Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Supporting Information

Nitrogen Polishing in a Partial Denitrification/Anammox MBBR Using Glycerol, Acetate, and Methanol

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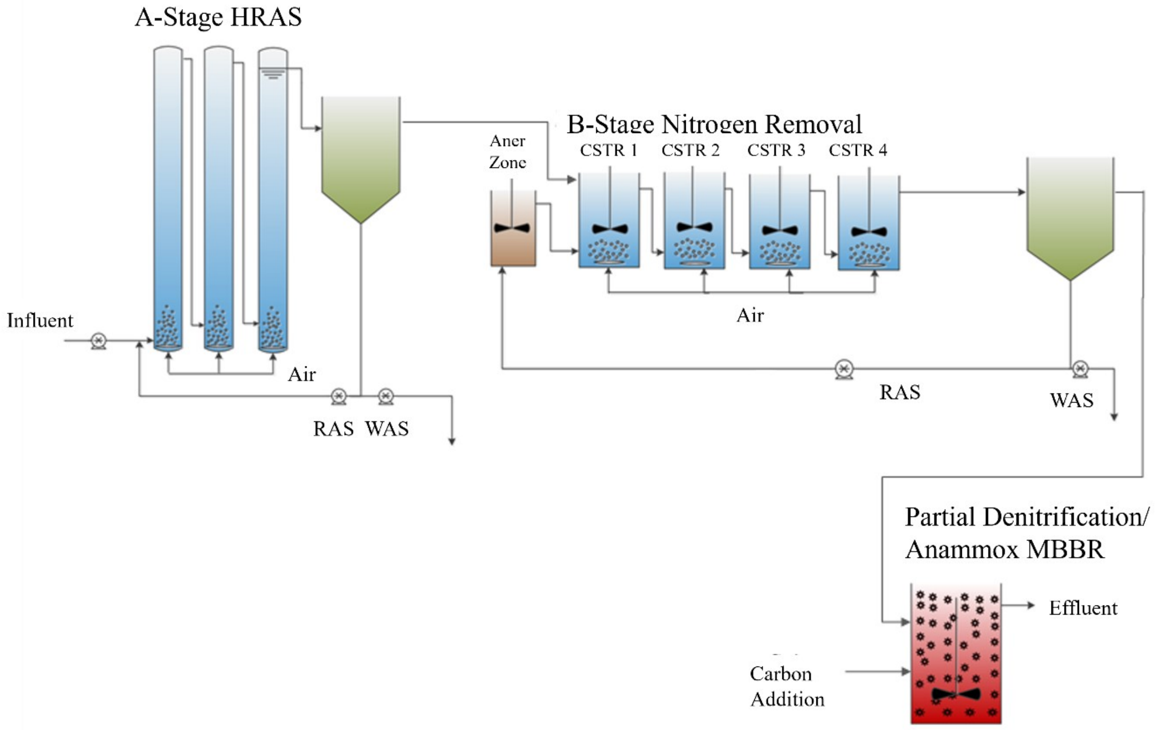

Figure S1: Pilot process flow diagram

Table S1: 16S rRNA-targeted oligonucleotide probes used in this study.

Probe	Sequence (5' to 3')	FA (%)	Specificity	Reference
AOB Mix				
NEU	CCC CTC TGC TGC ACT CTA	35	Most halophilic and halotolerant Nitrosomonas spp.	Wagner et al. 1995
CTE (NEU Competitor)	TTC CAT CCC CCT CTG CCG	35	Unlabeled together with NEU Comamonas spp., Acidovorax spp., Hydrogenophaga spp., Aquaspirillum spp.	Wagner et al. 1995
Nso1225	CGC CAT TGT ATT ACG TGT GA	35	Ammonia-oxidizing β-proteobacteria	Mobarry et al. 1996
Cluster6a192	CTT TCG ATC CCC TAC TTT CC	35	Nitrosomonas oligotropha lineage (Cluster 6a)	Adamczyk et al. 2003
Cluster6a 192 Competitor	CTT TCG ATC CCC TGC TTT CC	35	Competitor for Cluster6a192	Adamczyk et al. 2003
Nitrospira Mix				
Ntspa662	GGA ATT CCG CGC TCC TCT	35	Genus Nitrospira	Daims et al. 2001
Ntspa662 Competitor	GGA ATT CCG CTC TCC TCT	35	Competitor for Ntspa662	Daims et al. 2001
Ntspa712	CGC CTT CGC CAC CGG CCT TCC	35	Most members of the phylum Nitrospirae	Daims et al. 2001
Ntspa712 Competitor	CGC CTT CGC CAC CGG TGT TCC	35	Competitor for Ntspa712	Daims et al. 2001
Anammox Mix				
Amx820	AAA ACC CCT CTA CTT AGT GCC C	35	Brocadia anammoxidans Kuenenia stuttgartiensis	Schmid et al. 2000
Bfu 613	GGA TGC CGT TCT TCC GTT AAG CGG	35	Brocadia fulgida"	Kartal et al. 2008
NON Eub	ACT CCT ACG GGA GGC AGC	35	Negative control for nonspecific binding	Wallner et al. 1993

