Toxicity and Biodegradation of Formaldehyde in Anaerobic Methanogenic Culture

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Abstract: Formaldehyde is present in several industrial wastewaters including petrochemical wastes. In this study, the toxicity and degradability of formaldehyde in anaerobic systems were investigated. Formaldehyde showed severe toxicity to an acetate enrichment methanogenic culture. As low as 10 mg/L (0.33 m*M*) of formaldehyde in the reactor completely inhibited acetate utilization. Formaldehyde however, was, degraded while acetate utilization was inhibited. Degradation of formaldehyde (Initial concentration $\leq$30 mg/L) followed Monod model with a rate constant, k, of 0.35–0.46 d^{-1}. At higher initial concentrations ($\geq$60 mg/L), formaldehyde degradation was inhibited and partial degradation was possible. The initial formaldehyde to biomass ratio, S_0/X_0, was useful to predict the degradation potential of high formaldehyde concentrations in batch systems. When $S_0/X_0 \leq 0.1$, formaldehyde was completely degraded with initial concentration of up to 95 mg/L; when $S_0/X_0 \geq 0.29$, formaldehyde at higher than 60 mg/L was only partially degraded. The inhibition of formaldehyde degradation in batch systems could be avoided by repeated additions of low concentrations of formaldehyde (up to 30 mg/L). Chemostats (14-day retention time) showed degradation of 74 mg/L-d (1110 mg/L) of influent formaldehyde with a removal capacity of 164 mg/g VSS-day. A spike of 30 mg/L (final concentration in the chemostat) formaldehyde to the chemostat caused only a small increase in effluent acetate concentration for 3 days. But a spike of 60 mg/L (final concentration in the chemostat) formaldehyde to the chemostat resulted in a dramatic increase in acetate concentration in the effluent. The results also showed that the acetate enrichment culture was not acclimated to formaldehyde even after 226 days. *Biotechnol Bioeng* **55**: 727–736, 1997.

Keywords: acetate; anaerobic; biodegradation; formaldehyde; methanogenic; toxicity

INTRODUCTION

Formaldehyde (HCHO) is found in wastewaters from resin manufactures (Goeddertz et al., 1990) and petrochemical plants (Sharma et al., 1994). Formaldehyde is also used as an active ingredient in preservatives and disinfectants. Its disposal after use may impair sewage treatment plant performance, due to its antimicrobial effects. The demonstration of its carcinogenicity in laboratory animals led to a heightened concern that formaldehyde may present a similar carcinogenic risk to humans (Swenberg et al., 1980).

Formaldehyde can inhibit the growth of aerobic bacteria at very low concentrations. As low as 1–2 mg/L of formaldehyde inhibited *Pseudomonas fluorescens* and *E. coli* growth on glucose (Verschueren, 1983). The inhibition threshold of formaldehyde for *Pseudomonas putida* growth was 14 mg/L (Bringman and Kuhn, 1980). Thirty mg/L formaldehyde inhibited oxygen consumption of activated sludge (Gerike and Gode, 1990). In spite of the inhibition, formaldehyde is degradable in aerobic cultures. Gerike and Gode (1990) reported formaldehyde degradation at 2–5 mg/L in activated sludge. Bonastre et al. (1986) observed the partial degradation of formaldehyde at 2100 mg/L in activated sludge. *Pseudomonas putida* A2 degraded 250 mg/L of formaldehyde as the sole carbon source (Adroer et al., 1990). *Halomonas* sp. MA-C degraded 125 mg/L formaldehyde in a salt medium (Azachi et al., 1995).

Anaerobic degradation is an alternative to aerobic degradation for formaldehyde removal from wastewaters. However, limited information is found in the literature on anaerobic formaldehyde degradation. The reported minimal formaldehyde concentration that caused inhibition of methanogenesis was 10–180 mg/L (Chou et al., 1978b; Hickey, et al., 1987; Hovious, et al., 1973; Parkin et al., 1983; Parkin and Specce, 1982). The extent of the inhibition in methane production is proportional to formaldehyde concentrations. Formaldehyde concentrations that resulted in 50% reduction in methanogenesis activity varied from 20 to 254 mg/L (Chou et al., 1978a, b; Hickey, et al., 1987; Hovious, et al., 1973; Pearson et al., 1980; Todini and Pol, 1992). Both complete and partial inhibitions on methane production in anaerobic systems were reported. Formaldehyde exhibited reversible toxicity in methanogenic cultures and recovery in methanogenesis was observed (Parkin et al., 1983; Parkin and Speece, 1982; Hickey et al., 1987). Many previous studies on formaldehyde toxicity were limited to measurement of only the gas production; the concentrations of

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formaldehyde in the cultures were not measured in those studies. Therefore, it was not always clear whether the recovery of the methanogens was due to acclimation or to degradation of formaldehyde. Effects of continuous feeding of formaldehyde on anaerobic systems were studied by Parkin et al. (1983) and Bhattacharya and Parkin (1988). An anaerobic filter tolerated up to 400 mg/L of formaldehyde in the feed. However, 7 days of feeding 500 mg/L of formaldehyde (in the feed) to the filter caused the cessation of gas production (Parkin et al., 1983). No degradation of formaldehyde in the filter was reported. Methanogenic chemostats with a 40-day solids retention time (SRT) tolerated 400 mg/L of influent formaldehyde, while chemostats with a 15-day-SRT tolerated only 100 mg/L of influent formaldehyde. Thirty-five to 80 percent of formaldehyde was removed by the methanogenic chemostats with 15 to 40 days of SRT (Bhattacharya and Parkin, 1988).

Applications of anaerobic systems for treatment of actual formaldehyde wastewaters were reported by Goeddertz et al. (1990) and Sharma et al. (1994). A methanogenic system achieved >95% COD reduction and 95–98% removal of 375 mg/L of formaldehyde in the feed when treating a formaldehyde wastewater from a chemical plant (Sharma et al., 1994). A pilot-scale, anaerobic, biological activated carbon system was tested to treat a high strength wastewater containing phenol, formaldehyde and methanol. The system tolerated 2–3 g/L of influent formaldehyde and showed a 98% COD removal (Goeddertz et al., 1990). However, the formaldehyde removal efficiency in the system was not reported.

