Supplementary Material

Resuscitation-promoting factor accelerates enrichment of highly active PCE/PCB-dechlorinating cultures

Running Title: Rpf enhances anaerobic microbial dechlorination

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Table S1 PCE removal efficiencies (E_{3d}) and simulation results with apparent first-order reaction kinetics (k and R^2).

Groups	Removal efficiencies (%) in day 3 (E_{3d})	Kinetics simulation	
		k (d^{-1})	R^2
TG1	78.0 ± 0.1	0.428	0.943
CG1	28.1 ± 0.6	0.111	0.971
TG2	73.7 ± 1.7	0.431	0.961
CG2	30.7 ± 1.7	0.134	0.909
TG3	79.1 ± 0.7	0.729	0.918
CG3	56.5 ± 1.1	0.135	0.926

Table S2 Retention time, mass, peak intensity and concentration change of final dechlorination products of Aroclor 1260 in TG4 and CG4 after 50 days of incubation. The products were analyzed by gas chromatography-mass spectrometry (GC-MS) based on the standards of Aroclor 1260 and Aroclor 1242.

Product (s)	Retention time (min)	Number of chlorine	Mass of derivative	Peak intensity	Change in concentration after 50 d (μM)	
					TG4	CG4
PCB 52	19.375	4	291.92	33993	0.484	0.360
PCB 49	20.676	4	291.92	22161	1.632	0.556
PCB 47	20.707	4	291.92	23309	3.161	0.374
PCB 64	23.659	4	291.92	159578	1.836	0.409
PCB 95	23.713	5	325.88	18089	1.492	0.634
PCB 99	24.894	5	325.88	81178	1.820	0.973
PCB 90/91/101	25.295	5	325.88	26106	3.387	0.804
PCB 110/118	27.889	5	325.88	13291	1.140	0.231
PCB 151	28.767	6	359.84	20244	-0.610	-0.390
PCB 135	29.088	6	359.84	29632	-0.406	-0.355
PCB 149	29.624	6	359.84	18634	-1.207	-1.016
PCB 146	30.936	6	359.84	28955	-1.363	-1.062
PCB 153	31.364	6	359.84	31718	-0.521	-0.484
PCB 141	32.277	6	359.84	27318	-0.347	-0.271
PCB 130	32.947	6	359.84	52297	-1.583	-0.898
PCB 138	33.353	6	359.84	26312	-0.215	-0.314
PCB 178	34.373	7	393.80	17926	-1.575	-0.925
PCB 187	34.580	7	393.80	17073	-0.992	-0.976
PCB 183/185	34.938	7	393.80	18874	-0.371	-0.255
PCB 174	36.264	7	393.80	19114	-0.898	-0.702
PCB 175	36.655	7	393.80	21302	-0.804	-0.642
PCB 172	37.785	7	393.80	18026	-0.255	0.000
PCB 180	38.252	7	393.80	185862	-2.938	-1.758
PCB 156	38.558	6	359.84	24574	-0.269	-0.199
PCB 193	39.135	7	393.80	19253	-1.798	-1.443
PCB 170	40.370	7	393.80	19284	-1.134	-0.750
PCB 198	40.985	8	429.76	14900	-1.425	-1.008
PCB 199	41.368	8	429.76	13333	-0.688	-0.868
PCB 194	45.369	8	429.76	10445	-0.043	-0.188
PCB 206	48.964	9	463.72	8479	-0.392	-0.339

Table S3 Information and quality of Illumina high throughput sequencing data from PCE/PCB-fed cultures with and without Rpf amendment (TGs and CGs) at stage 1 (0.3 mM PCE), stage 2 (0.4 mM PCE), stage 3 (0.5 mM PCE) and stage 4 (26.88 μM Aroclor 1260).

Sample	Raw reads	Clean reads	OTUs	*CleanQ20	*CleanQ30
TG1-1	44386	39036	1279	96.86	90.98
TG1-2	79572	71090	2102	96.77	90.55
TG1-3	107935	97633	1748	97.35	92.10
CG1-1	143743	131264	2482	97.36	92.10
CG1-2	170475	156872	2233	97.65	92.77
CG1-3	30104	25919	1492	96.91	91.10
TG2-1	126419	103077	1672	96.35	89.76
TG2-2	56030	49288	1470	96.89	91.10
TG2-3	87165	77000	1155	96.92	91.17
CG2-1	80851	71458	1678	96.98	91.27
CG2-2	426776	391727	2331	97.15	91.60
CG2-3	65243	57731	1243	97.02	91.29
TG3-1	104528	92465	829	96.64	90.33
TG3-2	200344	173217	1085	96.86	91.07
TG3-3	189482	173673	1097	97.14	91.62
CG3-1	148401	136533	1055	97.28	91.93
CG3-2	38588	34469	552	97.02	91.31
CG3-3	101635	91304	729	96.73	90.52
TG4-1	68253	60638	807	97.12	91.55
TG4-2	200756	188543	658	97.36	91.97
TG4-3	205768	193867	633	97.47	92.27
CG4-1	110293	103988	595	97.52	92.28
CG4-2	367005	339336	1113	97.06	91.03
CG4-3	161820	152697	831	97.54	92.36

*****Clean Q20 or Q30: the percentage of bases with a phred value of >20 or 30.

Table S4 Microbial alpha diversity in the PCE/PCB-fed cultures with and without Rpf amendment (TGs and CGs) at stage 1 (0.3 mM PCE), stage 2 (0.4 mM PCE), stage 3 (0.5 mM PCE) and stage 4 (26.88 μM Aroclor 1260).

Sample	Chao1	Dominance	Equitability	Richness	Simpson	Shannon_2
TG1	2322.75±344.86	0.98±0.01	0.71±0.03	1925.00±250.32	0.024±0.00	7.74±0.23
CG1	2212.10±565.83	0.99±0.01	0.78±0.06	1862.50±523.97	0.010±0.01	8.55±1.04
TG2	1930.10±136.47	0.97±0.01	0.65±0.05	1571.00±142.83	0.026±0.01	6.92±0.42
CG2	1774.30±290.62	0.92±0.05	0.58±0.07	1460.50±307.59	0.079±0.05	6.07±0.93
TG3	1473.05±157.76	0.95±0.01	0.55±0.02	1091.00±8.49	0.044±0.01	5.58±0.24
CG3	906.20±103.66	0.92±0.00	0.54±0.26	640.50±125.16	0.081±0.00	5.02±0.85
TG4	998.67±82.47	0.70±0.19	0.36±0.14	699.33±94.08	0.29±0.19	3.46±1.37
CG4	1004.45±139.51	0.56±0.03	0.29±0.00	713.00±166.88	0.44±0.25	2.73±0.14

Table S5 Literature reports on the chlorine atoms and removal rate of biphenyl in the final product of Aroclor 1260 dechlorination culture.

