

Supplemental Material

**Efficient metabolic exchange and electron transfer within a syntrophic TCE degrading  
co-culture of *Dehalococcoides mccartyi* 195 and *Syntrophomonas. wolfei***

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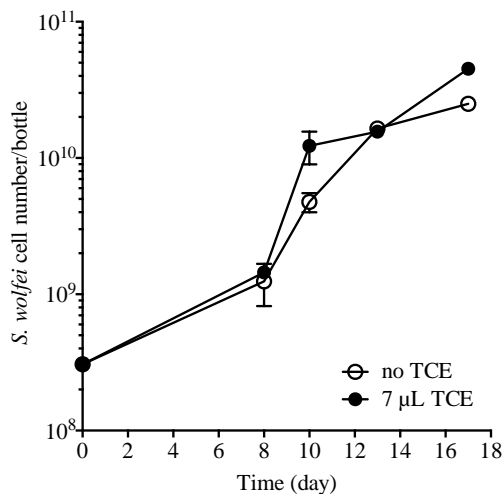
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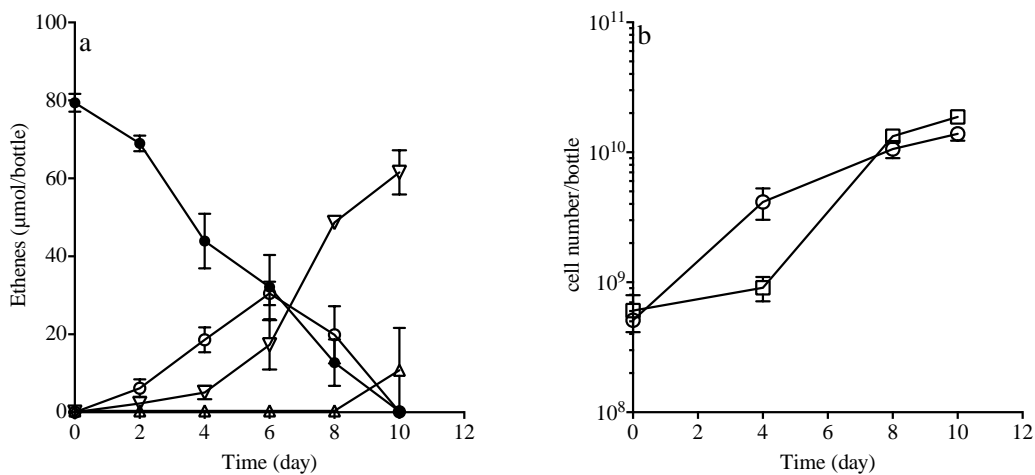
## Supplemental material. Part 1.

Effect of TCE on *S. wolfei* growth.



**Fig. S1.** Increase in the cell numbers of *S. wolfei* growing in pure culture with 10 mM crotonate with or without TCE amendment.

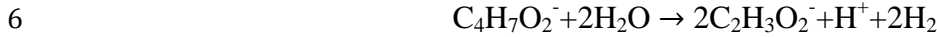
Time course of TCE degradation and cell growth in strain 195 and *S. wolfei* co-culture growing on crotonate as the sole electron donor. The experiment was carried out after 80 transfers (5% vol/vol inoculation, around 1,600 generations) of the original set-up co-culture.



**Fig. S2.** a) Time course of TCE removal and production of TCE-reduced metabolites in strain 195 and *S. wolfei* co-culture growing on 5 mM crotonate (● TCE, ○ *cis*-DCE, ▽ VC, ▲ ETH), and b) Cell growth of ○ *S. wolfei* and □ strain 195 growing on 5 mM crotonate. The cell numbers were normalized to 16S rRNA gene copy numbers. The symbols indicate the averages based on biological triplicate determinations. The error bars indicate standard deviation.

1 **Supplemental material. Part 2.**

2 Calculation of (i) standard Gibbs free energy and entropy changes of acetogenic butyrate  
3 fermentation catalyzed by *S. wolfei* and (ii) Gibbs free energy of acetogenic fermentative  
4 degradation of butyrate in the course of TCE dechlorination by the strain 195 and *S. wolfei* co-  
5 culture.



$$\Delta G = \Delta G^\circ + RT \ln K = \Delta G^\circ + RT \ln \frac{[\text{Acetate}]^2 [\text{H}^+] p_{\text{H}_2}^2}{[\text{Butyrate}]} = \Delta G_{\text{pH}}^\circ + RT \ln \frac{[\text{Acetate}]^2 p_{\text{H}_2}^2}{[\text{Butyrate}]}$$

7 
$$\Delta G_{\text{pH}, 298.15\text{K}}^\circ = \Delta G^\circ + nRT \ln [\text{H}^+] = \Delta G^\circ - 2.3026nRT \times \text{pH} = \Delta G^\circ - 5.708n \times \text{pH}$$

8 
$$R = 0.00831451 \text{ kJ mol}^{-1} \text{ K}^{-1} = 0.083451 \text{ L bar mol}^{-1} \text{ K}^{-1}.$$

9  $n$  = number of protons.

