Supporting Information

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

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Supporting Information

Preliminary Calculations for Cost Estimations

In addition to the sample calculation above, an estimation of sludge handling cost was used in the preparation of this manuscript. The calculations involved in that estimation are given below:

Table S1. Preliminary Calculations for Cost Estimations			
Description	***Value***	***Units***	***Citation or Calculation***
Sludge Handling Costs			
Typical Sludge Solids Content	21.50%		[1]
Mass of sludge to landfill	4.65	kg wet sludge to landfill	1 / % Solids Content
Cost to landfill sludge	11	\$/Mg wet	[1]
Cost to landfill 1 kg biomass produced	\$ (0.05)	\$/kgVSS produced	Landfilled Wet Sludge x Cost/Mg to Landfill / 1000
Plant Flowrate	60	10^3 m^3/day	This study
	15.9	MGD	This study
Cost of electricity to the plant	0.078	\$/kWh	[2]
Fraction of Energy for Solids Handling in a Typical Plant	0.3	unitless	[3]
Typical Fraction of VSS in TSS, Mass Basis	0.75	unitless	[4]
Conventional Plant Energy Demand, 7-16 MGD on Volume Basis	2000	kWh/MG	[3]
Conventional Plant Energy Demand, Daily Basis	31701	kWh/day	Plant Energy Demand Volume Basis x Plant Flowrate
Conventional Plant Sludge Production, Volume Basis	248	kgTSS/10^3 m^3	[4]
Conventional Plant Sludge Production, Daily Basis	14880	kgTSS/day	Plant Sludge Production (flowrate basis) x Plant Flowrate
Conventional Plant VSS Production, Daily Basis	11160	kgVSS/day	Plant Sludge Production (daily basis) x Fraction VSS in TSS

Table S1 (cont). Preliminary Calculations for Cost Estimations			
Conventional Plant Sludge from Digester, Daily Basis	4241	kgVSS/day	Daily VSS Production x Fraction VSS to Landfill
Energy Required for Solids Handling, Daily Basis	9510	kWh/day	Daily Plant Energy Demand x Typical Fraction of Energy for Biosolids Handling
Energy Required for Solids Handling, VSS Basis	*2.24*	*kWh/kgVSS*	*Daily Energy Required for Solids Handling / Daily VSS from digester*
Cost of Energy for Solids Handling	$ (0.17)	$/kgVSS	Electricity Cost x Energy Required for Solids Handling
Total Cost of Solids Handling	**$ (0.23)**	**$/kgVSS**	**Energy Cost + Cost to Landfill**
Cost of Oxygen Supply			
Energy Demand of Aeration	*1.5*	*kWh/kgO$_2$ Dissolved*	[4]
Cost of Aeration	**$ (0.12)**	**$/kgO$_2$**	**Energy Demand of Aeration x Cost of Electricity**
Cost of Energy for AnMBR Operation			
Energy Demand of AnMBR Operation	*190*	*kWh/ 10^3 m^3*	*[5]*
Cost of Energy for AnMBR Operation	**$(14.82)**	**$/10^3 m^3**	**AnMBR Energy Demand x Flowrate x Cost of Electricity**
Cost of Anaerobic Mixing			
Typical Energy Demand of Biological Nitrogen Removal Mixing, 10 MGD Plant	1100	kWh/d	[3]
Typical Energy Demand of Biological Nitrogen Removal Mixing, 20 MGD Plant	2100	kWh/d	[3]
Interpolated BNR Mixing Energy Demand of This Plant, daily basis	1580	kWh/d	Interpolate between 10 & 20 MGD values at 15.8 MGD
Anaerobic Mixing Energy, volume basis	*26.3*	*kWh/th m^3*	*BNR Mixing Energy Demand / Flowrate, assumed to be similar for all anaerobic tanks*
Cost of Energy for Anaerobic Tank Mixing	**$(2.05)**	**$/10^3 m^3**	**AnMBR Energy Demand x Flowrate x Cost of Electricity**

Table S1 (cont). Preliminary Calculations for Cost Estimations			
Cost of External COD			
Specific Weight of Methanol	0.786	kgCH3OH/L	[6]
Cost of Methanol	$1.30	$/gal	[7]
COD in Methanol, weight basis	1.5	kgCOD/kgCH3OH	Stoichiometry of Combustion Reaction
CO_2 released oxiding methanol, COD basis	*0.92*	*$kgCO_2/kgCOD$*	*Stoichiometry of Combustion Reaction*
COD in Methanol, volume basis	4.46	kgCOD/gal CH3OH	COD in Methanol x Specific Weight x 3.78 L/gal
Cost of External COD, Methanol (Scenario A, Base Case Only)	**$ (0.29)**	**$/kgCOD as CH3OH**	**Cost of Methanol x COD in Methanol, volume basis**
COD in Methane, weight basis	4	$kgCOD/kgCH_4$	Stoichiometry of Combustion Reaction
CO_2 released oxiding methane, COD basis	*0.69*	*$kgCO_2/kgCOD$*	*Stoichiometry of Combustion Reaction*
Cost of Natural Gas	$5.04	$\$/th\ ft^3$	[8]
Density of Methane Gas at 0 °C	0.717	kg/m^3	[9]
Cost of COD, Methane (Scenarios D&E, ndamo only)	**$ (0.03)**	$/kgCOD	**Cost of Natural gas x Density of Methane x 0.0353 th ft^3/m^3 / COD in 1 kg CH_4**
Cost Saved through Methane Recovery			
Concentration Methane in Biogas	0.65	Volume fraction	[4]
Biogas Density at STP	0.86	kg/m^3	[4]
Lower Heating Value of Biogas	22400	kJ/m^3	[4]
	6.22	kWh/m^3	Lower Heating Value of Biogas x 0.00028 kWh/kJ
Cogen Plant Efficiency	20%	unitless	[4]
Mass Methane in Biogas	0.56	$kgCH_4/m^3$ biogas	Concentration Methane in Biogas x Biogas Density
Energy recovered from cogen plant, Volume basis	1.24	kWh/m^3 biogas	Lower Heating Value of Biogas x Plant Efficiency
Energy recovered from cogen plant, mass methane basis	*2.2*	*$kWh/kgCH_4$*	*Energy recovered from cogen plant, volume basis / Mass methane in Biogas*
Cost saved through methane recovery	**$ 0.17**	**$/kgCH4 produced**	Cogen energy per kg CH_4 x Electricity Cost

Description of Models & Sample Calculations

The following document is intended to describe in detail the models created for the five carbon and nitrogen removal scenarios (Modified Luzak-Ettinger, HRAS/anammox, AnMBR/anammox, HRAS/anammox & n-damo, and AnMBR/anammox & n-damo) described in detail in figure 1 of the manuscript. Equations are provided with the associated R script file name and line number in the code to better enable model users to interact with the code directly. (e.g. ScenarioX.R line #) The following key metrics are returned from these models:

- Total oxygen demand
- Sludge discharge volume
- Methane available for energy recovery
- External Carbon Addition Required
- Greenhouse Gas Emissions

The models were written in R code using RStudio IDE operating on MacOSX High Sierra. The code for these models is available online with instructions on how to run these models here:

https://github.com/cogerk/ndamo-econ

Definitions

In the definitions given below, **Z** represents a constituent (e.g. N for total nitrogen, COD for chemical oxygen demand, O2 for oxygen, etc.) and **Y** represents an organism or process (e.g. AOB for ammonium oxidizing bacteria or AnMBR for anaerobic membrane bioreactor, etc.)

Q, WWTP capacity, [th m^3/day]
$c_{\boldsymbol{Z}in}$, Concentration of **Z** in influent, [g-**Z**/ m^3]
$L_{Z_{stream}}$, Total load of **Z** in a given stream (i.e. influent, centrate), [kg-**Z**/d]
$MW_{\boldsymbol{Z}}$, Molecular weight of **Z**
$S_{\boldsymbol{Z}_Y}$, Stoichiometric coefficient of **Z** in metabolism of organism **Y,** [mol-**Z**]
Y_Y, Biomass yield of organism **Y,** [g-VSS/g-Substrate]
f_{Z_Y}, Fractional conversion of constituent **Z** by organism **Y**
O_{2_Y}, Total oxygen demand by organism **Y,** [kg-O_2/d]
$COD_{req'd}$, mass of COD required for heterotrophic organisms, [kg-COD/d]
COD_{bal}, COD required for nitrogen removal minus COD available, [kg-COD/d]
$CO_{2_{\boldsymbol{Y}}}$, mass of carbon dioxide produced by organism or process **Y**, [kg-CO_2/d]
$CH_{4_{prod}}$, Mass of methane produced from anaerobic digestion, [kg-CH_4/d]
$CH_{4_{burn}}$, Mass of methane available to burn for energy regeneration, [kg-CH_4/d]
$CH_{4_{MOB,ox}}$, Mass of methane oxidized by methanotrophs,
$V_{\boldsymbol{X}}$, Volumetric measure of component **Z**, [m^3/d]
$\rho_{\boldsymbol{X}}$, Density of component **Z,** [kg/m3]
$c_{\boldsymbol{l}}^*$, Concentration of component **Z** in liquid at gas-vapor equilibrium
e_Y, Electrical demand of process **Y** on weight or volume basis, [kWh/kg-**Z**] or [kWh/m^3]
E_Y, Electrical demand/production due to process **Y** on daily basis, [kWh/d]
η_Y, Efficiency of process **Y**, [%]
ΔH_{C_Z}, Heat of combustion or energy density of **Z**, [kWh/kg-**Z**]
C_Y, Cost per unit of a given metric calculated in this study, [$/cost factor unit]
x_Z, Volume fraction of **Z** in gas [%]
BG, Biogas production from sludge [m^3/kgVSS destroyed]

Model Assumptions

The following assumptions were made in this model:

- pH and temperature were assumed to be ideal for microbial reactions, meaning this is a stoichiometry-based model where:
  - 100% of COD was removed
  - Scenarios A, C, and E achieved a nitrogen removal of 100%.
  - In scenarios B and D, the maximum amount of nitrogen was removed, but some nitrate remained in the effluent according to following Anammox stoichiometry (defined per 1N-mol):

$$NH_4^+ + 1.3\ NO_2^- \rightarrow N_2 + 0.3\ NO_3^- \qquad \text{(reaction SI-1)}$$

$$\frac{2\ mol - N_2N}{1\ molNH_4^+ - N + 1.3\ molNO_2^- - N} = 87\%$$

- The biomass yield of n-damo archaea was assumed to be the same as n-damo bacteria as calculated by Winkler et al.[13]
- According to literature recommendation 59% of sludge by weight was reduced in anaerobic sludge digestion (AD).[4]

$$f_{x_{AD}} = 0.59$$

- Biogas production from the anerobic digester was assumed to be:[4]

$$BG = 0.75 \frac{m^3\ biogas}{kgVSS\ destroyed}$$

- Biogas density is assumed to be: [4]

$$\rho_{BG} = 0.86 \frac{kg}{m^3}$$

- Methane concentration in biogas is assumed to be:[4]

$$x_{CH_4} = 62\ \%\ by\ vol$$

- Dissolved methane concentration from the AnMBR was assumed to be 1.5 times that of the saturation concentration calcuated with Henry's law with constant H = 0.0015 mol/kg•atm.[14], [15] Assuming 62%v/v methane concnentration in the biogas,[4] this yielded a dissolved methane concentration of:

$$1.5 \times c_l^* = 1.5HPx_{biogas_{CH_4}}MW_{CH_4}\rho_{H_2O} =$$

$$1.5 \times 0.0015 \frac{mol}{kg\ atm} \times 1\ atm \times 0.62 \times 16 \frac{gCH_4}{mol\ CH_4} \times 1000 \frac{kg}{m^3} =$$

$$22.4 \frac{gCH_4}{m^3} = 0.0224 \frac{kg}{m^3}$$

(equation SI-1)
scenarioC.R&
scenarioE.R
line 40

- To reflect a "worse case" for GHG emissions, if COD concentration was so low such that less than 22.4 gCH_4/m^3 was produced from biogas, it was assumed all produced methane is dissolved even though this is not in line with equilibrium concentration predictions.
- Centrate was returned to the mainstream from anaerobic sludge digestion and according to literature it was assumed to contain 25% of the total nitrogen load[4].

