

Supporting Online Material for

**Extending the Carbon Chain: Hydrocarbon Formation Catalyzed by**

**Vanadium/Molybdenum Nitrogenases\***

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**This PDF file includes:**

Materials and Methods

Figs. S1 to S4

Table S1

## MATERIALS AND METHODS

Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Natural abundance  $^{12}\text{CO}$  (99.5% purity) was purchased from Airgas (Lakewood, CA). All isotope-labeled compounds ( $\geq 98\%$  isotopic purity) were purchased from Cambridge Isotopes (Andover, MA).

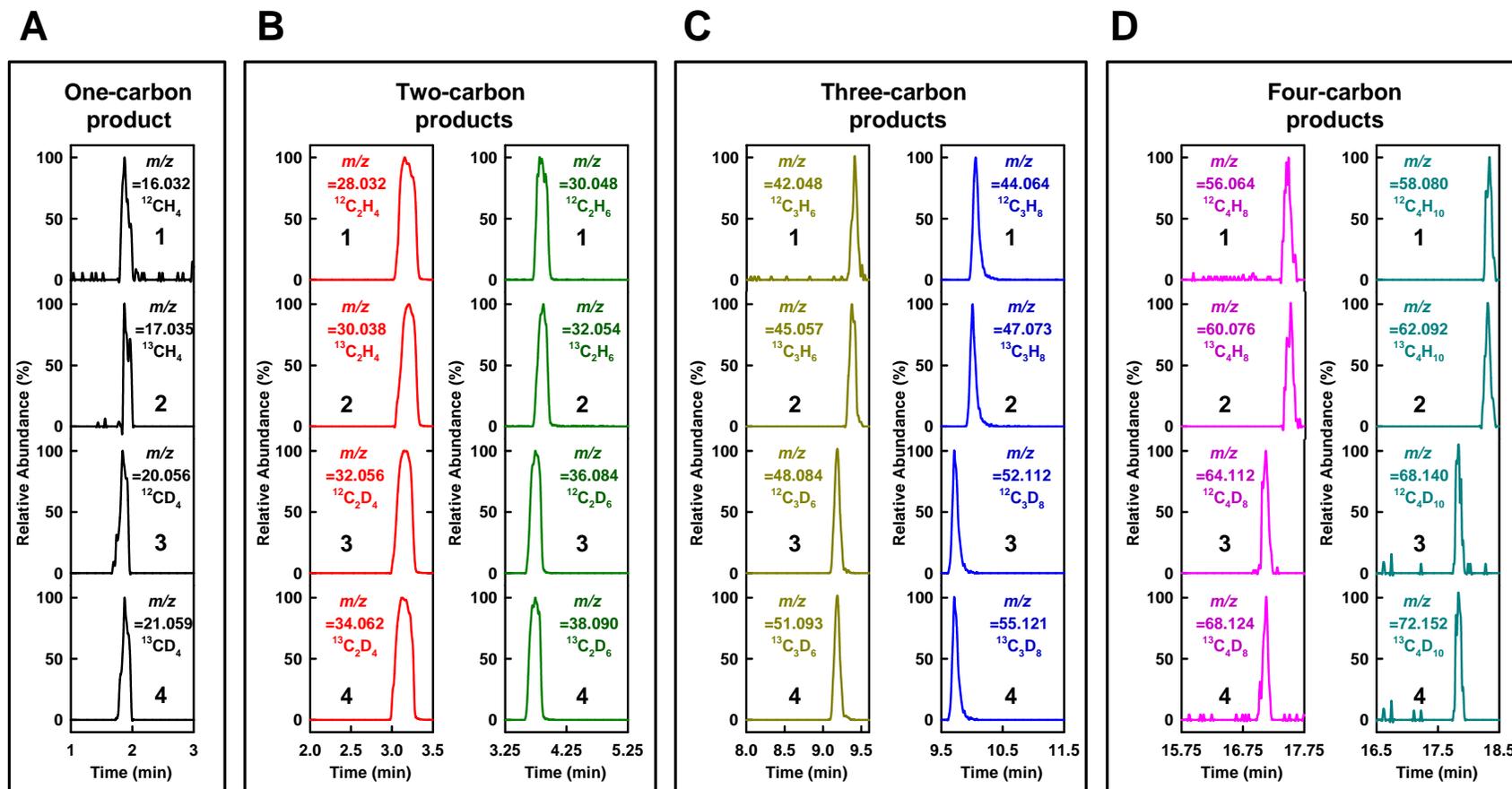
**Protein Purification.** *Azotobacter vinelandii* strains expressing wild-type VFe and MoFe proteins, and *vnfH*- and *nifH*-encoded Fe proteins were grown as described elsewhere (4). Published methods were used for the purification of these nitrogenase proteins (4).

