Supporting Information

Effect of Influent Carbon Fractionation and Reactor Configuration on Mainstream Nitrogen Removal and NOB Out-Selection

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Table S1: Summary of primers utilized for qPCR analysis

Target Gene	qPCR Primer	Nucleotide Sequence (5'-3')	Base Pairs	Reference
Universal 16S rRNA	1055F	ATGGCTGTCGTCAGCT	353	Ferris et al, 1996
	1392R	ACGGGCGGTGTGTAC		
amoA	amoA-1F	GGGGTTTCTACTGGTGGT	491	Rotthauwe et al, 1997
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC		
Nitrospira 16S rRNA	NSPRA-675f	GCGGTGAAATGCGTAGAKATCG	67	Kindaichi et al, 2006
	NSPRA-746r	TCAGCGTCAGRWAYGTTCCAGAG		
	Nspra-723Taq	CGCCGCCTTCGCCACCG		
Nitrobacter 16S rRNA	Nitro-1198f	ACCCCTAGCAAATCTCAAAAAACCG	227	Graham et al, 2007
	Nitro-1423r	CTTCACCCCAGTCGCTGACC		
	Nitro-1374Taq	AACCCGCAAGGGAGGCAGCCGACC		

Thermal cycling profile for qPCR

Thermal cycling for universal 16S rRNA gene was carried out with an initial denaturation step at 94°C for 3 min, which was followed by 40 cycles of denaturation at 94°C for 30 s, primer annealing at 54°C for 40 s, and elongation at 72°C for 45 s. Melt curve analysis was performed from 50-95 °C with 0.5 °C increments, each for 10s.

Thermal cycling for *amoA* gene was carried out with an initial denaturation step at 94°C for 2 min, which was followed by 40 cycles of denaturation at 94°C for 30 s, primer annealing at 57°C for 40 s, and elongation at 72°C for 30 s. Melt curve analysis was performed from 50-95 °C with 0.5 °C increments, each for 10s.

Thermal cycling for *Nitrospira* 16S rRNA gene was carried out with an initial denaturation step at 94°C for 10 min, which was followed by 40 cycles of denaturation at 94°C for 30 s, primer annealing at 58°C for 30 s, and elongation at 72°C for 40 s.

Thermal cycling for *Nitrobacter* 16S rRNA gene was carried out with an initial denaturation step at 94°C for 5 min, which was followed by 40 cycles of denaturation at 94°C for 20 s, primer annealing at 58°C for 40 s, and elongation at 72°C for 40 s.

Explanation of Nitrification and Denitrification Rate Test Calculations

During nitrification, all NO_3^- produced was once NO_2^-. Similarly, during denitrification, all NO_3^- must first be reduced to NO_2^- before N_2 gas. So it is assumed that all NO_3^- reduced to N_2 gas (full denitrification), is at some point NO_2^-. In the AOB/NOB test if nitrite is accumulating then AOB rate is faster than NOB rate. Similarly, in the denitrification batch test, if nitrite is accumulating then the denitr**a**tation rate (NO_3^- to NO_2^-) is faster than the denitr**i**tation rate (NO_2^- to N_2 gas). See figure S1 for a graphical representation of the potential batch test results.

Nitrification Rate Test Calculations:

AOB (nitr**i**tation) and NOB (nitr**a**tation) rates are measured simultaneously under aerobic conditions, without substrate limitation. NOB rates are measured as the change in NO_3^- over time and AOB rates are measured as the change in NO_x (NO_3^- +NO_2^-) over time.