10  $\Delta G^\circ$  = Standard Gibbs free energy change of reaction when all reactants and products are present  
11 at unit activity at a specified standard state (i.e., 298.15 K, 100 kPa = 1bar).

12  $p_{\text{H}_2}$  = Hydrogen partial pressure (bar);  $[\text{H}_2(\text{aq})] = \frac{p_{\text{H}_2(\text{g})}}{k_{\text{H}}}$ .

13  $k_{\text{H}}(\text{H}_2, 298.15\text{K}) = 1.299038 \times 10^3 \text{ bar L mol}^{-1}$  ( $1.236747 \times 10^3 \text{ bar L mol}^{-1}$  at 307.15 K) (1).

14 The pH during syntrophic butyrate fermentation and TCE dechlorination was maintained at 7.3  
15 by a dual buffer bacterial growth medium ( $\text{NaHCO}_3^-$  and TES) (i.e.,  $[\text{H}^+] = 5 \times 10^{-8} \text{ mol L}^{-1}$ ).

16 The standard Gibbs free energy change of reaction ( $\Delta G_r^\circ$ ) for acetogenic butyrate fermentation  
17  $\text{C}_4\text{H}_7\text{O}_2^- + 2\text{H}_2\text{O} \rightarrow 2\text{C}_2\text{H}_3\text{O}_2^- + \text{H}^+ + 2\text{H}_2$  is calculated using the Hess's law and the standard molar  
18 Gibbs energy of formation (Table S1).

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26 **Table S1.** Standard molar Gibbs energy of formation ( $\Delta_f G_i^\circ$ ), standard molar enthalpy of  
 27 formation ( $\Delta_f H_i^\circ$ ) and standard molar entropy ( $S_i^\circ$ ) values (at 298.15 K, unit activity) used for the  
 28 calculation of  $\Delta G_r^\circ$ ,  $\Delta H_r^\circ$  and  $\Delta S_r^\circ$  of butyrate fermentation reaction.

Reactant/product	$\Delta_f G_i^\circ$ (kJ mol <sup>-1</sup> )	$\Delta_f H_i^\circ$ (kJ mol <sup>-1</sup> )	$S_i^\circ$ (kJ mol <sup>-1</sup> K <sup>-1</sup> ) <sup>(a)</sup>
Butyric acid (ionized form), pK <sub>a</sub> = 4.821	-352.6 <sup>(b)</sup>	-536 <sup>(c)</sup>	0.1358 <sup>(d)</sup>
Acetic acid (ionized form), pK <sub>a</sub> = 4.757	-369.4 <sup>(b)</sup>	-486.0	0.0866
H <sup>+</sup> (pH = 0)	0	0	0
H <sub>2</sub> O	-237.17	-285.8	0.070
H <sub>2</sub> (g)	0	0	0.1307
H <sub>2</sub> (aq)	17.8 <sup>(e)</sup>	-4.16 <sup>(f)</sup>	0.0577 <sup>(f)</sup>

29 (a) Data obtained from reference (2) and (3); (b) Thermodynamic values for ionized forms of  
 30 butyric acid and acetic acid from reference (4); (c) Calculated from reference (5); (d) reference  
 31 (6); (e) Calculated from  $\Delta_f G_{i(aq)}^\circ = \Delta_f G_{i(g)}^\circ + RT \ln k_{H,i}$ , as described in reference (7); (f) values  
 32 are from reference (4).

$$33 \Delta G_r^\circ = (2 \times \Delta_f G_{\text{CH}_3\text{COO}^- (aq)}^\circ) + (\Delta_f G_{\text{H}^+ (aq)}^\circ) + (2 \times \Delta_f G_{\text{H}_2 (g)}^\circ) - [(2 \times \Delta_f G_{\text{H}_2\text{O} (aq)}^\circ) +$$

$$34 (\Delta_f G_{\text{C}_4\text{H}_7\text{O}_2^- (aq)}^\circ)]$$

$$35 = [2 \times (-369.4 \text{ kJ mol}^{-1}) + (0 \text{ kJ mol}^{-1}) + 2 \times (0 \text{ kJ mol}^{-1})] - [2 \times (-237.17 \text{ kJ mol}^{-1}) + (-352.63 \text{ kJ mol}^{-1})]$$

$$36 = 88.17 \text{ kJ mol}^{-1}$$

37 For the calculation of the standard Gibbs free energy of reaction using the standard Gibbs free  
 38 energy of formation of hydrogen in the aqueous phase (i.e., all reactants and products are in  
 39 dissolved or liquid state,  $\Delta G_{r,(aq)}^\circ$ ),

$$40 \Delta G_{r,(aq)}^\circ = [2 \times (-369.4 \text{ kJ mol}^{-1}) + (0 \text{ kJ mol}^{-1}) + 2 \times (17.8 \text{ kJ mol}^{-1})] - [2 \times (-237.17 \text{ kJ mol}^{-1}) + (-352.63 \text{ kJ}$$

$$41 \text{ mol}^{-1})] = 123.7 \text{ kJ mol}^{-1}$$

42 Because the experiments are carried out at 307.15 K, the standard Gibbs free energy change of  
 43 reaction is corrected for the desired incubation temperature using the Gibbs-Helmholtz equation.

