Supporting Information:

Synchronic Biotransformation of 1,4-Dioxane and 1,1-Dichloroethylene by a Gram-negative Propanotroph *Azoarcus* sp. DD4

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S1 Material and Methods

S1.1 Chemicals

1,4-Dioxane (anhydrous, 99.8%) was purchased from Sigma-Aldrich Company (Milwaukee, WI). 1,1-Dichloroethylene (99.0%) was supplied by EMD Millipore Corporation (Billerica, MA). Instrument grade propane (99.5%) and atomic absorption grade acetylene (99.6%) were purchased from Airgas® (Oakland, NJ). $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ and mercuric thiocyanate were purchased from Sigma-Aldrich Company (Milwaukee, WI). Bovine serum albumin (BSA) were purchased from Thermo Scientific (Rockford, IL). All other chemicals and solvents used in the study were of the highest purity available.

S1.2 Bacteria Strain and Media

Azoarcus sp. DD4 was isolated as described below. The nitrate mineral salts (NMS) medium contains (per liter) $MgSO_4 \cdot 7H_2O$ 0.1 g, $CaCl_2 \cdot 6H_2O$ 0.02 g, KNO_3 0.1 g, 3.8% (w/v) Fe-EDTA solution 0.1 mL, 0.1% (w/v) $Na_2MoO_4 \cdot 4H_2O$ 0.5 mL and 1 mL of trace elements solution. The trace element solution contains (per liter) EDTA 250.0 mg, $FeSO_4 \cdot 7H_2O$ 500.0 mg, $ZnSO_4 \cdot 7H_2O$ 400.0 mg, $MnCl_2 \cdot 7H_2O$ 20.0 mg, H_3BO_3 15.0 mg, $CoCl_2 \cdot 6H_2O$ 50.0 mg, $NiCl_2 \cdot 6\ H_2O$ 10.0 mg. The NMS medium was adjusted to pH 7.0 and autoclaved at 121°C for 15 minutes, and then 10 mL phosphate solution stock was added after the medium cooled down to room temperature. The phosphate solution stock (per liter) contains KH_2PO_4 26 g, and $Na_2HPO_4 \cdot 7H_2O$ 62 g. The chloride-free NMS buffer contains (per liter) $MgSO_4 \cdot 7H_2O$ 0.1 g, KNO_3 0.1 g, 3.8 % (w/v) Fe-EDTA solution 0.1 mL, 0.1% (w/v) $Na_2MoO_4 \cdot 4H_2O$ 0.5 mL and 1 mL of trace elements solution. The trace element solution contains (per liter) EDTA 250.0 mg, $FeSO_4 \cdot 7H_2O$ 500.0 mg, $ZnSO_4 \cdot 7H_2O$ 400.0 mg, H_3BO_3 15.0 mg. The buffer was adjusted

to pH 7.0 and autoclaved at 121 ℃ for 15 minutes, and then 10 mL phosphate solution stock was added after the medium cooled down to the room temperature. The phosphate solution stock contains (per liter) KH_2PO_4 26 g, and $Na_2HPO_4 \cdot 7H_2O$ 62 g.

S1.3 Isolation and Identification of Azoarcus sp. DD4

An active sludge sample was obtained from a local wastewater treatment plant in northern New Jersey. Prior to the enrichment, 2.0 g of sludge (wet weight) was washed three times with sterile phosphate buffer solution (PBS, 20 mM, pH 7.2) to remove the dissolved natural organic carbon sources. The washed sludge was suspended in 20 mL NMS in a 120-mL serum bottle supplemented with dioxane (88 mg/L), 1,1-DCE (1.0 mg/L), and propane (3% in headspace) as the carbon source, and incubated on a rotary shaker at 160 rpm and 30 ℃. To suppress the growth of protozoa in the enrichment culture, the medium was amended with 10 mg/L cycloheximide (Amresco, Solon, OH). Bi-weekly, 0.2 mL of the culture was transferred into 20 mL of fresh NMS for further enrichment. Degradation of propane, 1,1-DCE and dioxane was monitored during the enrichment. After two months of incubation, the final enrichment culture exhibiting fast propane and dioxane removal rates was diluted and plated onto R2A agar plates. After incubation at 30 ℃ three days, morphologically distinct colonies were obtained. Individual colonies were transferred to 20 mL of NMS amended with propane and dioxane to verify the co-metabolism of dioxane. The dioxane degrading isolates were subjected to further 1,1-DCE degradation screening. A bacterial strain grown on propane and co-metabolize both dioxane and 1,1-DCE was selected for further characterization study. The selected isolate was identified by 16S rRNA gene sequencing analysis. The strain was designated as *Azoarcus* sp. DD4.

S1.4 SEM Imaging

After grown on NMS medium with propane as the growth substrate, DD4 cells were harvested via centrifugation at 6000 rpm for 5 min at 4 °C. Cells were washed three times with 0.1 M phosphate buffer solution (PBS, pH 7.4) and then fixed in 0.1 M PBS with 4% glutaraldehyde overnight at 4 °C. The cells were dehydrated in a series of ethanol/water solutions (20, 40, 60, 80, 100%, v/v) for 15 min and treated with a mixture of ethanol/tert-butanol (1:1, v/v) for 15 min. After freeze drying, cells were coated with gold/palladium using a sputter coater under vacuum and then examined using a LEO 1530VP-FE SEM (LEO Electron Microscopy Inc., Thornwood, NY) with the in-lens detector.

S1.5 Bacteria Growth and Cell Yield with Propane

A total of 20 mL of cell suspension at an OD_{600} of 0.01 was inoculated into 150 mL serum bottle and supplemented with 4 mL propane. The bottles were then capped with butyl rubber stoppers and incubated with rotary shaking at 30 °C. Propane depletion and cell growth of DD4 were monitored by measuring the residual substrate and total biomass based on the OD_{600}, respectively. The cell yield on propane was then calculated using increase of the total protein mass normalized to the consumption of the substrate (i.e., propane).[1] Doubling time was estimated by using the first-order bacterial growth model to fit with the biomass data obtained within the exponential growth phase of DD4.

