Supplementary Information (SI) for Environmental Science: Water Research & Technology.

Supporting Information:

Comparison of 1-Propanol and Propane as Auxiliary Substrates on 1, 4-Dioxane Biodegradation via Bioaugmentation with *Azoarcus* sp. DD4 at a Landfill Site

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Materials and Method

S1. Calculation of propane and 1-propanol dosing concentrations

The reaction of aerobic oxidation of propane is outlined below.

$$C_3H_8 + 5O_2 \longrightarrow 3CO_2 + 4H_2O$$

$$44.097 \text{ g} \quad 160 \text{ g}$$

There is 21% of oxygen in air. Thus, the microcosm headspace (140 mL) contains 0.0294 L of oxygen.

Considering the density of oxygen at 25°C at 1 atm is 1.308 g/L, the total available oxygen in the headspace is 0.0384 g.

For propane

$$\frac{44 \text{ g propane}}{160 \text{ g oxygen}} = \frac{X \text{ g propane}}{0.0384 \text{ g oxygen}}$$

$$X = 0.01056 \text{ g (mass of propane)}$$

Density of propane at 25°C at 1 atm = 1.808 kg/m^3, Volume of propane (Vp) = 6.08 mL

To ensure aerobic conditions in the microcosms, we assume only half of the oxygen in headspace could be consumed for propane oxidation. Thus, the corrected propane volume (Vp) = 3.04 mL

In 140 mL of the microcosm headspace, Vp stands for 2.1%, which is equal to the lower explosive limit (LEL) 2.1% of propane. (1)

If we use a safety factor of 3 for LEL to avoid explosion concerns, Vp = 2.1/3 = 0.7%

Therefore, in 140 mL of the headspace, 140 × 0.007 = 0.98 mL of propane. Thus, ~1 mL propane is amended.

To estimate an equimolar quantity of 1-propanol to 1 mL propane (=1.808 mg) amended to the microcosms, calculations are outlined below.

1 mol of propane = 44×10^3 mg

$$1.808 \text{ mg of propane} = \frac{1 \text{ mol} \times 1.808\text{mg}}{44 \times 10^3 \text{ mg}} = 4.1 \times 10^{-5} \text{ mol}$$

1 mol of 1-propanol = 60×10^3 mg

$$4.1 \times 10^{-5} \text{ mol of 1-propanol} = \frac{60 \times 10^3 \text{ mg} \times 4.1 \times 10^{-5}}{1 \text{ mol}} = 2.46 \text{ mg of 1-propanol}$$

Thus, the concentration of 1-propanol in the 60 mL groundwater = 2.46 mg/60 mL = 41 mg/L.

S2. Calculation of the amount of DD4 cells inoculated in microcosms

Total biomass inoculated = 0.17 mg protein

Final protein concentration = 0.17 mg protein/60 mL

= 2.83 μg protein/mL

According to (2), DD4 inoculum 0.010 mg protein = 6.6×10^2 cfu

DD4 inoculum 2.83 μg protein/mL = 4.6×10^3 cfu/mL

The initial inoculated viable cell number for DD4 is 4.6×10^3 cfu/mL

S3. Calculation of total bacterial biomass and DD4 concentration based on qPCR results

Total bacterial biomass and DD4 concentrations (cells/mL) in Table S4 and S5 were estimated by using the following formula

$$\text{cells/mL} = \frac{\text{16S rRNA or } \textit{tmoA} \text{ gene copies} \times \text{DNA elution volume}}{\text{n} \times \text{qPCR DNA volume} \times \text{volume of extracted samples}}$$

DNA elution volume =100 μL, n = 4.2 as average 16S rRNA copies per genome (3) or 1 for *tmoA* gene copy number per genome of DD4, qPCR DNA volume = 1 μL, DNA extracted Volume for original = 100 mL, volume of extracted samples= 50 mL for bioaugmented samples and 100 mL for original groundwater samples

Table S1. Sequences of primers and probes used for qPCR and microbial community analysis.

Target Gene	Primer Name	Sequence (5’-3’)	Reference
16S rRNA	341F	CCTACGGGAGGCAGCAG	(4)
	534R	ATTACCGCGGCTGCTGG	
	806R	GGACTACNNGGGTATCTAAT	
tmoA	*tmoA*_F	GGCGGATGGCTGTACTCAACAGAATG	(2)
	*tmoA*_R	AAATCGCCGGAAAGCTTGGGC	
	*tmoA*_probe	/FAM/CGACCTGGC/ZEN/CAGGAGTACGAAC/IABkFQ/	

Table S2. Comparison of zero-order dioxane degradation rates (µg/L/day).