$$\left( \frac{\partial(\Delta G/T)}{\partial T} \right)_p = - \frac{\Delta H}{T^2}$$

$$44 \Delta H_r^\circ = [2 \times (-486 \text{ kJ mol}^{-1}) + (0 \text{ kJ mol}^{-1}) + 2 \times (0 \text{ kJ mol}^{-1})] - [2 \times (-285.8 \text{ kJ mol}^{-1}) + (-536 \text{ kJ mol}^{-1})]$$

45  $=135.6 \text{ kJ mol}^{-1}$

46  $\Delta H_{r(\text{aq})}^{\circ}=[2 \times (-486 \text{ kJ mol}^{-1})+(0 \text{ kJ mol}^{-1})+2 \times (-4.16 \text{ kJ mol}^{-1})]-[2 \times (-285.8 \text{ kJ mol}^{-1})+(-536 \text{ kJ mol}^{-1})]=127.28 \text{ kJ mol}^{-1}$

48  $\Delta S_r^{\circ}=[2 \times (0.0867 \text{ kJ mol}^{-1} \text{ K}^{-1})+(0 \text{ kJ mol}^{-1} \text{ K}^{-1})+2 \times (0.1307 \text{ kJ mol}^{-1} \text{ K}^{-1})]-[2 \times (0.07 \text{ kJ mol}^{-1} \text{ K}^{-1})+(0.1358 \text{ kJ mol}^{-1} \text{ K}^{-1})]=0.159 \text{ kJ mol}^{-1} \text{ K}^{-1}$

50  $\Delta S_{r(\text{aq})}^{\circ}=[2 \times (0.0867 \text{ kJ mol}^{-1} \text{ K}^{-1})+(0 \text{ kJ mol}^{-1} \text{ K}^{-1})+2 \times (0.0577 \text{ kJ mol}^{-1} \text{ K}^{-1})]-[2 \times (0.07 \text{ kJ mol}^{-1} \text{ K}^{-1})+(0.1358 \text{ kJ mol}^{-1} \text{ K}^{-1})]=0.013 \text{ kJ mol}^{-1} \text{ K}^{-1}$

52 Using the Gibbs-Helmholtz equation,

53  $\Delta G_{307.15}^{\circ} = 307.15 \left( \frac{\Delta G_{298.15}^{\circ}}{298.15} \right) + \left( \frac{\Delta H^{\circ} (298.15-307.15)}{298.15} \right) = 86.74 \text{ kJ mol}^{-1}$

54 Using another form of the Gibbs-Helmholtz equation,

$$\Delta G_{307.15}^{\circ} = \Delta G_{298.15}^{\circ} - \Delta S^{\circ} (307.15 - 298.15)$$

55  $=88.17 \text{ kJ mol}^{-1} - [0.159 \text{ kJ mol}^{-1} \text{ K}^{-1} \times 9 \text{ K}] = 86.74 \text{ kJ mol}^{-1}$

56 When all reactants and products are in dissolved or liquid state,

57  $\Delta G_{307.15(\text{aq})}^{\circ} = 307.15 \left( \frac{\Delta G_{298.15(\text{aq})}^{\circ}}{298.15} \right) + \left( \frac{\Delta H_{(\text{aq})}^{\circ} (298.15-307.15)}{298.15} \right) = 123.59 \text{ kJ mol}^{-1}$

58 A minimum of free energy about  $-20 \text{ kJ mol}^{-1}$  is required by a bacterium to exploit the free  
59 energy change in a reaction and support growth (8). Therefore, each data point of Gibbs free  
60 energy change of reaction was calculated based on the measurement of each compound  
61 concentration at specific time (9). Table S2 presents an example of calculation of Gibbs free  
62 energy available for *S. wolfei* during syntrophic growth with strain 195 in the presence of  
63 butyrate as the sole electron donor.

