Emerging Models for Nitrogen Removal in Treatment Wetlands

Scott Wallace, P.E., M.S.
David Austin, P.E., M.S.

Abstract

Engineering textbooks tell a simple story about nitrification and denitrification. Classic nitrification-denitrification theory begins with the bacterial genera *Nitrosomonas* and *Nitrobacter* performing ammonia and nitrite oxidation, respectively. Then facultative or obligate anaerobic bacteria denitrify by oxidizing organic carbon with nitrate. Recent advances in environmental microbiology have revealed previously unknown bacteria and pathways in the nitrogen cycle that tell a far more complex story. Classic theory has been successful for technologies that employ fast-growing bacteria, such as activated sludge, for almost a century. In contrast, nitrogen transformations in treatment wetlands are only partially explained by classic theory because they are ideal environments for slow-growing bacteria. Recently discovered bacterial processes, such as Anammox and heterotrophic nitrification, can be native to treatment wetlands. Other known nitrogen-cycle bacteria in nature occupy ecological niches similar to those that can exist in treatment wetlands, but their role in denitrification remains unexplored in a treatment context. The experience of treatment wetlands demonstrates that classic theory is no longer valid as a general model. We propose a broader model of nitrogen transformations in treatment wetlands that integrates recent discoveries. This general model is intended as a conceptual tool for those working with nitrogen pollution abatement.

Introduction

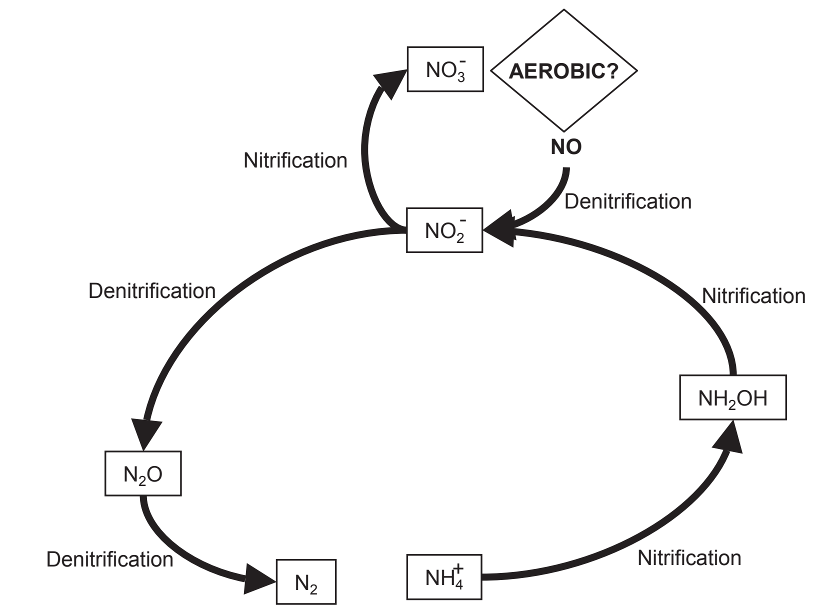
Engineering textbooks tell a simple story about nitrification and denitrification (Figure 1, Table 1). First, the bacterial genera *Nitrosomonas* and *Nitrobacter* (or *Nitrospira*) carry out ammonia (NH_4^+) oxidation to nitrite (NO_2^-) and then to nitrate (NO_3^-). Hydroxylamine (NH_2OH) is an important intermediate product between ammonia and nitrite. Then facultative or obligate anaerobic bacteria denitrify by oxidizing organic carbon with nitrate and nitrite. Nitrous oxide (N_2O) is an important intermediate product between nitrite and atmospheric nitrogen (N_2). This model has been successful for conventional wastewater technology and design (Grady, Daigger, & Lim, 1999; Tchobanoglous, Burton, & Stensel, 2003). Recent developments in science and technology, however, reveal that the "classic model" is specific to the treatment technologies that use it, and thus can no longer be considered a general model for treatment wetlands (Kadlec & Wallace, 2008; Wallace & Knight, 2006).

