

What is the Best Biological Process for Nitrogen Removal: When and Why?

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Supporting Information

ABSTRACT: Many different aerobic and anaerobic biological processes and treatment schemes are available for transforming organics and/or removing nitrogen from domestic wastewaters. Significant reductions in oxygen requirements and absence of a need for organics for nitrogen reduction are often indicated as advantageous for using the newer anammox organism approach for nitrogen removal rather than the traditional nitrification/denitrification method, the most common one in use today. However, treatment schemes differ, and there are some in which such suggested advantages may not hold. When nitrification/denitrification is used, an anoxic tank is now commonly used first and the nitrate formed by nitrification later is recycled back to that tank for oxidation of wastewater organics. This greatly reduces oxygen requirements and the need for adding organics. So when are such claims correct and when not? What factors in wastewater composition, regulatory requirements, and treatment flow sheet alter which treatment process is best to use? As an aid in making such judgments under different circumstances, the stoichiometry of the different biological processes involved and the different treatment approaches used were determined and compared. Advantages of each as well as imitations and potential opportunities for research to prevent them are presented.



INTRODUCTION

The earliest and still most prevalent biological process for removing nitrogen species from wastewaters has been nitrification/denitrification, that is nitrification of ammonia to nitrate, followed by organic reduction of the nitrate to diatomic nitrogen.¹ Out of concerns for sustainability, particularly with the energy needed to supply oxygen and the amount of organic matter needed, which might otherwise be used for producing methane fuel, other potential nitrogen removal processes have emerged. Among these is the SHARON process² in which ammonia is oxidized only to nitrite followed by denitrification. This might reduce both the oxygen and organic material requirements for denitrification. Also, in the middle 1990s, a unique group of bacteria called anammox was discovered that anaerobically converts ammonia to dinitrogen gas while using nitrite as an electron acceptor.^{3,4} In this process, only about half of the ammonia is first oxidized to nitrite by the usual ammonia oxidizing bacteria. The anammox bacteria then under anoxic conditions use this nitrite as the electron acceptor for oxidation of the other half of the ammonia, converting both to dinitrogen gas. This has been called the completely autotrophic nitrogen removal over nitrite process or CANON.⁵

While the anammox CANON process provides similar end results to the nitrification/denitrification and SHARON processes, it could offer a significant advantage. Third et al.⁵ indicated the CANON “process consumes 63% less oxygen and 100% less reducing agent than traditional nitrogen removal

systems.” This suggested advantage has been promoted actively ever since.^{6–9} However, Daigger et al.¹⁰ indicated that if the oxidized nitrogen species are used for organic oxidation, then these claimed advantages disappear. What then are the real advantages and limitations of the different approaches, under what circumstances do they apply, and how might the disadvantages be reduced by other approaches or through further research? These issues need better clarification as we move forward in the design of wastewater treatment systems for the future and determine the most optimum processes for nitrogen removal under different situations. In order to understand differences better, a typical base-case municipal wastewater composition was assumed, and mass-balance stoichiometric analyses for a variety of conventional and nonconventional nitrogen removal processes and systems were conducted for comparison. Advantages and limitations of each are then addressed, including how the limitations might be circumvented through alternative approaches or research.

Biological Nitrogen Removal Treatment Systems Considered. Mass-balance results were examined for the four nitrogen-removal treatment systems illustrated in Figure 1. Also, seven different biological processes were used in one or more of these systems, including aerobic and anaerobic (methanogenesis) for organic removal, nitrification to either nitrite or nitrate and denitrification of the resulting waters, and