$$N_{cent} = 0.25$$

- Carbon dioxide consumption by autotrophs was considered negligible.
- Biogenic CO2 emissions from heterotrophs were not considered according to IPCC guidelines.[16]

- According to literature results from Daelman et al. driven by the kinetics of methane oxidizing bacteria, it was assumed that aerobic methanotrophs were only active when methane concentration exceeded 5 mgCOD/L and that they remove 90% of methane present.[17]

$$c_{\min_{CH_4}} = 5\frac{mgCOD}{L};\ x_{CH_{4_{ox}}} = 0.9$$

- Methane stripped from the nitrification reactor was assumed to be too dilute for energy recovery and was considered a greenhouse gas emission.
- CO2 emissions due to electrical demand were determined with the most recent United States Electrical Profile published by the U.S. Energy and Information Administration (EIA) where the emission of carbon dioxide is given as 1,041 lbsCO_2/MWh or:[2]

$$\frac{kgCO_2}{kWh} = 0.4722\frac{kgCO_2}{kWh}$$

- The global warming potential of methane was considered to be34 times that of carbon dioxide as per IPCC guidelines.[16]

$$CO_{2_{\mathrm{eq}_{CH_4}}} = 34$$

- Cost factors for all metrics considered were estimated using calculations outlined in table S1 and summarized again here as variables:

Table S2. Summary of cost factors from table S1.		
Description	**Variable**	**Value, unit**
Anaerobic Mixing	C_{ana}	\$ (2.18), \$/th m^3
Sludge Handling Costs	C_{solids}	\$ (0.23), \$/kgVSS
Oxygen Demand	C_{O_2}	\$ (0.12) \$/kg O_2 Dissolved
AnMBR Operation	C_{AnMBR}	\$ (14.82), \$/th m^3
COD Addition, Methanol	C_{CH_3OH}	\$ (0.29), \$/kgCOD
COD Addition, Methane	$C_{CH_4,added}$	\$ (0.03), \$/kgCOD
Methane Prodcution	$C_{CH_4,prod}$	\$ 0.17

Organism Characteristics

Table S3. Key Metabolism stoichiometric constants and biomass yields used in this model.			
Process	**Key stoichiometric coefficients, S_{Z_Y}**	**Biomass Yield, Y_Y**	**Reference**
Heterotrophic Denitrification[4]	5 gCOD/gN	0.30 gVSS/gCOD	(Tchobanoglous G, Burton FL, & Stensel HD, 2014)
Heterotrophic Oxidation[4]	1 gO_2/gCOD	0.45 gVSS/gCOD	(Tchobanoglous G, Burton FL, & Stensel HD, 2014)[4]
Ammonium Oxidation[4]	1.5 $molO_2/molNH_4$	0.12 gVSS/gNH_4-N	(Tchobanoglous G, Burton FL, & Stensel HD, 2014)[4]
Nitrite Oxidation[4]	0.5 $molO_2/molNO_2$	0.05 gVSS/ gNO_2-N	(Tchobanoglous G, Burton FL, &

			Stensel HD, 2014)[4]
Anammox[4]	0.3 $molNO_3/molNH_4$[a] 1.3 $molNO_2/molNH_4$[a]	0.13 gVSS/ gNH_4-N[b]	(a) (Tchobanoglous, Burton FL, & Stensel HD, 2014)[4] (b) (Strous, Kuenen, & Jetten, 1999)[18]
N-Damo Archaea	0.25 mol CH_4/ $molNO_2$[a]	0.071 gCOD/ gCOD[b]	(a) (Haroon et al.,, 2014) [19] (b) (Winkler et al., 2015) [13]
Anaerobic Membrane Bioreactor	N/A	0.036 gCOD/ gCOD	(Gouveia et al., 2015)[20]

Constants

Table S4. Additional constants required for model calculations.		
Description	**Value**	**Reference**
Biomass conversion of VSS to COD, n	1.48 gVSS/gCOD	(Marais & Ekama, 1976)[10]
Universal gas constant, R	$0.082\,\frac{m^3\ atm}{kmol\ K}$	

Sample Calculations

The following sample calculation was performed for all four scenarios given the following conditions:

$$Q = 40\,\frac{th\ m^3}{d}$$

$$c_{N_{in}} = 40\,\frac{mgN}{L}$$

$$c_{COD_{in}} = 100\,\frac{mgCOD}{L}$$

Scenario A – Conventional Nitrification/Denitrification

In scenario A, a traditional MLE system, it was assumed that 100% of the influent nitrogen is completely nitrified and denitrified for 100% nitrogen removal and 100% of COD was consumed by heterotrophs.

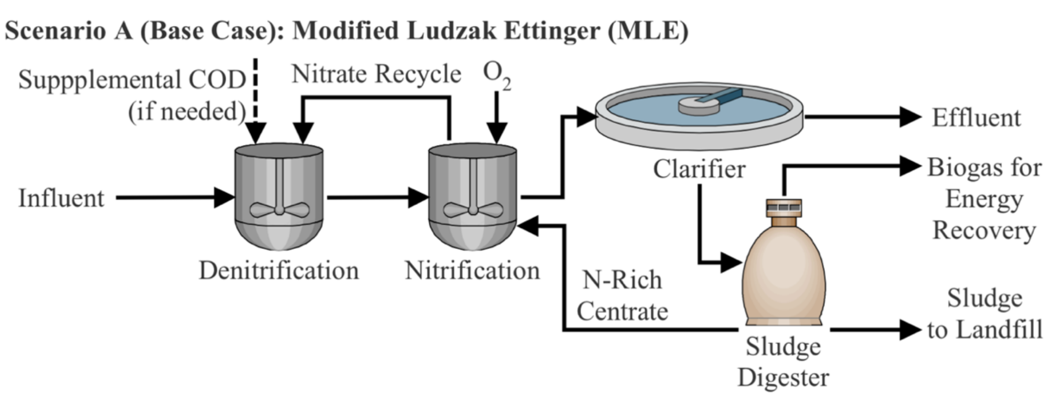

Figure S1. Replication of Scenario A taken from Figure 1 of the manuscript

The fractional conversion of constituent **Z** by organism **Y** in this scenario**,** f_{Z_Y}, were given in the table 3 below:

Table S5. Fractional conversions for scenario A, f_{Z_Y}			
Organism, Y	**AOB**	**NOB**	**Denitrifying Heterotrophs (Y is HET)**
Nitrogen (Z is N)	1	1	1
Scenario A.R Line #	20	21	28

Step 1) Total Contaminant Load

The COD load in the influent was calculated as:

$$L_{COD} = c_{COD_{in}} Q = \qquad \text{(equation SI-2)}$$

$$100 \frac{mgCOD}{L} \times 40 \frac{th\, m^3}{d} = 4000 \frac{kgCOD}{d} \qquad \text{scenarioA.R line 8}$$

The nitrogen load in the influent was calculated as the total nitrogen load in the influent.

$$L_{N_{in}} = c_{N_{in}} \times Q = \qquad \text{(equation SI-3)}$$

$$40 \frac{mgN}{L} \times 40 \frac{ML}{d} = 1600 \frac{kgN}{d} \qquad \text{scenarioA.R line 9}$$

The centrate from the anaerobic sludge digester (AD) was returned to the nitrification reactor for additional removal. It was assumed that 25% of total nitrogen load of the system resides in the centrate sidestream:

$$L_{N_{cent}} = N_{cent} \times L_{N_{in}} = \qquad \text{(equation SI-4)}$$

$$0.25 \times 1600 \frac{kgN}{d} = 400 \frac{kgN}{d} \qquad \text{scenarioA.R line 10}$$

Therefore:

$$L_N = L_{N_{in}} + L_{N_{cent}} = \qquad \text{(equation SI-5)}$$

$$1600 \frac{kgN}{d} + 400 \frac{kgN}{d} = 2000 \frac{kgN}{d} \qquad \text{scenarioA.R line 11}$$

Step 2) Nitrification

Oxygen demand by AOB and NOB is calculated as:

$$O_{2_{AOB}} = f_{N_{AOB}} L_N s_{O_{2_{AOB}}} \frac{MW_{O_2}}{MW_N} = \qquad \text{(equation SI-6) scenarioA.R line 22}$$

$$1 \times 2000 \frac{kgN}{d} \times 1.5 \frac{kmol\ O_2}{kmol_N} \times \frac{32 kg/kmol}{14\ kg/kmol} = 6857 \frac{kgO_2}{d}$$

$$O_{2_{NOB}} = f_{N_{NOB}} L_N s_{O_{2_{NOB}}} \frac{MW_{O_2}}{MW_N} =$$

$$1 \times 2000\ \frac{kgN}{d} \times 0.5 \frac{kmol\ O_2}{kmol_N} \times \frac{32kg/kmol}{16kg/kmol} = 2286 \frac{kgO_2}{d}$$

(equation SI-7)
scenarioA.R line 23

Sludge production by AOB and NOB is calculated as:

$$p_{x_{AOB}} = f_{N_{AOB}} L_N Y_{AOB} =$$

$$1 \times 2000\ \frac{kgN}{d} \times 0.12 \frac{kgVSS}{kgN} = 240 \frac{kgVSS}{d}$$

(equation SI-8)
scenarioA.R line 24

$$p_{x_{NOB}} = f_{N_{NOB}} L_N Y_{NOB} =$$

$$1 \times 2000\ \frac{kgN}{d} \times 0.05 \frac{kgVSS}{kgN} = 240 \frac{kgVSS}{d}$$

(equation SI-9)
scenarioA.R line 25

Step 3) Denitrification

In order to remove nitrogen, COD was required in a 1:5 N/C ratio ($s_{COD_{DENIT}}$) for denitrification and therefore the total COD required was calculated as:

$$COD_{req'd} = f_{N_{DENIT}} L_N s_{COD_{DENIT}} =$$

$$1 \times 2000\ \frac{kgN}{d} \times 5 \frac{gCOD}{gN} = 10000 \frac{kgCOD}{d}$$

