Review

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Prospects for Biological Nitrogen Removal from Anaerobic Effluents during Mainstream Wastewater Treatment

Jeseth Delgado Vela, Lauren B. Stadler, Kelly J. Martin, Lutgarde Raskin, Charles B. Bott, Nancy G. Love*

* Corresponding author
Department of Civil and Environmental Engineering, University of Michigan, 2350 Hayward Street, Ann Arbor, MI 48109, USA
Phone: 734-663-9664, Fax: 734-664-4292
E-mail: *nglove@umich.edu*

Abstract

Growing interest in anaerobic treatment of domestic wastewater requires a parallel focus on developing downstream technologies that address nitrogen pollution, especially for treatment systems located in eutrophication-impacted watersheds. Anaerobic effluents contain sulfide and hydrogen sulfide (a corrosive gas), dissolved methane (a potent greenhouse gas), ammonium and residual organic carbon predominantly in the form of volatile fatty acids. Conventional approaches to nitrogen removal are energy- and chemical-intensive, and are not appropriate for nitrogen removal from anaerobic effluents. Innovative, energy efficient nitrogen removal processes are developing and involve several novel chemotrophic processes. This review provides information on these processes, identifies how to control and retain the most desirable microorganisms, and considers the impact of reactor configuration on performance. Given the complexity of the technologies under development that remove nitrogen from anaerobically-treated domestic wastewater, we conclude that computational models can support their development, and that sensor-mediated controls are essential to achieving energy efficiency.

1. Introduction

The domestic wastewater treatment industry in the United States is in a period of major change motivated by two movements that are inspiring technological innovation in the industry: a need to renew infrastructure that was built in response to the 1972 Clean Water Act and is reaching the end of its design life, and a drive toward more sustainable practices including energy recovery and efficiency. At the same time, increasingly stringent effluent nutrient guidelines are being implemented across the highly populated coastal margins of the United States[1-3], fueled mostly by a need to mitigate eutrophication by managing nitrogen and phosphorus. Nutrient recovery technologies applied to domestic wastewater exist[4] or are under development[5-7]. While

desirable from a sustainability perspective, nutrient recovery is typically not practical for treatment plants located far from agricultural land or where direct water reuse is not practiced because conveying liquid nutrients is economically infeasible[8].

Although both nitrogen and phosphorus management are important, this review focuses on mainstream biological nitrogen removal, which has been identified as a priority for innovation and development by the water industry. This prioritization has been motivated by performance, energy and space inefficiencies associated with existing nitrogen removing technologies that evolved as "add ons" to conventional activated sludge processes. Within these traditional systems, multiple microbial groups coexist across a range of redox environments to achieve carbon and nitrogen conversions that are not optimized for any one group. Motivated by the desire to develop cost-effective and energy-efficient technologies that achieve net energy neutral (or positive) domestic wastewater treatment, the water industry has prioritized the need to develop a new generation of mainstream (as opposed to sidestream) nitrogen removing technologies that are suitable for treating effluents from anaerobic systems receiving domestic wastewater.

Anaerobic treatment of domestic wastewater is gaining attention for enhancing energy recovery from wastewater[9, 10] because it: eliminates aeration, produces methane, and reduces sludge handling requirements[10]. Several demonstrations have met USEPA's secondary effluent standards[11-14] even at temperatures as low as 6°C[15]; however, anaerobic processes produce effluents with high ammonia-nitrogen, organic-nitrogen and dissolved methane concentrations which constrain wide-scale adoption of domestic anaerobic wastewater treatment[16]. Advancing anaerobic treatment of domestic wastewater in nutrient-sensitive regions where total nitrogen removal regulations exist or are anticipated requires implementing novel, energy efficient

mainstream nitrogen removal technologies that avoid nullifying energy savings realized from the anaerobic process, while also mitigating greenhouse gas emissions, maintaining or reducing the footprint, and reducing sludge handling requirements.

This review synthesizes past studies of biological nitrogen removal from anaerobic effluents[17-23] and rapidly developing energy efficient approaches for nitrogen removal that have not been included in previous reviews[24, 25]. We review microbial metabolic processes that are likely to be active in nitrogen removing treatment systems receiving anaerobic effluents, and emphasize that computational process modeling can help us understand how these complex metabolisms are likely to behave under various treatment approaches. We consider tradeoffs that exist between reducing energy demand, greenhouse gas emissions, space requirements and effluent nitrogen concentration, all of which are strongly influenced by reactor configuration and operational control strategy. Throughout the review, we highlight key areas where innovative research is needed to develop solutions that meet increasingly stringent nitrogen regulations and move the wastewater industry toward net energy neutral (or positive) treatment.