In nature, nitrification and denitrification is not a simple story. Ammonia-oxidizing bacteria, classically regarded as obligate aerobes, are now known to have an alternate path of nitrogen transformation during transient periods of anoxia that results in nitrogen loss from ammonia (Schmidt et al., 2002). Recently discovered biogeochemical processes also play important roles in the nitrogen cycle: anaerobic ammonia oxidation (Anammox) (van Loosdtrecht & Jetten, 1998), heterotrophic nitrification (Robertson & Kuenen, 1990), aerobic denitrification (Robertson & Kuenen, 1990), methanotrophic denitrification (Raghoebarsing et al., 2006), and denitrifying oxidization by nitrate or nitrite of sulfide (Gevertz, Telang, Voordouw, & Jenneman, 2000), ferrous iron (Straub, Benz, Schink, & Widdel, 1996), manganese(II) (Tebo, Johnson, McCarthy, & Templeton, 2005), and hydrogen (Smith, Ceazan, & Brooks, 1994). The global mass flux of nitrogen through these microbial pathways is not known, but it is probably large for Anammox bacteria (Op den Camp et al., 2006; Zehr & Ward, 2002). These processes appear to be ubiquitous in marine, freshwater, and estuarine sediments. Because a large denitrification mass flux from these sediments exists (Galloway et al., 2004), these newly discovered microbial pathways are probably fundamental constituents of the global nitrogen cycle.

New general models are needed to address a global nitrogen pollution problem barely considered until the late 20th century. Human production of reactive nitrogen exceeds natural fixation approximately by a factor of two (Galloway & Cowling, 2002). Vast "dead zones," such as in coastal areas of the Baltic Sea, the northern Gulf of Mexico, and the northwestern shelf of the Black Sea, are consequences of coastal eutrophication caused by widespread application of nitrogen fertilizers (Rabalais, Turner, & Wiseman, 2001). Resolution of an environmental problem of this magnitude is highly complex in terms of policy, politics,

FIGURE 1

Classic Wastewater Nitrification and Denitrification



Intermediate products hydroxylamine (NH_2OH) and nitrous oxide (N_2O) are depicted. Others are omitted for clarity.

TABLE 1

Classic Nitrification-Denitrification Stoichiometry*

Process	Stoichiometry
Ammonia oxidation *(Nitrosomonas)*	$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$
Nitrite oxidation *(Nitrospira and Nitrobacter)*	$NO_2^- + 0.5O_2 \rightarrow NO_3^-$
Denitrification (many bacteria genera)	$5(CH_2O) + 4NO_3^- \rightarrow H_2CO_3 + 4HCO_3^- + 2N_{2(g)} + H_2O$

* Nitrification is comprised of autotrophic ammonia oxidation plus nitrite oxidation. Ammonia and nitrite are the carbon donors. Oxygen is the electron acceptor. The bicarbonate ion (HCO_3)is used for cell growth, and is consumed by hydrogen ions in a neutralization reaction, but is not an electron donor. Denitrification is heterotrophic. Organic carbon (CH_2O) is used for cell growth and is also the electron donor. Nitrate is the electron acceptor.

science, and engineering. From a technical perspective, the classic nitrogen model is not useful to address this issue because of the large energy requirements of nitrification and organic carbon requirements for denitrification. Environmental professionals will recognize technical opportunities to improve water quality in the face of unprecedented global nitrogen loading only if they have more complete models of nitrogen microbial transformation as part of their conceptual and design "tool kit."

Engineering applications of some of these novel processes are already emerging. The Anammox process is in commercial development (Jetten et al., 2005), and it also appears to be native to certain engineered wetland systems (Dong & Sun, 2007; Sun & Austin, 2007). Heterotrophic nitrification was discovered in a wastewater treatment system (Robertson & Kuenen, 1990) and is undergoing commercial development in engineered wetland systems (Maciolek & Austin, 2006). In addition to pollution abatement, the sharply reduced energy demand for nitrogen removal through Anammox and heterotrophic nitrification (Jetten et al., 2005; Maciolek & Austin, 2006) is a significant potential advantage to society of technologies based on these processes.

Nitrogen Removal in Treatment Wetlands

Constructed wetlands employ biogeochemical processes found in natural wetland environments. Common forms of treatment wetlands include surface flow systems (essentially man-made equivalents of natural marshes) and subsurface flow systems (where the flow is designed to pass through the root zone of the wetland vegetation growing in aggregate or engineered soils).