Measured Rates:

$rNO_2^- = nitrite\ rate\ measured\ in\ batch\ test$

$rNO_3^- = nitrate\ rate\ measured\ in\ batch\ test$

$rNO_x = rNO_2^- + rNO_3^-$

Unknown Rates:

$rNO_{2\ NOB}^- = rate\ of\ nitrite\ consumed\ by\ NOB$

$rNO_{2\ AOB}^- = rate\ of\ nitrite\ produced\ by\ AOB$

$rNO_{3\ NOB}^- = rate\ of\ nitrate\ produced\ by\ NOB$

Assume:

NO_3^- can only be produced by NOB, so $rNO_3^- = rNOB_{NO3}$

NO_2^- is produced by AOB and consumed by NOB

$rNO_{3\ NOB}^- = rNO_{2\ NOB}^-$

Calculations:

From equations above: $rNO_3^- = rNO_{3\ NOB}^- = -rNO_{2\ NOB}^-$

Therefore $rNO_3^- = -rNO_{2\ NOB}^-$ (EQ 1)

$rNO_2^- = rNO_{2\ AOB}^- + rNO_{2\ NOB}^-$ (EQ 2)

By substituting EQ 1 into EQ 2: $rNO_{2\ AOB}^- = rNO_2^- + rNO_3^-$

Therefore: $rNO_{2\ AOB}^- = rNO_x$

Denitrification Rate Test Calculations:

The denitr**a**tation (NO_3^- to NO_2^-) rates and denitr**i**tation (NO_2^- to N2 gas) rates are measured simultaneously under anoxic conditions, without substrate limitation. Denitr**a**tation rates are measured as the change in NO_3^- over time and denitr**i**tation rates are measured as the change in NO_x (NO_3^- +NO_2^-) over time.

Measured Rates:

$rNO_2^- \;=\; nitrite\ rate\ measured\ in\ batch\ test$
$rNO_3^- \;=\; nitrate\ rate\ measured\ in\ batch\ test$
$rNO_x \;=\; rNO_2^- + rNO_3^-$

Unknown Rates:
$rNO_{2\ denNO2-N2}^- \;=\; rate\ of\ nitrite\ consumed\ by\ denitritation$

$rNO_{2\ denNO3-NO2}^- = \; rate\ of\ nitrite\ produced\ by\ denitratation$

$rNO_{3\ denNO3-NO2}^- = \; rate\ of\ nitrate\ consumed\ by\ denitratation$

Assume:

NO_3^- can only be consumed by denitr**a**tation, so $rNO_3^- \;=\; rNO_{3\ denNO3-NO2}^-$

NO_2^- is produced by denitr**a**tation and consumed by denitr**i**tation

$rNO_{3\ denNO3-NO2}^- \;=\; rNO_{2\ denNO3-NO2}^-$

Calculations:

From equations above: $-rNO_3^- \;=\; rNO_{3\ denNO3-NO2}^- = \; rNO_{2\ denNO3-NO2}^-$

Therefore $-rNO_3^- \;=\; rNO_{2\ denNO3-NO2}^-$ (EQ 1)

$rNO_2^- \;=\; rNO_{2\ denNO3-NO2}^- + \; rNO_{2\ denNO2-N2}^-$ (EQ 2)