The literature shows that anaerobic systems are applicable for treatment of formaldehyde wastewaters. However, a wide range of reported formaldehyde inhibitory concentrations, a limited understanding of formaldehyde toxicity, and the lack of anaerobic formaldehyde degradation data make further research necessary for appropriate design of anaerobic systems for treatment of wastewaters containing formaldehyde. The objectives of the present research were to investigate the toxicity and degradability of formaldehyde in acetate enrichment methanogenic cultures.

MATERIALS AND METHODS

Methanogenic Culture

The acetate enrichment culture for subsequent serum bottle and chemostat studies was developed in a 2-L (culture volume 1.5-L) chemostat (control or seed) at 35 ± 1°C at a designed 14-day hydraulic retention time (HRT) with an acetate loading rate of 500 mg/L-d. This chemostat was seeded from a stock acetate enrichment culture which has been maintained by fill-and-draw method for about 10 years with nutrients and acetate as the sole carbon source. The nutrient solution added to the stock culture and the seed chemostat contained (in mg/L): NH_4Cl, 1200; $MgCl_2$, 500; KCl, 400; $CaCl_2 \cdot 2H_2O$, 25; $(NH_4)_2HPO_4$, 80; $FeCl_2 \cdot 4H_2O$, 40; $CoCl_2 \cdot 6H_2O$, 2.5; Kl, 2.5; $(NaPO_3)_6$, 10; $MnCl_2 \cdot 4H_2O$, 0.5; NH_4VO_3, 0.5; $ZnCl_2$, 0.5; $Na_2MoO_4 \cdot 2H_2O$, 0.5; H_3BO_3, 0.5; $NiCl_2 \cdot 6H_2O$, 0.5; Cysteine, 10; $NaHCO_3$, 6000. For the seed chemostat, to avoid precipitation in the feed lines, $(NH_4)_2HPO_4$, $FeCl_2 \cdot 4H_2O$, $(NaPO_3)_6$, cysteine and $NaHCO_3$ were prepared in a separate solution and added by daily manual injections (10 mL). Feed solution to the seed chemostat was prepared in a 1 L volumetric flask by adding acetic acid to the nutrient solution (without $(NH_4)_2HPO_4$, $FeCl_2 \cdot 4H_2O$, $(NaPO_3)_6$, cysteine and $NaHCO_3$) using a 10 mL pipette. The acetate, biomass measured as volatile suspended solids (VSS), pH in the chemostat and feed flow rate were monitored. The average methane production from the seed chemostat was about 274 mL/d (methane was about 55% of total gas volume), which was about 95% of the calculated stoichiometric production.

Serum Bottle Studies

Formaldehyde Toxicity and Degradation in the Unacclimated Acetate Enrichment Culture

Formaldehyde toxicity and degradation were first tested in batch serum bottles. The serum bottles (150 mL) were first filled with tap water, which was then displaced with a 20% CO_2: 80% N_2 gas mixture and capped with rubber stoppers. For tests with low biomass concentrations (VSS = 220–360 mg/L), the acetate enrichment culture (50–75 mL) from the seed chemostat was anaerobically transferred to the serum bottles; for tests with high biomass concentrations (VSS ~1,000 mg/L), the stock acetate enrichment culture (50–75 mL) was anaerobically transferred to the serum bottles. The acetate enrichment culture in the serum bottles was fed 500 mg/L of acetic acid daily for 4–7 days, until the variation in daily total gas production was less than 10%, before spiking formaldehyde (37% formaldehyde with 10–15% methanol, Sigma Chemical CO., St. Louis, MO). After spiking formaldehyde and acetic acid to serum bottles, initial biomass (VSS), formaldehyde, and acetate concentrations were measured. All the tests were conducted in duplicates. During the tests, 1–2 mL of the culture were withdrawn from the bottles at selected time intervals. The samples were filtered immediately through a 0.45 μm membrane filter and stored at 4°C for subsequent analysis of acetate and formaldehyde. The abiotic test was performed after autoclaving the culture at 120°C for 20 minutes.

Biochemical Methane Potential (BMP) Assay

The methane production from degradation of formaldehyde in the acetate enrichment culture was determined following the method described by Owen et al. (1979). Four hundred mL of the acetate enrichment stock culture was anaerobically transferred to 550 mL serum bottles. The bottles were incubated at 35°C for one day to let the culture utilize any residual acetate. Then the bottles were flushed with the 20% CO_2:80% N_2 gas mixture for a few minutes to remove pos-

sible residual methane. Headspace samples were analyzed to ensure that methane was below detection limit. Twenty two μL of formaldehyde was spiked to the bottles for five times. The gas was sampled with glass syringes (5 mL) and the methane content was measured each time. Methane production during the interval between two sampling was calculated using the following formula:

$$(V_h + V_{i,\ i-l})\ m_i - V_h m_{i-l}$$

Where, V_h is the headspace volume of the serum bottle (mL), $V_{i,\ i-l}$ is the gas produced between the ith and $i - l$th sampling (mL), m_i and m_{i-l} are the methane percentages in the headspace at the ith and $i - l$ sampling, respectively. Total methane production was the sum of methane produced in all sampling intervals.

Formaldehyde Toxicity and Degradation in the Acetate Enrichment Culture after Acclimation

The ability of the acetate enrichment culture to acclimate to formaldehyde was investigated by monitoring both acetate and formaldehyde degradation in the serum bottles by the acetate enrichment culture (50–75 mL) from Chemostats I and II after respectively, 226 days and 84 days of acclimation, as described in the next section. All these tests were conducted in duplicates or triplicates.