Table S2: Summary of qPCR primers.

Target Gene	qPCR Primer	Nucleotide Sequence (5'-3')	Base Pairs	Reference
Universal 16S rRNA	1055F	ATGGCTGTCGTCAGCT	353	Ferris et al, 1996
	1392R	ACGGGCGGTGTGTAC		
Anammox 16S rRNA	Pla46F	GGATTAGGCATGCAAGTC	674	van der Star et al, 2007
	Amx667R	ACCAGAAGTTCCACTCTC		

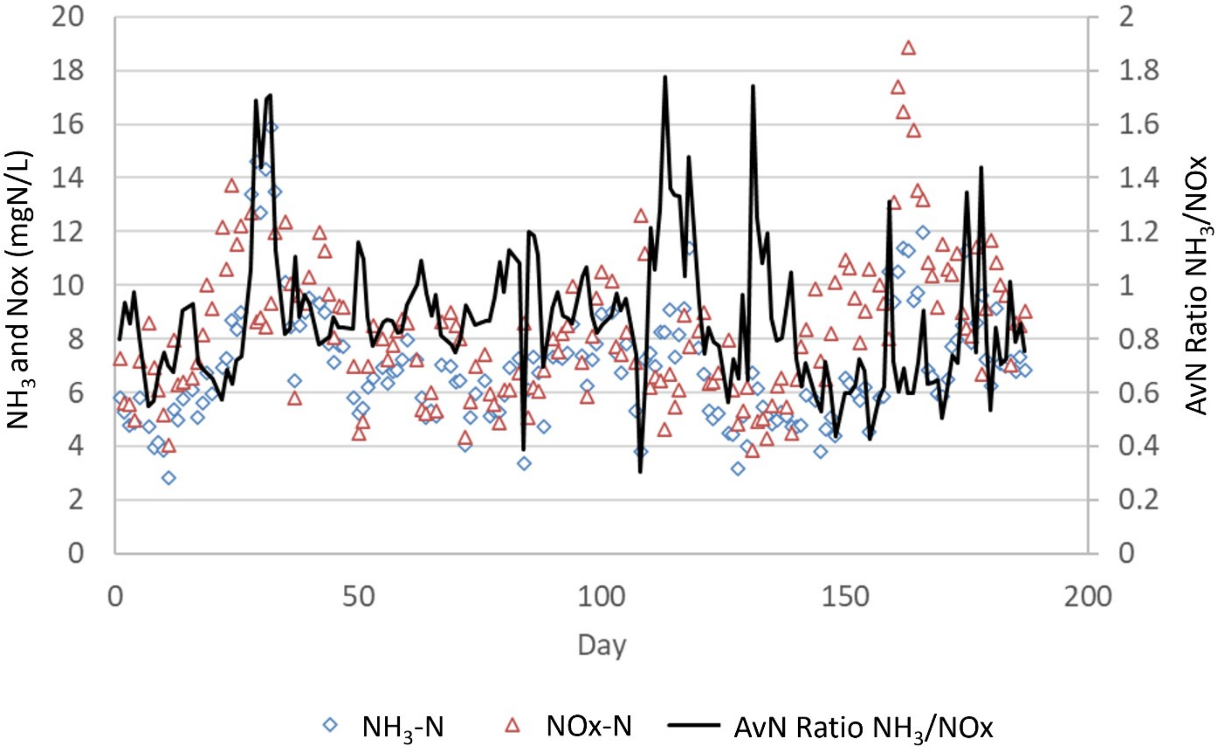

Figure S2: MBBR influent NH_3-N, NO_x-N, and AvN ratio (NH_3-N/NO_x-N). Phase I: Day 1 – 60, Phase II: Day 61 – 108, Phase III: Day 109 – 116, Phase IV: Day 117 – 128, Phase V: Day 129 – 187.

