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THE EFFECT OF TEMPERATURE ON THE METABOLISM OF HYDROGEN AND BUTYRATE IN A TEMPERATE SWAMP ECOSYSTEM

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The effects of substrate concentrations on temperature sensitivity of methanogenesis and butyrate metabolism was investigated in sediment slurries from a permanently waterlogged swamp. Temperature sensitivity decreased with decreasing substrate concentrations parallelling results obtained with axenic methanogenic cultures. H_2 concentrations decreased with decreasing temperatures while the concentration of volatile fatty acids remained fairly unaffected by incubation temperature. The possibility of butyrate metabolism at *in situ* conditions was verified by temperature compensated thermodynamic calculations.

Key words: Methanogenesis, microbiology, wetland ecology

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INTRODUCTION

Natural wetlands of the Northern hemisphere are considered important contributors of atmospheric methane. In these ecosystems, methanogenesis is controlled by physical, chemical, and biological factors and processes among which, substrate availability and seasonal variations in temperature are regarded important when the concentration of inorganic electron acceptors other than CO_2 is low (Conrad et al. 1987, Westermann & Ahring 1987).

Acetate and H_2 concentrations have previously been shown to affect the temperature sensitivity of *Methanosarcina barkeri* increasing the activation energy of acetate and H_2 metabolism with increasing substrate concentration (Westermann et al. 1989).

Besides dependence upon substrate and product concentrations, the ΔG of a reaction is temperature dependent according to the Van´t Hoff equation implicating that the energy yield of exergonic reactions increases with decreasing temperature (as the equilibrium is displaced to the right). This might affect syntrophic degradation of volatile fatty acids such as butyrate, which under standard conditions is an endergonic process.

The purpose of this study was to test whether a temperature compensating mechanism is operative during long-term incubations of wetland slurries, and to investigate whether the substrate and product concentrations measured during these conditions fitted to calculated thermodynamics of butyrate oxidation and methanogenesis from H_2.

MATERIALS AND METHODS

The wetland studied is a 1 ha stand of red alder (*Alnus glutinosa*) located in the Dyrehaven forest north of Copenhagen. The site is permanently waterlogged and alder leaves and twigs constitute the primary carbon and energy source (litter dry mass 335g $m^{-2}a^{-1}$ (Kjøller & Struwe 1980)). Sediments were sampled with corers and brought to the laboratory, diluted with anoxic pore-water (1:1), blended under N_2, and then distributed into 50 ml N_2-flushed serum vials.

Volatile fatty acids and CH_4 were measured by flame ionization gas chromatography. H_2 was measured in the headspace by a Hg vapor atom absorption analyzer (Trace Analytical).

The effect of temperature, substrate and product concentrations on the free energy available to the bacteria carrying out the process:

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$$

was calculated at various butyrate concentrations and temperatures to estimate permissible H_2 partial pressures giving a $\Delta G = 0$. This was carried out by inserting the equation for Gibbs free energy:

$$\Delta G^0 = \Delta H^0 - \Delta S^0 \times T$$

instead of ΔG^0 in the Nernst equation:

$$\Delta G = (\Delta H_0 - \Delta S_0 \times T) + RT \times \ln\left(\frac{[CH_3COO^-]^2 \times [H^+] \times [H_2]^2}{[CH_3CH_2CH_2COO^-]}\right)$$

Solving this equation for $\Delta G = 0$ and with respect to $[H_2]$ leads to:

$$[H_2] = \sqrt{\left(\frac{e^{\left(\frac{-\Delta H^0 + \Delta S^0 \times T}{R \times T}\right)} \times [CH_3CH_2CH_2COO^-]}{[CH_3COO^-]^2 \times [H^+]}\right)}$$

Where ΔH^0 is the standard reaction enthalpy, ΔS^0 is the entropy change of the reaction. T is the temperature in Kelvin, and R is the gas constant (0.008315 kJ x $^0C^{-1}$ x mol^{-1}).

ΔG values of methanogenesis from H_2 and butyrate degradation were calculated by inserting measured *in vitro* concentrations of the relevant compounds in the temperature compensated Nernst equation.

RESULTS

The effect of temperature on the concentration of dissolved H_2, butyrate, and acetate in the swamp slurries is shown in Table 1. The H_2 partial pressure increased with increasing temperature while the differences between volatile fatty acid concentrations were less pronounced although having an opposite tendency compared to the H_2 concentrations.

Table 2 shows Q_{10} values and activation energies for methanogenesis and concentrations of butyrate, acetate and H_2 at two incubation intervals. Q_{10} and hence activation energies decreased with incubation time parallelling the decrease in H_2, acetate and butyrate concentrations.

The effects of temperature on the H_2 partial pressures and butyrate concentrations at which $\Delta G = 0$ (calculated by equation 3) is shown in Fig. 1. With decreasing temperature, either a decrease in H_2 partial pressure or an increase in butyrate concentration is necessary to ensure a negative ΔG of syntrophic butyrate degradation.

