Upscaling the Zeolite-Anammox Process: Treatment of Secondary Effluent

Permalink
https://escholarship.org/uc/item/8ns518qh

Journal
WATER, 10(3)

ISSN
2073-4441

Authors
Collison, Robert S
Grismer, Mark E

Publication Date
2018-03-01

DOI
10.3390/w10030236

Peer reviewed
The Zeolite-Anammox Treatment Process for Nitrogen Removal from Wastewater – A Review

M. E. Grismer* and R. S. Collison
Biological & Agricultural Engineering, UC Davis
*Corresponding author: megrismer@ucdavis.edu

Abstract
Water quality in San Francisco Bay has been adversely affected by nitrogen loading from wastewater treatment plants (WWTPs) discharging around the periphery of the Bay. While there is documented use of zeolites and anammox bacteria in removing ammonia and possibly nitrate during wastewater treatment, there is little information available about the combined process. Though relatively large, zeolite beds have a finite ammonium adsorption potential and require periodic re-generation depending on the wastewater nitrogen loading. Use of anammox bacteria reactors for wastewater treatment have shown that ammonium (and to some degree, nitrate) can be successfully removed from the wastewater, but the reactors require careful attention to loading rates and internal redox conditions. Generally, their application has been limited to treatment of high-ammonia strength wastewater at relatively warm temperatures. Moreover, few studies are available describing commercial or full-scale application of these reactors. We briefly review the literature considering use of zeolites or anammox bacteria in wastewater treatment to set the stage for description of an integrated zeolite-anammox process used to remove both ammonium and nitrate without substrate regeneration from mainstream WWTP effluent or anaerobic digester filtrate at ambient temperatures.

Keywords: anammox bacteria, wastewater treatment, nitrification, denitrification, zeolite

Introduction
As with many estuaries associated with population centers around the world, San Francisco Bay (SFB) water quality is adversely affected by nitrogen and phosphorous inputs from multiple anthropogenic sources, the greatest being nitrogen loads from wastewater treatment plant (WWTP) discharges on the Bay periphery. Nitrogenous waste (consisting primarily of ammonia and/or nitrate) is of particular concern in SFB, especially in the more shallow reaches subject to tidal flooding/draining
processes. Ammonia is directly toxic to fish and marine life, while nitrate stimulates algal growth that depletes dissolved oxygen (DO) levels at night resulting in suffocation of oxygen-breathing organisms. While, SFB has shown some resistance to the classic symptoms of nutrient over-enrichment, recent observations suggest that SFB's resistance to nutrient enrichment is weakening. It appears that SFB may be trending toward, or already experiencing, adverse impacts due to high nutrient loads, thereby requiring greater regulation of WWTP nitrogen loading to the Bay (SFEI, 2016). Thus, discharge permitting at WWTPs may require greater removal of both reduced and oxidized nitrogen species. This review considers the development of zeolite and anammox domestic wastewater treatment methods during the past two decades to set the stage for possible commercial development of the integrated zeolite-anammox treatment process capable of transforming WWTP effluent nitrogen loads to nitrogen gas prior to effluent disposal.

"Traditional" nitrogen removal in WWTPs rely on a two-step treatment process of nitrification and denitrification. The nitrification process employs nitrifying bacteria to oxidize ammonia to nitrate using available dissolved oxygen, while denitrification uses denitrifying bacteria to reduce the nitrate to nitrogen gas. Nitrification occurs only under aerobic conditions at dissolved oxygen (DO) concentrations of >1.0 mg/L where Nitrosomonas-type bacteria convert ammonium to nitrite; then Nitrobacter-type bacteria convert nitrite to nitrate. Nitrification is sensitive to inhibition by high organic concentrations because of bacterial competition and is typically represented by the equation;

\[
\text{NH}_4^+ + 2.5\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}_2\text{O}.
\]

Denitrification is an anaerobic process occurring at DO levels <0.5 mg/L where facultative heterotrophic bacteria reduce nitrate to nitrogen gas that volatilizes to the atmosphere. It requires a carbon source as an electron donor, uses nitrate as an electron acceptor and is represented by the simplified equation;

\[
\text{NO}_3^- + \text{CH}_3\text{N} \rightarrow \text{N}_2(g) + \text{CO}_2(g) + \text{H}_2\text{O}.
\]
During the past two decades, new approaches to nitrogen treatment methods have developed in the laboratory and some tested in pilot-scale treatment plants; two of the more promising methods include use of zeolite aggregates and anammox bacteria. Zeolites are a relatively commonly found deposit around the world whose aggregates have relatively low density, some internal porosity and unusually large cation-exchange capacity (CEC) for the type of mineral. Some research has explored use of the zeolite aggregates as an ammonium adsorption substrate. Anammox bacteria were discovered in WWTP anaerobic digesters and in several marine environments. They were key towards closing nitrogen balance estimates in WWTP and estuary-marine studies and found to readily convert ammonia ions using nitrite to nitrogen gas. Anammox bacteria prefer anaerobic environments and are relatively slow growing; some ten times slower than nitrifiers for example. Presumably, anammox bacteria congregate at aerobic-anaerobic interfaces where they can combine available nitrite and ammonia to form nitrogen gas with some residual nitrate following the reaction (Paredes, 2007):

$$\text{NH}_4^+ + 1.32 \text{NO}_2^- + 0.066 \text{HCO}_3^- + 0.13 \text{H}^+ \rightarrow 1.02 \text{N}_2(\text{g}) + 0.26 \text{NO}_3^- + 2.03 \text{H}_2\text{O} + 0.066 \text{CH}_2\text{O}_{0.03}\text{N}_{0.15}$$

As anammox bacteria are capable of direct conversion of oxidized and reduced forms of nitrogen in WWTP discharge to nitrogen gas with little sludge production, they provide an interesting opportunity to reduce WWTP nitrogen loads to sensitive receiving waters; however, there are only limited reports of commercial application of this integrated process.

**Literature Review**

This literature review considers the wastewater treatment aspects associated with use of zeolite aggregate as a reactor substrate and cultivation of anammox bacteria for transformation of dissolved aqueous nitrogen species (i.e. nitrate, nitrite and ammonia) found in WWTP discharge to nitrogen gas thereby reducing nitrogen loading to receiving waters. We direct this review towards increasing the
development and evaluation of zeolite-anammox treatment systems for commercial-scale applications to improve receiving water quality wherever adversely impacted by WWTP discharges.