S1.6 Growth of DD4 at Varying Temperatures, pHs, and Salinities

To facilitate the bioremediation implementation of DD4, the survival of DD4 and its adaptability to the diverse environmental conditions were assessed. DD4 was cultured under

different pH, temperature, and salinity conditions using propane as the sole carbon and energy source. The pHs of the NMS media were adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 with appropriate amount of 1 M HCl or 1 M NaOH. With neutral NMS, culturing of DD4 was conducted under 10, 15, 20, 25, 30, 35, 40, and 45°C. The salinities of the NMS media were adjusted to 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5% (w/v) with NaCl. Each test was initially inoculated with 0.038±0.002 mg protein of DD4 and cultured under 30 °C (unless for temperature tests) for 72 h while shaking at 150 rpm. The biomass in the bacterial suspensions was estimated as the light absorption at the wavelength of 600 nm with spectrometer.

S1.7 Co-metabolism of Dioxane Using Propane as the Primary Substrate

The ability of strain DD4 to co-metabolize dioxane using propane as primary substrate was evaluated with bench tests. DD4 cells were grown on propane in NMS at 30 °C while shaking at 150 rpm. Initial concentrations of 20 mg/L dioxane and 100 mg/L propane in headspace were added and the cultures were inoculated with a single colony of DD4 in 150-ml serum vials sealed with Teflon-lined septa and an aluminum crimp cap. The disappearance of propane and dioxane was monitored for 96 h. All degradation experiments were conducted in triplicate. Control treatments were prepared with DD4 cells killed by autoclaving at 121°C for 15 minutes.

S1.8 Viability Test of DD4 after Condensation and Cooling Storage

DD4 cells were aerobically grown in VM-ethanol medium overnight at 30 °C and harvested via centrifugation at 6000 rpm at 4 °C for 5 min. After washing with PBS for three times, cells were resuspended to an initial OD_{600} of 2.0 using PBS and VM-ethanol,

respectively. The concentrated cultures were then incubated at 4 °C for 11 days. At selected days, culture samples were collected and serially diluted to count the number of viable colonies formed using VM-ethanol agar plates. VM-ethanol medium which consisted of (per liter) (l) 0.6 g K_2HPO_4, 0.4 g KH_2PO_4, 0.5 g NH_4Cl, 0.2 g $MgSO_4 \cdot 7H_2O$, 1.1 g NaCl, 0.026 g $CaCl_2 \cdot 2H_2O$, 0.01 g $MnSO_4 \cdot H_2O$, 0.002 g $Na_2MoO_4 \cdot 2H_2O$, 0.066 g Fe(III)-EDTA, 1 g yeast extract, 3 g Bacto-Tryptone, pH 6.8; (2) 6 mL ethanol sterilized by filtration.

S1.9 Nitrogen Fixation Assay

The nitrogen fixation assay was conducted using nitrogen-free MNF medium which consists of (per liter) 900 mL of A Solution and 100 mL of B solution.[2] A Solution contains (g/900 mL) 0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.1 g NaCl, 28 mg $Na_2FeEDTA$, and 25 mg $Na_2MoO_4 \cdot 2H_2O$, 100 mg yeast extract, pH 7.0; B Solution contains (g/100 mL) 0.2 g $MgSO_4 \cdot 7H_2O$ and 0.06 g $CaCl_2 \cdot 2H_2O$. The two solutions were autoclaved separately and then vitamins and trace elements solutions were added. DD4 cells were aerobically grown in VM-ethanol medium overnight, and then the cells were harvested, washed twice, and resuspended in nitrogen-free MNF medium. Propane was amended as the sole carbon and energy source. Cultures were grown aerobically and the propane depletion was monitored using GC-FID, and the biomass accumulation was spectrophotometrically monitored at OD_{600}.

S1.10 Insoluble Phosphate Solubilization Assay

DD4 cells were aerobically grown in VM-ethanol medium overnight. Grown cells were pelleted by centrifugation at 6000 × *g* for 10 min and resuspended in MALP medium. MALP medium (MA modified medium with low phosphate) contains (per liter) 0.2 g KH_2PO_4, 0.2 g Na_2HPO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.1 g NH_4Cl, 5 g $Ca_3(PO_4)_2$, and 1.5% agar for solid medium.

After autoclaving, the $CaCl_2$, vitamins and trace elements were added. Trace element solution contains (per liter): 0.10 g $ZnSO_4 \cdot 7H_2O$, 0.03 g $MnCl_2 \cdot 4H2O$, 0.30 g H_3BO_3, 0.20 g $CoCl_2 \cdot 6H_2O$, 0.01 g $CuCl_2 \cdot 2H_2O$, 0.02 g $NiCl_2 \cdot 6H_2O$, and 0.03 g $Na_2MoO_4 \cdot 2H_2O$. The droplets of 10 μL and 20 μL of the resuspended cell solutions were placed onto the surface of a MALP agar plate. The halo formation around the colonies was visualized after 3 days of incubation of plates at 30°C, reflecting the ability of the bacteria to solubilize insoluble phosphate.[2]

S1.11 Transformation Capacity

The transformation capacities for dioxane and 1,1-DCE were measured using the resting cells of DD4. The washed propane pre-grown cells were exposed to 87.8±3.3 mg/L dioxane and 8.04±0.44 mg/L 1,1-DCE in aqueous phase, respectively. Transformation capacities were calculated as the difference between the initial dioxane/1,1-DCE concentration and the concentration remaining in vials that were incubated for an additional 24 h after the cessation of biotransformation activities, which was subsequently normalized to the initial protein concentration.[3]

S1.12 Evaluation of Inhibitory Effects of Dioxane and 1,1-DCE on DD4 Biotransformation

To exploit the degradation behavior of DD4 with the presence of both of dioxane and 1,1-DCE, the resting cell assessment was conducted to exclude the influence derived from the presence of the primary substrate propane. DD4 resting cells were exposed to dioxane and 1,1-DCE at the initial concentration of 20.9±0.3 mg/L and 5.02±0.36 mg/L, respectively. The initial DD4 biomass was 1.18±0.03 mg total protein per vial. For comparison, treatments were

prepared with only dioxane or 1,1-DCE, as well as the abiotic control. At the selected incubation time, aliquots (0.7 mL) of liquid phase and 100 μL headspace samples were removed for the GC-FID analysis of dioxane and 1,1-DCE, respectively.