Treatment	Zero-order Degradation Rate		R^2 Value	
	MW-A	MW-B	MW-A	MW-B
DD4 + Propane	2.23	0.72	0.81	0.95
DD4 + 1-Propanol	1.94	0.57	0.77	0.85

Table S3. Comparison of first-order dioxane degradation rates (day^{-1}).

Treatment	First-order Degradation Rate		R^2 Value	
	MW-A	MW-B	MW-A	MW-B
DD4 + Propane	0.73	0.42	0.79	0.79
DD4 + 1-Propanol	0.63	0.45	0.59	0.47

Table S4. Calculation of total bacterial biomass concentrations in cells/mL

Treatment	**16S rRNA gene copies**		**Total bacterial biomass (cells/mL)**	
	MW-A	**MW-B**	**MW-A**	**MW-B**
Original GW	4.6×10^7	3.1×10^7	1.1×10^7	7.3×10^6
DD4 + propane	3.2×10^7	1.04×10^7	7.6×10^6	2.4×10^6
DD4 + 1-propanol	1.7×10^7	4.4×10^6	4.0×10^6	1.0×10^6

Table S5. Calculation of DD4 concentrations in cells/mL

Treatment	***tmoA* gene copies**		**DD4 biomass (cells/mL)**	
	MW-A	**MW-B**	**MW-A**	**MW-B**
Original GW	ND*	ND*	ND*	ND*
DD4 + propane	7.8×10^4	3.3×10^4	1.6×10^5	6.6×10^4
DD4 + 1-propanol	8.7×10^2	1.4×10^4	1.7×10^3	2.7×10^4

* = Not detectable

Table S6. Microbial community diversity analysis.

Sample	Shannon	Simpson
MW-A original	6.14	0.99
DD4 + propane	4.07	0.89
DD4 + 1-propanol	4.35	0.95
MW-B original	6.29	0.99
DD4 + propane	4.95	0.95
DD4 + 1-propanol	4.96	0.97

Table S7. Detection of heavy metals present in both groundwater samples

Sample	Lead (µg/L)	Manganese (µg/L)	Mercury (µg/L)	Nickel (µg/L)	Selenium (µg/L)	Vanadium (µg/L)	Zinc (µg/L)
MW-A	1	2400	0.2	13	6.5	3.8	18
MW-B	3.4	260	0.2	4.9	2.9	8.3	18

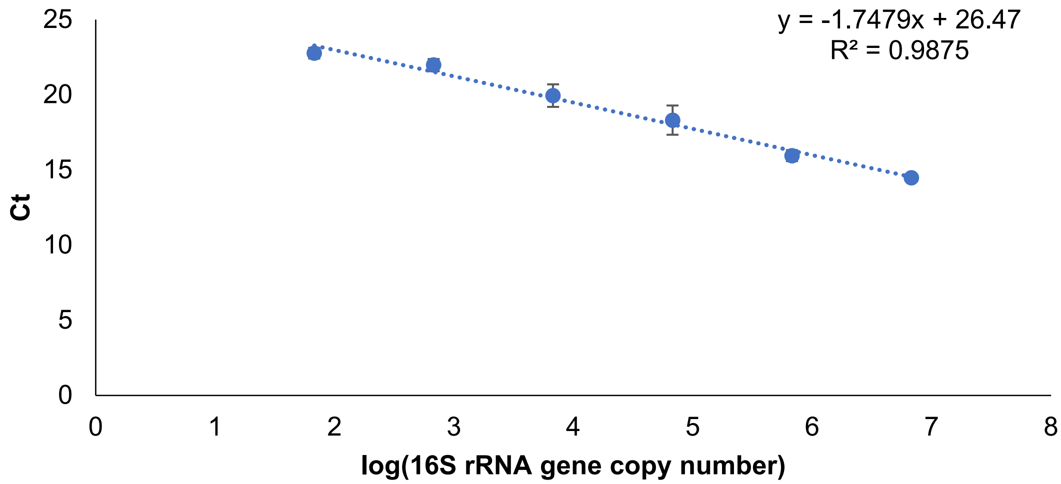

Figure S1. Calibration curve for 16S rRNA gene by qPCR using the SYBR Green qPCR assay.