Zeolites & Wastewater treatment

In the late 1950's, enormous beds of zeolite-rich sediments, formed by the alteration of volcanic ash in lake and marine waters, were discovered in the western United States and elsewhere around the world notably in Australia, Canada, China, South America and Turkey, (Mumpton, 1999). Zeolites are characterized by extensive internal porosity, very large surface areas (i.e. both internal and external), and correspondingly high CECs (Bowman, 2003). Zeolites are classified as inclusion compounds of hydrated aluminosilicates having three-dimensional tetrahedral networks of SiO$_4$ and AlO$_4$, linked by the shared oxygen atoms (Rehakova et al., 2004). Partial substitution of Al$^{3+}$ for Si$^{4+}$ results in excess negative charge offset by alkali and earth alkaline cations. These cations, along with the water molecules, are located in cavities and channels inside the aluminosilicate macro-anion framework enabling zeolites to function as effective natural ion exchangers. During the past 20 years, there has been a substantial amount of research and application of natural zeolites in environmental remediation schemes that capitalize on their ready availability and ion-exchange properties (Misaelides, 2011).

Several proposed wastewater treatment methods exploit the ammonium adsorption abilities of zeolites across a range of scales, from commercial WWTPs to development of patents for modified septic systems using zeolites (e.g. Rose, 2003). Wang and Peng (2010) reviewed studies of natural zeolites from around the world and found varying ion-exchange capacity for ammonium, some anions and organics, and heavy metal ions. Of the 21 zeolites considered, 18 were clinoptilolites with SiO$_2$ and Al$_2$O$_3$ fractions that ranged from 56-71% and 7.5-15.8%, respectively, while CECs ranged from 0.6-2.3 meq/mg. Similarly, at temperatures ranging from 20-70 C (when reported), the corresponding ammonium adsorption capacities of the different clinoptilolites ranged from 23-3 mg/g with higher values reported using Canadian forms while the USA-derived clinoptilolite value reported was 18.5 mg/g. Widiastuti et al
(2008 & 2011) studied use of Australian zeolite for greywater treatment and similar to that reported by others found zeolite ammonium removal capacity increases with increasing initial ammonium concentration (e.g. Sarioglu, 2005), presumably as a result of greater aqueous to adsorbed phase concentration gradients. It appears that the ammonium ions can migrate from the external surface to the internal micro-pores of the zeolite within a given contact time. Several studies indicated that the adsorption or ion-exchange process is quite rapid and can be modeled by typical Langmuir and Freundlich isotherms (e.g. Rozic et al., 2000; Du et al., 2005; Englert and Rubio, 2005; and Motsi et al., 2009). Solution pH affected ammonium removal efficiency by the zeolite as well because the nitrogen dissociation form (NH$_3$ or NH$_4^+$) depends on pH. For example, ammonium removal efficiency from a 50 mg/L NH$_4^+$ solution increased as pH increased from 2 to 5 peaking at about pH 5 and declining thereafter. Similarly, Jorgensen et al. (1976) found that zeolite was more selective at pH 5. Conversely, Du et al. (2005) reported that an optimal ammonium removal efficiency was achieved at pH 6 while Ji, Z-Y et al. (2007) using Ca$^{2+}$-formed clinoptilolite found a maximum adsorption capacity of 82% at pH 7 and Saltali et al. (2007) reported 75% ammonium removal at pH 7 and nearly 79% at pH 8 for Turkish (Yildizeli) zeolite. Together with Karadag et al. (2006), Ji et al. (2007) and Saltali et al. (2007) found the adsorption process to be exothermic and removal efficiency improved with decreasing temperatures. Studies have also considered the influence of other ions or compounds in solution on ammonium uptake by zeolites. Jorgensen and Weatherley (2003) found that in most cases studied, the presence of organic compounds enhanced ammonium ion uptake. Similarly, considering adsorption from aqueous solutions having ammonium concentrations of 0–200 mg/L in the presence of Ca, K, Mg and Cl ions, Weatherley and Miladinovic (2004) found only minor changes on ammonium uptake by mordenite and clinoptilolite. This was a rather unexpected result since most other work to date had shown clinoptilolite exhibiting a greater affinity for potassium as compared to the ammonium ion. Calcium ions in solution had the greatest effect upon ammonium ion uptake, followed by potassium ions while magnesium ions had the
least effect. Most studies considering zeolite ion-exchange properties were conducted using laboratory-scale reactors with controlled environments, though some work has involved larger-scale applications in wastewater treatment.

Misaelides (2011) noted in a short review that in addition to the ion-exchange properties of zeolites, zeolite aggregates demonstrated the ability to harbor bacteria that can increase sludge activity in WWTPs. The apparent drawback of this use was the slow formation of the bacteria layer on the zeolite surface, which does not become immediately effective, requiring bacterial growth establishment times of 1-2 weeks in the digesters. The modification of zeolites by cation-active polyelectrolytes accelerated the interaction among the bacteria with the zeolite surface further increasing the sludge activity. By 2011, zeolite was recognized for its high CEC and for its ability to preferentially remove ammonium ions from wastewater. Use of zeolite for ammonium removal increased because of its wide availability and low-costs where available, and because ammonium-saturated zeolite can be relatively easily regenerated and re-used. High-strength brine was traditionally the preferred method of regeneration (Ji, 2007), but concerns about high levels of dissolved solids in the spent regenerant liquor led to development of other methods. An electrochemical method of regeneration was also established and used in several applications (Lei, 2009). One of the more promising methods explored more recently, however, is biological regeneration using microbial action to strip the ammonium from the cation exchange sites.

There are few commercial scale applications of zeolite adsorption reactors to remove ammonium from wastewater. Facing strict regulations associated with treated wastewater disposal to a pristine river, the Truckee Sanitation District deployed a zeolite reactor to remove residual ammonium prior to discharge. Using a relatively short contact time of several hours, the zeolite reactor successfully removed the ammonium from the treated wastewater. However, the zeolite reactor required near daily regeneration using saline water that eventually was disposed with the treated wastewater.
Unfortunately, the regenerant addition to the discharge stream increased the salinity beyond acceptable disposal levels to the river and the reactor was decommissioned.

Early discovery of biological regeneration of zeolite by nitrifying bacteria by researchers in Israel (Green, 1996; and Lahav, 1998) suggested a two-stage process where brine removed ammonium from zeolite, followed by brine regeneration using nitrifying bacteria. Later processes exploited the ability of these bacteria to strip the ammonium from the zeolite, thereby simplifying the process (Jung, 2004). In Norway, “zeolite containing expanded clay aggregate filter media” was used to remove ammonia from domestic wastewater by a combination of nitrification and ion exchange. No chemical regeneration was necessary in addition to the biological regeneration during the four-month experimental period (Gisvold, 2000). Zeolites used for stripping ammonium in reactors are typically sand-sized aggregates combining relatively large exterior surface area with ease of handling. The bacteria presumably could not strip ammonium from exchange sites within the zeolite aggregates since their cells are approximately 1000 times larger than the pores formed by the zeolite lattice structure. Nitrifying biofilm-enhanced zeolite also appears to provide a dampening effect on shocks to digesters associated with peak or variable loads (Inan, 2005; McVeigh, 1999; Hedstrom, 2001). Such early studies considering nitrifying bacteria combined with older knowledge about anammox bacteria found in marine environments led to the possibility of combining these processes with zeolites to enhance nitrogen removal rates from domestic wastewater.

