Table 1 The chemical and isotopic compositions of CO2 and short-chain alkanes during preservation at 20 oC. Unit for chemical compositions: mmol/mol. Unit for δ13C values: ‰ -vPDB and for δ2H values: ‰ -vSMOW.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site | Sampling date | Sample number | Institute\* | Preservation time (months) | CO2 | C1 | C2 | C3 | C1/CO2$ | δ13C-CO2 | δ13C-C1 | δ13C-C2 | δ13C-C3 | δ2H-C1 |
| vent A | 2012/5/5 | 012a1 | *a* | 3±0.1 | 1.10 | 126.9 | 0.500 | 0.100 | 115.4 | -13.4 | -15.1 | -22.0 | n.a. | n.a. |
| vent A | 2014/4/29 | 014a1 | *a* | 1±0.1 | 0.60 | 166.1 | 0.820 | 0.150 | 276.8 | -28.7 | -15.9 | -19.4 | n.a. | n.a. |
| vent A | 2014/4/29 | 014a2 | *a* | 1±0.1 | 1.10 | 117.3 | 0.580 | 0.110 | 106.6 | -23.8 | -15.2 | -19.4 | n.a. | n.a. |
| vent A | 2014/4/30 | 014a3 | *a* | 1±0.1 | 0.85 | 158.6 | 0.800 | 0.160 | 186.6 | -18.5 | -16.5 | -20.1 | n.a. | n.a. |
| vent A | 2014/9/22 | 014b1 | *a* | 13±1.0 | 4.20 | 158.6 | 0.733 | 0.085 | 38.0 | -21.7 | -16.8 | n.a. | n.a. | -61 |
| vent A | 2014/9/22 | 014b1 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 30.8 | n.a. | -15.7 | -19.8 | -17.5 | n.a. |
| vent A | 2014/9/22 | 014b1 | *c* | 26±1.0 | 2.10 | 37.8 | 0.108 | 0.016 | 18.3 | -22.3 | 0.9 | -10.5 | -18.5 | n.a. |
| vent A | 2014/9/22 | 014b3 | *a* | 13±1.0 | 19.8 | 109.5 | 0.494 | 0.062 | 5.50 | -17.5 | -14.8 | -19.3 | -18.2 | -55 |
| vent A | 2014/9/22 | 014b4 | *a* | 13±1.0 | 7.40 | 127.4 | 0.605 | 0.066 | 17.30 | -13.1 | -14.8 | -19.4 | -14.6 | -60 |
| vent A | 2014/9/22 | 014b4 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 7.20 | n.a. | -14.8 | -19.6 | -17.8 | n.a. |
| vent A | 2014/9/22 | 014b4 | *c* | 26±1.0 | 3.70 | 0.300 | 0.006 | 0.003 | 0.10 | -15.4 | 113.7 | 12.8 | -18.0 | n.a. |
| vent A | 2014/9/22 | 014b5 | *a* | 13±1.0 | 9.20 | 145.4 | 0.670 | 0.066 | 15.80 | -14.9 | -14.3 | -19.6 | -13.7 | -62 |
| vent A | 2014/9/22 | 014b5 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 7.50 | n.a. | -15.7 | -20.0 | -17.9 | n.a. |
| vent A | 2014/9/22 | 014b5 | *c* | 26±1.0 | 7.20 | 17.0 | 0.088 | 0.020 | 2.40 | -20.2 | 16.1 | -6.8 | -19.6 | n.a. |
| vent A | 2014/9/23 | 014b6 | *a* | 13±1.0 | 22.2 | 81.1 | 0.408 | 0.062 | 3.70 | -19.1 | -6.7 | -17.6 | n.a. | 33 |
| vent A | 2014/9/23 | 014b6 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 0.40 | n.a. | 26.6 | -13.8 | -17.0 | n.a. |
| vent A | 2014/9/23 | 014b6 | *c* | 26±1.0 | 21.9 | 2.80 | 0.022 | 0.020 | 0.10 | -19.1 | 79.3 | 8.4 | -22.0 | n.a. |