**Activity Determination.** All nitrogenase activity assays were carried out in the presence of 100% CO at ambient temperature and pressure as previously described (7), except that the assays were scaled up by 130-fold from a standard reaction (4, 6, 7). In a 25 mL glass vial, each H<sub>2</sub>O-based assay had a total volume of 13 mL and contained 25 mM Tris buffer (pH 8.0), 25 mM ATP, 52.5 mM MgCl<sub>2</sub>, 300 mM phosphocreatine, 1.35 mg/mL phosphocreatine kinase, 20 mM dithionite, 200 mg VnfH or NifH and 20 mg VFe or MoFe protein. The D<sub>2</sub>O-based assay had the same composition as the H<sub>2</sub>O-based assay, except that all components were dissolved in 25 mM (D11)-Tris (*i.e.*, (DOCD<sub>2</sub>)<sub>3</sub>CND<sub>2</sub>) buffer and that all protein samples were exchanged into the same deuterated buffer. The pD of this buffer was adjusted to 8.0 with DCl and NaOD, determined by the previously established equation [pD = measured pH + 0.40 (24)], and further verified by pH indicator strips. Simultaneous determination of the hydrocarbon products was performed on an alumina F-1 column (Deerfield, IL). The products CH<sub>4</sub>, C<sub>2</sub>H<sub>4</sub>, C<sub>2</sub>H<sub>6</sub>, C<sub>3</sub>H<sub>6</sub>,

C<sub>3</sub>H<sub>8</sub>,  $\alpha$ -C<sub>4</sub>H<sub>8</sub>, and *n*-C<sub>4</sub>H<sub>10</sub>, as well as their deuterated counterparts, were quantified using a previously published method (4, 25) as follows. First, varying amounts of purchased standards (0-1%) were determined by a GC-FID equipped with an alumina F-1 column. Then, a linear standard curve ( $R \geq 0.97$ ) was constructed by plotting the known amounts of standards versus their respective, integrated areas. Finally, the samples were measured by the same GC-FID setup and the abundance of each given species in the samples was determined from the standard curve. The gas pressure of each injection was carefully normalized to obtain a consistent volume of analysis.

