Complete Nutrient Removal Coupled to Nitrous Oxide Production as a Bioenergy Source by Denitrifying Polyphosphate Accumulating Organisms

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SUPPORTING INFORMATION

Synthetic municipal wastewater preparation

25 The synthetic wastewater medium (without COD and N) contained 66.79mg/L $MgSO_4$, 14mg/L

26 $CaCl_2 \cdot 2H_2O$, 44.52 mg/L $NaH_2PO_4 \cdot H_2O$, 56.19mg/L K_2HPO_4, 1mg/L yeast extract, and 0.1 g/L

27 $NaHCO_3$. Each liter of mineral medium also contained 0.3mL of trace elements solution (1.5 g/L

28 $FeCl_3 \cdot 6H_2O$, 0.15 g/L H_3BO_3, 0.03 g/L $CuSO_4 \cdot 5H_2O$, 0.18 g/L KI, 0.06 g/L $Na_2MoO_4 \cdot 2H_2O$,

29 0.12 g/L $ZnSO_4 \cdot 7H_2O$, 0.15 g/L $CoCl_2 \cdot 6H_2O$ and 10g/L EDTA).

30

31 **PHA measurements**

32 For measurement of PHAs in reactor biomass, 80-100 mg of freeze-dried biomass was placed

33 into PTFE-lined screw-topped glass tubes. Biomass was suspended in 2mL of acidic methanol

34 solution (3% H_2SO_4) with 0.25 mg/ml benzoic acid as internal standard and 2mL of chloroform,

35 and heated to 100 °C for 20h. After cooling to room temperature, 1mL of 1M NaCl solution was

36 added, and the solution was shaken vigorously for 30s. 1mL of the bottom organic layer was

37 transferred into a GC vial after settling for 15 minutes for phase separation. An Agilent 7890A

38 GC-MS (Agilent Technologies, USA) with HP5-MS column was used for the analysis. A 0.2 μL

39 sample was injected into the GC with 25:1 split ratio under constant pressure of 30.9 psi. The

40 following temperature program was used: initial, 80°C for 2 mins, 8°C/min up to 176°C,

41 10°C/min up to 300°C, and holding at 300°C for 2.6 mins.

42

43 **PCR amplification for Amplicon sequencing**

44 Amplification and barcoding were performed using the Fluidigm Biomark multiplex PCR

45 strategy, based on the protocol provided by the University of Illinois, Chicago DNA Services

46   Facility. First, the V4 region of the bacterial 16S rRNA gene was amplified using forward primer

47   CS1515f (5'-GTGCCAGCMGCCGCGGTAA) and reverse primer CS2806r (5'-

48   GGACTACHVGGGTWTCTAAT)[1]. A 20 μL PCR reaction was performed per DNA extract,

49   using 2× Epicentre Premix F PCR mastermix (Epicentre, Madison, WI), 3.5U Expand HiFidelity

50   Taq (Roche Diagnostics, Indianapolis IN), 200 nM primer, and 1 μL genomic DNA. PCR

51   amplification followed a temperature profile of 95 °C for 5 minutes, followed by 28 cycles of:

52   95 °C (30 s), 55 °C (45 s), and 68 °C (30 s); and a final elongation step at 68°C for 7 minutes.

53   For each timepoint, two DNA extracts were used for amplification. A second PCR step was then

54   conducted using Fluidigm primers with sequencing adapters and a sample-specific barcode.

55   Amplicons from replicate PCRs were pooled, and the PCR reaction was performed using 2×

56   Accuprime Supermix (ThermoFisher, Carslbad CA), 50 μM forward and reverse primers

57   (Fluidigm, South San Francisco CA), and 1 μL of template from the first round of PCR using the

58   following temperature program: 95 °C for 5 minutes, 95 °C for 30s, 60°C for 30s, and 68 °C for

59   30s in a total of 8 cycles. The resulting amplicons were processed with a Qiagen PCR

60   purification kit and sequenced on the Illumina MiSeq platform.

61　**List of Supplementary Tables and Figures:**

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72　**Table S6.** Phylogenetic affiliations of OTUs (97% identity level) that significantly correlated to

73　genera *Zoogloea* or Accumulibacter in the CANDO+P reactor. Phylogenetic inferences are

74　based on the Greengenes database and sequence alignments in QIIME.

75　**Figure S1.** Conceptual schematic of CANDO+P SBR operation.

76　**Figure S2**. Dynamics in alpha diversity metrics over time: (a) Chao1, (b) Shannon index and (c)

77　observed OTUs. All metrics were calculated after rarifying 10 times to the lowest sampling

78　depth, and error bars indicate standard deviations.

