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Reducing Cost and Environmental Impact of Wastewater Treatment with Denitrifying Methanotrophs, Anammox, and Mainstream Anaerobic Treatment

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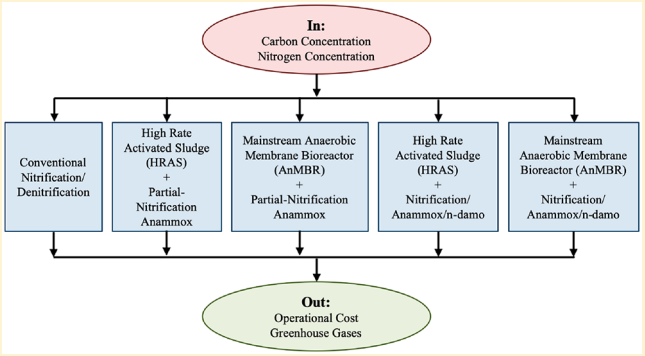
Supporting Information

ABSTRACT: In water resource recovery facilities, sidestream biological nitrogen removal via anaerobic ammonium oxidation (anammox) is more energy and cost efficient than conventional nitrification-denitrification. However, under mainstream conditions, nitrite oxidizing bacteria (NOB) out-select anammox bacteria for nitrite produced by ammonium oxidizing bacteria (AOB). Therefore, nitrite production is the bottleneck in mainstream anammox nitrogen removal. Nitrate-dependent denitrifying anaerobic methane oxidizing archaea (n-damo) oxidize methane and reduce nitrate to nitrite. The nitrite supply challenge in mainstream anammox implementation could be solved with a microbial community of AOB, NOB, n-damo, and anammox with methane from anaerobic sludge digestion or a mainstream anaerobic membrane bioreactor (AnMBR). The cost and environmental impact of traditional nitrification/dentrification relative to AOB/anammox and AOB/anammox/n-damo systems, with and without an AnMBR, were compared with a stoichiometric model. AnMBR implementation reduced costs and emission rates at moderate to high nutrient loading by lowering aeration and sludge handling demands while increasing methane available for cogeneration. AnMBR/AOB/anammox systems reduced cost and GHG emission by up to \$0.303/d/m^3 and 1.72 kg equiv. CO_2/d/m^3, respectively, while AnMBR/AOB/anammox/n-damo systems saw a similar reduction of at least \$0.300/d/m^3 and 1.65 kg equiv. CO_2/d/m^3 in addition to alleviating the necessity to stop nitrification at nitrate, allowing easier aeration control.



INTRODUCTION

Wastewater treatment accounts for 2% of the U.S energy budget.[1] Two major costs of water resource recovery facility (WRRF) operation are sludge handling, which can account for up to 60% of total operational cost, and aeration, which makes up 40−60% of energy usage.[2,3] Aeration demand is related to oxygen requirements of aerobic organisms consuming ammonium (NH_4-N) and organic carbon (measured in chemical oxygen demand or COD) in the influent. Sludge handling requirements are driven by the biomass yield of organisms involved in the treatment process. Additionally, some WRRFs require external carbon addition for complete nitrogen removal if the COD/N influent ratio is too low for complete denitrification. Meanwhile, 0.5% of greenhouse gas (GHG) emissions produced in the U.S. are contributed by WRRFs.[4] Two primary GHGs of concern in WRRF operation are methane (CH_4) and carbon dioxide (CO_2).[5] Although nitrous oxide (N_2O) is also a contributor to GHG emissions from WRRF (with a global warming potential 298 times that of CO_2), it is difficult to include N_2O into stoichiometric modeling as the microbial origin, amounts emitted, and conditions triggering its emission are controversial.[6−10] CH_4 has a global warming potential of approximately 34 times that of CO_2. Therefore, even small quantities can greatly impact atmospheric quality.[11] Thus, the reduction of GHG emissions and energy consumption by wastewater treatment while maintaining high effluent water quality will be imperative to ensure long-term air, water, and energy security.

In order to reduce the cost and carbon footprint of WRRF operation, much effort has been put into developing technologies that (1) demand minimal oxygen supply, (2) produce little sludge, and (3) require no external carbon addition for nutrient removal, while (4) reducing GHG emissions.[12] Anaerobic ammonia oxidizing (anammox) bacteria provide one such solution in nitrogen removal. Anammox

