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Effects of 2-Bromoethanesulfonic Acid and 2-Chloroethanesulfonic Acid on Acetate Utilization in a Continuous-Flow Methanogenic Fixed-Film Column

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2-Bromoethanesulfonic acid (BESA) and 2-chloroethanesulfonic acid (CESA) have been reported to be potent inhibitors of methane formation during methanogenic decomposition in batch cultures. However, in a laboratory-scale continuous-flow methanogenic fixed-film column containing a predominance of acetate-decarboxylating methanogens, BESA at 6×10^{-4} M produced only a 41% inhibition of acetate utilization, and CESA at 5.4×10^{-4} M produced a 37% inhibition of acetate utilization. BESA and CESA concentrations were not monitored in the effluent, so their fate is unknown. The organisms in the column were capable of degrading trace halogenated aliphatic compounds (~30 μg/liter) with acetate (100 mg/liter) as the primary substrate. Previous exposure of the cells to halogenated organic compounds may have conferred resistance to BESA and CESA. Degradation of the inhibitor compounds is another possible explanation for the observed effects.

As structural analogs of coenzyme M (2-mercaptoethanesulfonic acid), 2-bromoethanesulfonic acid (BESA) and 2-chloroethanesulfonic acid (CESA) have been reported to competitively inhibit the methyl transfer reaction at the terminal reductive step during methane formation in methanogens using H_2 and CO_2 (2). A 50% inhibition of methyl reductase was observed with 10^{-6} M BESA and 10^{-5} M CESA. BESA has also been shown to inhibit methane formation by an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium (7, 8). At a concentration of 5×10^{-7} M BESA, methane formation by the acetate-cleaving organism attained only one-half of its normal rate. Nearly complete inhibition of methane formation occurred at 10^{-5} M BESA. BESA was used by Healy et al. (3) to inhibit gas production and enhance the buildup of organic intermediates in batch cultures of a mixed culture of bacteria capable of degrading ferulic acid at a concentration of 1,000 mg/liter to methane and carbon dioxide under strict anaerobic conditions. Partial inhibition of methane formation occurred at 10^{-7} to 10^{-5} M BESA, and 10^{-3} M BESA completely stopped methane production. Oremland (5) found that addition of BESA (~10^{-3} M) completely inhibited methane and ethane evolution in anaerobic sediments incubated under batch conditions. These findings were obtained from batch studies and indicate that very low concentrations of BESA and CESA can block the pathway of methane formation. In contrast, we report here that concentrations of BESA or CESA of 1×10^{-4} to 6×10^{-4} M only partially inhibited the activity of methanogens using acetate in a continuous-flow methanogenic fixed-film reactor.

A continuous-flow, laboratory-scale column system with anaerobic fixed-film bacteria was used to study the transformations of one- and two-carbon halogenated aliphatic compounds (chloroform, bromodichloromethane, dibromochloromethane, bromoform, carbon tetrachloride, 1,2-dichloroethane, 1,1,1-trichloroethane, and tetrachloroethylene) at low concentrations (~30 μg/liter) with acetate (100 mg/liter) as the primary substrate (1). Nearly complete degradation of the trace halogenated aliphatic compounds occurred in this methanogenic column. Chloroform, carbon tetrachloride, and 1,2-dichloroethane were almost completely oxidized to CO_2, confirming removal by biooxidation. CO_2 was the only electron acceptor available (O_2, NO_3^-, and SO_4^{2-} were not present in the feed), so an enrichment culture of methanogens

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capable of using acetate developed in the column. Epifluorescence and scanning electron microscopy indicated a predominance of one organism that resembled the acetate-decarboxylating methanogen *Methanothrix soehngenii* as described by Zehnder et al. (8).