S1.13 Acetylene Inhibition Assays

Acetylene is an irreversible suicide substrate that inactivate bacterial MOs[4]. To demonstrate the involvement of MOs in propane, dioxane and 1,1-DCE degradation in DD4, inhibition assays were conducted using propane-grown DD4 resting cells. The washed resting cells were exposed with 10% headspace volume of acetylene for 20 minutes prior to the amendment of substrates. For propane degradation inhibition, the initial concentration was determined as 6.55±0.04 mg/L in gaseous phase. For dioxane and 1,1-DCE degradation inhibition, the initial aqueous concentrations were determined as 20.3±0.3 mg/L and 4.85±0.12 mg/L, respectively. The inhibition effect was evaluated by monitoring the depletion of test substrates over time with or without the acetylene. Triplicate abiotic controls were prepared in parallel. The propane, dioxane and 1,1-DCE depletion were monitored by GC-FID analysis.

S1.14 16S rRNA and SDIMO Gene Analysis

Total genomic DNA of DD4 was extracted using the UltraClean Microbial DNA isolation kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's instruction. The concentration and quality of the extracted DNA were determined using SpectraMax Plus 384 Microplate Reader with the SpectraDrop Micro-Volume Microplate (Molecular Devices, Sunnyvale, CA). PCR of the 16S rRNA gene was performed using the genomic DNA as the

template and the primer pair, 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-CGG TTA CCT TGT TAC GAC TT-3')[5]. To amplify the SDIMO genes, the degenerate primers NVC65 (5'-CAR ATG YTN GAY GAR GTN CGN CA-3'), NVC57 (5'-CAG TCN GAY GAR KCS CGN CAY AT-3'), NVC66 (5'-CCA NCC NGG RTA YTT RTT YTC RAA CCA-3'), and NVC58 (5'-CGD ATR TCR TCD ATN GTC CA-3') were used for the PCR reaction[6]. Each 50 μL PCR reaction consisted of 1× Green GoTaq® Flexi Buffer, 200 nM of dNTPs, 500 nM of each primer, 0.25 u of GoTaq® G2 Hot Start Polymerase (Promega, Madison, WI), and 5 ng of DD4 genomic DNA. Thermocycling conditions were: 98 ºC for 5 min, then 30 cycles of (98 ºC for 20s, 55 ºC 30s, and 72 ºC for 1 min), then 72 ºC for 7 min. The amplicons were checked for the correct product size and then gel-purified using a Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Irvine, CA). The amplified fragments of the 16S rRNA and SDIMO genes were sequenced in both directions by Eton Bioscience Sequencing Center (Union, NJ) and have been deposited in the GenBank database under the accession numbers MF614847 and MF624252, respectively.

S1.15 Analytical Methods

Biomass Quantification. Cell biomass was quantified as total protein concentration using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, IL) and bovine serum albumin was used as a standard following the manufacturer's instruction. Spectral absorbance at 660 nm was measured using the SpectraMax Plus 384 Microplate Reader (Molecular Devices, Sunnyvale, CA).

Gas Chromatography (GC). Concentrations of dioxane in aqueous samples, and propane and 1,1-DCE in headspace were measured using a Trace 1300 gas chromatograph (GC) equipped with a Flame Ionization Detector (FID) via direct injection of 1 μL of the filtered aqueous phase or 100 μ L of the headspace gas using a gas-tight syringe. The injector and detector temperatures were set at 220 °C and 250 °C, respectively. The carrier gas flow rate was 2 mL/min. For dioxane measurement, the oven temperature was initially held at 110°C for 1 min, ramped to 200 °C at the rate of 20 °C/min, and held for 1 min at the end. For 1,1-DCE detection, the oven temperature program ran from 150°C for 2 min, ramped to 200 °C at the rate of 15 °C/min, and further held for 3 min. Propane was detected at a constant oven temperature of 150 °C. The propane, 1,1-DCE and dioxane peaks were observed at a retention time of 2.18 min, 4.22 min, and 7.61 min, respectively. Calibration curves were prepared from standards generated over concentration ranges spanning those of the experiments, and the method detection limits (MDLs) for propane, dioxane and 1,1-DCE were estimated as 0.01 mg/L, 0.1 mg/L and 0.02 mg/L, respectively.

Gas Chromatography Mass Spectrometry (GC/MS). In microcosm assays using groundwater samples collected at the CA site, aqueous samples from day 8 to 14 were extracted by the liquid/liquid frozen micro-extraction method with dichloromethane as the solvent[7]. Dioxane at parts-per-billion levels was detected by a Trace 1300 gas chromatograph (GC) equipped with an ISQ mass spectrum (MS) detector (Thermo, Waltham, MA). To detect the intermediates of dioxane cometabolism by DD4, the filtered samples were subjected to acidification with formic acid to transform 2-hydroxyethoxyacetic acid (HEAA) into 1,4-dioxan-2-one (PDX) as previously described[7].

Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS). To identify the metabolites of 1,1-DCE transformation, aliquots of liquid culture were filtered and analyzed using a Agilent 1290 Infinity II HPLC in tandem with Agilent 6470A triple quadrupoles mass spectrometer (Agilent, Santa Clara, CA). Aliquots (20 μL) were analyzed by the LC/MS/MS system with a Symmetry C18 column (I.D. 2.1 mm, length 100 mm, particle size 3.5 μm) (Waters, Milford, MI) with a flow rate of 0.250 mL/min. The mobile phase initially consisted of 100% solvent A (5 mM ammonia acetate in 10% methanol) (v/v) and isocratic for 3.75 min, and then decrease to 75% A with 25% solvent B (pure methanol) for 5.9 min, and changed back to 100% A over the last 4.5 min. Triple quadrupoles mass spectrometer was set in the positive-ion electrospray mode. Full scan from 50-700 amu with the dwell time of 200 ms was employed to obtain the complete mass spectra of potential metabolites of 1,1-DCE. The fingerprint ions of 2-*S*-glutathionyl acetate include m/z 237 and 366[8] with the retention time of 3.5 min. For characterizing the relative increase of 2-*S*-glutathionyl acetate during 1,1-DCE decomposition, m/z 237 was extracted via MS2Scan/SIM. The nebulizer pressure was set at 25 psi with a capillary voltage of 2.5 kV. The desolvation gas temperature was 350 °C with a flow rate of 10 L/min.

S1.16 Microscope Images of Growing Cells

DD4 was grown in NMS media fed with propane. CB1190 and PH-06 were cultured in AMS media fed with dioxane. Bacterial growth was initiated from single colony inoculant and incubated at 30 °C at a rotary speed of 150 rpm for 2 to 4 days. The bacterial cells were collected at exponential phase and washed using 1× PBS. The cells were mounted onto glass slides and subjected to bright-field microscopic observation using an EVOS FL Cell Imaging System

(Thermo Fisher Scientific, Bothell, WA) equipped with a × 100 oil immersion objective (Olympus, Center Valley, PA).

S2 Results and Discussion

S2.1 DD4 Exhibits Superior Physiological Properties for Remedial Applications

Little aggregation. To date, the majority of the extensively investigated dioxane degraders are gram-positive Actinomycetes (Figure 1A). These bacteria, such as *Pseudonocardia* sp. ENV478[9], *Pseudonocardia dioxanivorans* CB1190[10], *Mycobacterium dioxanotrophicus* PH-06[11] tend to aggregate and form clumps during growth preventing their distribution and dispersion in the subsurface. As a gram-negative bacterium, DD4 possesses a practical advantage over these gram-positive dioxane degraders since DD4 can propagate to a significant high cell density without the observation of aggregation. The less tendency of clumping formation for DD4 was evident by our microscopic observation. After grown with propane to the exponential phase, most DD4 cells were free floating in the medium (Figure S1A). In contrast, clumps of cell aggregates were observed for CB1190 and PH-06 (Figure S1B and S1C).

Fast growth. DD4 also exhibited a fast specific growth rate (1.95±0.01 day^{-1}) and high cell yield (0.25±0.003 mg protein/mg propane) when fed with propane (Figure S2). The doubling time was estimated as 8.5±0.02 h during its exponential phase. As propane is very inexpensive and commercially available, it is well suited for biosparging or other applications to mitigate the large impacted areas formed by dioxane and chlorinated solvents.

Adaptability to a wide range of temperatures, pHs, and salinities. Growth test under varying conditions (Figure S6) indicated that DD4 exhibited high versatility to adapt to diverse environments. DD4 can survive in relative cold environment at the temperature as low as 10 ^{o}C, and tolerate the salinity as high as 3% (as NaCl, w/v%). DD4 can also grow in relatively acid (pH 5) or alkaline (pH 9) solutions. It is also worth mentioning that dioxane biodegradation activities were observed along with the biomass accumulation of DD4 under various tested conditions (data not shown). These tests cover a wide span of conditions representing non-extreme aquifer and estuarine environments, suggesting that DD4 is promising to be applied for *in situ* remediation.

Nitrogen fixation and phosphate solubilization. As other *Azoarcus* species, DD4 is capable of fixing nitrogen in the atmosphere when grown in nitrogen-deficient medium with propane as the sole carbon source (Figure S7). Absence of readily accessible nitrogen sources (e.g., ammonia and nitrate) greatly limited the growth of DD4 with the doubling time increased from 8.5±0.02 h to 53.5±3.8 h. Such retarded cell growth of DD4 is probably due to the extensive energy demand for nitrogen fixation.[12] In addition, DD4 exhibited the capability of mobilizing insoluble inorganic phosphate compounds enhancing phosphate availability to support bacterial and plant growth in nutrient-limiting environment. After 3 days of culturing, clearing zones (Figure S8) were formed surrounding DD4 colonies in agar media containing insoluble mineral phosphates (mostly tricalcium phosphate or hydroxyapatite), indicating that DD4 is able to transform insoluble phosphorous.

Long-lasting viability after concentration and storage. For field application, microbial cells cultured in the lab are typically subjected to condensation at a high centrifugal force to

separate cells from the liquid media and subsequent refrigerated storage during delivery. As these operations may lead to physical damage of bacterial cells and/or limit their growth, it is of practical value to assess if DD4 remains viable after the handling and delivery prior to the injection at the field. Figure S9 indicated storage in the nutrient-rich medium (i.e., VM-ethanol medium) significantly enhanced the viability of DD4 after the aggressive condensation and low-temperature storage procedures in comparison with DD4 cultured in the nutrient-poor medium (i.e., PBS). After storage in the VM-ethanol medium at 4 °C for 3 days, over 82% of the initial DD4 cells can be revived based on the plate counting results (Figure S9). The viability rate reduced to 65% when the storage was prolonged to 5 days. Our results indicated high viability of DD4 can be achieved using conventional culture handling and shipment approaches (e.g., second day delivery with refrigeration).