(equation SI-10)
scenarioA.R line 29

The biomass yield of denitrifying heterotrophs was calculated as:

$$p_{x_{DENIT}} = COD_{req'd} Y_{DENIT} =$$

$$10000 \frac{kgCOD}{d} \times 0.3 \frac{kgVSS}{kg} = 3000 \frac{kgVSS}{d}$$

(equation SI-11)
scenarioA.R line 30

In order to determine whether additional COD needs to be externally supplied to remove the nitrogen in the influent a COD balance was preformed:

$$COD_{bal} = COD_{req'd} - L_{COD} =$$

$$10000 \frac{kgCOD}{d} - 4000 \frac{kgCOD}{d} = 6000 \frac{kgCOD}{d}$$

(equation SI-12)
scenarioA.R line 31

At these conditions, ($COD_{bal} > 0$) additional COD was required, therefore:

$$COD_{added} = COD_{bal} = 6000 \frac{kgCOD}{d}$$

(equation SI-13)
scenarioA.R line 33

However, if $COD_{bal} < 0$ (at higher COD/N ratios) there is more COD than can be removed by denitrification ($COD_{bal} < 0$), and:

$$COD_{added} = 0$$

(equation SI-14)
scenarioA.R line 32

Furthermore, if $COD_{bal} < 0$, additional oxygen will be required for heterotrophic oxidation of the remaining COD (after denitrification):

$$O_{2_{HET}} = COD_{bal}$$ (equation SI-15)
scenarioA.R lines 35-36

Finally, the the biomass yield of aerobic heterotrophs was calculated as follows:

$$p_{x_{HET}} = COD_{bal}Y_{HET}$$ (equation SI-16)
scenarioA.R line 37-38

Step 4) Anaerobic Digestion

The total sludge load into the AD was the sum of all biomass yields from all organisms:

$$p_{x_{TOT}} = \sum p_{x_Y} =$$ (equation SI-17)
scenarioA.R line 62

$$240 + 100 + 3000 + 0 = 3340 \frac{kgVSS}{d}$$

The sludge load from the AD (total sludge handling demand of the MLE system) was determined using the assumed sludge reduction factor defined above:

$$p_{x_{out}} = p_{x_{TOT}} \times \left(1 - f_{x_{AD}}\right) =$$ (equation SI-18)
scenarioA.R line 63

$$3340 \frac{kgVSS}{d} \times (1 - 0.59) = 1369 \frac{kgVSS}{d}$$

Digested COD was assumed to be coverted to biogas in a ratio of 0.74 m^3/kgVSS destroyed so volume of biogas produced is calculated as:

$$Biogas_{vol} = \left(p_{x_{TOT}} - p_{x_{out}}\right) \times BG$$ (equation SI-19)
scenarioA.R lines 64

$$(3340 - 1369) \frac{kgVSS}{d} \times 0.75 \frac{kgCH_4}{kgCOD}$$

$$= 1478 \frac{m^3 biogas}{d}$$

As calculated in Table S1, mass of methane in biogas is estimated given biogas density, ρ_{BG}=0.86 kg/m^3, and methane concentration in biogas, x_{CH_4}=0.62

$$CH_{4_{burn}} = V_{biogas} \times \rho_{BG} \times x_{CH_4} =$$ (equation SI-20)
scenarioA.R lines 65

$$1478 \frac{m^3 biogas}{d} \times 0.62 \frac{m^3\ CH_4}{m^3\ biogas} \times 0.86 \frac{kg}{m^3}$$

$$= 788 \frac{kg\ CH_4}{d}$$

Step 5) Electrical Demand

The electrical demand due to sludge thickening and aeration were considered. The electrical demand for aeration was based on the total oxygen demand of the system

$$O_{2_{TOT}} = \sum O_{2_Y} =$$ (equation SI-22)
scenarioA.R line 76

$$6857 + 2286 = 9143 \frac{kgO_2}{d}$$

From this the electrical demand due to aeration was estimated as follows:

$$E_{O_2} = O_{2,TOT}\, e_{O_2} = 9143 \frac{kgO_2}{d} \times 1.5 \frac{\mathrm{kWh}}{\mathrm{kgO_2}} = 13700 \frac{kWh}{d}$$ (equation SI-23) scenarioA.R line 80

The electrical demand from the sludge dewatering system was based on the mass of sludge leaving the digester:

$$E_{Solids} = p_{x_{out}} e_{solids} = 1369 \frac{kgVSS}{d} \times 2.24 \frac{\mathrm{kWh}}{\mathrm{kgVSS}} = 3070 \frac{kWh}{d}$$ (equation SI-24) scenarioA.R line 81

The electrical demand required for mixing the denitrification tank was from biogas in the combined heat and power plant (CHP) is based on the mass of methane produced in the digester and estimated as follows:

$$E_{mix} = Q \times e_{base} = = 40 \times 10^3 \frac{m^3}{d} \times 28 \frac{kWh}{10^3 m^3} = 1120 \frac{kWh}{d}$$ (equation SI-25) scenarioA.R line 82

The electricity recovered from biogas in the combined heat and power plant (CHP) is based on the mass of methane produced in the digester and estimated as follows:

$$E_{CHP} = -CH_{4_{burn}} e_{cogen} = -788 \frac{kgCH_4}{d} \times 2.2 \frac{kWh}{kgCH_4} = -1730 \frac{kWh}{d}$$ (equation SI-26) scenarioA.R line 84

The total GHG emission is calculated as the non-biogenic CO_2 produced from electrical consumption as reported by the U.S. Energy & Information Administration and the biogenic CO_2 produced from external COD added. The amount of CO_2 produced per kg methanol added is calculated in table S1:

$$CO_{2_{TOT}} = \frac{kgCO_2}{kWh} \sum E_Y + COD_{added} \times s_{CH_3OH/CO_2} = (13700 + 3070 + 1120 - 1730) \frac{kWh}{d} \times 0.47 \frac{kgCO_2}{kWh} + 6000 \times 0.92 = 13100 \frac{kgCO_2}{d}$$ (equation SI-27) scenarioA.R line 86

Step 6) Scenario A Cost Estimation

The key cost factors were combined together by multiplying them each by an estimated cost per units calculated in table S1. They are then added together to provide a base cost factor. This does not represent the operational cost of the plant, but instead provides a value that can be compared to the other scenarios to estimate how much would be saved using the other theoretical systems examined in this study. In this scenario, methanol is used as the additive COD if required for denitrification.

$$Cost_A = CH_{4_{burn}} \times C_{CH_4,prod} + COD_{added} \times C_{COD_{CH_3OH}} + p_{x_{out}} \times C_{solids} + O_{2_{TOT}} \times C_{O_2} + C_{ana} \times Q =$$ (equation SI-28) scenarioA.R line 89

$$788 \frac{kgCH_4}{d} \times \frac{\$0.17}{kgCH_4} + 6000 \frac{kgCOD}{d} \times -\frac{\$0.29}{kgCOD_{CH_3OH}} + 1369 \frac{kgVSS}{d} \times -\frac{\$0.23}{kgVSS} + 9143 \frac{kgO_2}{d} \times -\frac{\$0.12}{kgO_2} + \frac{\$2.18}{th\,m^3} \times 60 \frac{th\,m^3}{d} = -\frac{\$3080}{d}$$

Step 7) Scenario A Summary

Table S6. The following cost and equivalent GHG emissions are reported from ScenarioA.R when using the input used in this sample calculation

Metric	**Combined Cost of Key Metrics**	**GHG Emissions**
Variable	$Cost_{compare,A}$	$CO_{2_{TOT}}$
ScenarioA.R Line #(s)	98	97
Value	-\$3080/d	13100 kgCO$_2$/d

Scenario B – HRAS/Anammox

In scenario B it was assumed that all COD was removed aerobically by heterotrophs. Furthermore, it was assumed enough of the influent nitrogen was partially nitrified from ammonium to nitrite by AOB in order to supply anammox with a stoichiometric ratio of nitrite:ammonium, calculated with the anammox metabolic reaction:

$$NH_4^+ + 1.3\,NO_2^- \rightarrow N_2 + 0.3\,NO_3^-$$ (reaction SI-1)

The fraction of influent ammonium undergoing partial nitrification by AOB is then calculated as:

$$f_{N_{AOB}} = \frac{1.3\;kgNO_2^- - N}{1\;kgNH_4^+ - N + 1.3kgNO_2^- - N} = 0.565$$ (equation SI-29)

The rest of the influent ammonium is then anaerobically oxidized by anammox, therefore:

$$f_{N_{ANAMX}} = 1 - f_{N_{AOB}} = 1 - 0.565 = 0.435$$ (equation SI-30)

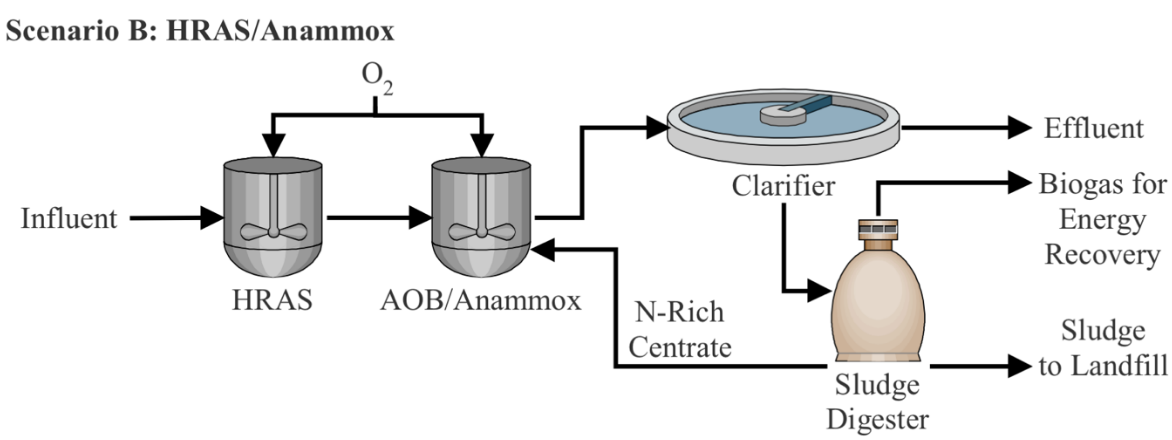

Figure S2. Replication of Scenario B taken from Figure 1 of the manuscript

The fractional conversion of constituent **Z** by organism **Y** in this scenario**,** f_{Z_Y}, are given in the table 5 below:

Table S7. Fractional conversions for scenario B, f_{Z_Y}				
Organism, Y	**AOB**	**NOB**	**Anammox (Y is ANAMX)**	**Heterotrophs (Y is HET)**
Nitrogen (Z is N)	0.565	0	0.435	N/A
COD	N/A	N/A	N/A	1
Scenario B.R Line #	20	21	28	16

Step 1) Total Contaminant Load

Calculated identically to scenario A using equations SI-1 thru SI-4 (scenarioB.R lines 8-11).

Step 2) High Rate Activated Sludge for COD Removal

Biomass yield and oxygen demand were calculated with equations SI-15 and SI-16,respectively (but using total COD load L_{COD} instead of the COD balance).

$$p_{x_{HET}} = L_{COD}Y_{DENIT} = 4000\frac{kgCOD}{d} \times 0.45\frac{kgVSS}{kgCOD} = 1800\frac{kgVSS}{d}$$ scenarioB.R line 16

$$O_{2_{HET}} = L_{COD} = 4000\frac{kgO_2}{d}$$ scenarioB.R line 17

Step 2) Nitrification

Biomass yield and oxygen demand were calculated by equations SI-6 thru 9 utilizing the fractional conversion calculated in SI-29 and given in table S7:

$$O_{2_{AOB}} = f_{N_{AOB}}L_N s_{O_{2_{AOB}}}\frac{MW_{O_2}}{MW_N} = 0.565 \times 2000 \times 1.5 \times \frac{32}{14}$$ scenarioB.R line 22

$$= 3874\frac{kgO_2}{d}$$

$$p_{x_{AOB}} = f_{N_{AOB}}L_N Y_{AOB} = 0.565 \times 2000 \times 0.12 = 136\frac{kgVSS}{d}$$ scenarioB.R line 24

Because it was assumed there is no NOB activity in this scenario, $O_{2_{NOB}} = 0$, $p_{x_{NOB}} = 0$ (scenarioB.R line 23 & 25)

Step 3) Anammox

Sludge production by anammox was calculated according to equation SI-8:

$$p_{x_{ANAMX}} = f_{N_{ANAMX}}L_N Y_{ANAMX} =$$ scenarioB.R line 32

$$0.435 \times 2000\frac{kgN}{d} \times 0.13\frac{kgVSS}{kgN} = 113\frac{kgVSS}{d}$$

Step 4) Anaerobic digester

This system was identical to scenario A. Sludge handling demand and methane available for energy recovery were calculated with equations SI-17 thru SI-20:

$$p_{x_{out}} = 839\ kgVSS/d$$ scenarioB.R line 62 & 63

$$V_{biogas} = 907\frac{m^3}{d}$$ scenarioB.R line 64 & 6

$$CH_{4_{burn}} = 483\frac{kgCH_4}{d}$$

Step 5) Electrical Demand

The electrical demands were considered in the same way as done in scenario A with equations SI-21 thru SI-27.

$$O_{2_{TOT}} = 7870\frac{kgO_2}{d}$$ scenarioB.R line 75

$$E_{O_2} = 11800\frac{kWh}{d}$$ scenarioB.R line 79

$$E_{solids} = 1880\frac{kWh}{d}$$ scenarioB.R line 80

$$E_{CHP} = -1060\frac{kWh}{d}$$ scenarioB.R line 82

$$CO_{2_{TOT}} = 5940\ \frac{kgCO_2}{d}$$ scenarioB.R line 83

Step 6) Cost Estimation

As in scenario A, the key cost factors were combined with a modified version of equation SI-28. Because there is no denitrification, no external COD will be added in this scenario, so that term was not included.