Engineered wetlands build upon the experience of the previous generation of passive, constructed subsurface flow wetlands by several methods to oxidize ammonia, including vertical flow flood and drain systems (Behrends, Houke, Bailey, Jansen, & Brown, 2001), vertical pulsed flow system (Molle, Lienard, Boutin, Merlin, & Iwema, 2005), and use of supplemental aeration in horizontal and vertical flow systems (Wallace, Higgins, Crolla, Bachand, & Verkuijl, 2006). Although it was once thought that wetland plant roots would provide sufficient oxygen to oxidize ammonia, it is now clear that the root zone in passive constructed wetlands is best viewed as at least a partially anaerobic system (Kadlec & Wallace, 2008).

Many surface flow wetlands have been designed for tertiary treatment of domestic wastewater. In these systems, nitrogen loss is not accompanied by formation of nitrite or nitrate on a mass balance basis as would be expected in the classic model (Bishay & Kadlec, 2005; Kadlec & Knight, 1996; Kadlec & Wallace, 2008).

In literature on treatment wetlands, the "missing" nitrogen problem is common. On a mass balance basis, classic nitrification and denitrification is often only partially demonstrable because a large fraction of influent ammonia either "disappears" or clearly follows non-classic pathways (Kadlec & Wallace, 2008; Maciolek & Austin, 2006; Shipin, Koottatep, Khanh, & Polprasert, 2005; Sun & Austin, 2007; Sun, Gray, Biddlestone, Allen, & Cooper, 2003).

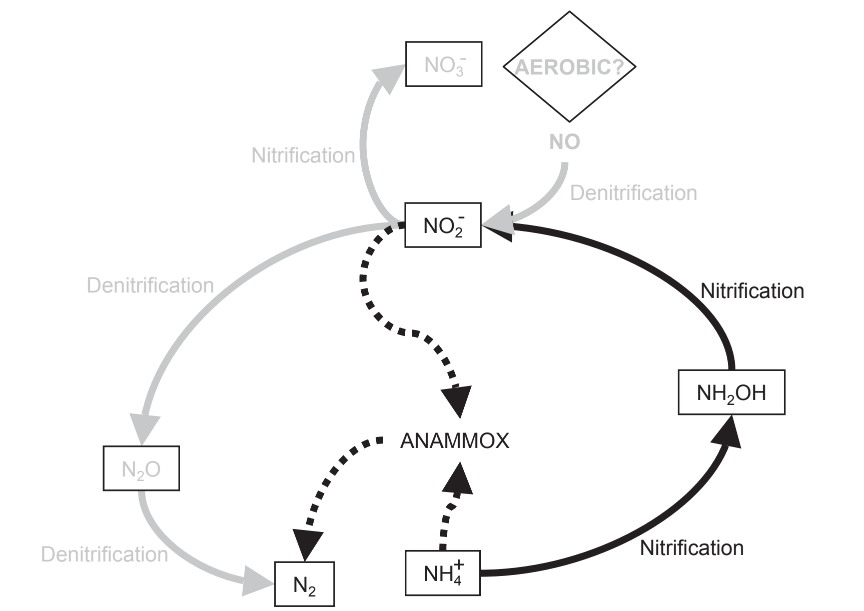
The simplest explanation for data not conforming to the classic model is that treatment wetlands and conventional wastewater technologies have fundamental differences of microbial ecology. Mean cell residence time (MCRT) is a measure of how long an average bacterium remains within a treatment system. Conventional technologies, such as activated sludge, rely on bacteria with fast-growth life strategies in which nitrifying and denitrifying bacteria grow suspended in the water column. In conventional systems the MCRT is seldom more than three weeks and is typically less (Tchobanoglous, Burton, & Stensel, 2003). In contrast, wetland bacteria grow in biofilms attached to aggregate and plants. The wetland is a low fluid shear environment and biofilms are very stable. Using a Monod growth and decay model developed for subsurface flow wetlands (Austin, Maciolek, Wallace, & Davis, 2007) the MCRT in a subsurface flow wetland is calculated to be greater than 200 days. Because these non-classic nitrogen processes are based on slow-growing bacteria, such as Anammox or heterotrophic nitrifiers (Jetten et al., 2005; Robertson & Kuenen, 1990), they can only become established in long MCRT environments, some examples of which are soils, sediments, and treatment wetlands.

