435N_2 + 0.13NO_3^- + 1.3H_2O + 1.4H^+ \quad (12)$$

The interaction of aerobic and anaerobic ammonium-oxidizing bacteria under oxygen-limited conditions results in an almost complete conversion of ammonium to dinitrogen gas. Small amounts of nitrate is also produced.

A dissolved oxygen (DO) concentration of up to 0.5 mg/l has no effect on ammonia oxidation, but nitrite oxidation is strongly inhibited in suspended growth reactors (Hanaki et al., 1990). In the oxygen-limited conditions, nitrite oxidizers have to compete for oxygen with the aerobic ammonia oxidizers and for nitrite with anaerobic ammonia oxidizers. Possible inhibition of nitrite oxidizers by free ammonia has been suggested (Abeling and Seyfried, 1992). Considering this, ANAMMOX processes are feasible at low bulk oxygen concentrations. ANAMMOX bacteria are reversibly inhibited by low (0.5% air saturation) concentration of oxygen (Strous et al., 1997b). The combined process (Eq. (12)) can occur under oxygen-limited conditions.

The effect of ammonium limitation in the CANON system was investigated at the laboratory scale in two different reactor types (sequencing batch reactor and chemostat). The lower limit of effective and stable nitrogen removal to dinitrogen gas was 0.1 kg N/$m^3$·day. At this loading rate, 92% of the total nitrogen was removed. If the influx of nitrogen is lower than the critical $NH_4^+$ influx, the stoichiometry of the CANON reaction is affected, and this causes a temporary decrease of nitrogen removal from 92% to 57%.

In studies with a sequencing batch reactor operated with an ammonium-rich wastewater under oxygen-limited conditions at a suitable loading rate with aerobic nitrifying bacteria and ANAMMOX bacteria, a nitrogen removal rate of up to 0.3 kg N/$m^3$·day has been reported for the CANON process. In this reactor, heterotrophic denitrification did not occur, and no aerobic nitrite-oxidizing bacteria were detected (Sliekers et al., 2002). The CANON process has been carried out in gas lift reactors. Nitrogen removal rates up to 1.5 kg N/$m^3$·day were achieved. This removal rate was 20 times higher compared to the removal rates achieved in the laboratory previously (Sliekers et al., 2003). Gaslift reactors are easy to operate stably, and a lot of information has become available for designing them (Chisti, 1989, 1998).

The CANON process is an economic and efficient option for wastewater treatment, especially for wastewaters rich in ammonium but devoid of organic carbon (COD). The CANON process is completely autotrophic and therefore requires no added COD. In addition, the entire nitrogen removal can be achieved in a single reactor with little aeration. This greatly reduces the space and energy requirements. The autotrophic process

Table 1
A comparison of the new processes of nitrogen removal and conventional nitrification/denitrification (Jetten et al., 2002)

| System | Conventional nitrification/ denitrification | SHARON | ANAMMOX | CANON |
|---|---|---|---|---|
| Number of reactors | 2 | 1 | 1 | 1 |
| Feed | wastewater | wastewater | ammonium + nitrite | wastewater |
| Discharge | $NO_2^-$, $NO_3^-$; $N_2$ | $NH_4^+$, $NO_2^-$ | $NO_3^-$, $N_2$ | $NO_3^-$, $N_2$ |
| Conditions | oxic; anoxic | oxic | anoxic | oxygen limited |
| Oxygen requirement | high | low | none | low |
| pH control | yes | none | none | none |
| Biomass retention | none | none | yes | yes |
| COD requirement | yes | none | none | none |
| Sludge production | high | low | low | low |
| Bacteria | nitrifiers + various heterotrophs | aerobic $NH_4^+$ oxidizers | planctomycetes | aerobic $NH_4^+$ oxidizers + planctomycetes |

consumes 63% less oxygen and 100% less reducing agents than does a conventional nitrogen removal process (Kuai and Verstraete, 1998).

## 4. Concluding remarks

As discussed in this review, the newer microbial processes for nitrogen removal offer important advantages compared to the traditional nitrogen removal that is based on autotrophic nitrification and heterotrophic denitrification. A summary comparison of the various processes and the conventional method is presented in Table 1 (Jetten et al., 2002). The new processes—the SHARON, the ANAMMOX, combined SHARON and ANAMMOX and CANON processes—reduce energy demand, the need for added chemicals and produce less sludge in relation to the conventional treatment. These processes represent a significant step forward in the contribution of biotechnology in remediation of nitrogen pollution.

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