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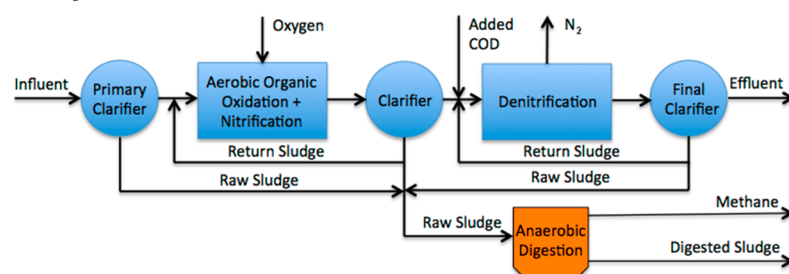
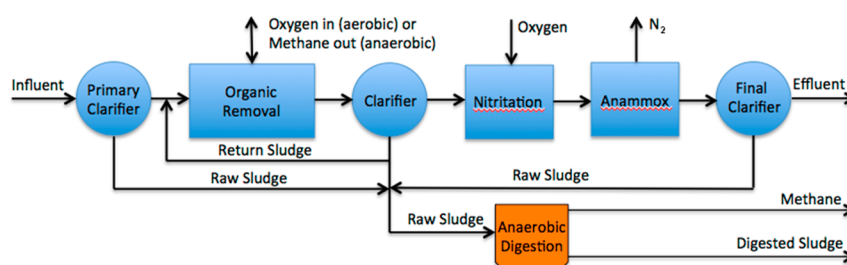
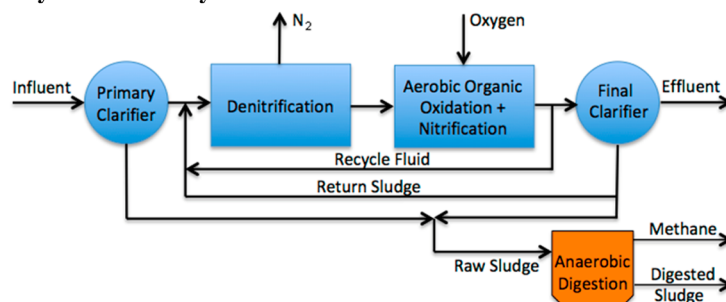
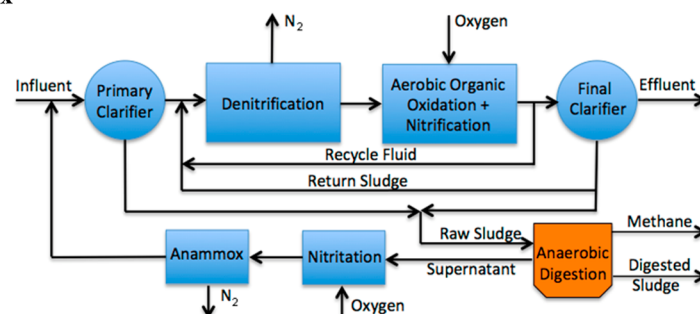
Treatment System A – Direct Line Nitrification/Denitrification**Treatment System B – Mainstream Anammox****Treatment System C – Recycle Nitrification/Denitrification****Treatment System D – Recycle Nitrification/Denitrification Plus Side-Stream Anammox**

Figure 1. Schematic figures of nitrogen-removal treatment systems evaluated.

anammox, either in the side stream,¹¹ which is often used today, or in the main stream. Treatment System A represents the historical approach using a series or direct-line nitrification/denitrification processes in which aerobic organic oxidation and nitrification are accomplished first, followed by anoxic denitrification. Here, additional organic matter, commonly methanol, is added to supply the electron donor requirement for denitrification.

Treatment System B represents the new approach being explored for mainstream anammox treatment. Examined with mainstream treatment are both aerobic and anaerobic organic removal (methanogenesis) followed by anammox treatment for nitrogen removal. While neither mainstream methanogenesis nor anammox treatment may have yet reached a practical full-

scale stage, the significant potential advantages of doing so needs to be explored. Treatment Systems C and D represent the common mainstream biological nitrogen removal processes with anoxic denitrification as the first stage followed by aerobic organic and nitrogen oxidation, and then recycle of oxidized nitrogen species back to the anoxic reactor for denitrification to N_2 . System D processes also include side-stream anammox treatment.

Mass balances for the biological processes involved were obtained using oxidation–reduction half reactions and biological reaction stoichiometry as outlined elsewhere.¹ Details of equation construction are outlined in the [Supporting Information \(SI\)](#). The assumed characteristics of the typical municipal wastewater used in the base-case analyses are

Table 1. Base-Case Assumptions for Wastewater Characteristics, Biological Reactions, Organism Growth Rate Parameters, and Biological Treatment Efficiencies

base case wastewater characteristics	
characteristic	concentration
BOD _L	400 mg/L
VSS	200 mg/L
VSS Org-N	20 mg/L
NH ₃ -N	30 mg/L
COD/VSS ratio	1.42 gCOD/g VSS

Base Case Biological Assumptions^aA. the empirical formula for biomass is C₅H₇O₂NB. primary settling removes 65% of volatile suspended solids (VSS) and 35% of BOD_L

