Supplementary Information

Nitrous Oxide Is a Potent Inhibitor of Bacterial Reductive Dechlorination

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This supplementary file contains: 6 tables and 3 figures on 7 pages.

Table S1. Inhibition models used in whole cell suspension assays.

Michaelis-Menten Equation: $v_0 = \frac{V_{\max}[S]}{K_m+[S]}$ (1)	Noncompetitive inhibition: $v_0 = \frac{V_{\max}[S]}{\alpha(K_m+[S])}$ (3)
Competitive inhibition: $v_0 = \frac{V_{\max}[S]}{(\alpha K_m+[S])}$ (2)	Uncompetitive inhibition: $v_0 = \frac{V_{\max}[S]}{(K_m+\alpha[S])}$ (4)
For simplification, the inhibitor concentrations and inhibition constants in equations (2), (3) and (4) are expressed as α, whereby $\alpha = 1 + \frac{[I]}{K_i}$	

Table S2. Initial PCE-to-*c*DCE dechlorination rates versus PCE concentrations in *Geo* strain SZ cell suspension assays in the presence of 0, 10 and 60 μM N_2O.

PCE (μM)	***V*** [a]	**PCE (μM)**	***V*** [a]	**PCE (μM)**	***V*** [a]
No N_2O		10 μM N_2O		60 μM N_2O	
3.6	4.1	10.9	19.3	4.9	5.3
6.3	11.7	13.1	24.7	9.5	8.5
11.7	24.0	18.5	25.3	15.3	13.6
15.7	28.5	29.0	28.5	52.7	20.0
42.2	48.4	41.3	36.8	59.4	22.1
60.8	60.3	57.0	41.2	69.5	24.3
83.6	63.9	63.6	44.7	87.5	24.5
97.8	65.0	74.1	45.0	110.4	25.5
110.2	61.3	91.6	51.9	124.3	25.4
122.0	58.4	109.7	47.6	146.5	25.2
133.9	68.0				
153.9	56.7				

[a] Initial dechlorination rate (nmol Cl^- released min^{-1} mg $protein^{-1}$).

Table S3. Initial *c*DCE-to-VC dechlorination rate versus *c*DCE concentrations in *Dhc* strain BAV1 cell suspension assays in the presence of 0, 10 and 60 μM N_2O.

***c*DCE (μM)**	***V*** [a]	***c*DCE (μM)**	***V*** [a]	***c*DCE (μM)**	***V*** [a]
No N_2O		10 μM N_2O		60 μM N_2O	
2.7	15.4	1.9	10.3	1.7	2.4
4.4	27.6	5.0	16.9	5.4	4.9
7.5	48.6	8.4	23.6	10.1	7.2
17.5	53.2	17.5	36.4	17.5	14.2
31.7	79.5	31.7	38.5	29.5	18.5
64.5	93.1	63.4	54.6	63.4	24.7
174.9	110.3	161.8	75.4	208.8	39.6
250.3	106.5	211.0	80.5	225.2	42.6
377.1	111.4	488.6	86.4	424.6	42.1

[a] Initial dechlorination rate (nmol Cl^- released min^{-1} mg $protein^{-1}$).

Table S4. Initial VC-to-ethene dechlorination rate versus VC concentrations in *Dhc* strain BAV1 cell suspension assays in the presence of 0, 15 and 50 μM N_2O.

VC (μM)	***V*** [a]	**VC (μM)**	***V*** [a]	**VC (μM)**	***V*** [a]
No N_2O		15 μM N_2O		50 μM N_2O	
4.4	18.5	3.2	7.9	4.1	3.0
13.9	53.3	11.4	19.2	11.3	7.0
23.5	72.4	22.0	25.2	21.7	10.9
56.3	89.9	51.4	34.4	47.3	14.2
98.5	104.3	87.9	39.2	72.6	16.7
116.0	107.6	118.0	42.6	113.3	17.1
149.0	107.6	126.0	43.5	140.8	20.9

[a] Initial dechlorination rate (nmol Cl^- released min^{-1} mg $protein^{-1}$).