Practitioners with an interest in treatment wetlands need a more comprehensive nitrogen microbiology model. To develop this model, even specialists must carefully pick their way through the often vexing complexity of the subject. Complicating the effort is that the fast pace of new discoveries in nitrogen microbiology may not abate for some time to come. Nevertheless, enough is now known to extract useful concepts for a non-specialist interested in engineering applications.

Two key concepts are needed to understand any pathway of nitrogen transformation: carbon source and electron transfer. Heterotrophic bacteria use organic carbon (e.g., sugar or methanol) to build new cells. Autotrophic bacteria use inorganic carbon from the bicarbonate ion ($HC0_3^-$), which is continually replenished in natural water systems from the atmospheric pool of carbon dioxide. Mixotrophic bacteria can switch carbon sources. Electron transfer creates energy for respiration and growth. An electron donor (reduced compound) and an electron acceptor (oxidized compound) are needed. For example, in the case of a bonfire, organic carbon in wood is an electron donor and oxygen is an electron acceptor. Of relevance to nitrogen microbiology, oxygen, nitrate, and nitrite are terminal electron acceptors. A range of reduced compounds (e.g, organic carbon, ammonium—NH_4^+, or ferrous iron—Fe[II]) are electron donors. Most bacteria require a specific electron donor and acceptor pair. Some bacteria, however, are more physiologically flexible and can use more than one electron donor and electron acceptor.

FIGURE 2

Anammox Pathway in Context of Classic Model



Residual nitrate produced by Anammox not depicted. Note that the Anammox process (dotted lines) relies upon aerobic ammonia oxidation (solid lines), but competes with nitrite oxidation.

TABLE 2

Anammox Stoichiometry*

Process	Stoichiometry
Ammonia oxidation	$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$
Anammox	$NH_4^+ + 1.3NO_2^- \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O$

* Partial oxidation of ammonia provides sufficient nitrite for oxidation of remaining ammonia. Ammonium is the electron donor and nitrite is the electron acceptor in the Anammox step. The bicarbonate ion is the carbon source.

Anammox Model in Wetlands

Anammox is a two-step process in which ammonia-oxidizing bacteria (*Nitrosomonas* sp.) partially oxidize ammonia to nitrite (via hydroxylamine) and then Anammox bacteria (multiple candidate species) use nitrite to oxidize the remaining ammonia directly to atmospheric nitrogen. (Figure 2, Table 2) (van Loosdrecht & Jetten, 1998). Ammonia is the electron donor and nitrite is the electron acceptor. These bacteria are slow-growing autotrophs. A large advantage of the Anammox process is that it does not require an organic carbon source to remove nitrogen from water. Although Anammox bacteria have a slow growth rate, their rate of nitrogen removal is high once biomass has been established. It is this combination of a high nitrogen removal rate operating on partial oxidation of ammonia that enables commercial application of Anammox processes while having only about 20% of the oxygen demand of conventional nitrification-denitrification (Jetten et al., 2005). A reduction in oxygen demand is directly related to reduction in process energy requirements and reduction of greenhouse gas emissions.

The commercial application, known as SHARON-ANAMMOX, is a physically two-stage, suspended-growth process in which ammonia in warm wastewater (30°C–40°C) is first par-