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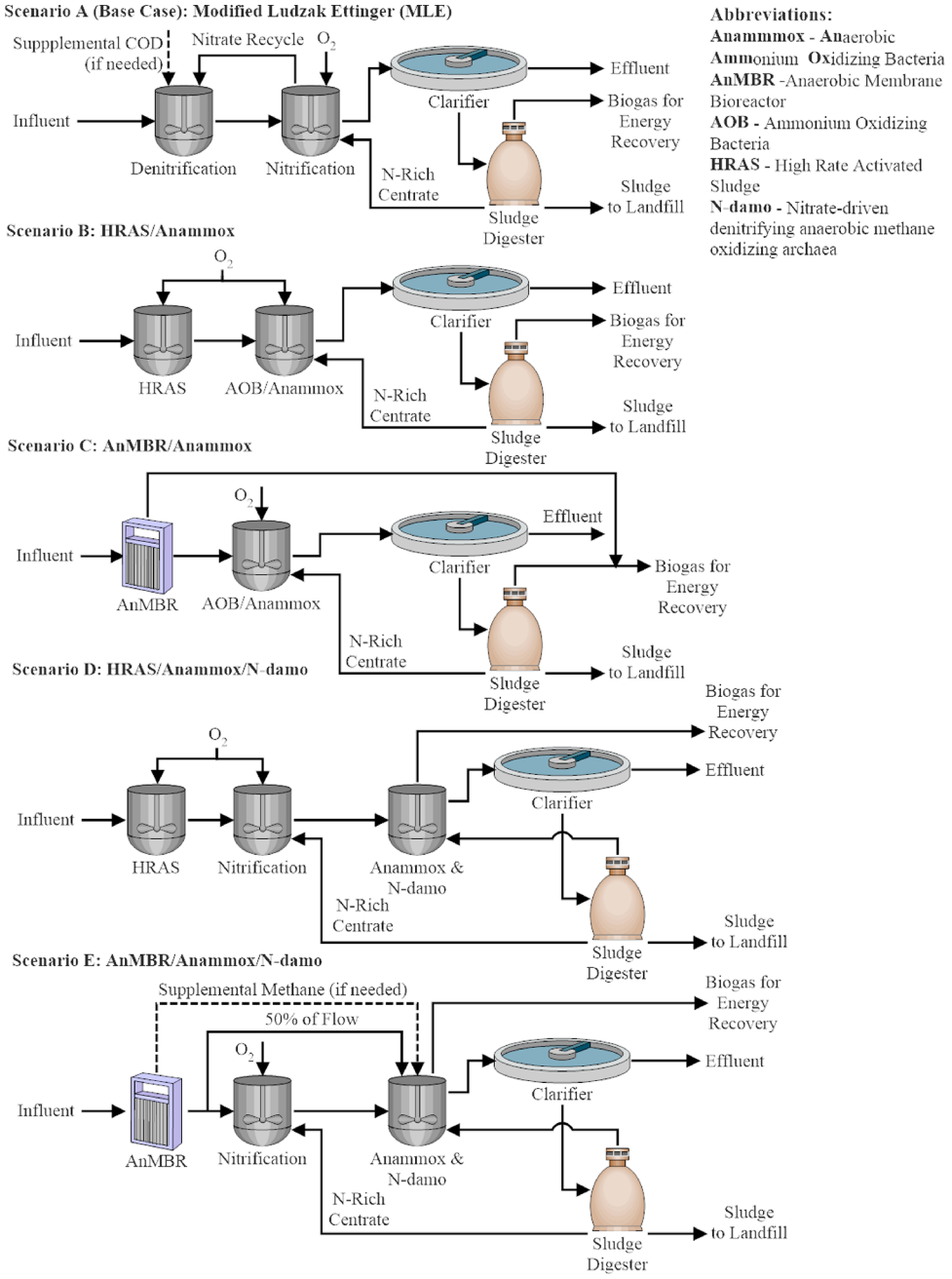

Figure 1. (A, the base case) traditional nitrification/denitrification. (B) High Rate Activated Sludge (HRAS) and AOB/anammox for nitrogen removal. (C) Anaerobic Membrane Bioreactor (AnMBR) and AOB/anammox. (D) HRAS and nitrification/anammox/n-damo for nitrogen removal. (E) AnMBR and nitrification/anammox/n-damo.

bacteria are autotrophs that anaerobically oxidize ammonium to dinitrogen gas utilizing nitrite as an electron acceptor, reducing aeration demands by nearly 60%, sludge production by 90%, and eliminating the need for external carbon.[13−15] Anammox has been implemented for nitrogen removal in the side stream (1% of total volumetric flow) at many full-scale installations treating warm (>35 °C) ammonium (NH_4^+) rich digester reject water.[16] Ammonium oxidizing bacteria (AOB) supply the nitrite (NO_2^-) for anammox while the competing activity of nitrite oxidizing bacteria (NOB) are suppressed by low dissolved oxygen levels or free ammonia.[17−19] Implementation of anammox for mainstream nitrogen removal (99% of volumetric flow) with high-rate activated sludge carbon removal is gaining attention because it is theoretically posited to be an energy-neutral system as shown by other stoichiometric models.[20] However, anammox has been almost entirely limited to sidestream centrate treatment because of the difficulty limiting NOB activity at colder mainstream temperatures, while also meeting stringent nitrogen removal requirements.[21−23] A more robust configuration is needed to combine anammox with a process that can supply nitrite to anammox without relying on NOB out-selection.

A metabolic pathway that could be utilized to supply anammox with nitrite without NOB competition is nitrate-driven anaerobic methane oxidation (n-damo), in which a newly discovered archaea, *Ca. Methanoperedens nitroreducens*, are capable of using methane as an electron donor to reduce nitrate to nitrite[24]

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$$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O$$

Previous work has demonstrated a sustained coculture dominated by anammox and n-damo archaea capable of removing nitrogen,[24,25] and bench scale studies have demonstrated complete high-rate nitrogen removal, outcompeting the nitrite-driven anaerobic methane oxidizing bacteria (*Ca. Methylomirabilis oxyfera*).[26−28] The n-damo bacteria and anammox are in direct competition for nitrite, but modeling work has shown that n-damo bacteria are only able to survive in the presence of excess nitrite because they have a lower affinity for nitrite than anammox.[29] In a well-controlled n-damo archaea/anammox system, nitrite would be fully consumed, eliminating the environmental niche required for n-damo bacteria. Therefore, n-damo archaea/anammox nitrogen removal systems could theoretically be scaled up for mainstream nitrogen removal.[30,31] In this concept, half of influent ammonium is oxidized to nitrate, reducing oxygen demand by 50%. The nitrate- and ammonium-rich streams are then fed to an anaerobic anammox/n-damo reactor supplied with methane. The n-damo archaea reduce nitrate with methane, and the resulting nitrite is available for anammox bacteria to convert the remaining ammonium to dinitrogen (N_2) gas. In such a system, methane could be supplied into the n-damo reactor either dissolved in solution or as a gas from biogas produced by anaerobic digestion.[25,26]