Acclimation and Formaldehyde Degradation Studies in Chemostats

Three chemostats with the acetate enrichment culture were developed in this study to investigate the effects of continuous addition of formaldehyde on the acetate enrichment culture, acclimation of the culture to formaldehyde, and formaldehyde removal in continuous reactors. All three chemostats were inoculated with the acetate enrichment culture from the seed chemostat and were initially operated under the same conditions as the seed chemostat. Feed solutions were prepared in a 1 L volumetric flask by adding acetic acid and formaldehyde to the nutrient solution using 10 mL and 1 mL pipettes, respectively. The effluent acetate, formaldehyde, pH, volatile suspended solids, and flow rate were monitored. The flow rate varied within 10% of the designed value and pH varied between 6.8–7.2. Chemostat I was maintained with a feed of 7.5 g/L of acetic acid and nutrients for 103 days. On day 104, 100 mg/L of formaldehyde was added to the feed solution. The feed formaldehyde was increased in steps up to 1110 mg/L during the 226 days of the experiment. Chemostat II was maintained with a feed of 7.5 g/L of acetic acid for the first 37 days before feeding 370 mg/L of formaldehyde for 22 days. From day 60, it was fed 740 mg/L of formaldehyde for 71 days. The feed formaldehyde concentrations for Chemostat II were selected based on the results from Chemostat I. Chemostat III was used to study the effect of formaldehyde shock loading on the performance of the chemostat. After 93 days of operation, Chemostat III was spiked with 30 and 60 mg/L of formaldehyde (final concentrations in the chemostat).

Analytical Methods

Biomass was measured as volatile suspended solids (VSS) following Standard Methods (2540E) (APHA, 1992). Acetic acid was measured with a Shimadzu 14A GC equipped with a flame ionization detector and a 2 mm i.d. × 2 m glass column packed with 80/120 Carbopack B-DA/4% Carbowax 20 M (Supelco, Bellefonte, PA). The column temperature was set at 175°C and the injector and detector temperatures were both set at 200°C; the carrier gas was zero grade helium. The filtered samples, acidified with solid oxalic acid to pH < 2, were injected into the GC. The detection limit for acetic acid was 1 mg/L.

Formaldehyde was measured with HPLC after derivatization with 2,4-dinitrophenylhydrazine (DNPH) (Kieber and Mopper, 1990). The reagent was prepared by dissolving 20 mg of recrystallized DNPH in 15 mL of concentrated HCl (~12 M), water, and acetonitrile (2:5:1 by volume). Carbonyl contamination contained in the DNPH reagent solution was removed by extraction with 2 mL of carbon tetrachloride. Samples were first properly diluted to the range of 0.05 − 1.0 mg/L and then derivatized. Fifty μL of reagent solution was added to a 1 mL diluted sample in a 4 mL glass vial. The capped vials were shaken briefly and the reaction was allowed for 1 hour at ambient temperature (~25 °C). A 20-μL aliquot was injected into the HPLC system (Waters Model 428) with a UV detector and a Nova-Pak C_{18} column (Millipore Corporation, Milford, MA). The eluent was acetonitrile (40%) and deionized water (60%) mixture. The flow rate was 1 mL/min and the detection wavelength was 365 nm. The detection limit for formaldehyde was 0.05 mg/L.

Methane in gas phase was analyzed with a Shimadzu 8A GC equipped with a thermal conductivity detector and a 8′ × 1/8″ stainless steel column packed with 100/120 HayeSep D (Supelco, Bellefonte, PA). The oven temperature was set at 50°C and the injector and detector temperatures were both set at 140°C; the carrier gas was zero grade helium. The detection limit for methane was 0.05%.

RESULTS AND DISCUSSION

Formaldehyde Toxicity and Degradation in Serum Bottles with the Unacclimated Culture

In the unacclimated culture with a VSS of 203–240 mg/L, as low as 10 mg/L (0.33 m*M*) of formaldehyde in the serum bottle inhibited acetate utilization (Figure 1a). With higher formaldehyde (i.e., 20 and 27 mg/L in the serum bottle), the inhibition time was longer. This observation was similar to other studies (Hickey et al., 1987; Parkin et al., 1983; Parkin and Speece, 1982). Although acetate degradation was inhibited, the formaldehyde was degraded without any lag

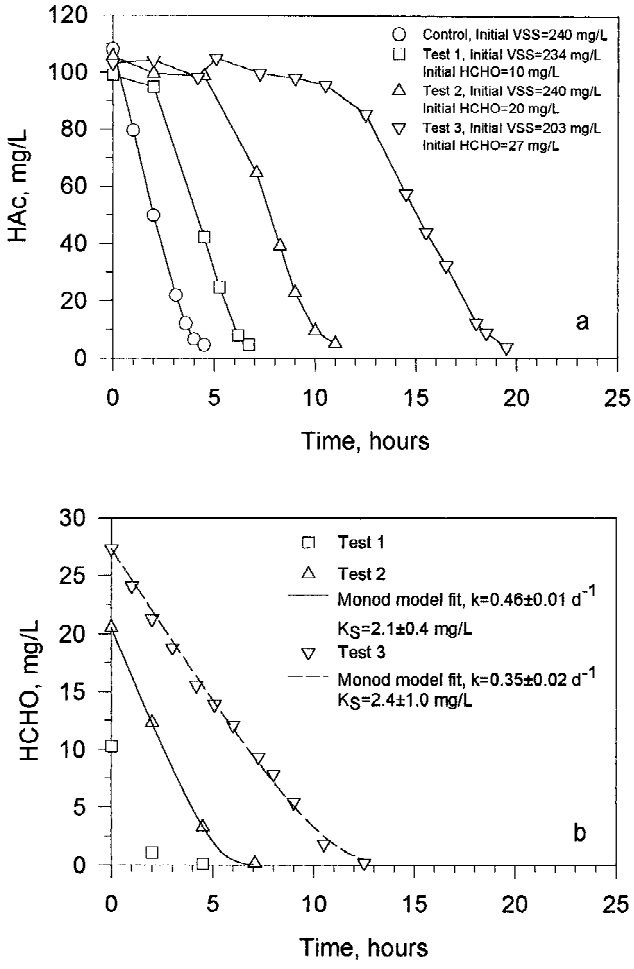

Figure 1. (a) Inhibition of acetate degradation by formaldehyde in the unacclimated acetate enrichment culture. (b) Formaldehyde degradation in the unacclimated acetate enrichment culture.