Table S5. Statistical parameters (R^2, AICc and Sy.x values) used for determining the best-fit inhibition model and inhibition constants in cell suspensions amended with N_2O as inhibitor.

Culture	Substrate	Inhibitor	Tested models	Statistical Parameters			K_I (μM)
				R^2	AICc	Sy.x	
			Noncompetitive	**0.971**	**81.338**	**3.350**	**40.8 ± 3.8**
Geo strain SZ	PCE	N_2O	Uncompetitive	0.966	86.477	3.639	29.1 ± 3.1
			Competitive	0.944	102.041	4.678	9.2 ± 1.9
			Noncompetitive	**0.968**	**107.060**	**6.422**	**21.2 ± 3.5**
Dhc strain BAV1	*c*DCE	N_2O	Competitive	0.952	117.928	7.853	2.3 ± 0.5
			Uncompetitive	0.938	124.696	8.902	25.9 ± 2.9
			Noncompetitive	**0.996**	**43.791**	**2.386**	**9.6 ± 0.4**
Dhc strain BAV1	VC	N_2O	Uncompetitive	0.986	69.426	4.393	7.0 ± 0.6
			Competitive	0.974	82.243	5.961	1.6 ± 0.3

R^2, the Coefficient of Determination, gives information about the fit of the measured data to the different models tested, and the model with highest R^2 value provides the best data fit.

The AICc (i.e., corrected Akaike's Information Criterion) offers an estimate of the relative quality of tested models, and the model with the lowest AICc value represents the relative best fit among the tested models.

The Sy.x represents the Standard Deviation of the Residuals, and the model with the lowest Sy.x value provides best prediction of the data.

In all cell suspensions assays, the noncompetitive model (highlighted in bold) gave the highest R^2 and the lowest AIC and Sy.x values.

Table S6. Initial versus final amounts of N_2O measured of *Geo* strain SZ and *Dhc* strain BAV1 cultures growing with chlorinated ethenes as electron acceptors in 160-mL vessels with 60 mL headspace.

Culture	e^- Acceptor	Inhibitor N_2O (μM)	Inhibitor N_2O (total μmol/vessel)	
			Initial	Final
Geo strain SZ	PCE	9.5	2.1 ± 0.1	2.1 ± 0.3
		19.1	4.1 ± 0.1	3.9 ± 0.5
		57.3	12.4 ± 1.0	12.6 ± 1.4
Dhc strain BAV1	*c*DCE	9.5	2.1 ± 0.2	2.1 ± 0.3
		29.0	6.3 ± 0.5	6.8 ± 0.8
		57.3	12.4 ± 0.5	12.2 ± 0.4
Dhc strain BAV1	VC	2.9	0.6 ± 0.1	0.6 ± 0.1
		5.7	1.2 ± 0.1	1.2 ± 0.2
		19.1	4.1 ± 0.6	4.1 ± 0.4

Error values represent the standard deviation based on measurements of triplicate cultures.