Non-pathogenic endophyte. Azoarcus strains are widely distributed Proteobacteria that are abundant in diverse environments and known with versatile degradation capabilities[13]. In general, *Azoarcus* can be categorized into two major groups based on their taxonomic and ecophysiological properties (Figure 1A). Group I *Azoarcus* species are mostly aerobic diazotrophs living a commensal life with terrestrial plants[13, 14], while group II strains are free-living bacteria that are capable of degrading aromatic pollutants under both aerobic and denitrifying conditions[15, 16]. Though isolated from suspended activated sludge samples from a municipal wastewater treatment plant, DD4 exhibits high phylogenetical synteny with group I plant-associated species (Figure 1A) and many traits supporting plant growth (e.g., nitrogen

fixation and phosphate solubilization), suggesting its potential value for synergistic bacteria-plant remediation.

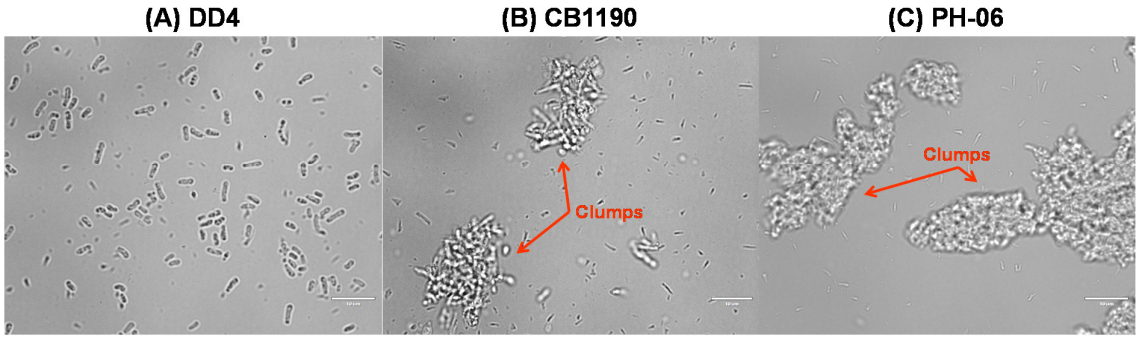

Figure S1. Microscopic images of propane-grown DD4 (A), dioxane-grown CB1190 (B), and dioxane-grown PH-06 (C). Clumps formed by CB1190 and PH-06 are indicated by red arrows. The scale bars label the length of 10 μm.

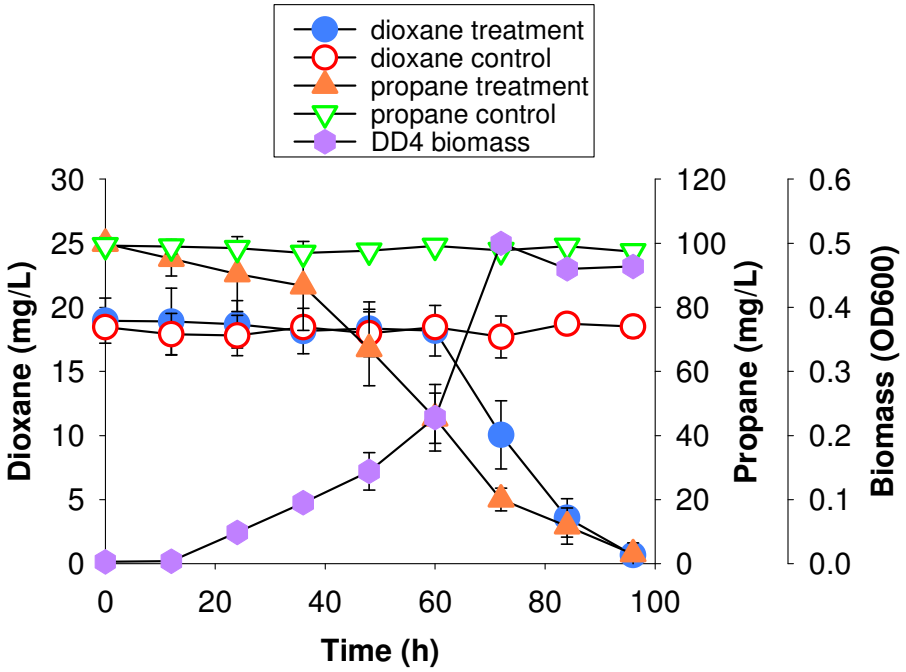

Figure S2. Growth of DD4 with propane and cometabolism of dioxane.

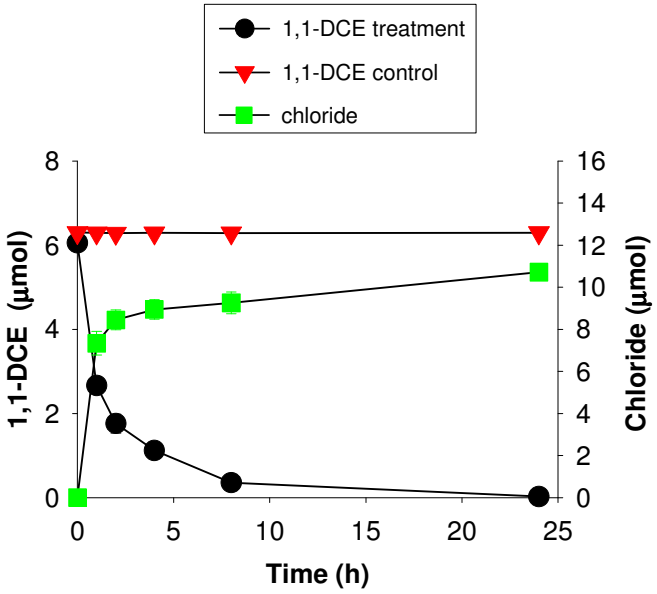

Figure S3. Release of free chloride during the co-oxidation of 1,1-DCE by DD4.