(Figure 1b). The degradation rate fit the Monod model $dS/dt = -kSX/(K_s + S)$ (where, S and X are formaldehyde and biomass concentrations, respectively), with the maximum specific rate constant k of 0.35–0.46 day^{-1} and Monod half-saturation constant K_s of 2.1–2.4 mg/L. The results showed that the acetate degradation was inhibited in these systems (VSS = 203–240 mg/L) when the formaldehyde in the culture exceeded about 5 mg/L. After the formaldehyde was almost removed from the culture, the acetate degradation started even though the rates of degradation were lower than that in the control bottles. The average acetate degradation rates after inhibition were 2.7, 2.2, 1.9, and 1.4 mg HCHO/mg VSS-day in the control and test bottles with 10, 20, and 27 mg/L of formaldehyde, respectively. This showed that the acetate enrichment culture did not fully recover in the short period after the removal of formaldehyde.

Several researchers reported reversible inhibition of formaldehyde on methanogenesis. Parkin and Speece (1982) reported that methane production from acetate was completely inhibited by 150 mg/L of formaldehyde for the first 40 days and fully recovered in 50 days. Hickey et al. (1987) also found that 10 mg/L of formaldehyde inhibited the methane production rate for 4 hours in an anaerobic sludge. The inhibition time and recovery for 10, 20, and 30 mg/L of formaldehyde in their study were very similar to that in this study. They suggested that low concentrations of formaldehyde (<30 mg/L) inhibited only the hydrogen to methane pathway resulting in accumulation of hydrogen gas whereas high concentrations of formaldehyde (>50 mg/L) also inhibited methanogenesis from acetate. Our results show that low concentrations of formaldehyde (as low as 10 mg/L) did inhibit methanogenesis from acetate. Our data showed that a long time (i.e., 4.5, 7.1, and 12.5 hrs) was needed to degrade high concentrations of formaldehyde (i.e., 10, 20, and 27 mg/L). The recovery of the acetate enrichment culture started only after almost complete degradation of formaldehyde as shown in Figure 1.

Abiotic tests were conducted to determine the extent of formaldehyde removal by sorption, volatilization, and chemical transformation. Figure 2 shows that only 10% of 30 mg/L of formaldehyde was removed in the triplicate abiotic test bottles and almost all of 26 and 62 mg/L of formaldehyde was removed in the bottles with the acetate enrichment culture, indicating biodegradation as the main mechanism of formaldehyde removal.

At initial concentrations of up to 27 mg/L, formaldehyde degradation rates were similar and complete degradation occurred (Figures 1b and 2). However, the formaldehyde degradation rate decreased when the initial concentration was increased to 62 mg/L. Degradation rate further decreased and formaldehyde was only partially degraded when its initial concentration was increased to 95 mg/L (Figure 2). To avoid the inhibition of formaldehyde degradation, it seemed necessary to maintain the formaldehyde concentration below 30 mg/L in the serum bottles. Figure 3 shows the formaldehyde degradation with repeated additions of low concentrations (about 20 mg/L in the reactor) of formaldehyde. The total amount of formaldehyde degraded was about 100 mg/L over a period of about 105 hours with a VSS of 340 mg/L. In contrast, as discussed later, when 100 mg/L of formaldehyde was added as a single dose to the acetate enrichment culture, less than 50% of the formaldehyde was degraded with a VSS of 330 mg/L (Figure 5b). These results demonstrated the ability of the acetate enrichment culture to degrade large amounts of formaldehyde when it was repeatedly added at low concentrations. Adroer et al. (1990) also reported that step additions of low concentrations of formaldehyde (<200 mg/L) prevented the decline of *Pseudomonas* cell population during formaldehyde degradation and resulted in high degradation rates.

BMP Results

To determine the ultimate fate of formaldehyde in the acetate enrichment culture, methane production from formaldehyde was determined. Figure 4 shows that 26 and 28 mL methane were produced from total 41 mg of formaldehyde in the two test bottles. Only 2.6 and 4.7 mL of methane were

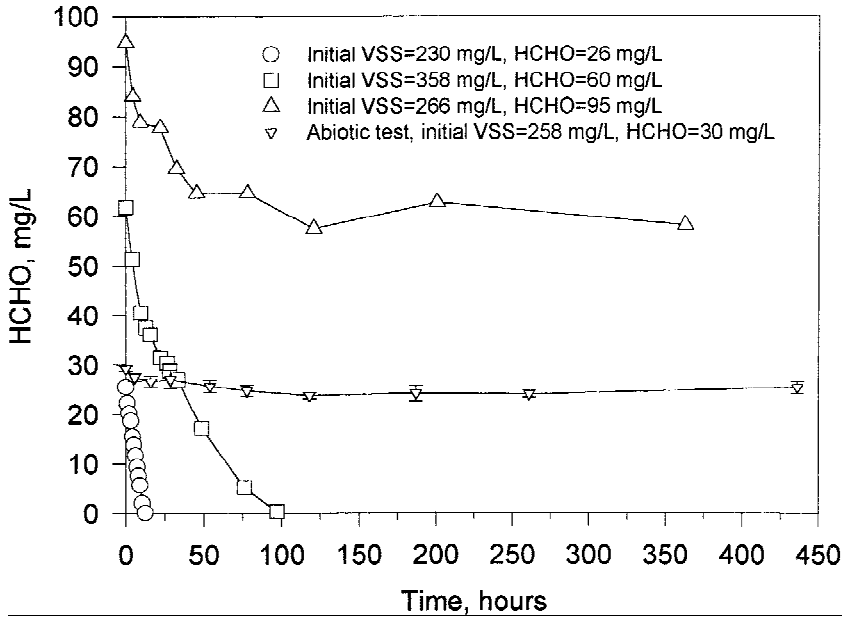

Figure 2. Formaldehyde degradation and effects of its initial concentrations on the unacclimated acetate enrichment culture.

