Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information:

A Comparative Study of Microbial Dynamics and Phosphorus Removal for two Side-Stream Wastewater Treatment Processes

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MiSeq. The forward primer was constructed with the Illumina i5 adapter (5′-3′) (AATGATACGGCGACCACCGAGATCTACAC), an 8–10 bp barcode, a primer pad (Forward: TATGGTAATT), and the 28F-GAGTTTGATCNTGGCTCAG primer. The reverse fusion primer was constructed with (5′-3′) the Illumina i7 adapter (CAAGCAGAAGACGGCATACGAGAT), an 8–10 bp barcode, a primer pad (Reverse: AGTCAGTCAG), and the reverse primer (388R-TGCTGCCTCCCGTAGGAGT). Reactions were performed on ABI Veriti thermocyclers (Applied Biosytems, Carlsbad, California, USA) under the following thermal profile: 95 °C for 5 min, then 35 cycles of 94 °C for 30 sec, 54 °C for 40 sec, 72 °C for 1 min, followed by one cycle of 72 °C for 10 min and 4°C hold. Amplification products were then pooled equimolar and each pool was size selected in two rounds using AgencourtAMPure XP (Beckman Coulter, Indianapolis, Indiana, USA) in a 0.7 ratio for both rounds. Size selected pools were then quantified using the Quibit 2.0 Fluorometer (Life Technologies) and loaded on an Illumina MiSeq (Illumina, Inc. San Diego, California, USA) 2 × 300 flow cell at 10 pM. After denoising (USEARCH application) and chimera removal (UCHIIME in de novo mode), the sequences were clustered into operational taxonomic units (OTU) clusters with 100% identity (0% divergence) using USEARCH for taxonomic identification.

Table S1. Characteristics of influent wastewaters at different stages of operation

Major parameters (mg/L)	Stage 1‡		Stage 2†	Stage 3†
	Phase I	Phase II		
COD (dissolved)	100-120	124-130	360-410	360-420
Total P	6.9-7.8	7.5-7.8	3.9-4.9	20.6-22.2
Reactive P	5.9-6.5	6.9-7.2	3.6-4.6	19.6-20.9
NH_3-N	45.4-50.1	31.2-35.4	22.1-26.4	22.2-23.6
NO_2-N	<0.01	<0.01	<0.03	<0.4
NO_3-N	0.5-1.0	0.5-1.0	1.0-2.0	<1.0
Alkalinity	420	458	436	497
pH (unitless)	6.85	6.71	6.86	4.97

‡Stage 1: The raw municipal wastewater in Goldbar wastewater treatment plant was used for the operation of two systems in Stage 1.

Phase I: The wastewater was collected before primary clarifier on March 08, 2015.

Phase II: The wastewater was collected before primary clarifier on May 12, 2015.

†Stage 1 & 2: Mixed wastewater was prepared after mixing of treated wastewater and raw wastewater collected from Goldbar plant. Sodium acetate was added to increase the COD of simulated wastewater.

Table S2. Volumetric flow rate in each reactor for Modified and Denitrifying EBPR processes

	Modified		Denitrifying	
	Flow rate, mL/min	Recycle%	Flow rate, mL/min	Recycle%
Feed/influent	4	-	4	-
Internal return to anoxic	-	-	12	300
Sludge to anaerobic tank	2.1	-	2.1	-
Return anaerobic sludge	1.4	35	1.4	35
Effluent	3.4	-	3.4	-
Conc. P effluent	0.6	-	0.6	-

Table S3. Target genes and primers for qPCR analyses of total bacteria and the different clades from Accumulibacter ppk1 gene [1]