FIGURE 3

Heterotrophic Nitrification and Aerobic Denitrification

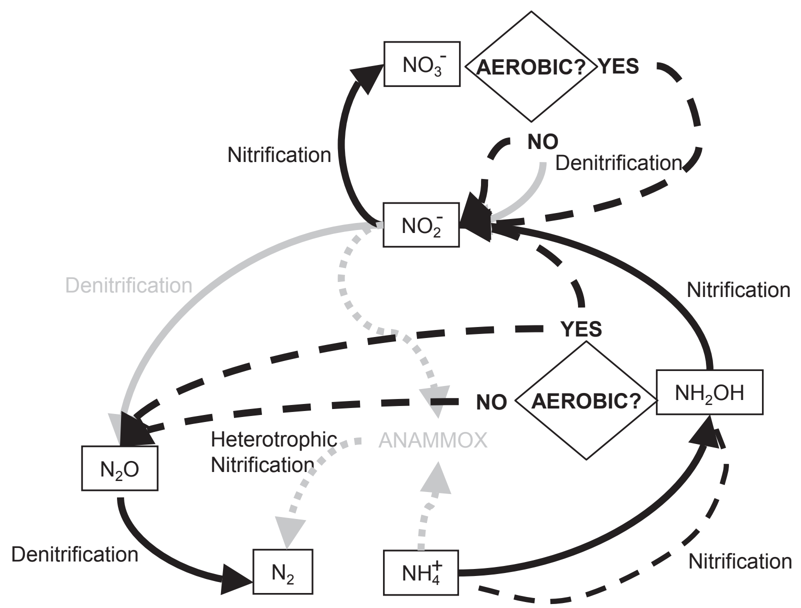


Figure adapted from Richardson (2000) and Moir, Wehrfritz, Spiro, & Richardson (1996). For both heterotrophic nitrification (dashed lines) and aerobic denitrification (dashed lines) to occur, the environment must frequently switch between aerobic and anoxic conditions. Autotrophic nitrification (solid lines) can occur in competition with heterotrophic nitrification. Aerobic denitrification requires nitrite oxidation by other bacteria *(Nitrospira* and *Nitrobacter).*

TABLE 3

Heterotrophic Nitrification Stoichiometry*

Process	Stoichiometry
Ammonia oxidation	$NH_4^+ + 0.5O_2 \rightarrow NH_2OH + 3H^+$
Anoxic hydroxylamine oxidation	$2NH_2OH \rightarrow N_2O + H_2O + 4H^+$
Aerobic hydroxylamine oxidation	$NH_2OH + O_2 \rightarrow \begin{cases} \alpha N_2O \\ \beta NO_2^- \end{cases}$

* Ammonia oxidation to hydroxylamine begins the process. Under anoxic conditions the exclusive product of hydroxylamine oxidation is nitrous oxide (Moir, Wehrfritz, Spiro, & Richardson, 1996). The electron acceptor is cytochrome C550. Under aerobic conditions both nitrite and nitrous oxide are end products in unknown proportions, hence stoichiometry is left unbalanced.

tially oxidized to nitrite (SHARON) and then passed on to another reactor in which Anammox bacteria oxidize the remaining ammonia with nitrite (Hellinga, Schellen, Mulder, van Loosdrecht, & Heijnen, 1998). The SHARON-ANAMMOX process is limited to warm, ammonia-rich wastewater, as found in the supernatant of municipal sludge digestors.

A more broadly applicable Anammox process is known as CANON (Complete Autotrophic Nitrogen-removal Over Nitrite). It is a physically single-stage, attached-growth process that can operate at low temperatures (Third, Sliekers, Kuenen, & Jetten, 2002). Under conditions of limited oxygen and high ammonia loading (>0.12 kg NH_4-N $m^{-3}d^{-1}$) Anammox bacteria out-compete nitrite oxidizing bacteria (*Nitrobacter* and *Nitrospira*) in a CANON reactor. At low ammonia loading, nitrite oxidizing bacteria out-compete Anammox bacteria and the process reverts to classic nitrification. The process is reversible and spontaneous with a lag period associated with bacteria growth (Third, Sliekers, Kuenen, & Jetten, 2002). Thus Anammox and classic nitrification occupy neighboring ecological niches within an attached growth system.

The aggregate bed of a subsurface flow wetland creates an attached-growth reactor similar to the CANON process. Dong and Sun (2007) modified a vertical flow wetland treating domestic wastewater to create a shallow (25 cm), unsaturated surface layer over a deeper (55 cm), saturated layer. Pulsed influent dosing to the unsaturated zone provided limited aeration of wastewater that percolated into the anoxic saturated layer. Under conditions of low-ammonia loading (<0.05 kg NH_4-N $m^{-3}d^{-1}$) and oxygen limitation, treatment performance was consistent with nitrogen loss by Anammox. Biomolecular probes confirmed the presence of Anammox bacteria. Sun and Austin (2007) demonstrated the CANON process by mass balance in one of four wetland columns treating leachate. Oxygen was not limiting, but the ammonia loading was high (0.11 kg NH_4-N $m^{-3}d^{-1}$). It is interesting to note that some columns retained classic nitrification and denitrification. The switch of one column to Anammox at a transitional ammonia loading rate could be attributed to its use in previous experiments with high-ammonia wastewater. In those experiments, a large fraction of ammonia "disappeared" according to the classical model (Sun, Gray, Biddlestone, Allen, & Cooper, 2003).