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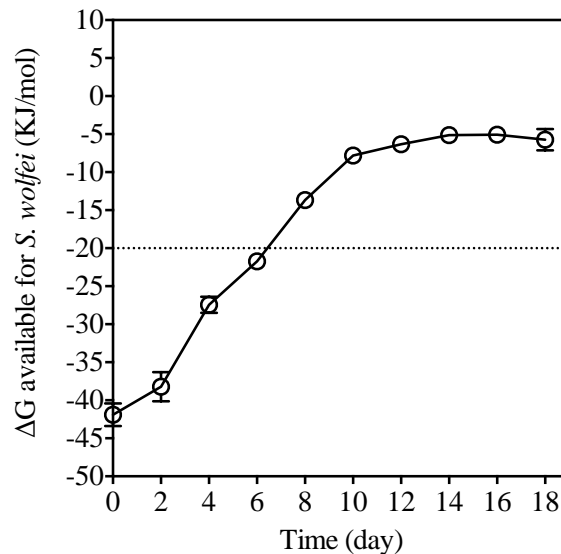
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**Table S2.** Sample calculation for free Gibbs energy available for *S. wolfei*<sup>(a)</sup>

Time (day)	H <sub>2</sub> partial pressure ( $\times 10^{-5}$ bar)	H <sub>2(aq)</sub> (nM) <sup>(b)</sup>	Acetate concentration (M)	H <sup>+</sup> (M)	Butyrate Concentration (M)	$\Delta G^{\circ}_{307.15K}$ (kJ mol <sup>-1</sup> )	Final delta G (kJ mol <sup>-1</sup> )
0	2.7	21.8	$9.0 \times 10^{-5}$	$5 \times 10^{-8}$	$4.3 \times 10^{-3}$	86.7	-43.6 <sup>(c)</sup>
2	5.9	47.5	$1.5 \times 10^{-4}$	$5 \times 10^{-8}$	$4.6 \times 10^{-3}$	86.7	-37.1
4	15.3	123.8	$3.1 \times 10^{-4}$	$5 \times 10^{-8}$	$4.5 \times 10^{-3}$	86.7	-28.5
6	21.9	177.0	$7.5 \times 10^{-4}$	$5 \times 10^{-8}$	$4.2 \times 10^{-3}$	86.7	-22.0
8	42.9	347.0	$1.7 \times 10^{-3}$	$5 \times 10^{-8}$	$3.7 \times 10^{-3}$	86.7	-13.9
10	126.6	1023.7	$2.0 \times 10^{-3}$	$5 \times 10^{-8}$	$3.4 \times 10^{-3}$	86.7	-7.5
12	118.2	955.5	$2.3 \times 10^{-3}$	$5 \times 10^{-8}$	$3.5 \times 10^{-3}$	86.7	-7.2
14	152.4	1232.4	$2.54 \times 10^{-3}$	$5 \times 10^{-8}$	$3.5 \times 10^{-3}$	86.7	-5.4
16	160.8	1300.1	$2.61 \times 10^{-3}$	$5 \times 10^{-8}$	$3.5 \times 10^{-3}$	86.7	-5.0
18	192.4	1555.5	$2.32 \times 10^{-3}$	$5 \times 10^{-8}$	$3.2 \times 10^{-3}$	86.7	-4.5

72 (a) The calculation summarized in the table is for one biological replicate in the feeding cycle, (b)

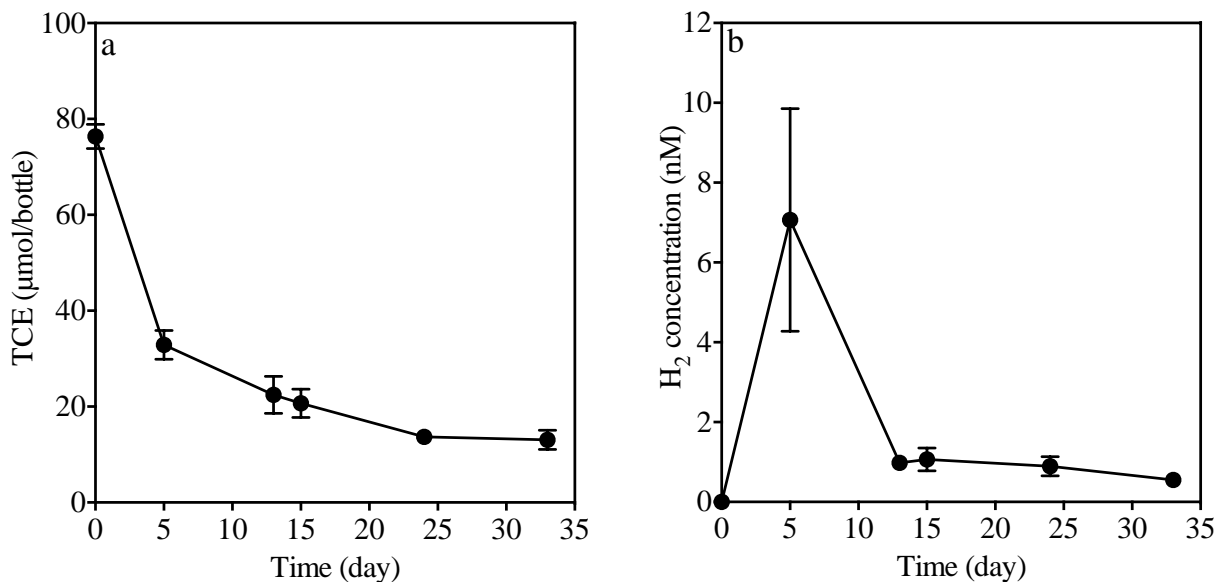
73 Henry's law constant of hydrogen gas at 307.15 K =  $1.236747 \times 10^3$  bar L mol<sup>-1</sup>, (c) If we use74 aqueous concentration of H<sub>2</sub>,  $\Delta G = \Delta G^{\circ}_{(aq)} + RT \ln \frac{[\text{Acetate}]^2 [\text{H}^+] [\text{H}_2]^2}{[\text{Butyrate}]} = 123.59 \text{ kJ mol}^{-1} +$ 75  $(0.00831451 \text{ kJ mol}^{-1} \text{ K}^{-1} \times 307.15 \text{ K}) \ln \frac{((9 \times 10^{-5} \text{ M})^2 (5 \times 10^{-8} \text{ M}) (21.8 \times 10^{-9} \text{ M})^2)}{(4.3 \times 10^{-3} \text{ M})} = -43.1 \text{ kJ mol}^{-1}$ 