C. the assumed biodegradable portion of VSS is 0.35(400)/(0.65(1.42)(200)) = 75.8%

D. operational criteria for different biological reactions are as given below

reaction	f_s	b d ⁻¹	SRT d	f_s net	treat. eff. %
Aerobic Organic Oxidation					
B1 System	0.555	0.05	6	0.449	95
A, C, and D systems	0.555	0.05	12	0.385	95
organic methanogenesis	0.080	0.03	70	0.037	95
ammonia oxidation to nitrite	0.090	0.05	12	0.063	90
nitrite oxidation to nitrate	0.070	0.05	12	0.049	90
ammonia oxidation to nitrate	0.080	0.05	12	0.056	90
Denitrification of Nitrite or Nitrate to N ₂					
A systems	0.500	0.05	6	0.408	95
C and D systems	0.500	0.05	12	0.350	95
anammox	0.080	0.05	20	0.0480	95

^a f_s represents the maximum fraction of donor substrate converted to cells and f_s net represents an actual decreased value of that fraction depending upon SRT, the solids retention time, and b , the assumed organism decay rate.

contained in Table 1. Rather than using process kinetics, efficiencies of treatment were assumed for conversions by each biological process, and of suspended solids and BOD removals by the primary and secondary clarifiers. For the base case, a wastewater flow rate of 4,000 m³/d was taken along with an influent ultimate biochemical oxygen demand (BOD_L) of 400 mg/L. This rather than the traditional 5-day BOD (BOD₅) was used in order to make mass balances directly. This BOD_L corresponds to a BOD₅ of 273 mg/L assuming a typical first order conversion rate 0.23 d⁻¹. An influent total nitrogen concentration of 50 mg/L was used, yielding an influent BOD_L/N ratio of 8.0.

Base-Case System Comparisons. Of particular interest for each system examined are the total nitrogen removal efficiency, the effluent nitrogen concentrations, the oxygen requirements for nitrogen and organic transformations, the waste digested biosolids production, the methane gas production, and the organic mass (COD) added if needed for denitrification. Also included is the ratio of the energy resulting from methane combustion to that required for supplying oxygen (CH₄/O₂ energy ratio). Values used were 9.92 kWh/m³ (STP) for methane combustion¹² and 1.00 kWh/kg for oxygen usage.¹³

Results for the base cases with both 65 and 100% volatile suspended solids (VSS) removal through primary sedimentation are contained in Table 2. Jetten et al.¹⁴ in 1997 suggested use of 100% chemically enhanced primary removal for aerobic mainstream biological treatment in order to achieve maximum methane production along with efficient nitrogen removal. There are other approaches used or proposed to enhance mainstream organic capture for anaerobic digestion, for

example the use of a first or A-stage high-rate activated sludge treatment, which is then applied with a second or B-stage conventional activated sludge^{15,16} or with contact stabilization.¹⁷ Either system might also be combined with anammox treatment. These other processes reduce the need for chemicals for enhanced settling, but come with perhaps more uncertainty in treatment efficiency. Based upon reported results there appear to be relatively little difference between them in organics captured, and so 100% primary treatment was selected here to represent this group because of greater ease in calculation.

Cases A through D represent the four different systems depicted in Figure 1. For cases A, C, and D, results for nitrification to nitrate (nitrification) and nitrite (nitrification) are shown in Table 2, while for Case B, results for aerobic and anaerobic mainstream organic oxidation together with anammox treatment are listed. Not listed are calculated BOD removals, which were over 98% for all. A Cases with direct line nitrification/denitrification are the least environmentally sound, require large quantities of added COD, have the greatest oxygen demand, and produce the most digested biosolids for handling and disposal. A sole advantage is that System A1 has the highest percentage of total nitrogen removal among the systems. While perhaps easier to operate reliably, the high capital, operating, and environmental costs make A systems the most undesirable choices.

A Systems A1/B1 comparison illustrates the advantage often claimed for anammox treatment. The need for COD addition is removed, total oxygen requirement is reduced 25–40% and digested biosolids production by 8–19%, benefits that are offset somewhat by the 6–13% lower methane production and the lower total nitrogen removal.