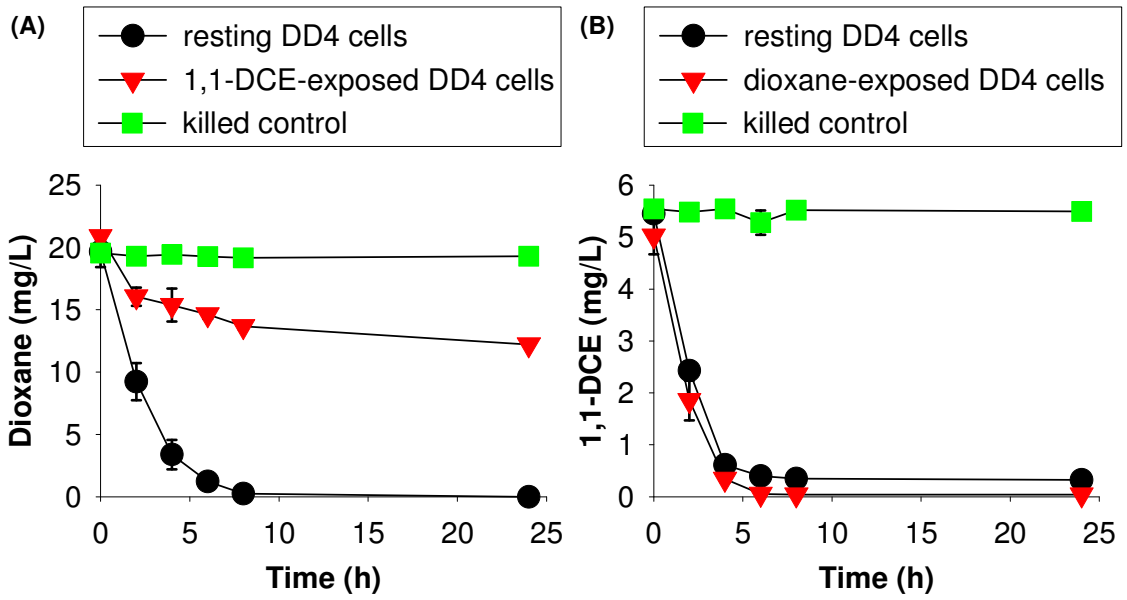

Figure S4. Significant inhibition of 1,1-DCE to dioxane degradation by DD4 (A) and no observable effect of dioxane to 1,1-DCE biotransformation (B).

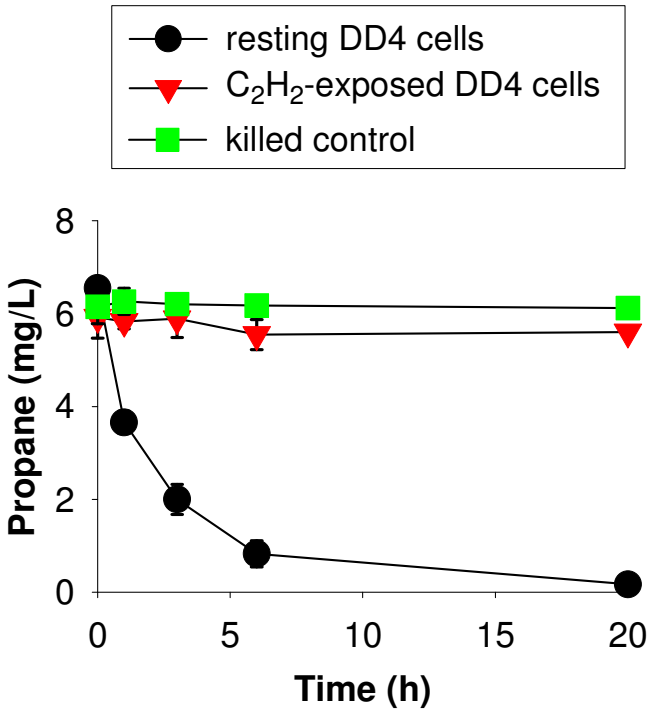

Figure S5. Degradation of propane by DD4 and complete inhibition by the acetylene exposure.