| vent A | 2014/9/23 | 014b7 | *a* | 13±1.0 | 11.1 | 73.3 | 0.414 | 0.064 | 6.60 | -18 | -10.0 | -17.4 | -13.2 | n.a. |
| vent A | 2014/9/23 | 014b7 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 2.50 | n.a. | -8.3 | -18.1 | -17.1 | n.a. |
| vent A | 2014/9/23 | 014b7 | *c* | 26±1.0 | 4.50 | 0.0126 | 0.00045 | 0.00028 | 0.003 | -21.9 | 243.0 | n.a. | n.a. | n.a. |
| vent A | 2016/5/20 | 016z1# | *c* | 7±1.0 | 0.17 | 140.2 | 0.570 | 0.119 | 844.1 | -14.0 | -17.1 | -7.0 | -14.0 | -86 |
| vent A | 2016/5/20 | 016z1# | *a* | 29±0.5 | 1.44 | 77.7 | 0.337 | 0.055 | 54.1 | n.a. | -17.7 | n.a. | n.a. | -81 |
| vent A | 2016/5/20 | 016z3# | *c* | 7±1.0 | 0.51 | 170.7 | 0.569 | 0.116 | 337.7 | -14.6 | -17.8 | -16.6 | -27.6 | -88 |
| vent A | 2016/5/20 | 016z3# | *a* | 29±0.5 | 0.75 | 75.8 | 0.392 | 0.069 | 100.5 | n.a. | n.a. | n.a. | n.a. | -72 |
| vent A | 2016/5/20 | 016z4# | *c* | 7±1.0 | 0.27 | 158.3 | 0.568 | 0.111 | 589.4 | -15.2 | -16.2 | -15.2 | -15.3 | -89 |
| vent A | 2016/5/21 | 016z5# | *c* | 7±1.0 | 0.22 | 156.4 | 0.553 | 0.114 | 714.8 | -14.3 | -16.4 | -15.0 | -25.2 | n.a. |
| vent A | 2016/5/21 | 016z6 | *c* | 7±1.0 | 0.07 | 155.7 | 0.559 | 0.115 | 2110.7 | -10.3 | -16.9 | -12.9 | -22.8 | n.a. |
| vent A | 2016/5/21 | 016z7# | *c* | 7±1.0 | 0.14 | 142.6 | 0.657 | 0.131 | 1043.0 | -13.7 | -17.2 | -16.8 | -16.9 | n.a. |
| vent A | 2016/5/21 | 016z8# | *c* | 7±1.0 | 0.08 | 148.3 | 0.688 | 0.137 | 1785.9 | -10.1 | -16.2 | -13.3 | -22.6 | -89 |
| vent A | 2016/5/22 | 016z9# | *c* | 7±1.0 | 0.21 | 112.4 | 0.710 | 0.145 | 532.9 | -8.3 | -15.9 | -16.5 | -26.0 | -89 |
| vent A | 2016/5/22 | 016z10 | *c* | 7±1.0 | 0.20 | 168.7 | 0.571 | 0.112 | 856.3 | -10.9 | -16.4 | -15.6 | -21.3 | n.a. |
| vent A | 2016/5/22 | 016z10 | *a* | 29±0.5 | 0.46 | 151.1 | 0.652 | 0.101 | 332.0 | n.a. | -16.8 | n.a. | n.a. | -81 |
| vent A | 2016/5/22 | 016z11# | *c* | 7±1.0 | 0.13 | 32.5 | 0.124 | 0.024 | 254.9 | -9.3 | -17.8 | -15.2 | -24.9 | -89 |
| vent A | 2018/10/30 | 18ZG1 | *a* | 2.5±0.2 | 0.86 | 170.4 | 0.836 | 0.066 | 197.7 | n.a. | -17.7 | n.a. | n.a. | n.a. |
| vent A | 2018/10/30 | 18ZG2 | *a* | 2.5±0.2 | 4.13 | 82.0 | 0.386 | 0.029 | 19.85 | -21.7 | -15.2 | n.a. | n.a. | n.a. |
| vent A | 2018/10/30 | 18ZG3 | *a* | 2.5±0.2 | 1.14 | 172.6 | 0.827 | 0.064 | 152.01 | n.a. | -16.8 | n.a. | n.a. | n.a. |
| vent A | 2018/10/30 | 18ZG4 | *a* | 2.5±0.2 | 1.20 | 165.5 | 0.775 | 0.055 | 138.1 | n.a. | -15.8 | n.a. | n.a. | n.a. |