Primer	Sequence (5'-3')	Target	Annealing temperature (°C)
341f 534r	CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	Bacterial 16S rRNA genes	60
Acc-ppk1-763f Acc-ppk1-1170r	GACGAAGAAGCGGTCAAG AACGGTCATCTTGATGGC	Acc-I ppk 1	61
Acc-ppk1-893f Acc-ppk1-997r	AGTTCAATCTCACCGAGAGC GGAACTTCAGGTCGTTGC	Acc-IIA ppk 1	61
Acc-ppk1-870f Acc-ppk1-1002r	GATGACCCAGTTCCTGCTCG CGGCACGAACTTCAGATCG	Acc-IIB ppk1	61
Acc-ppk1-254f Acc-ppk1-460r	TCACCACCGACGGCAAGAC CCGGCATGACTTCGCGGAA G	Acc-IIC ppk1	66
Acc-ppk1-375f Acc-ppk1-522r	GGGTATCCGTTTCCTCAAGC G GAGGCTCTTGTTGAGTACAC GC	Acc-IID ppk1	63
Acc-ppk1-355f Acc-ppk1-600r	CGAACTCGGCGAAAGCGAG TA ATCGCCTCCGAGCAACTGTT C	Acc-IIF ppk1	70

Table S4. Target genes and primers for qPCR analyses of nitrifiers and denitrifiers

Function	Target gene	Primer	Sequence	Reference
Nitrification	AOB *amoA*	amoA-1F	GGGGTTTCTACTGGTGGT	2
		amoA-2R	CCCCTCKGSAAAGCCTTCTTC	
	Nitrospira spp. 16S rDNA	NSR 1113f	CCTGCTTTCAGTTGCTACCG	3
		NSR 1264r	GTTTGCAGCGCTTTGTACCG	
	Nitrobacter spp. 16S rDNA	Nitro 1198f	ACCCCTAGCAAATCTCAAAAAACCG	
		Nitro 1423r	CTTCACCCCAGTCGCTGACC	
Denitrification	*narG* gene	narG 1960 m2f	TAYGTSGGGCAGGARAAACTG	
		narG 2050 m2r	CGTAGAAGAAGCTGGTGCTGTT	
	nirS gene	nirS 1f	TACCACCCSGARCCGCGCGT	
		nirS 3r	GCCGCCGTCRTGVAGGAA	
	nirK gene	nirK 876	ATYGGCGGVCAYGGCGA	
		nirK 1040	GCCTCGATCAGRTTRTGGTT	
	nosZ gene	nosZ 2f	CGCRACGGCAASAAGGTSMSSGT	
		nosZ 2r	CAKRTGCAKSGCRTGGCAGAA	

Table S5. qPCR amplification programs for total, nitrifying and denitrifying bacteria

Target gene	Initial denaturation	Cycles				Final extension (72 °C)
		Cycles	Denaturation	Annealing	Extension (72 °C)	
AOB *amoA*	95 °C, 15 min	45	95 °C, 1 min	54 °C, 1 min	1 min	10 min
Nitrospira spp. 16S rDNA	50 °C, 2 min; 95 °C, 10 min	50	95 °C, 30 s	60 °C, 60 s	-	-
Nitrobacter spp. 16S rDNA	50 °C, 2 min; 95 °C, 10 min	50	94 °C, 20 s	58 °C, 60 s	40 s	-
narG gene	95 °C, 30 s	35	95 °C, 15 s	58 °C, 30 s	31 s	-
nirS gene	95 °C, 30 s	30	95 °C, 15 s	60 °C, 20 s	31 s	-
nirK gene	95 °C, 30 s	30	95 °C, 15 s	58 °C, 30 s	31 s	-
nosZ gene	95 °C, 30 s	30	95 °C, 15 s	60 °C, 30 s	31 s	-

Table S6. Steady-state nutrient concentrations (mg/L) in influent and effluent streams for the conventional and denitrifying EBPRs.