79　**Figure S3.** Presence of denitrification genes in near complete Accumulibacter genomes

80　representing Clades IA and IIC assembled by Skennerton et al. and Flowers et al.[45,46] Gene

81　presence is shown in dark red and gene absence is shown in light grey.

82　NAP: periplasmic nitrate reductase; NAR: respiratory nitrate reductase; NIR: nitrite reductase;

83　NOR: nitric oxide reductase; NOS: nitrous oxide reductase.

Table S1. Operational parameters and phases of the CANDO+P reactor operation.

	SBR operation				Initial COD (mg/L)	Initial NO_2^- (mg-N/L)	Initial PO_4^{3-} (mg-P/L)	COD/P ratio
	Anaerobic (min)	Anoxic (min)	Aerobic (min)	Total cycle (h)				
phase I (acclimation period, day 0 to 120)	280	300	60	12	<80	<20	25	<3
phase II (day 121 to 158)	90	120	60	6	45 - 120	15 - 45	25	<5
phase III (day 159 to 181)	90	120	60	6	120 - 160	35 - 45	25	~5
phase IV (day 182 to 219)	70	160	60	6	120 - 160	34 - 45	15	~10

Table S2. Endpoint PCR and qPCR primers and associated PCR conditions used for amplification of 16S rRNA genes and various *ppk1* gene clades. The temperature program of endpoint PCR assays for Accumulibacter clade-specific *ppk1* genes consisted of denaturation at 95 ˚C for 4 min; 35 cycles of 95 ˚C for 30s, annealing temperature for 30s, and 72 ˚C for 40s; and finally extension at 72 ˚C for 10 min. PCR products were detected by 2% agarose gel electrophoresis. qPCR temperature programs for Accumulibacter clade-specific *ppk1* genes, Accumulibicter 16S rRNA genes, and total bacterial 16S rRNA genes are as follows: 95 °C for 3 min, 40 cycles of 95°C for 10-15s, annealing temperature for 30-60s (the fluorescent signals are collected in this step); and a melt curve automatically generated on the qPCR instrument.

Target gene	Genes	Primers	Primer Sequence	Length (bp)	Annealing Temperature (°C)	Denaturing Time (s)	Annealing Time (s)	Reference
ppk1 gene	Clade IA	Acc-ppk-1974f	TGATGCGCGACAATCTCAAATTCAA	140	58	15	60	2
		Acc-ppk-11113r	AATGATCGGATTGAAGCTCTGGTAG					
	Clade IB	Acc-ppk-1372f	TGAAGGCATTCGCTTCCT	282	52	20	60	2
		Acc-ppk-1653r	AAGCAGTATTCGCTGTC					
	Clade ID	Acc-ppk-1634f	TGCGACAGCGAATACAG	215	60	20	60	2
		Acc-ppk-1848r	ACTTCGAGGCGGACG					
	Clade IIA	Acc-ppk-1893f	AGTTCAATCTCACCGAGAGC	105	61	15	60	3
		Acc-ppk-1997r	GGAACTTCAGGTCGTTGC					
	Clade IIB	Acc-ppk-1870f	GATGACCCAGTTCCTGCTCG	133	61	15	60	3
		Acc-ppk-11002r	CGGCACGAACTTCAG					
	Clade IIC (IIC1)	Acc-ppk-1254f	TCACCACCGACGGCAAGAC	207	62	20	60	3
		Acc-ppk-1460r	CCGGCATGACTTCGCGGAAG					
	Clade IIC excluding OTU NS D3 (IIC2)	Acc-ppk-1123f	GAACAGTCCGCCAACGACC	254	60	20	60	3
		Acc-ppk-11376r	ACGATCATCAGCATCTTGGC					
	Clade IID	Acc-ppk-1375f	GGGTATCCGTTTCCTCAAGCG	148	60	15	60	3
		Acc-ppk-1522r	GAGGCTCTTGTTGAGTACACGC					
	CladeIIE	Acc-ppk-1757f	TTCGTGGACGAGGAAGA	373	55	30	75	2
		Acc-ppk-1129r	ATTGTTCGAGCAACTCGATG					
	CladeIIG	Acc-ppk-1410f	CCGAGCAACGCGAATGG	105	60	15	60	2