2. Anaerobic effluent composition

Direct anaerobic treatment of domestic wastewater generates an effluent (Table 1) with a significantly different composition than that of conventional aeration-based treatment. For example, ammonium and phosphate concentrations increase during anaerobic treatment due to ammonification and biotic[24] or abiotic[24, 25] phosphate release. In contrast, conventional aerobic treatment decreases ammonium and phosphate concentrations due to nitrification and uptake for biomass growth. Sulfate concentrations in domestic wastewater vary considerably, depending upon factors such as industrial wastewater discharges, coagulant used for drinking water treatment, and drinking water sources. During anaerobic treatment, sulfate is reduced by sulfate

reducing microorganisms to sulfide and hydrogen sulfide gas, undesirable products that are corrosive, malodorous with an odor threshold of 0.04 ppmv, dangerous to human health at concentrations above 100 ppmv[26], and inhibitory to nitrification[21] and methanogenesis[27, 28]. Anaerobic treatment converts the organic carbon in domestic wastewater to gaseous products (carbon dioxide and methane) and dissolved products (organic acids[29, 30] and methane[11, 31]). The concentration of dissolved methane in anaerobic effluents varies seasonally and increases when temperature decreases. While the average measured effluent concentration of dissolved methane is approximately 1.1 times saturation (Table 1), this value is highly variable and values as high as 4.1 times saturation[15, 32] have been reported. Methane is a potent greenhouse gas and its release represents a significant potential environmental impact[16] that should be prevented. At present, technologies to recover dissolved methane either produce biogas with low methane content or recover insufficient methane to offset their energy demands[33-36]. Considering the undesirable impacts of sulfide and dissolved methane in anaerobic effluents, it makes sense to consider the efficacy of using either or both as novel electron donors for nitrogen removal when stringent nitrogen discharge limits apply[19, 37].

Conventional nitrogen removal systems located in regions that require high levels of total nitrogen removal tend to be electron donor limited due to use of primary clarifiers and aerobic loss of organic carbon as CO_2. Consequently, many full scale systems require exogenous electron donor to achieve effluent nitrogen limits. In contrast, primary clarifiers are not used when mainstream anaerobic treatment is deployed. Furthermore, the electron donor equivalents typically present in anaerobic effluents are preserved in solution as organic COD not removed during anaerobic treatment, dissolved methane and sulfide, and far exceed what is necessary to achieve nitrogen removal via nitrite or nitrate (Table 1). This assumes these electron donors have

not been aerobically oxidized and are available for denitrification, a challenge that can be addressed through reactor configuration design (highlighted in Section 5). The COD balance also indicates that the electron donors will compete for nitrite or nitrate, and underscores the importance of applying reactor flow models, reaction rate kinetics and stoichiometry to ascertain which competing metabolic groups will thrive during nitrogen removal from anaerobic effluents.

Table 1: Summary of effluent characteristics reported in studies of anaerobic domestic wastewater treatment, and electron donor requirements (expressed as COD equivalents) for complete denitrification from nitrate to N_2.

	Average ± Standard Deviation	Range	References[a]
Ammonium (mg N/L)	36±17	9-67	4, 17, 22, 23, 38-46
Phosphate (mg P/L)	6±7	1-20	4, 22, 38, 40, 41, 44, 45
Sulfide (mg COD/L)	62±83	3-184	4, 17, 34, 40, 41, 44, 47
Methane (mg COD/L)	91±50	42-204	31, 33-35, 40, 41, 46, 48
Soluble[b] COD (mg COD/L)	99±46	46-201	17, 23, 31, 34, 35, 38, 40-43, 45, 46
Total electron donor available[c] (mg COD/L)	252±107		
Electron donor consumed for denitrification via nitrate[d] (mg COD/L)	135±47		
Electron donor required for denitrification via nitrite[d] (mg COD/L)	98±32		

[a] Only studies that treated real domestic wastewater were used to generate this table.

[b] Soluble COD definition determined by the pore size of filters used: 0.45 μm[17, 35, 43, 45], 0.7 μm[38], Unreported[23, 31, 34, 40-42, 46]

[c] The theoretical oxygen demand from ammonium is not considered in this calculation. Total electron donor is calculated as the sum of soluble COD, dissolved methane as COD, and sulfide as COD. Methane does not react in the COD test[34], and it was assumed that sulfide is oxidized or stripped in the filtration/sample acidification process prior to COD measurement.

[d] The electron donor consumed incorporated demands for both respiration and cell growth, and considers loss of ammonium to support cell growth. Yields were assumed for nitrifiers (0.1 g COD_x/g N, Table 3), for heterotrophic denitrifiers applied only to the soluble COD fraction (0.39 g COD_x/g COD[49]). Growth of other organisms was assumed to be insignificant.

3. Metabolic Pathways for Biological Nitrogen Removal from Anaerobic Effluents

A number of metabolic processes directly achieve or support nitrogen removal (Table 2) and deserve consideration, although some produce environmentally undesirable byproducts like

nitrous oxide, a greenhouse gas 300 times more potent than carbon dioxide over 100 years[50] (discussed further in Sections 3.2 and 4). Kinetic and stoichiometric parameters for many of the reactions in Table 2 were compiled from the literature (see Table 3) and can be used with process modeling to predict the distinct microbial community structures that are likely to form when attempting nitrogen removal in anaerobic effluents. The majority of these parameters were derived across a broad range of growth conditions; therefore, values vary considerably (see Table S1), making predictions about competition between pathways challenging. Nevertheless, relative values provide insight into competitive growth conditions that will occur in nitrogen removal systems treating anaerobic effluents. When coupled with process flow models, the information in Table 3 can help assess nitrogen removal treatment technologies under development, and prioritize research or technology development needs for meeting nitrogen removal treatment goals.