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77 **Fig. S3.** Gibbs free energy available for *S. wolfei* during syntrophic fermentation of butyrate with  
78 strain 195.

79 **Supplemental material. Part 3.**

80 The method used to determine H<sub>2</sub> threshold in the co-culture was based on the method described  
81 by Löffler (10). Briefly, triplicate 100-mL co-cultures were inoculated (2%, vol/vol) from active  
82 dechlorinating cultures that had completely reduced all of the TCE present to ethene. One set of  
83 the triplicate cultures were amended with 7 μL neat TCE (~ 78 μmol), and 25 μL 1M butyrate  
84 stock solution (0.25 mM butyrate) while the other set did not receive an electron acceptor. The  
85 concentrations of chlorinated compounds were determined weekly, and the H<sub>2</sub> concentration was  
86 measured accordingly. Values for H<sub>2</sub> threshold were assessed when the H<sub>2</sub> concentration  
87 remained stable.



88  
89 **Fig. S4.** a) Time course of TCE removal and b) Aqueous H<sub>2</sub> concentration in the bottle while co-  
90 culture strain 195 and *S. wolfei* was fed with 0.25 mM butyrate and 78 μmol TCE. 5μL butyrate  
91 (0.05 mM) was re-spiked to the bottle (on day 15) when TCE removal significantly decreased  
92 (no peak of H<sub>2</sub> was observed because of the long delay of sampling). The measured values  
93 correspond to the averages based on biological triplicate determinations. The error bars indicate  
94 standard deviation.

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100 **Supplemental material. Part 4.**

101 5.1 Calculation of allowed interspecies distance for butyrate fermentation in strain 195 and *S.*  
102 *wolfei* co-culture by using Fick's diffusion law.

103 
$$J_{H_2} = D_{H_2} \times \frac{\Delta C_{H_2}}{d_{sw-195}}$$

104

105 
$$d_{sw-195} = D_{H_2} \times \frac{\Delta C_{H_2}}{J_{H_2}}$$

106

107 1.  $J_{H_2}$  = H<sub>2</sub> flux (pmol μm<sup>-2</sup> cell d<sup>-1</sup>) across the total surface area ( $A_{S,tot}$ ) of H<sub>2</sub>-producing *S.*  
108 *wolfei*.

109 The hydrogen flux  $J_{H_2}$  in the co-culture experiment was calculated on the basis of the oxidation  
110 rate of butyrate by *S. wolfei* at a specific interval time and the hydrogen consumption rate of  
111 strain 195.

112 2.  $A_{S,tot}$ : total surface area over which hydrogen diffuses (total surface area of H<sub>2</sub>-producing  
113 *S. wolfei*) (μm<sup>2</sup>).

114

115 Surface area of *S. wolfei*: assume diameter= 0.25μm, length= 2.5 μm

116 
$$A_S = \pi dl + \frac{1}{2} \pi d^2 = 2.1 \mu m^2 \text{ cell}^{-1}$$

117

$$A_{S,tot} = A_S \times \text{cell number}$$

118 3.  $D_{H_2}$  = molecular diffusion coefficient in water for hydrogen at 35 °C,  $6.31 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} =$   
119  $6.31 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$  (2).

120

121 4.  $\Delta C_{H_2}$  is the maximum difference of hydrogen concentration at the outside cell surface  
122 between the H<sub>2</sub>-producing *S. wolfei* and H<sub>2</sub>-consuming strain 195, taking into account the  
123 highest H<sub>2</sub> level at which *S. wolfei* can ferment butyrate and the lowest H<sub>2</sub> level at which  
124 strain 195 can dechlorinate TCE (H<sub>2</sub> threshold for strain 195).

