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Supplementary material for

Evaluation of Electron Donors for Biological Perchlorate Removal Highlights the Importance of Diverse Perchlorate-Reducing Populations

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Figure S1 Setup of the FBR and fixed bed reactors, which were fed $200 \ \mu g/L \ ClO_4^-$ and $15 \ mg/L \ NO_3^--N$ and operated using acetate for the first 100 days; then acetate was replaced with MicroC4000TM, a proprietary, carbohydrate-based electron donor.²⁸



Figure S2 Correlation between effluent soluble non-purgeable organic carbon (sNPOC) and effluent soluble chemical oxygen demand (sCOD) in the high-perchlorate FBR.



Figure S3 Relative abundance of phyla in (A) the high-perchlorate FBR, (B) the low-perchlorate FBR and (C) the low-perchlorate fixed bed reactor. Truncated y axes are used to accentuate changes in abundance.

<i>Azospira</i> OTU0051	600660
A.suillum_PS	<mark>CC</mark> TGGGAA <mark>C</mark> TG <mark>C</mark> GTTTGTGA <mark>C</mark> TG <mark>C</mark> GAGG <mark>C</mark> TAGAGTA <mark>C</mark> GG <mark>C</mark> AGAGGGGGGGTAGAATT <mark>CC</mark> A <mark>C</mark>
<i>Azospira</i> _OTU0051 <i>A.suillum</i> _PS	⁶⁸⁷ TGGAGGAATA <mark>CC</mark> GATGG <mark>C</mark> GAAGG <mark>C</mark> AGCCCCCTGG GTGTAG <mark>C</mark> AGTGAAATG <mark>C</mark> GTAGAGATGTGGAGGAATA <mark>CC</mark> GATGG <mark>C</mark> GAAGG <mark>C</mark> ACCCCCTGG
Azospira_OTU0051 A.suillum_PS	721 GTTAGTA <mark>C</mark> TGA <mark>C</mark> G <mark>CTC</mark> ATG <mark>CAC</mark> GAAAG <mark>C</mark> GTGGGGGA GCAAACAGGAT TAGATACCCTGG GTTAGTA <mark>C</mark> TGA <mark>C</mark> GCTCATG <mark>CAC</mark> GAAAG <mark>C</mark> GTGGGGGA GCAAACAGGAT TAGATACCCTGG
<i>Azospira</i> _OTU0051 A.suillum_PS	⁷⁸¹ TAGT <mark>CCACGCCC</mark> TAAA <mark>C</mark> GATGT <mark>CAAC</mark> TAGGTGTTGGAAGGGTTAAA <mark>CC</mark> TTTTAGTA <mark>CCGC</mark> TAGT <mark>CC</mark> AC <mark>GCCC</mark> TAAA <mark>C</mark> GATGT <mark>C</mark> AA <mark>C</mark> TAGGTGTTGGAAGGGTTAAA <mark>CC</mark> TTTTAGTA <mark>CC</mark> GC
Azospira_OTU0051 A.suillum_PS	⁸⁴¹ AG <mark>C</mark> TAACGCGTGAAGTTGA <mark>CCGCC</mark> TGGGGAGTACGGCCGCAAGGTTAAA AG <mark>C</mark> TAACGCGTGAAGTTGA <mark>CCGCC</mark> TGGGGAGTACGGCCGCAAGGTTAAAAC <mark>TC</mark> AAAGGAA
Azospira_OTU0051 A.suillum_PS	⁹⁰¹ ⁹⁶⁰ TTGA <mark>C</mark> GGGGA <mark>CCC</mark> G <mark>C</mark> AC <mark>AAGC</mark> GGTGGATGATGTGGATTAATT <mark>C</mark> GATG <mark>C</mark> AA <mark>C</mark> GCGAAAAA <mark>C</mark>
Azospira_OTU0051 A.suillum_PS	¹⁰⁶¹ ¹⁰²⁰ CTTACCTACCCTTGACATGCCAGGAACCTTTCCCAGAGAGTGGATTGGTGCCCGAAAGGGAGC
Azospira_OTU0051 A.suillum_PS	¹⁰²¹ ¹⁰⁸⁰ CTGGACACAGGTGCTGCATGCTGCGTGCGTGCGTGAGGTGGGTTAAGTCC
Azospira_OTU0051 A.suillum_PS	¹⁰⁸¹ 1140 CGCAACGAGCGCAACCCTTGTCATTAATTGCCCATCATTCAGTTGGGCACTTTAATGAGAC
Azospira_OTU0051 A.suillum_PS	114 <u>1</u>
Azospira_OTU0051 A.suillum_PS	¹²⁰¹ ¹²⁶⁰ AGGG <mark>C</mark> TT <mark>C</mark> AC <mark>AC</mark> GT <mark>C</mark> ATA <mark>C</mark> AATGGTCGGTACCAGAGGGTTGCCAAGCCGCGAGGTGGAGCC
Azospira_OTU0051 A.suillum_PS	¹²⁶¹ 1320 AAT <mark>CCC</mark> AGAAAG <mark>CC</mark> GAT <mark>C</mark> GTAGT <mark>CC</mark> GGATCGCAGTCGGAACTCGACTGCGTGAAGTCGG
Azospira_OTU0051 A.suillum_PS	¹³²¹ 1380 AAT <mark>C</mark> GCTAGTAATCGCGGATCAGCATGTCGCGGGTGAATACGTTCCCGGGGTCTTGTACACA
<i>Azospira</i> _OTU0051 A.suillum_PS	¹³⁸¹ 1424 CCGCCCGTCACACCATGGGAGTGGGTTCCTACCAGAAGTAGTTAG