126 **Table 2:** Potential metabolisms present in a nitrogen removal system when sulfide, methane,
127 acetate (a volatile fatty acid prominent in anaerobic effluents), and ammonium are present.

Relevant Processes		Metabolism	Catabolic Stoichiometric Equation	Free Energy of Reaction (1 bar, 25°C)[a]
Nitrification	Nitritation	Ammonium Oxidation[53]	$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$	-190 kJ/mol NH_4^+
		Nitrite Oxidation[53]	$NO_2^- + 0.5O_2 \rightarrow NO_3^-$	-79 kJ/mol NO_2^-
Denitrification	Denitritation	Anaerobic Ammonium Oxidation[b] (anammox)[54]	$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$	-360 kJ/mol NH_4^+
		Denitrifying anaerobic methane oxidation (damo)[55]	$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 4N_2 + 3CO_2 + 10H_2O$	-1050 kJ/mol CH_4
		Sulfide-based denitritation[56]	$3HS^- + 5H^+ + 8NO_2^- \leftrightarrow 3SO_4^{2-} + 4N_2 + 4H_2O$	-990 kJ/mol HS^-
		Heterotrophic denitritation[53]	$3CH_3COO^- + 8NO_2^- + 11H^+ \leftrightarrow 4N_2 + 6CO_2 + 10H_2O$	-1100 kJ/mol CH_3CHOO^-
	Denitratation	damo[57]	$5CH_4 + 8NO_3^- + 8H^+ \rightarrow 4N_2 + 5CO_2 + 14H_2O$	-830 kJ/mol CH_4
		Sulfide-based denitrification[56]	$5HS^- + 3H^+ + 8NO_3^- \leftrightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$	-770 kJ/mol HS^-
		Heterotrophic denitrification[53]	$5CH_3COO^- + 8NO_3^- + 13H^+ \leftrightarrow 4N_2 + 10CO_2 + 14H_2O$	-910 kJ/mol CH_3CHOO^-
Aerobic processes		Methane oxidation[58]	$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	-820 kJ/mol CH_4
		Sulfide oxidation[59]	$HS^- + 2O_2 \leftrightarrow SO_4^{2-} + H^+$	-760 kJ/mol HS^-
		Heterotrophic oxidation[60]	$CH_3COO^- + 2O_2 + H^+ \leftrightarrow 2CO_2 + 2H_2O$	-890 kJ/mol CH_3CHOO^-
		Sulfate reduction[61]	$CH_3COO^- + SO_4^{2-} + 2H^+ \leftrightarrow HS^- + 2CO_2 + 2H_2O$	-140 kJ/mol CH_3CHOO^-

128

[a] The free energy of reaction, which was calculated using[51], can be used to determine the true growth yield for each process[52].
[b]Nitrate is produced from anammox as a result of anabolic reactions.

Table 3: Median kinetic (maximum specific growth rate, μ_{max}, half-saturation constants for electron donor, K_{donor}, and acceptor, $K_{acceptor}$) and stoichiometric (cell growth yield) parameters for relevant biological processes. Underlined values were obtained with pure cultures. With the exception of † which have a temperature range between 20-38° C, all kinetic and stoichiometric parameters were determined between 17-30° C. For all references and ranges see supplementary information.

Organism	μ_{max} (day^{-1})	Observed Yield		K_{donor}			$K_{acceptor}$		
	Median	Median	Units	Median	Donor	Units	Median	Acceptor	Units
Ammonium Oxidizing Microorganisms (AOM)	1.16[62]	<u>0.12</u>[63]	g COD_x/g N	<u>0.7</u>[64]	NH_4^+	mg N/L	0.8[49]	O_2	mg O_2/L
Nitrite Oxidizing Bacteria (NOB)	0.65	0.08[49]	g COD_x/g N	0.9	NO_2^-	mg N/L	1[60]	O_2	mg O_2/L
Methane Oxidizing Bacteria	<u>3.0</u>[65]	0.06[66]	g COD_x/g COD_s	<u>0.44</u>[67]	CH_4	mg COD/L	0.05	O_2	mg O_2/L
Sulfide Oxidizing Bacteria	3.25	0.32[61]	g COD_x/g COD_s	21	HS^-	mg S/L	3[59, 68]	O_2	mg O_2/L
Nitrite-damo	0.035	0.038	g COD_x/g COD_s	0.32[69]	CH_4	mg COD/L	0.6[70]	NO_2^-	mg N/L
Nitrate-damo†	0.8	0.09	g COD_x/g COD_s	5.44[71]	CH_4	mg COD/L	0.11[72]	NO_3^-	mg N/L
Anammox†	0.121	0.11	g COD_x/g N	0.1[73]	NH_4^+	mg N/L	0.27	NO_2^-	mg N/L
Sulfide-based denitrification	3.24[74]	1.4	g COD_x/g S	1.1	HS^-	mg S/L	5.5	NO_3^-	mg N/L