By substituting EQ 1 into EQ 2: $rNO_{2\ denNO2-N2}^- = \; -rNO_3^- + rNO_2^-$

Therefore: $rNO_{2\ denNO2-N2}^- = \; -rNO_x$

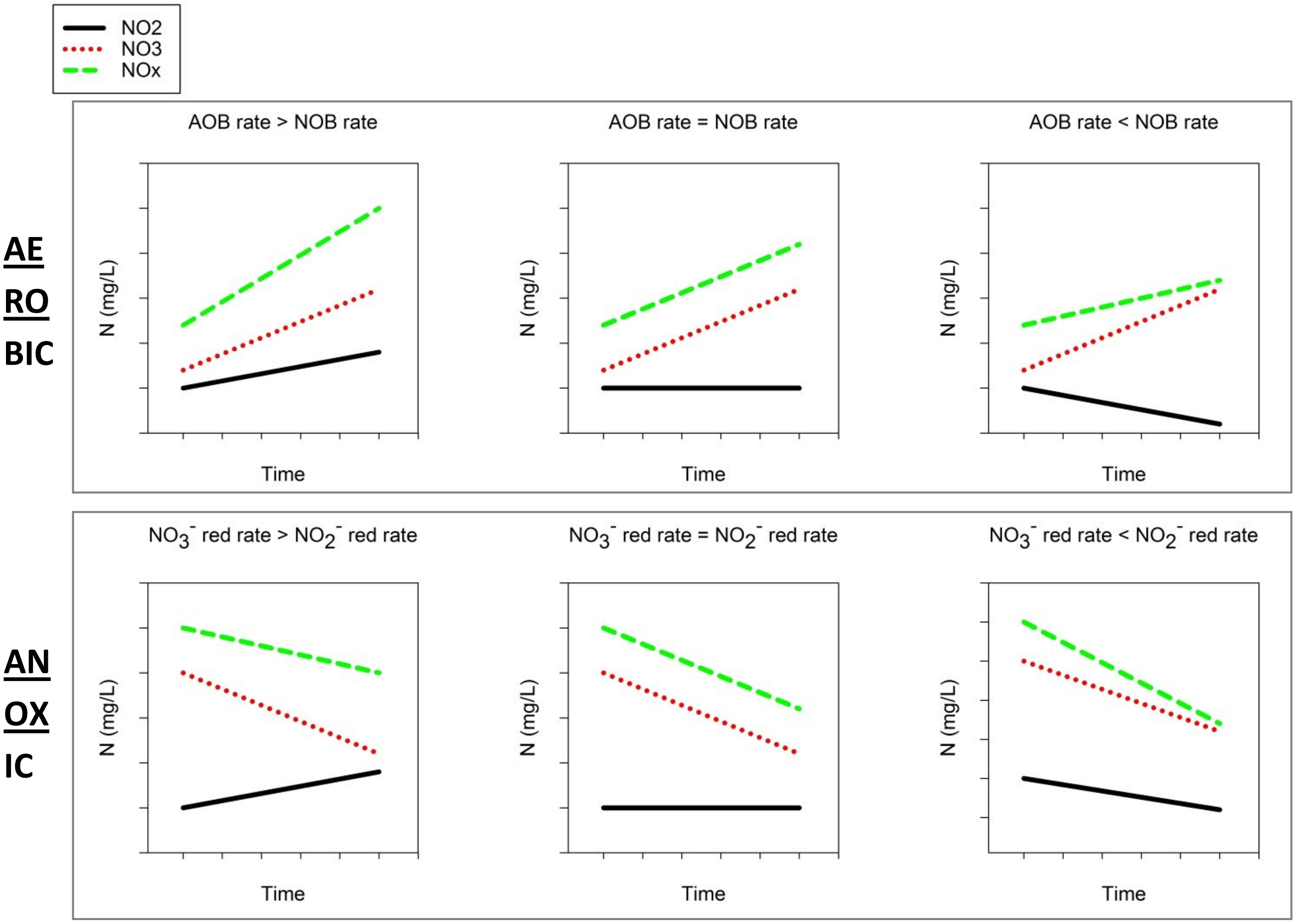

Figure S1: Examples of theoretical batch tests with varying AOB/NOB and NO_3^-/NO_2^- reduction rates