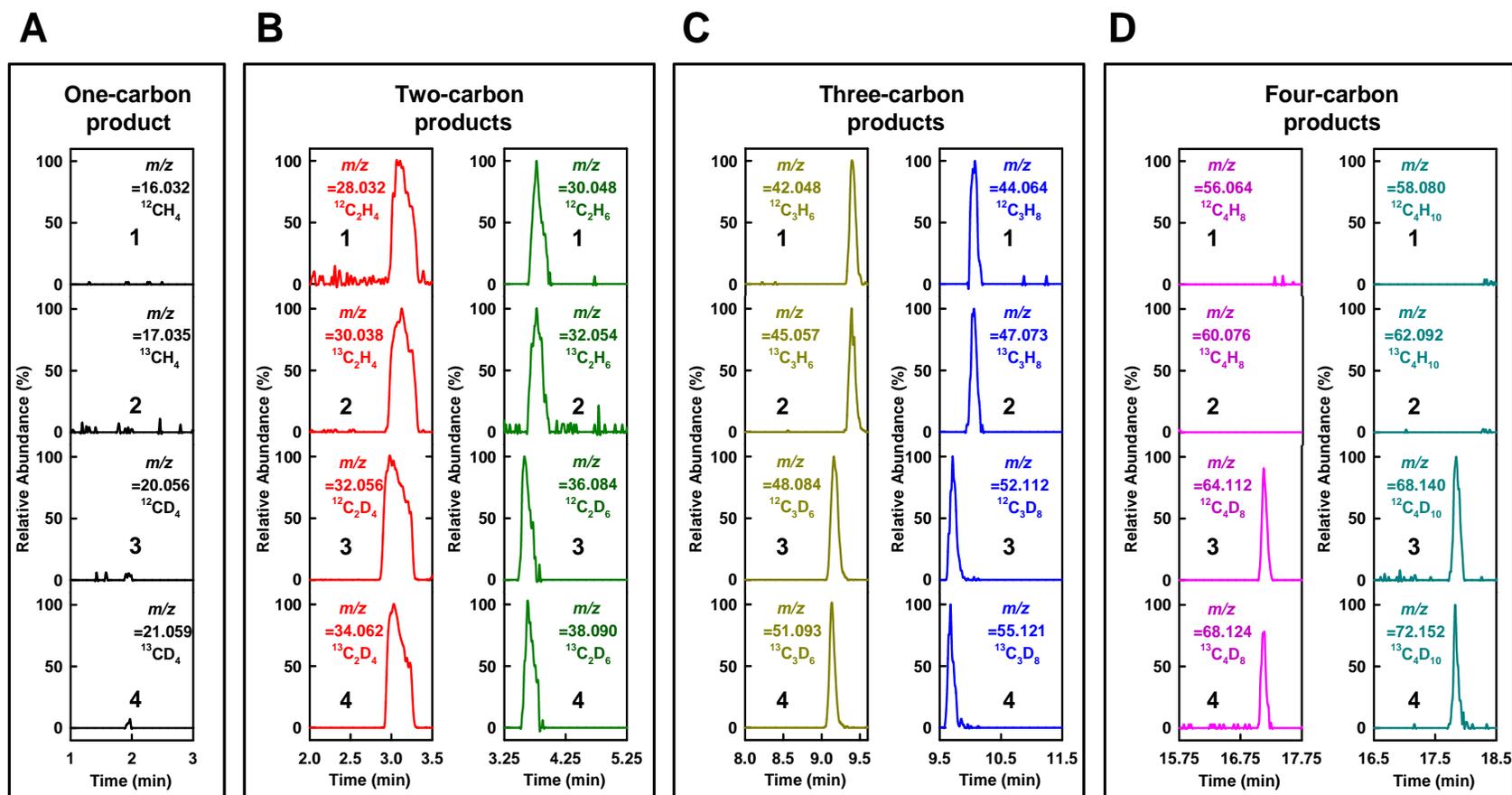
**GC-MS Analysis.** Samples were prepared as above, except that the reactions were terminated after 5 hr. GC-MS analysis was performed on an Agilent 6890 GC system coupled to a Waters GCT-Premier time-of-flight mass spectrometer. For each sample, 50  $\mu$ L of gas was injected into a split/splitless injector, which was operated at 125<sup>0</sup>C in split mode (30:1 split ratio). Gas separation was achieved with a PLOT-Q capillary column (0.320 mm ID x 30 m length), which was held at 40°C for one min, increased to 120°C at a rate of 5°C/min, and held for an additional 3 min at 120°C. Carrier He gas was passed through the column at 1.1 mL/min. The mass spectrometer was operated in electron impact ionization mode at 7000 resolution and calibrated over a range of 18 to 614 *m/z* using reference H<sub>2</sub>O, N<sub>2</sub>, O<sub>2</sub>, Ar, and CO<sub>2</sub> in addition to ions from the mass reference compound tris(perfluoro-tributyl) amine. The calibrated mass axis was locked to the CF<sub>3</sub><sup>+</sup> ion at 68.995 *m/z*.

