

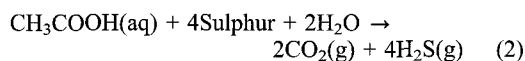
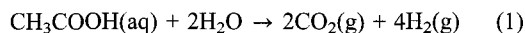
## Metabolic energy from acetic acid catabolism in the hot springs of Vulcano Island, Southern Italy

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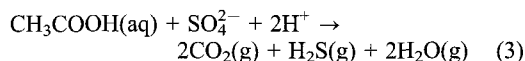
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Low molecular weight aqueous carboxylic acids and/or their anions are the dominant organic compounds in many natural surface and subsurface waters. Concentrations of these acids, also referred to as volatile fatty acids (VFAs), rarely exceed 1–2 ppm in uncontaminated surface waters, but may approach 1,000–10,000 ppm in saline waters of subsurface origin. VFAs, in particular acetic acid/acetate, are well-known substrates for and products of microbial metabolism in diverse aqueous environments, including those at elevated temperatures. Nevertheless, few analytical studies are available that report the concentrations of these compounds in hydrothermal fluids where thermophilic organisms are known to live. Analyses of this kind are a prerequisite, however, to determine the energetics of potential VFA catabolism in high temperature ecosystems. Here I report the results of organic and inorganic chemical analyses from shallow hot springs known for their prolific thermophilic activity on the Island of Vulcano, southern Italy, together with thermodynamic calculations of the energy released by acetic acid catabolism at the temperature (98.4 °C) and solution chemistry that obtain in this system.

Anaerobic chemotrophic microorganisms readily catabolize acetic acid (CH<sub>3</sub>COOH) to carbon dioxide (CO<sub>2</sub>) or CO<sub>2</sub> plus methane (CH<sub>4</sub>), and the exact mechanisms involved in these conversions, including the specific functions of essential enzymes, have now been resolved (Thauer *et al.*, 1989). The known overall metabolic reactions of anaerobic microbial acetic acid oxidation include

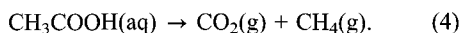


and



Numerous species, including *Desulfuromonas acetoxidans*, *Desulfobacter postgatei*, and

*Desulfotomaculum acetoxidans*, gain metabolic energy from the processes represented by reactions (1)–(3). In addition, anaerobic microbes are known to mediate the disproportionation of acetic acid, represented by



Members of the genera *Methanosarcina* and *Methanotherix*, among others, gain energy from reaction (4). However, at present, no species are known that can harness energy from any of these forms of acetic acid catabolism at temperatures above approximately 55 °C. This may be an artifact of the small number of hyperthermophiles (organisms able to grow at temperatures ≥ 90 °C) currently in culture and the even smaller number of complete metabolic studies on these organisms, rather than the inability of hyperthermophiles to catalyze these reactions. On the other hand, perhaps anaerobic acetic acid oxidation and disproportionation yield insufficient amounts of energy under the environmental conditions that exist in high temperature ecosystems such as hot springs. To explore the notion that acetic acid catabolism is inadequate as an energy-yielding process for hyperthermophiles, values of the overall Gibbs free energy (ΔG<sub>r</sub>) of reactions (1)–(4) were determined.

The amounts of energy associated with reactions (1)–(4) in any environment of interest can be calculated from the familiar relation:

$$\Delta G_r = \Delta G_r^0 + RT \ln Q_r \quad (5)$$

where ΔG<sub>r</sub> is as defined above, ΔG<sub>r</sub><sup>0</sup> stands for standard state Gibbs free energy of reaction *r*, *R* and *T* denote the gas constant and temperature in Kelvin, respectively, and *Q<sub>r</sub>* represents the activity or fugacity product. Values of *Q<sub>r</sub>* required to evaluate ΔG<sub>r</sub> with Eqn. (5) can be determined with the expression

$$Q_r = \Pi a_i^{\nu_{i,r}} \quad (6)$$

where *a<sub>i</sub>* denotes the activity of the *i*th species, and *ν<sub>i,r</sub>* represents the stoichiometric reaction coefficient

of the  $i$ th species in reaction  $r$ , which is negative for reactants and positive for products. In the case of gases, activities are replaced by fugacities ( $f_i$ ) in Eqn. (6).