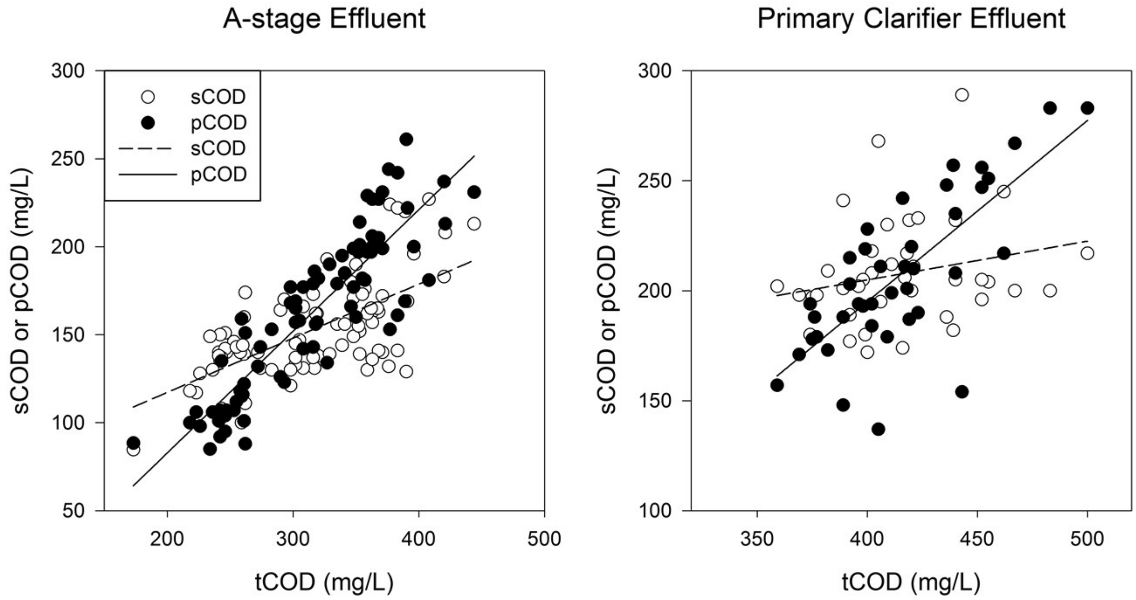

Figure S2: Particulate COD (pCOD) and soluble COD (sCOD, 1.5μm filtered) vs. total COD (tCOD) for A-stage effluent and primary clarifier effluent.

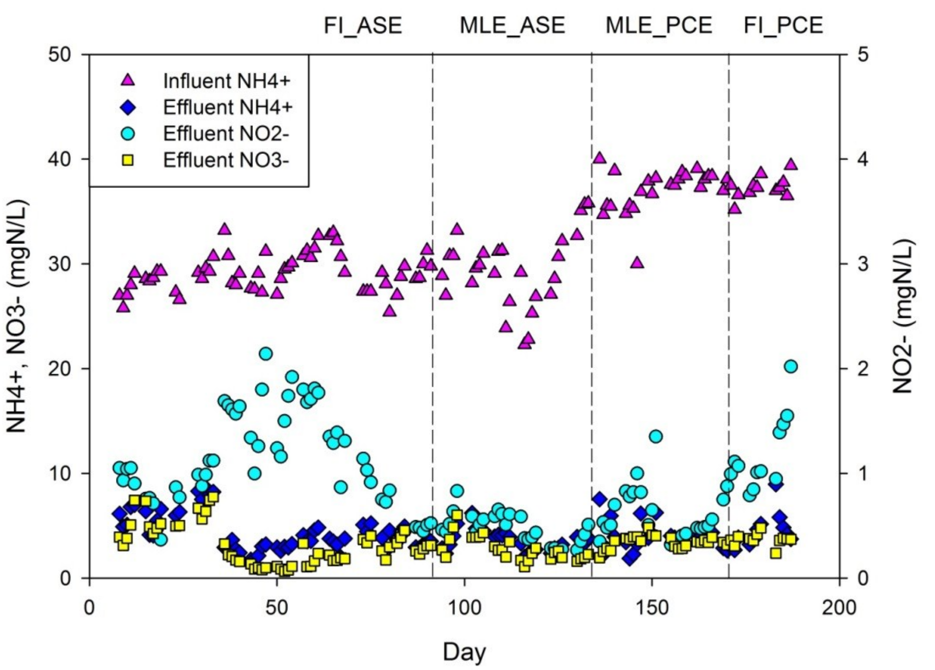

Figure S3: Concentration of influent and effluent ammonia, effluent nitrite, and effluent nitrate over time