Table 2. Base Case Results for 65 and 100% Primary Suspended Solids Removal

		N rem.	add. COD	O ₂ used	dig. bio- solids	CH ₄ m ³ /d	CH ₄ /O ₂	effluent N – mg/L			
sys.	treatment	%	kg/d	kg/d	kg/d	STP	ER	Org	NH ₃	NO ₂ [–]	NO ₃ [–]
A. 65% Primary Suspended Solids Removal											
Direct Line Nitrification/Denitrification											
A1	nitratification	98	724	1272	468	297	2.3	0.9	0.0	0.0	0.0
A2	nitritification	94	420	1112	424	280	2.5	0.7	0.6	1.8	0.0
Mainstream Anammox											
B1	aer. org. oxid.	93		832	388	258	3.1	0.6	0.4	1.0	1.4
B2	methanogenesis	93		320	252	526	16.3	0.2	0.5	1.1	1.5
Recycle Nitrification/Denitrification											
C1	nitratification	85		960	372	252	2.6	0.1	0.7	0.0	6.8
C2	nitritification	85		928	372	252	2.7	0.1	0.7	6.8	0.0
Recycle Nitrification/Denitrification Plus Side-Stream Anammox											
D1	nitratification	90		936	372	252	2.7	0.1	0.5	0.0	4.6
D2	nitritification	90		916	372	252	2.7	0.1	0.5	4.5	0.0
B. 100% Primary Suspended Solids Removal											
Direct Line Nitrification/Denitrification											
A1	nitratification	99	744	1120	452	375	3.3	0.0	0.0	0.0	0.0
A2	nitritification	94	432	936	408	358	3.8	0.3	0.6	1.9	0.0
Mainstream Anammox											
B1	aer. org. oxid.	93		672	364	336	5.0	0.4	0.6	1.0	1.4
B2	methanogenesis	93		308	268	526	16.9	0.1	0.7	1.1	1.5
Recycle Nitrification/Denitrification											
C1	nitratification	85		784	352	330	4.2	0.1	0.8	0.0	6.9
C2	nitritification	85		752	352	330	4.4	0.1	0.8	6.9	0.0
Recycle Nitrification/Denitrification Plus Side-Stream Anammox											
D1	nitratification	91		752	352	330	4.4	0.1	0.4	0.0	4.1
D2	nitritification	91		732	352	330	4.5	0.1	0.4	4.0	0.0

The most environmentally friendly is System B2 with mainstream anaerobic organics removal followed by anammox treatment. Here, oxygen requirement is only 38–46% of that for System B1 and digested biosolids production is only 65–74%, while methane for energy production is more than twice that of any other B through D system. On the negative side, efficient full-scale mainstream anaerobic treatment of domestic wastewater in temperate climates has not yet been demonstrated, and neither has anammox treatment. But there appear to be no insurmountable hurdles for accomplishing this.

While full-scale anaerobic domestic wastewater treatment is being used in tropical regions, the organics removal is typically only 60–80%,¹⁸ requiring further effluent treatment. But efficient mainstream anaerobic treatment of dilute wastewaters has now been demonstrated at pilot scale using membrane bioreactors, even at temperatures down in the 10 °C range.¹⁹ Similar comments can be made for the anammox process. Lower oxygen and biosolids production with increased methane production should lower operational costs. Construction costs are likely to be reduced as well because of the reduced size needed for anaerobic digestion of biosolids, and the absence of need with a membrane bioreactor for final

clarifiers and filtration. For both systems, further research directed toward system optimization, improvement in reliability, and cost reduction is necessary.

Additional concerns with mainstream anaerobic treatment are the need for lower energy membrane fouling control, increased membrane flux, and management of effluent dissolved methane.¹⁵ With low influent COD concentrations in the 250 mg/L range, dissolved methane may represent 40–60% of the total methane production. The higher the influent COD and temperature, the less dissolved methane that results. Methane is a greenhouse gas with warming potential 25 times that of CO₂. Economical and energy-efficient dissolved methane removal and use must be found for this system to be environmentally sound. Methane is a poorly soluble gas and there are several potential methods for its removal²⁰ – the best approach environmentally is yet to be determined.

Systems C and D represent the traditional nitrification/denitrification approach in wide use today. Systems C1 and D1 provide the usual approach of nitrification to nitrate, whereas C2 and D2, that to nitrite. The nitrogen removal efficiency in these systems is a function of the recycle rate, the higher the recycle the higher the removal efficiency. However, recycle

