

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

Supplementary Materials

Method S1 Analytical methods for 1,4-dioxane

1,4-Dioxane (1–1000 µg/L) was measured on an Agilent 6890 chromatograph equipped with an Agilent 5973 mass spectrometer (GC-MS) with the Supelco SPB-1 Sulfur column (30 m × 0.32 mm id × 4 µm). The collected aqueous samples were prepared for GC-MS analysis using a frozen micro-extraction procedure as previously described (Li et al., 2011), and 5 µL of extracted samples were injected into the GC-MS. The program setup with a pulsed split-less injection with an inlet temperature of 150°C, 77 kPa, a pulsed pressure of 170 kPa for 2 min, and a purge flow of 15.0 mL/min. The oven temperature was initially held constantly at 35°C for 5 min, then increased to 100°C at a rate of 20°C/min, and further increased to 275°C at a rate of 50°C/min. The mass-selective detector (MSD) was operated with an electron multiplier (EM) offset of 400 and EM voltage of 2000. The MS quadrupole was set to 150°C with a source for 230°C (Zhang et al., 2016).

1,4-Dioxane concentrations over 1000 µg/L were measured using a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector (GC-FID) (Hewlett-Packard, Atlanta, GA) with a Restek® Stabilwax-DB capillary column (30 m x 0.53 mm ID x 1 µm; Restek, Bellefonte, PA) as described in Pornwongthong et al. (2014). Briefly, liquid samples (100 µL) were collected and filtered through 0.2-µm-pore Fisherbrand nylon syringe filters. 2 µL of the filtrate was directly injected into the GC-FID. The injector and detector were maintained at a temperature of 220°C and 250°C, respectively. The oven was programmed to rise from 80°C to 140°C at a ramp rate of 20°C/min, and held for 1 min at 140°C. The 1,4-dioxane retention time was approximately 3.56 min.

Table S1 Groundwater concentrations in the vicinity of the demonstration area (Courtesy: AECOM 2014)

COC	Groundwater Concentrations (July–August 2013) (µg/L)					
	TW43	TW44	TW45	8MNW17	8MNW20	8MNW54*
TCE	14700/15700	486 J	7.12	7080	17400/17000	1310
TCA	164/156	< 0.5	< 0.5	67.3	4.23/4.18	91.5
1,4-DX	620/680	91	62/63	270	147/145	351 J
cDCE	120/119	0.822 F	19.9/18.9	69.1	31.4/30.6	105 M
1,1-DCE	3600/3630	7.07	18.6/17.1	1720	2330/2910 J	296 F
1,1-DCA	7.26/6.54	5.77	68.2/65	8.59	3.96/3.87	6.81
1,2-DCA	< 5	4.04 F	< 5	13.2	37.3/36.3	24.4

Notes: *Monitoring well 8MNW54 installed adjacent to location of temporary well TW43;
/ denotes duplicate sample;
DCA dichloroethane; cDCE cis-1,2-dichloroethene;
F qualifier indicating concentration in excess of detection limit and below reporting limit;
J qualifier indicating estimated concentration;
M qualifier indicating matrix effect

26
27
28
29

Table S2 Initial 1,4-dioxane and CVOCs concentrations under each condition. The 1,4-dioxane concentration were measured using GC-MS and GC-FID, while the CVOCs were calculated prior to the study based on dilutions

Conditions	1,4-Dioxane	TCE	1,1,1-TCA	1,1-DCE	1,2-cDCE
High DX/High CVOCs	6.75 mg/L	~5 mg/L	~5 mg/L	~5 mg/L	~5 mg/L
High DX/Low CVOCs	7.36 mg/L	~0.5 mg/L	~0.5 mg/L	~0.5 mg/L	~0.5 mg/L
Low DX/Low CVOCs	1.11 mg/L	~0.5 mg/L	~0.5 mg/L	~0.5 mg/L	~0.5 mg/L
Low DX Only	1.00 mg/L	0	0	0	0

30
31
32

Table S3 Optimization of hydrogen peroxide dose for 1,4-dioxane oxidation

8MNW54 + TW1

Hydrogen Peroxide Added	Percent 1,4-Dioxane Degradation
77.62 mmol/L	> 99.0%
38.81 mmol/L	> 99.0%
19.40 mmol/L	> 99.0%
9.55 mmol/L	> 99.0%
4.78 mmol/L	> 99.0%
1.19 mmol/L	90.0%
1.07 mmol/L	> 99.0%
0.96 mmol/L	71.4%
0.96 mmol/L	59.4%
0.84 mmol/L	47.7%
0.48 mmol/L	30.8%
0.06 mmol/L	0