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		Acc-ppk-1514r	TGTTGAGTACGCGCGGGA					
	CladeIIIH	Acc-ppk-1701f	ACTCCTTCGTATTCCTCTCT	228	60	20	60	2
		Acc-ppk-1928r	TCATCGCTTCGGAGCA					
	CladeIII	Acc-ppk-1688f	AGTGATTATGCTTTCGTCTTTC	259	60	20	60	2
		Acc-ppk-1946r	TGAACTGTCCGAGCAGGA					
16S rRNA genes	Accumulibacter	16S rRNA 518f	CCAGCAGCCGCGGTAAT	351	60	30	75	3
		16S rRNA 846r	GTTAGCTACGGCACTAAAAGG					
	Universal Bacteria	16S rRNA 341f	CCTACGGGAGGCAGCAG	194	55	20	60	3
		16S rRNA 534r	ATTACCGCGGCTGCTGG					

Table S3. Quality control parameters for qPCR assays. Duplicate real-time PCR assays were performed for the decimally diluted standard plasmids to obtain the standard curves, together with all samples and negative controls.

Gene Type	Gene Target	Standard curve correlation efficiency (R^2)	Amplification Efficiency (%)	Limit of detection (copies/µL DNA solution)
16S rRNA genes	Total Bacteria	0.999	101.3	1000
	Total Accumulibacter	0.99	89.0	100
ppk1 gene	Clade IA	0.999	98.6	10
	Clade IIA	0.999	96.2	10
	Clade IIB	0.99	99.7	10
	Clade IIC (IIC1)	0.999	97.2	10
	Clade IID	0.99	95.1	100

Table S4. Relative abundances and standard deviations of different Accumulibacter clades detected in the CANDO+P reactor biomass.

Day	Accumulibacter/EUB (%)	IA/EUB (%)	IIA/EUB (%)	IIB/EUB (%)	IIC1/EUB (%)	IID/EUB (%)
0	9.35 ± 1.46	0.14 ± 0.04	0.02 ± 0.01	0.61 ± 0.10	6.12 ± 0.18	0.01 ± 0.00
23	15.77 ± 0.27	1.37 ± 0.45	0.05 ± 0.02	0.23 ± 0.05	12.89 ± 0.30	0.07 ± 0.01
51	4.55 ± 0.06	0.04 ± 0.01			0.88 ± 0.17	
73	28.61 ± 0.14	2.73 ± 0.46			3.08 ± 0.55	
87	58.63 ± 0.83	6.31 ± 0.73			4.81 ± 0.47	
103	96.02 ± 5.53	21.72 ± 4.98	0.02 ± 0.01	0.03 ± 0.01	29.40 ± 0.93	0.03 ± 0.01
117	72.71 ± 0.41	13.00 ± 1.97			7.34 ± 1.24	
144	55.47 ± 12.26	7.88 ± 0.12			1.92 ± 0.38	
172	44.68 ± 0.63	3.21 ± 0.52			4.51 ± 0.80	
201	73.05 ± 4.46	10.67 ± 2.65			12.72 ± 2.61	

Table S5a. Pairwise Pearson and Spearman Correlation coefficients between the core OTUs (average relative abundance > 0.8%) and genera Accumulibacter or *Zoogloea*.

OTU	Accumulibacter_Spearman	*Zoogloea*_Spearman	Accumulibacter_Pearson	*Zoogloea*_Pearson	Average relative abundance (%)[**]
OTU10	-0.84	-[*]	-0.84	-	4.28±2.67
OTU17	0.89	-0.99	0.83	-	1.75±1.98
OTU5	-0.94	0.95	-0.73	0.78	1.57±1.83
OTU19	0.89	-0.96	0.92	-	1.47±2.37
OTU7	-0.7	-	-0.7	-	1.39±1.41
OTU9	-0.63	-	-0.65	-	1.34±0.51
OTU13	-0.84	0.78	-0.7	-	1.28±0.76
OTU24	0.82	-0.89	0.8	-0.77	0.99±0.70
OTU12	-0.63	-	-0.69	-	0.87±0.49
OTU14	-0.9	0.95	-0.87	0.83	0.84±1.30
OTU64	-0.82	0.91	-0.63	0.82	0.82±0.71

*Coefficients with p-value > 0.05 were marked as '-' in the table.

**Average and standard deviation were calculated based on 11 time-series samples from day 0 to day 131 (phase I and II).

*Zoogloea*_Spearman: Spearman correlation coefficient between core OTUs and *Zoogloea*.

Accumulibacter_Spearman: Spearman correlation coefficient between core OTUs and Accumulibacter.

*Zoogloea*_ Pearson: Pearson correlation coefficient between core OTUs and *Zoogloea*.

Accumulibacter_ Pearson: Pearson correlation coefficient between core OTUs and Accumulibacter.