Anammox & Wastewater treatment

As nitrogen removal processes and models were refined, WWTP operators and marine environment researchers became aware that nitrogen mass-balance “errors” indicated an unexplained nitrogen loss. Though existence of microorganisms capable of anaerobic ammonium oxidation using nitrite or nitrate as the electron acceptor was predicted in the 1970s (Jetten, 2009), they were not discovered until around 1992 in a WWTP in Delft, The Netherlands (Jetten, 1999; Sliekers, 2002;
Dalsgaard et al., 2005), when they were named “anaerobic ammonium oxidation” or “anammox” bacteria. At the same time, the importance of anammox bacteria towards nitrogen cycling in the marine environment was well understood and researchers explored isolation of these bacteria from freshwater and marine environments for other applications. However, it was difficult to isolate this process in the laboratory until Mulder et al. (1995) developed laboratory denitrifying fluidized-bed reactors capable of removing nitrogen under anaerobic conditions. As anaerobic autotrophs, it remains difficult to isolate and raise pure cultures of anammox bacteria in the laboratory; DNA-sequencing of the bacteria is largely limited to university and research institute laboratories. However, study of highly enriched cultures obtained from WWTP anaerobic digesters has enabled some understanding of the bacterial cell biology and biochemistry (Dalsgaard et al., 2005). By 2005, the three genera of anammox bacteria described were quite small (<1 µm) and all shared a similar cellular structure that includes a membrane-bound compartment, known as the anammoxosome, where the anammox process is believed to occur. This membrane is composed of ladderane lipids in part that form a tight proton diffusion barrier, thereby enhancing ATP production within the cell. By 2010, Bae et al. (2010) using PCR (polymerase chain reaction) methods identified six anammox genera in activated sludges taken from WWTPs; three freshwater, two marine environment and one mixed species are also generally acknowledged. With discovery of more species and habitats, we anticipate that more versatile species will be identified, but their overall diversity remains relatively unknown (Jetten, 2009). Though surprisingly widespread, anammox bacteria discovered within each ecosystem appear to be dominated by a single anammox genus, indicating specialization for distinct ecological niches (Boumann, 2009; Kartal, 2007b). Some have speculated that up to 50% of atmospheric nitrogen is a result of widespread anammox activity (see Mansell, 2011).

Employment of anammox bacteria can revolutionize domestic wastewater treatment because of their ability to simplify removal of nitrogenous waste at significantly lower costs and with less sludge
production than that of conventional WWTP nitrification-denitrification processes. Liu and Ni (2015) among others (Jetten et al., 2005) consider the anammox process "as one of the most sustainable alternatives to the conventional costly nitrification-denitrification biological nitrogen removal process" in wastewater treatment, particularly for high nitrogen low BOD wastewater streams. The autotrophic anammox process directly oxidizes ammonium to nitrogen gas utilizing nitrite as the electron acceptor without the need for an organic carbon source as required by heterotrophic denitrification processes (Hao & van Loosdrecht, 2004). Further, oxygen demand is reduced as the ammonium is only required to be nitrified to nitrite instead of nitrate (Hao et al., 2005). As a result, anammox bacterial biomass yield is very low, creating a small amount of excess sludge production and thus lower operational costs (Strous et al., 1997; and Ni et al., 2012). Overall, the anammox process can reduce oxygen and exogenous carbon source demand by 64% and 100%, respectively, while reducing sludge production by 80–90% as compared to conventional WWTP nitrogen removal processes (Bi et al., 2014).

At this point, there are numerous anammox pilot plants currently operating or under construction, however, anammox processes at these plants are limited to treatment of high-ammonium strength wastewater (500 to 3000 mg/L) and operated at relatively warm temperatures (30-40 C), though marine anammox are known to function at much cooler temperatures (10-15 C).

Relatively slow growth rates of anammox are seemingly linked to the environments from which they were obtained (Dalsgaard et al., 2005). For example, anammox exhibit bacterial growth doubling times of about 9-12 days under optimal temperature conditions associated with their origin (Li, 2009); that is, about 37 C for those cultures obtained from wastewater treatment plants while those from cooler anoxic marine environments prefer 12-15 C. This slow growth rate has limited commercial applications using anammox bacteria at WWTPs (Liu and Ni, 2015). Anammox bacterial growth can be very sensitive to WWTP operational conditions such as dissolved oxygen, temperature, pH and organic matter content thereby requiring considerable direct management or manipulation at the WWTP. While
originally thought that nitrate was the oxidant for ammonium by anammox bacteria, nitrogen-isotope labeling experiments confirmed that the bacteria are using the nitrite form where presumably nitrate-reducing bacteria in the environment are converting the nitrate to nitrite prior anammox conversion to $N_2$ gas. As denitrifying bacteria have much greater growth rates as a competitive advantage over anammox bacteria, the presence of oxygen drastically inhibits the anammox process, though the inhibition process appears to be reversible and the anammox process resumes when anoxic conditions are restored. On the other hand, addition of reduced forms of manganese or iron, as an essential substrate for anammox bacteria, can facilitate growth of anammox bacteria (Liu and Ni, 2015), and such additions have been used for culturing anammox sludge (Van de Graaf et al., 1996).

Another important process in possible WWTP applications is linked to anammox ability for dissimilatory nitrate reduction to ammonium (DNRA). This is a microbiologically mediated pathway transforming nitrate to ammonium and traditionally thought to be involved with fermentation or sulfur oxidation (Burgin, 2007) and is a critical process (Giblin et al., 2013) in nitrogen cycling at coastal marine environments. Recently at least one genus of anammox bacteria appears capable of DNRA, even in the presence of 10 mM ammonium (Kartal, 2007a; Francis, 2007). It now appears that through DNRA anammox bacteria can also produce nitrogen gas from nitrate, even in the absence of a carbon source (organic or inorganic). Figure 1, taken from Giblin et al. (2013), summarizes the key nitrogen transformation processes associated with DRNA as well as the likely associated enzymes.
Figure 1. Nitrogen cycle pathways important to the DNRA process and some of the enzymes known to be involved (taken from Giblin et al., 2013). Nap = Periplasmic nitrate reductase. Nrf = Cytochrome C nitrite reductase. NosZ = Nitrous oxide reductase.