$$Cost_B\ =\ CH_{4_{burn}} \times C_{CH_4,prod} + p_{x_{out}} \times C_{solids} + O_{2_{TOT}} \times C_{O_2} =$$ scenarioB.R line 88

$$483\frac{kgCH_4}{d} \times \frac{\$0.17}{kgCH_4} +\ 839\frac{kgVSS}{d} \times -\frac{\$0.23}{kgVSS}$$
$$+ 7870\frac{kgO_2}{d} \times -\frac{\$0.12}{kgO_2} = \frac{\$1031}{day}$$

The most effective way to consider this number is in comparison to the combined cost metrics from a conventional nitrification/denitrification system at the same conditions (flowrate, nitrogen and carbon concentration). That is done as follows:

$$Cost_{compare} = -(Cost_A - Cost_B) = -\left(\frac{-\$3080}{d} - \frac{-\$1030}{day}\right)$$ (equation SI-31) masterrun.R line 111

$$= \frac{\$2050}{day}(\$\ are\ saved)$$

Therefore, we roughly estimate that operating an HRAS/anammox system as the same conditions would save $2050. The same comparison is made for GHG emissions:

$$GHG_{compare} = -(GHG_A - GHG_B)$$ masterrun.R line 106

$$= -\left(\frac{13100\ kg\ CO_2}{d} - \frac{5940\ kg\ CO_2}{day}\right)$$

$$= -7180\ (emissions\ averated)$$

Step 7) Scenario Summary

Table S8. The following metrics are reported from ScenarioB.R when using the input used in this sample calculation				
Metric	**Combined Cost of Key Metrics**	**Comparitive Cost**	**GHG Emissions**	**Comparitive GHG Emissions**
Variable	$Cost_B$	$Cost_{compare}$	GHG_b	$GHG_{compare}$
Location in Code	ScenarioB.R Line 95	masterrun.R Line 111	ScenarioB.R Line 94	masterrun.R Line 106
Value	-$1030/d	$2050/d (saved)	5940 $kgCO_2/d$	7180 $kgCO_2/d$ (GHG Redcued)

Scenario C – AnMBR/Anammox

In scenario C, nitrogen removal was calculated identically to the AOB/Anammox scenario in scenario B. 100% COD removal was assumed to be achieved by an AnMBR system.

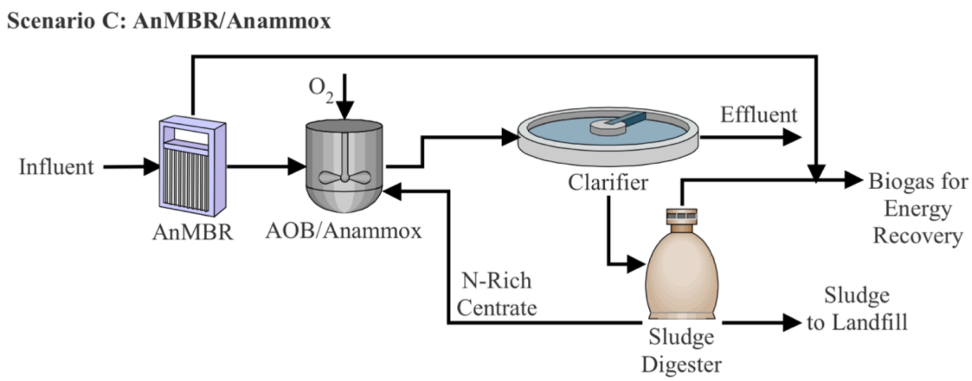

Figure S3. Replication of Scenario C taken from Figure 1 of the manuscript

Table S9. Fractional conversions for scenario C, f_{Z_Y}				
Organism, Y	**AOB**	**NOB**	**Anammox (Y is ANAMX)**	**AnMBR**
Nitrogen (Z is N)	0.565	0	0.435	N/A
COD	N/A	N/A	N/A	1
Scenario C.R Line #	20	21	28	35

Step 1) Total Contaminant Load

This was calculated identically to scenario A using equations SI-1 thru SI-4 (scenarioC.R lines 8-11).

Step 2) Nitrification & Anammox for Nitrogen Removal

Biomass yield and oxygen demand were calculated identically to scenario B with equations SI-6 thru 9 and the fractional conversion calculated in SI-29 and given in table 7, therefore $O_{2_{AOB}} = 3874 \frac{kgO_2}{d}$, $p_{x_{AOB}} = 136 \frac{kgVSS}{d}$, & $p_{x_{ANAMX}} = 113 \frac{kgVSS}{d}$ (scenarioC.R lines 22, 24 & 29)

Step 3) AnMBR for COD Removal

Sludge production from the AnMBR was calculated by equation SI-8 as follows:

$$p_{x_{AnMBR}} = f_{COD_{AnMBR}} L_{COD} Y_{AnMBR} n = \qquad \text{scenarioC.R line 36}$$
$$1 \times 4000 \frac{kgCOD}{d} \times 0.036 \frac{kgCOD}{kgCOD} \times 1.48 \frac{kgVSS}{kgCOD}$$
$$= 213 \frac{kgVSS}{d}$$

COD was assumed to be converted to methane by equation as follows:

$$CH_{4_{prod}} = f_{COD_{AnMBR}} L_{COD} (1 - Y_{AnMBR}) \frac{kgCH_4}{kgCOD} = \qquad \text{(equation SI-32) scenarioC.R line 39}$$
$$1 \times 4000 \frac{kgCOD}{d} \times \left(1 - 0.036 \frac{kgCOD}{kgCOD}\right) \times 4$$
$$= 15424 \frac{kgCH_4}{d}$$

Step 4) Dissolved vs. Gaseous Methane & CO_2 Production

The mass of dissolved methane was determined using the saturated dissolved methane concentration determined in equation SI-1 and the following:

$$L_{CH_{4_{diss,sat}}} = Qc_l^* = \qquad \text{(equation SI-33) scenarioC.R line 41}$$
$$40 \frac{ML}{d} \times 0.0224 \frac{kgCH_4}{m^3} \times 10^3 \frac{m^3}{ML} = 896 \frac{kgCH_4}{d}$$

The amount of methane available for energy recovery from the AnMBR was adjusted by removing the fraction of methane that was dissolved:

$$CH_{4_{burn_{AnMBR}}} = CH_{4_{prod_{AnMBR}}} - L_{CH_{4_{diss}}} = \qquad \text{(equation SI-34) scenarioC.R line 42}$$
$$15424 \frac{kgCH_4}{d} - 896 \frac{kgCH_4}{d} = 14528 \frac{kgCH_4}{d}$$

NOTE: If less methane was produced than could be dissolved at equilibrium ($CH_{4_{prod}} < L_{CH_{4_{diss}}}$) , it was assumed that all methane was captured in the dissolved phase for simplicity in order to simulate a "worst case scenario" for comparison to the base case.

$$L_{CH_{4_{diss}}} = CH_{4_{prod_{AnMBR}}}; \; CH_{4_{burn_{AnMBR}}} = 0 \qquad \text{(equation SI-35) scenarioC.R line 43-44}$$

Step 5) Fate of Dissolved Methane in Nitrification Reactor

Based on results from Daelman et al., 2014[17] it was assumed that at low methane concentrations ($c_{CH_4} < 5 \frac{mgCOD}{L}$), methane would be 100% stripped from the reactor. At high methane concentrations, methane would be 90% oxidized by aerobic methanotrophs and the remaining 10% would be stripped out[17].

$$CH_{4_{MOB,ox}} = 0.9 \times L_{CH_{4_{diss,sat}}} = \qquad \text{(equation SI-36) scenarioC.R line 52}$$
$$0.9 \times 896 \frac{kgCH_4}{d} = 806 \frac{kgCH_4}{d}$$

The residual dissolved methane was then calculated as:

$$L_{CH_{4_{diss}}} = L_{CH_{4_{diss,sat}}} - CH_{4_{MOB,ox}} = \qquad \text{(equation SI-37) scenarioC.R line 56}$$

$$896\frac{kgCH_4}{d} - 806\frac{kgCH_4}{d} = 90\frac{kgCH_4}{d}$$

Sludge produced by MOBs was calculated with a modified version of equation SI-8, $p_{x_{MOB}} = 56.7\frac{kgVSS}{d}$ (scenarioC.R line 58).

Oxygen demand of MOBs was determined by converting methane consumed to chemical oxygen demand (COD).

$$O_{2_{MOB}} = CH_{4_{MOB,ox}} s_{O_{2_{MOB}}} = 806\frac{kgCH_4}{d} \times 0.25\frac{kgO_2}{kgCH_4} = 201\ kg\ O_2$$ (equation SI-38) scenarioC.R line 57

Step 6) Anaerobic digester

This system was calculated identically to scenario A. Sludge handling demand and methane available for energy recovery were calculated with equations SI-17 thru SI-20.

$$p_{x_{out}} = 213\ kgVSS/d$$ scenarioC.R line 63

$$V_{biogas} = 229\frac{kgCH_4}{d}; CH_{4_{prod_{AD}}} = 122\frac{kgCH_4}{d}$$ scenarioC.R line 64 & 65

with the addendum that the total methane available for energy regeneration was the sum of methane from both the AnMBR and the AD:

$$CH_{4_{burn}} = CH_{4_{prod_{AD}}} + CH_{4_{burn_{AnMBR}}} = 122 + 14500 = 14700\frac{kgCH_4}{d}$$ (equation SI-39) scenarioC.R line 66

Step 7) Electrical Demand

The electrical demand is considered in the same way as in scenario A with equations SI-21 thru SI-27.

$$O_{2_{TOT}} = 4080\frac{kgO_2}{d}$$ scenarioC.R line 76

$$E_{O_2} = 6110\frac{kWh}{d}$$ scenarioC.R line 80

$$E_{solids} = 7600\frac{kWh}{d}$$ scenarioC.R line 81

$$E_{CHP} = -32200\frac{kWh}{d}$$ scenarioC.R line 84

In addition to these demands, the electrical energy for scouring and mixing of the AnMBR was determined on a volumetric basis:

$$E_{AnMBR} = Q \times e_{AnMBR} = 40\frac{10^3m^3}{d} \times 190\frac{kWh}{10^3m^3} = 7600\frac{kWh}{d}$$ (equation SI-40) scenarioC.R line 83

The total emissions were then calculated as the sum of the electrical demands and sources calculated above plus the amount of dissolved methane exiting the system adjusted to CO2 equivalents.

$$CO_{2_{TOT}} = \frac{kgCO_2}{kWh} \sum E_Y + \frac{CO_2}{CH_{4_{eq}}} L_{CH_{4_{diss}}}$$

(equation SI-41)
scenarioC.R line 85

$$= (-18000)\frac{kWh}{d} \times 0.47\frac{kgCO_2}{kWh} + 90 \times 34\frac{kgCO_2}{kgCH_4}$$

$$= -5450\frac{kgCO_2}{d}$$

Step 8) Cost Estimation
As in scenario A, the key cost factors were combined with a modified version of equation SI-28. Because there is no denitrification, no external COD will be added in this scenario, so that term is not included. The cost of electricity used operating an AnMBR is included.

