

Article

Efficacies of Nitrogen Removal and Comparisons of Microbial Communities in Full-Scale (Pre-Anoxic Systems) Municipal Water Resource Recovery Facilities at Low and High COD:TN Ratios

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Abstract: At a low COD:TN ratio (≤ 5) in influent, maintaining a longer HRT (≥ 9 h) and longer SRT (≥ 30 d) are suggested to improve higher N removal efficiency in case of operation at low DO (Dissolved oxygen) level (0.9 ± 0.2 mg-O₂/L). However, in case of operation at high DO level (4.0 ± 0.5 mg-O₂/L), short HRT (1 h) and typical SRT (17 d) make it possible to achieve nitrogen removal. On the other hand, at a high COD:TN ratio (≥ 8.4), a typical HRT (9–15 h), SRT (12–19 d), and DO level (1.3 – 2.6 mg-O₂/L) would be applied. Microbial distribution analysis showed an abundance of AOA (Ammonia-oxidizing archaea) under conditions of low DO (≤ 0.9 mg-O₂/L). *Nitrosomonas* sp. are mostly found in the all investigated water resource recovery facilities (WRRFs). *Nitrospira* sp. are only found under operating conditions of longer SRT for WRRFs with a low COD:TN ratio. In comparison between abundances of *Nitrobacter* sp. and *Nitrospira* sp., abundances of *Nitrobacter* sp. are proportional to low DO concentration rather than abundance of *Nitrospira* sp. A predominance of *nosZ*-type denitrifiers were found at low DO level. Abundance of denitrifiers by using *nirS* genes showed an over-abundance of denitrifiers by using *nirK* genes at low and high COD:TN ratios.

Keywords: pre-anoxic; COD:TN; nitrogen removal; microbial communities

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Table S1. Efficiency, slope and r^2 values of individual real-time PCR assays.

Target group	Target gene	Primer set	Linearity range	Efficiency		
				%	Slope	r^2
All bacteria	EUB	338F/518R	10 ² –10 ⁹	106.1	−3.1	0.993
AOA	Ammonium monooxygenase (<i>amoA</i>)	Arch-amoAF/AR	10 ¹ –10 ⁸	102.4	−3.5	0.999
AOB	Ammonium monooxygenase (<i>amoA</i>)	amoA1F/2R	10 ¹ –10 ⁸	99.6	−3.4	0.993
NOB	16S rRNA <i>Nitrobacter</i> sp.	Nb1000F/1387R	10 ¹ –10 ⁸	105.4	−3.2	0.997
	16S rRNA <i>Nitrospira</i> sp.	NSR1113F/1264R	10 ¹ –10 ⁸	104.4	−3.0	0.996
	Nitrite reductase (<i>nirK</i>)	nirScd3AF/R3cd	10 ¹ –10 ⁸	99.8	−3.3	0.993
DNB	Nitrite reductase (<i>nirS</i>)	nirKF1aCu/R3Cu	10 ¹ –10 ⁸	98.7	−3.3	0.999
	N ₂ O reductase (<i>nosZ</i>)	nosZ2F/Z2R	10 ¹ –10 ⁸	98.3	−3.3	0.995

Table S2. Overall gene abundance of the with and without pre-anaerobic plants by multiple mean comparisons of one-way ANOVA test.