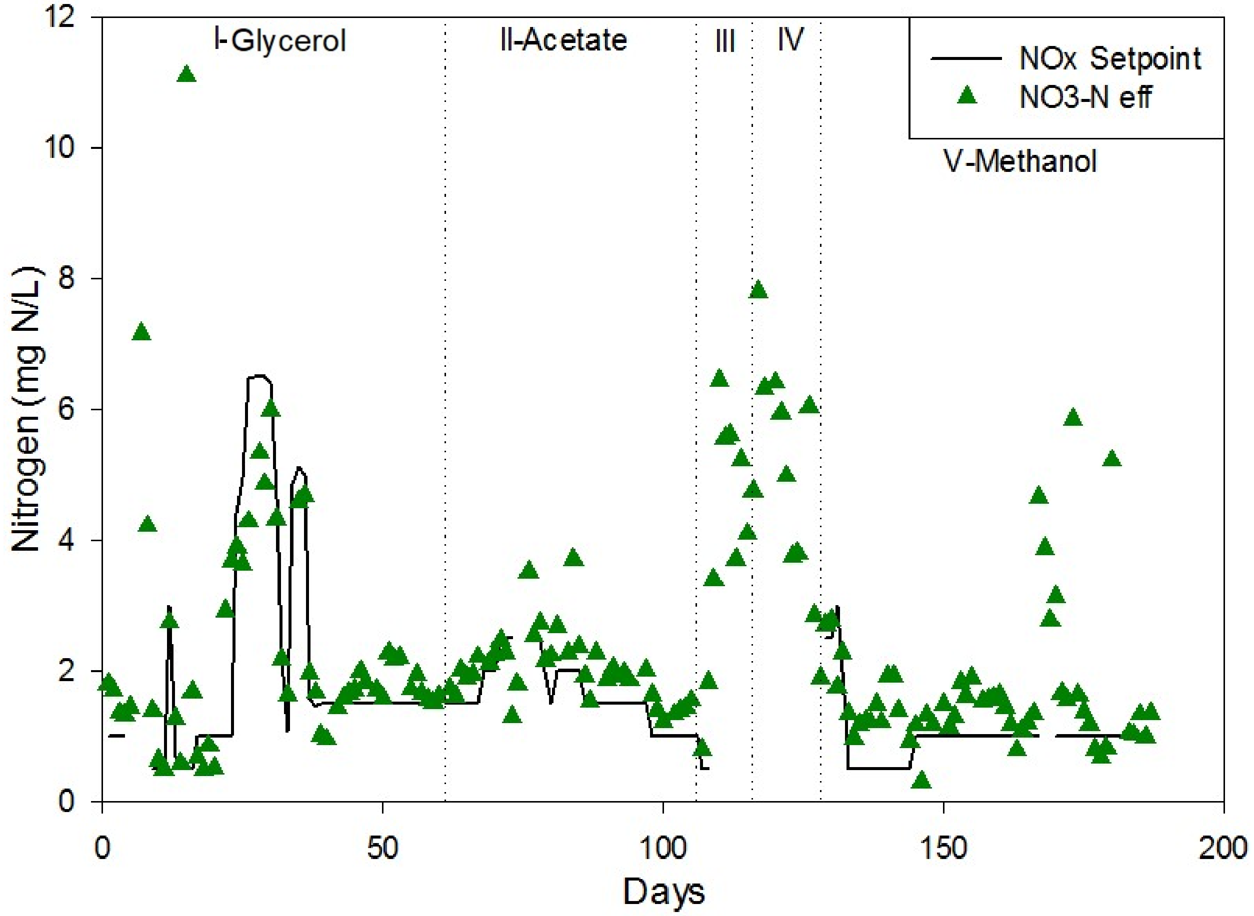

Figure S3: MBBR effluent NO_3-N composite values and MBBR effluent NOx setpoint. If no NOx setpoint is shown, then the carbon dosing pump was in manual control at a constant rate. Vertical blue lines indicate start and end dates for each phase.