| vent A | 2018/10/30 | 18ZG5 | *a* | 2.5±0.2 | 1.71 | 123.8 | 0.579 | 0.041 | 72.48 | -21.9 | -16.9 | n.a. | n.a. | n.a. |
| vent A | 2018/10/30 | 18ZG6 | *d* | 2.5±0.2 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | -16.25 | n.a. | n.a. | -78.77 |
| vent A | 2018/10/30 | 18ZG7 | *a* | 2.5±0.2 | 3.47 | 133.9 | 0.591 | 0.042 | 38.57 | -28.1 | -16.1 | n.a. | n.a. | n.a. |
| vent A | 2018/10/30 | 18ZG8 | *a* | 2.5±0.2 | 3.45 | 163.1 | 0.748 | 0.060 | 47.30 | n.a. | -16.8 | n.a. | n.a. | n.a. |
| vent A | 2018/10/30 | 18ZG9 | *a* | 2.5±0.2 | 4.16 | 121.8 | 0.574 | 0.044 | 29.31 | -25.6 | -17.4 | n.a. | n.a. | n.a. |
| vent A | 2018/10/30 | 18ZG10 | *d* | 2.5±0.2 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | -16.57 | n.a. | n.a. | -80.49 |
| vent B | 2012/5/5 | 012a2 | *a* | 3±0.1 | 7.90 | 88.9 | 0.200 | 0.100 | 11.25 | -18.5 | -10.7 | -13.4 | n.a. | n.a. |
| vent B | 2014/4/29 | 014w1 | *a* | 1±0.1 | 2.90 | 47.1 | 0.089 | 0.042 | 16.24 | -16.6 | -8.1 | n.a. | n.a. | n.a. |
| vent B | 2014/4/30 | 014w2 | *a* | 1±0.1 | 3.40 | 49.1 | 0.082 | 0.041 | 14.44 | -13.3 | -7.5 | n.a. | n.a. | n.a. |
| vent B | 2014/9/22 | 014s2 | *a* | 13±1.0 | 3.80 | 31.9 | 0.066 | 0.040 | 8.45 | -12.9 | -12.9 | n.a. | n.a. | n.a. |
| vent B | 2014/9/22 | 014s2 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 2.80 | n.a. | -10.7 | -24.7 | -23.2 | n.a. |
| vent B | 2014/9/22 | 014s2 | *c* | 26±1.0 | 7.90 | 10.6 | 0.017 | 0.014 | 1.35 | -17.5 | 6.5 | -16.71 | -19.3 | n.a. |
| vent B | 2014/9/22 | 014s3 | *a* | 13±1.0 | 3.10 | 14.6 | 0.045 | 0.020 | 4.72 | -13.2 | -10.5 | n.a. | n.a. | -55 |
| vent B | 2014/9/22 | 014s3 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 1.90 | n.a. | -9.5 | -12.9 | -17.4 | n.a. |
| vent B | 2014/9/22 | 014s3 | *c* | 26±1.0 | 8.30 | 7.3 | 0.005 | 0.004 | 0.88 | -19.0 | -9.5 | 10.51 | -12.2 | n.a. |
| vent B | 2014/9/22 | 014s4 | *a* | 13±1.0 | 9.10 | 46.9 | 0.062 | 0.113 | 5.18 | -14.6 | -7.1 | -15.1 | n.a. | n.a. |
| vent B | 2014/9/22 | 014s4 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 2.8 | n.a. | -8.1 | -17.5 | -28.3 | n.a. |
| vent B | 2014/9/22 | 014s4 | *c* | 26±1.0 | 16.3 | 43.1 | 0.046 | 0.011 | 2.64 | -23.0 | -7.4 | 3.65 | -22.5 | -20 |
| vent B | 2014/9/23 | 014s6 | *a* | 13±1.0 | 5.80 | 49.8 | 0.113 | 0.053 | 8.54 | -17.2 | -9.9 | n.a. | n.a. | n.a. |
| vent B | 2014/9/23 | 014s6 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 5.30 | n.a. | -8.7 | -14.0 | -16.0 | n.a. |