FIGURE S1



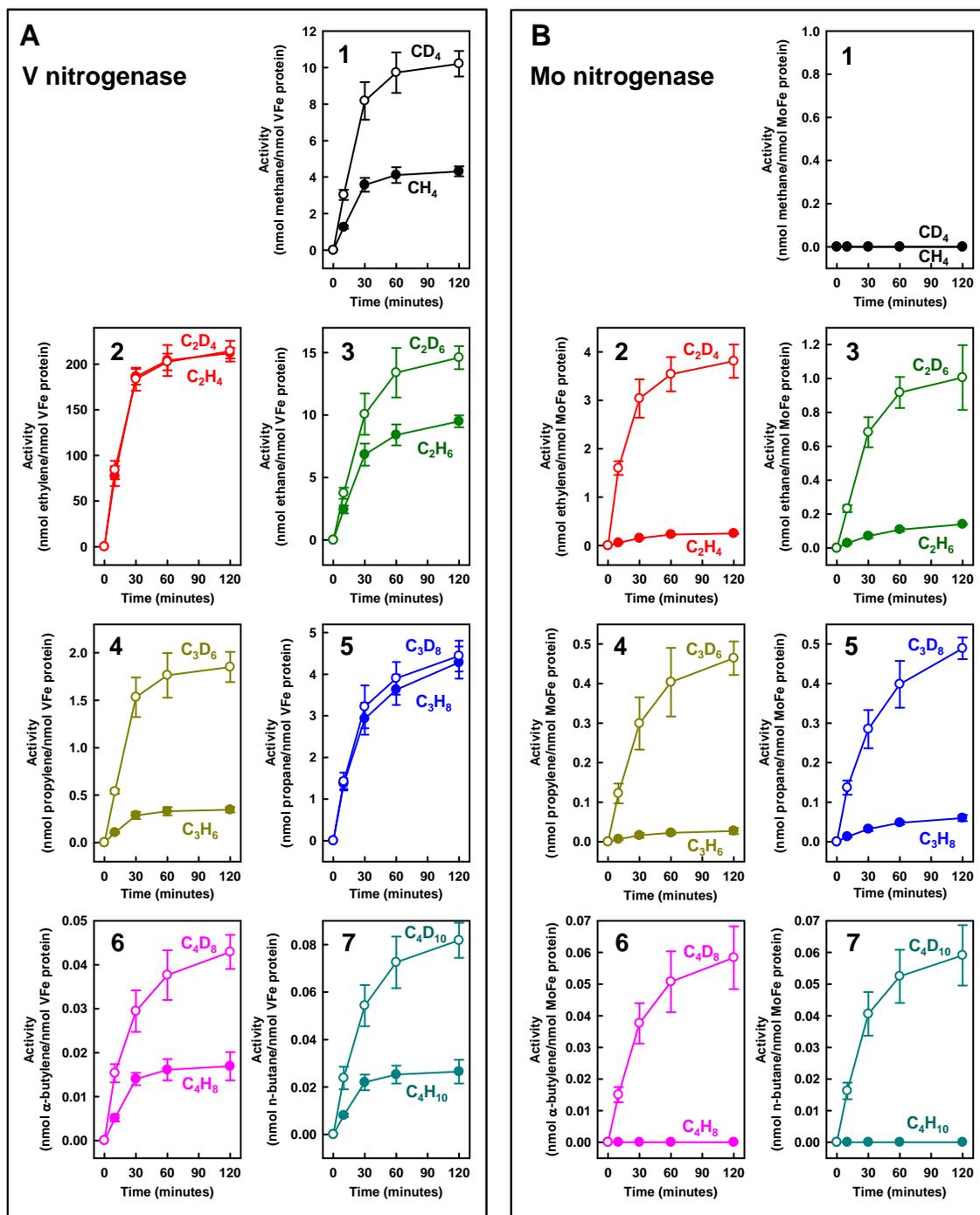
**Fig. S1.** Identities of hydrocarbons formed by V nitrogenase. GC-MS analyses of one (A)-, two (B)-, three (C)-, and four (D)-carbon products formed in the presence of 100% CO. The products were generated in the presence of H<sub>2</sub>O (1 and 2) or D<sub>2</sub>O (3 and 4) with <sup>12</sup>CO (1 and 3) or <sup>13</sup>CO (2 and 4) as the substrate. The mass-to-charge ( $m/z$ ) ratios at which the products were traced are indicated in the figure. See Fig. S3 for the GC-based activity analyses of product formation and Fig. S4 for the representative GC traces of product distribution.