Wastewater Treatment Systems using Anammox

Although anammox bacteria exist in the nitrification/denitrification "environment" of conventional WWTPs, they seem constrained to micro-sites and are of marginal importance; the slow-growing anammox bacteria are likely out-competed by the faster-growing organo-heterotrophs. The anammox process is primarily anaerobic, though in the absence of DRNA process, enough oxygen must be present to create the nitrite needed to react with NH$_4^+$-N to form N$_2$ gas. Originally thought to be inhibited by organic matter, some anammox species are less inhibited by carbon (Trimmer, 2003; Sabumon, 2007) and some of the most recently discovered species flourish when organic matter is present. Kindaichi (2008) postulated that anammox was inhibited by COD; but probably a result of species, pH, temperature, type of carbon, and C:N ratio. Molinuevo’s work appeared to indicate that organic matter at high COD concentrations (100 to 250 mg COD/L) negatively affected the anammox process and facilitated heterotrophic denitrification, but at COD concentrations <100 mg/L, anammox
bacteria successfully converted ammonium to nitrogen gas suggesting that anammox removal of nitrogen of already treated wastewater having low COD is quite possible. Dong (2003) considered anaerobic digestion of poultry manure and detected active anammox bacteria but determined they were unable to effectively compete with denitrifiers at high CODs (between 2200 and 5400 mg/L COD). Sensitivity to organic matter may be related to the C:N ratio, and wastewater with a BOD₅/N <1.0 appears to be suitable for anammox treatment. Furukawa (2009) successfully treated wastewater having concentrations of 600-800 mg/L BOD, 500-700 mg/L TN, 30-70mg/L NH₄-N and 4000-4500 mg/L COD. Subsequently, anammox bacteria were found to be much more flexible and capable of competing for organic compounds and nitrate in the environment (Kartal, 2007a), and may be mixotrophic (Guven, 2005). For example, Kartal (2007b) reported that anammox bacteria could use organic acids as electron donors to reduce nitrate and nitrite, and then successfully compete with denitrifiers for use of these compounds. There are also examples of denitrifying bacteria and anammox bacteria existing in dynamic equilibrium to achieve simultaneous nitrogen and COD removal in anaerobic systems (Chen, 2009).

Other research has indicated that anammox bacteria usually find specialized niche environments though their growth can be inhibited by compounds such as acetylene, phosphate, oxygen, methanol, sulfide at concentrations greater than 1mM, and organic matter combined with high nitrite concentrations (Graaf, 1996; Guven, 2005; Molinuevo, 2009). There is some research directed at overcoming the relatively slow growth rates of anammox that can delay the full treatment capability of larger-scale systems. Several studies (Liu and Bi, 2015, Qiao et al., 2012 & 2013, Waki et al., 2013, and Zhang et al., 2012) suggest utilizing external energy fields and/or addition of MnO₂ or ferrous iron to the wastewater stream treated to accelerate anammox growth, though such laboratory-scale augmentations have yet to be validated at the commercial scale. Practically, addition of manganese or iron to the wastewater treatment process, much less large electrical fields, may constitute a substantial cost to the WWTP, especially as uncertainty remains as to the required type of iron or manganese, their related
concentration, and the duration supplemental metal additions are needed to maintain desired nitrogen removal.

Much of the anammox process understanding developed from various commercial applications designed to exploit the capability of anammox bacteria (e.g. Van Dongen et al., 2001; Van Loosdrecht et al., 2004). Many of these systems involve optimization of a two-step process in which the first reactor, or system employs partial nitritation of the available ammonia to nitrite to achieve the ‘optimal’ 1.2:1 nitrite to ammonia ratio feedstock for the second anammox reactor step converting these to nitrogen gas. Lackner et al. (2014) notes the rapid expansion of the partial nitration-anammox process to more than 100 WWTPs worldwide and outlines the operational and process control aspects and concerns described by surveys at 14 installations. The primary commercial systems include the CANON, DEMON and SHARON processes. The CANON process employs natural or engineered wetland systems treating wastewater with high ammonia and low BOD (Sun, 2007). Under excess ammonium conditions, the cooperation between aerobic (nitrosomonas-like) and anaerobic (planctomycetes) ammonium oxidizing bacteria leave no oxygen or nitrite for aerobic (nitrospira-like) nitrite oxidizing bacteria (Third, 2001; Sliekers, 2002). The DEMON process removes nitrogen from anaerobic co-digestion of urban and industrial sludge liquor using an anammox pathway with aerobic/anaerobic cycling inside a single bioreactor and the DEMON plant in The Netherlands has been operational since 2009. The SHARON process (Single reactor system for High activity Ammonium Removal Over Nitrite) has been developed specifically to treat liquor containing high ammonia concentrations (van Dongen et al., 2001). This is a partial nitrification process where bacteria in the reactor oxidize ammonium to nitrite at temperatures of 30 to 40 C. An anaerobic ammonium-oxidation process follows this where anammox use the nitrite to oxidize ammonia and produce nitrogen gas. Gonzalez-Martinez et al. (2013 & 2014) describe the success of the SHARON process and found a broad range of microbial species completing the nitrogen conversions. In general, such combined partial nitration – anammox reactors have operated successfully
and Schmidt et al., (2003) and Lackner outline their particular operational advantages or challenges. Overall, the interrelationships between N-removing microbial consortia including nitrifiers, denitrifiers, and anammox have also been documented (e.g. Shipin, 2005) in wastewater treatment wetlands. Shipin (2005) described the role of *Nitrobacter* species in dissimilatory reduction of nitrate to nitrite, providing a major nitrite source for anammox. Clearly interest in applications of anammox bacteria to wastewater treatment continues to grow as Lackner et al. (2014) underscored that the number of research publications related to anammox applications in wastewater treatment is also growing rapidly and now to a rate of ~10 articles/year since 2016.