Cultures	Concentration of Aroclor 1260 (μM)	Incubation time (day)	Dechlorination rate (μM/d)	Cl/ biphenyl	Reference
TG4	26.88	50	1.15	4.24	This study
CG4	26.88	50	0.76	4.96	This study
Sediment microcosm	53.80	120	-	6.19	(1)
WAS	26.88	180	-	6.06	(2)
DS	26.88	180	-	6.26	(2)
DEH10	134.4	400	-	5.3	(3)
DF-1	3.49	120	-	4.1	(4)
BH	268.8	100	-	6.3	(5)
BH+SF1+ DEH10	268.8	100	-	5.6	(5)
BH+*o*-17+DF-1	268.8	100	-	6.1	(5)
BH+SF1+DEH10+*o*-17+DF-1	268.8	100	-	5.5	(5)
AD14-PCE	80.65	210	0.20	5.87	(6)
PE	67.2	32		6.04	(7)
PP	67.2	32		6.03	(7)
PS	67.2	32		5.96	(7)

Table S6 Basic chemical properties of the soil collected from the PCB-contaminated site.

Parameters	Soil sample	Parameters	Soil sample
Total PCBs (μg/g)	78.64 ± 4.78	OrgC (g/kg)	37.53 ± 2.13
Di-CBs (μg/g)	1.37 ± 0.83	TN (g/kg)	1.08 ± 0.08
Tri-CBs (μg/g)	21.92 ± 0.48	TP (g/kg)	2.45 ± 0.35
Tetra-CBs (μg/g)	8.77 ± 0.83	Cu (mg/kg)	368.91 ± 36.79
Penta-CBs (μg/g)	28.57 ± 1.99	Pb (mg/kg)	281.27 ± 56.25
Hexa-CBs (μg/g)	15.7 ± 0.65	Zn (mg/kg)	387.20 ± 25.93
Hepta-CBs (μg/g)	2.31 ± 0.20	Cd (mg/kg)	33.40 ± 1.90
pH	6.84 ± 0.07	Ni (mg/kg)	77.45 ± 2.69

Table S7. Mineral salts medium compositions used in this study.

Reagents	Amount (1L)
	Unit (g)
Trace elements solution A	
$FeCl_2 \cdot 4H_2O$	1.5
$CoCl_2 \cdot 6H_2O$	0.19
$MnCl_2 \cdot 4H_2O$	0.1
$ZnCl_2$	0.07
H_3BO_3	0.006
$Na_2MoO_4 \cdot 2H_2O$	0.036
$NiCl_2 \cdot 6H_2O$	0.024
$CuCl_2 \cdot 2H_2O$	0.002
Trace elements solution B	
$Na_2SeO_3 \cdot 5H_2O$	0.03
$Na_2WO_4 \cdot 2H_2O$	0.08
Salt solution	
NaCl	1.0
$MgCl_2 \cdot 6H_2O$	0.5
KH_2PO_4	0.2
NH_4Cl	0.3
KCl	0.3
$CaCl_2 \cdot 2H_2O$	0.015
Vitamin solutions	
D-biotin	0.02
Folic acid	0.02
Pyridoxine hydrochloride	0.1
Riboflavin	0.05
Thiamin hydrochloride	0.05
Nicotinic acid	0.05
DL-calcium pantothenate	0.05
P-aminobenzoic acid	0.05
Thioctic acid	0.05
Vitamin B12	0.01
1, 4-naphthoquinone	0.04
Nicotinamide	0.1
Hemin	0.01
Others	
Resazurin	0.001
$NaHCO_3$	2.52

Table S8 PCB congeners assignment to main peaks in gas chromatography (GC) analysis of Aroclor 1260.

Peak number	PCB congeners	The number of chlorine	Position of substituent	Weight percent contribution (%)
1	PCB 52	4	2,2′,5,5′-	0.288
2	PCB 70	4	2,3′,4′,5-	0.211
3	PCB 95	5	2,2′,3,5′,6-	1.803
4	PCB 92	5	2,2′,3,5,5′-	0.441
5	PCB 90	5	2,2′,3,4′,5-	2.493
	PCB 101	5	2,2′,4,5,5′-	
6	PCB 87	5	2,2′,3,4,5′-	0.806
7	PCB 135	6	2,2′,3,3′,6,6′-	1.227
8	PCB 110	5	2,3,3′,4′,6-	1.669
9	PCB 151	6	2,2′,3,5,5′,6-	2.934
10	PCB 144	6	2,2′,3,4,5′,6-	1.803
11	PCB 123	5	2,3′,4,4′,5′-	5.217
	PCB 149	6	2,2′,3,4′,5′,6-	
12	PCB 118	5	2,3′,4,4′,5-	0.499
13	PCB 114	5	2,3,4,4′,5-	0.115
14	PCB 146	6	2,2′,3,4′,5,5′-	1.285
15	PCB 153	6	2,2′,4,4′,5,5′-	5.946
16	PCB 105	5	2,3,3′,4,4′-	2.551
	PCB 130	6	2,2′,3,3′,4,6′-	
17	PCB 141	6	2,2′,3,4,5,5′-	3.241
18	PCB 179	7	2,2′,3,3′,5,6,6′-	2.110
19	PCB 176	7	2,2′,3,3′,4,6,6′-	1.132
20	PCB 138	6	2,2′,3,4,4′,5′-	
	PCB 163	6	2,3,3′,4′,5,6-	6.310
	PCB 164	6	2,3,3′,4′,5′,6-	
21	PCB 158	6	2,3,3′,4,4′,6-	1.323
22	PCB 126	5	3,3′,4,4′,5-	1.362
23	PCB 178	7	2,2′,3,3′,5,5′,6-	0.422
24	PCB 187	7	2,2′,3,4′,5,5′,6-	4.910
25	PCB 183	7	2,2′,3,4,4′,5′,6-	3.088
26	PCB 128	6	2,2′,3,3′,4,4′-	0.940
27	PCB 185	7	2,2′,3,4,5,5′,6-	1.343
28	PCB 174	7	2,2′,3,3′,4,5,6′-	5.044
29	PCB 177	7	2,2′,3,3′,4,5′,6′-	2.915
30	PCB 171	7	2,2′,3,3′,4,4′,6-	2.014
31	PCB 201	8	2,2′,3,3′,4,5′,6,6′-	0.690
32	PCB 172	7	2,2′,3,3′,4,5,5′-	1.266
33	PCB 180	7	2,2′,3,4,4′,5,5′-	9.762
34	PCB 156	6	2,3,3′,4,4′,5-	0.422
35	PCB 193	7	2,3,3′,4′,5,5′,6-	0.729
36	PCB 200	8	2,2′,3,3′,4,5,6,6′-	0.153
37	PCB 170	7	2,2′,3,3′,4,4′,5-	5.025
38	PCB 190	7	2,3,3′,4,4′,5,6-	0.384
39	PCB 198	8	2,2′,3,3′,4,5,5′,6-	3.165
40	PCB 199	8	2,2′,3,3′,4,5,5′,6′-	3.414
41	PCB 203	8	2,2′,3,4,4′,5,5′,6-	0.288
42	PCB 195	8	2,2′,3,3′,4,4′,5,6-	1.688
43	PCB 208	9	2,2′,3,3′,4,5,5′,6,6′-	0.192
44	PCB 194	8	2,2′,3,3′,4,4′,5,5′-	3.126
45	PCB 205	8	2,3,3′,4,4′,5,5′,6-	0.288
46	PCB 206	9	2,2′,3,3′,4,4′,5,5′,6-	0.882
Unidentified peaks	-	-	-	3.511