Surface flow wetlands create shallow water-sediment interfaces in which Anammox bacteria are found in nature (Zehr & Ward, 2002). Anammox bacteria have been identified in surface flow wetlands by biomolecular probes (Shipin, Koottatep, Khanh, & Polprasert, 2005) and by mass balance (Bishay & Kadlec, 2005). Neither of these wetlands received a high specific mass loading of ammonia. Because sediments in surface flow wetlands tend to be anaerobic just below the water-sediment interface, it is likely that oxygen limitation induced Anammox activity.

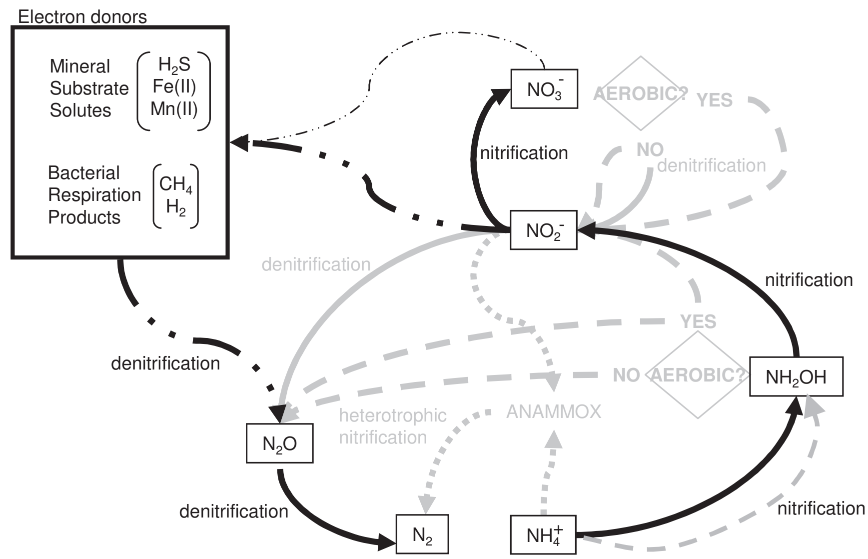
Heterotrophic Nitrification Model in Treatment Wetlands

Heterotrophic nitrification is a complex process that is coupled to *aerobic* denitrification (Figure 3, Table 3). The model organism is *Paracoccus denitrificans* (Richardson, 2000). In contrast to classic nitrification, which is an autotrophic, energy-yielding reaction, heterotrophic nitrification does not yield energy. Energy yield comes out of a physiological strategy known as aerobic denitrification that uses both oxygen and nitrate simultaneously as terminal electron acceptors (Moir, Wehrfritz, Spiro, & Richardson, 1996; Richardson, 2000; Robertson & Kuenen, 1990). Classic denitrifying bacteria use either oxygen or nitrogen but have a lag (resting) period of some hours to switch between the two. Where rapid oscillation between aerobic and anoxic conditions is the norm, aerobic denitrifying bacteria have a competitive advantage even though their growth rate is comparatively much slower. (The tortoise wins the race if the hare is short of breath.) Heterotrophic nitrification appears to be a mechanism to dump excess ammonia which would otherwise interfere with the internal energy balance (redox) of the bacterium cell (Richardson, 2000). By analogy, heterotrophic nitrification is "house cleaning," whereas these bacteria "earn a living" by oxidizing carbon with nitrate or oxygen. The energy balance is complex. Under anaerobic conditions ammonia is oxidized only to hydroxylamine and then exclusively to nitrous oxide, bypassing nitrite altogether (Richardson, 2000). Under aerobic conditions hydroxylamine is both oxidized to nitrite and nitrous oxide. The proportions of this split are unknown.

Heterotrophic nitrification has been observed in flood and drain (tidal flow) treatment wetlands (Austin, Wolf, & Strous, 2006; Maciolek & Austin, 2006). Tidal flow wetlands flood and drain several times per day. Biofilms growing on aggregate and roots saturate with oxygen shortly after the bed drains but are anoxic when flooded (Behrends, Houke, Bailey, Jansen, & Brown, 2001). Nitrification occurs as ammonium ions adsorbed to media and biofilms during the flooded phase rapidly nitrify upon exposure to atmospheric oxygen during the drained phase (Tanner, D'Eugenio, McBride, Sukias, & Thompson, 1999). As a result, nitrate is continually pumped into the water column. In this environment, the ability to use simultaneously oxygen and nitrate as a terminal electron acceptor appears to favor aerobic denitrification and, because it is physiologically coupled, heterotrophic nitrification (Austin, Wolf, & Strous, 2006). The advantage of this process is that it efficiently uses organic carbon already present in the wastewater for denitrification within the same volume used for nitrification. Also, a sharply reduced energy demand exists because of the efficiency of the pumping that drives the flood and drain process as compared to mechanical aeration of bulk water, such as in activated sludge (Maciolek & Austin, 2006).