In an effort to determine whether acetate-decarboxylating methanogens were responsible for the transformation of the halogenated aliphatic compounds, BESA and CESA (Aldrich Chemical Co.) were added to the influent of the methanogenic column at concentrations between 1×10^{-4} and 6×10^{-4} M to inhibit the activity of methanogens and block the utilization of acetate to test the effect on biodegradation of the halogenated compounds. The application of each concentration level of BESA or CESA to the continuous-flow methanogenic column was maintained for 3 weeks. BESA and CESA at the concentrations used reduced acetate utilization by a maximum of 41% (Table 1). The radioactivity in the effluent was composed of nondegraded acetate and CO_2. The acetate radioisotope was labeled only on the carboxyl carbon, so methanogenic decomposition yielded only $^{14}CO_2$ and no radioactive methane (8). The extent of removal of the halogenated aliphatic compounds was not affected. Gas production was observed at all concentrations of BESA and CESA used. Less than 0.5 ml of gas was produced each day, so that gas composition was not determined. BESA or CESA addition at concentrations greater than 6×10^{-4} M (125 mg/liter) was not administered.

These results show that concentrations of BESA and CESA that have been reported to significantly inhibit methane formation in batch cultures caused only partial inhibition of acetate utilization in the continuous-flow methanogenic column. The active bacterial concentration was estimated to be 0.05 mg of cells per ml of liquid, or a total of 2.3 mg of cells within the column. This estimate was computed by assuming a growth yield of 0.035 g of cells per g of acetate (4), a first-order endogenous decay coefficient of 0.03 day^{-1} (4), and an acetate loading rate of 2 mg/day. The substrate and organism concentrations in the methanogenic column system were much lower than those typically reported in batch experiments, for which BESA or CESA was a powerful inhibitor of methanogenesis. The column system was operated as a plug flow reactor, so the toxicity response to the BESA or CESA should have been similar to that of a batch system. The small amount of gas produced provided little mixing. A thin biofilm (one or two cells deep) developed on the glass beads, so mass transfer limitation with these inhibitory compounds was not a likely explanation for the limited inhibition of methanogenesis in the column. A thin biofilm was the result of low organic loading and a low bacterial growth yield under anaerobic conditions.

TABLE 1. Removal of acetate in a methanogenic fixed-film column in the presence of BESA or CESA

Inhibitor	Inhibitor concn (M)	Effluent acetate concn (mg/liter)	Acetate removal[a] (%)	Inhibition of acetate utilization (%)
BESA	0[b]	37 ± 2	63 ± 3	0
	1×10^{-4}	38 ± 2	62 ± 3	2
	6×10^{-4}	63 ± 3	37 ± 4	41
CESA	5.4×10^{-4}	60 ± 3	40 ± 4	37

[a] The influent acetate concentration was 100 ± 5 mg/liter.

[b] Steady-state condition before the addition of BESA or CESA to the methanogenic column influent.

Smith and Mah (6) found that a single exposure of *Methanosarcina* sp. strain 227 to 24 μM BESA was sufficient to produce cultures resistant to 240 μM BESA. Hence, a possible explanation for the partial inhibition of acetate utilization when the inhibitor compounds were administered to the column was that previous exposure of the methane bacteria to low concentrations of halogenated aliphatic compounds resulted in a greater tolerance to BESA or CESA. Because the organisms in the column were capable of degrading trace chlorinated aliphatic compounds to mineralized end products, another possibility was that BESA or CESA was degraded in the column. BESA and CESA concentrations were not monitored in the effluent, so their fate in unknown. Bromide concentrations in the effluent, measured with a bromide-specific electrode, were below detectable levels (<0.1 mg/liter) both before and during BESA application. This indicated that degradation of the inhibitor compound to mineralized end products was unlikely. However, transformation of BESA to other brominated organic intermediates cannot be ruled out. A single transformation may have been sufficient to eliminate the inhibitory effects of BESA and CESA on methanogenic activity.

In summary, concentrations of BESA or CESA in the range of 1×10^{-4} to 6×10^{-4} M caused only partial inhibition of the activity of methanogens using acetate at a concentration of 100 mg/liter in a continuous-flow methanogenic fixed-film column operated with a 2-day actual detention time. These concentrations of BESA or CESA have been reported to cause nearly complete inhibition of methanogenic activity under batch conditions (2, 7) with significantly higher substrate and organism concentrations than used here. Trace halogenated aliphatic

compounds were almost completely degraded in the methanogenic column, so previous exposure of the cells to these compounds may have conferred resistance to BESA and CESA. Therefore, caution must be exercised when using BESA or CESA to bring about inhibition of methanogens in a continuous-flow operation, especially if the organisms have been previously exposed to halogenated organic compounds, to ensure that high enough concentrations are used and that the extent of inhibition is closely monitored.

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