"-" means not available in references.

Table S9 PCB congeners assignment to main peaks in gas chromatography (GC) analysis of Aroclor 1242.

Peak number	PCB congeners	The number of chlorine	Position of substituent	Weight percent contribution (%)
1	PCB 4	2	2,2′-	1.719
2	PCB 7	2	2,4-	1.023
3	PCB 6	2	2,3′-	1.135
4	PCB 8	2	2,4′-	3.765
5	PCB 19	3	2,2′,6-	0.734
6	PCB 18	3	2,2′,5-	5.338
7	PCB 17	3	2,2′,4-	2.854
8	PCB 24	3	2,3,6-	3.809
9	PCB 16 PCB 32	3 3	2,2′,3- 2,4′,6-	1.124
10	PCB 26	3	2,3′,5-	5.286
11	PCB 25	3	2,3′,4-	4.083
12	PCB 31	3	2,4′,5-	5.107
13	PCB 28	3	2,4,4′-	12.905
14	PCB 33	3	2,3′,4′-	5.567
15	PCB 22	3	2,3,4′-	0.463
16	PCB 45	4	2,2′,3,6-	3.301
17	PCB 47	4	2,2′,3,6′-	1.458
18	PCB 52	4	2,2′,5,5′-	0.462
19	PCB 49	4	2,2′,4,5′-	3.665
20	PCB 45 PCB 46	4 4	2,2′,3,6- 2,2′,3,6′-	3.318
21	PCB 44	4	2,2′,3,5′-	3.184
22	PCB 37 PCB 42	3 3	3,4,4′- 2,2′,3,4′-	5.275
23	PCB 41 PCB 64	3 4	2,2′,3,4- 2,3,4′,6-	2.265
24	PCB 40	4	2,2′,3,3′-	1.209
25	PCB 100 PCB 67	5 4	2,22,4,42,6- 2,3′,4,5-	1.739
26	PCB 74	4	2,4,4′,5-	4.134
27	PCB 70	4	2,3′,4′,5-	1.94
28	PCB 66	4	2,3′,4,4′-	1.674
29	PCB 56 PCB 60	4 4	2,3,3′,4′- 2,3,4,4′-	2.532
30	PCB 101	5	2,2′,4,5,5′-	0.664
31	PCB 99	5	2,2′,4,4′,5-	0.594
32	PCB 97	5	2,2′,3,4′,5′-	1.492
33	PCB 87	5	2,2′,3,4,5′-	0.755
34	PCB 85	5	2,2′,3,4,4′-	0.704
35	PCB 110	5	2,3,3′,4′,6-	0.64
36	PCB 82	5	2,2′,3,3′, -	0.434
37	PCB 118	5	2,3′,4,4′,5-	0.463
38	PCB 153	6	2,2′,4,4′,5,5′-	0.938
Unidentified peaks	-	-	-	2.248

"-" means not available in references.

Table S10 The primer sets of real-time quantitative PCR used in this study

Primers	Target	Orientation	Sequence	Temperature	Reference
Eub338	Bacterial 16S rRNA gene	Forward	ACTCCTACGGGA GGCAGCAG	58 °C	(8)
Eub518	Bacterial 16S rRNA gene	Reverse	ATTACCGCGGCT GCTGG	58 °C	(8)
F515	Archaeal 16S rRNA gene	Forward	GTGCCAGCMGC CGCGGTAA	57 °C	(9)
R806	Archaeal 16S rRNA gene	Reverse	GGACTACVSGGG TATCTAAT	57 °C	(9)
DhcF	*Dehalococcoides*16S rRNA gene	Forward	GGTAATACGTAG GAAGCAAGCG	58 °C	(6)
DhcR	*Dehalococcoides*16S rRNA gene	Reverse	CCGGTTAAGCCG GGAAATT	58 °C	(6)