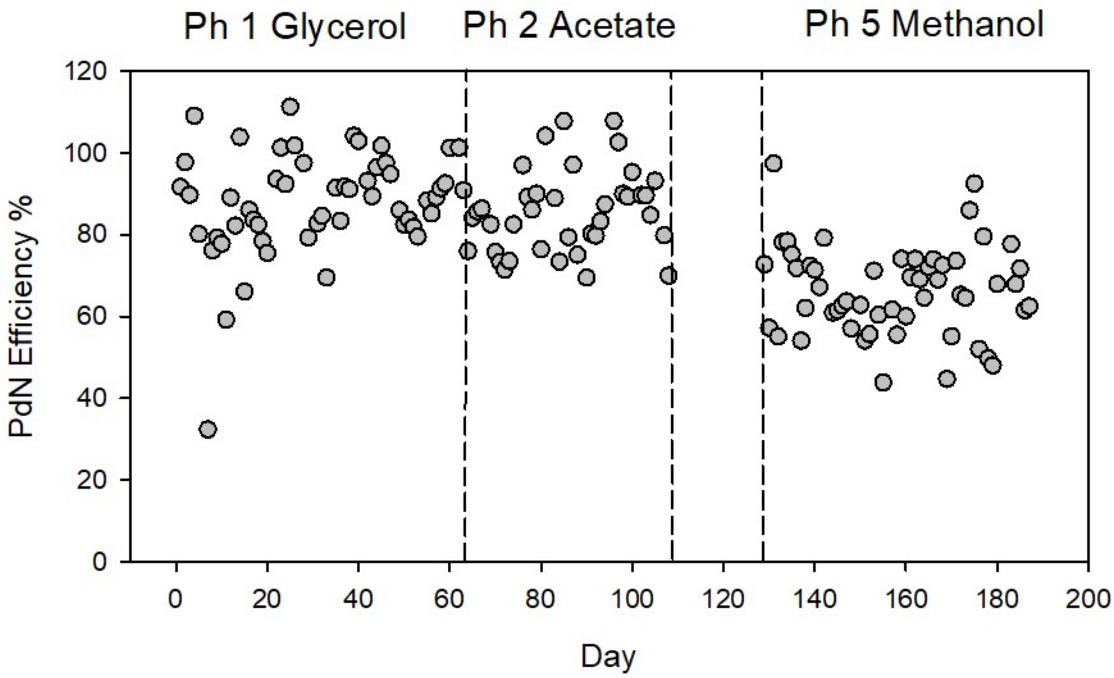

Figure S4: PdN efficiency. Phases 3 and 4 not shown because little to no carbon was added during these phases.