125  $\Delta C_{H_2} = C_{H_2-sw} - C_{H_2-195} = 3.494 \times 10^{-1} \mu M$

126  $C_{H_2-sw} = 0.35 \pm 0.1 \mu M$

127  $C_{H_2-195} = 0.6 \times 10^{-3} \mu M$

128 Calculated from Fig. 1b-c of the manuscript.

129 \* Incubation period:  $t_{\text{day2-day4}} = 2$  days



130 \* H<sub>2</sub> produced in the defined time interval by *S. wolfei* (day4-day2) was  $3.4 \times 10^7$  pmol. The  
 131 number was calculated from theoretical hydrogen production by butyrate fermentation using  
 132 Equation 2 in Table 1 of the manuscript. Because hydrogen production (from butyrate  
 133 fermentation) is directly linked to generation of energy in *S. wolfei* cells, hydrogen will be  
 134 formed during bacterial growth. The theoretical yield of hydrogen from biomass  $Y_{H_2/X}$  can be  
 135 calculated from the measured amount of hydrogen produced during the incubation time  $\Delta t$  per  
 136 unit of biomass formed.

137 Biomass formation during incubation period (day2 and day4) =  $2.7 \times 10^8$  cells.

138 Cell number of *S.wolfei* on day 2 =  $7.4 \times 10^7$  cells.

139 Cell number of *S.wolfei* on day 4 =  $3.4 \times 10^8$  cells.

140

$$141 \quad Y_{H_2/X} = \frac{3.4 \times 10^7 \text{ pmol}}{2.7 \times 10^8 \text{ cells}} = 0.1259 \text{ pmol cell}^{-1}$$

142

143 \* H<sub>2</sub> consumed in the defined time interval by strain 195 (day4-day2) was  $2.8 \times 10^7$  pmol.  
 144 Hydrogen consumption during the targeted incubation time is mainly due to TCE dechlorination  
 145 activity of strain 195. The number was calculated from the Cl<sup>-</sup> production rate based on direct GC  
 146 measurements using Equations 3-5 in Table 1 of the manuscript. This number is slightly lower  
 147 than the H<sub>2</sub> produced, due to part of the electrons went to biosynthesis.

148 The hydrogen flux is calculated using the following equation:

149

$$J_{H_2} = \frac{\text{H}_2\text{produced (pmol) in the defined time interval } \Delta t \text{ by } S. \text{ wolfei}}{\text{total surface area of growing } S. \text{ wolfei in } \Delta t \times \Delta t}$$

150

$$151 \quad J_{H_2} = \frac{3.4 \times 10^7 \text{ pmol}}{2.7 \times 10^8 \text{ cell} \times 2.1 \times 10^{-12} \frac{\text{m}^2}{\text{cell}} \times 1.728 \times 10^5 \text{ s}} = 3.47 \times 10^5 \text{ pmol m}^{-2} \text{ s}^{-1}$$

152

$$d_{\text{sw-strain 195}} = D_{H_2} \times \frac{C_{H_2-\text{sw}} - C_{H_2-\text{strain 195}}}{J_{H_2}}$$

153

$$154 \quad d_{\text{sw-strain 195}} = \frac{6.31 \times 10^{-9} \frac{\text{m}^2}{\text{s}} \times \frac{3.494 \times 10^5 \text{ pmol}}{10^{-3} \text{ m}^3}}{3.47 \times 10^5 \frac{\text{pmol}}{\text{m}^2 \times \text{s}}} = 6.3 \text{ } \mu\text{m}$$

155 On day 4, *S.wolfei* cell number was  $3.4 \times 10^8$  per bottle and strain195 cell number was  $4.7 \times 10^9$   
 156 per bottle (i.e.  $5.04 \times 10^9$  total cells / bottle containing 100-mL culture medium). In a previous  
 157 study, the calculation showed *S. wolfei* (H<sub>2</sub> producer) could only exert an influence on local H<sub>2</sub>  
 158 concentrations within 10 $\mu$ m of its surface (13).

159 There are two scenarios of cell distribution in the bottle:

160 Scenario 1: Cell aggregation between strain 195 and *S. wolfei*

161 Cell-cell distances in cell aggregates <1 μm. Previous studies calculated the cell-cell distance of  
162 aggregated cells to be 0.08~2 μm in propionate degrading co-cultures (14,15).

163 Scenario 2: Equal distribution of cells growing in planktonic state:

164 Assuming the cells were evenly dispersed in the bottle, the average cell-cell distance will be  
165 27.1 μm.

166 
$$d_{\text{cell-cell}} = \frac{1\text{cm}}{\sqrt[3]{5.04 \times 10^7}} \times \frac{10^4 \mu\text{m}}{1\text{cm}} = 27.1 \mu\text{m}$$

167 This distance is larger than the predicted distance (6.3 μm) that can support interspecies  
168 hydrogen transfer at the measured butyrate oxidation rate (calculated above).