**Wastewater Treatment using Combined Zeolite-Anammox systems**

Collison (2010) reported on bench and pilot-scale linear-channel reactor (wetland flumes) studies investigating several aspects associated with the effects of constructed wetland (CW) substrate and wastewater characteristics on COD and nitrogen removal rates. Collison and Grismer (2014) focused more specifically on the role of zeolites in nitrogen removal from these gravity-flow linear reactors. They found that in the zeolite substrate system, the wastewater NH$_4^+$-N was nearly completely removed midway along the first reactor channel prior to an aeration tank leading to the second channel. In the other three aggregate substrate systems, only about a quarter of the NH$_4^+$-N was removed prior to an aeration tank with the remaining NH$_3^-$-N removed in the aeration tank. That is, the zeolite CW system appeared to remove 98% of the influent nitrogen without using the nitrification-denitrification process. Though zeolite ability to adsorb NH$_4^+$-N cations was undoubtedly occurring in the zeolite CW flume, based on the measured zeolite CEC, the calculated mass of NH$_4^+$-N ions that could be adsorbed was less than half that added to the system as influent. The failure of ammonium ions to saturate the zeolite adsorption sites indicated that other processes were occurring - most likely biological stripping of the NH$_3^-$-N from the aggregate surfaces by anammox bacteria. The ability of anammox to compete effectively
in an anaerobic flume with significant organic matter content seemed contentious but promising in terms of developing an efficient long-term nitrogen removal system for domestic wastewater treatment. As both anammox and nitrifiers bacteria are several orders of magnitude larger (1 to 5 µm) than zeolite pore sizes (0.7 to 1.0 nm), only NH$_4$ ions can travel to internal CEC sites within the zeolite suggesting that only the NH$_4$ ions on the aggregate surfaces are available for the bacterial processes. It is also probable that such related bacterial biofilms are very thin, possibly as rudimentary as individual bacteria adhering to the aggregate surface. Quite possibly, influent NH$_4$ ions can diffuse through the water to the zeolite surface where they were adsorbed at ion-exchange sites and/or ingested by the bacteria. This relatively rapid and efficient process thus only relies on diffusion through water, and neither diffusion through the biofilm or through the aggregate particle is required. Collison and Grismer (2014) postulated that the unique performance of the zeolite CW systems in removing nitrogen was a function of the zeolite’s ability to rapidly capture NH$_4$ ions, coupled with the anammox bacteria’s ability to strip the NH$_4$ and regenerate the surface layer of the zeolite substrate. Environmental conditions for the anammox bacteria were further enhanced by the zeolite aggregate ability to soak up water and create an extensive aerobic/anaerobic interface (oxycline), thereby providing conditions where anammox has access to both the nitrite and ammonium ions needed to produce nitrogen gas. We found application of such an approach at the larger scale reported by Pei et al. (2013) who created a riparian wetland system that employed a zeolite-anammox treatment process and identified that three primary anammox genera were present and operational when flowrates were such that anaerobic conditions prevailed in the zeolite substrate.

Commercial Upscaling of the Zeolite-Anammox Wastewater Treatment Process

While considerable laboratory-scale work related to use of zeolite or anammox to remove nitrogen species from various wastewaters has provided insight into the various treatment mechanisms associated with the ion-exchange and autotrophic anammox processes, there has been little work until
recently considering the combined processes, especially at the commercial domestic WWTP scale (e.g. Kassab et al., 2010). Building on the proof-of-concept benchtop-scale zeolite-anammox treatment system described by Collison and Grismer (2014), Collison and Grismer (2018a) successfully upscaled this process to remove 25-75 mg/L ammonia-N in secondary WWTP effluent to final discharge ammonia and nitrate concentrations less than 1 and 3 mg/L, respectively. Secondary-treated effluent from east San Francisco Bay region WWTPs was pumped to trailers housing parallel linear-channel reactors assembled from channel sections about 3.7 m long by 0.7 m wide and 0.17 m deep. The channel sections were nearly filled with 20 mm zeolite aggregate and seeded at 3-4% by volume with either anaerobic digester effluent containing anammox bacteria or ‘bio-zeolite’ (zeolite aggregate having nitrifier/anammox bacteria biofilm) cultured in other reactors. Following a period of several weeks for complete colonization of the reactors, steady flows through the linear channels submerged the lower half of the zeolite substrate maintaining anaerobic conditions, while the upper half was passively aerated through capillary rise, or wicking action by the aggregate. During a roughly one-year period, they found that approximately 22 m of total reactor length was needed to reduce outlet ammonia concentrations to <1 mg/L; moreover, that these gravity-flow systems required little maintenance and operated across a range of ambient temperatures (10-22 ºC). Overall, at inflow rates from about 40 to 110 Lph, the linear-channel reactors removed 21 to 42 g NH₃-N/m³/day on a bulk-reactor-volume basis (about 1.5 m³) from the secondary treated wastewater with the greater value associated with the higher nitrogen loading rate. On a total nitrogen mass basis, this removal rate exceeded the zeolite adsorption capacity by more than an order-of-magnitude and could not have occurred by denitrification because there was insufficient carbon in the secondary effluent (i.e. very low BOD/COD) for this process. Determination of the linear channel degradation factors was critical towards development of constructed wetland designs for this tertiary treatment prior to discharge to sensitive waters on the Bay periphery.
In an effort to reduce the zeolite-anammox reactor ‘footprint’ or total volume and to explore the possibility of using this process to treat much greater ammonia strength wastewater, Collison and Grismer (2018b and 2018c) investigated use of active aeration methods on nitrogen removal. This effort stemmed in part from needs of the San Francisco Bay area WWTPs and observations from controlled laboratory studies that anammox bacteria based reactors (e.g., Kotay et al., 2013) were capable of roughly 1 kg NH$_3$-N/m$^3$/day removal when supplied optimal nitrite:ammonia concentration ratio wastewater. In these two studies, Collison and Grismer employed tank reactors using recirculating trickling-filter (RTF) and blown, or forced countercurrent airflow designs to remove ammonia from both secondary-treated effluent and high-strength anaerobic digester (AD) filtrate (~500 mg/L ammonia-N). Nitrogen removal from the AD filtrate can significantly reduce total nitrogen loading in the WWTP facilitating achievement of low effluent discharge targets, however, the AD filtrate treatment posed other problems associated with the very high and variable TSS loading. With the project goal of reducing WW ammonia concentrations to <100 mg/L, Collison and Grismer (2017b) first deploy parallel 210 L barrel RTF reactors to assess the feasibility of AD filtrate treatment and investigate effects of aggregate size on ammonia removal. The reactors were operated such that the lower 2/3 of the reactor depth remained submerged facilitating anammox bacterial growth and function, while the top 1/3rd of the reactor aggregate remained desaturated. The barrel reactors successfully removed about 400 mg/L ammonia from the AD filtrate resulting in discharge concentrations of roughly 70 and 90 NH$_3$-N mg/L and 100 and 120 NO$_3$-N mg/L, respectively, for the smaller (10 mm) and larger (20 mm) aggregates. Next, they upscaled the RTF reactor design to a ~68-m$^3$ (18,000 gal) intermediate-scale ‘Baker tank’ reactor for treatment of about 10% of the WWTP AD filtrate sidestream. When operated using the two-layer system for an 8-month period, the Baker tank reactor achieved an ~80% removal fraction with a nearly one-day retention time, successfully reducing the average inlet ammonia concentration from about 460 mg/L to about 85 NH$_3$-N mg/L and 90 NO$_3$-N mg/L, despite variable inlet ammonia concentrations
ranging from 250-710 mg/L. Such a removal rate was equivalent to what Mansell (2011) achieved with a two-stage partial-nitritation anammox laboratory reactor treating AD filtrate using a 220 day retention time. On a total reactor volume basis, the RTF tank design resulted in an ammonia degradation factor about an order-of-magnitude greater than that in the linear-channel reactors (i.e. 192 to 226 gm NH$_3$N/m$^3$/day for the barrel and Baker tank reactors, respectively). The large and highly variable TSS loading associated with the AD filtrate was problematic and contributed to aggregate pore clogging and some flow ‘short-circuiting’ during testing; not surprisingly, this effect was more apparent in the smaller-aggregate barrel reactors. Efforts to use settling tanks were of limited success and the authors proposed that backflush capabilities be included in the RTF tank reactor designs.