Another new technology proposed for energy-neutral wastewater treatment is the anaerobic membrane bioreactor (AnMBR), which can provide a high methane yield while efficiently removing organic carbon with minimal sludge production.[32−35] AnMBR treatment holds great potential for the future of wastewater treatment, as it is predicted to produce more energy and thus have lower greenhouse gas emissions than high-rate activated sludge (HRAS) systems.[36] Organic carbon is converted into methane rich biogas for energy generation instead of being aerobically oxidized with high energy costs in HRAS.[37] Despite immense potential for lower operational costs, recent life cycle analysis research reveals that one major disadvantage to AnMBRs is downstream GHG emissions due to the high concentrations of dissolved methane in the effluent.[32] Yet, because n-damo archaea require a source of dissolved methane, AnMBRs could have a powerful synergy with n-damo/anammox systems, where simultaneous methane and ammonium removal is achieved anaerobically.

There has been little attempt to compare financial and environmental benefits of anammox paired with AnMBR or n-damo to existing secondary treatment technologies.[38] In this study, theoretical mainstream technologies (HRAS/anammox, AnMBR/anammox, HRAS/anammox/n-damo, and AnMBR/anammox/n-damo) were compared to a traditional nitrification/denitrification system for carbon and nitrogen removal. The impact of aeration demand, sludge handling, external carbon addition, and methane for energy generation on both economic and environmental feasibility was considered.

MATERIALS AND METHODS

Five scenarios were investigated in this study: (A, base case) traditional nitrification/denitrification, (B) High Rate Activated Sludge (HRAS) COD removal followed by AOB/anammox (aerobic and anaerobic ammonium oxidation), (C) mainstream AnMBR followed by AOB/anammox, (D) HRAS followed by nitrification/anammox/n-damo, and (E) mainstream AnMBR (anaerobic membrane bioreactor) followed by nitrification/anammox/n-damo (Figure 1). Because the objective of this study was to examine theoretical nitrogen removal technologies and nitrogen removal is most challenging at low COD/N ratios, a wide range of typical nitrogen concentrations were explored (0.6−60 mgN-NH_4/L) while a moderate to low range of COD influent concentrations were explored (4−400 mg-COD/L). All calculations were done assuming a nominal plant flow rate of 60 th m^3/day. All COD was presumed 100% biologically degradable. All results were reported in comparison to the base case as a percent increase or decrease.

The model estimated how changes in oxygen demand, sludge production, biogas available for energy recovery, and external carbon addition (if applicable) influenced operational costs. Oxygen demand was estimated as the total oxygen required to remove maximum possible nitrogen and COD. The rate of biological decay can vary significantly, especially when comparing a highly controlled bench experiment to a full scale WRRF. An overestimation of this value would lead to the calculation of an unrealistically low sludge production rate. Therefore, the rate of decay was not included in calculations to ensure that sludge handling demands would not be underestimated. Biomass produced by all organisms involved in each scenario were considered to be waste activated sludge (WAS) fed to anaerobic digestion. A volatile solids reduction of 59% in the digester was assumed.[37] Methane production was assumed to be equivalent to 100% of the COD reduced in anaerobic sludge digestion and AnMBR. Biomass yield from sludge digestion was not considered. External carbon addition was only required if not enough COD was available for nitrogen removal.

Calculating Differences in Greenhouse Gas Emissions and Cost. The primary drivers of both cost and GHG emissions were assumed to be external COD added, oxygen demand, sludge produced, and methane produced. These metrics were combined together into an equivalent kg CO_2 emitted or cost in USD. Resulting GHG emission rates and costs were used as a direct comparison of the theoretical technologies explored in scenarios B−E to a conventional nitrification/denitrification plant by taking the difference in the calculated combined metrics ($kgCO_2$ or USD per day per m^3) in scenario A and subtracting it from the combined metrics calculated in scenarios B−E. The resulting value represents the relative difference in GHG emissions or cost between theoretical and conventional technologies (Figures 2 and 3). Literature values for a plant with a nominal flow rate of 60 th m^3/day were used to estimate the contribution external COD added, oxygen demand, sludge produced, and methane produced to greenhouse gas emissions in equivalent kg CO_2 and operational cost in USD. The estimated calculations are provided in the Supporting Information, Table S1. It was assumed other factors contributing to cost and GHG emission (i.e., pumping demand, chemicals added, etc.) varied much less than those factors considered in this study.

GHG emission was estimated with CO_2 emissions originating from plant electrical demand, CO_2 released from external carbon added, and residual CH_4 emissions released from the plant. Energy demand due to aeration was calculated given the typical energy demand of an aeration blower to be 1.5 kWh/kg dissolved O_2.[37,39] Solids handling energy demand of the anaerobic digester and dewatering system were inferred to be 2.24 kWh/kgVSS given typical values.[1,37] Reproducible calculations for this value are provided in Supporting

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Information, Table S1.[1,37] Electrical demand of 190 kWh/th m^3 for AnMBR operation in scenarios C and E and 26 kWh/th m^3 for mixing an anaerobic compartment in scenarios A, D, and E were also taken from literature and included.[37,40] Finally, the energy recovered from the cogeneration plant was calculated on a kg CH_4 produced basis given the lower heating value of biogas of 22400 kJ/m^3 and a cogeneration plant efficiency of 30%.[37] Additional electrical demands, like pumping requirements, were not included as they were assumed to be a minor contributor to operational costs and greenhouse gas emissions. The potential impact of this assumption on the results of this study was explored in a sensitivity study where the pumping demand of the theoretical scenarios B thru E was varied between 50 and 500% of the base case, scenario A. The impact of pumping demand on our results was determined to have only a minor impact (see Supporting Information, Figures S9 and S10). The electrical demand was converted to offsite CO_2 emission using recent aggregate U.S. emission factors.[41] CO_2 from biogas cogeneration was not included. Biogenic CO_2 was only included if it originated from oxidizing externally added COD, as the methanol and methane purchased by WRRFs typically originates from fossil fuels.[42] CH_4 emissions were estimated from the amount of residual methane emitted from the system and converted to equivalent CO_2.[11,43] N_2O is known to be a significant contributor to GHG, but N_2O metabolic pathways are too complex to be accurately reflected by a stoichiometric model.[6−8] It was therefore decided to omit N_2O production and consumption from this analysis.