169 Therefore, in order to accomplish syntrophic butyrate oxidation at the rate observed, the average  
170 interspecies distance should be much less than the distance between randomly dispersed cells.

171

172 5.2. Allowed interspecies distance in another syntrophic co-culture *Desulfovibrio vulgaris*  
173 Hildenborough (DvH) with strain195 growing on lactate.

174 A comparison of the allowed interspecies distances is summarized below:

175 **Table S3** Parameters in Fick's equation and allowed interspecies distance calculation.<sup>(a)</sup>

	<i>S. wolfei</i> with strain 195 on butyrate	DvH with strain 195 on lactate
As (μm <sup>2</sup> )	2.1	1.3 <sup>(b)</sup>
ΔCell <sub>syn</sub> (day4-day2) <sup>(c)</sup>	2.7 × 10 <sup>8</sup>	1.1 × 10 <sup>9</sup>
C <sub>H<sub>2</sub>-syn</sub> μM	0.35±0.1	38.9 <sup>(d)</sup>
J <sub>H<sub>2</sub></sub> (pmol m <sup>-2</sup> s <sup>-1</sup> )	3.5 × 10 <sup>5</sup>	3.2 × 10 <sup>5</sup>
d <sub>syn-strain195</sub> (μm)	6.3	755

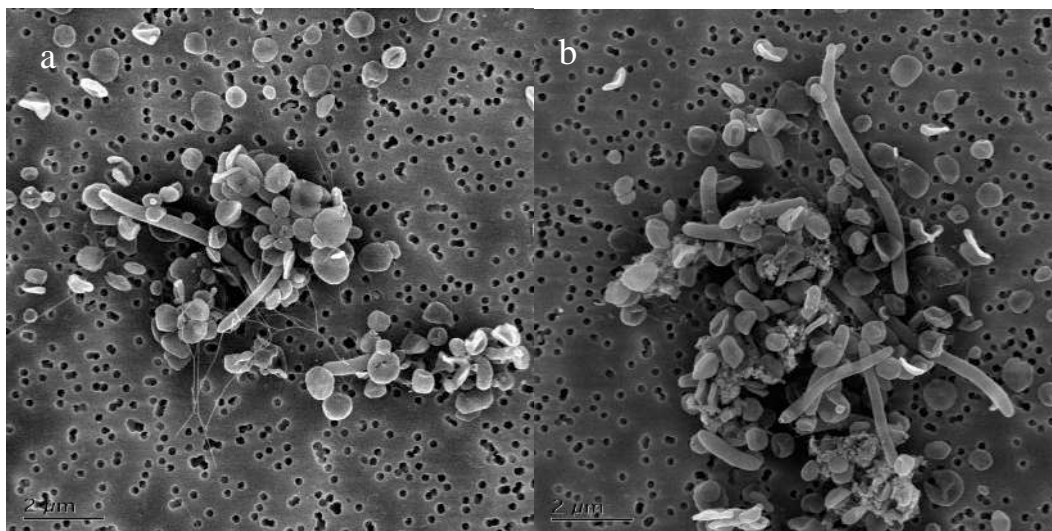
176 (a) The values were calculated in a time interval from day 2 to day 4, at 307.15 K, (b) Surface  
177 area of DvH: assume diameter= 0.25 μm, length= 1.5 μm, (c) Syntroph cell number increase  
178 from day 2 to day4. DvH cell number increase was calculated from unpublished data, (d) The  
179 highest H<sub>2</sub> level at which DvH can ferment lactate was calculated from Figure 3a. reference (16).

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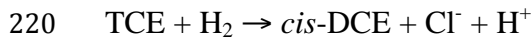


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**Fig. S5.** Cell aggregates observed on a) day 4 and b) day 6 in co-culture *S.wolfeyi*/strain 195 growing on butyrate during exponential growth phase.

217 **Supplemental material. Part 5.**

218 The Gibbs free energy available for strain 195 to dechlorinate TCE to *cis*-DCE has been  
219 calculated at different H<sub>2</sub> concentrations.



221 The standard free energy of reductive dechlorination of TCE to *cis*-DCE is calculated using the  
222 Hess's law and the standard molar Gibbs energy of formation (Table S3).

223 **Table S4.** Standard free energy of formation of reactants and products.