produced in the controls without formaldehyde. It should be noted that the 37% formaldehyde solution contains an average of 12.5% of methanol as mentioned in the Material and Methods section. So 14 mg of methanol was also fed along with 41 mg of formaldehyde. The stoichiometric methane production (Speece, 1996) from 14 mg of methanol is 8 mL at 35°C and 1 atm (1.5 mg COD/mg methanol × 14 mg methanol × 0.395 mL CH_4/mg COD = 8 mL CH_4). So the calculated methane production from 41 mg formaldehyde in the two test bottles was 15 mL (i.e. 26 mL − 2.6 mL − 8 mL = 15 mL) and 15 mL (i.e. 28 mL − 4.7 mL − 8 mL = 15 mL). These values are 88% of the stoichiometric methane from 41 mg formaldehyde (1.067 mg COD/mg HCHO × 41 mg HCHO × 0.395 mL CH_4/mg COD = 17 mL). The results demonstrated that formaldehyde was converted to methane by the acetate enrichment culture.

Initial Formaldehyde to Biomass Ratio

Formaldehyde degradation was tested at different biomass concentrations and the results are shown in Figure 5. With

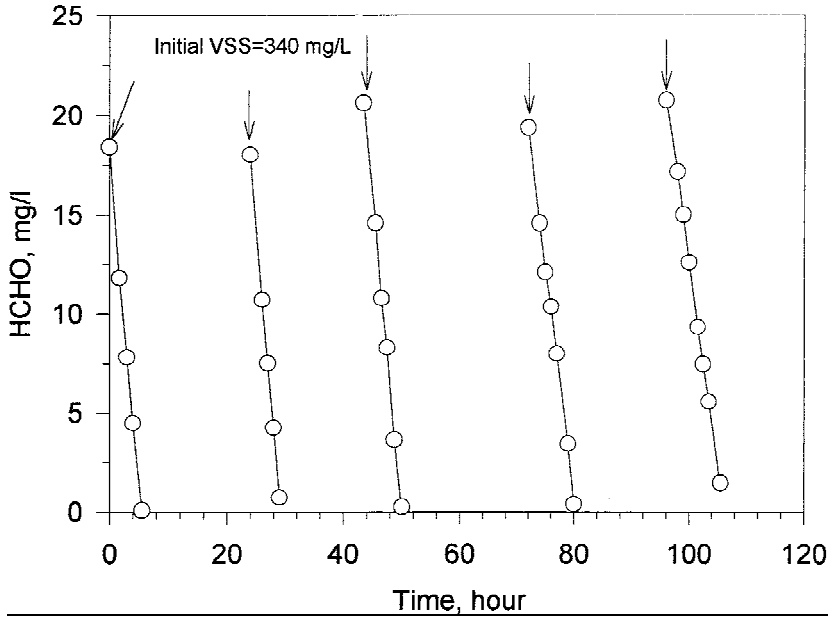

Figure 3. Formaldehyde degradation by repeated spiking to the unacclimated acetate enrichment culture. Each arrow represents a 20 mg/L addition of formaldehyde.

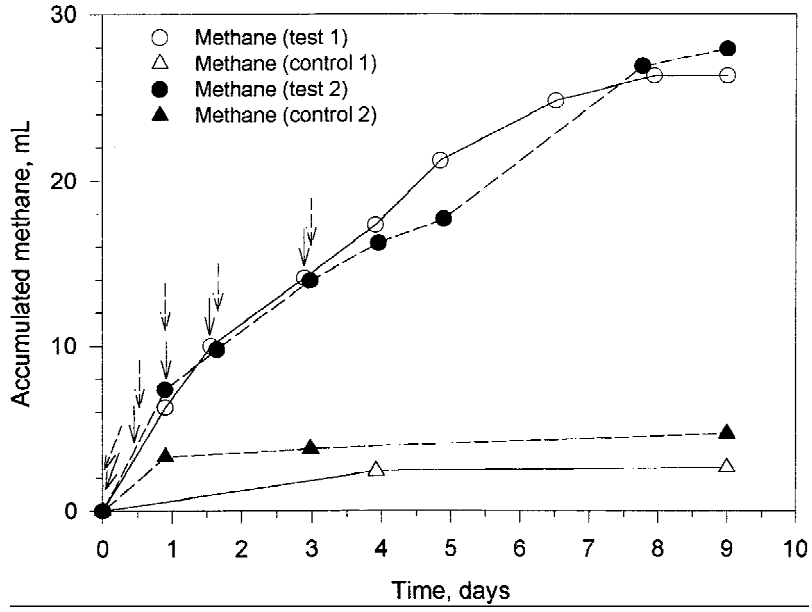

Figure 4. Methane production from formaldehyde degradation in the unacclimated acetate enrichment culture. Each arrow represents a spike of 8.14 mg formaldehyde along with 2.75 mg methanol. The solid and broken arrows represent spikes in test 1 and test 2, respectively.

230 mg/L of VSS, the acetate enrichment culture did not completely degrade 68 mg/L of formaldehyde in 420 hours. With 358 mg/L of VSS, however, 62 mg/L of formaldehyde was completely degraded in about 100 hours. Similarly, the acetate enrichment culture with a low biomass (330 mg VSS/L) only partially degraded about 100 mg/L of formaldehyde in 384 hours; with a high biomass (990 mg VSS/L), complete degradation of almost the same concentration of formaldehyde was observed in about 200 hours. For higher formaldehyde concentrations (180–220 mg/L), although formaldehyde degradations were not completed at both biomass concentrations (224 and 1030 mg VSS/L), more formaldehyde was degraded with the higher biomass.