$$Cost_C = CH_{4_{burn}} \times C_{CH_4,prod} + p_{x_{out}} \times C_{solids} + O_{2_{TOT}} \times C_{O_2} + Q \times C_{AnMBR} =$$

scenarioC.R line 88

$$14700\frac{kgCH_4}{d} \times \frac{\$0.17}{kgCH_4} + 212\frac{kgVSS}{d} \times -\frac{\$0.23}{kgVSS} + 4080\frac{kgO_2}{d} \times -\frac{\$0.12}{kgO_2} + 40\frac{th\,m^3}{d} \times -\frac{\$14.82}{th\,m^3}$$

$$= \frac{\$1400}{d}$$

This value is positive because so much energy is generated from biogas cogeneration from mainstream anaerobic digestion. It does not suggest that this WRRF would make money or that it is energy positive, because these calculations only include the key metrics that would be most greatly affected by the theoretical technologies examined in this study. The most effective way to consider this number is in comparison to the combined cost metrics from a conventional nitrification/denitrification system at the same conditions (flowrate, nitrogen and carbon concentration). That is done as follows:

$$Cost_{compare} = Cost_A - Cost_C = -\left(\frac{-\$3080}{d} - \frac{\$1400}{day}\right)$$

masterrun.R line 112

$$= \frac{\$4470}{day}\ (cost\ saved)$$

The same comparison is made for GHG emissions:

masterrun.R line 107

$$GHG_{compare} = -(GHG_A - GHG_C)$$

$$= -\left(\frac{13100\ kg\ CO_2}{d} - \frac{5450\ kg\ CO_2}{day}\right)$$

$$= 18565\ (emissions\ averated)$$

Step 9) Scenario Summary

Table S10. The following metrics are reported from ScenarioC.R when using the input used in this sample calculation

Metric	Combined Cost of Key Metrics	Comparitive Cost	GHG Emissions	Comparitive GHG Emissions
Variable	$Cost_C$	$Cost_{compare}$	GHG_C	$GHG_{compare}$
Location in Code	ScenarioC.R Line 97	masterrun.R Line 112	ScenarioC.R Line 96	masterrun.R Line 107
Value	-$3080/d	$4470/d ($ saved)	-5450 $kgCO_2$/d	-18565 kgCO2/d (GHG Reduced)

Scenario D – HRAS/Anammox & N-Damo

In scenario D it was assumed that all COD was removed aerobically by heterotrophs. Furthermore, it was assumed enough of the influent nitrogen underwent nitrification in order to supply anammox and n-damo with a stoichiometric ratio of nitrate and ammonium, calculated with the anammox and n-damo metabolic reactions:

$$Anammox: NH_4^+ + 1.3\ NO_2^- \rightarrow N_2 + 0.3\ NO_3^- \qquad \text{(reaction SI-1)}$$
$$N - damo:\ 0.3\ NO_3^- \rightarrow\ 0.3\ NO_2^- \qquad \text{(reaction SI-2)}$$
$$Overall:\ NH_4^+ + NO_2^- \rightarrow N_2$$

The fraction of influent ammonium undergoing nitrification was then calculated as:

$$f_{N_{NOB}} = f_{N_{AOB}} = \frac{1\ kgNO_2^- - N}{1\ kgNH_4^+ - N + 1\ kgNO_2^- - N} = 0.5 \qquad \text{(equation SI-42)}$$

scenarioD.R line 21

The rest of the influent ammonium was then anaerobically oxidized by anammox, therefore:

$$f_{N_{ANAMX}} = 1 - f_{N_{AOB}} = 0.5 \qquad \text{(equation SI-43)}$$

scenarioB.R line 29

N-damo will reduce all nitrate produced from the nitrification reactor as well as the nitrate produced by anammox (stoichiometric coefficient in reaction SI-1 of 0.3).

$$f_{N_{NDAMO}} = f_{N_{NOB}} + 0.3 = 0.8 \qquad \text{(equation SI-44)}$$

scenarioC.R line 30

Table S11. Fractional conversions for scenario D, f_{Z_Y}

Organism, Y	AOB	NOB	Anammox (Y is ANAMX)	N-damo (Y is NDAMO)	Heterotrophs (Y is HET)
Nitrogen (Z is N)	0.5	0.5	0.435	0.8	N/A
COD	N/A	N/A	N/A	N/A	1
Scenario D.R Line #	20	21	28	29	15

Scenario D: HRAS/Anammox/N-damo

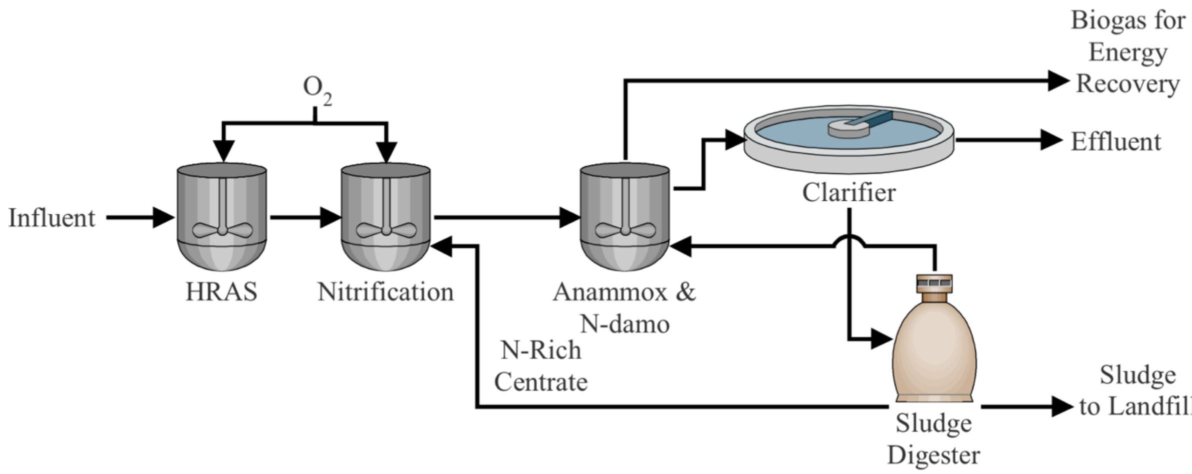

Figure S4. Replication of Scenario D taken from Figure 1 of the manuscript

The addition of n-damo will increase the total oxygen demand by 16% as compared to an anammox system. In partial nitritation/anammox system, 1 mole nitrogen requires 0.84 mole O_2 in order for AOB to convert 56% ($f_{N_{AOB,scenario\ B}}$, table S9) to nitrite for anammox. (Recall AOB require 1.5 molO_2/molNH_4, table S3):

$$0.84\ \frac{mol\ O_2}{mol\ NH_4} = 1\ mol\ NH_4 \times 0.56\ \times \frac{1.5\ mol\ O_2}{1\ mol\ NH_4} \qquad \text{(equation SI-45)}$$

Meanwhile, in an nitrification/anammox/n-damo system, 1 mole nitrogen will require 1 mol O2 in order for AOB & NOB to completely convert 50% ($f_{N_{AOB,}}$& $f_{N_{NOB}}{}_{scenario\ D}$, table S11) of influent ammonium to nitrate for n-damo. (Recall NOB requires an additional 0.5 molO_2/molNH_4, table S3 and therefore complete nitrification requires 2 molO_2/molNH_4)

$$1\ \frac{mol\ O_2}{mol\ NH_4} = 1\ mol\ NH_4 \times 0.50\ \times \frac{2\ mol\ O_2}{1\ mol\ NH_4} \qquad \text{(equation SI-46)}$$

Therefore, there is a 16% increase in oxygen demand with the addition of n-damo:

$$16\% = \frac{1 - 0.84}{1} \qquad \text{(equation SI-47)}$$

Step 1) Total Contaminant Load
Calculated identically to scenario A using equations SI-1 thru SI-4 (scenarioD.R lines 8-11).
Step 2) High Rate Activated Sludge for COD Removal
Calculated identically to scenario B using equations SI-15 thru SI-16. (scenarioD.R lines 16-17).
Therefore: $p_{x_{HET}} = 1800\frac{kgVSS}{d}$, $O_{2_{HET}} = 4000\frac{kgO_2}{d}$.

Step 3) Nitrification
Given the above defined fractional conversions, biomass yield and oxygen demand were calculated for each nitrfier (AOB and NOB) via equations SI-6 thru 9:

$$O_{2_{AOB}} = 3429\frac{kgO_2}{d}, \qquad O_{2_{NOB}} = 1143\frac{kgO_2}{d}$$

scenarioD.R lines 22 & 23

$$p_{x_{AOB}} = 120\frac{kgVSS}{d}, \qquad p_{x_{NOB}} = 50\frac{kgVSS}{d}$$

scenarioD.R lines 24 & 25

Step 4) Anammox & N-Damo
Biomass yield of anammox was calculated via modifying equation SI-8:

$$p_{x_{ANAMX}} = 130\frac{kgVSS}{d}$$

scenarioD.R line 31

The above defined fractional conversion for n-damo and the n-damo stoichiometric coefficient of methane was used to determine the total mass of methane consumed as well as sludge produced by n-damo:

$$L_{CH_{4_{cons}}} = L_N f_{N_{NDAMO}} s_{CH_{4_{NDAMO}}} \frac{MW_{CH_4}}{MW_N} = 2000 \times 0.8 \times 0.25 \times \frac{16}{14} = 457\frac{kgCH_4}{d}$$

(equation SI-48)
scenarioD.R line 30

$$p_{x_{NDAMO}} = \frac{L_{CH_{4_{cons}}} Y_{ANAMX}}{\frac{kgCH_4}{kgCOD}} n = \frac{457\frac{kgCH_4}{d} \times 0.071\frac{kgCOD}{kgCOD}}{4} \times 1.48\frac{kgVSS}{kgCOD} = 12.1\frac{kgVSS}{d}$$

(equation SI-49)
scenarioD.R line 32

Step 5) Anaerobic Digester
This system was calculated identically to scenario A. Sludge handling demand and methane available for energy recovery were calculated with equations SI-17 thru SI-20.

$$p_{x_{out}} = 866\ kgVSS/d$$

scenarioD.R line 62 & 63

$$V_{biogas} = 936\frac{m3}{d};\ CH_{4_{prod}} = 498\frac{kgCH_4}{d}$$

scenarioD.R line 64

Step 6) Methane Balance
Methane available for energy recovery must be adjusted since n-damo will consume some as an electron donor:

$$CH_{4_{burn}} = CH_{4_{prod}} - L_{CH_{4_{cons}}} =$$

(equation SI-50)
scenarioD.R line 69

$$3368 - 457 = 41 \frac{kgCH_4}{d}$$

In this example calculation, there was enough methane available for n-damo ($CH_{4_{burn}} > 0$) and:

$$COD_{added} = 0$$ (equation SI-51) scenarioD.R line 71

NOTE: At low COD/N ratios, this may not be the case. Therefore if $CH_{4_{burn}} < 0$:

$$COD_{added} = \frac{-CH_{4_{burn}}}{\frac{kgCH_4}{kgCOD}}$$ (equation SI-52) scenarioD.R line 72

$$CH_{4_{burn}} = 0$$ (equation SI-53) scenarioD.R line 73

Step 7) Electrical Demand

The electrical demands were considered in the same way as in scenario A with equations SI-21 thru SI-27.

$$E_{base} = 10160 \frac{kgO_2}{d}$$ scenariD.R line 79

$$O_{2_{TOT}} = 8571 \frac{kgO_2}{d}$$ scenarioD.R line 76

$$E_{O_2} = 12900 \frac{kWh}{d}$$ scenarioD.R line 80

$$E_{solids} = 140 \frac{kWh}{d}$$ scenarioD.R line 81

$$E_{mix} = 1120 \frac{kWh}{d}$$ scenarioD.R line 82

$$E_{CHP} = -90.5 \frac{kWh}{d}$$ scenarioD.R line 84

$$CO_{2_{TOT}} = 7440 \frac{kgCO_2}{d}$$ scenarioD.R line 85

Step 8) Cost Estimation

Key cost factors were combined with a modified version of equation SI-27. If external COD addition is required, it would be in the form as methane in natural gas, so that term is modified for the cost of natural gas.

$$Cost_D = CH_{4_{burn}} \times C_{CH_4,prod} + p_{x_{out}} \times C_{solids} + O_{2_{TOT}} \times C_{O_2} + COD_{added} \times C_{CH_4,added} =$$ scenarioD.R line 88

$$41.1 \frac{kgCH_4}{d} \times \frac{\$0.17}{kgCH_4} + 866 \frac{kgVSS}{d} \times -\frac{\$0.19}{kgVSS} + 8571 \frac{kgO_2}{d} \times -\frac{\$0.12}{kgO_2} + 0 \frac{kgCOD}{d} \times -\frac{\$0.13}{kgCOD} = \frac{-\$1280}{d}$$

As with all other scenarios, this is compared to the base case as follows:

$$Cost_{compare} = Cost_A - Cost_D = \frac{-\$3080}{d} - \frac{-\$1280}{day}$$ masterrun.R line 113
$$= \frac{\$1790}{day}\ (cost\ saved)$$

The same comparison is made for GHG emissions: masterrun.R line 108

$$GHG_{compare} = -(GHG_A - GHG_B)$$
$$= -\left(\frac{13100\ kg\ CO_2}{d} - \frac{7440\ kg\ CO_2}{day}\right)$$
$$= -5680\ (emissions\ averated)$$

Step 9) Scenario Summary

Table S12. The following metrics are reported from ScenarioD.R when using the input used in this sample calculation

Metric	**Combined Cost of Key Metrics**	**Comparitive Cost**	**GHG Emissions**	**Comparitive GHG Emissions**
Variable	$Cost_D$	$Cost_{compare}$	GHG_D	$GHG_{compare}$
Location in Code	ScenarioD.R Line 97	masterrun.R Line 113	ScenarioD.R Line 96	masterrun.R Line 108
Value	-$1280/d	$4470/d ($ saved)	7440 $kgCO_2$/d	5680 kgCO2/d (GHG Reduced)

Scenario E – AnMBR/Anammox & N-Damo

In scenario E it was assumed 100% of COD was removed by an AnMBR system, and 100% of nitrogen was removed with n-damo and anammox identically to scenario D.