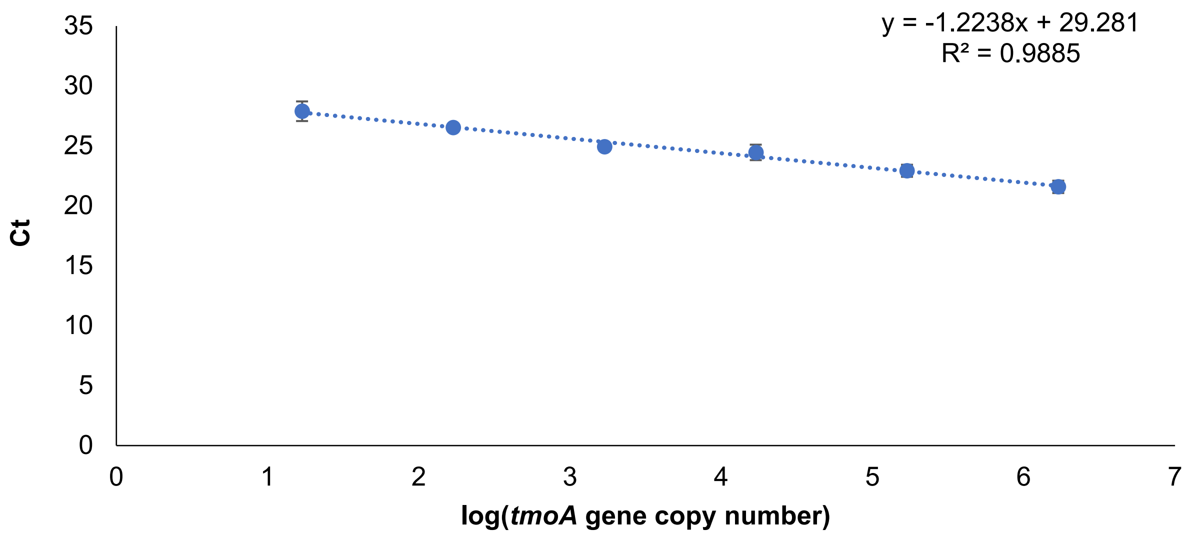

Figure S2. Calibration curve for the DD4 *tmoA* gene by qPCR with the Taqman qPCR assay.

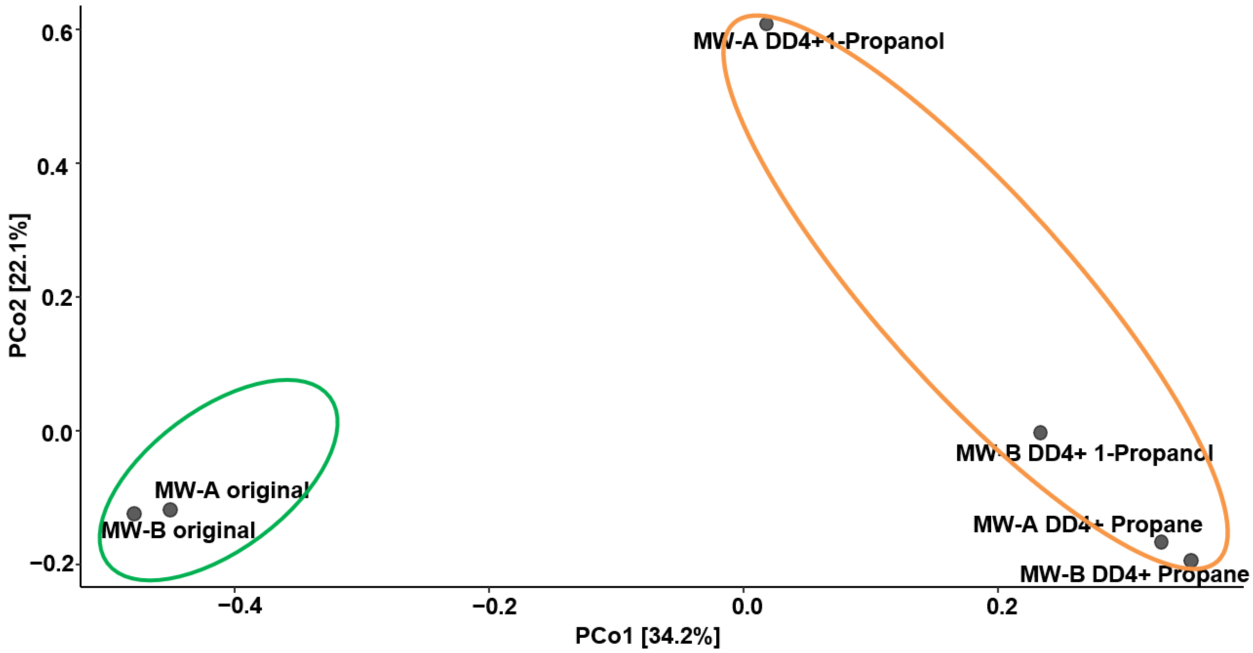

Figure S3. Principal coordinate analysis (PCoA) plot showing two distinct clusters of microbial communities in original groundwater samples and those that have received bioaugmentation treatments.

References

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