3.1 Competition for oxygen in downstream nitrogen removal systems

The reduced nitrogen in anaerobic effluents has to be oxidized to produce nitrite or nitrate to achieve anaerobic biological nitrogen removal. Directing nitrification processes through nitrite[75-77] (nitritation) as opposed to nitrate (nitratation) is preferable because it reduces aeration demand by 25% based on stoichiometric estimates. If partial nitritation is desired, where about half of the ammonium is oxidized to nitrite so that both are present to support anammox, then stringent control strategies or novel reactor configurations are needed, as discussed in section 4 and 5. Once oxygen is introduced, however, other competing microbially-mediated aerobic reactions also occur. For instance, dissolved methane coupled with aeration supports the growth of methanotrophic bacteria. Information in Table 3 suggests that aerobic methanotrophs (median K_{O2}=0.05 mg/L) will out-compete ammonia oxidizing microorganisms (AOM) (median K_{O2}=0.8 mg/L) for oxygen, which has been experimentally corroborated[78, 79]. Although aerobic

147 methanotrophy represents an aeration (and, therefore, energy) demand that does not contribute to
148 nitrogen removal, it also mitigates greenhouse gas emissions by preventing methane stripping.
149 Sulfide exerts an abiotic oxygen demand because sulfide is rapidly oxidized in the presence of
150 trace metals[80, 81] but sulfide oxidizing bacteria are not expected to compete for oxygen with
151 nitrifiers and methane oxidizing bacteria, based on the values in Table 3. Finally, any residual
152 VFA will be rapidly oxidized by heterotrophic bacteria under aerobic conditions at a faster rate
153 than all other biological processes listed in Table 3[49].

154 Multiple factors (in addition to aeration) can be used to control the growth competition between
155 AOM and NOB (see Section 4). Furthermore, bioreactor design (discussed in Section 5) can
156 influence which metabolic processes succeed. Overall, it is clear that there are complex
157 interactions between a number of aerobic microbial metabolic processes that occur when treating
158 methane- and sulfide-ladened anaerobic effluents and will contribute to or interfere with the goal
159 of nitrogen removal. This strengthens the argument for using computational models of new
160 treatment technology concepts to elucidate likely competitive responses, and the need for good
161 kinetic and stoichiometric estimates to do so.

162 **3.2 Potential denitrification pathways**

163 Biological nitrogen removal processes use an electron donor to reduce nitrite or nitrate,
164 preferably to innocuous N_2 gas. Conventional wastewater treatment systems use organic matter
165 as the electron donor for heterotrophic denitrification to achieve nitrogen removal. Excellent
166 nitrogen removal (i.e., total nitrogen concentrations below 4 mg N/L) can be achieved when the
167 influent COD/NH_3 ratio is > twelve[49]. In many instances where conventional treatment
168 approaches are used, wastewater streams are organic carbon-limited and supplemental organic
169 matter must be provided at an economic and environmental life cycle cost[82, 83]. Conversely,

nitrogen removal from anaerobic effluents can proceed via multiple electron donors that include dissolved methane, ammonium, and sulfide in addition to residual biodegradable organic matter that exists mostly as volatile fatty acids (VFAs)[29, 30]. As noted in Table 1, anaerobic effluents have very high COD/NH_3 ratios and, therefore, typically have sufficient electron donor to meet excellent total nitrogen removal goals. Note, however, that methane and sulfide represent well more than 50% of the electron donor in these effluents, although full-scale systems have not yet been designed that use them to achieve mainstream nitrogen removal. Herein lies an opportunity for innovative nitrogen removal via one or more of these novel electron donors.

Anammox (ammonium as electron donor) and damo (methane as electron donor) are oxygen-sensitive[84, 85] anaerobic microbial metabolic processes that can achieve nitrogen removal. While our understanding of how to use anammox for denitritation in mainstream applications is growing[76, 77, 86-89], damo-based mainstream nitrogen removal is in its infancy[19, 20]. Nitrite or nitrate can be used as electron acceptor to oxidize methane (damo) while meeting nitrogen removal goals and reducing methane emissions. Nitrous oxide emissions from damo have not been reported[90] and, in the case of nitrite-damo, are unlikely to be produced because the enzymatic denitritation pathway does not involve nitrous oxide[91]. Similarly, nitrous oxide emissions are diminished in anammox systems[92, 93]. Nitrite-based damo has a particularly slow maximum specific growth rate relative to nitrate-based damo and anammox (Table 3), and both damo and anammox have slow maximum specific growth rates relative to heterotrophic denitrification, suggesting that long solids residence times (SRTs) are needed to retain the former. Between the demand for long SRTs and the dilute biomass concentrations expected in mainstream damo or anammox applications versus side stream (high strength) applications,

biofilm treatment process configurations should be used[94]. Indeed, attempts to limit biomass washout of nitrite-damo with biofilms showed improved nitrite reduction rates[20].