Table S5b. Pairwise Pearson and Spearman Correlation coefficients between the rare OTUs (average relative abundance < 0.8%) and genera Accumulibacter or *Zoogloea*.

OTU	Accumulibacter_Spearman	*Zoogloea*_Spearman	Accumulibacter_Pearson	*Zoogloea*_Pearson	Average relative abundance (%)[**]
OTU30	0.81	-0.82	0.78	-0.64	0.66±0.53
OTU191	-0.82	0.71	-0.64	-[*]	0.65±0.80
OTU29	-	-0.69	-	-0.68	0.59±0.46
OTU640	-0.8	0.92	-	0.78	0.54±1.20
OTU21	-0.85	0.74	-0.78	-	0.48±0.40
OTU18	-0.91	0.75	-0.9	-	0.47±0.35
OTU49	-0.66	-	-0.68	-	0.47±0.17
OTU16	-	0.93	-	0.74	0.46±0.74
OTU505	-0.81	0.72	-0.82	-	0.45±0.32
OTU121	-0.9	0.96	-0.87	0.81	0.43±0.37
OTU25	-0.95	0.92	-0.78	-	0.42±0.38
OTU26	0.97	-	-	0.91	0.39±0.63
OTU42	-0.67	0.73	-0.69	-	0.38±0.35
OTU33	-0.65	-	-0.62	-	0.38±0.34
OTU50	-0.92	0.96	-	0.93	0.38±0.50
OTU288	0.92	-0.97	0.81	-	0.35±0.62
OTU34	-0.94	0.79	-0.89	-	0.30±0.21
OTU254	-0.71	-	-0.88	-	0.29±0.12
OTU261	-0.91	0.98	-0.61	0.95	0.27±0.34
OTU40	-0.94	0.93	-0.86	-	0.26±0.26
OTU67	0.85	-0.94	0.84	-0.64	0.26±0.22

OTU65	0.65	-0.83	0.8	-0.76	0.26±0.08
OTU37	-0.85	0.9	-	0.92	0.25±0.47
OTU48	-0.98	0.85	-0.86	-	0.23±0.17
OTU36	-0.73	0.61	-0.61	-	0.23±0.30
OTU46	-0.96	-	-0.83	-	0.22±0.23
OTU935	-0.9	0.94	-	0.88	0.21±0.36
OTU98	0.61	-0.61	-	-0.67	0.17±0.14
OTU54	-0.82	0.64	-0.8	-	0.17±0.12
OTU72	0.75	-0.78	-	-0.64	0.17±0.12
OTU508	-0.75	-	-0.75	-	0.16±0.13
OTU132	0.93	-0.94	0.9	-	0.16±0.17
OTU91	0.88	-0.91	0.84	-0.77	0.15±0.10
OTU1039	-0.65	-	-0.65	-	0.15±0.10
OTU71	-0.97	0.87	-0.78	-	0.15±0.15
OTU669	-	0.95	-	0.8	0.15±0.26
OTU73	-0.88	0.88	-0.73	-	0.14±0.16
OTU355	-0.8	0.9	-0.7	0.8	0.14±0.15
OTU206	-0.8	0.8	-0.85	0.63	0.14±0.08
OTU60	-0.94	0.84	-0.7	-	0.14±0.11
OTU146	-0.69	-	-0.64	-	0.13±0.09
OTU53	-0.69	-	-0.73	-	0.13±0.12
OTU78	-0.92	0.83	-0.75	-	0.12±0.08
OTU62	-0.73	-	-0.81	-	0.11±0.07
OTU74	-0.84	0.71	-0.75	-	0.10±0.11
OTU61	-0.93	0.96	-0.75	0.79	0.09±0.11
OTU77	-0.88	0.95	-0.78	0.76	0.09±0.10
OTU152	-	-0.78	0.69	-0.71	0.09±0.07