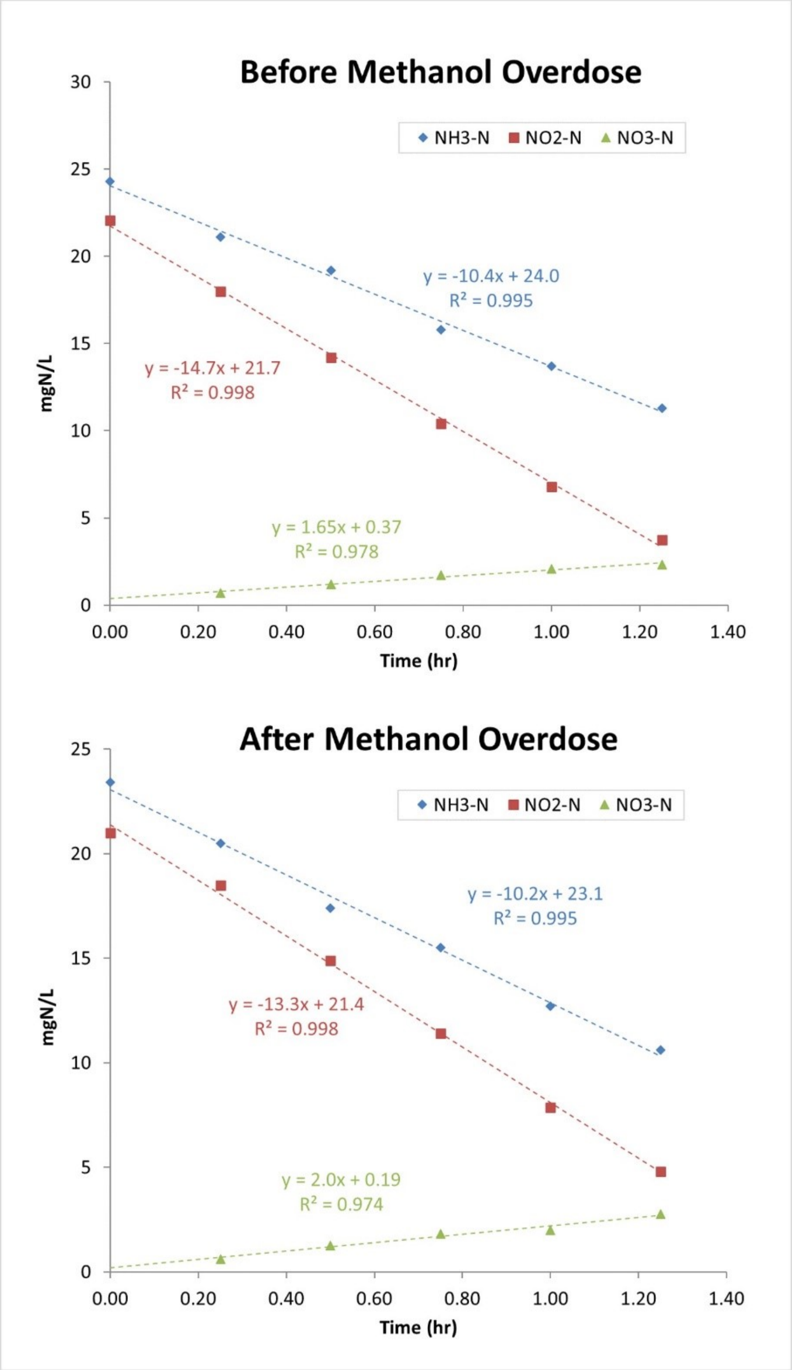

Figure S5: MBBR overdosing of methanol experiment results. Top - AMX max activity before overdosing of methanol. Bottom - AMX max activity after methanol overdosing.

Heterotrophic Assimilation Calculation

Example calculation for methanol assuming anoxic yield for methanol = 0.4 gCOD/gCOD and 20 mgCOD/L added. A yield of 0.55 gCOD/gCOD was used for glycerol, and 0.43 gCOD/gCOD was used for acetate [1,2]. This represents the maximum amount of ammonia that could have been assimilated assuming no decay.

Molecular weight of biomass, $C_5H_7O_2N$ = 113 g/mol

Molecular weight of O_2 = 32 g/mol

$C_5H_7O_2N + 5O_2 \leftrightarrow 5CO_2 + 2H_2O + NH_4$

(5 mol O_2 * 32 g O_2/mol O_2) / (1 mol $C_5H_7O_2N$ * 113 g $C_5H_7O_2N$ /mol $C_5H_7O_2N$) = 1.42 g COD/g biomass as VSS

Nitrogen content of biomass = 1 mol N/mol $C_5H_7O_2N$

(1 mol N * 14 g N/mol N)/113 g $C_5H_7O_2N$/ mol $C_5H_7O_2N$ = 0.124 g NH_4-N assimilated/g biomass VSS produced

(0.124 g NH_4-N assimilated/g biomass VSS) X (1 g biomass VSS/1.42 g COD) X (20 mg COD/L X 0.4 g/g) =

0.70 mg NH_4-N/L assimilated.

1 S. Ledwell, M. Fabiyi and G. Farmer, Optimizing Denitrification with non-Methanol Carbon Sources in Deep-Bed Denitrification Filter Technologies, *Optimizing Denitrification with non-Methanol Carbon Sources in Deep-Bed Denitrification Filter Technologies*, 2010, **2010**, 548–565.

2 C. P. L. Grady, G. T. Daigger and H. C. Lim, *Biological wastewater treatment / edited by C. P. Leslie Grady, Jr., Glen T. Daigger, Henry C. Lim.*, Marcel Dekker, New York, Second edition revised and expanded., 1999.