The results demonstrated that both the initial formaldehyde and the biomass concentrations could be critical for anaerobic degradation of high formaldehyde concentrations. Higher biomass resulted in a large quantity of formaldehyde being degraded. When formaldehyde concentrations are higher than 60 mg/L, the initial formaldehyde to biomass ratio (S_0/X_0) can be used to predict the percentage of formaldehyde degradation (Table I). When $S_0/X_0 \geq 0.29$ ($S_0 \geq$ 68 mg/L), formaldehyde was partially degraded; when $S_0/X_0 = 0.10$ ($S_0 = 95$ mg/L) formaldehyde was almost completely degraded. It can be seen that at similar S_0/X_0 ratios (0.17 and 0.18; 0.29 and 0.30), higher initial formaldehyde concentrations led to lower percent degradation.

Acclimation

Acclimation of the acetate enrichment culture to formaldehyde was studied in Chemostats I and II by gradually increasing the influent formaldehyde to 1110 (74 mg/L-d) and 740 mg/L (49 mg/L-d), respectively. After 226 and 84 days of acclimation in Chemostats I and II, respectively, the culture from the chemostats was transferred to serum bottles to determine the acclimation potential to formaldehyde. The results showed that formaldehyde still caused severe inhibition on acetate utilization after the acclimation (Figure 6). Thirty mg/L of formaldehyde significantly inhibited the acetate degradation for 4 and 8 hours in the culture from Chemostats I and II, respectively. The culture in Chemostat I had a higher VSS. As seen earlier in the unacclimated acetate enrichment culture, formaldehyde was degraded while acetate degradation was inhibited. Only after almost complete degradation of formaldehyde, the acetate degradation started. The formaldehyde degradation rate at low concentration (30 mg/L) fit the Monod model with the k of 0.35–0.42 day^{-1} and K_s of 0.8–3.7 mg/L, respectively, which were close to the values prior to acclimation. Again, as prior to acclimation, the increase in initial formaldehyde concentration caused a decrease in formaldehyde degradation rate (Figure 7). When the initial formaldehyde concentration was doubled from 30 to 60 mg/L, the formaldehyde degradation rate decreased. When the initial formaldehyde concentration was increased to 100 mg/L, the degradation rate further decreased and only 20% of the formaldehyde was eventually degraded. The effects of S_0/X_0 ratio on formaldehyde degradation after the acclimation period were similar to those prior to the acclimation. With $S_0/X_0 = 0.3$ and $S_0 = 60$ mg/L, formaldehyde was only partially degraded. When $S_0/X_0 = 0.09$ and $S_0 = 30$ mg/L, formaldehyde was completely degraded. When $S_0/X_0 = 0.17$, complete degradation occurred with an initial formaldehyde concentration of 60 mg/L.

It appears that even the long-term exposure (84 and 226 days) to low concentrations of formaldehyde at SRT of 14

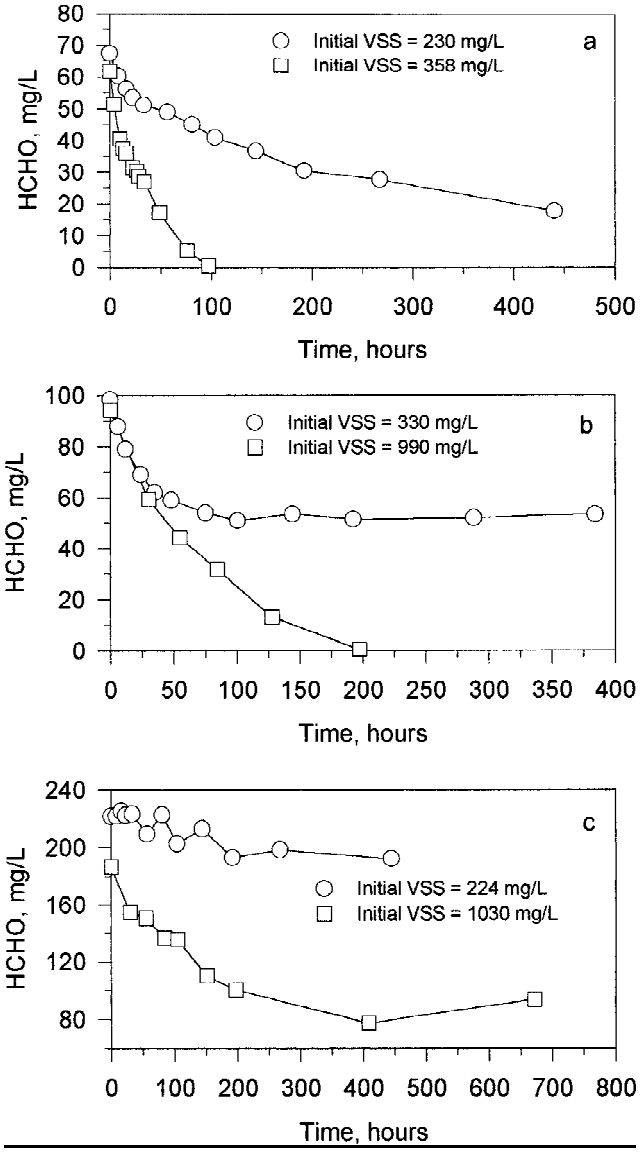

Figure 5. Effect of biomass concentration on formaldehyde degradation in the unacclimated acetate enrichment culture.

days did not acclimate the acetate enrichment culture to formaldehyde (Figures 8 and 9). Earlier serum bottle results showed that repeated short-term exposures to 20 mg/L of formaldehyde did not improve the potential of acclimation either (Figures 3). The results reported in the literature on methanogenic cultures' potential for acclimation to formaldehyde are different. Pearson et al. (1980) conducted acclimation tests in semi-continuous reactors and found that the domestic wastewater sludge was not acclimated to formaldehyde. Bhattacharya and Parkin (1988), however, showed that an acetate enrichment culture was acclimated to formaldehyde at higher SRTs (15, 25, and 40 days). They also reported that systems with high SRTs could tolerate higher concentrations of formaldehyde when added continuously.