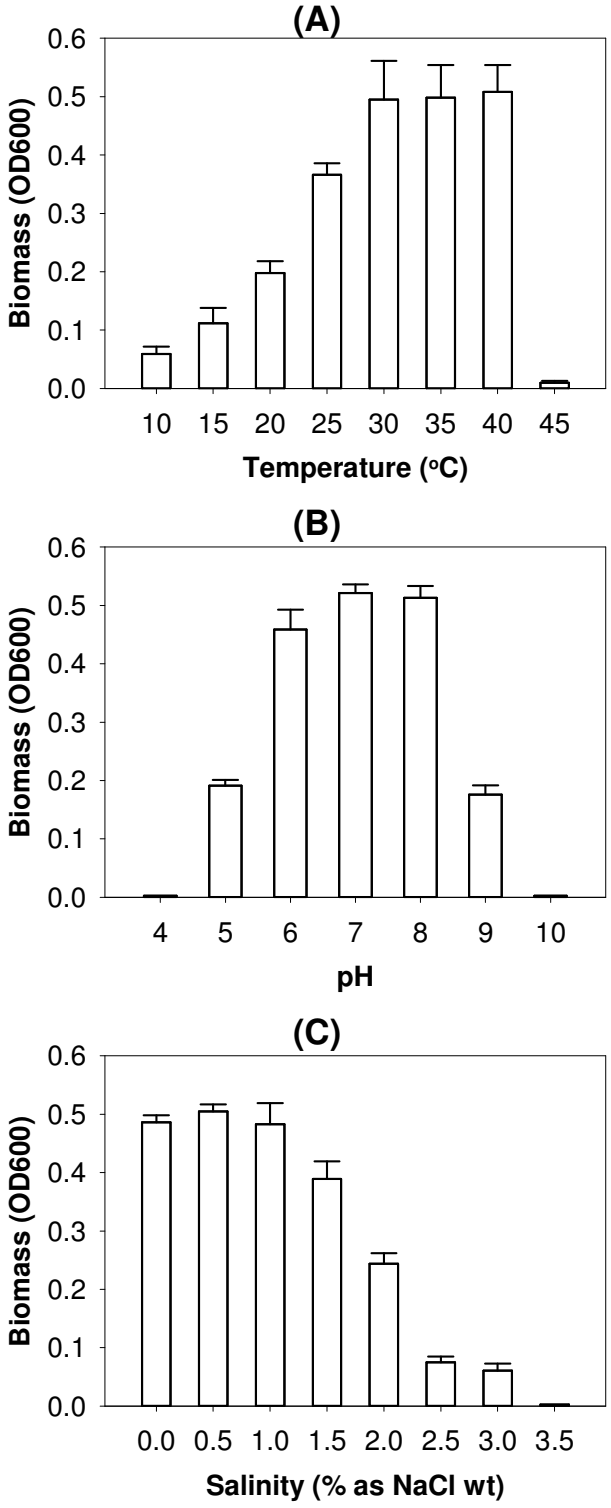

Figure S6. DD4 growth under varying temperatures (A), pHs (B), and salinities (C).

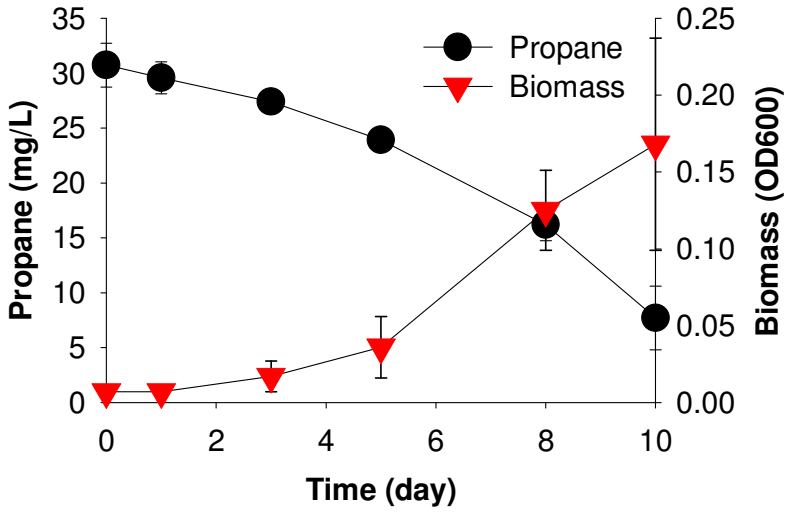

Figure S7. Growth of DD4 with propane in nitrogen-deficient medium.

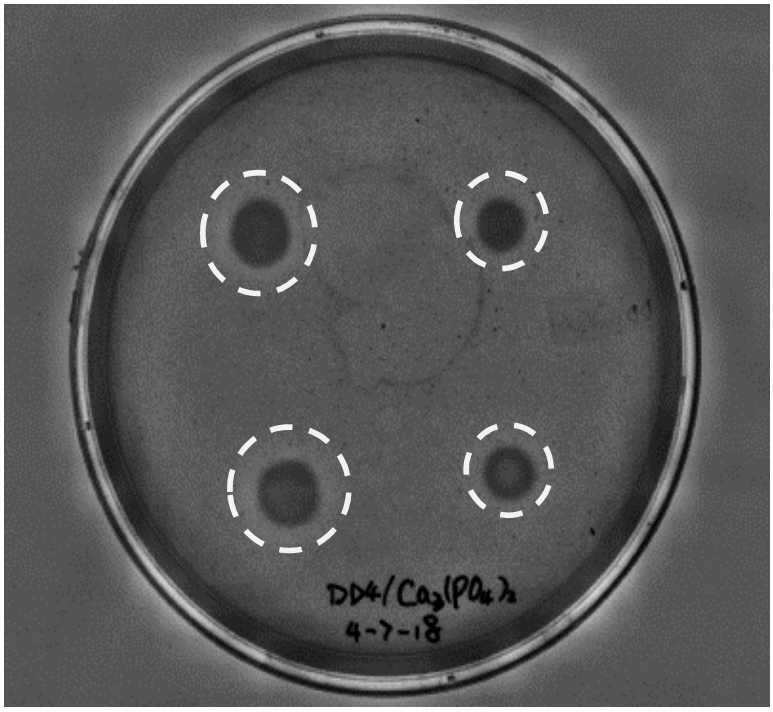

Figure S8. Solubilization of insoluble inorganic phosphate by DD4. Halos indicated by dotted cycles were observed after 3 days of incubation on solid MALP medium at 30°C. The two larger colonies formed on the left side of the plates were initially inoculated with 20 µL of DD4 cell resuspension; the other two on the right were inoculated with 10 µL of DD4 cell resuspension.