Both experiments and modeling exercises offer insight into how damo and anammox may compete in systems treating anaerobic effluents. Early enrichments of suspended growth co-cultures of anammox and nitrite-dependent damo suggested they would only coexist under ammonium-limited conditions[95], which slow anammox growth. Modeling results support this finding and show that anammox out-competes damo for nitrite at higher ammonium concentrations[70]. Other suspended and biofilm enrichment studies found that nitrate- and to a lesser extent nitrite-dependent damo and anammox coexist[96, 97]. Nitrate-dependent damo and anammox can coexist because they do not compete for substrates; furthermore, damo can use the nitrate produced by anammox[96]. In an interesting twist, aerobic methanotrophs can support heterotrophic denitrification by converting dissolved methane into methanol, which can be used as an electron donor (reviewed by[98]). One study found an aerobic methanotroph that reduced nitrate to nitrous oxide under low oxygen conditions[99]. Others showed that aerobic methanotrophs can oxidize ammonium to nitrite[100] and emit nitrous oxide in the process[101, 102]; however, it is unclear if this process can successfully compete with AOMs to achieve partial nitritation of anaerobic effluents.

While the competition between anammox and damo is complex and multifaceted, addition of sulfide oxidation makes it more so. Microbial sulfide oxidation via nitrite and nitrate is very rapid relative to other processes that use these same electron acceptors (reviewed by [103]). For example, denitrification rates are faster with sulfide as the electron donor than with methanol[37]. Additionally, sulfide can be partially oxidized to elemental sulfur or sulfite, and both can be used as an electron donor for denitrification[104, 105]. In one study of industrial wastewater, 90% total

nitrogen removal when anammox and sulfide-based denitrification via nitrate (produced by the anammox process) were coupled[106]. Interestingly, sulfide inhibits AOM and NOB at concentrations around 3 and 1 mg S/L[107], respectively; therefore, differential sulfide-driven inhibition of NOB over AOM could be a strategy for achieving nitritation[108]. Sulfide also inhibits anammox, but studies are conflicted over how sensitive it is since inhibition half saturation constants (K_I) range from 0.3[109] to 30[110] mg S/L. Finally, sulfide inhibits heterotrophic reduction of nitrous oxide to nitrogen gas and can lead to increased nitrous oxide emissions[111-113]. In total, sulfide plays a very complex yet important role during nitrogen removal from anaerobic effluents and also offers interesting possibilities as a useful electron donor; in turn, it is a high priority for further study.

4. Aeration Control to Sustain Nitritation

Removing nitrogen from anaerobic effluents using nitrite as the electron acceptor can save energy by reducing aeration, but requires a strategy that out-selects (or prevents growth of) NOB. Out-selection strategies have been demonstrated for side-stream treatment of anaerobic digester reject waters and include using elevated temperatures[114, 115], high free ammonia (FA) concentrations[116-118], high free nitrous acid concentrations[117-119], and high pH[120]. However, these strategies are unsuitable for mainstream processes, which have ammonium concentrations up to 50 times lower than anaerobic digesters that treat waste sludge, have large volumes that are impractical to heat, and are too dilute for FA inhibition. Therefore, alternative strategies tailored to mainstream applications are needed.

Given the higher relative median oxygen affinity (lower K_{O2}) of AOM over NOB (Table 3), dissolved oxygen (DO) control has been used to achieve stable nitritation in sidestream[121] and

mainstream[122] processes. The actual DO used to achieve NOB out-selection is complicated by the fact that oxygen affinity varies among different genera of NOB[76, 123, 124]. Several mainstream studies have successfully out-selected NOB at DO concentrations at or greater than 1.5 mg/L[23, 76, 86, 88, 125]. It has also been shown that NOB exhibit a period of reduced growth rate following anoxic disturbances[125, 126] (called transient anoxia), and this phenomenon has been used to achieve NOB out-selection[76]. Using transient anoxia to support nitrite-mediated nitrogen removal from anaerobic effluents requires consideration of the dissolved VFAs, methane and sulfide present in anaerobic effluents, which can aid in achieving rapid anoxia when they are biotically or abiotically oxidized via DO.

A key development that has enhanced the application of nitritation in mainstream processes is sensor-mediated online control systems that are reliable and durable. Newer, more robust sensor technologies for monitoring ammonia[76, 127], DO[76, 127], nitrite[128] and nitrate[76, 127, 128] are being used successfully in full-scale systems and increasingly for nitritation control[76, 129, 130]. Although online control for mainstream nitrogen removal from anaerobic effluents is in its infancy with no published studies to date, it has been shown that sensor-mediated control can help achieve stable, mainstream anammox downstream of aerobic carbon removal[76]; these experiences can inform strategies for sensor-mediated online control of nitrogen removal from anaerobic effluents. An example of sensor- mediated online control is ammonia-based aeration control where on-line ammonia sensors are used to control the duration of aeration[86] or DO setpoint[85] to achieve NOB out-selection and ensure partial nitritation where only a fraction of the ammonium is oxidized to nitrite so that both nitrite (electron acceptor) and ammonium (electron donor) coexist to support anammox. In one pilot scale study[76], the duration of aeration was decreased when the ammonium to nitrite plus nitrate ratio was less than one. Subsequently, aeration was gradually