Eventual pore clogging and problems with the recirculation pump in the Baker tank reactor provided the opportunity to operate the tank as a largely anaerobic system for cultivation of biozeolite for other reactors and chance to explore nitrate scavenging potential of the anammox biofilms using DRNA processes. Decreased vertical flows through the top aerated media layer from pore clogging during this stage of the Baker tank reactor experiment, decreased aeration of the lower layer that in turn increased anammox bacterial growth and initially impaired ammonia oxidation in the submerged layer. As described above, had there been an adequate organic food supply, the lower anaerobic layer would have facilitated denitrifying bacterial growth, but the small reactor effluent BOD concentrations (<5 mg/L) indicated that nitrate removal by denitrification was insignificant in this layer. Rather, the absence of nitrate and excess ammonia promoted dissimilatory nitrate reduction to ammonium (DNRA) processes that converted the nitrate back to nitrite. Thus, the anammox bacteria removed about half of the inlet ammonia but practically all influent nitrate such that tank effluent nitrate-N concentrations were averaged ~0.1 mg/L.

Collison and Grismer (2018c) again explored active aeration methods in the zeolite-annamox process as above, but for treatment of secondary-treated WWTP effluent. Unfortunately, during most of
the project period (~13 months), they failed to recognize that the secondary-treated effluent lacked sufficient ferrous iron necessary for anammox bacterial growth because the particular WWTP employed sludge incineration methods that precluded the need to add iron to AD processes to preserve WWTP plumbing infrastructure. As a result, for reactor inlet ammonia and nitrate concentrations of ~30 mg/L and 1 mg/L, reactor discharge ammonia and nitrate concentrations from the RTF and blown-air tank reactors remained disturbingly high at ~3 mg/L and ~25 mg/L, respectively, indicating poor anammox activity and treatment. In the last few months of the project, additions of ferric and chelated iron to the secondary effluent had no effect on treatment, though in the very last month, addition of ferrous iron almost immediately resulted in increased anammox activity as reactor discharge nitrate concentrations fell below 4 mg/L. Ultimately, they identified that zeolite aggregate coated with ‘black’ biofilms was a good indicator that sufficient iron was present in the wastewater to encourage and maintain the anammox bacterial populations in the biofilms necessary for adequate wastewater treatment.

Summary & Conclusions

During the past two decades, new approaches to nitrogen treatment methods that include use of available zeolite aggregates as an adsorptive substrate and various strains of newly discovered anammox bacteria capable of converting ammonia to nitrogen gas. Zeolites are a relatively commonly found deposit around the world whose aggregates have relatively low density, internal porosity and unusually large cation-exchange capacity (CEC). Discovered in WWTP anaerobic digesters and in several marine environments, anammox bacteria were key towards closing nitrogen balance estimates in estuary-marine studies. These slow-growing bacteria prefer anaerobic environments and presumably congregate at aerobic-anaerobic interfaces where they can combine available nitrite and ammonia to form nitrogen gas with some residual nitrate, however, in the past few years they appear capable of direct conversion of ammonium to nitrogen gas via \( \text{H}_2\text{N}_2 \) production. As anammox bacteria appear
capable of direct conversion of oxidized and reduced forms of nitrogen in WWTP discharge to nitrogen gas, they are an exciting opportunity to reduce WWTP nitrogen loads; however, only limited reports of commercial application zeolites and anammox in domestic wastewater treatment are available. Only recently have reports from Collison and Grismer that build on their previous lab work from 2010 become available describing applications of a zeolite-anammox treatment process in commercial WWTPs of the San Francisco Bay region of California.

Of course, additional laboratory and applied process work remains before the combined capabilities of zeolite substrates and anammox bacteria can be fully exploited at the full-scale domestic WWTP setting. As anammox bacteria are difficult to culture, currently there are no standardized techniques for sampling, preservation and transport of anammox bacterial biofilms from sediment, aggregates or reactor surfaces of practical benefit to facilitate identification of particular strains and DNA sequencing. Bacteria identification and DNA sequencing of what anammox samples are collected are largely limited to university or research institute labs as analytical costs at the very few commercial labs capable of these analyses are prohibitive in practice. No doubt, with such information, several more strains of anammox bacteria may be identified from diverse WWTP and marine environments that could be cultivated for wastewater treatment applications. Lacking such analyses, as a practical measure Collison and Grismer (2017c) suggest that presence of ‘black’ biofilms on the aggregate surfaces within WWTP reactors coupled with clear removal of both oxidized and reduced forms of nitrogen from the wastewater is a clear indication of adequate anammox bacteria activity. However, such observation provides little opportunity to identify which anammox strains are present and active.

At the WWTP scale, several operational parameters associated with successful removal of nitrogen species using the zeolite-anammox process remain ambiguous. These operational aspects requiring better definition include bio-zeolite seeding rates in reactors and associated effective start-up times, effective operating temperature ranges, optimal supplemental oxidation rates, and preferred Mn
Fe species supplementation to facilitate anammox growth rates, among others. At the most basic design level, simple gravity-flow zeolite-substrate channel reactors successfully removed nitrogen from secondary treated effluent with little energy or maintenance costs; however, it is not clear that such reactors would function as well at greater flow and nitrogen loading rates. Supplemental aeration through blown-air or recirculating trickling-filter designs appear capable of greater nitrogen removal rates for a particular reactor volume (i.e. greater ammonia degradation factors), but greater operational attention is required to maintain pumps and aerobic-anaerobic layers within the reactors. Nonetheless, preliminary upscaling results thus far are quite promising and additional applied research at the WWTP scale should better refine desirable operational parameters.