Costs of (1) electrical demand on a per kWh basis, (2) landfilling sludge on a per kgVSS basis, and (3) external COD addition on a per kgO_2 basis were calculated from literature and industry provided values and are presented in the Supporting Information, Table S1. The programming language R (version 3.5.2) in RStudio (1.1.419) was used for simulations, and a detailed explanation of the models are given in the Supporting Information. The code used for this analysis can be accessed via the repository located at: https://github.com/cogerk/ndamo-econ.

Scenario A: Conventional Nitrification/Denitrification. Scenario A represents the base case scenario and describes a nitrification/denitrification system.[37] Influent was fed into an anoxic denitrification reactor, which received recycled nitrate from the nitrification reactor. If the COD/N in the influent was too low to remove all nitrogen, then supplemental COD was added. Conversely, if not enough nitrogen was present to remove all COD, residual COD was oxidized aerobically in the nitrification reactor.

Scenario B: High Rate Activated Sludge and AOB/Anammox. The anammox system in Scenario B was based on the system described by Sliekers et al.[16] currently in use at WRRFs worldwide for centrate treatment. The HRAS system was added to aerobically respire COD to CO_2 similar to the theoretical energy-neutral system described by Kartal et al.[20] In order to meet the anammox bacteria metabolic demands, 57% of influent nitrogen must undergo partial nitrification to generate 1.3 mol of nitrite per mole of ammonium (see SI).

Scenario C: AnMBR and AOB/Anammox. Scenario C simulated a mainstream anaerobic membrane bioreactor (AnMBR) for the removal and recovery of organic carbon as biogas. Sludge input to anaerobic digestion was the sludge production from AnMBR and anammox/AOB reactors. The 190 kWh/th m^3 factor included for AnMBR operation represents electrical demand required for the scouring, mixing, and transmembrane pressure application of an AnMBR system.[40]

Dissolved methane concentration from AnMBR effluent was assumed to be 1.5 times the saturation concentration assuming the headspace is 62% v/v methane.[43] Results from Daelman et al. were used to approximate the fate of dissolved methane in the aerated AOB/anammox reactor. A binary scheme based on the biological kinetics of methanotrophs was used. At methane concentrations above the methanotroph affinity, (>5 mgCOD/L), methane would be 90% consumed by methanotrophs and the remaining 10% would be stripped. Below that threshold, 100% of methane would be stripped. It was assumed stripped methane exiting the AOB/anammox reactor was too dilute for energy recovery and escaped, contributing to GHG emissions. Residual dissolved methane not consumed by methanotrophs was also included in GHG emission calculations. All methane emissions were multiplied by 34 CO_2 equivalents based on the potency of methane as a GHG.[11]

Scenario D: High Rate Activated Sludge, Anammox, and n-Damo. In this scenario, COD was removed with HRAS followed by nitrification where half of ammonium was oxidized to nitrate. The nitrate/ammonium rich effluent was fed into a n-damo/anammox reactor in which n-damo archaea supplied anammox bacteria with nitrite to remove ammonium. Methane required for nitrate reduction was supplied to n-damo archaea from digester biogas, and in all scenarios considered, enough methane was available for 100% nitrogen removal; however if that were not the case, methane could be externally purchased.

Scenario E: AnMBR, Anammox, and n-Damo. In scenario E, COD was removed anaerobically via AnMBR. Half of AnMBR effluent was fed to a nitrification reactor where ammonium was converted to nitrate. The remaining half was fed into a n-damo/anammox reactor where dissolved methane was available for n-damo. Oxygen consumption included nitrification and methane oxidation by aerobic methanotrophs. The amount of methane stripped from the nitrification reactor was calculated using the same assumption described in scenario C. Residual dissolved methane not consumed by methanotrophs or stripped from the nitrification reactor was included in the GHG emission calculation by multiplying the amount of methane by 34 CO_2 equivalents based on the potency of methane as a GHG.[11]

RESULTS

The differences in GHG emission in kg equiv. CO_2 and operational cost in USD between each scenario (HRAS/Anammox, AnMBR/anammox, HRAS/anammox/n-damo, AnMBR/anammox/n-damo) and the base case (conventional nitrification/denitrification) are shown in Figures 2 and 3, respectively. Similar comparisons of the oxygen demand, sludge production, external COD added, and methane production are available in the Supporting Information (Figures S6−S8).