Supplementary Figures

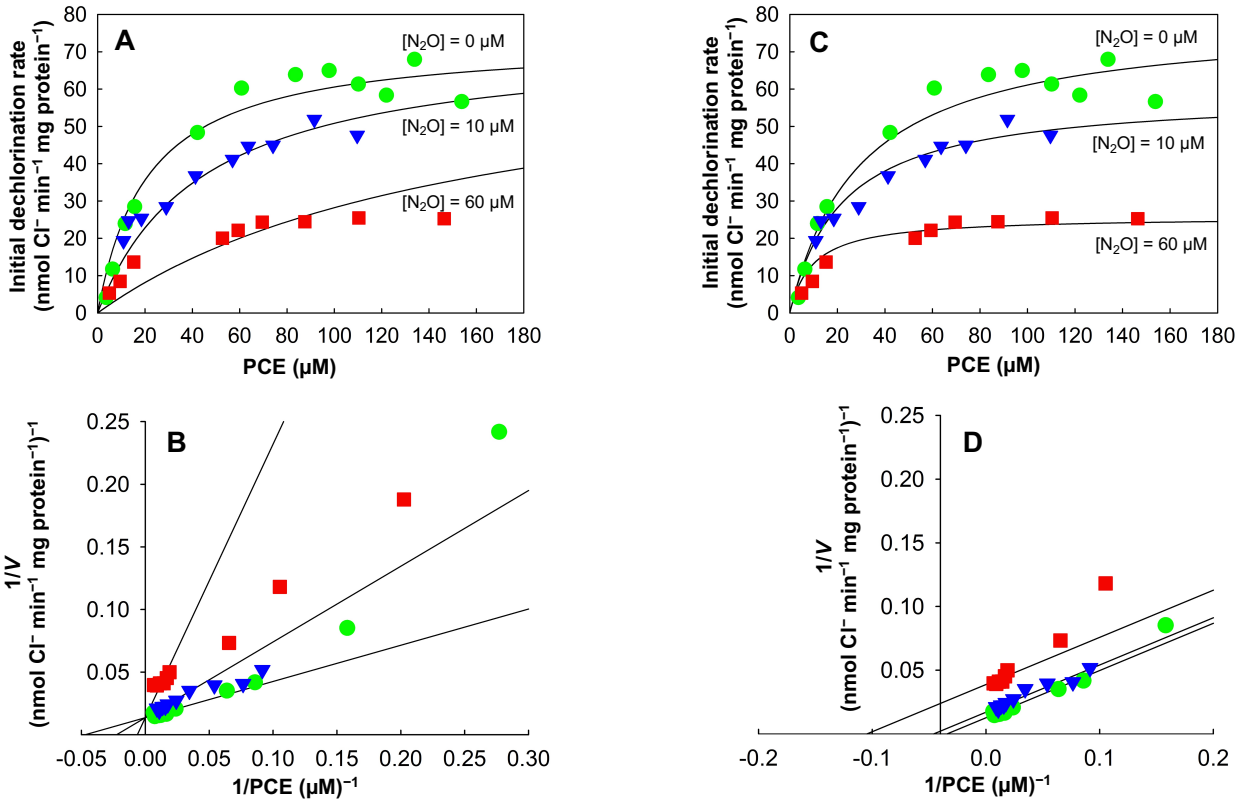

Figure S1. Competitive and uncompetitive N_2O inhibition kinetics of PCE-to-*c*DCE reductive dechlorination in *Geo* strain SZ cell suspensions. **Panels A and B** show Michaelis-Menten and Lineweaver-Burk plots, respectively, of competitive N_2O inhibition of PCE-to-*c*DCE reductive dechlorination in *Geo* strain SZ cell suspensions without and in the presence of 10 and 60 μM N_2O. **Panels C and D** depict Michaelis-Menten and Lineweaver-Burk plots, respectively, of uncompetitive N_2O inhibition of PCE-to-*c*DCE reductive dechlorination in *Geo* strain SZ cell suspensions without and in the presence of 10 and 60 μM N_2O. Solid lines represent the model simulation to each data set based on the nonlinear regression using the SigmaPlot 13 Enzyme Kinetic Module. Solid green circles represent rate data measured in the absence of N_2O; solid blue triangles show rate data measured in the presence of 10 μM N_2O; and solid red squares show rate data measured in the presence of 60 μM N_2O.