FIGURE S2



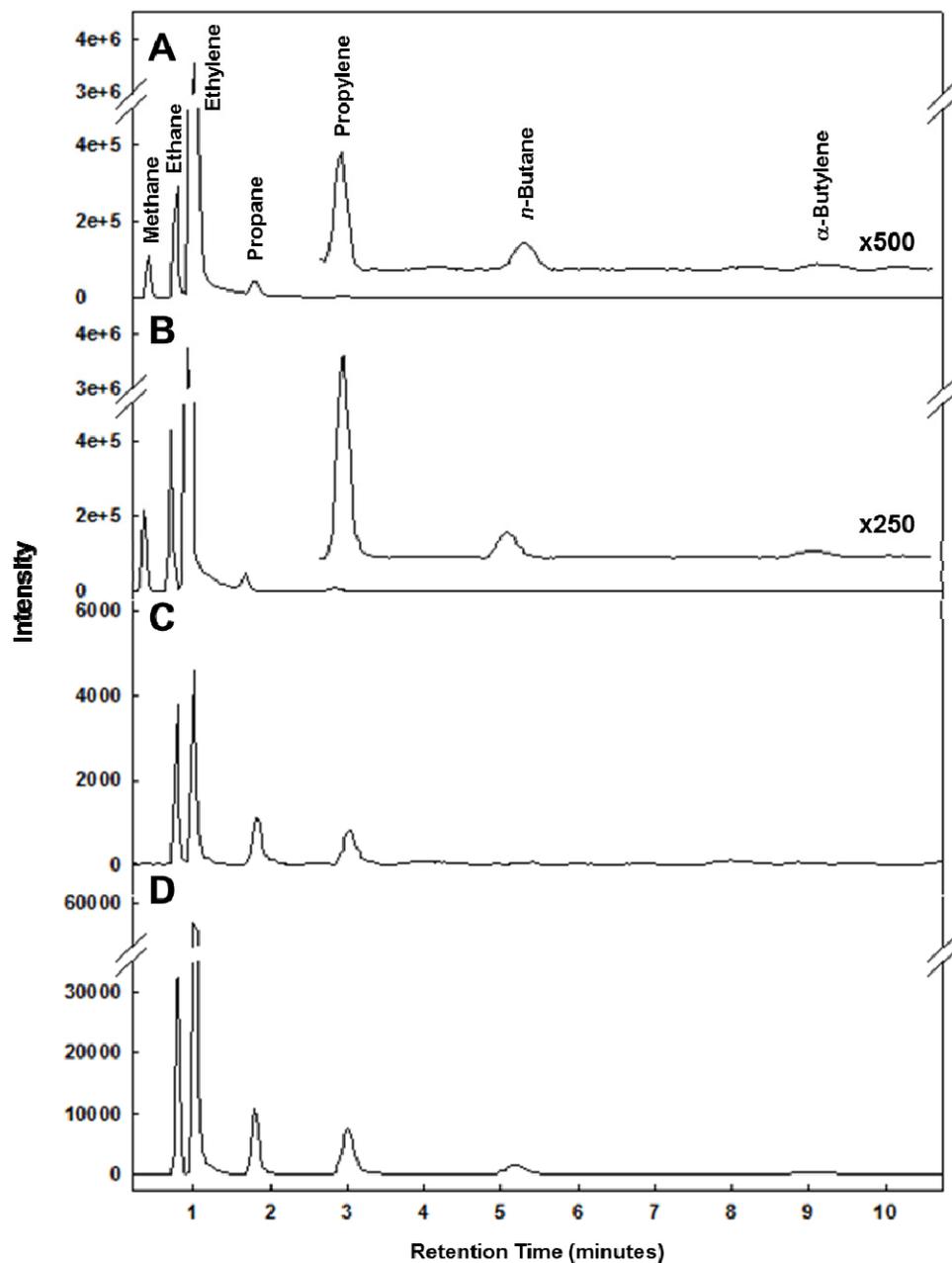
**Fig. S2.** Identities of hydrocarbons formed by Mo nitrogenase. GC-MS analyses of one (A)-, two (B)-, three (C)-, and four (D)-carbon products formed in the presence of 100% CO. The products were generated in the presence of H<sub>2</sub>O (1 and 2) or D<sub>2</sub>O (3 and 4) with <sup>12</sup>CO (1 and 3) or <sup>13</sup>CO (2 and 4) as the substrate. The mass-to-charge (*m/z*) ratios at which the products were traced are indicated in the figure. See Fig. S3 for the GC-based activity analyses of product formation and Fig. S4 for the representative GC traces of product distribution.