As compared to traditional nitrification-denitrification WWTP processes, the primary benefits include possibly greater nitrogen removal and far smaller sludge production rates that reduce WWTP operating costs. As compared to the partial-nitritation two-stage reactor systems, the single reactor zeolite-anammox systems successfully remove nitrogen across a greater temperature range and wastewater strength variability while also being easier to maintain and operate as they do not require continuous adjustments for wastewater characteristics. On the other hand, as a fixed media bed system, the zeolite-anammox reactors are subject to possible pore clogging and some attention must be given to either pretreatment removal of recalcitrant solids, or backflushing capability within the reactor bed. Finally, from the perspective of WWTP greenhouse-gas generation, anammox bacterial conversions of nitrogen species either directly to nitrogen gas via DRNA processes, or through combination of ammonium and nitrite as outlined in the stoichiometric equations above, bypasses production of CO$_2$ gas occurring in the traditional nitrification-denitrification treatment process and represents a significant advantage over traditional WWTP processes. However, this aspect also needs further investigation that includes monitoring of the WWTP gases generated by each unit operation across the plant.
References

Bae, H., K-S Park, Y-C Chung and J-Y Jung. 2010. Distribution of anammox bacteria in domestic WWTPs and their enrichments evaluated by real-time quantitative PCR. Process Biochemistry 45:323-334.

Bi, Z., Qiao, S., Zhou, J., Tang, X. & Zhang, J. 2014. Fast start-up of Anammox process with appropriate ferrous iron concentration. Bioresour. Technol. 170, 506–512.

Burgin, A. and Hamilton, S. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front. Ecol. Environment 5(2): 89-96.

Chen, H., Sitong Liu, Fenglin Yang, Yuan Xue, Tao Wang. 2009. The development of simultaneous partial nitrification, anammox and denitrification (SNAD) process in a single reactor for nitrogen removal. Bioresource Technology 100:1548-1554.

Collison, R.S. 2010. Effects of porous media and plants in the performance of subsurface flow treatment wetlands. PhD Dissertation in Biological Systems Engineering, UC Davis. March.

Collison, R.S. and M.E. Grismer. 2014. Nitrogen and COD Removal from Septic Tank Wastewater in Subsurface Flow Constructed Wetlands: 3. Substrate (CEC) Effects. Water Environment Research 86(4):314-323.

Collison, R.S. and M.E. Grismer. 2018a. Upscaling the Zeolite-Anammox process: Treatment of secondary effluent. Water In-review.

Collison, R.S. and M.E. Grismer. 2018b. Upscaling the Zeolite-Anammox process: Treatment of high-strength anaerobic digester filtrate. Water In-review.

Collison, R.S. and M.E. Grismer. 2018c. Upscaling the Zeolite-Anammox process: Effects of Aeration on treatment of secondary effluent. Water In-review.

Dalsgaard, T., B. Thamdrup and D.E. Canfield. 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. Res. In Microbiology. 156:457-464.

Dong, Z. & Sun, T. 2007. A potential new process for improving nitrogen removal in constructed wetlands – Promoting coexistence of partial-nitrification and ANAMMOX. Ecological Engineering 31:69-78.

Du, Q., Liu, S., Cao, Z., Wang, Y. 2005. Ammonia removal from aqueous solution using natural Chinese clinoptilolite. Separation & Purification Tech., 44(3), 229-234.

Englert, AH and J. Rubio. 2005. Characterization and environmental application of a Chilean natural zeolite. Int. J. Miner. Process. 75:21–29.
Francis, C., J M Beman and MMM Kuypers. 2007. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archael ammonia oxidation. ISME Journal 1:19-27.

Giblin, A.E., C.R. Tobias, B. Song, N. Weston, G.T. Banta, and V.H. Rivera-Monroy. 2013. The importance of dissimilatory nitrate reduction to ammonium (DNRA) in the nitrogen cycle of coastal ecosystems. Oceanography 26(3):124–131, http://dx.doi.org/10.5670/oceanog.2013.54.

Gisvold, B., H. Odegaard and M. Follesdal. 2000. Enhancing the removal of ammonia in nitrifying biofilters by the use of a zeolite containing expanded clay aggregate filtermedia. Water Science & Technology 41(9):107-114.

Gonzalez-Martinez A, Calderon K, Albuquerque A, Hontorio E, Gonzalez-Lopez J, Guisado IM, Osorio F. 2013. Biological and technical study of a partial-SHARON reactor at laboratory scale: effect of hydraulic retention time. Bioprocess Biosyst Eng 36:173–184

Gonzalez-Martinez A, Rodriguez-Sanchez A, Munoz-Palazon, B., Garcia-Ruiz MJ, Osorio F, M.C.M. van Loosdrecht, and Gonzalez-Lopez J. 2014. Microbial community analysis of a full-scale DEMON bioreactor. Bioprocess & Biosystems Engr. DOI 10.1007/s00449-014-1289-z

Graaf, AA., P . de Bruijn, LA. Robertson, MS.M. Jetten and J.G Kuenen. 1996. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology 142:2197-2196.

Güven, D., A. Dapena, B. Kartal, M.C. Schmid, B. Maas, Ka van de Pas-Schoonen, S. Sozen, R. Mendez, H.J.M. Op den Camp, MS.M. Jetten, M. Strous and I. Schmidt. 2005. Propionate Oxidation by and Methanol Inhibition of Anaerobic Ammonium-Oxidizing Bacteria. Applied & Environmental Microbiology, p 1066-1071.

Hao, X. & van Loosdrecht, M. 2004. Model-based evaluation of COD influence on a partial nitrification-Anamox biofilm (CANON) process. Water Sci. Technol. 49, 83–90.

Jetten, MSM., M. Strous, K.T. van de Pas-Schoonen, J. Schalk, Udo G.J.M. van Dongen, A.A. van de Graaf, S. Logemann, G. Muyzer, M.C.M. van Loosdrecht, J. G. Kuenen. 1999. The anaerobic oxidation of ammonium. FEMS Microbiology Review 22:421-437.

Jetten, MSM., I. Cirpus, B. Kartal, L. van Niftrik, K.T. van de Pas-Schoonen, O. Sliekers, S. Haaijer, W. van der Star, M. Schmid, J.van de Vossenberg, I. Schmidt, H.Harhangi, M. van Loosdrecht, J. Gijs Kuenen, H. Op den Camp, M. Strous. 2005. 1994-2004: 10 years of research on the anaerobic oxidation of ammonium. Biochemical Society Transactions 33(1):119-123.
Lei, X., M. Li, Z. Zhang, C. Feng, W. Bai, N. Sugiura. 2009. Electrochemical regeneration of zeolites and the removal of ammonia. J. Hazardous Materials 169:746-750.