Table I. Effects of initial formaldehyde and biomass concentrations on degradation of formaldehyde.

Initial HCHO S_0 (mg/L)	Initial biomass X_0 (mg/L)	S_0/X_0 ratio	Formaldehyde degraded (%)
95	990	0.10	99
62	358	0.17	99
187	1030	0.18	50
68	230	0.29	74
99	330	0.30	46
95	266	0.36	39
220	224	0.98	13

Formaldehyde Degradation in Chemostats

Figures 8 and 9 show the removal of formaldehyde in Chemostats I and II with a 14-day retention time. Chemostat I was initially fed 100 mg/L (7 mg/L-d) of formaldehyde to avoid possible inhibition of acetate utilization. The feed formaldehyde concentration to Chemostat I was increased to about 400 mg/L on day 173. Chemostat II was initially fed relatively high formaldehyde (370 mg/L). However, the results from the two chemostats were similar, showing that 370 mg/l of formaldehyde in the feed can be completely degraded with no acclimation period. Increasing the feed formaldehyde concentration to 740 mg/L did not increase the acetate concentration above 50 mg/L in Chemostat II. In Chemostat I, with 740 mg/L of formaldehyde, the acetate levels were higher but were still less than 200 mg/L during days 230–280; eventually, the acetate level dropped to less than 50 mg/L. Almost complete removal of formaldehyde was observed in both the chemostats. After 300 days of operation, the final influent formaldehyde concentration was increased to 1110 mg/L in Chemostat I. Even with this high feed formaldehyde, the effluent formaldehyde concentration was very low (<1 mg/L). This corresponds to a formaldehyde removal rate of 164 mg/g VSS-d.

The feeding of formaldehyde to the chemostats had little effect on the effluent acetate concentrations as shown in Figures 8 and 9. However, significant increases in biomass concentrations (VSS) in the chemostats were observed. The VSS concentration in Chemostat I before feeding formaldehyde was 194 ± 5 mg/L. When feeding 740 and 1110 mg/L of formaldehyde (along with 270 and 405 mg/L of methanol), the VSS concentrations were 341 ± 59 and 444 ± 45 mg/L, showing increases of 147 and 250 mg/L, respectively. The VSS concentration in Chemostat II increased from 236 ± 29 mg/L (before feeding formaldehyde) to 357 ± 18 mg/L (after feeding 740 mg/L of formaldehyde, along with 270 mg/L of methanol). These increases in VSS and the earlier measured methane data (Figure 4) are strong indications of formaldehyde biodegradation.

The effect of formaldehyde shock loading is shown in Figure 10. Thirty mg/L of formaldehyde in Chemostat III caused an immediate inhibition, leading to the increase of acetate concentration to 284 mg/L. The inhibition on acetate utilization lasted only a day because of complete degradation of the 30 mg/L of formaldehyde within 24 hours. When 60 mg/L of formaldehyde was spiked to Chemostat III, a

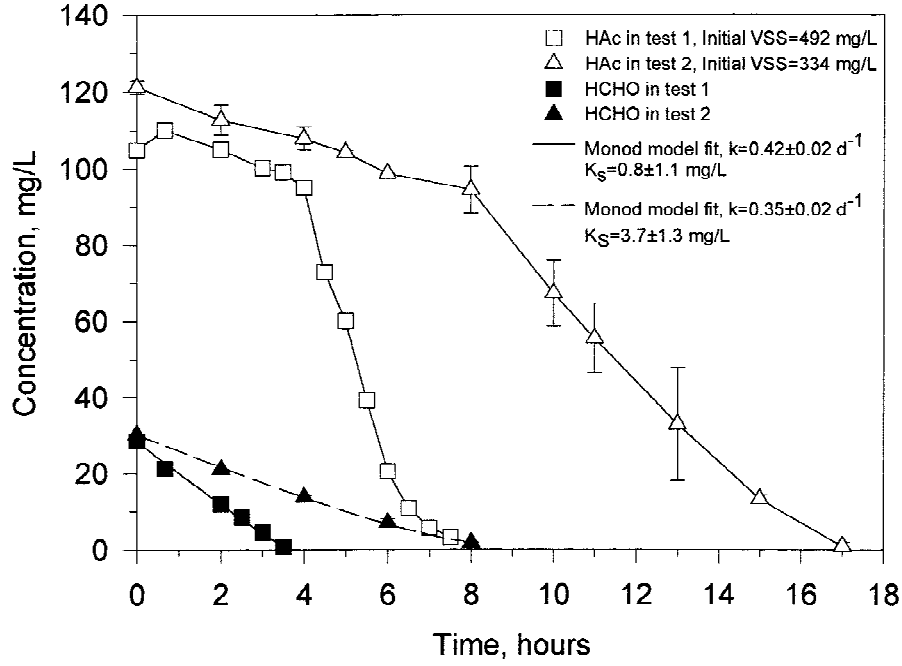

Figure 6. Inhibition of acetate utilization by formaldehyde after acclimation. Test 1 was conducted using the acetate enrichment culture from Chemostat I after 226 days of acclimation (Day 330). Test 2 was conducted using the acetate enrichment culture from Chemostat II after 84 days of acclimation (Day 122).

longer inhibition period (6.5 days) was observed. Effluent acetate concentration increased to 2550 mg/L in 6.5 days (almost at the same rate as the acetate loading 500 mg/L-d). The 60 mg/L of formaldehyde was completely degraded in 4 days. After 6.5 days, the acetate enrichment culture began to recover and the effluent acetate began to decrease. It took 20 days for the acetate concentration to decrease to 70 mg/L. Chou et al. (1978a) reported that a spike of 100 mg/L of formaldehyde to a completely mixed anaerobic reactor (10-day HRT) caused the cessation of methane production for 26 days.