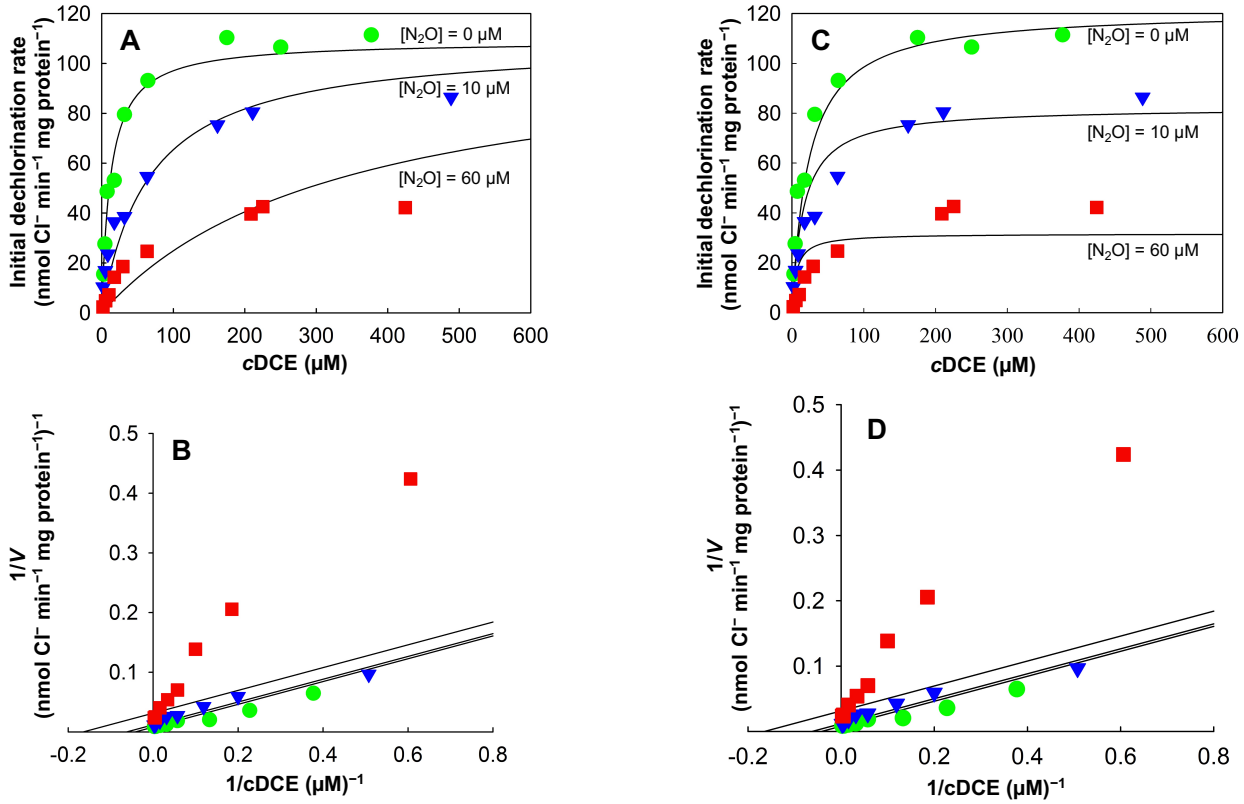

Figure S2. Competitive and uncompetitive N_2O inhibition kinetics of *c*DCE-to-VC reductive dechlorination in *Dhc* strain BAV1 cell suspensions. **Panels A and B** show Michaelis-Menten and Lineweaver-Burk plots, respectively, of competitive N_2O inhibition of *c*DCE-to-VC reductive dechlorination in *Dhc* strain BAV1cell suspensions without and in the presence of 10 and 60 μM N_2O. **Panels C and D** depict Michaelis-Menten and Lineweaver-Burk plots, respectively, of uncompetitive N_2O inhibition of VC-to ethene reductive dechlorination in *Dhc* strain BAV1 cell suspensions without and in the presence of 10 and 60 μM N_2O. Solid lines represent the model simulation to each data set based on the nonlinear regression using the SigmaPlot 13 Enzyme Kinetic Module. Solid green circles represent rate data measured in the absence of N_2O; solid blue triangles show rate data measured in the presence of 10 μM N_2O; and solid red squares show rate data measured in the presence of 60 μM N_2O.