Li, X-R., B. Du, H-X. Fu, R-F. Wang, J-H Shi, Y Wang, MSM. Jetten, Z-X Quan. 2009. The bacterial diversity in an anaerobic ammonium-oxidizing (anammox) reactor community. Systematic & Applied Microbiology 32:278-289.

Mansell, B.L. 2011. Side-stream treatment of anaerobic digester filtrate by anaerobic ammonia oxidation. MS Thesis in Civil & Environmental Engineering at Univ. of Utah. May.

Misaelides, P. 2011. Application of natural zeolites in environmental remediation: A short review. Microporous & Mesoporous Materials 144:15–18.

Molinuevo, B., M. Cruz-Garcia, D. Karakashev, I. Angelidaki. 2009. Anammox for ammonia removal from pig manure effluents: Effect of organic matter content on process performance. Bioresource Technology 100: 2171-2175.

Motsi, T., NA Rowson, MJH Simmons. 2009. Adsorption of heavy metals from acid mine drainage by natural zeolite. Int. J. Miner. Process. 92: 42–48.

Ni, B.-J., Ruscalleda, M. & Smets, B. F. 2012. Evaluation on the microbial interactions of anaerobic ammonium oxidizers and heterotrophs in Anammox biofilm. Water Res. 46, 4645–4652.

Paredes, D., P. Kuschk, F. Stange, R.A. Muller and H. Koser. 2007. Model experiments on improving nitrogen removal in laboratory scale subsurface constructed wetlands by enhancing the anaerobic ammonia oxidation. Water Science & Technology, 56(3):145-150.

Pei Y, Wang J, Wang Z, and Tian B. 2013. Anammox bacteria community and nitrogen removal in a strip-like wetland in the riparian zone. Water Sci & Technol. 67(5):968-975.

Qiao, S., Bi, Z., Zhou, J., Cheng, Y., Zhang, J. 2013. Long term effects of divalent ferrous ion on the activity of anammox biomass. Bioresour. Technol., 142, 490–497.

Qiao, S., Bi, Z., Zhou, J., Cheng, Y., Zhang, J., Bhatti, Z. (2012) Long term effect of MnO2 powder addition on nitrogen removal by anammox process. Bioresour. Technol., 124, 520–525.

Rodriguez-Sanchez A, Gonzalez-Martinez A, Martinez-Toledo MV, Garcia-Ruiz MJ, Osorio F, Gonzalez-Lopez J. 2014. The effect of influent characteristics and operational conditions over the performance and microbial community structure of partial nitration reactors. Water 6:1905–1924.

Rose, JA. 2003. Zeolite bed leach septic system and method for wastewater treatment. US Patent No. 6531063 B1. March.
Rozâ Icâ, M., Sî Cerjan-Stefanovicâ, S. Kurajica, V. Vancâ Ina and E. Hodzî Icâ. 2000. Ammoniacal Nitrogen Removal from Water by Treatment with Clays and Zeolites. Water Res. 34(14):3675-3681.

Saltali, K., A. Sari, M. Aydîn. 2007. Removal of ammonium ion from aqueous solution by natural Turkish (Yildizeli) zeolite for environmental quality. J. Hazardous Materials 141:258–263.

Sarioglu, M. 2005. Removal of ammonium from municipal wastewater using Turkish (Dogantepe) zeolite. Separation & Purification Technology 41:1-11.

SFEI. 2016. SF Bay Nutrient Management Strategy Science Plan. March. 68 p.

http://sfbaynutrients.sfei.org/sites/default/files/2016_NMSSciencePlan_Report_Sep2016.pdf

Shipin, O., T. Koottatep, N.T.T. Khanh and C. Polprasert. 2005. Integrated natural treatment systems for developing communities: low-tech N-removal through the fluctuating microbial pathways.

Water Science & Technology (12):299-306.

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

Schmidt, I.; Sliekers, O.; Schmid, M.; Bock, E.; Fuerst, J.; Kuenen, J. G.; Jetten, M. S. M.; Strous, M. 2003. New concepts of microbial treatment processes for the nitrogen removal in wastewater. FEMS Microbiology Reviews, 27:481-492.

Strous, M., Van Gerven, E., Zheng, P., Kuenen, J.G.&Jetten, M. S. M. 1997. Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (anammox) process in different reactor configurations. Water Res. 31:1955–1962.

Third, K., AO. Sliekers, J.G. Kuenen and M.S.M. Jetten. 2001. The CANON System (completely autotrophic nitrogen-removal over nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. System. Appl. Microbiol. 24:588-596.

Trimmer, M., Joanna C. Nicholls, and Bruno Deflandre. 2003. Anaerobic ammonium oxidation measured in sediments along the Thames estuary, United Kingdom. Applied & Environmental Microbiology, p. 6447-6454.

Van de Graaf, A. A., de Bruijn, P., Robertson, L.A., Jetten, MSM. And Kuenen, J. G. 1996. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology 142, 2187–2196.

Van Dongen U, Jetten MSM, & Van Loosdrecht MCM. 2001. The SHARON-Anammox process for treatment of ammonium rich wastewater. Water Sci Technol 44(1):153-160.

Van Loosdrecht MCM, Hao X., Jetten MSM. & Abma W. 2004. Use of anammox in urban wastewater treatment. Water Sci Technol: Water Supply 4(1) p87-94.
Waki, M., Yasuda T., Fukumoto, Y., Kuroda, K., Suzuki, K. 2013. Effect of electron donors on anammox coupling with nitrate reduction for removing nitrogen from nitrate and ammonium. Bioresour. Technol., 130, 592-598.

Weatherley, LR and N.D. Miladinovic. 2004. Comparison of the ion exchange uptake of ammonium ion onto New Zealand clinoptilolite and mordenite. Water Res. 38:4305-4312.

Widiastuti, N., H. Wu, HM Ang, D Zhang. 2011. Removal of ammonium from greywater using zeolite. Desalination 277:15-23.

Widiastuti, N., H. Wu, HM Ang, D Zhang. 2008. The potential application of natural zeolite for greywater treatment. Desalination 218:271-280.

Zekker I, Rikmann E, Tenno T, Saluste A, Tomingas M, Menert A, Loorits L, Lemmiksoo V, Tenno T. 2012. Achieving nitritation and anammox enrichment in a single moving-bed biofilm reactor treating reject water. Environ Technol 33(6):703–710.

Zhang, J.X., Zhang, Y.B., Li, Y., Zhang, L., Qiao, S., Yang, F.L., Quan, X. 2012. Enhancement of nitrogen removal in a novel anammox reactor packed with Fe electrode. Bioresour. Technol., 114, 102–108.