| vent B | 2014/9/23 | 014s6 | *c* | 26±1.0 | 10.5 | 37.4 | 0.031 | 0.020 | 3.56 | -27.5 | -9.4 | -11.6 | -12.8 | -23 |
| vent B | 2014/9/23 | 014s8 | *a* | 13±1.0 | 16.3 | 33.4 | 0.060 | 0.045 | 2.04 | -14.3 | -9.5 | n.a. | n.a. | 41 |
| vent B | 2014/9/23 | 014s8 | *b* | 20±0.5 | n.a. | n.a. | n.a. | n.a. | 1.70 | n.a. | -7.8 | -13.3 | -16.0 | n.a. |
| vent B | 2014/9/23 | 014s8 | *c* | 26±1.0 | 14.5 | 28.4 | 0.025 | 0.013 | 1.96 | -23.9 | -7.7 | -10.2 | -12.1 | n.a. |
| vent B | 2016/5/20 | 016w1# | *c* | 7±1.0 | 3.56 | 50.1 | 0.036 | 0.035 | 14.06 | -12.2 | -6.3 | -11.2 | -37.9 | 17 |
| vent B | 2016/5/20 | 016w1# | *a* | 29±0.5 | 1.73 | 33.9 | 0.040 | n.a. | 19.56 | n.a. | -3.8 | n.a. | n.a. | 31 |
| vent B | 2016/5/20 | 016w2 | *c* | 7±1.0 | 2.36 | 51.1 | 0.036 | 0.029 | 21.62 | -8.4 | -6.0 | -7.8 | -31.2 | 18 |
| vent B | 2016/5/20 | 016w3# | *c* | 7±1.0 | 3.13 | 49.0 | 0.042 | 0.029 | 15.66 | -10.5 | -6.7 | -11.2 | -27.5 | 14 |
| vent B | 2016/5/20 | 016w3# | *a* | 29±0.5 | 1.84 | 45.4 | 0.057 | 0.025 | 24.74 | n.a. | -8.1 | n.a. | n.a. | 16 |
| vent B | 2016/5/20 | 016w4# | *c* | 7±1.0 | 2.18 | 48.0 | 0.037 | 0.028 | 22.07 | -8.6 | -7.2 | -8.6 | -26.4 | n.a. |
| vent B | 2016/5/21 | 016w5# | *c* | 7±1.0 | 3.96 | 46.5 | 0.033 | 0.026 | 11.74 | -11.7 | -6.5 | -8.2 | -21.4 | n.a. |
| vent B | 2016/5/21 | 016w6 | *c* | 7±1.0 | 2.95 | 47.1 | 0.034 | 0.028 | 15.97 | -12.4 | -6.6 | -9.2 | -21.8 | n.a. |
| vent B | 2016/5/21 | 016w7# | *c* | 7±1.0 | 3.58 | 47.1 | 0.041 | 0.030 | 13.14 | -12.1 | -7.2 | -10.8 | -13 | 11 |
| vent B | 2016/5/21 | 016w7# | *a* | 29±0.5 | 1.48 | 40.1 | 0.052 | 0.023 | 27.08 | n.a. | -8.8 | n.a. | n.a. | 4 |
| vent B | 2016/5/21 | 016w8# | *c* | 7±1.0 | 2.36 | 43.2 | 0.033 | 0.028 | 18.27 | -12.5 | -6.8 | -8.4 | -12.9 | 11 |
| vent B | 2016/5/22 | 016w9# | *c* | 7±1.0 | 4.34 | 46.5 | 0.035 | 0.027 | 10.72 | -13.3 | -6.2 | -7.6 | -13.0 | 10 |
| vent B | 2016/5/22 | 016w10 | *c* | 7±1.0 | 3.68 | 43.3 | 0.030 | 0.026 | 11.77 | -14.2 | -6.4 | -6.3 | -13.6 | n.a. |
| vent B | 2016/5/22 | 016w11# | *c* | 7±1.0 | 3.59 | 47.0 | 0.035 | 0.027 | 13.10 | -14.8 | -7.1 | -6.3 | -13.0 | n.a. |
| vent B | 2018/10/30 | 18WG3 | *d* | 2.5±0.2 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | -10.52 | n.a. | n.a. | -27.15 |
| vent B | 2018/10/30 | 18WG4 | *d* | 2.5±0.2 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | -10.17 | n.a. | n.a. | -20.02 |