33
34
35
36
37
38

Note: The instrument detection limit for 1,4-dioxane was 4 µg/L

39

40

Table S4 Timeline of the experiments

Time points	Operations	Sample collections	Treatment states	Labels
0 h	Microcosms setup, start oxidation	Yes	Original	OC0/original
12 h	End of oxidation, start catalysis with more amount of peroxide	Yes	Post-oxidation	OC1
36 h	End of catalysis	Yes	Post-catalysis	OC2

41

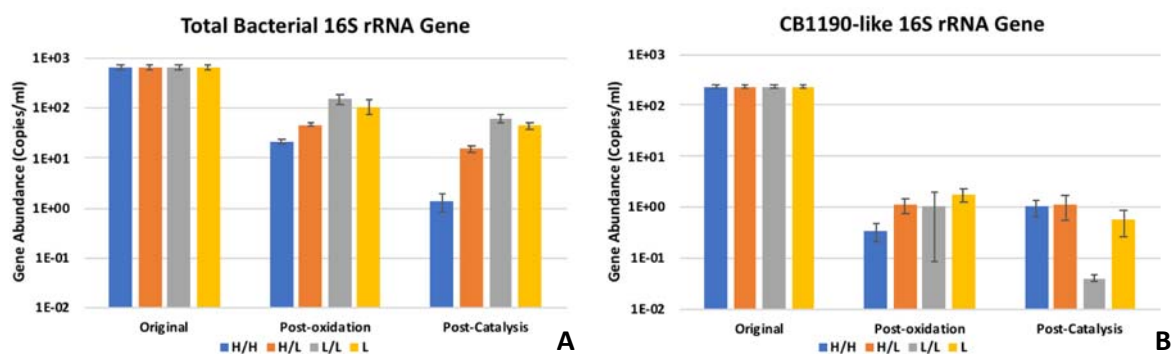
42

Table S5 Primer sequences and annealing temperatures

Gene	For-Primer (5'→3')	Rev-Primer (5'→3')	Annealing temperature (°C)	References
CB1190-like 16S rRNA	CCAAACGGGCGTC AGTCAT	AGAACGTGCGCTC CCAAAG	60	Gedalanga et al. (2014)
Universal 16S rRNA	ATGGCTGTCGTCA GCT	ACGGGCGGTGTGT AC	50	Harms et al. (2003)
16S rRNA gene V3-V4 region	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGCCTACG GGNGGCWGCAG	GTCTCGTGGGCTC GGAGATGTGTATA AGAGACAGGACTA CHVGGGTATCTAA TCC	55	Klindworth et al. (2013)

43

44



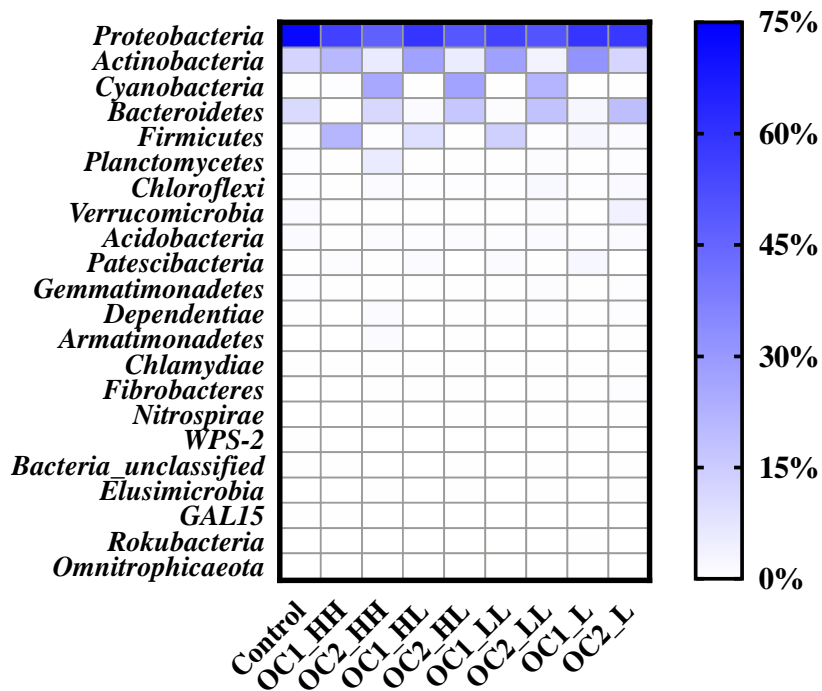
45

46

47

Fig. S1 Total bacterial 16S rRNA gene (a) and CB1190-like 16S rRNA gene (b) abundance under different conditions during oxidation-catalysis process. Y-axis is in log scale