OTU138	-0.67	-	-0.74	-	0.09±0.07
OTU104	-0.91	0.97	-0.79	0.73	0.09±0.09
OTU116	-0.91	0.87	-0.83	0.68	0.08±0.06
OTU122	-0.64	-	-0.69	-	0.08±0.06
OTU107	-0.78	0.61	-0.71	-	0.08±0.08
OTU111	-0.78	0.85	-0.78	0.66	0.08±0.08
OTU239	-	-0.65	0.8	-	0.08±0.06
OTU164	-0.9	0.88	-0.75	-	0.08±0.07
OTU82	-0.85	0.63	-0.7	-	0.08±0.06
OTU133	-0.83	0.89	-0.86	0.77	0.08±0.18
OTU270	0.84	-0.92	0.88	-0.69	0.07±0.05
OTU106	-0.9	0.97	-	0.78	0.07±0.17
OTU114	-0.93	0.94	-0.88	-	0.07±0.07
OTU108	-0.86	0.68	-0.75	-	0.07±0.06
OTU186	-0.85	0.75	-0.66	0.62	0.07±0.04
OTU79	-0.89	0.98	-0.62	0.97	0.07±0.11
OTU90	-0.83	0.66	-0.7	-	0.07±0.08
OTU95	-0.97	0.87	-0.85	-	0.07±0.06
OTU174	-0.94	0.9	-0.65	0.71	0.07±0.09
OTU169	0.8	-0.8	0.72	-0.75	0.07±0.05
OTU87	-0.84	0.64	-0.84	-	0.07±0.05
OTU159	-0.92	0.72	-0.84	-	0.07±0.04
OTU81	-0.97	0.87	-0.83	-	0.06±0.05
OTU113	-0.63	-	-0.62	-	0.06±0.04
OTU94	-0.92	0.83	-0.8	-	0.06±0.04
OTU118	-0.88	0.95	-0.82	0.87	0.06±0.06
OTU89	-0.8	0.65	-0.69	-	0.06±0.06

OTU117	-0.91	0.91	-0.64	0.71	0.06±0.08
OTU149	-0.98	0.91	-0.8	-	0.06±0.06
OTU263	0.72	-0.79	0.67	-0.66	0.06±0.05
OTU292	-0.61	-	-0.72	-	0.06±0.04
OTU136	-0.77	-	-0.67	-	0.06±0.05
OTU112	-0.88	0.78	-0.64	-	0.06±0.05
OTU110	-0.97	0.92	-0.85	-	0.06±0.06
OTU144	-0.77	-	-0.82	-	0.06±0.04
OTU123	-0.83	0.66	-0.74	-	0.05±0.05
OTU151	-0.96	0.89	-0.8	-	0.05±0.05
OTU148	-0.83	0.9	-	0.87	0.05±0.09
OTU265	-0.63	0.71	-0.74	-	0.05±0.03
OTU141	-0.85	0.64	-0.74	-	0.05±0.04
OTU276	-0.81	0.94	-0.62	0.75	0.05±0.06
OTU140	-0.87	0.89	-0.78	-	0.04±0.04
OTU182	-0.85	0.66	-0.73	-	0.04±0.03
OTU154	-0.8	0.66	-0.73	-	0.04±0.03
OTU329	-0.9	0.88	-0.81	-	0.04±0.04
OTU155	-0.93	0.94	-0.8	0.68	0.04±0.04
OTU328	-0.9	0.93	-0.75	0.7	0.04±0.03
OTU127	-0.92	0.88	-0.93	-	0.04±0.03
OTU135	-0.87	0.88	-0.75	0.63	0.04±0.04

*Coefficients with p-value > 0.05 were marked as '-' in the table.

**Average and standard deviation were calculated based on 11 time-series samples from day 0 to day 131 (phase I and II).

*Zoogloea*_Spearman: Spearman correlation coefficient between rare OTUs and *Zoogloea*.

Accumulibacter_Spearman: Spearman correlation coefficient between rare OTUs and Accumulibacter.

*Zoogloea_* Pearson: Pearson correlation coefficient between rare OTUs and *Zoogloea*.

Accumulibacter_ Pearson: Pearson correlation coefficient between rare OTUs and Accumulibacter.

1 **Table S6.** Phylogenetic affiliations of OTUs (97% identity level) that significantly correlated to genera *Zoogloea* or Accumulibacter
2 in the CANDO+P reactor. Phylogenetic inferences are based on the Greengenes database and sequence alignments in QIIME.
3

OTU	Phylum	Class	Order	Family	Genus
OTU10	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	
OTU17	*Chloroflexi*	*Anaerolineae*	*Anaerolineales*	*Anaerolinaceae*	*Longilinea*
OTU5	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Intrasporangiaceae*	
OTU19	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Chitinophagaceae*	*Sediminibacterium*
OTU7	*Proteobacteria*	*Gammaproteobacteria*	*Thiotrichales*	*Thiotrichaceae*	*Thiothrix*
OTU9	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	
OTU13	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Rhodobacter*
OTU24	*Chlorobi*	SJA-28			
OTU12	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Chitinophagaceae*	
OTU14	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Dechloromonas*
OTU64	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	
OTU30	*Chlorobi*	*Ignavibacteria*	*Ignavibacteriales*	*Ignavibacteriaceae*	
OTU191	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Sulfuritalea*
OTU29	*Chlorobi*	*Ignavibacteria*	*Ignavibacteriales*	*Ignavibacteriaceae*	
OTU640	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Hydrogenophaga*
OTU21	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*		
OTU18	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Saprospiraceae*	
OTU49	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Cryomorphaceae*	
OTU16	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Carnobacteriaceae*	
OTU505	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Propionivibrio*
OTU121	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	
OTU25	*Proteobacteria*	*Betaproteobacteria*	*Methylophilales*	*Methylophilaceae*	*Methylotenera*
OTU26	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Saprospiraceae*	
OTU42	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Dechloromonas*