increased again once a predetermined minimum duration was reached. In essence, this strategy controlled the aerobic solids residence time (SRT) to only allow ammonium oxidation, and was combined with transient anoxia to improve NOB out-selection. The innovative combination of ammonia-based control of aerobic SRT and transient anoxia should also help achieve NOB out-selection in anaerobic effluents. Another control strategy for nitrite-mediated nitrogen removal in mainstream applications involves pH-based control[131]. For example, pH-based control of feeding and aeration was applied to a lab-scale sequencing batch reactor system modeled after the DEMON® process[132] but adapted for mainstream applications[77]. It was deemed successful because higher total nitrogen removal was achieved and less air was used than a system with time-based aeration control. Both of these studies of sensor-mediated control were operated at ambient temperatures (20°C and 25°C); NOB out-selection at lower temperatures is an area of on-going research[133, 134].

While transient anoxia may be used to out-select NOB, some studies have found it promotes nitrous oxide emissions[135] (reviewed by [136]). If transient anoxia is to be implemented, frequent aerobic-anoxic cycling may be needed because it decreases nitrous oxide emissions[137]. There is limited understanding of how process control can be used to minimize nitrous oxide emissions, which is confounded by the dynamic nature of nitrous oxide emissions from wastewater treatment plants[138]. In general, high DO concentrations and one-step systems (one reactor is used with multiple redox zones to achieve all biological treatment goals) instead of multi-step systems (wastewater passes between reactors and the biomass in each is retained and not mixed) reduce nitrous oxide[139, 140]. These findings suggest that nitrous oxide emissions and energy consumption to achieve nitrogen removal from anaerobic effluents are inter-related and should be evaluated simultaneously when reviewing system control strategies.

5. Reactor Configurations

Achieving mainstream nitrogen removal from anaerobic effluents offers unique challenges that demand innovative solutions. First, because anaerobic effluents contain significant amounts of dissolved gases that are difficult to recover for beneficial purposes and damaging if stripped, successful configurations should minimize off-gassing. Second, innovative, energy efficient, mainstream nitrogen removal can occur by either one-step (nitritation plus denitritation together) or two-step (nitritation and denitritation occur separately) processes. These reactor configurations utilize multiple complex microbial metabolisms that achieve nitrogen removal, support NOB out-selection, reduce energy consumption, prevent greenhouse (including nitrous oxide) and corrosive gas emissions, and reduce space requirements. For treatment plants that use aerobic treatment to manage organic carbon, the most energy efficient means to achieve denitrification is by anammox[141]; both one- and two-step technologies are being developed around this strategy[76, 86, 87, 142]. However, for systems that treat organic carbon anaerobically, multiple electron donors capable of denitrification are present and dissolved gases may replace or supplement ammonia as an electron donor.

5.1 Designing reactor configurations to minimize gaseous stripping

The dissolved gases in anaerobic effluents are prone to off-gassing. Bubbling air into a reactor where influent concentrations of dissolved gasses are highest makes a system more vulnerable to gas stripping[143], and prevents use of the dissolved gases as electron donors. Reactor configurations that avoid use of bubbles or extensive agitation during anaerobic nitrogen removal are needed and some promising options are discussed in Sections 5.2 and 5.3. Furthermore, advances in technologies that recover dissolved methane from anaerobic effluents in an energy efficient manner without diluting the gas with carbon dioxide and hydrogen sulfide are greatly

needed but, to date, do not exist[33]. If a less energy intensive method for recovering dissolved methane is developed, enough electron donor is likely to remain in anaerobic effluents to support nitrogen removal from domestic wastewater via sulfide and VFA (161 ± 95 mg/L COD electron donor available, well above nitrite or nitrate-based denitrification demand as given in Table 1); however, gas stripping should still be avoided to prevent loss of hydrogen sulfide. Consequently, reactor configurations that prevent gas stripping and encourage biological reactions that consume dissolved gases are needed.

5.2 One-step nitrogen removal systems

We contend that biofilms are preferred for one-step systems designed to achieve anaerobic nitrogen removal because slow growing and low yielding anammox, damo and anaerobic sulfur oxidizing microorganisms would be retained more effectively than in suspended culture systems. Because nitrite is the preferred electron acceptor for energy efficient nitrogen removal, one-step systems must be configured so that off-gassing of dissolved electron donors is prevented even though aeration is needed to achieve nitritation. Furthermore, AOM must coexist with anaerobic nitrogen removing microorganisms in one-step systems; unfortunately, biofilm systems make NOB out-selection difficult, but not impossible[86, 87, 92], to achieve. Some biofilm systems are co-diffusional, where the electron donor and electron acceptor diffuse into the biofilm in the same direction. In these systems, aeration to support biological nitritation occurs in the bulk liquid and creates an aerobic outer biofilm layer and anoxic inner layer. To prevent off-gassing of precious electron donors from the bulk liquid, an anoxic phase is needed at the beginning of the process where feed is introduced. We suspect that sequencing between anoxic and aerobic conditions will prevent damo from establishing in co-diffusional one-step systems in lieu of other denitrifiers and that methane oxidation and stripping may still occur during the nitritation phase.