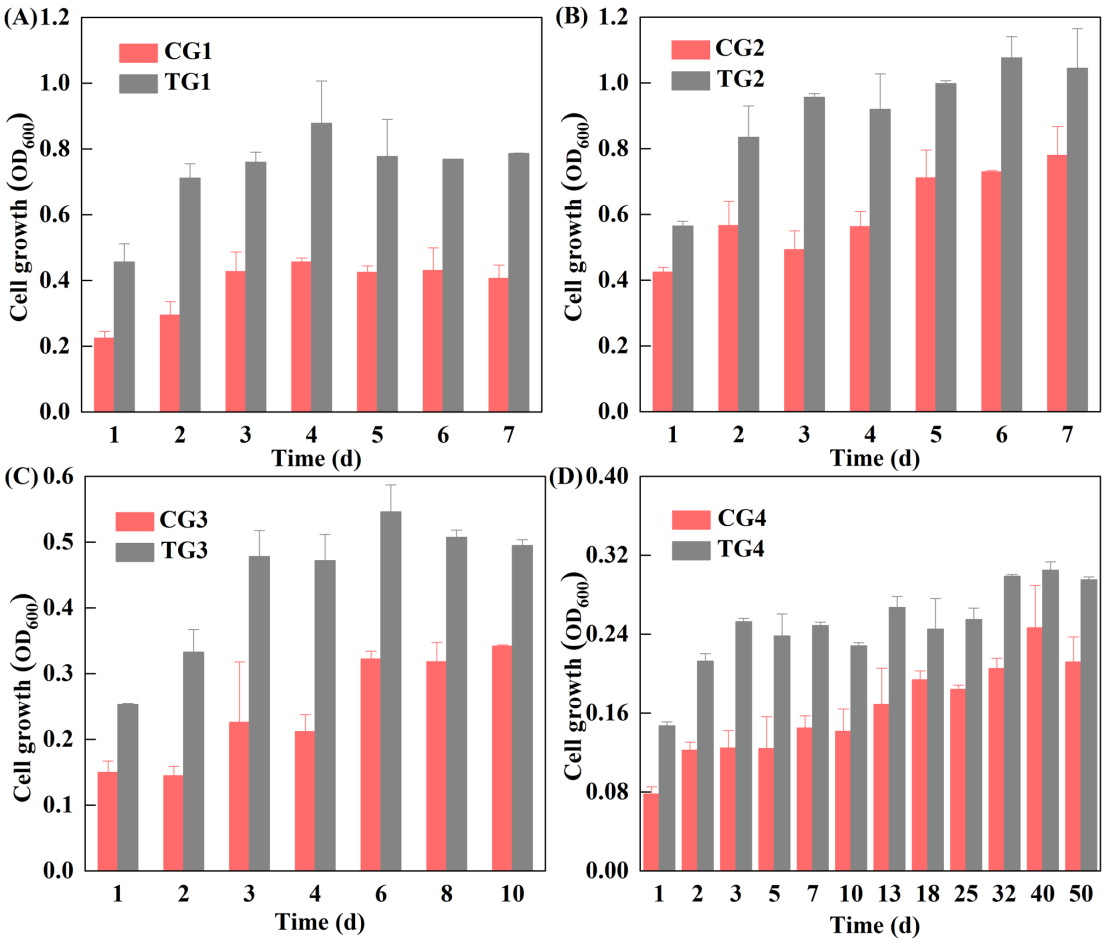

Fig. S1 Cell growth of PCE/PCB-fed cultures with and without Rpf amendment (TGs and CGs) at stage 1 (A, 0.3 mM PCE), stage 2 (B, 0.4 mM PCE), stage 3 (C, 0.5 mM PCE) and stage 4 (D, 26.88 μM Aroclor 1260).

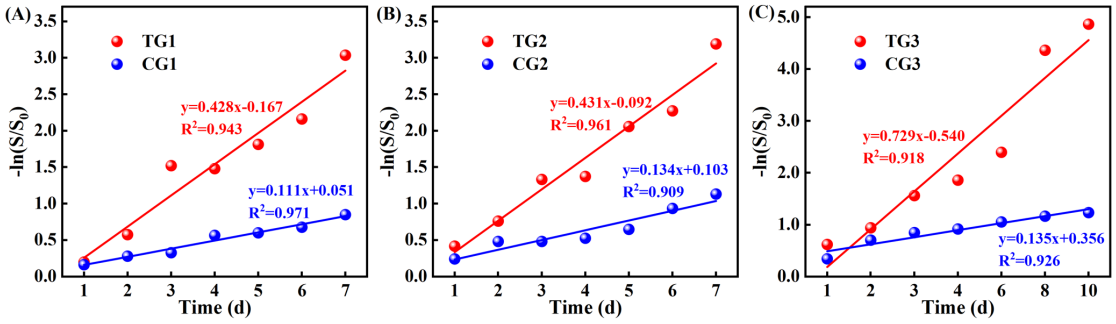

Fig. S2 Pseudo-first-order kinetics for PCE dichlorination in PCE-fed cultures with and without Rpf amendment (TGs and CGs) at stage 1 (0.3 mM PCE, A), stage 2 (0.4 mM PCE, B), stage 3 (0.5 mM PCE, C).

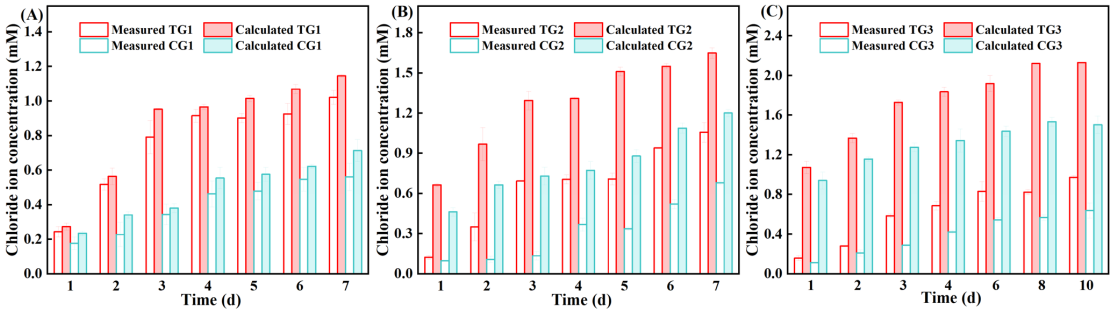

Fig. S3 Chloride ion accumulation in PCE-fed cultures during PCE dechlorination at initial concentrations of 0.3 (A), 0.4 (B) and 0.5 mM (C). The chloride ion in cultures was analyzed by ion chromatography. Calculated values were obtained based on the PCE dechlorination shown in Fig. 2.