To determine the amounts of energy available from reactions (1)–(4) at the temperature (98.4°C), pressure (1 bar), and chemical composition of a Vulcano hot spring (Punto 1), values of  $\Delta G_r$  for these metabolic processes were computed with Eqn. (5). The standard state terms,  $\Delta G_r^0$ , at this temperature and pressure, given in Table 1, were determined using thermodynamic properties and parameters for the revised Helgeson-Kirkham-Flowers equation of state together with the SUPCRT92 computer code. Values of  $f_{\text{CO}_2}$  ( $2.79 \times 10^{-2}$ ),  $f_{\text{CH}_4}$  ( $2.76 \times 10^{-4}$ ),  $f_{\text{H}_2\text{S}}$  ( $4.74 \times 10^{-4}$ ),  $f_{\text{H}_2}$  ( $4.80 \times 10^{-5}$ ),  $a_{\text{SO}_4^{2-}}$  ( $1.44 \times 10^{-1}$ ), and  $a_{\text{H}^+}$  ( $5.37 \times 10^{-4}$ ) used to evaluate  $Q_1$ ,  $Q_2$ ,  $Q_3$ , and  $Q_4$  were taken from Gurrieri *et al.* (1998), and the value of  $a_{\text{CH}_3\text{COOH}}$  ( $3.05 \times 10^{-6}$ ) is given by Amend *et al.* (1998). In this latter study, water samples were collected from various hot springs on Vulcano and analysed for VFAs using liquid chromatography. Activities of VFAs ranged from  $1.41 \times 10^{-6}$  –  $3.05 \times 10^{-6}$  formic,  $2.21 \times 10^{-6}$  –  $3.06 \times 10^{-6}$  acetic, and  $<1.35 \times 10^{-6}$  –  $2.59 \times 10^{-6}$  propanoic acids. The activities of acetic acid were generally higher than both those of formic and propanoic acids which is consistent with analyses in a saline spring near the Dead Sea reported by Kawamura and Nissenbaum (1992) as well as with thermodynamic calculations by Helgeson *et al.* (1993) for equilibrium activities of  $n$ -carboxylic acids in oil-field waters.

Values of  $\Delta_r$  for reactions (1)–(4) at the conditions prevailing in the Punto 1 hot spring on Vulcano are given in Table 1, together with corresponding values of  $\Delta G_r^0$  and  $RT\ln Q_r$  required to calculate  $\Delta G_r$ . Depending on the metabolic pathway chosen, between 14.6 and 44.7 kcal of energy per mole of acetic acid catabolized are available to an organism living at  $\sim 100^\circ\text{C}$  in this hot spring system. Acetic acid oxidation to  $\text{CO}_2$  coupled to the reduction of elemental sulphur (reaction 2) or sulphate (reaction 3) to hydrogen sulphide yields about 2–3 times as much metabolic energy as acetic acid oxidation by dehydrogenation (reaction 1) or disproportionation (reaction 4). Nevertheless, in the hot springs of Vulcano, all four metabolic 'strategies' are strongly exergonic and

TABLE 1. Values of  $\Delta G_r^0$ ,  $RT\ln Q_r$ , and  $\Delta G_r$  (in kcal  $\text{mol}^{-1}$ ) for reactions (1)–(4) given in the text at 98.4°C and 1 bar in the hot spring (Punto 1) on Vulcano

Reac.	$\Delta G_r^0$	$RT\ln Q_r$	$\Delta G_r$
1	8.8	-25.3	-16.5
2	-26.2	-18.5	-44.7
3	-47.9	11.0	-36.9
4	-15.3	0.7	-14.6

probably represent sufficient energy sources for anaerobic microbial metabolism. That even 14.6 kcal  $\text{mol}^{-1}$  is sufficiently exergonic is supported by the fact that  $\Delta G_r$  is *only*  $-7.1$  kcal  $\text{mol}^{-1}$  for the experimentally verified energy-yielding sulphur reduction reaction ( $\text{H}_2 + \text{S} \rightarrow \text{H}_2\text{S}$ ) mediated by *Pyrodicticum occultum*, an anaerobic hyperthermophile isolated from this same hot spring on Vulcano (Amend, 1998). Although we currently lack evidence verifying anaerobic acetic acid catabolism by hyperthermophiles in hydrothermal ecosystems, it seems unlikely that such copious amounts of metabolic energy would go unutilized. I postulate that the isolation and characterization of novel hyperthermophiles using growth media based on chemical analyses of the host environment combined with thermodynamic calculations of potential energy-yielding reactions will provide the missing evidence to confirm this heretofore unknown metabolic type in hyperthermophiles.

## References

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