Two types of co-diffusional, one-step biofilm technologies that show great promise to achieve energy efficient nitrogen removal from anaerobic effluents are highlighted: granular sludge systems[144] select for dense biofilm aggregates in a sequencing batch reactor that is exposed to high shear forces and managed with short settling and decant times, and moving bed biofilm reactors (MBBRs) [145] contain an inert carrier that supports biofilm formation. Both technologies are compact have been successfully applied at full-scale for nitrogen removal from centrate[146, 147], and are being evaluated for use in mainstream systems that couple aerobic management of carbon, nitritation and anammox[86, 142, 148]; therefore their use for nitrogen removal from anaerobic effluents would be a new application of the technology.

In contrast to granular sludge systems and MBBRs, membrane biofilm reactors (MBfRs) are one-step biofilm systems that are ideally suited to treat anaerobic effluents. In these systems, oxygen diffuses through biofilm-coated hollow fiber membranes and creates a bubbleless aerobic zone adjacent to the membrane and an anaerobic zone at the biofilm surface. If used with an anaerobic effluent, the electron donors would diffuse from the anaerobic bulk liquid into the biofilm across anaerobic then aerobic zones. In contrast, electron acceptors diffuse from the membrane lumen or are biological formed and then diffuse into the biofilm in the opposite direction; hence, these systems are counter-diffusional[149]. The biofilm-coated membrane provides bubbleless aeration, which prevents gas stripping[150, 151], and the counter-diffusion configuration allows for separate control of electron donor mass loading rates and aeration rates via the membrane lumen. These systems achieve efficient aeration; one study showed oxygen transfer efficiencies of 20-35%[152]. With one exception[153], nitrogen removal from wastewater using MBfRs has been limited to lab-scale demonstrations of high strength wastewater[92, 154, 155]

and therefore the technology is still under development; however; it holds great promise as long as reliable NOB out-selection[149, 156, 157] can be achieved.

5.3 Two-step nitrogen removal systems

Separating nitritation and denitritation steps offers simpler control, allows for a combination of suspended and/or biofilm processes to be used, and may be the easiest to deploy by retrofitting existing infrastructure; however, it requires a larger treatment footprint. To prevent loss of dissolved electron donor gases, a denitritation tank can precede the nitritation tank and achieve nitrogen removal via recycled or internally recirculated nitrite using a configuration that resembles a Modified Ludzack Ettinger (MLE) system. Although internal recirculation provides more nitrite flux and higher levels of total nitrogen removal[49] it comes at an energy cost; one study of an MLE process requiring 10 mg/L effluent total nitrogen found that the energy required for recirculation pumping comprised 9% of the total energy needed to operate the process[158]. For the denitritation step, a biofilm system will retain the slow-growing denitrifying microorganisms best along with VFA-consuming denitrifiers. A number of energy efficient configurations could be used, including MBBRs or fluidized bed systems, where an inert carrier supports biofilm formation.

6. Moving Forward

Coupling nitrogen removal with anaerobic treatment of domestic wastewater requires improved understanding of the key microbial metabolic processes involved and presents opportunities to create novel reactor configurations and sensor-based control strategies. We contend that bioprocess models, which can be validated in lab- and pilot-scale studies, are an integral step to developing these treatment technologies because they help us understand how different reactor

configurations and operating strategies control the complex microbial population interactions within them. Significant research efforts are underway to identify functional and reliable reactor configuration and operational strategy combinations that support the microbial metabolisms needed to achieve energy efficient nitrogen removal goals. Advancing these technologies is critical to making energy neutral or positive wastewater treatment via anaerobic mainstream treatment economically viable in many populated regions around the world where total nitrogen regulations are in place or anticipated for wastewater treatment systems.

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Supporting Information Available

The Supporting Information includes complete kinetic and stoichiometric parameter literature review and schematics of the reactor configurations considered. This material is available free of charge via the Internet at http://pubs.acs.org/journal/estlcu.

The authors declare no competing financial interest.