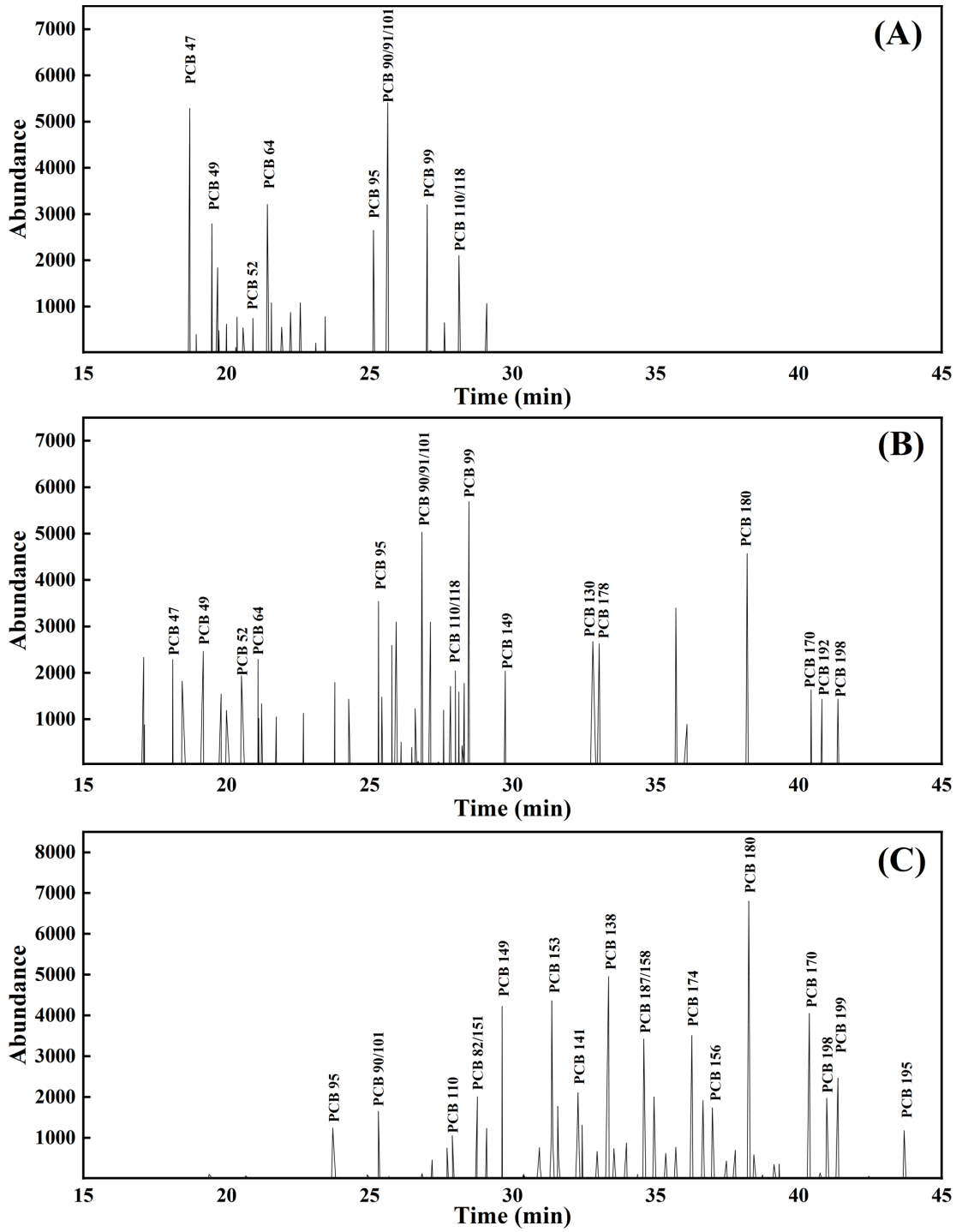

Fig. S4 Aroclor 1260 dechlorination in PCB-fed cultures after 50 days of incubation. Dechlorination of Aroclor 1260 in TG4 (A), CG4 (B) and abiotic control (C) analysis by gas chromatography (GC).

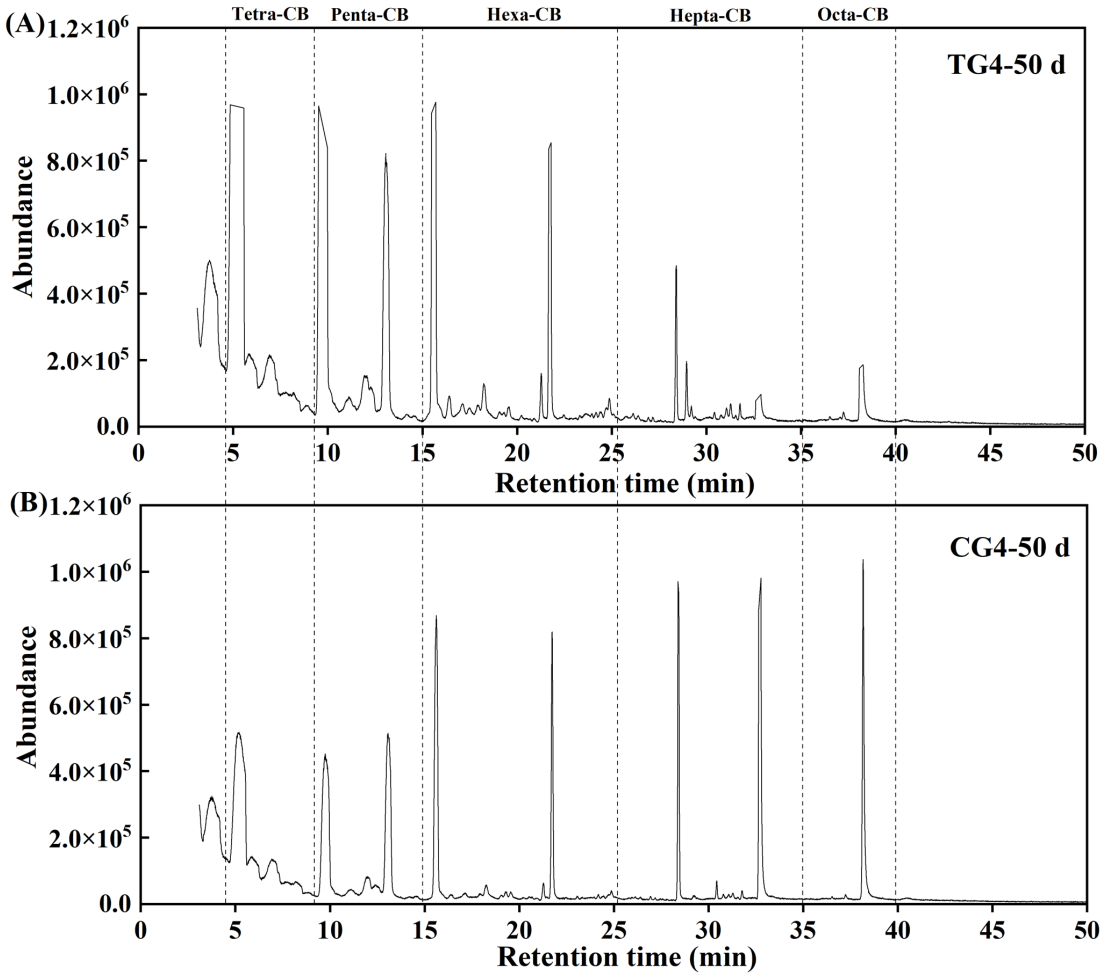

Fig. S5 Gas chromatography-mass spectrometry (GC-MS) graphs of PCBs in PCB-fed cultures with and without Rpf amendment (TG4 and CG4) on days 50 of incubation (the abundance on the y-axis is 1.0×10^6 for all samples).