48



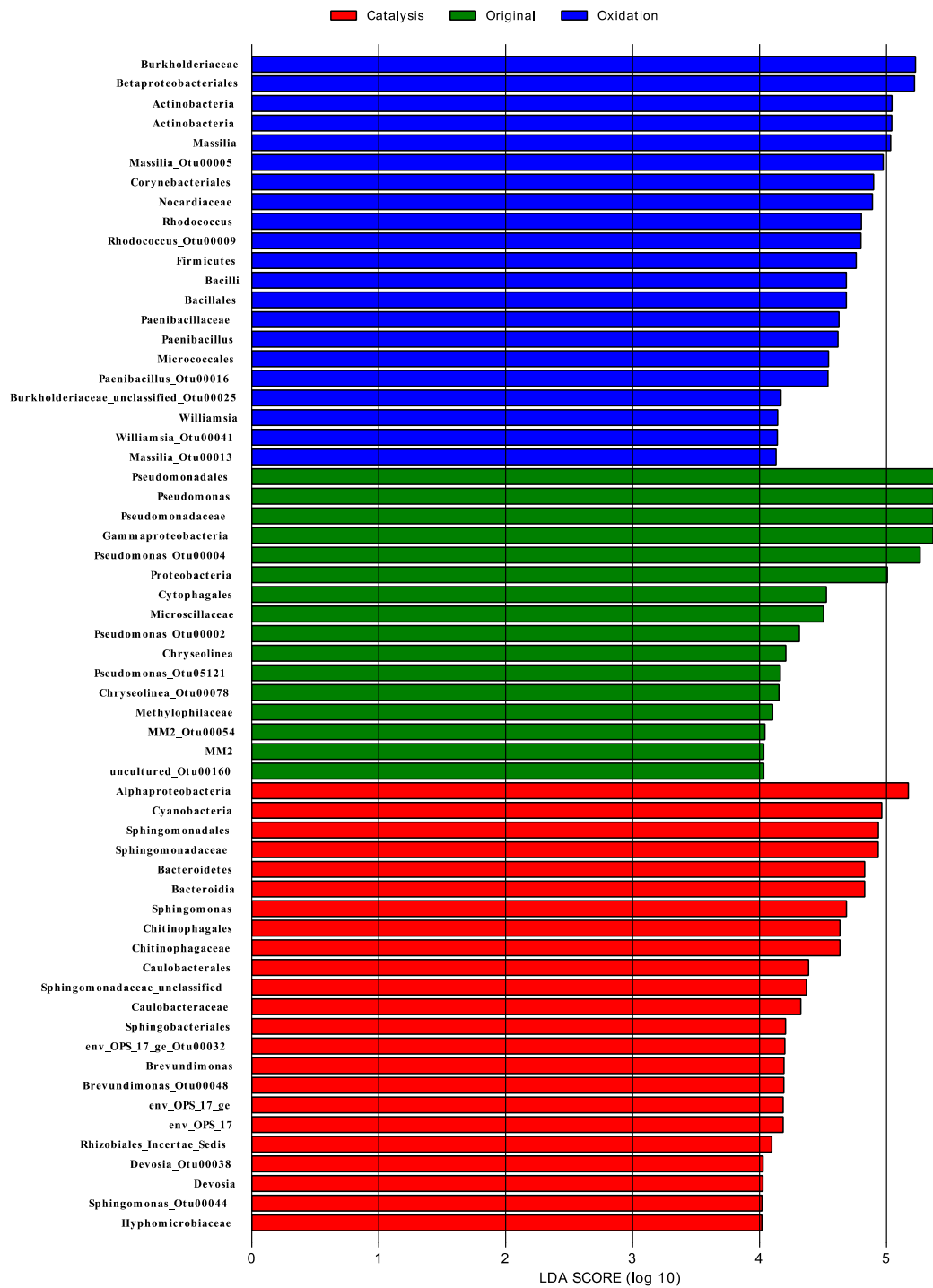
49

50

51

52

Fig. S2 Relative abundance of all taxa on phylum level at each time points under all conditions. Control is original state, OC1 is post-oxidation state, and OC2 is post-catalysis state