OTU33	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	
OTU50	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Dechloromonas*
OTU288	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Aquimonas*
OTU34	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Chitinophagaceae*	
OTU254	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	
OTU261	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Rhodoferax*
OTU40	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	
OTU67	*Proteobacteria*	*Alphaproteobacteria*			
OTU65	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Dokdonella*
OTU37	*Bacteroidetes*	*Cytophagia*	*Cytophagales*	*Cytophagaceae*	
OTU48	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*		
OTU36	*Proteobacteria*	*Alphaproteobacteria*	BD7-3		
OTU46	*Bacteroidetes*	*Sphingobacteriia*	*Sphingobacteriales*		
OTU935	*Proteobacteria*	*Betaproteobacteria*	SBla14		
OTU98	*Chlorobi*	*Ignavibacteria*	*Ignavibacteriales*	*Melioribacteraceae*	
OTU54	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	
OTU72	*Proteobacteria*	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Mycoplana*
OTU508	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	
OTU132	*Chloroflexi*	*Anaerolineae*	envOPS12		
OTU91	*Chloroflexi*	*Anaerolineae*	SBR1031	A4b	
OTU1039	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	
OTU71	*Bacteroidetes*	*Sphingobacteriia*	*Sphingobacteriales*		
OTU669	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	
OTU73	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*	
OTU355	*Proteobacteria*	*Betaproteobacteria*	*Thiobacterales*		
OTU206	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	
OTU60	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	
OTU146	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobulbaceae*	*Desulfobulbus*

OTU53	*Spirochaetes*	*Spirochaetes*	*Spirochaetales*	*Spirochaetaceae*	*Treponema*
OTU78	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	*Devosia*
OTU62	*Bacteroidetes*	*Sphingobacteriia*	*Sphingobacteriales*		
OTU74	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*		
OTU61	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Saprospiraceae*	
OTU77	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Cryomorphaceae*	
OTU152	*Chloroflexi*	*Anaerolineae*	*Anaerolineales*	*Anaerolinaceae*	
OTU138	*Spirochaetes*	*Spirochaetes*	*Spirochaetales*	*Spirochaetaceae*	*Treponema*
OTU104	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Hyphomonadaceae*	
OTU116	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Saprospiraceae*	
OTU122	*Proteobacteria*	*Gammaproteobacteria*	*Alteromonadales*	125ds10	
OTU107	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	Blvii28
OTU111	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*		
OTU239	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	
OTU164	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Mycobacteriaceae*	*Mycobacterium*
OTU82	*Firmicutes*	*Clostridia*	*Clostridiales*		
OTU133	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Saprospiraceae*	*Haliscomenobacter*
OTU270	*Proteobacteria*	*Betaproteobacteria*	SC-I-84		
OTU106	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	
OTU114	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Cryomorphaceae*	
OTU108	*Proteobacteria*	*Alphaproteobacteria*	*Rhodospirillales*	*Rhodospirillaceae*	
OTU186	*Proteobacteria*	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	
OTU79	*Cyanobacteria*	4C0d-2	MLE1-12		
OTU90	*Acidobacteria*	*Holophagae*	*Holophagales*	*Holophagaceae*	
OTU95	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Chitinophagaceae*	
OTU174	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	
OTU169	*Chloroflexi*	*Anaerolineae*	*Anaerolineales*	*Anaerolinaceae*	
OTU87	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Chitinophagaceae*	

