Acetate Inhibition of Methanogenic, Syntrophic 
Benzoate Degradation†

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Acetate inhibited benzoate degradation by a syntrophic coculture of an anaerobic benzoate degrader (strain 
BZ-2) and Methanospirillum strain PM-1; the apparent Kₐ for acetate was approximately 40 mM. The addition 
of acetate resulted in a decrease in the hydrogen concentration in the coculture, indicating that phenomena 
related to interspecies hydrogen transfer affected this value and that the effect of acetate on the benzote-
degrading partner was probably greater than the apparent Kₐ for the coculture suggests.

The availability of nitrate or sulfate as an electron acceptor makes it possible for benzoate to be completely mineralized by a single species despite the absence of molecular oxygen (1, 10, 13). In the absence of such exogenous electron acceptors, the anaerobic oxidation of benzoate is an acetogenic process (2), producing 3 mol of acetate per mol of benzoate oxidized. In addition to this stoichiometry, 6 mol of reducing equivalents are also produced, which probably take the form of 3 mol of H₂.

benzoate⁻ + 7H₂O → 3 acetate⁻ + 3H⁺ + HCO₃⁻ + 3H₂

ΔG°' = 53 kJ (11) (1)

So far, attention has been focused on the hydrogen-producing aspect of this and other acetogenic dehydrogenations. Defined cultures of acetogenic benzoate degraders are routinely grown in the presence of hydrogen-scavenging organisms, which are usually sulfate reducers or methanogens (6, 7, 9, 12). The rationale for this approach is that the chemical equilibrium of equation 1 is strongly to the left, which makes the continuous removal of the reaction products a necessity if the reaction is to sustain prolonged growth of the benzoate-degrading organisms. It has indeed been shown that accumulation of hydrogen greatly inhibits benzoate oxidation (6; manuscript in preparation) and that the phenomenon of interspecies hydrogen transfer (14) is associated equally with benzoate oxidation as it is with propionate or butyrate oxidation (2).

Since acetate and hydrogen are both produced in a stoichiometry of 3 mol of each per mol of benzoate oxidized, they are thermodynamically equivalent as products which inhibit benzoate oxidation. So far, the possibility that these oxidation products are physiologically equivalent has not been experimentally addressed. In the present study, the sensitivity of the acetogenic oxidation of benzoate to acetate is described. Studies were conducted with a syntrophic coculture of a benzoate degrader (strain BZ-2) with a methanogen, Methanospirillum strain PM-1 (9). The coculture was grown under strictly anaerobic conditions in vitamin-amended mineral medium (9) at 35°C with benzoate (10 mM, unless indicated otherwise) as the sole carbon and energy source. Benzoate, acetate, hydrogen, and methane concentrations were determined by chromatography (3, 8).

Benzoate degradation was tightly and stoichiometrically coupled to methane production (unpublished data). Because it was easier and (for us) more accurate to measure methane production than to measure benzoate degradation, we chose methane production as an indirect method of quantifying benzoate degradation.

The influence of acetate on the rate of benzoate degradation was determined by adding various amounts of sodium acetate to actively metabolizing cultures and measuring benzoate degradation (methane production) over time. Late-exponential-phase cultures were used as inoculum (10%, vol/vol), and cultures were incubated for 3 days with substrate before amendments were made to the media. This procedure resulted in acetate concentrations of less than 5 mM before the amendments were made. After the methane production rate over 24 h had been established for each individual culture, sodium acetate from anaerobic stock solutions was added and the methane production rate was determined over the next 24 to 36 h. Thus, the measurements were well within the 3-day doubling time of the coculture (4). These methane production rates were divided by the rates measured before the amendments were made, and the resulting value was divided by a similarly obtained value for the unamended controls, which had received anaerobic distilled water instead of acetate. Each treatment was done in at least two bottles. This approach allowed for an accurate estimation of the influence of acetate on the rate of benzoate degradation. Control experiments were performed similarly, but sodium chloride or choline chloride was added instead of sodium acetate.

The results indicate that sodium acetate inhibited the rate of benzoate degradation. The addition of 20 mM sodium acetate resulted in a 40% inhibition of the benzoate oxidation rate (Fig. 1A). Since acetate in these experiments had been added as a sodium salt, experiments were conducted to distinguish between the impacts of sodium and acetate. The results indicate that NaCl concentrations of up to 50 mM do not significantly affect the rate of benzoate degradation (Fig. 1B). To evaluate possible antagonistic effects between chloride and sodium in the NaCl experiment, we also included an experiment with choline chloride; chloride concentrations of up to 50 mM had no effect on the rate of benzoate degradation (Fig. 1C.).
Here this may have occurred, since the hydrogen partial pressure values in acetate-amended cultures dropped to as much as 30% below those in the unamended controls (Fig. 1A, inset). This phenomenon can be explained by the fact that the hydrogen concentration in the coculture is a function of the activities of both the hydrogen-producing and the hydrogen-consuming partners in the consortium. Inhibition of the hydrogen-producing partner, therefore, resulted in excess hydrogen removal capacity (because the hydrogen consumption potential of *Methanospirillum* strain PM-1 was not affected by the presence of 50 mM sodium acetate [unpublished data]) and hence resulted in a new, lower steady-state concentration of hydrogen in the coculture. Since hydrogen is an inhibitor of the rate of benzoate degradation, this lowering of the hydrogen concentration allows for a somewhat higher benzoate degradation rate, thus partially offsetting the negative effect of the presence of acetate. As a result of this interplay, the apparent $K_i$ for acetate estimated for the consortium could be different from the apparent $K_i$ for acetate estimated for the benzoate degrader alone.

Determinations of the half-saturation constants of hydrogen-producing partners in syntrophic cocultures may be subject to the same source of uncertainty; thus these constants should be measured at constant hydrogen concentrations. If this precaution is not taken, measured apparent half-saturation constants can only be valid for the coculture as a whole. We found that the rate of benzoate degradation in the coculture was independent of the benzoate concentration at and above benzoate concentrations of 200 mM and that at those concentrations the hydrogen concentration was independent of the benzoate concentration. This indicates that the apparent half-saturation constant for benzoate degradation was below 200 μM.

The thermodynamic equivalence of acetate and hydrogen, combined with the inhibitory effect of acetate on benzoate degradation at ecologically and physiologically realistic concentrations, makes it tempting to speculate upon the feasibility of an interspecies-acetate-transfer-based strategy to grow the benzoate oxidizer in the absence of a hydrogen-scavenging system. The main concern for the success of such an approach is whether the $\mu_{\text{max}}/K_i$ ratio for acetate of an acetate-scavenging organism will be high enough to allow the organism to bring the acetate concentration down to the micromolar level and still grow.

While $H_2$ and acetate are thermodynamically equivalent (i.e., for the conditions of this coculture, any order of magnitude change in either product changes the $\Delta G'$ by 17.8
VOL. 54, 1988

kJ), this equivalence cannot be directly extrapolated to apply to physiological equivalence. Although both products of benzoate oxidation act at a physiological level as inhibitors, the concentrations at which these products have comparable effects are vastly different; the apparent \( K_a \) for acetate is approximately 40 mM, while the apparent \( K_a \) for \( H_2 \) is lower by a factor of more than 100 (manuscript in preparation).

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LITERATURE CITED