53

54

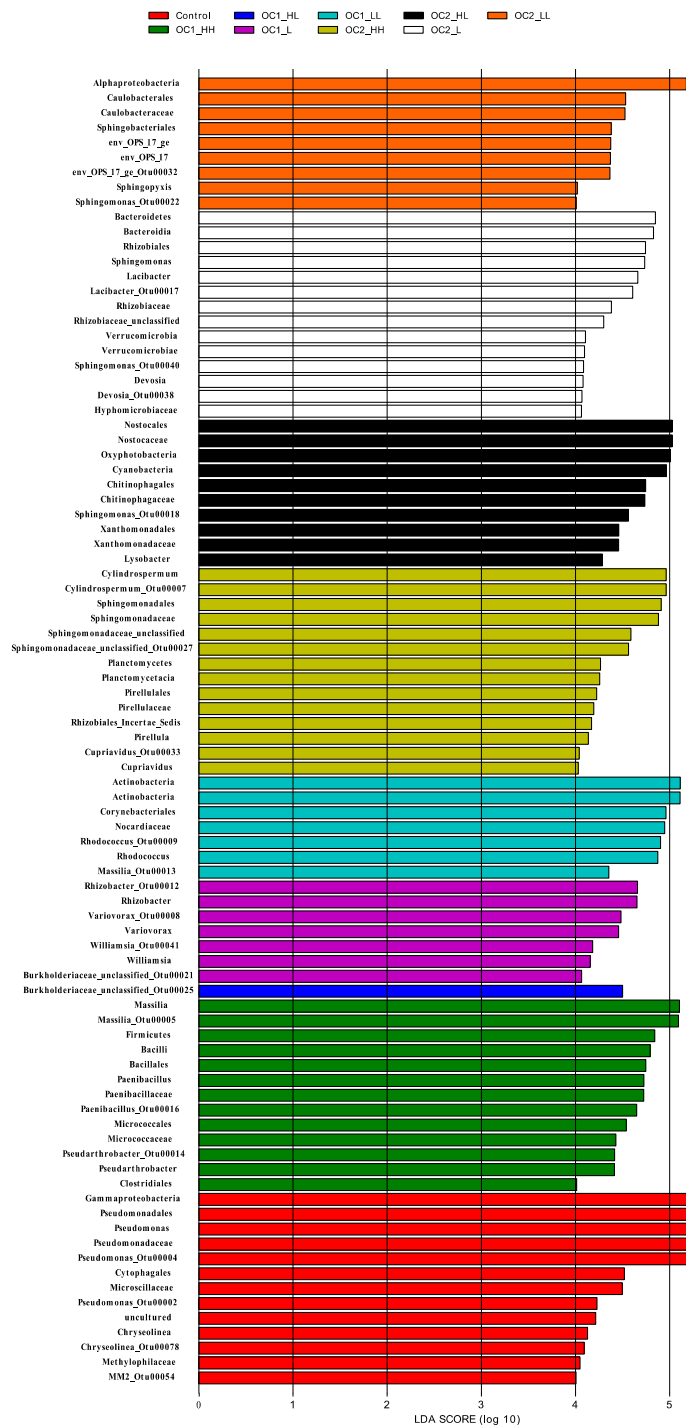
55

56

57

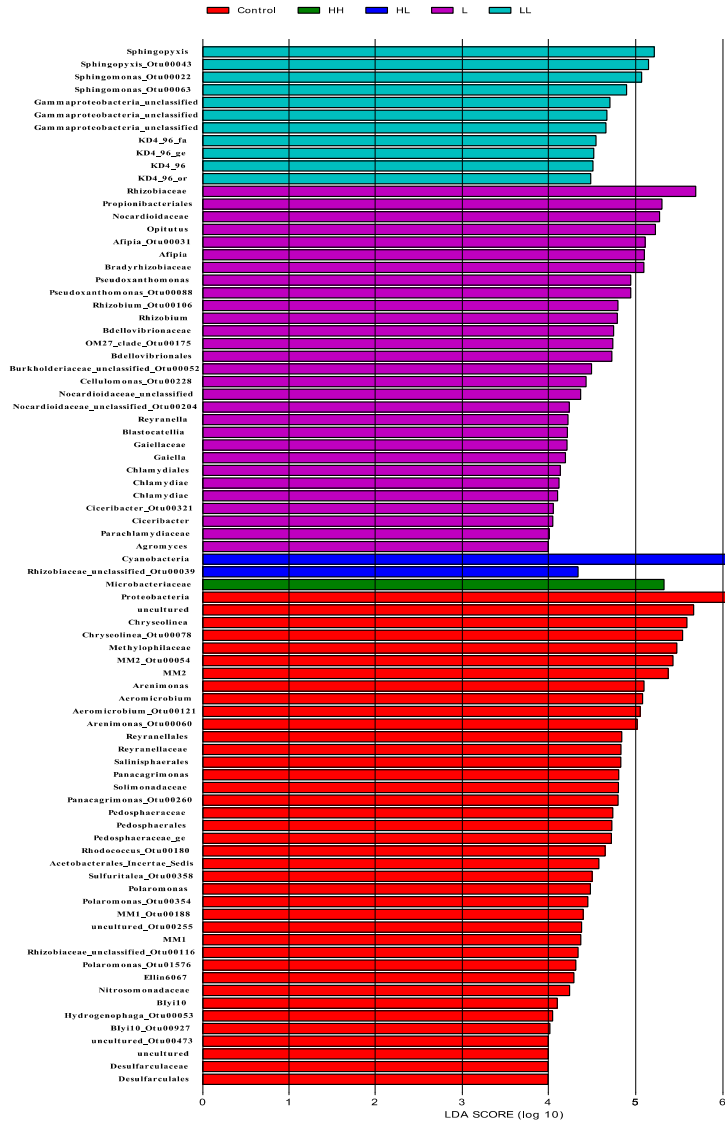
58

Fig. S3 Histogram of the LDA scores computed for taxon differentially abundant among treatment processes including original, oxidation and catalysis. LEfSe scores can be interpreted as the degree of consistent difference in relative abundance between taxa in the two groups of analyzed microbial communities. Only the taxa with meeting a significant LDA threshold value of > 4 are shown



59
60
61
62
63

Fig. S4 Histogram of the LDA scores computed for taxon differentially abundant among specific conditions after each treatment state. LEfSe scores can be interpreted as the degree of consistent difference in relative abundance between taxa in the two groups of analyzed microbial communities. Only the taxa with meeting a significant LDA threshold value of > 4 are shown



64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79

Fig. S5 Histogram of the LDA scores computed for taxon differentially abundant among conditions. LefSe scores can be interpreted as the degree of consistent difference in relative abundance between taxa in the two groups of analyzed microbial communities. Only the taxa with meeting a significant LDA threshold value of > 4 are shown