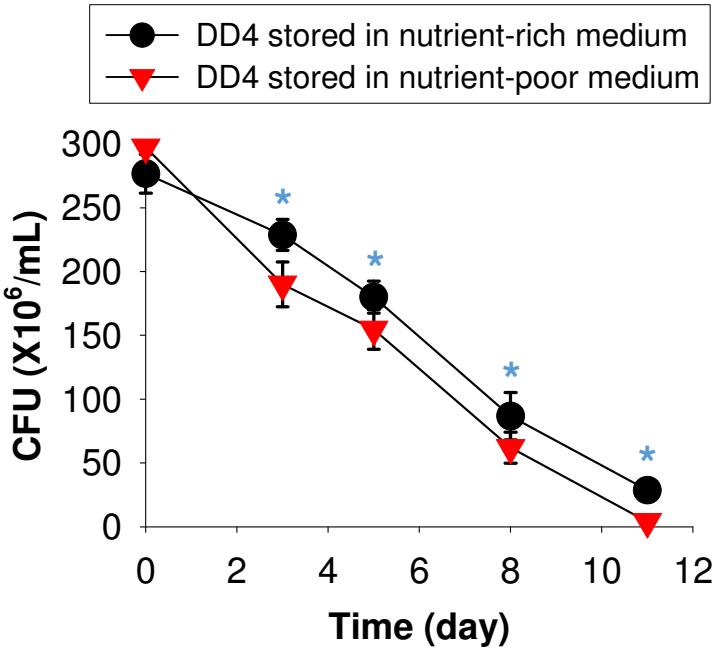

Figure S9. Viability test of DD4 cells after stored at 4 °C in nutrient-rich (i.e., VM-ethanol) or nutrient-poor (i.e., PBS) medium. The blue asterisks indicate the numbers of viable DD4 cells in the two media are statistically different ($p < 0.05$).

References

1. Sei, K.; Miyagaki, K.; Kakinoki, T.; Fukugasako, K.; Inoue, D.; Ike, M., Isolation and characterization of bacterial strains that have high ability to degrade 1,4-dioxane as a sole carbon and energy source. *Biodegradation* **2013,** *24*, 665-674.
2. Fernández, H.; Prandoni, N.; Fernández-Pascual, M.; Fajardo, S.; Morcillo, C.; Díaz, E.; Carmona, M., *Azoarcus* sp. CIB, an anaerobic biodegrader of aromatic compounds shows an endophytic lifestyle. *PloS one* **2014,** *9*, e110771.
3. Chang, H. L.; Alvarez-Cohen, L., Transformation capacities of chlorinated organics by mixed cultures enriched on methane, propane, toluene, or phenol. *Biotechnology and bioengineering* **1995,** *45*, 440-449.
4. Prior, S.; Dalton, H., Acetylene as a suicide substrate and active site probe for methane monooxygenase from *Methylococcus capsulatus* (Bath). *FEMS microbiology letters* **1985,** *29*, 105-109.
5. Lane, D., 16S/23S rRNA sequencing. *Nucleic acid techniques in bacterial systematics*. In E. Stackebrandt and M. Goodfellow (ed.), John Wiley & Sons, New York **1991**. p. 115-175.
6. Coleman, N. V.; Bui, N. B.; Holmes, A. J., Soluble di-iron monooxygenase gene diversity in soils, sediments and ethene enrichments. *Environmental Microbiology* **2006,** *8*, 1228-1239.
7. Deng, D.; Li, F.; Li, M., A Novel Propane Monooxygenase Initiating Degradation of 1, 4-Dioxane by *Mycobacterium dioxanotrophicus* PH-06. *Environmental Science & Technology Letters* **2018**, 5, 86-91.
8. Simmonds, A. C.; Reilly, C. A.; Baldwin, R. M.; Ghanayem, B. I.; Lanza, D. L.; Yost, G. S.; Collins, K. S.; Forkert, P.-G., Bioactivation of 1, 1-dichloroethylene to its epoxide by CYP2E1 and CYP2F enzymes. *Drug metabolism and disposition* **2004,** *32*, 1032-1039.
9. Vainberg, S.; McClay, K.; Masuda, H.; Root, D.; Condee, C.; Zylstra, G. J.; Steffan, R. J., Biodegradation of ether pollutants by *Pseudonocardia* sp. strain ENV478. *Applied and Environmental Microbiology* **2006,** *72*, 5218-5224.
10. Grostern, A.; Sales, C. M.; Zhuang, W. Q.; Erbilgin, O.; Alvarez-Cohen, L., Glyoxylate metabolism is a key feature of the metabolic degradation of 1,4-dioxane by *Pseudonocardia dioxanivorans* strain CB1190. *Applied and Environmental Microbiology* **2012,** *78*, 3298-3308.
11. Kim, Y. M.; Jeon, J. R.; Murugesan, K.; Kim, E. J.; Chang, Y. S., Biodegradation of 1,4-dioxane and transformation of related cyclic compounds by a newly isolated *Mycobacterium sp.* PH-06. *Biodegradation* **2009,** *20*, 511-519.
12. Thauer, R. K.; Jungermann, K.; Decker, K., Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological reviews* **1977,** *41*, 100-180.
13. Faoro, H.; Rene Menegazzo, R.; Battistoni, F.; Gyaneshwar, P.; do Amaral, F. P.; Taulé, C.; Rausch, S.; Gonçalves Galvão, P.; de los Santos, C.; Mitra, S., The oil-contaminated soil diazotroph *Azoarcus olearius* DQS-4T is genetically and phenotypically similar to the model grass endophyte *Azoarcus* sp. BH72. *Environmental microbiology reports* **2017,** *9*, 223-238.
14. Krause, A.; Ramakumar, A.; Bartels, D.; Battistoni, F.; Bekel, T.; Boch, J.; Böhm, M.; Friedrich, F.; Hurek, T.; Krause, L., Complete genome of the mutualistic, N2-fixing grass endophyte *Azoarcus* sp. strain BH72. *Nature biotechnology* **2006,** *24*, 1384-1390.

15. Fries, M. R.; Zhou, J.; Chee-Sanford, J.; Tiedje, J. M., Isolation, characterization, and distribution of denitrifying toluene degraders from a variety of habitats. *Applied and Environmental Microbiology* **1994,** *60*, 2802-2810.
16. Kasai, Y.; Kodama, Y.; Takahata, Y.; Hoaki, T.; Watanabe, K., Degradative capacities and bioaugmentation potential of an anaerobic benzene-degrading bacterium strain DN11. *Environmental Science & Technology* **2007,** *41*, 6222-6227.