Figure S4 Alignment of partial 16S rRNA gene sequences from *Azospira* OTU0051 and *Azospira suillum* PS. The full 16S rRNA gene sequence for *Azospira suillum* PS was downloaded from the Integrated Microbial Genome database.⁸⁰

Chemical	Amount to		Influent	Influent Metal	
	prepare 1 L of		Chemical	Conce	entration
	trace metal mix in		Concentration	(n	ng/L)
	distilled water		(mg/L)		
HCl (25%; 7.7 M)	10	mL			
$FeCl_2 imes 4H_20$	1.5	g	1.5	0.421	as Fe ²⁺
ZnCl ₂	70	mg	0.07	0.034	as Zn ²⁺
MnCl ₂ X 4H ₂ 0	100	mg	0.1	0.028	as Mn ²⁺
H ₃ BO ₃	6	mg	0.006	0.001	as B ³⁺
CoCl ₂ X 6H ₂ 0	190	mg	0.19	0.047	as Co ²⁺
CuCl ₂ X 2H ₂ 0	2	mg	0.002	0.001	as Cu ²⁺
NiCl ₂ X 6 H ₂ 0	24	mg	0.024	0.006	as Ni ²⁺
Na ₂ MoO ₄ X 2 H ₂ 0	36	mg	0.036	0.014	as Mo ⁶⁺

 Table S1 Composition of Trace Element Solution SL-10

Table S2 Estimated biomass yields for different electron donor operating conditions distinguished by composition or influent COD concentration. The first 80 days of reactor operation (data not shown) were for startup and optimization of reactor operating conditions. Yield estimates were determined for periods until day 210, the last day for which sCOD or sNPOC measurements were available.

Operating	Period	Electron	Measured	Period	COD	COD	COD	Estimated
condition	(days)	donor	influent	(days)	requirement	used	used for	yield (mg
	reported		COD	included	for observed	(mg/L)	biomass	COD _{biomass} /mg
			(mg/L)	for yield	reduction of		(mg/L)	COD _{electron}
				calculations	electron			donor) [¥]
					acceptors			
					(mg/L)			
1	81-114	Acetate	200	102-114	118	180	62	0.34
1	81-114	Acetate	200	81-114	119	182	64	0.35
2	115-142	Glycerol	216	120-142	98	205	107	0.52
		product						
2	115-142	Glycerol	216	130-142	95	211	116	0.55
		product						
3	143-197	Glycerol	484	178-189	125	322	197	0.62
		product						
3	143-197	Glycerol	484	186-196	129	325	195	0.60
		product						
4	198-222	Glycerol	442	200-210	124	401	276	0.69
		product						