Table 3. Systems C and D Minimum BOD_L/N Ratios with 65 and 100% Primary VSS Removal Efficiencies, 50 mg/L of Influent total N Concentration, And Recycle Ratio of 5:1

system	treatment	65% primary settling			100% primary settling		
		N rem. %	minimum BOD _L mg/L	influent BOD _L /N ratio	N rem. %	minimum BOD _L mg/L	influent BOD _L /N ratio
Recycle Nitrification/Denitrification							
C1	nitrification	84	258	5.2	84	326	6.5
C2	nitritification	83	164	3.3	83	208	4.2
Recycle Nitrification/Denitrification Plus Side-Stream Anammox							
D1	nitrification	86	222	4.4	87	260	5.2
D2	nitritification	85	148	3.0	86	179	3.6

requires energy, brings back dissolved oxygen from the aeration reactors, decreasing the COD that can be used for denitrification, and other factors. Ekama and Wentzel²¹ discussed such limitations, suggesting a typical optimal recycle rate of 5, the one used in this analysis. But an approach has been suggested by Kartal et al.²² to reduce the energy requirements associated with recycle—that is the use of an aerobic/anoxic sequencing batch reactor with readily settling granular sludge, with results recently reported for a full-scale system.²³

The Table 2 comparison between mainstream aerobic organic oxidation with anammox System B1 and all of the C and D systems indicates much less benefit of mainstream anammox treatment than commonly indicated in the literature. While System B1 offers an oxygen reduction benefit of 12–17%, it offer no significant advantage in either digested biosolids production, methane production, or absence of need for added COD. System B1 does benefit from a higher total nitrogen removal capability (93 versus 85–90%). But it has many disadvantages such as slow growth rate of organisms, sensitivity to high nitrite and other environmental conditions, and difficulty in maintaining just the right amount of oxygen. With too little oxygen, ammonia removal becomes limiting, with too much oxygen, nitrate production results in low removal as well. With high daily variations in influent nitrogen, adjusting oxygen delivery to just the right nitrogen concentration becomes difficult. However, this is not a significant problem with the traditional nitrification/denitrification process with recycle.

Comparisons between normal and enhanced primary VSS removal results for Systems C and D (Table 2) illustrates the potential advantages of enhanced primary removal and also of side stream anammox treatment. Sending 100% of influent suspended solids to the digester reduces oxygen consumption by 18 to 20%, digested biosolids production by 6–7%, and increases methane production by 28–30%. But while side-stream anammox treatment increases the amount of total nitrogen removal by 5 or 6%, changes for comparable nitrification systems in digested biosolids, methane, and oxygen are not significant.

Table 2 data for effluent nitrogen concentrations illustrate the expected formation of effluent nitrate in systems with nitrification and nitrite in systems with nitrification. Nitrite is an unstable and inhibitory nitrogen species, and also its presence can lead to the production of nitrous oxide, a significant greenhouse gas. While perhaps offering some advantages, the potential greenhouse gas formation from use of nitrification for the mainstream needs to be examined. Also of note is that when anammox treatment is used, both effluent nitrite and nitrate result. Nitrite is the electron acceptor used in the process, while nitrate is formed as anammox organisms

oxidize some nitrite to nitrate while reducing carbon dioxide to organic carbon during synthesis.⁵

Table 2 also contains CH₄/O₂ Energy Ratios for all processes. Efficiency of methane combustion to form electricity is 25–35%. For example, a ratio of 3.3 would result if the efficiency were 30%. Such an energy ratio would thus be needed to produce enough electricity to satisfy the need for oxygen production. That would be met in all cases with 100% primary settling, but not quite with 65% settling, except with anaerobic System B2. However, total energy costs for treatment may be twice that, in which case only System B2 would meet the total need as its energy ratio is above 16. However, this assumes that an energy efficient method for recovery of dissolved methane is developed. Also assumed is an influent with no sulfate, otherwise its presence and reduction would reduce the amount of organics available for methanogenesis, another complexity that requires evaluation when making choices.

Best Processes for Use With Low Influent BOD_L to Nitrogen Ratios. The above comparisons apply only for the base case BOD_L/N ratio of 8.0 in which there is sufficient organics to satisfy the need for traditional nitrification/denitrification. But the major advantage of anammox treatment comes when the influent BOD_L/N ratio is too low to satisfy that need. What is too low is the significant question to be answered, but seldom is adequately addressed. Daigger¹⁰ illustrated that the theoretical required BOD_L/N ratio would be 3.4–4.0 with nitrification, 2.0–2.5 with nitrification and 0.5 with anammox. These are useful values for guidance, but both wastewater compositions and biological treatment systems are complex. What ratios then apply? How might side-stream anammox treatment affect these values? Also, wastewater contains other forms of nitrogen than ammonia, especially organic nitrogen, which is only partially available for biotransformation, adding to the complexity. Influent organics do not affect nitrogen removals in Systems A and B, only affected are Systems C and D. In order to examine the limiting BOD_L/N ratio for these Systems, the total nitrogen concentration was maintained at 50 mg/L, while the influent BOD_L was lowered until it became limiting. Also assumed is that the influent ratio of VSS/BOD_L was maintained at 0.5 and the ratio of organic nitrogen to VSS ratio was maintained at 0.1 as in the base case. This resulted in an increase in ammonia nitrogen as the BOD_L/N ratio was lowered.