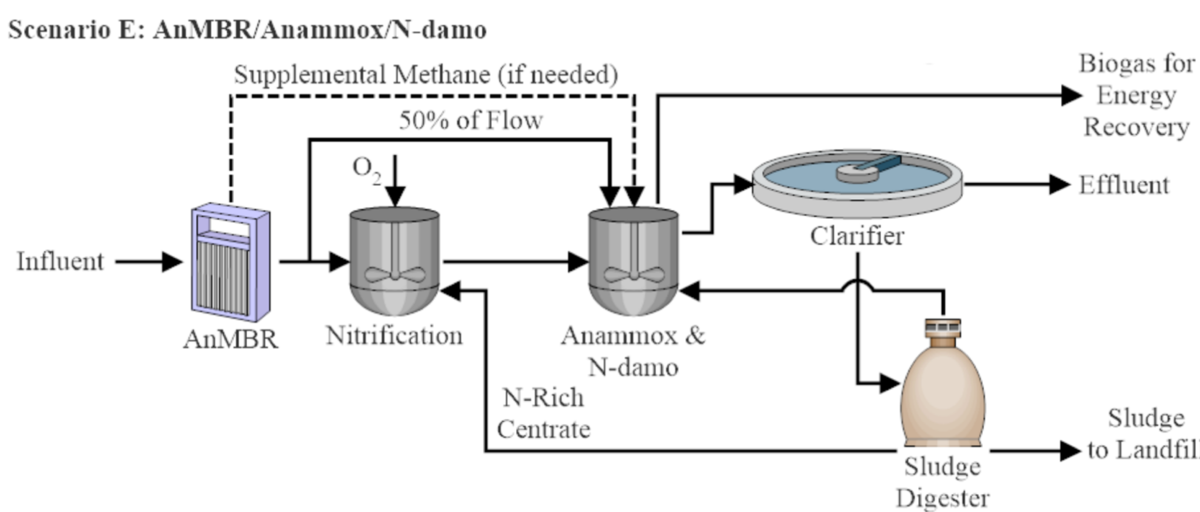

Figure S5. Replication of Scenario E taken from Figure 1 of the manuscript

Table S13. Fractional conversions for scenario E, f_{Z_Y}

Organism, Y	AOB	NOB	Anammox (Y is ANAMX)	N-damo (Y is NDAMO)	AnMBR
Nitrogen (Z is N)	0.5	0.5	0.435	0.8	N/A
COD	N/A	N/A	N/A	N/A	1
Scenario E.R Line #	20	21	28	29	35

Step 1) Total Contaminant Load
This was calculated identically to scenario A using equations SI-1 thru SI-4 (scenarioC.R lines 8-11).

Step 2) Nitrification, Anammox & N-damo (Nitrogen Removal)
The nitrification, anammox and n-damo sludge production and oxygen/methane consumption were identical to scenario D and calculated via equations SI-6 thru 8 and SI-36 thru SI-38 (scenarioE.R lines 22-25 & 30-32) and thus $O_{2_{AOB}} = 3429\frac{kgO_2}{d}$; $O_{2_{NOB}} = 1143\frac{kgO_2}{d}$; $p_{x_{AOB}} = 120\frac{kgVSS}{d}$; $p_{x_{NOB}} = 50\frac{kgVSS}{d}$; $L_{CH_{4_{cons}}} = 457\frac{kgCH_4}{d}$; $p_{x_{ANAMX}} = 130\frac{kgVSS}{d}$; $p_{x_{NDAMO}} = 12\frac{kgVSS}{d}$.

Step 3) Mainstream Anaerobic Membrane Bioreactor
The sludge and methane production from the AnMBR is calculated identically to scenario C with equations SI-8 & SI-32 (scenarioE.R lines 36 & 37), and thus: $p_{x_{AnMBR}} = 213\frac{kgVSS}{d}$;
$CH_{4_{prod_{AnMBR}}} = 15424\frac{kgCH_4}{d}$.

Step 4) Dissolved vs. Gaseous Methane & CO2 Production
Calculated nearly identically to scenario C with equations SI-33 thru SI-35. (scenarioE.R lines 42-44): $L_{CH_{4_{diss}}} = 893\frac{kgCH_4}{d}$; $CH_{4_{burn_{AnMBR}}} = 14500\frac{kgCH_4}{d}$

However, unlike scenario C, 50% of the flow from the AnMBR is diverted to anammox/n-damo reactor, and half was diverted to the nitrification reactor as per the fractional conversions defined in table 9. Dissolved methane into the nitrification and anammox/n-damo reactor was therefore calculated as:

$$L_{CH_{4_{diss_{NIT}}}} = f_{NOB_N} L_{CH_{4_{diss}}} = 0.5 \times 893\frac{kgCH_4}{d} = 446.5\frac{kgCH_4}{d}$$

(equation SI-54) scenarioE.R line 45

$$L_{CH_{4_{diss_{AMX}}}} = L_{CH_{4_{diss}}} - L_{CH_{4_{diss_{NIT}}}} = 2976\frac{kgCH_4}{d} - 1488\frac{kgCH_4}{d} = 446.5\frac{kgCH_4}{d}$$

(equation SI-55) scenarioE.R line 46

Step 6) Fate of Dissolved Methane in Nitrification Reactor (50% of AnMBR Flow)
The same assumption regarding methane stripping and consumption by MOBs from scenario C was used here, therefore equations SI-8 and SI-34 thru SI-38 were used to calculate the methane/oxygen consumed by MOBs, the sludge produced by MOBs, and the residual dissolved

methane from the nitrification reactor (scenarioE.R lines 52, 57 thru 59): $CH_{4_{MOB,ox}} = 1339\frac{kg}{d}$; $L_{CH_{4_{diss_{From\ NIT}}}} = 45\frac{kg}{d}$; $O_{2_{MOB}} = 100\ kg\ O_2$; $p_{x_{MOB}} = 28\frac{kg}{d}$;

Step 7) Anaerobic Digester

This system was identical to scenario C with sludge handling demand and methane available for energy recovery were calculated with equations SI-17 thru SI-20 and SI-39 (scenarioE.R lines 62 thru 66) as follows: $p_{x_{out}} = 227\frac{kgVSS}{d}$; $CH_{4_{dig}} = 131\frac{kg}{d}$;

Step 8) Methane Balance

It was assumed that n-damo would first consume methane from the already dissolved methane available from the AnMBR before consuming methane gas, therefore the methane balance was calculated as:

$$L_{CH_4} = \left(L_{CH_{4_{diss_{AMX}}}}\right) - L_{CH_{4_{cons}}} = 446 - 457 = -10\frac{kgCH_4}{d}$$ (equation SI-56) scenarioE.R line 68

Additional methane was provided from the anaerobic digester, and there is no residual methane available in the liquid phase:

$$CH_{4_{burn}} = CH_{4_{burn}} + L_{CH_4} = 14700\frac{kgCH_4}{d}$$
$$L_{CH_4} = 0$$
(equation SI-57) scenarioE.R lines 66-71

NOTE: If additional methane was required beyond what the anaerobic digester can provide ($CH_{4_{burn}} < 0$), it was added externally and calculated as:

$$COD_{added} = \frac{-CH_{4_{burn}}}{s_{O_{2_{burn}}}}$$
$$CH_{4_{burn}} = 0$$
scenarioE.R lines 72-73

If there was enough methane available in the liquid phase ($L_{CH_4} < 0$), The residual methane was then treated like a greenhouse gas.

Step 8) Electrical Demand

The electrical demand was determined identically to in scenario C with equations SI-21 thru SI-27 & SI-40.

$$O_{2_{TOT}} = 4670\frac{kgO_2}{d}$$ scenarioE.R line 76

$$E_{O_2} = 7000\frac{kWh}{d}$$ scenarioE.R line 79

$$E_{Solids} = 508\frac{kWh}{d}$$ scenarioE.R line 80

$$E_{mix} = 1120\frac{kWh}{d}$$ scenarioC.R line 82

$$E_{AnMBR} = 7600\frac{kWh}{d}$$ scenarioE.R line 81

$$E_{CHP} = -32200\frac{kWh}{d}$$ scenarioB.R line 82

The total emissions were then calculated as the sum of the electrical demands and sources calculated above plus the amount of dissolved methane exiting the system and the amount of stripped methane leaving the nitrification reactor adjusted to CO_2 equivalents.

$$CO_{2_{TOT}} = \frac{kgCO_2}{kWh}\sum E_Y + \frac{CO_2}{CH_{4_{eq}}}\left(L_{CH_{4_{diss}}} + L_{CH_{4_{diss_{From\,NIT}}}}\right)$$ (equation SI-58) scenarioE.R line 83

$$= (-16000)\frac{kWh}{d} \times 0.47\frac{kgCO_2}{kWh} + (0+45)\times 34\frac{kgCO_2}{kgCH_4} = -6000\frac{kgCO_2}{d}$$

Step 9) Cost Estimation

Key cost factors were combined with a modified version of equation SI-28. If external COD addition is required, it would be in the form as methane in natural gas, so that term is modified for the cost of natural gas. The cost of electricity required to operate an AnMBR is also included.

$$Cost_E = CH_{4_{burn}} \times C_{CH_4,prod} + p_{x_{out}} \times C_{solids} + O_{2_{TOT}} \times C_{O_2} + COD_{added} \times C_{CH_4,added} + Q \times C_{AnMBR} =$$ scenarioE.R line 88

$$14700\frac{kgCH_4}{d} \times \frac{\$0.17}{kgCH_4} + 254\frac{kgVSS}{d} \times -\frac{\$0.23}{kgVSS} + 4670\frac{kgO_2}{d} \times -\frac{\$0.12}{kgO_2} + 0\frac{kgCOD}{d} \times -\frac{\$0.13}{kgCOD} + 40\frac{th\,m^3}{d} \times -\frac{\$14.82}{th\,m^3} + 40\frac{th\,m^3}{d} \times -\frac{\$2.18}{th\,m^3} = \$1240$$

As with all other scenarios, this is compared to the base case as follows:

$$Cost_{compare} = Cost_A - Cost_E = \frac{-\$3080}{d} - \frac{\$1240}{day} = \frac{\$4310}{day}\ (cost\ saved)$$ masterrun.R line 97