	Stages	Time	COD			NH_4^+-N			Total P (phosphorus)		
		d	influent	effluent	% removal	influent	effluent	% removal	influent	effluent	% removal
Modified	1, I	0-56	113.4 ± 5.2	67.6 ± 9.6	86.9 ± 1.9	46.3 ± 2.4	23.4 ± 7.3	47.4 ± 7.8	7.3 ± 0.2	4.8 ± 0.5	35.2 ± 3.4
	1, II	57-80	129.2 ± 2.9	56.9 ± 6.6	89.7 ± 2.2	33.6 ± 0.8	5.8 ± 1.2	83.4 ± 3.2	7.2 ± 1.6	3.0 ± 0.0	57.4 ± 3.6
	2	81-100	377.2 ± 17.7	62.7 ± 4.6	91.9 ± 0.5	24.9 ± 1.7	0.1 ± 0.1	99.5 ± 0.4	4.2 ± 0.3	1.3 ± 0.1	68.4 ± 0.9
	3	100-120	396.2 ± 10.3	61.3 ± 9.3	92.3 ± 1.1	22.2 ± 0.7	0.2 ± 0.3	99.0 ± 1.1	20.7 ± 0.3	9.0 ± 0.8	56.7 ± 4.4
Denitrifying	1, I	0-56	113.4 ± 5.2	62.8 ± 7.3	88.2 ± 1.4	46.3 ± 2.4	1.3 ± 2.1	96.1 ± 4.9	7.3 ± 0.2	2.4 ± 0.3	69.2 ± 2.8
	1, II	57-80	129.2 ± 2.9	51.2 ± 5.8	90.2 ± 1.1	33.6 ± 0.8	0.3 ± 0.2	99.1 ± 0.7	7.2 ± 1.6	1.8 ± 0.2	75.0 ± 1.2
	2	81-100	377.2 ± 17.7	58.3± 5.1	92.6 ± 0.9	24.9 ± 1.7	0.1 ± 0.1	99.7 ± 0.3	4.2 ± 0.3	1.0 ± 0.3	78.6 ± 3.3
	3	100-120	396.2 ± 10.3	49.3± 7.9	93.9 ± 0.9	22.2 ± 0.7	0.1 ± 0.3	99.3 ± 0.9	20.7 ± 0.3	6.7 ± 0.9	67.9 ± 2.1

Note: The COD removal was calculated based on the following equation:

Removal = 1-(effluent COD)/(influent COD+NaOAc COD entering anaerobic reactor)

Table S7. Abundance (%) of nitrifiers and denitrifers in terms of OTUs in the modified and denitrifying EBPRs

		Stage	AOB	NOB		Denitrifiers			
			amoA	***Nitrospira***	***Nitrobacter***	***nirK***	***nirS***	***narG***	***nosZ***
Modified	**Contact**	1	0.11	0.02	1.69	6.42	4.16	0.31	13.45
		2	0.06	0.01	1.55	5.19	1.56	0.64	20.36
		3	0.13	0.02	1.94	6.89	2.18	1.24	35.53
	Stabilization	1	0.20	0.02	1.90	7.18	4.52	0.31	13.79
		2	0.07	0.01	1.95	5.92	2.69	0.84	31.18
		3	0.13	0.02	1.94	6.39	2.20	1.12	33.34
	Anaerobic	1	0.09	0.01	1.14	4.71	2.65	0.18	9.05
		2	0.09	0.02	2.10	6.75	3.01	0.72	23.09
		3	0.12	0.02	1.96	6.88	2.33	1.03	30.23
Denitrifying	**Aerobic**	1	0.09	0.01	2.14	8.18	3.65	0.49	17.40
		2	0.14	0.01	2.05	9.17	3.98	0.73	27.03
		3	0.15	0.02	2.39	7.72	2.65	0.89	33.15
	Anoxic	1	0.08	0.01	1.86	5.78	2.52	0.36	12.41
		2	0.12	0.01	1.85	6.88	2.81	0.51	22.63
		3	0.18	0.02	2.60	8.83	2.95	1.11	36.29
	Anaerobic	1	0.14	0.01	3.16	11.85	4.41	0.58	18.33
		2	0.09	0.01	1.51	6.01	2.72	0.42	20.70
		3	0.15	0.01	2.35	7.47	2.97	0.97	27.19

Table S8. Abundance (%) of *Dechloromonas* related PAOs in terms of OTUs in different reactors of the modified and denitrifying EBPRs

	Stage	Modified			Denitrifying		
		Contact	Stabilization	Anaerobic	Anoxic	Aerobic	Anaerobic
Dechloromonas denitrificans	1	0.495	0.361	0.425	0.433	0.424	0.593
	2	0.028	0.118	0.000	0.045	0.259	0.231
	3	0.006	0.090	0.129	0.341	0.472	0.326
***Dechloromonas* sp.**	1	6.254	4.684	6.406	6.594	5.797	7.509
	2	0.634	2.304	2.199	2.728	4.395	5.429
	3	1.677	2.668	3.591	1.349	3.331	1.611

References

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