Process	Target, copies-DNA/g-MLVSS															
	EUB		<i>amoA</i> -AOA		<i>amoA</i> -AOB		NSR		Nitrob		<i>nirS</i>		<i>nirK</i>		<i>nosZ</i>	
	Avg	Group	Avg	Group	Avg	Group	Avg	Group	Avg	Group	Avg	Group	Avg	Group	Avg	Group
w/o pre-anaerobic	L1															
	Anx 4.16×10 ¹¹	<i>a</i>	6.19×10 ⁷	<i>b</i>	6.10×10 ⁶	<i>a</i>	1.38×10 ⁶	<i>c</i>	8.09×10 ⁸	<i>a</i>	4.21×10 ⁹	<i>a</i>	5.07×10 ⁷	<i>c</i>	5.12×10 ¹⁰	<i>a</i>
	Aer 4.31×10 ¹¹	<i>a</i>	8.89×10 ⁷	<i>a</i>	6.91×10 ⁶	<i>a</i>	3.21×10 ⁶	<i>c</i>	7.39×10 ⁸	<i>a</i>	4.02×10 ⁹	<i>a</i>	4.33×10 ⁷	<i>c</i>	8.23×10 ⁹	<i>c</i>
	H1															
w/- pre-anaerobic	Anx 1.67×10 ¹¹	<i>b</i>	1.57×10 ⁶	<i>c</i>	1.12×10 ⁶	<i>cde</i>	3.11×10 ⁶	<i>c</i>	2.39×10 ⁸	<i>c</i>	4.56×10 ⁸	<i>c</i>	6.12×10 ⁷	<i>c</i>	2.14×10 ¹⁰	<i>b</i>
	Aer 6.28×10 ¹⁰	<i>c</i>	6.06×10 ⁶	<i>c</i>	1.65×10 ⁶	<i>cd</i>	2.72×10 ⁶	<i>c</i>	3.85×10 ⁸	<i>b</i>	6.17×10 ⁷	<i>c</i>	1.08×10 ⁷	<i>d</i>	3.95×10 ⁹	<i>cd</i>
	L2															
	Ana 6.73×10 ⁹	<i>d</i>	2.29×10 ⁵	<i>c</i>	3.39×10 ⁵	<i>de</i>	1.03×10 ⁸	<i>c</i>	1.34×10 ⁷	<i>d</i>	5.41×10 ⁷	<i>c</i>	1.24×10 ⁷	<i>d</i>	1.26×10 ⁷	<i>d</i>
w/- pre-anaerobic	Anx 7.90×10 ⁹	<i>d</i>	5.05×10 ⁴	<i>c</i>	2.29×10 ⁵	<i>e</i>	6.21×10 ⁷	<i>c</i>	1.08×10 ⁷	<i>d</i>	5.86×10 ⁷	<i>c</i>	5.73×10 ⁷	<i>c</i>	1.55×10 ⁷	<i>d</i>
	Aer 4.85×10 ⁹	<i>d</i>	1.56×10 ⁵	<i>c</i>	3.35×10 ⁵	<i>de</i>	6.97×10 ⁷	<i>c</i>	7.46×10 ⁶	<i>d</i>	5.19×10 ⁷	<i>c</i>	4.65×10 ⁷	<i>c</i>	1.03×10 ⁷	<i>d</i>
	H2															
	Ana 3.99×10 ¹⁰	<i>cd</i>	1.95×10 ⁵	<i>c</i>	3.36×10 ⁶	<i>b</i>	6.21×10 ⁸	<i>a</i>	1.73×10 ⁷	<i>d</i>	1.35×10 ⁹	<i>b</i>	2.27×10 ⁸	<i>a</i>	3.71×10 ⁸	<i>d</i>
w/- pre-anaerobic	Anx 3.91×10 ¹⁰	<i>cd</i>	3.81×10 ⁵	<i>c</i>	1.27×10 ⁶	<i>cde</i>	5.05×10 ⁸	<i>b</i>	1.66×10 ⁷	<i>d</i>	1.12×10 ⁹	<i>b</i>	1.37×10 ⁸	<i>b</i>	2.31×10 ⁸	<i>d</i>
	Aer 3.48×10 ¹⁰	<i>cd</i>	1.39×10 ⁵	<i>c</i>	1.76×10 ⁶	<i>c</i>	4.05×10 ⁸	<i>b</i>	1.73×10 ⁷	<i>d</i>	9.60×10 ⁸	<i>b</i>	1.39×10 ⁸	<i>b</i>	2.23×10 ⁸	<i>d</i>

Remark: An italic letter is multiple mean comparisons significantly grouping by pairwise comparisons of plant zone (show small letter: *a* to *e*), the means that do not share a letter refer significantly different at $p > 0.05$.