References

Gedalanga P B, Pornwongthong P, Mora R, Chiang S Y D, Baldwin B, Ogles D, Mahendra S (2014). Identification of biomarker genes to predict biodegradation of 1,4-dioxane. *Applied and Environmental Microbiology*, 80(10): 3209–3218

Harms G, Layton A C, Dionisi H M, Gregory I R, Garrett V M, Hawkins S A, Robinson K G, Saylor G S (2003). Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. *Environmental Science & Technology*, 37(2): 343–351

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner F O (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41(1): e1

Li M Y, Conlon P, Fiorenza S, Vitale R J, Alvarez P J J (2011). Rapid analysis of 1,4-dioxane in groundwater by frozen micro-extraction with gas chromatography/mass spectrometry. *Ground Water Monitoring and Remediation*, 31(4): 70–76
doi:10.1111/j.1745-6592.2011.01350.x

- 80 Pornwongthong P, Mulchandani A, Gedalanga P B, Mahendra S (2014). Transition metals and organic ligands influence
81 biodegradation of 1,4-dioxane. *Applied Biochemistry and Biotechnology*, 173(1): 291–306
- 82 Zhang S, Gedalanga P B, Mahendra S (2016). Biodegradation kinetics of 1,4-dioxane in chlorinated solvent mixtures.
83 *Environmental Science & Technology*, 50(17): 9599–9607