

## 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

Supaporn Phanwilai <sup>1</sup>, Pongsak (Lek) Noophan <sup>2,\*</sup>, Chi-Wang Li <sup>3</sup> and Kwang-Ho Choo <sup>4</sup>

<sup>1</sup> Department of Knowledge of The Land for Sustainable, School of Integrated Science, Kasetsart University, Bangkok 10900 Thailand; supaporn.phan@ku.th

<sup>2</sup> Department of Environmental Engineering, Faculty of Engineering, Kasetsart University, Bangkok 10900 Thailand

<sup>3</sup> Department of Water Resources and Environmental Engineering, Tamkang University, New Taipei City 25137, Taiwan; chiwang@mail.tku.edu.tw

<sup>4</sup> Department of Environmental Engineering, Kyungpook National University, 80 Daehak-ro, Buk-gu, Daegu 702-701, Korea; chookh@knu.ac.kr

\* Correspondence: pongsak.n@ku.ac.th; Tel: +66-2797-0999 (ext 1008)

**Abstract:** At a low COD:TN ratio ( $\leq 5$ ) in influent, maintaining a longer HRT ( $\geq 9$  h) and longer SRT ( $\geq 30$  d) are suggested to improve higher N removal efficiency in case of operation at low DO (Dissolved oxygen) level ( $0.9 \pm 0.2$  mg-O<sub>2</sub>/L). However, in case of operation at high DO level ( $4.0 \pm 0.5$  mg-O<sub>2</sub>/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 ( $\geq 8.4$ ), a typical HRT (9–15 h), SRT (12–19 d), and DO level (1.3–2.6 mg-O<sub>2</sub>/L) would be applied. Microbial distribution analysis showed an abundance of AOA (Ammonia-oxidizing archaea) under conditions of low DO ( $\leq 0.9$  mg-O<sub>2</sub>/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

**Citation:** Phanwilai, S.; Noophan, P.; Li, C.-W.; Choo, K.-H. 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. *Water* **2022**, *14*, 720. <https://doi.org/10.3390/w14050720>