Compound	$\Delta_f G_i^\circ$ (g) (kJ mol <sup>-1</sup> )	$\Delta_f G_i^\circ$ (aq) (kJ mol <sup>-1</sup> )	$\Delta_f H_i^\circ$ (kJ mol <sup>-1</sup> ) <sup>(c)</sup>
TCE	19.9 <sup>(a)</sup>	25.53 <sup>(b)</sup>	-32.2
H <sub>2</sub>	0	17.8	0
<i>cis</i> -DCE	24.3 <sup>(a)</sup>	27.82 <sup>(b)</sup>	-26.9
Cl <sup>-</sup>	N.A.	-131.3	0
H <sup>+</sup>	N.A.	0	0

224 (a) reference (11), (b) Using  $\Delta_f G_{i(\text{aq})}^\circ = \Delta_f G_{i(\text{g})}^\circ + RT \ln k_{i,\text{H}}$ ,  $k_{\text{H}}$  (TCE, 298.15K)= 9.706935 bar  
225 L mol<sup>-1</sup> and  $k_{\text{H}}$  (TCE, 298.15K)=4.134060 bar L mol<sup>-1</sup> (12), (c) reference (2)

226  $\Delta G_r^\circ = -\Delta_f G_{\text{TCE,aq}}^\circ - \Delta_f G_{\text{H}_2}^\circ + \Delta_f G_{\text{cis-DCE,aq}}^\circ + \Delta_f G_{\text{H}^+}^\circ + \Delta_f G_{\text{Cl}^-}^\circ = -128.9 \text{ kJ mol}^{-1}$

227  $\Delta H_r^\circ = [(-26.9 \text{ kJ mol}^{-1}) + (0 \text{ kJ mol}^{-1}) + (0 \text{ kJ mol}^{-1})] - [(-32 \text{ kJ mol}^{-1}) + (0 \text{ kJ mol}^{-1})] = 5.3 \text{ kJ mol}^{-1}$

228 Using the Gibbs-Helmholtz equation,

229  $\Delta G_{307.15}^\circ = 307.15 \left( \frac{\Delta G_{298.15}^\circ}{298.15} \right) + \left( \frac{\Delta H^\circ (298.15 - 307.15)}{298.15} \right) = -132.95 \text{ kJ mol}^{-1}$

230 The Gibbs free energy available for strain 195 to dechlorinate TCE to *cis*-DCE is calculated  
231 using the following equation:

$$\Delta G = \Delta G^\circ + RT \ln K = \Delta G^\circ + RT \ln \frac{[\text{cDCE}] [\text{H}^+] [\text{Cl}^-]}{[\text{TCE}] p_{\text{H}_2}}$$

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**Table S5.** Calculation of Gibbs free energy of reductive dechlorination of TCE to *cis*-DCE in the presence of H<sub>2</sub><sup>(a)</sup>.

H <sub>2</sub> (× 10 <sup>-5</sup> bar)	H <sub>2(aq)</sub> <sup>(b)</sup> (M)	Cl <sup>-</sup> (M)	H <sup>+</sup> (M)	TCE (M)	<i>cis</i> -DCE (M)	ΔG <sub>r,TCE-cDCE</sub> <sup>o</sup> (kJ mol <sup>-1</sup> )	ΔG (kJ mol <sup>-1</sup> )
<b>7.4×10<sup>-2</sup></b>	6.0 × 10 <sup>-10</sup>	0.025	5×10 <sup>-8</sup>	9.6×10 <sup>-5</sup>	4.3×10 <sup>-4</sup>	-133.0	-145.5
<b>7.4×10<sup>-10</sup></b>	6.0 × 10 <sup>-18</sup>	0.025	5×10 <sup>-8</sup>	9.6×10 <sup>-5</sup>	4.3×10 <sup>-4</sup>	-133.0	-98.4
<b>7.4×10<sup>-18</sup></b>	6.0 × 10 <sup>-26</sup>	0.025	5×10 <sup>-8</sup>	9.6×10 <sup>-5</sup>	4.3×10 <sup>-4</sup>	-133.0	-51.4
<b>7.4×10<sup>-24</sup></b>	6.0 × 10 <sup>-32</sup>	0.025	5×10 <sup>-8</sup>	9.6×10 <sup>-5</sup>	4.3×10 <sup>-4</sup>	-133.0	-16.1
<b>7.4×10<sup>-28</sup></b>	6.0 × 10 <sup>-36</sup>	0.025	5×10 <sup>-8</sup>	9.6×10 <sup>-5</sup>	4.3×10 <sup>-4</sup>	-133.0	7.4

241 (a) TCE and *cis*-DCE concentrations were measured on day 33 under electron donor-limited  
242 condition (Fig. S4). Gas-liquid equilibrium was assumed for calculation. H<sub>2</sub> concentration on day  
243 33 was 0.6 nM (Fig. S4). Henry's law constants used for calculation at 307.15 K are 15.309744  
244 bar L mol<sup>-1</sup> (dimensionless value: 0.591) and 7.65337 bar L mol<sup>-1</sup> (dimensionless value: 0.216)  
245 for TCE and *cis*-DCE, respectively (12).

246 (b) Henry's law constant of H<sub>2</sub> at 307.15 K = 1.236747 × 10<sup>3</sup> bar L mol<sup>-1</sup>.

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