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Prospects for Biological Nitrogen Removal from Anaerobic Effluents during Mainstream Wastewater Treatment

Jeseth Delgado Vela, Lauren B. Stadler, Kelly J. Martin, Lutgarde Raskin, Charles B. Bott, Nancy G. Love*

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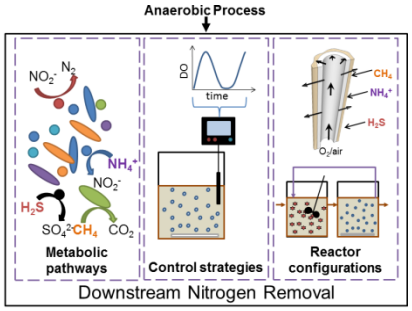

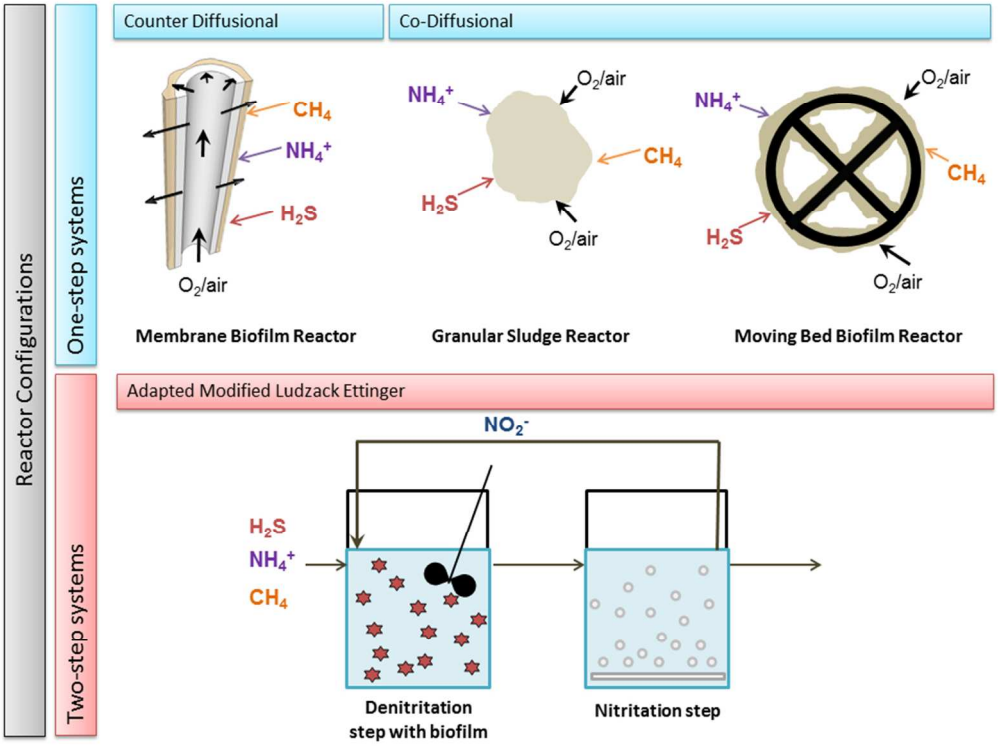

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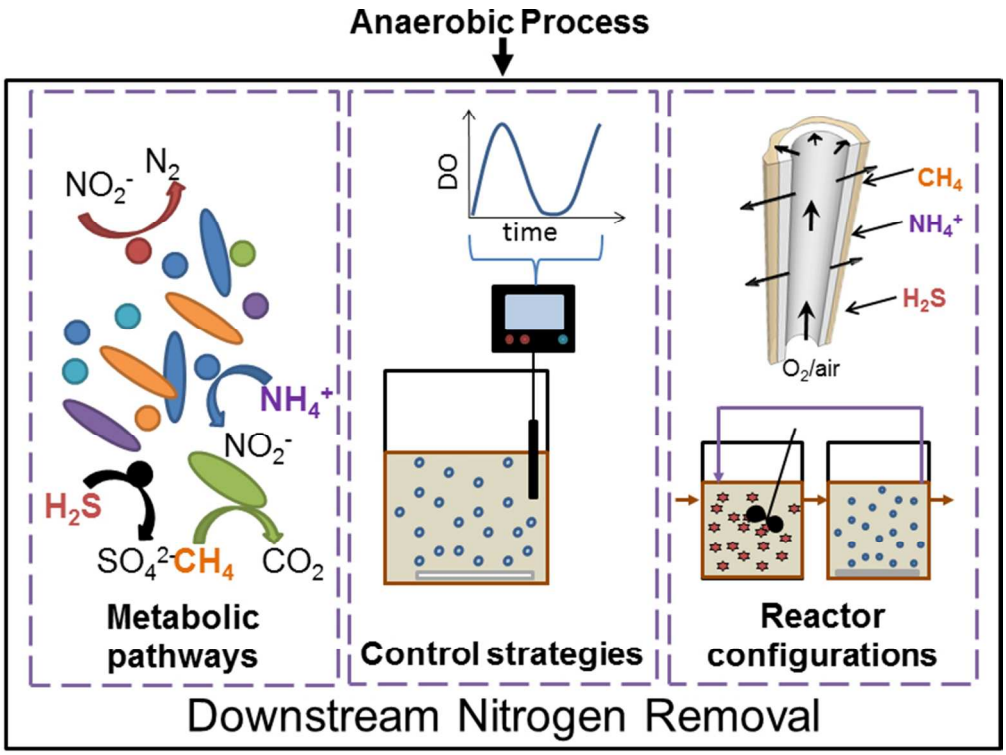

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