FIGURE 4

General Model



This model incorporates nitrogen pathways not yet demonstrated in engineered wetlands (emphasized here for clarity), but found in nature (Raghoebarsing, et al., 2006; Gevertz, Telang, Voordouw, & Jenneman, 2000; Straub, Benz, Schink, & Widdel, 1996; Tebo, Johnson, McCarthy, & Templeton, 2005; Smith, Ceazan, & Brooks, 1994). Reduced mineral substrates (e.g. F[II]) may themselves be respiration products of anaerobic bacteria. Nitrite is the preferred electron acceptor for electron donors in the box, but some bacteria can adapt to using nitrate. Most of the bacteria in the box are autotrophs. Some are mixotrophs.

General Model

The emerging picture from treatment wetlands is that they are environments in which very slow growing bacteria can become dominant players in nitrogen process microbiology. To date, Anammox and heterotrophic nitrification have been observed. No scientific reason exists as to why other nitrogen-cycle bacteria known in nature could not also play a significant role in treatment wetland systems. As long as oxidation of ammonia to nitrite occurs, several potential electron donors exist in wastewater that are compatible with known nitrogen pathways (Figure 4). Bacteria capable of using these electron donors are mainly slow-growing autotrophs that mostly prefer nitrite as a terminal electron acceptor. The mineral content of the source water will significantly influence which of these other electron donors will be present (e.g., reduced manganese—Mn[II]). Treatment wetland MCRTs are long enough to allow a bacterial community that relies upon a given electron donor and acceptor pair to occupy a significant niche in the microbial ecosystem. Research is needed to understand how a fully integrated nitrogen transformational model fits into treatment wetlands.

The proposed general model (Figure 4) is an overlay of Figures 1, 2, and 3 integrated with other pathways known in nature. As Figure 4 shows, a functional linkage exists between all processes presented in this article. The reason why one or more processes dominate nitrogen mass flux depends on MCRT, carbon sources (organic carbon or $HC0_3^-$), oxygen concentration (anoxic or aerobic or microaerobic), rate and frequency of oxygen concentration changes, mineral content of the water, and other factors. Engineering design and technology manipulates or takes advantage of these mechanisms. The utility of a new general model is that it allows recognition of opportunities to remove nitrogen from wastewater that are invisible if only the classic model (Figure 1) is accepted. Design practice will evolve accordingly.

Conclusion

Treatment wetlands are analogs of natural ecosystems, which create environments where slow-growing bacteria can thrive and significantly participate in nitrogen transformations. As a result, the classic model of nitrification and denitrification, which relies on comparatively fast-growing bacteria, does not fully describe process microbiology. Other processes, such as Anammox and heterotrophic nitrification, can be important. Rather than view these processes as exotic exceptions to the classic model, it is more useful to view them as integral to a complex ecosystem of nitrogen-cycle bacteria. Choice of technology can shift the dominance of one type of bacteria over another, but underlying functional relationships between these bacteria at least potentially remain. Consideration of the fate and flux of nitrogen compounds in treatment wetlands forces a more sophisticated process perspective than existing classic models currently found in engineering textbooks. Although simplicity is convenient, it can simply miss important opportunities to remove nitrogen from water.

Of course, the models described in this article are only a general overview when compared to the complexity of the subject. A great deal of research is needed to develop an overall engineering design theory around the many nitrogen pathways found in nature. Anammox and heterotrophic nitrification are just a start. We propose that treatment wetlands are ecosystem analogs that may be useful tools to help develop general nitrogen models that appropriately balance necessary engineering simplification with the complexity of nature to address problems of nitrogen pollution.

Corresponding Author: David Austin, Principal Technologist, Natural Treatment Systems, CH2M HILL, 1295 Northland Dr., Ste 200, Mendota Heights, MN 55120. E-mail: David.Austin@CH2M.com.

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