In Table 3 the Gibbs free energy from butyrate degradation and methanogenesis from H_2 in the alder swamp is shown. The values were calculated from the measured concentrations (Table 1). All processes are thermodynamically possible yielding a negative ΔG. Despite the variations in ΔG values with temperature, combination of the H_2-producing and H_2-consuming processes yielded almost constant ΔG values, independent of temperature.

Table 1. The effect of temperature on the concentration of important intermediates in the alder swamp. Concentrations were measured after 3–6 months of incubation under static conditions at the various temperatures. Values are means of 9 vials.

	5°C	10°C	15°C	20°C	30°C	37°C
Hydrogen (Pa)	0.16	0.25	0.35	0.32	0.83	1.66
Butyrate (μM)	0.89	0.84	0.65	0.40	0.30	0.19
Acetate (μM)	111	138	103	66.8	82.6	68.1

Table 2. The effect of incubation time on temperature sensitivity Q_{10}, and activation energies and acetate, butyrate, and hydrogen concentrations in the alder swamp. Data from Westermann (1992).

	Q_{10} values	
T °C Interval	2 days	51 days
5–15	6.4	1.5
10–20	5.5	1.8
15–35	3.6	3.1
20–30	2.3	2.2
25–35	1.9	1.8
Average	3.93	2.07
Acetate (μM)	205	50
Butyrate (μM)	60	0.7
Hydrogen (nM)	14.8	4.0
Activation energy (kJ x mol^{-1})	84.26	50.19

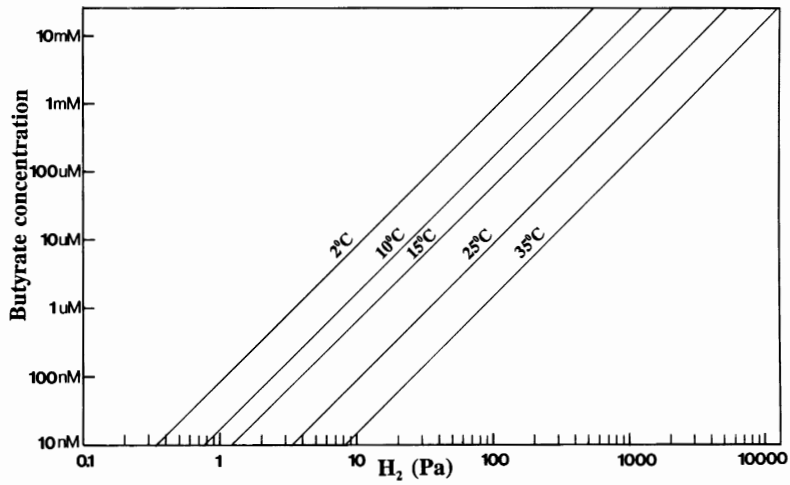

Fig. 1. Effects of temperature on H_2 partial pressures and butyrate concentrations giving a $\Delta G=0$.

Table 3. Gibbs free energy (kJ x mol H_2^{-1}) from butyrate degradation and methanogenesis from H_2 at *in situ* concentrations.

	Temperature					
	2°C	10°C	15°C	20°C	30°C	37°C
$\Delta G_{methanogenesis}$	–5.41	–4.72	–4.01	–2.78	–3.15	–3.53
$\Delta G_{butyrate\ oxidation}$	–6.86	–7.11	–8.27	–10.10	–9.46	–9.28
$\Delta G_{syntrophic\ butyrate\ degradation}$*	–12.27	–11.83	–12.28	–12.88	–12.61	–12.81

* Calculated by addition of ΔG for methanogenesis from H_2 and butyrate oxidation.

DISCUSSION

Cord–Ruwisch et al. (1988) demonstated that H_2 threshold concentrations in H_2-utilizing bacterial cultures decreased with increasing energy yield of the H_2-oxidation process. Therefore, in stable natural ecosystems where the size and composition of the bacterial populations are adjusted to the present conditions and substrates, an approximation between *in situ* and threshold concentrations could be expected. Based upon this assumption, a decrease in ΔG with decreasing temperature should result in a corresponding decrease in the concentrations of substrates under *in situ* conditions. This has been verified for axenic cultures of methanogenic and acetogenic bacteria (Conrad & Wetter 1990). The results from the alder swamp indicate that this mechanism also is operative in natural ecosystems, thereby creating a thermodynamic basis for syntrophic degradation of compounds such as butyrate at low temperatures.

As previously shown (Westermann 1992), methanogenesis in natural ecosystems show a substrate dependent capability to compensate for changing temperature. A model based upon achieved results covering both thermodynamic and kinetic aspects of temperature influence on anaerobic metabolism in temperate wetlands is presently under development.

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