Relative Difference in Greenhouse Gas Emissions. GHG emissions due to electrical consumption and escaped methane from WRRFs has a significant impact on total U.S. GHG emissions annually.[44] The GHG emissions produced due to electrical consumption, external COD addition, and escaped methane of scenarios B−E as they compared to the base case of conventional nitrification/denitrification were reported (Figure 2). As with the cost comparison, the two HRAS scenarios (Figure 2, 2.1 and 2.3) and two AnMBR scenarios

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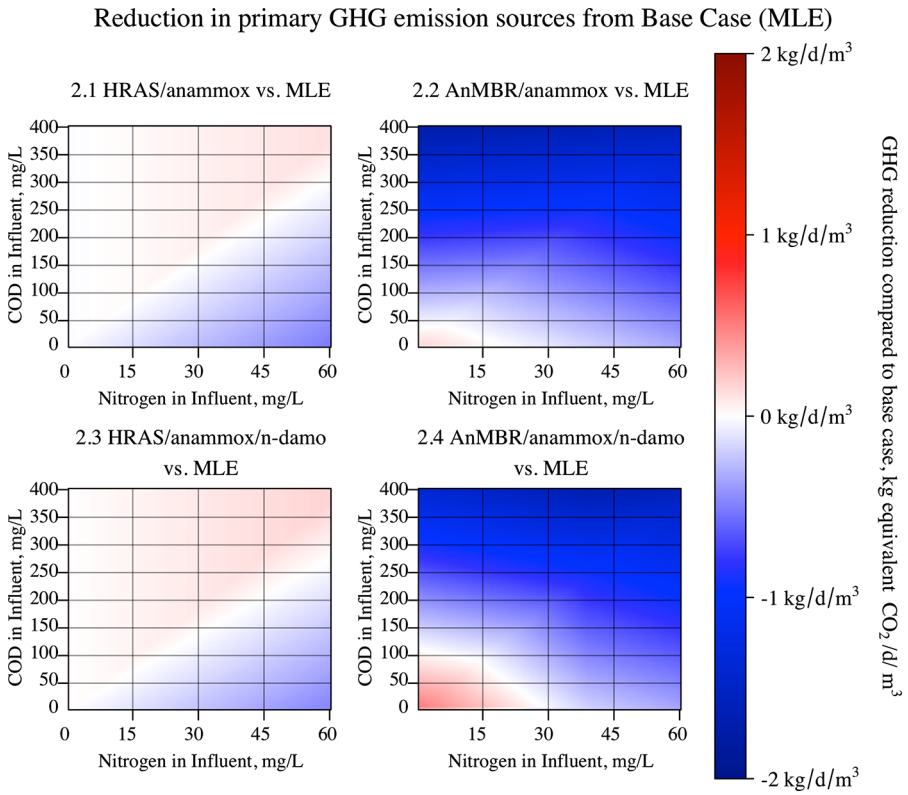

Figure 2. Reduction in GHG emissions possible by implementing four different theoretical treatment technologies: HRAS and partial nitritation-anammox (3.1), AnMBR and partial nitritation-anammox (3.2), HRAS and anammox/n-damo nitrogen removal (3.3), and AnMBR and anammox/n-damo nitrogen removal (3.4) versus Modified Ludzack-Ettinger, MLE, also referred to as conventional nitrification/denitrification. Calculations are performed assuming a plant flow rate of 60 th m^3/day. Greenhouse gases are reported in equivalent kg CO_2/m^3/day saved or additional kg CO_2/m^3/day. Reduction is shown in blue and increase is shown in red as defined by the color key.

(Figures 2, 2.2 and 2.4) appear to be almost identical suggesting that the economic driver for choosing either n-damo or anammox based processes is marginal. The addition of n-damo to an anammox system did not drastically change the GHG emission rate. Anammox systems release slightly fewer GHGs than their anammox/n-damo counterparts (0.07−0.04 kg equiv. $CO_2/d/m^3$ at most conditions) due to extra electrical demand required to supply additional oxygen for full nitrification by NOB to support n-damo with nitrate, thus increasing CO_2 emissions due to electrical demand. In addition, extra sludge production by NOB and n-damo slightly increased GHG emissions due growth yields of the NOB and n-damo populations.

In both the HRAS/anammox and HRAS/anammox/n-damo scenarios, the relative difference in GHG emissions was highly dependent on the COD/N ratios. Emissions were strongly reduced at low COD/N ratios (up to 0.512 fewer kg equiv. $CO_2/d/m^3$ in the HRAS/anammox system and 0.474 fewer kg equiv. $CO_2/d/m^3$ in the HRAS/anammox/n-damo) and slightly increased at COD/N ratios >5. At high ratios, relative emissions increased slightly with nitrogen loading rate (up to 0.122 more kg equiv. $CO_2/d/m^3$ in the HRAS/anammox system and 0.172 more kg equiv $CO_2/d/m^3$ in the HRAS/anammox/n-damo than the base case) due to the higher sludge production rate of aerobic heterotrophs (0.67 gVSS/gCOD) than denitrifying heterotrophs (0.44 gVSS/gCOD) and the additional oxygen demand required to aerobically remove COD that denitrification does not require. At high COD/N ratio and low nitrogen loading, there is no significant difference between the HRAS scenarios and the base case. At these conditions, in the conventional system, there is not enough COD available to support denitrification. Therefore, oxygen was used as an electron acceptor for COD oxidation like the HRAS system.

In both the AnMBR/anammox and AnMBR/anammox/n-damo scenarios (Figure 2, 2.2 and 2.4), difference in GHG emissions was dependent on the total nutrient loading. At moderate to high COD loading, enough methane for energy recovery was produced from the AnMBR to offset energy requirements and escaped methane emissions, resulting in dramatically lower GHG emissions than the base case (up to 1.72 fewer kg equiv. $CO_2/d/m^3$ in the AnMBR/anammox system and up to 1.65 fewer kg equiv. $CO_2/d/m^3$ in the AnMBR/anammox/n-damo system). At high nitrogen and low COD loading, less methane was produced for energy recovery, but less methane escaped and anammox-based nitrogen removal resulted in lower oxygen demand and sludge production, resulting in lower GHG emissions at high nitrogen loading (up to 0.371 fewer kg equiv. $CO_2/d/m^3$ in the AnMBR/anammox system and 0.362 fewer kg equiv. $CO_2/d/m^3$ in the AnMBR/anammox/n-damo system). At low nitrogen and COD loading, electrical demands and methane production are all minimal, so GHG emissions were dominated by escaped methane, resulting in higher emissions in both AnMBR systems as compared to the base case (up to 0.144 more kg equiv. $CO_2/d/m^3$ in the AnMBR/anammox system and 0.489 more kg equiv. $CO_2/d/m^3$). At low loading rates, ~0.35 more kg equiv. $CO_2/d/m^3$ was emitted from the AnMBR/anammox/n-damo system (Figure 2, 2.4) than the AnMBR/anammox system (Figure 2, 2.2) because n-damo did