The same comparison is made for GHG emissions: masterrun.R line 108

$$GHG_{compare} = -(GHG_A - GHG_E) = -\left(\frac{13100\,kg\,CO_2}{d} - \frac{-6000\,kg\,CO_2}{day}\right) = -19100\ (emissions\ averated)$$

Step 9) Scenario Summary

Table S12. The following metrics are reported from ScenarioE.R when using the input used in this sample calculation

Metric	Combined Cost of Key Metrics	Comparitive Cost	GHG Emissions	Comparitive GHG Emissions
Variable	$Cost_E$	$Cost_{compare}$	GHG_E	$GHG_{compare}$
Location in Code	ScenarioE.R Line 97	masterrun.R Line 114	ScenarioE.R Line 96	masterrun.R Line 109
Value	-$1240/d	$4310/d ($ saved)	-6000 kgCO_2/d	19100 kgCO2/d (GHG Reduced)

Individual Cost Factor Results from the Model

To dig into the contribution of individual cost factors on the overall cost comparion results for each scenario, plots were made comparing the relative % increase or decrease of each cost factor considered similar to Figures 4 & 5 in the study. Those plots are provided here:

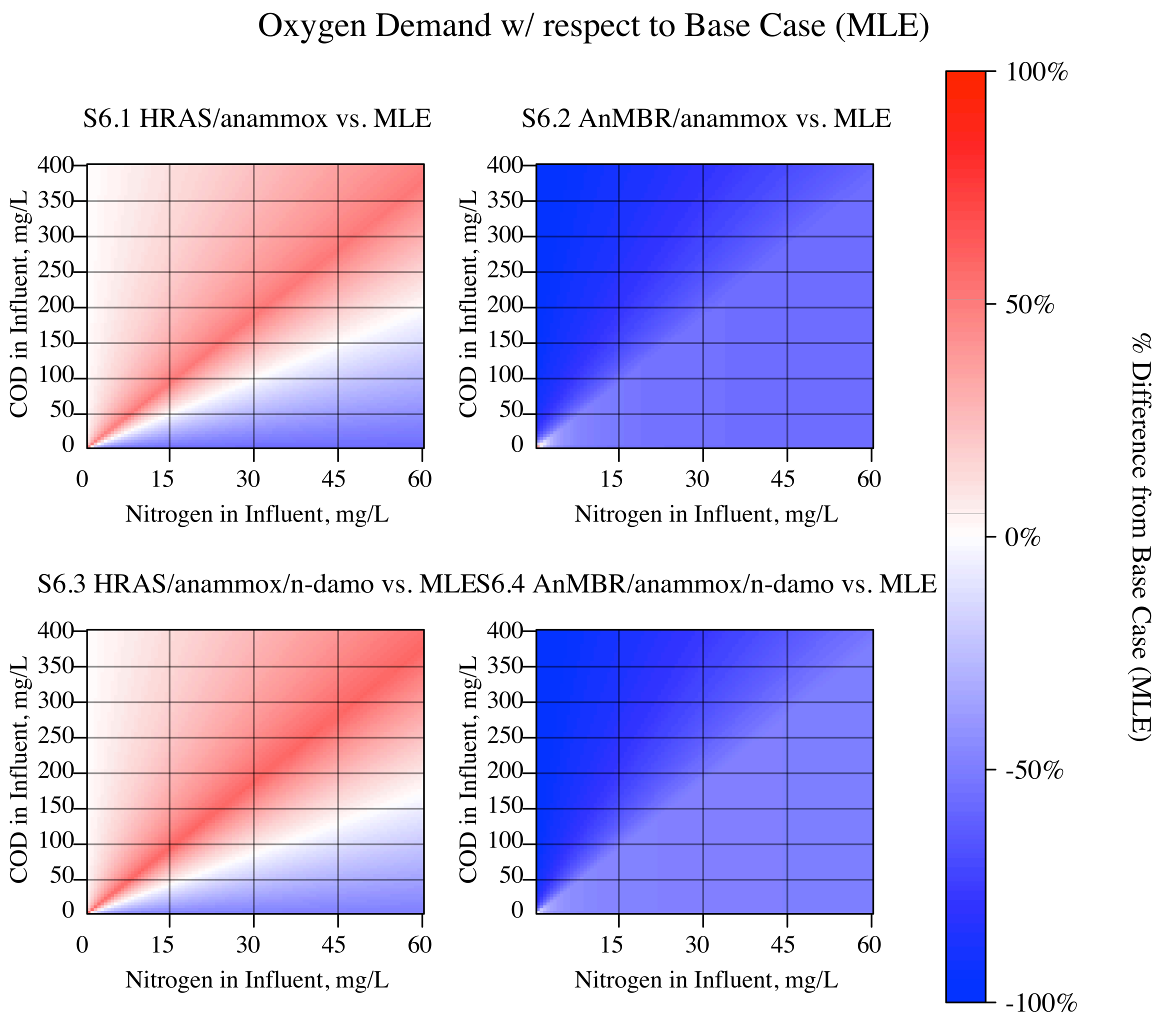

Figure S6. Comparison of oxygen demand in scenarios B (2.1), C (2.2), D (2.3), and E (2.4) to the base case scenario A (Modified Ludzack-Ettinger, MLE, also referred to as conventional nitrification/denitrification). Percentage increase or decrease in oxygen demand was shown in red (high) and blue (low) respectively as defined by the color key.

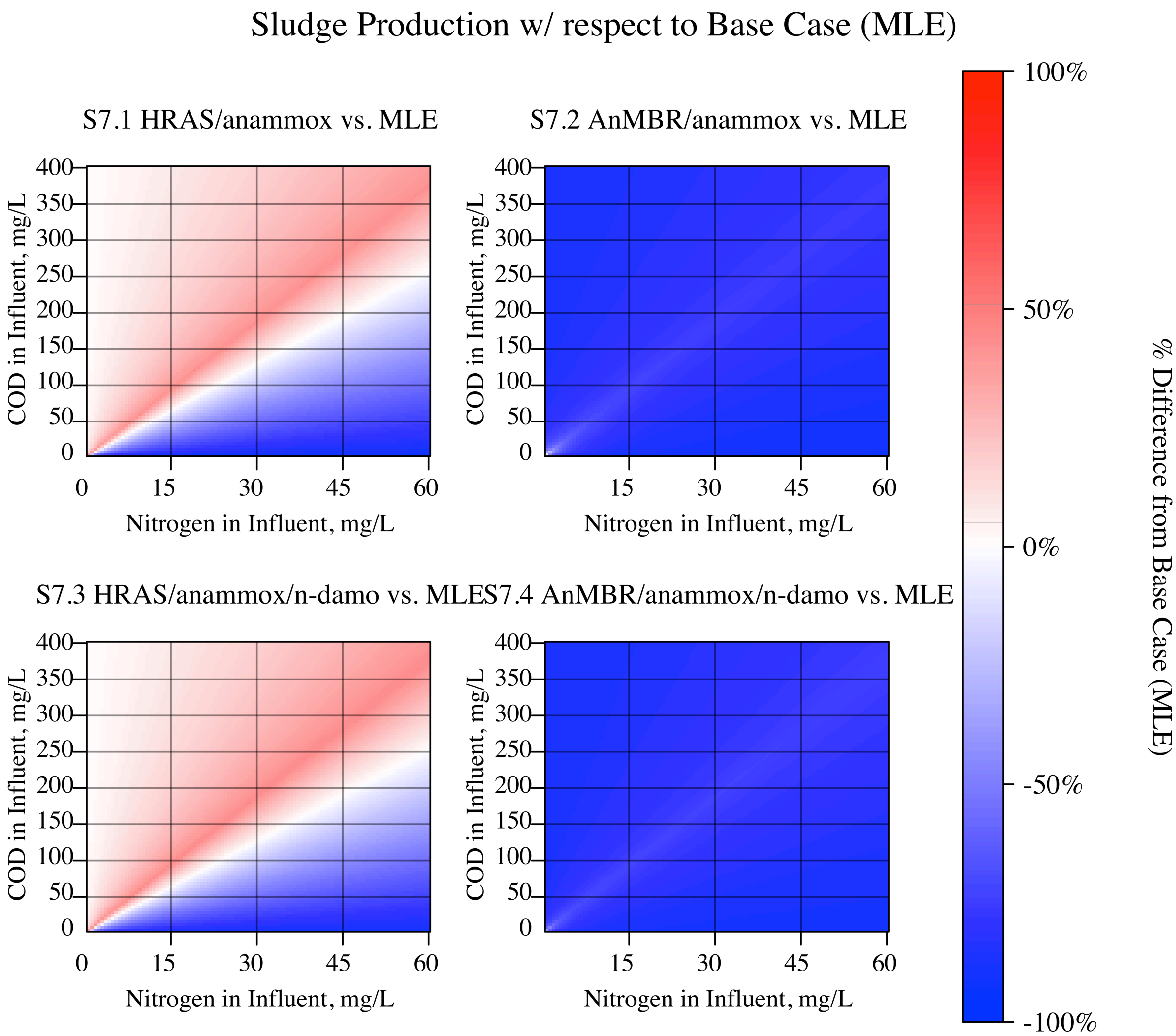

Figure S7. Comparison of sludge produced in Scenarios B (3.1), C (3.2), D (4.3), and E (3.4) to the base case scenario A (Modified Ludzack-Ettinger, MLE, also referred to as conventional nitrification/denitrification). Percentage increase or decrease in sludge discharge was shown in red (high) and blue (low) respectively as defined by the color key.

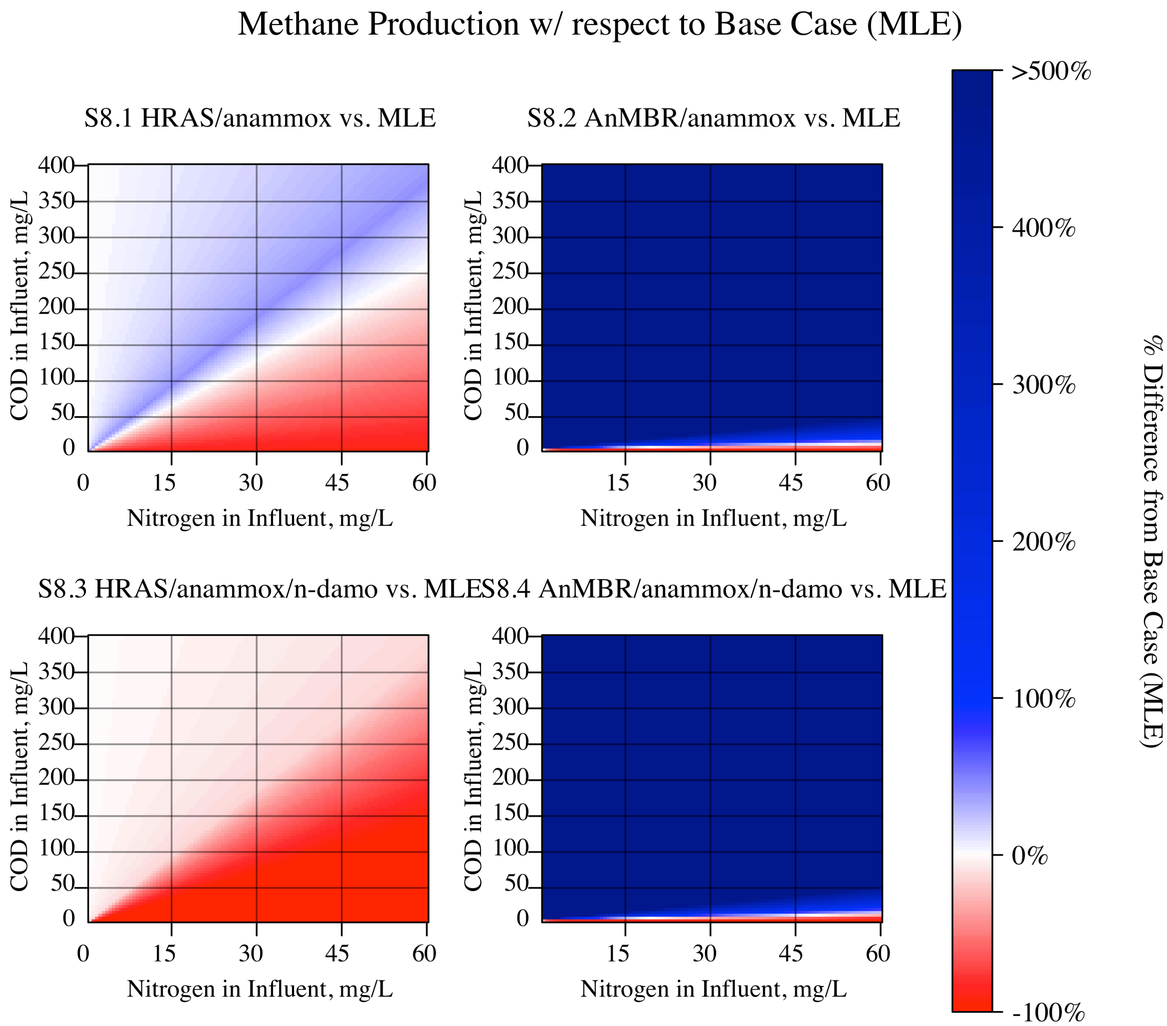

Figure S8. Comparison of methane production in Scenarios B (4.1), C (4.2), D (4.3), and E (4.4) to the base case scenario A (Modified Ludzack-Ettinger, MLE, also referred to as conventional nitrification/denitrification). Biogas production is of benefit to plant operational costs. Red is associated with a negative impact and therefore is chosen here to represent a percent decrease in methane production and blue is chosen to represent a percent increase in methane production as opposed to figures 2, 3, and 5.