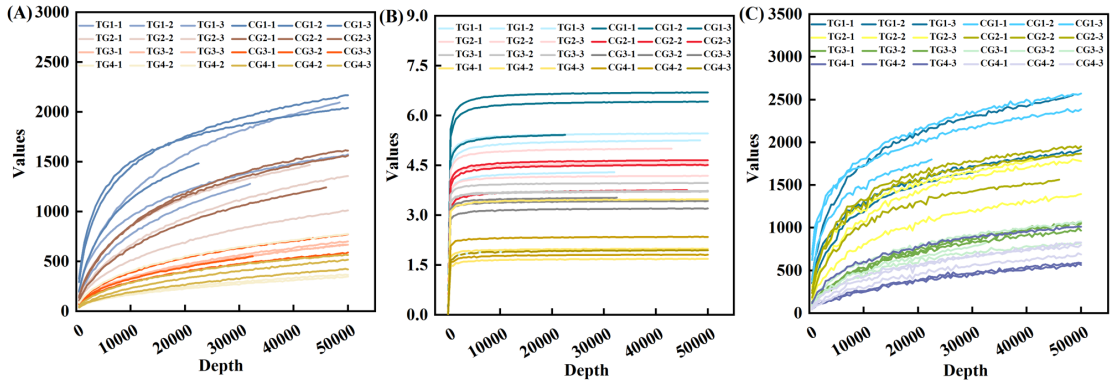

Fig. S6 Rarefaction curves based on observed richness (A), Shannon diversity (B) and Chao1 diversity (C).

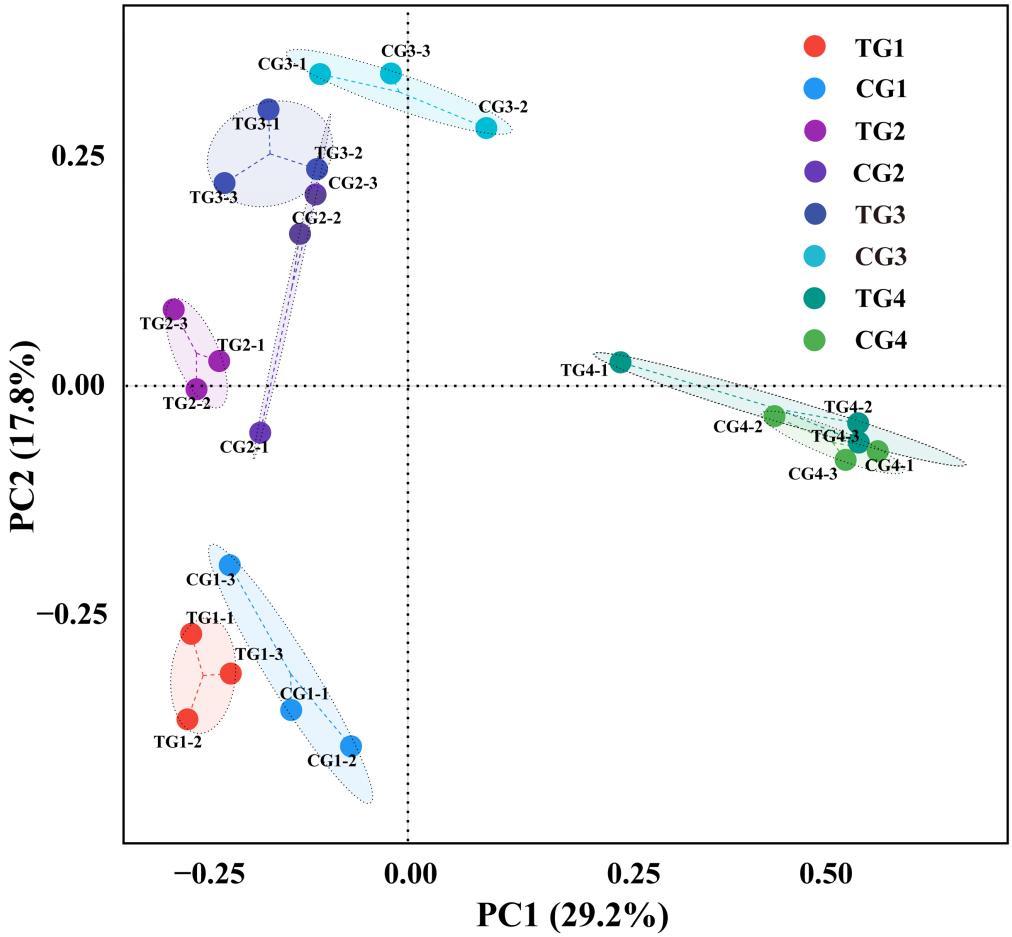

Fig. S7 Principal co-ordinates analysis (PCoA) using Bray-Curtis for the microbial community detected in PCE/PCB-fed cultures with and without Rpf amendment (TGs and CGs) from stage 1 to stage 4.