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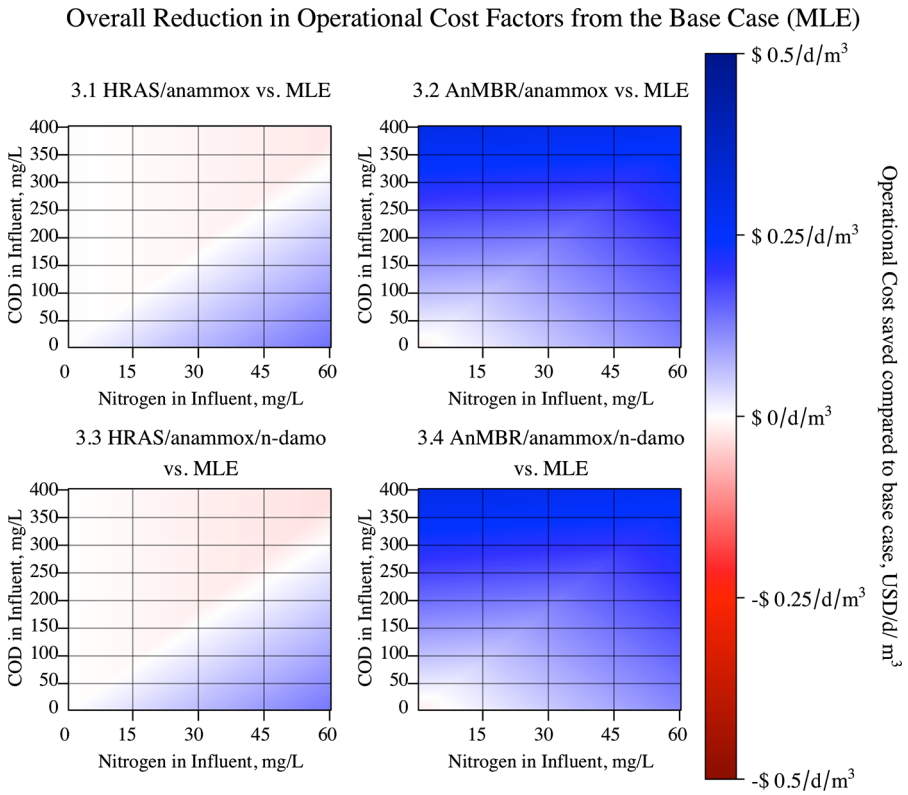

Figure 3. Estimated cost saved by implementing four different theoretical treatment technologies: HRAS and partial nitritation-anammox (2.1), AnMBR and partial nitritation-anammox (2.2), HRAS and anammox/n-damo nitrogen removal (2.3) and AnMBR and anammox/n-damo nitrogen removal (2.4) versus Modified Ludzack-Ettinger, MLE, also referred to as conventional nitrification/denitrification. Calculations are performed assuming a plant flow rate of 60 th m^3/day. USD saved or additional USD cost per m^3 per day shown in blue and red, respectively, as defined by the color key.

not consume all dissolved methane from the AnMBR effluent at low influent nitrogen concentrations, whereas that methane was oxidized by aerobic methanotrophs to CO_2 in AnMBR/anammox systems. The electrical demand required to operate an AnMBR or anaerobic compartments played only a minor role at nearly all conditions.

Relative Difference in Operational Cost. AnMBR, anammox, and n-damo have all been suggested as less expensive alternatives to conventional nitrification/denitrification.[20,30,31,43] The estimated relative difference in cost between all alternatives to a conventional system (shown in Figure 3) follows the same trends in relative difference in GHG emissions (Figure 2). This is understandable given that both operational cost and GHG emissions were assumed to have the same primary drivers: oxygen demand, sludge production, external COD addition, and methane production for cogeneration. However, unlike the GHG emission results reported in Figure 2, both AnMBR systems appear to be a better option than the conventional system at low nitrogen and carbon loading rates. At the lowest possible loading, 4 gCOD/m^3 and 0.6 gN/m^3 the AnMBR/anammox system (Figure 3, 3.2) cost only $0.13 more per day per m^3 and the AnMBR/anammox/n-damo (Figure 3, 3.4) system cost only up to $0.15 more per day per m^3. At these very low loading rates, a plant would likely not employ nutrient removal. Meanwhile, at high COD loading, the costs for AnMBR/anammox and AnMBR/anammox/n-damo are almost the same: up to $0.303 and $0.300 less per day per m^3, respectively.

The costs for the two HRAS systems were very dependent on COD/N ratio. At low COD/N ratios, the HRAS/anammox (Figure 3, 3.1) system cost up to $0.139 less per day while the HRAS/anammox/n-damo (Figure 3, 3.2) system cost up to $0.133 less per day to operate primarily because the base case required costly additional carbon addition at low COD/N ratios while the HRAS systems did not.

At COD/N ratios above 5, the conventional system did not require additional carbon addition, which made HRAS systems slightly more expensive to operate then the base case, and cost increased with total nitrogen loading (up to $0.021 more per day per m^3 in the HRAS/anammox system and up to $0.030 more per day per m^3 in the HRAS/anammox/n-damo system).