FIGURE S3



**Fig. S3.** Time-dependent formation of hydrocarbons by V (A)- and Mo (B)-nitrogenases. Formation of CH<sub>4</sub> (1), C<sub>2</sub>H<sub>4</sub> (2), C<sub>2</sub>H<sub>6</sub> (3), C<sub>3</sub>H<sub>6</sub> (4), C<sub>3</sub>H<sub>8</sub> (5),  $\alpha$ -C<sub>4</sub>H<sub>8</sub> (6) and *n*-C<sub>4</sub>H<sub>10</sub> (7) in the presence of H<sub>2</sub>O (●) or D<sub>2</sub>O (○) over a time period of 120 min. Data are presented as mean  $\pm$  SD ( $N = 5$ ).

FIGURE S4



**Fig. S4.** Gas chromatography of hydrocarbon products formed by V (A, B)- and Mo (C, D)-nitrogenase-catalyzed reactions in H<sub>2</sub>O (A, C) and D<sub>2</sub>O (B, D). The CO-background was subtracted from all traces.

**TABLE S1**

Table S1. Alkene/alkane ratios of V- and Mo-nitrogenases.

Products			V nitrogenase		Mo nitrogenase	
			H <sub>2</sub> O	D <sub>2</sub> O	H <sub>2</sub> O	D <sub>2</sub> O
			Alkene/ Alkane	Alkene/ Alkane	Alkene/ Alkane	Alkene/ Alkane
One-carbon product	Methane	CH <sub>4</sub> or CD <sub>4</sub>	--	--	--	--
Two-carbon products	Ethylene	C <sub>2</sub> H <sub>4</sub> or C <sub>2</sub> D <sub>4</sub>	31.78	22.46	2.00	6.92
	Ethane	C <sub>2</sub> H <sub>6</sub> or C <sub>2</sub> D <sub>6</sub>				
Three-carbon products	Propylene	C <sub>3</sub> H <sub>6</sub> or C <sub>3</sub> D <sub>6</sub>	0.08	0.38	0.50	0.89
	Propane	C <sub>3</sub> H <sub>8</sub> or C <sub>3</sub> D <sub>8</sub>				
Four-carbon products	$\alpha$ -Butylene	C <sub>4</sub> H <sub>8</sub> or C <sub>4</sub> D <sub>8</sub>	0.60	0.68	--	0.92
	<i>n</i> -Butane	C <sub>4</sub> H <sub>10</sub> or C <sub>4</sub> D <sub>10</sub>				

All ratios were calculated based on the data in Figure 2.