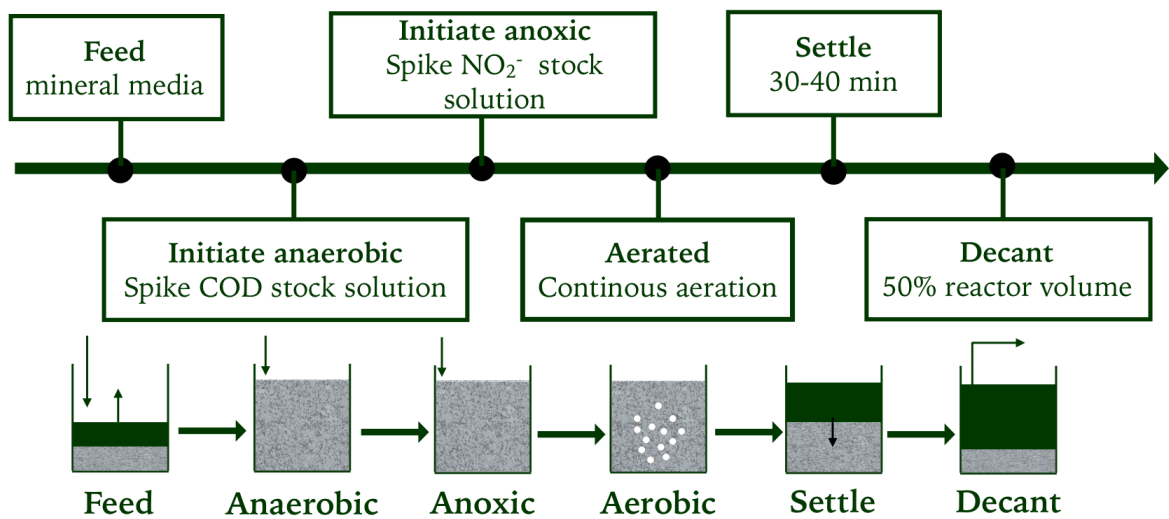| OTU159	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Bradyrhizobiaceae*	*Bradyrhizobium*
OTU81	*Gemmatimonadetes*	*Gemmatimonadetes*	*Gemmatimonadales*	*Gemmatimonadaceae*	*Gemmatimonas*
OTU113	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Sinobacteraceae*	
OTU94	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	SB-1	
OTU118	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*
OTU117	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*		
OTU149	*Actinobacteria*	*Acidimicrobiia*	*Acidimicrobiales*	*Microthrixaceae*	*Candidatus* Microthrix
OTU263	*Chloroflexi*	*Anaerolineae*	*Anaerolineales*	*Anaerolinaceae*	
OTU292	*Firmicutes*	*Clostridia*	*Clostridiales*	*Christensenellaceae*	
OTU136	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*		
OTU112	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*		
OTU110	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	
OTU144	*Proteobacteria*	*Gammaproteobacteria*	*Acidithiobacillales*		
OTU123	*Acidobacteria*	*Solibacteres*	*Solibacterales*		
OTU151	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Microbacteriaceae*	*Salinibacterium*
OTU148	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*
OTU265	*Bacteroidetes*	*Cytophagia*	*Cytophagales*	*Cytophagaceae*	
OTU141	*Actinobacteria*	*Acidimicrobiia*	*Acidimicrobiales*		
OTU276	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Novosphingobium*
OTU140	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Cryomorphaceae*	
OTU182	*Actinobacteria*	*Acidimicrobiia*	*Acidimicrobiales*		
OTU154	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*		
OTU329	*Proteobacteria*	*Gammaproteobacteria*	*Thiotrichales*	*Thiotrichaceae*	*Thiothrix*
OTU155	*Bacteroidetes*	*Saprospirae*	*Saprospirales*	*Saprospiraceae*	
OTU328	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	
OTU127	*Proteobacteria*	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	
OTU135	*Chloroflexi*	*Anaerolineae*	SBR1031	A4b	