DISCUSSION

At moderate to high COD loading rates, the AnMBR systems released fewer GHGs and cost less than the HRAS and base case scenarios, suggesting that a switch to an AnMBR/anammox scheme would save operational cost and lower GHG emissions for WRRFs at these high COD conditions. By direct conversion of COD to methane in the mainstream, enough methane could theoretically be produced to offset not only energy demands but also the impact of escaped methane. At low COD loading rates with low COD/N ratios, the HRAS systems were cheaper and released fewer GHGs than the base case and even AnMBR systems at some loading rates, suggesting that a switch to an HRAS/anammox system would save operational cost and lower GHG emissions for WRRFs at low COD/N ratios. This result is in line with other similar analysis performed on anammox/HRAS systems.[20] In both n-damo scenarios, enough methane was produced from either mainstream or sludge anaerobic digestion to supply n-

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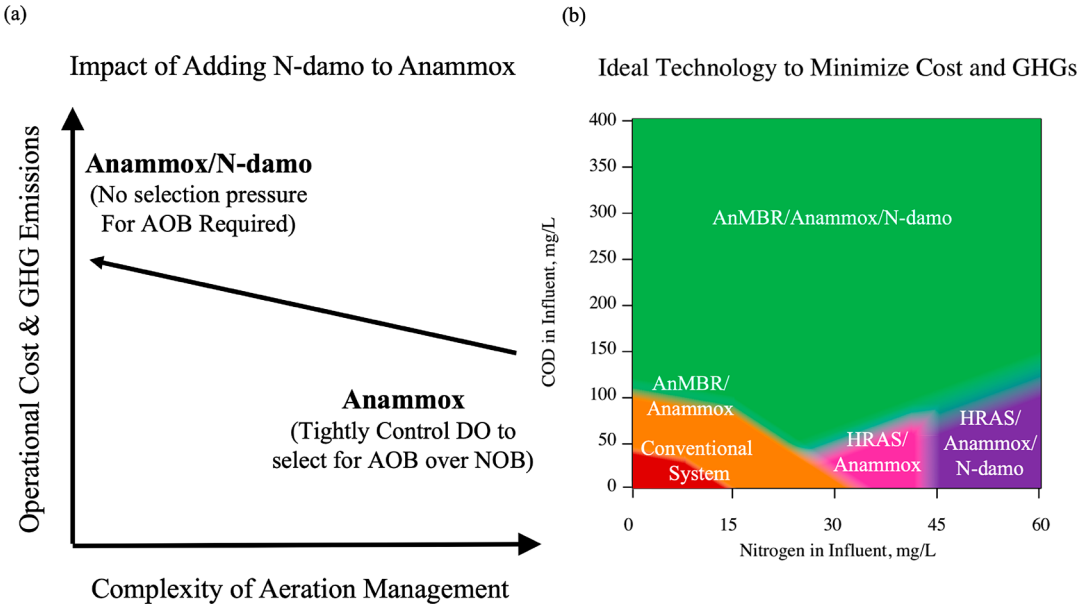

Figure 4. Conceptual diagrams describing the ideal use cases for the theoretical technologies in this study's results. (a) The AnMBR/anammox scenario had slightly lower cost metrics than the AnMBR/anammox/n-damo scenarios at nearly all conditions. However, due to the aeration management complexity of the anammox only system, the AnMBR/anammox/n-damo scheme may be the most beneficial to operational cost. (b) The ideal technology to minimize greenhouse gas emissions is variable and dependent on both nutrient ratios and influent concentrations.

damo enough carbon to convert all nitrate to nitrite. However, at COD/N ratios less than 5, the base case required the addition of external methanol, increasing both cost and GHGs as any added methanol would yield CO_2 that an autotrophic nitrogen removal scheme would not.

Anammox and anammox/n-damo systems perform very similarly because in both cases, about half of ammonium is anaerobically oxidized, thus reducing aeration demand. The n-damo systems demand slightly more oxygen because full nitrification instead of partial nitritation is required and together NOB and n-damo growth yields contribute to a slight increase in sludge production as well. However, these metrics do not take into account how anammox/n-damo nitrogen removal affects the control complexity through less strict aeration management. AnMBR/AOB/anammox systems reduced cost and GHG emission by up to \$0.303/d/m^3 and 1.72 kg equiv. CO_2/d/m^3, respectively, while AnMBR/AOB/anammox/n-damo systems saw a similar reduction of at least \$0.300/d/m^3 and 1.65 kg equiv. CO_2/d/m^3

Practical Control Concerns on Technology Selection. The n-damo/anammox nitrogen has yet to be tested at full or pilot scales but has been shown to remove nitrogen at a high rate at bench-scale.[24,27,45,46] Scale-up remains a source of uncertainty for any new technology, and n-damo is unlikely to be an exception. The ideal reactor design may be driven by the mass transfer rate of methane.[47] Additionally, it is unknown how impurities in the biogas, such as hydrogen sulfide, will limit n-damo.[37] However, this work illustrates the potential savings and GHG reduction possible by bringing anammox nitrogen removal to the mainstream with the help of n-damo archaea. Implementation of anammox under mainstream conditions has so far been largely impractical due to nitrate accumulation by NOB.[48] While n-damo has not been tested at full scale, n-damo systems would theoretically obviate the need for stringent oxygen control required to limit NOB activity with only a 16% increase in oxygen consumption as compared to anammox only systems (eqs SI-42 and SI-43 in page S17 in SI). While the AnMBR/AOB/anammox system resulted in reductions of GHG emissions, up to 1.72 kg equiv. CO_2/d/m^3, and cost reductions, up to \$0.303/d/m^3, as compared to the base case, n-damo systems do not require a stringent aeration control and still provide a substantial reductions of up to 1.65 kg equiv. CO_2/d/m^3 in GHGs and \$0.300/d/m^3 in cost as compared to the base case. Additionally, nitrate-consuming n-damo could prevent nitrate accumulation that occurs in AOB/anammox systems due to the stoichiometry of the anammox pathway limiting the maximum possible nitrogen removal efficiency to 87% (see reaction SI-1 on page S3 in SI).[15]