Academic Editors: Laura Bulgariu and Marco Guida

Received: 1 January 2022

Accepted: 18 February 2022

Published: 24 February 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**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 <sup>2</sup> –10 <sup>9</sup>	106.1	–3.1	0.993
AOA	Ammonium monooxygenase ( <i>amoA</i> )	Arch-amoAF/AR	10 <sup>1</sup> –10 <sup>8</sup>	102.4	–3.5	0.999
AOB	Ammonium monooxygenase ( <i>amoA</i> )	amoA1F/2R	10 <sup>1</sup> –10 <sup>8</sup>	99.6	–3.4	0.993
NOB	16S rRNA <i>Nitrobacter</i> sp.	Nb1000F/1387R	10 <sup>1</sup> –10 <sup>8</sup>	105.4	–3.2	0.997
	16S rRNA <i>Nitrospira</i> sp.	NSR1113F/1264R	10 <sup>1</sup> –10 <sup>8</sup>	104.4	–3.0	0.996
DNB	Nitrite reductase ( <i>nirK</i> )	nirScd3AF/R3cd	10 <sup>1</sup> –10 <sup>8</sup>	99.8	–3.3	0.993
	Nitrite reductase ( <i>nirS</i> )	nirKF1aCu/R3Cu	10 <sup>1</sup> –10 <sup>8</sup>	98.7	–3.3	0.999
	N <sub>2</sub> O reductase ( <i>nosZ</i> )	nosZ2F/Z2R	10 <sup>1</sup> –10 <sup>8</sup>	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	<b>L1</b>																
	Anx	4.16×10 <sup>11</sup>	<i>a</i>	6.19×10 <sup>7</sup>	<i>b</i>	6.10×10 <sup>6</sup>	<i>a</i>	1.38×10 <sup>6</sup>	<i>c</i>	8.09×10 <sup>8</sup>	<i>a</i>	4.21×10 <sup>9</sup>	<i>a</i>	5.07×10 <sup>7</sup>	<i>c</i>	5.12×10 <sup>10</sup>	<i>a</i>
	Aer	4.31×10 <sup>11</sup>	<i>a</i>	8.89×10 <sup>7</sup>	<i>a</i>	6.91×10 <sup>6</sup>	<i>a</i>	3.21×10 <sup>6</sup>	<i>c</i>	7.39×10 <sup>8</sup>	<i>a</i>	4.02×10 <sup>9</sup>	<i>a</i>	4.33×10 <sup>7</sup>	<i>c</i>	8.23×10 <sup>9</sup>	<i>c</i>
	<b>H1</b>																
Anx	1.67×10 <sup>11</sup>	<i>b</i>	1.57×10 <sup>6</sup>	<i>c</i>	1.12×10 <sup>6</sup>	<i>cde</i>	3.11×10 <sup>6</sup>	<i>c</i>	2.39×10 <sup>8</sup>	<i>c</i>	4.56×10 <sup>8</sup>	<i>c</i>	6.12×10 <sup>7</sup>	<i>c</i>	2.14×10 <sup>10</sup>	<i>b</i>	
Aer	6.28×10 <sup>10</sup>	<i>c</i>	6.06×10 <sup>6</sup>	<i>c</i>	1.65×10 <sup>6</sup>	<i>cd</i>	2.72×10 <sup>6</sup>	<i>c</i>	3.85×10 <sup>8</sup>	<i>b</i>	6.17×10 <sup>7</sup>	<i>c</i>	1.08×10 <sup>7</sup>	<i>d</i>	3.95×10 <sup>9</sup>	<i>cd</i>	
w/- pre-anaerobic	<b>L2</b>																
	Ana	6.73×10 <sup>9</sup>	<i>d</i>	2.29×10 <sup>5</sup>	<i>c</i>	3.39×10 <sup>5</sup>	<i>de</i>	1.03×10 <sup>8</sup>	<i>c</i>	1.34×10 <sup>7</sup>	<i>d</i>	5.41×10 <sup>7</sup>	<i>c</i>	1.24×10 <sup>7</sup>	<i>d</i>	1.26×10 <sup>7</sup>	<i>d</i>
	Anx	7.90×10 <sup>9</sup>	<i>d</i>	5.05×10 <sup>4</sup>	<i>c</i>	2.29×10 <sup>5</sup>	<i>e</i>	6.21×10 <sup>7</sup>	<i>c</i>	1.08×10 <sup>7</sup>	<i>d</i>	5.86×10 <sup>7</sup>	<i>c</i>	5.73×10 <sup>7</sup>	<i>c</i>	1.55×10 <sup>7</sup>	<i>d</i>
	Aer	4.85×10 <sup>9</sup>	<i>d</i>	1.56×10 <sup>5</sup>	<i>c</i>	3.35×10 <sup>5</sup>	<i>de</i>	6.97×10 <sup>7</sup>	<i>c</i>	7.46×10 <sup>6</sup>	<i>d</i>	5.19×10 <sup>7</sup>	<i>c</i>	4.65×10 <sup>7</sup>	<i>c</i>	1.03×10 <sup>7</sup>	<i>d</i>
<b>H2</b>																	
Ana	3.99×10 <sup>10</sup>	<i>cd</i>	1.95×10 <sup>5</sup>	<i>c</i>	3.36×10 <sup>6</sup>	<i>b</i>	6.21×10 <sup>8</sup>	<i>a</i>	1.73×10 <sup>7</sup>	<i>d</i>	1.35×10 <sup>9</sup>	<i>b</i>	2.27×10 <sup>8</sup>	<i>a</i>	3.71×10 <sup>8</sup>	<i>d</i>	
Anx	3.91×10 <sup>10</sup>	<i>cd</i>	3.81×10 <sup>5</sup>	<i>c</i>	1.27×10 <sup>6</sup>	<i>cde</i>	5.05×10 <sup>8</sup>	<i>b</i>	1.66×10 <sup>7</sup>	<i>d</i>	1.12×10 <sup>9</sup>	<i>b</i>	1.37×10 <sup>8</sup>	<i>b</i>	2.31×10 <sup>8</sup>	<i>d</i>	
Aer	3.48×10 <sup>10</sup>	<i>cd</i>	1.39×10 <sup>5</sup>	<i>c</i>	1.76×10 <sup>6</sup>	<i>c</i>	4.05×10 <sup>8</sup>	<i>b</i>	1.73×10 <sup>7</sup>	<i>d</i>	9.60×10 <sup>8</sup>	<i>b</i>	1.39×10 <sup>8</sup>	<i>b</i>	2.23×10 <sup>8</sup>	<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  $p > 0.05$ .