The results are summarized in Table 3. With nitrification, the minimum BOD_L/N ratio varied from 4.4 to 6.5, while with nitrification a lower ratio of 3.0–4.2 was obtained. These values are all about 50% higher than the theoretical values by Daigger¹⁰ as would be expected since much organics are removed through primary treatment. The more efficient the primary treatment, the higher will be the minimum BOD_L/N

ratio. Use of side stream anammox reduced the BOD_L/N ratio by 10–14% with 65% primary settling and 14–20% with 100% settling. Oxygen requirements are not shown in Table 3, nor are digested biosolids or methane productions, all of which would proportionately decrease with decreasing BOD_L/N ratio.

DISCUSSION

The question of what is the best biological processes to use for nitrogen removal for municipal wastewater treatment is important for future development as there are various emerging processes that may impact significantly on sustainability, particularly with respect to resource use and climate change impacts. By far the best process examined in this context is System B2, mainstream anaerobic treatment followed by mainstream anammox treatment. It far excels all the others in net energy production while greatly reducing biosolids handling needs. Nitrogen removal is also not affected in this process by a low influent BOD_L/N ratio. However, only recently has the anaerobic side of this system been demonstrated successfully at pilot scale with anaerobic membrane bioreactors. Before full-scale application, further research is needed to develop an energy-efficient method for dissolved methane recovery, to evaluate the best membranes for use, to reduce membrane fouling, and to address other concerns such as effluent sulfides. Also to consider are the less effective but already available and commonly mentioned alternative approaches of chemically enhanced primary treatment or high rate activated sludge. Mainstream anammox treatment could be attractive for nitrogen removal in these cases as well.

But efficient and reliable mainstream anammox treatment is yet to be demonstrated. Complexities here include slow organism growth, temperature effects, and the difficulties in balancing oxygen supply with the significant nitrogen diurnal variations that occur in the mainstream. Too little oxygen results in low ammonia removal efficiency, too much oxygen results in excess nitrate production. But because of the significant potential environmental benefits of this combination of newer processes, research to better speed full-scale development would appear to be an urgent need.

Use of mainstream anammox treatment together with aerobic organic oxidation is also often promoted with the claim that this reduces oxygen needs significantly as well as the need for organics for nitrogen reduction so that more would be available for methane production. That may be true if one were only considering the highly inefficient System A1 direct-line nitrification/denitrification process, but not nearly as true when one has a wastewater with a sufficiently high influent BOD_L/N ratio and uses one of the more sustainable nitrification/denitrification Systems C and D that are in common use today. What is a sufficiently high BOD_L/N ratio? The base case municipal wastewater considered here had a ratio of 8.0, which was sufficient even when coupled with enhanced primary settling of 100%. Here, even lower ratios, perhaps as low as 6.0 might be used if side-stream anammox treatment were used along with mainstream nitrification to nitrate. If ratios were even lower than that, then mainstream anammox treatment with aerobic organic oxidation would become attractive.

Another important feature of Systems C and D is that through modification they are being used successfully today for phosphate removal as well, something not yet demonstrated with mainstream anammox treatment. Chemically enhanced primary treatment also provides phosphorus removal as well, as can chemical treatment of some sort if added to any of the

treatment systems examined. Adding a denitrification and an aerobic stage after initial nitrification as in the four-stage Bardepheo process²⁴ can also be used to increase nitrogen removal efficiency over that demonstrated here as well as to reduce the recycle ratio.

Finally, whatever the system used, biological nitrogen removal is an energy-intensive process. Much better when the opportunity exists is to use the treated wastewater for agriculture and to leave the nitrogen in the ammonia form as is being practiced in Monterey, California for safe and reliable irrigation of raw vegetables. About 7% of the world's natural gas production in 1990 was used to convert atmospheric nitrogen to fertilizer.²⁵ Our use at wastewater treatment plants of energy through aeration to send the nitrogen back to the atmosphere appears wasteful if it can be avoided. More research on other approaches for using rather than losing this valuable resource is also needed.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b05832.

Development of stoichiometric equations for each biological process used (PDF)

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Notes

The author declares no competing financial interest.

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