External Carbon Addition

If there was not enough carbon for denitrification, the base case required exogenous carbon. In all other scenarios, nitrogen was removed via the autotrophic anammox metabolism and the only organic carbon source required, if any, was provided from biogas produced on-site. At all conditions considered in this study enough biogas was produced to supply n-damo with adequate methane for complete nitrogen removal, so no carbon addition was required for scenarios B-E, while at low COD/N ratios, carbon addition as methanol was required in scenario A. This binary conclusion resulted in a simple addition or exclusion of the cost of external carbon when calculating the cost factor and so this graph is omitted here.

Sensitivity Study of Pumping Demand on Results

Sensitivity Study of Pumping Demand on Results

A conventional nitrification/denitrification WRRF of the size in in this study (15.8 MGD, 60 th m^3/day) has a daily electrical demand between 2000-1700 kWh/MG and typically 52% and 30% of that electrical demand was devoted to the already accounted for aeration and biosolids processing, respectively.[1] The remaining 18%, representing other electrical demands such as pumping requirements, is roughly 90 kWh/th m^3. It was assumed that this value did not vary significantly when calculating the primary results in this model. In practice, the energy required for pumping could vary between scenarios. In this sensitivity study, the 90 kWh/th m^3 factor was included in calculating the electrical demand of the conventional system (base case, scenario A) while it was varied between 45-450 kWh/th m3 (50-500% of base case) in the other four scenarios examined (scenarios B-D).

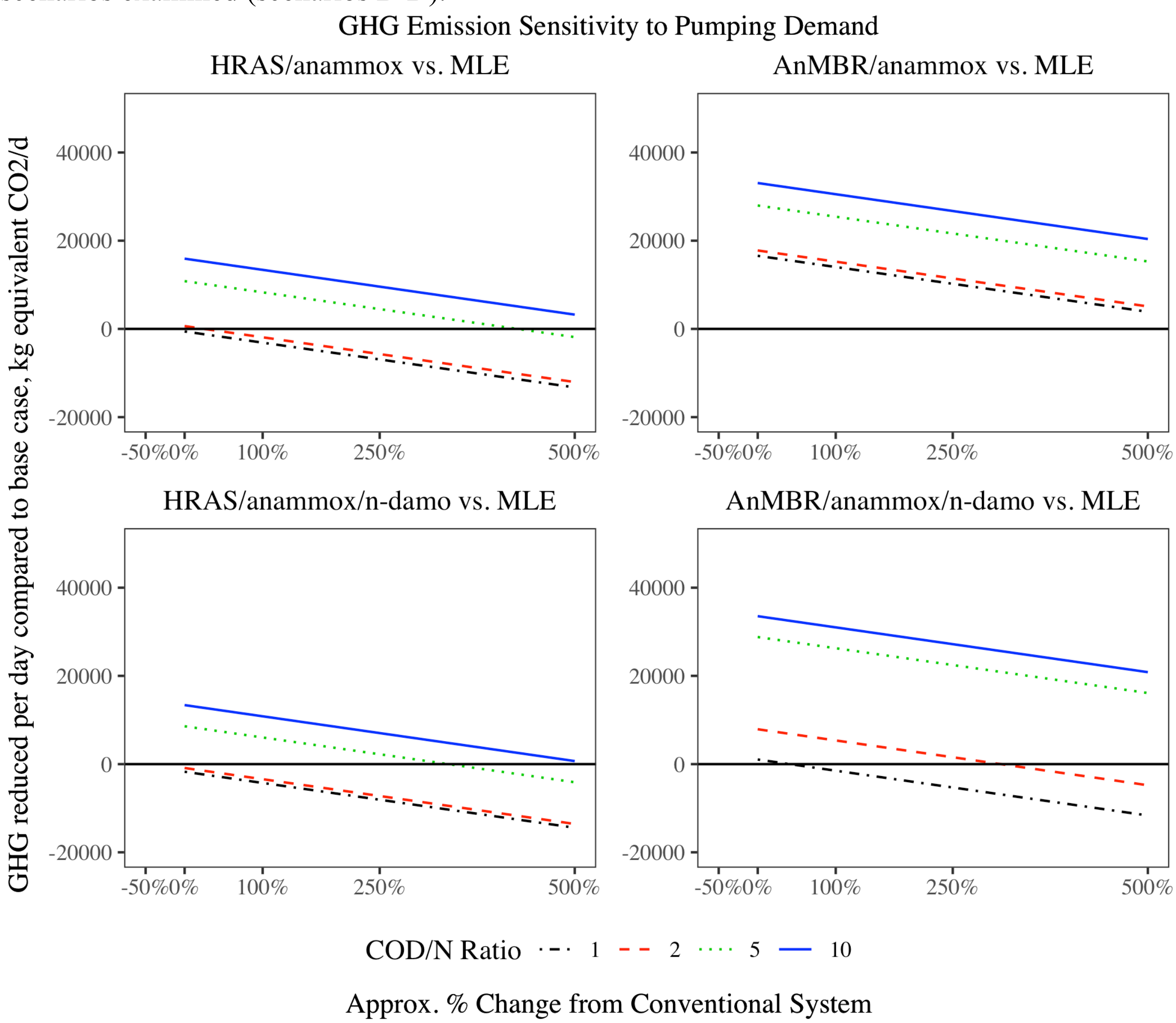

Figure S9. Sensitivity of GHG emissions to varying pumping demands in scenarios B-D. GHG emissions are measured as the kg CO_2 per day averted by utilizing one of the theoretical scenarios B-D instead of conventional nitrification/dentrification. This is plotted against the % increase or decrease in pumping demand as compared to the base case.

The difference in GHG emissions and operational cost between the base case and the four scenarios was compared at four different COD/N ratios and plotted in figures S9 & S10 respectively. At each COD/N ratio examined, COD concentration was held constant at 100 mg/L. As pumping demand increased, the relative GHG emissions averted or dollars saved decreased, which makes sense as both these values are tied to total electrical demand. It was found in both instances that at most COD/N ratios, the overall result (more or fewer GHGs than the base case) changed at the extremes of the sensitivity study if at all. The response of GHG emissions averted or dollars saved was only mildly sensitive to overall pumping demand. It can be inferred from this that the results in this study (figures 2 & 3 in the main manuscript) still hold value even though the pumping demand is not included in the cost as pumping demand would only have a mild impact on overall results.

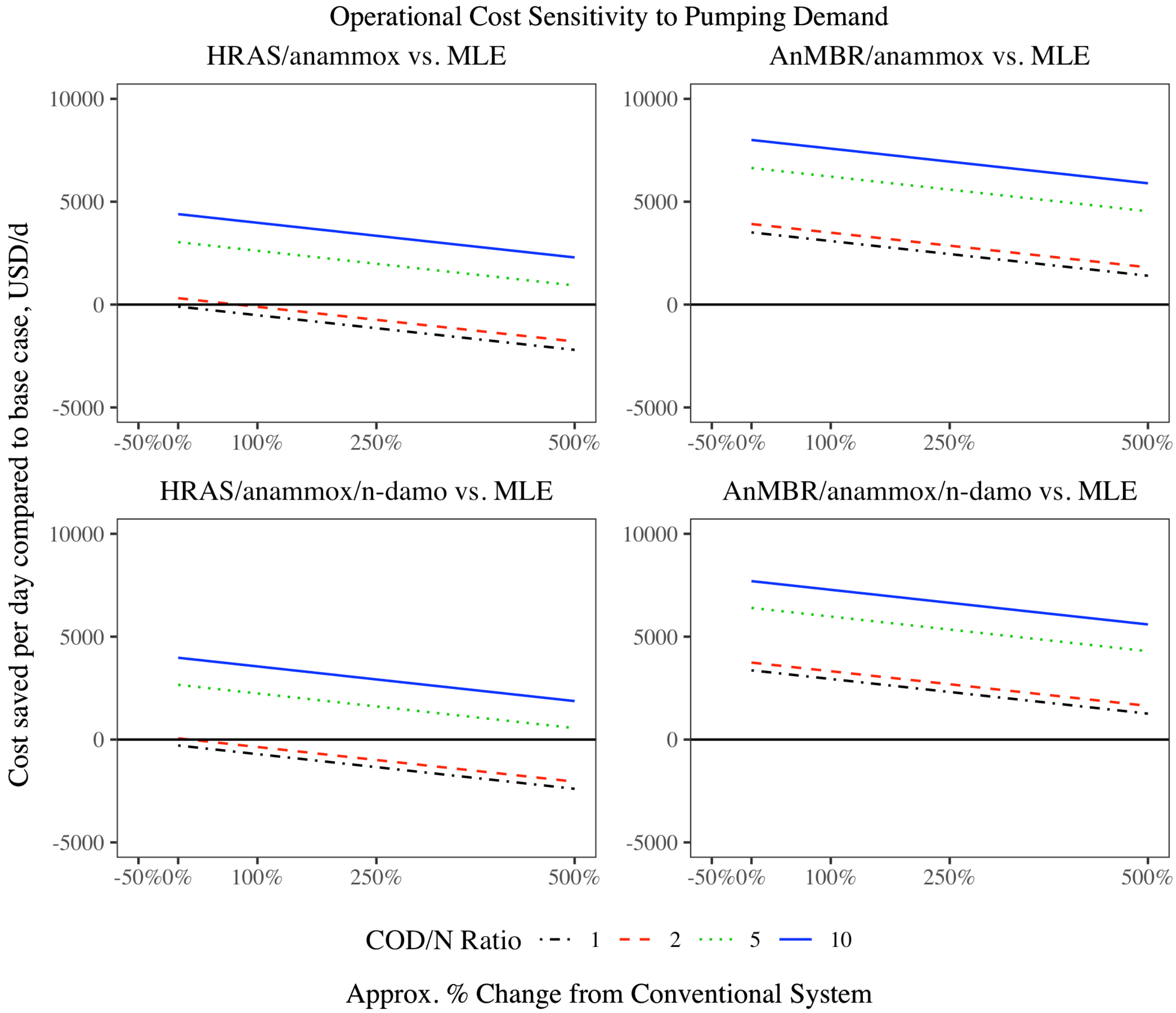

Figure S10. Sensitivity of GHG emissions to varying pumping demands in scenarios B-D. GHG emissions are measured as dollars saved per day averted by utilizing one of the theoretical scenarios B-D instead of conventional nitrification/dentrification. This is plotted against the % increase or decrease in pumping demand as compared to the base case.

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