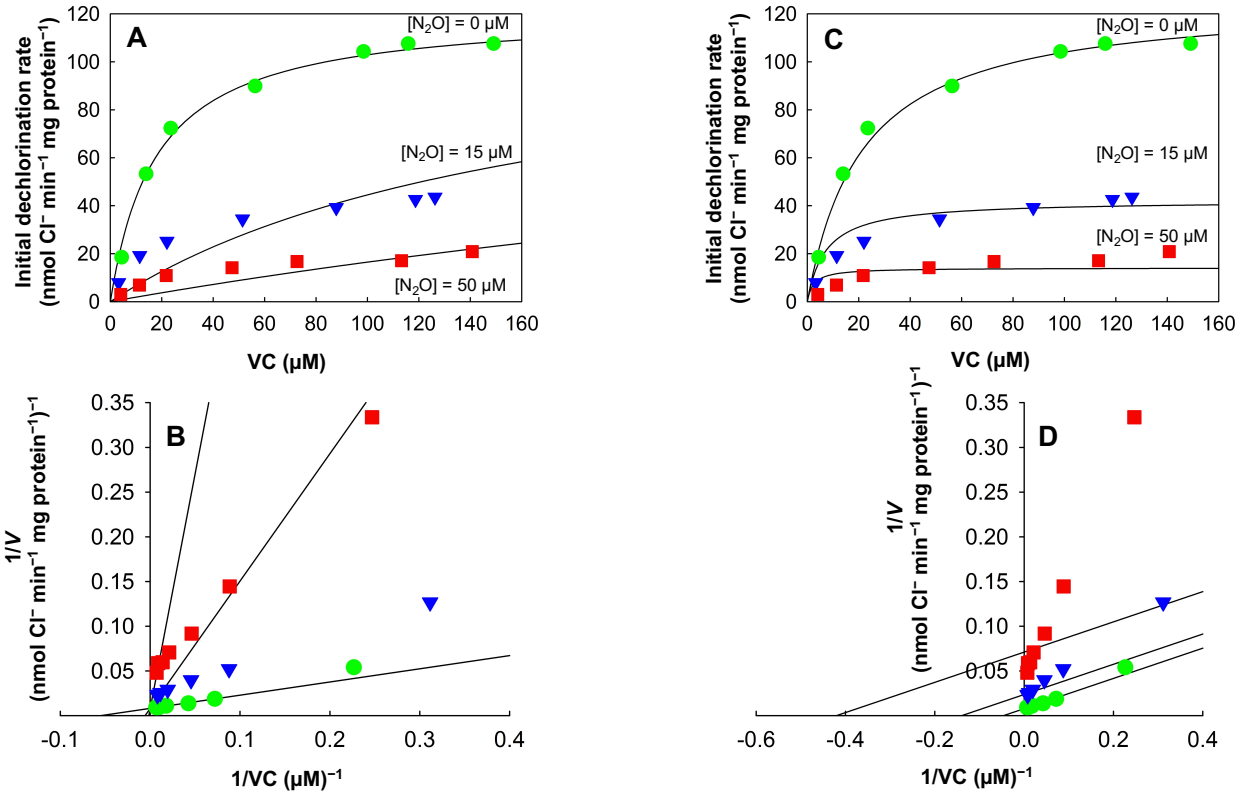

Figure S3. Competitive and uncompetitive N_2O inhibition kinetics of VC-to-ethene reductive dechlorination in *Dhc* strain BAV1cell suspensions. **Panels A and B** show Michaelis-Menten and Lineweaver-Burk plots, respectively, of competitive N_2O inhibition of VC-to-ethene reductive dechlorination in cell suspensions of *Dhc* strain BAV1 without and in the presence of 15 and 50 µM N_2O. **Panels C and D** depict Michaelis-Menten and Lineweaver-Burk plots, respectively, of uncompetitive N_2O inhibition of VC-to-ethene reductive dechlorination in *Dhc* strain BAV1 cell suspensions without and in the presence of 15 and 50 µM N_2O. Solid lines represent the model simulation to each data set based on the nonlinear regression using the SigmaPlot 13 Enzyme Kinetic Module. Solid green circles represent rate data measured in the absence of N_2O; solid blue triangles show rate data measured in the presence of 15 µM N_2O; and solid red squares show rate data measured in the presence of 50 µM N_2O.