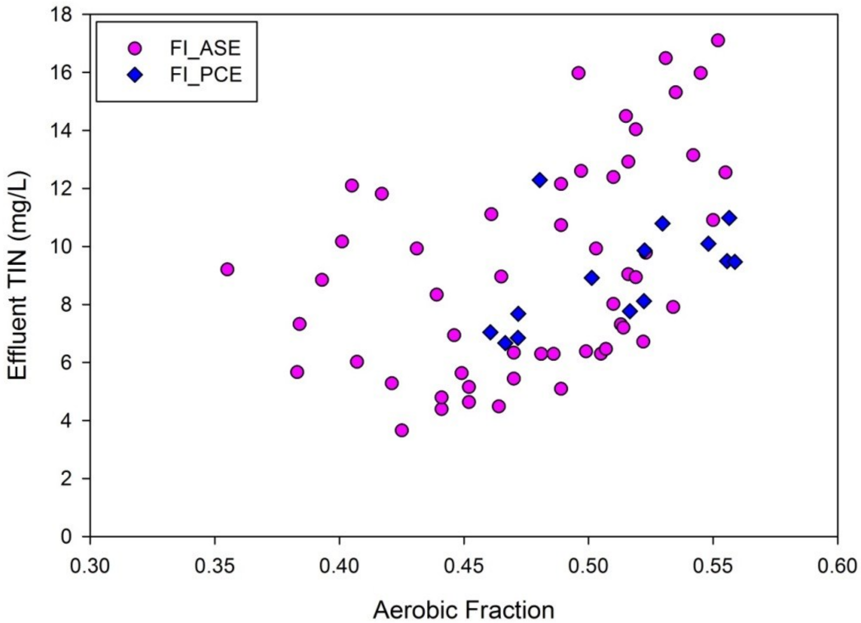

Figure S4: Effluent TIN vs. aerobic fraction for the fully intermittent scenarios (FI_ASE and FI_PCE).

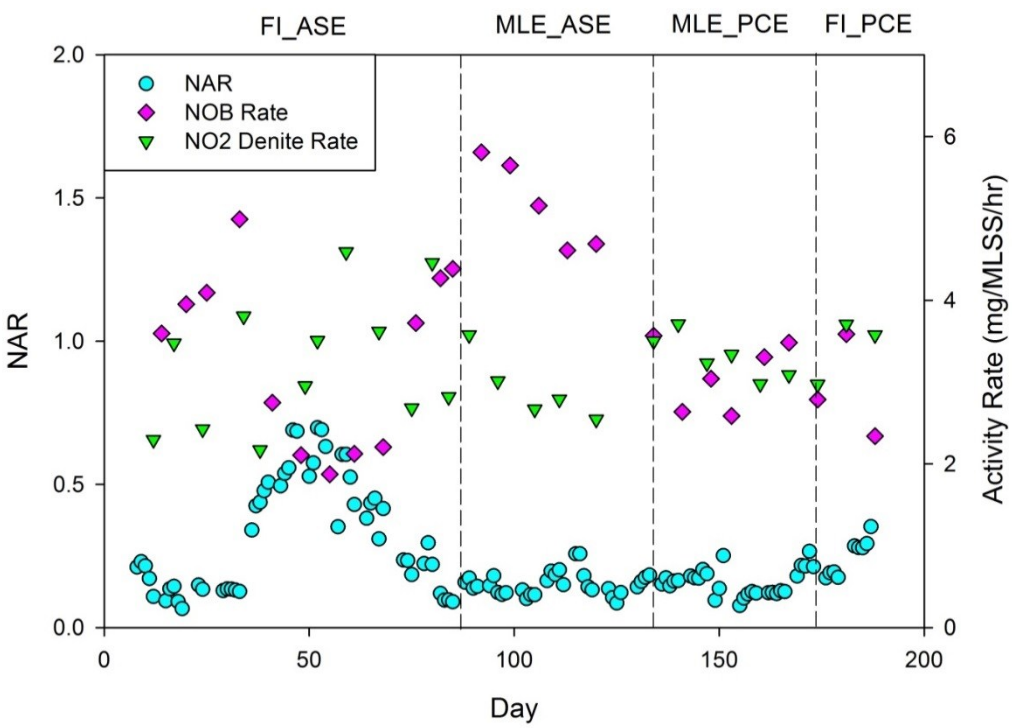

Figure S5: NOB rate and NO_2^- specific denitrification rates from ex-situ maximum activity rate tests in mg/MLSS/hr and nitrite accumulation ratio (NAR).