Impact of Nitrous Oxide Emissions on Results. N_2O remains a major factor of uncertainty in any quantification of GHG emissions from wastewater treatment due to both global warming potential (298 times more potent than CO_2) and variability in emission rates.[11] In conventional nitrification/denitrification, N_2O emissions can vary between 0 to 14.6% of influent nitrogen and can be affected by factors as diverse as COD/N ratio, pH, nitrite concentration, dissolved oxygen concentration, sudden increase in ammonium concentrations, and even size and scale of the nitrogen removal system.[10] In a conventional system, N_2O is produced through three mechanisms: nitrifier denitrification and hydroxylamine oxidation, both performed by autotrophic ammonium oxidizing organisms (AOB, ammonium oxidizing archaea, and complete ammonium oxidizing NOB) in the nitrification reactor, and incomplete denitrification, performed by heterotrophic denitrifying organisms.[9,43,49,50] N_2O emissions from denitrification at minimum COD/N ratios (~5) have been measured between 8 and 4% of influent nitrogen and decreases as COD/N ratios increase.[51] Meanwhile, N_2O emissions in AOB/anammox reactors like those in scenarios B and C range vary between 1 and 2% of influent nitrogen and should be independent of the COD/N ratio in plant influent as all COD is removed first to select for an autotrophic population.[52,53] Anammox alone cannot produce N_2O; however, supporting heterotrophic populations in an anammox reactor have been

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measured to release 0.056−0.6% of influent nitrogen as N_2O.[54,55] Most N_2O emissions in an AOB/anammox system are likely from AOB as these systems are controlled at low dissolved oxygen which has been shown to lead to increased N_2O emissions from AOB.[54,56,57]

As complete nitrification does not require low dissolved oxygen concentration to inhibit NOB, the n-damo based systems (scenarios D and E) might reduce emissions as compared to the partial nitritation/anammox systems (scenarios B and C). Moreover, n-damo/anammox nitrogen removal could possibly yield lower N_2O emissions than a conventional system, especially at low COD/N ratios. The n-damo genome includes a nitrous oxide reductase gene, but the role of this enzyme in situ and in N_2O emission rates from anammox/n-damo reactors has yet to be explored.[24] The current state of research on these next generation nitrogen removal technologies does not allow an accurate estimation of N_2O emissions and how it impacts the total GHG emissions from a WRRF. However, it is possible that at COD/N ratios near the minimum requirement for denitrification, N_2O emission rates from these systems could be comparable to those from anaerobic anammox/n-damo systems or even improved by the addition of an anaerobic anammox/n-damo system. However, due to a lack of understanding of N_2O emissions from anammox/n-damo systems, a clear conclusion cannot yet be made. Despite this limitation, these results show how vital a better understanding of these systems through pilot scale and full-scale studies could be to the reduction of GHG emissions from WRRFs.

Reduction in Greenhouse Gas Emissions and Cost. GHG emission and operational cost were found to depend on both nutrient loading and ratio. At high COD concentrations, both AnMBR scenarios with and without n-damo scenarios saw reduced GHGs and cost due to energy recovery from biogas production. Adding n-damo to an anammox process, while still a theoretical possibility, could reduce control complexity of aeration managment, so it is recommended as the preferred technology at high COD concentration independent of nitrogen concentration (Figure 4b). At low COD, AnMBR/anammox/n-damo saw more GHG emissions than the base case as n-damo was unable to consume all dissolved methane with available nitrogen in the AnMBR effluent. In the AnMBR/anammox scenario nearly all dissolved methane was oxidized to carbon dioxide by methane oxidizing bacteria in the nitrification reactor. Therefore, AnMBR/anammox is recommended as the preferred application at moderate to low nitrogen and moderate COD concentrations. At low COD/N ratios, HRAS scenarios had the lowest cost and GHG emissions as anammox lowered electrical demand (Figure 4a). The addition of n-damo only marginally increased greenhouse gas emissions while alleviating the necessity to stop nitrification at nitrite, allowing easier aeration control. The n-damo systems are therefore considered to be preferential at very high 587 nitrogen concentrations. Meanwhile, plants treating dilute wastewater streams will likely see minimal benefit from these new technologies and traditional nitrification/denitrification may be the best application if nutrient removal is required at these low concentrations.

This study demonstrates that AnMBR paired with n-damo/anammox nitrogen removal could be a powerful, albeit relatively unexplored, wastewater treatment scheme. Previous studies have demonstrated the potential of mainstream AnMBR treatment but have pointed out drawbacks associated with the GHG emissions from rogue dissolved methane emissions.[43] At high nutrient concentration, GHG emissions from AnMBR could be offset by energy recovery from biogas. With the addition of n-damo to an AnMBR/annamox system, greenhouse gases are lowered further as n-damo can simultaneously remove dissolved methane while supplying nitrogen-removing anammox with the required nitrite. Even though nitrogen removal by anammox/n-damo has not yet been accomplished at full or pilot scale, these results, along with the demonstrated success of anammox/n-damo at bench scale, begin to quantify the value of developing mainstream anammox and anammox/n-damo technologies at full scale.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b04764.

Description of models used, sample calculations, and estimating calculations, sensitivity studies, and additional results (PDF)

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