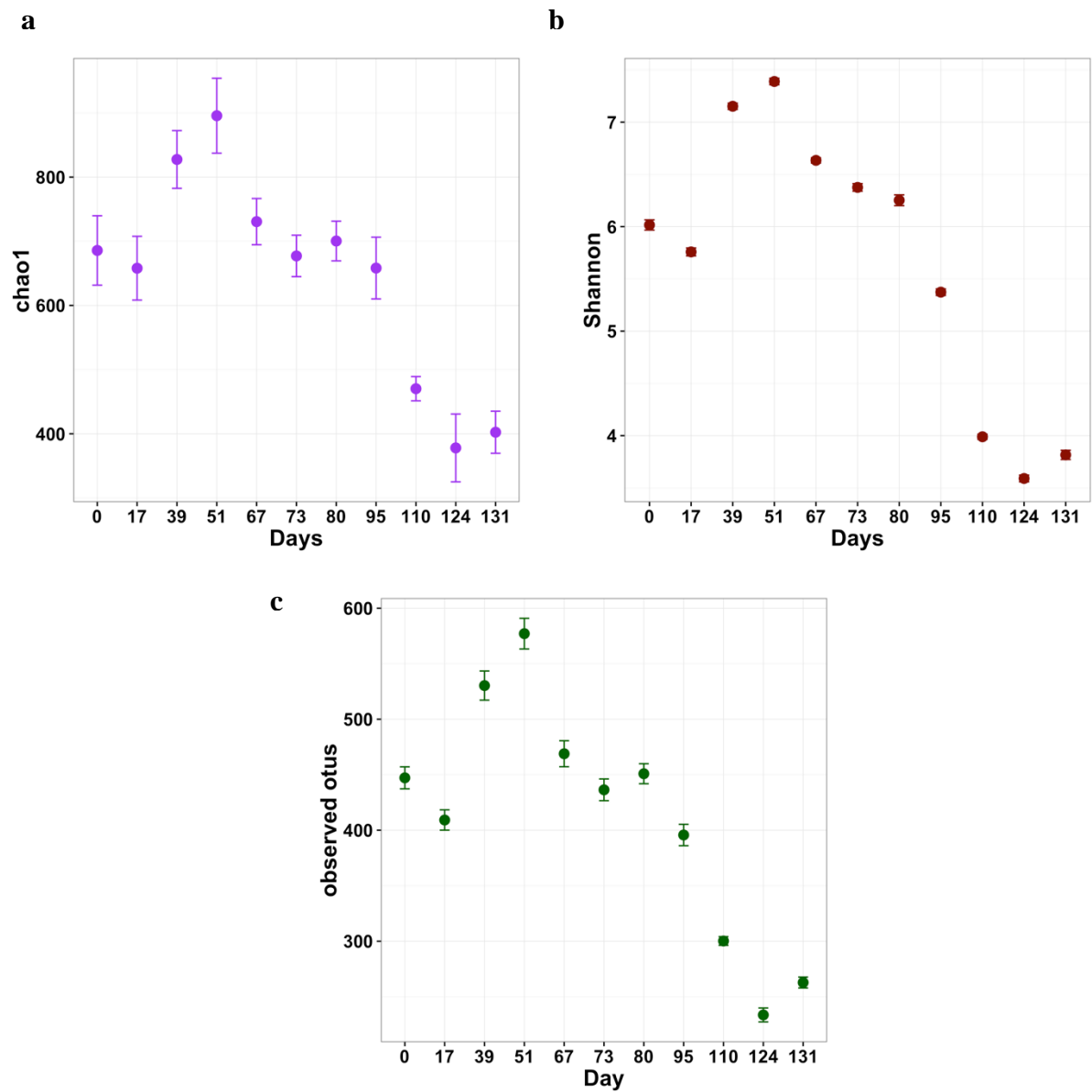**Figure S1.** Conceptual schematic of CANDO+P SBR operation.

Figure S2. Dynamics in alpha diversity metrics over time: (a) chao1, (b) Shannon index and (c) observed OTUs. All metrics were calculated after rarifying 10 times to the lowest sampling depth, and error bars indicate standard deviations.
Samples were rarefied to the lowest sequencing depth 10 times and averaged to calculate Shannon and chao1 alpha diversity indexes and numbers of observed OTUs.
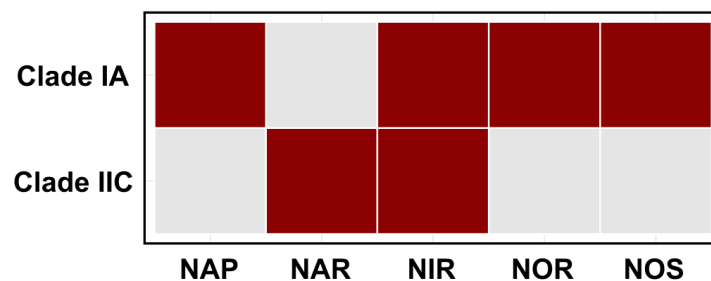
Figure S3. Presence of denitrification genes in near complete Accumulibacter genomes representing Clades IA and IIC assembled by Skennerton et al. and Flowers et al.[5,6] Gene presence is shown in dark red and gene absence is shown in light grey.

NAP: periplasmic nitrate reductase; NAR: respiratory nitrate reductase; NIR: nitrite reductase; NOR: nitric oxide reductase; NOS: nitrous oxide reductase.

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5. Flowers, J. J.; He, S.; Malfatti, S.; del Rio, T. G.; Tringe, S. G.; Hugenholtz, P.; McMahon, K. D., Comparative genomics of two Candidatus Accumulibacter clades performing biological phosphorus removal. *The ISME Journal* **2013,** *7*, (12), 2301-2314.