C1: methane; C2: ethane; C3: propane.

# These samples were sterilized by adding 1 mL 1% HgCl2 solution immediately after sampling.

\* Analyzing institutes:

*a*: the Key laboratory of Petroleum Resources Research, Institute of Geology and Geophysics, Chinese Academy of Science.

*b*: the Lab of stable isotopes, Goethe University Frankfurt.

*c*: The chemical and stable carbon isotope compositions were analyzed in Helmholtz-Zentrum für Ozeanforschung Kiel (GEOMAR). The hydrogen isotope compositions were measured at GEO-data GmbH.

*d*: Stable isotope laboratory of Massachusetts Institute of Technology.

$ The C1/CO2 ratios were measured directly at the Goethe University Frankfurt. Except for this, the C1/CO2 ratio was calculated from the absolute concentrations of C1 and CO2

n.a.: not analyzed or not detected.

Table 2 Overview of carbon and hydrogen isotope fractionation factors during microbial oxidation of short-chain alkanes.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **type** | **Samples/conditions** | **T (oC)** | **Carbon (*εC*)** | **Hydrogen (*εH*)** | ***Λ*** | **Reference** |
| Uncultured closed system experiment | Lutao hydrothermal gas | 20 | C1: -37.1 ± 7.5  C2: -12.8 ± 1.9  C3: -5.2 ± 0.8 | C1: -263 ± 60 | C1: 9.3 | This study |
| Cultured enrichment experiments | water sample and drip tray of ice-making machine | 26 | -24.6 ± 0.7 | -245.3 ± 25 | 12.9 | Coleman et al., 1981 |
| 11.5 | -12.8 ± 0.2 | -93.4 ± 6 | 7.9 |
| 26 | -23.8 ± 1.5 | -229.0 ± 2 | 12.2 |
| marine sediment collected from CA, USA | 15 | C1: -26.5 ± 3.9  C2: -8.0 ± 1.7  C3: -4.8 ± 0.9  C4: -2.9 ± 0.9 | C1: -238.1 ± 115.6  C2: -61.9 ± 8.3  C3: -15.1 ± 1.9 | C1: 11.5  C2: 8.2  C3: 3.2 | Kinnaman et al., 2007 |
| Hydrate Ridge, Pacific Ocean | 12 | -11.9 | -114.7 ± 19.3 | 10.8 | Holler et al., 2009 |
| Amon Mud Volcano, Mediterranean Sea | 20 | -20.6 ± 2 | -139.4 ± 16.7 | 7.7 |
| Black Sea microbial mat | 12 | -35.7 ± 2 | -229.6 ± 14.9 | 8.1 |
| *a* Anoxic sediment from marine hydrocarbon seep areas | 12 | C3: -5.9  C4: -1.6 |  |  | Kniemeyer et al., 2007 |
| 28 | C3: -5.2  C4: -1.6 |  |  |
| 60 | C3: -5.9 |  |  |
| Marine sediment from Bohai Bay, China: incubation iii | 28 | C1: -10.9 ± 2.9  C2: -3.2 ± 1.1  C3: -1.0 ± 0.3 | C1: -56.3 ± 8.4  C2: -135.6 ± 24.4  C3: -70.3 ± 23.1 | C1: 5.4  C2: 48.9  C3: 75.5 | Li et al., 2019 |
| Alluvial sand from Lake Geneva, Switzerland | 23 | C3: -10.8 ± 0.7  C4: -5.6 ± 0.1 |  |  | Bouchard et al., 2008 |
| Pure oxidizing experiments | *bMethylococcus capsulatus* | 45 | -27.9 ± 1.7 | -231.5 ± 30.5 | 10.5 | Feisthauer et al., 2011 |
| *cMethylococcus capsulatus* | 45 | -22.9 ± 3.2 | -192.0 ± 28.5 | 10.1 |
| *bMethylosinus sporium* | 30 | -18.8 ± 1.4 | -136.8 ± 20.1 | 8.3 |
| *cMethylosinus sporium* | 30 | -21.5 ± 2.7 | -182.6 ± 23.7 | 10.2 |
| *Methylocystis parvus* | 30 | -19.1 ± 1.0 | -168.2 ± 9.1 | 10.4 |
| *Methylomonas methanica* | 30 | -27.7 ± 2.3 | -225.5 ± 17.8 | 10.2 |
| *Methylocaldum gracile* | 30 | -14.8 ± 0.9 | -110.0 ± 11.5 | 8.2 |
| *a Methylomirabilis oxyfera* | 30 | -29.2 ± 2.6 | -227.6 ± 13.5 | 9.8 | Rasigraf et al., 2012 |
| *Methylococcus capsulatus* | 30 | -12 ± 0.3 | -105 ± 3 | 9.7 | Wang et al., 2006 |
| 37 | -22 ± 1 | -202 ± 10 | 11.3 |
| Field observations | *a* Hydrothermal gas emitted among the coast of the Baja California Peninsula |  | -13 | -97 | 8.2 | Batista Cruz et al., 2019 |
| Hydrothermal plume at Myojin Knoll Caldera, Izu-Bonin arc |  | -5.0 |  |  | Tsunogai et al., 2000 |
| Methane plums from Logatchev hydrothermal field |  | -14.8 |  |  | Keir et al., 2009 |
| Methane in an aging hydrothermal plume, Endeavour Segment |  | -7.16 - -7.67 |  |  | Cowen et al., 2002 |
| *a* Anoxic sediment of Skan Bay, Alaska |  | -8.7 ± 1 | -135.7± 23 | 17.8 | Alperin et al., 1988 |

*a* anaerobic

*b* pMMO

*c* sMMO

C1: methane; C2: ethane; C3: propane; C4: *n*-butane

The *Λ* values were calculated according to Equations (16 and 17).