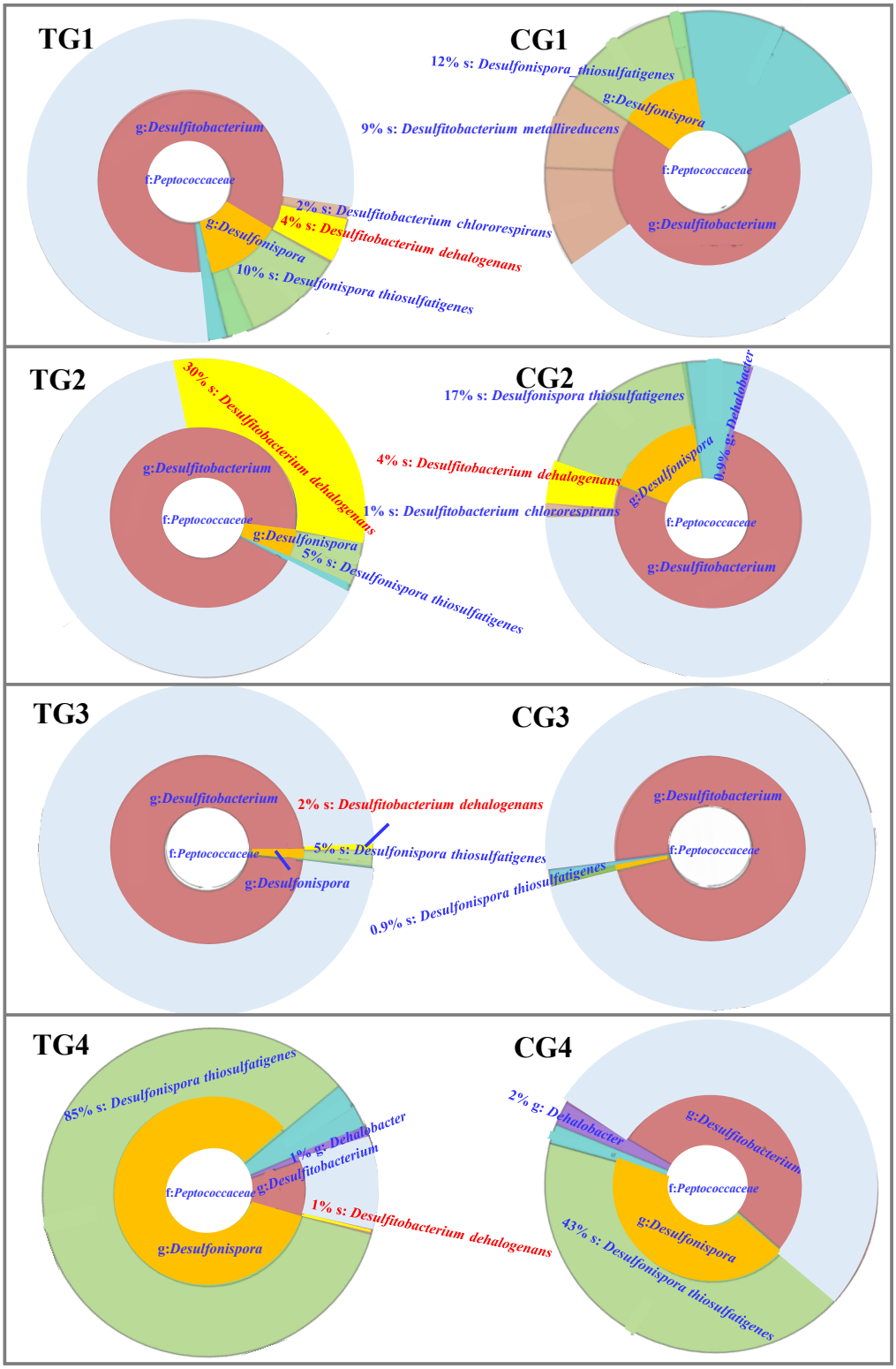

Fig. S8 Krona chart showing the relative abundance and diversity of family *Peptococcaceae* in PCE/PCB-fed cultures with and without Rpf amendment (TGs and CGs) from stage 1 to stage 4. The relative abundance of *Desulfitobacterium dehalogenans* was highlight in Red.

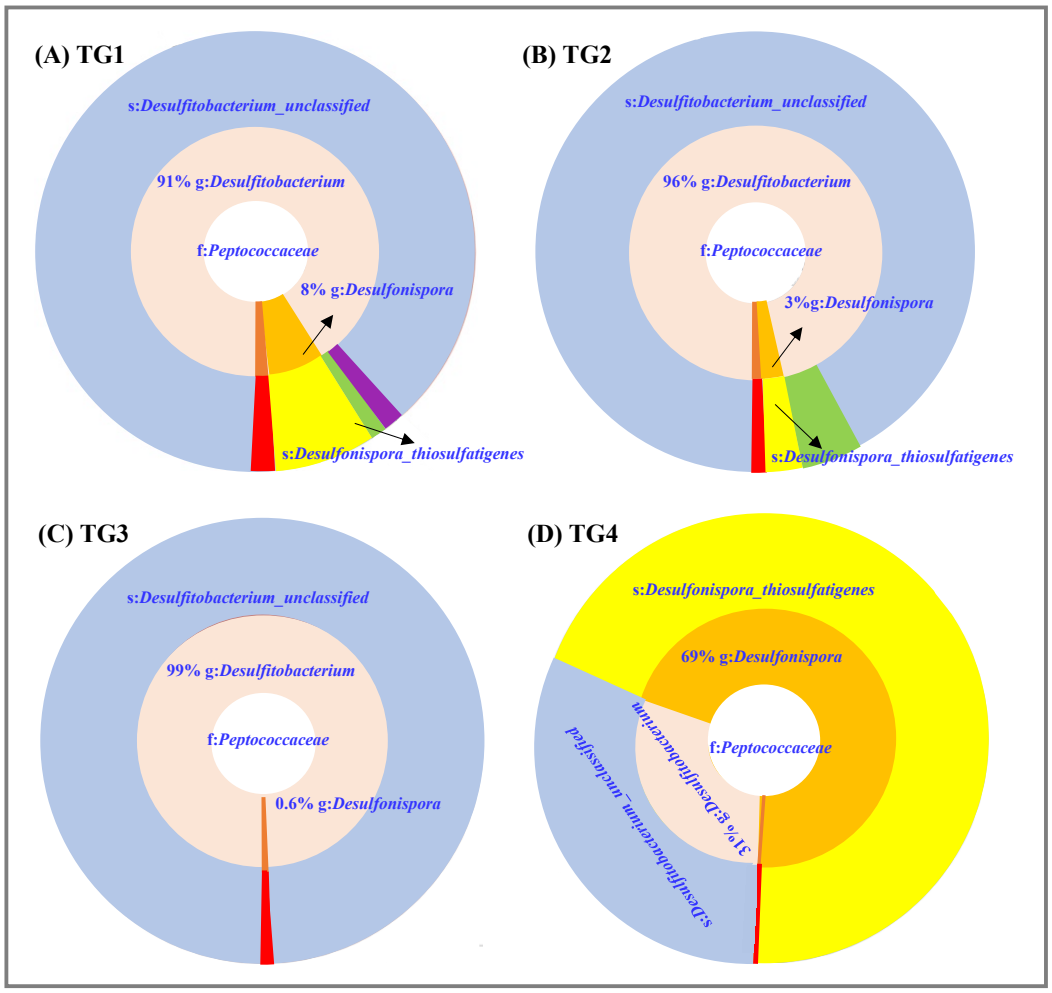

Fig. S9 Krona chart showing the relative abundance and diversity of genera *Desulfitobacterium* and *Desulfonispora* in PCE/PCB-fed cultures with Rpf amendment (TGs) from stage 1 to stage 4.

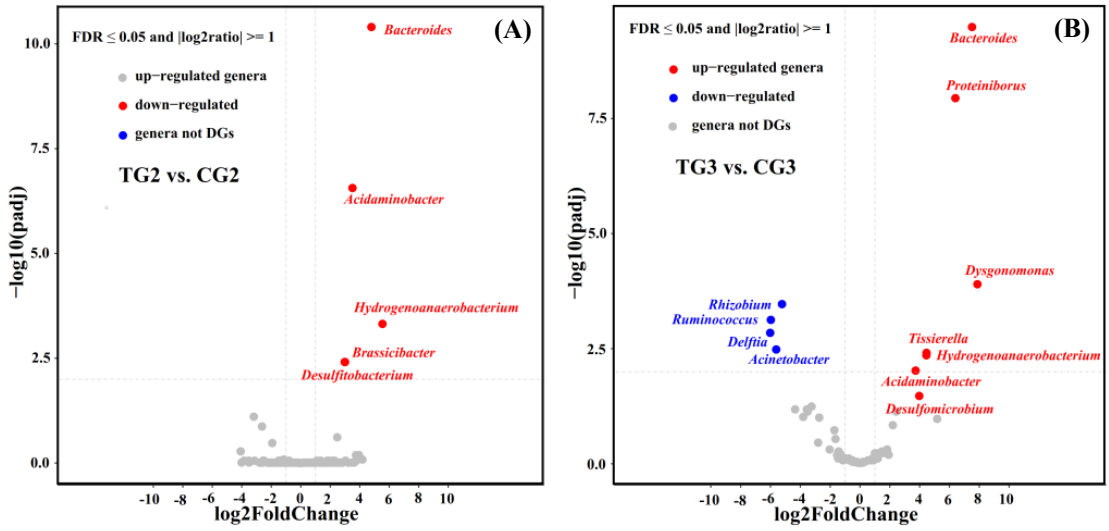

Fig. S10 Different abundance level of genera in cultures with and without Rpf amendment (TGs and CGs) at stage 2 (A) and stage 3 (B).

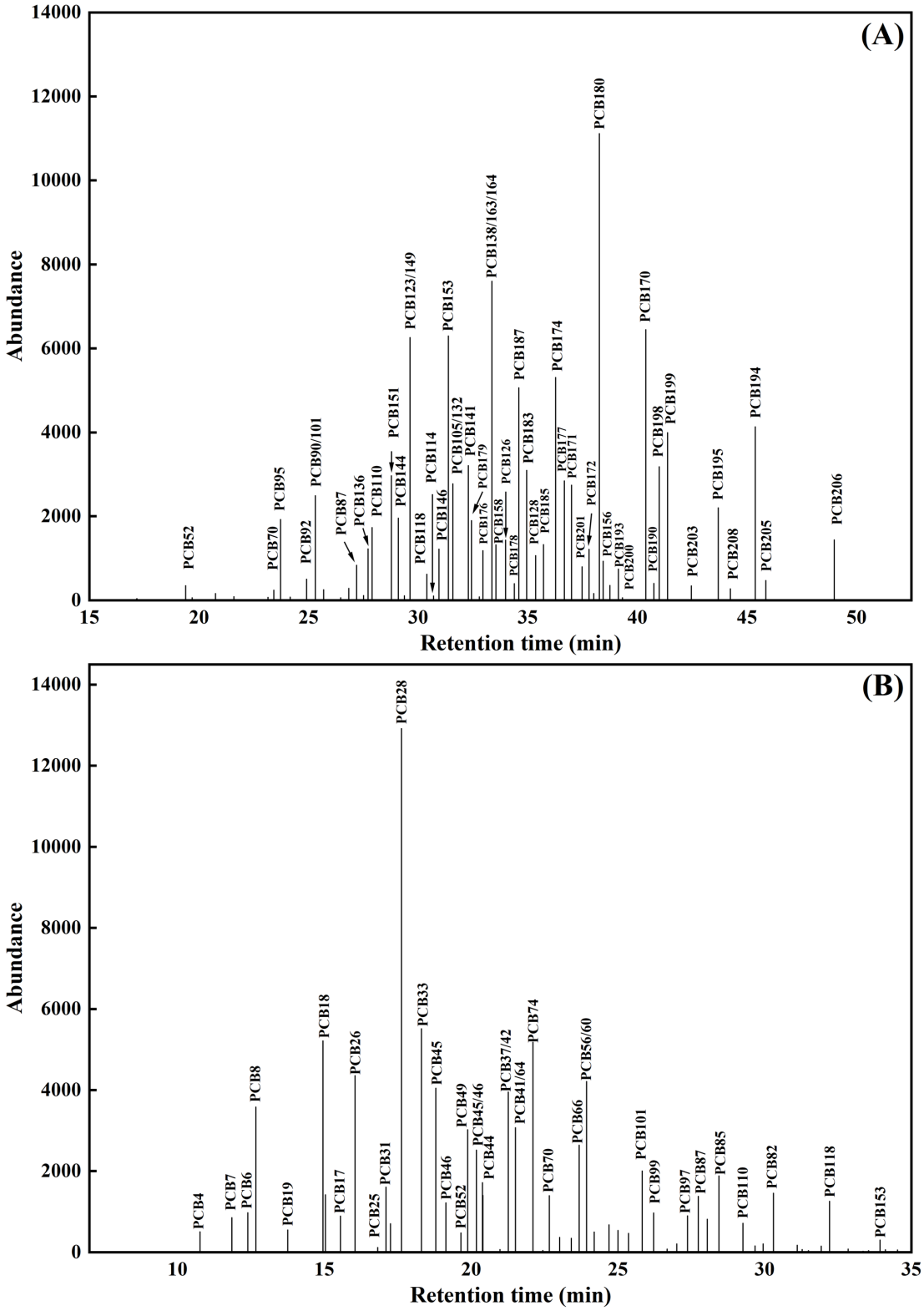

Fig. S11 PCB congeners in the standards of Aroclor 1260 (A) and Aroclor 1242 (B) analysis by gas chromatography (GC). The congeners were also listed in Table S8 (Aroclor 1260) and Table S9 (Aroclor 1242).

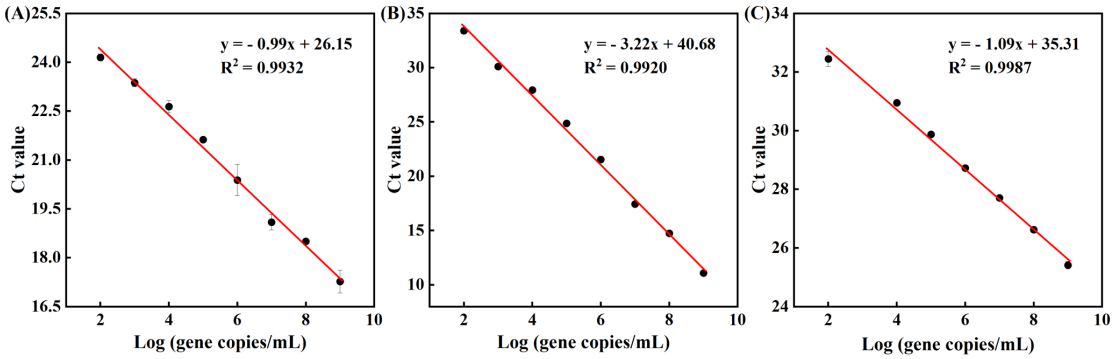

Fig. S12 Standard curves for bacterial 16S rRNA gene (A), archaeal 16S rRNA gene (B) and *Dehalococcoides*16S rRNA gene (C). Values are averages from three replicate experiments, and error bars represent standard deviations.

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