The acetate enrichment culture in the chemostats in this study tolerated and degraded much higher influent formaldehyde than that reported in the literature (Bhattacharya and

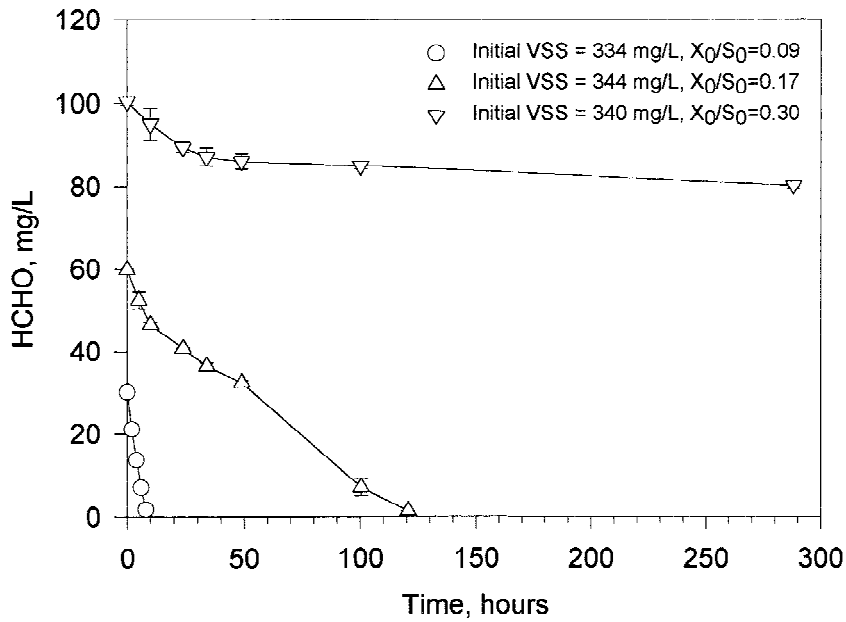

Figure 7. Effects of initial formaldehyde concentrations on its degradation in the acetate enrichment culture after 84 days of acclimation (Day 122) to formaldehyde in Chemostat II.

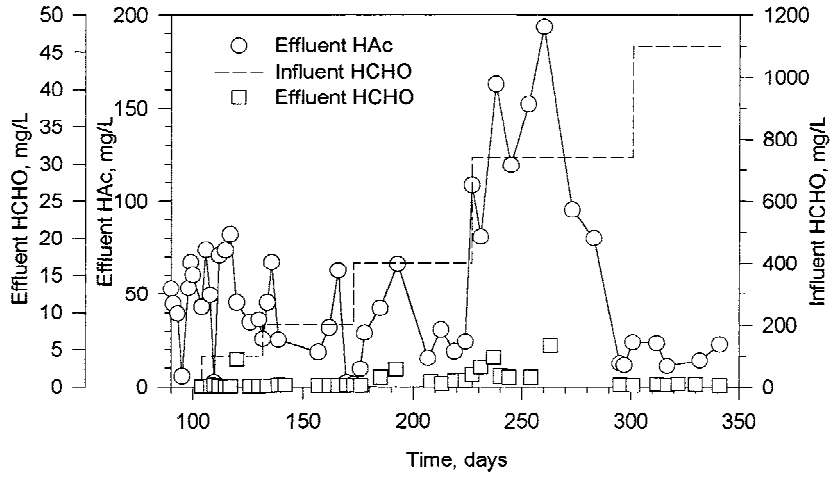

Figure 8. Formaldehyde degradation by the acetate enrichment culture in Chemostat I. SRT or HRT = 14 days, acetate loading rate = 500 mg/L-d.

Parkin, 1988; Parkin et al., 1983, Sharma et al., 1994). The chemostats in this study had a formaldehyde loading capacity of up to 74 mg/L-day compared to 12 and 11 mg/L-day reported in the literature (Bhattacharya and Parkin, 1988; Sharma et al., 1994). The biomass in the chemostats in this study was low (444 ± 45 mg/L) because these was no biomass recycle. In prototype anaerobic systems with biomass recycle, higher biomass can be maintained which could increase the formaldehyde removal capacity. The S_0/X_0 ratio and formaldehyde concentration can be used as a parameter to predict the performance of a methanogenic system after formaldehyde shock loading. If formaldehyde concentration is higher than 60 mg/L and the formaldehyde-to-biomass ratio in the system is greater than 0.3, it is likely that only a part of formaldehyde will be degraded. The remaining formaldehyde could inhibit acetate utilization leading to a system failure. If formaldehyde in the system after shock loading is less than 30 mg/L, the formaldehyde should be quickly and almost completely removed and the anaerobic treatment system can be expected to recover from the toxicity.

CONCLUSIONS

Up to 30 mg/L of formaldehyde can be completely degraded to methane by the acetate enrichment culture with a Monod rate constant k of 0.35–0.46 d^{-1}. At high initial formaldehyde concentration (≥60 mg/L), only a partial formaldehyde degradation can be expected. The initial formaldehyde to biomass ratio (S_0/X_0) and initial formaldehyde concentration are important for determining the extent of high formaldehyde degradation in batch anaerobic systems. The S_0/X_0 and formaldehyde concentration can also be used to predict the performance of a continuously-fed, complete-mix anaerobic system after formaldehyde loading. The acetate enrichment culture cannot be acclimated to formaldehyde in 226 days in chemostats (14-day-SRT) or by repeated spikings in batch systems. However, formaldehyde toxicity can be avoided by repeatedly adding low concentrations of formaldehyde to a batch system or by continuously adding formaldehyde to a complete-mix, anaerobic bioreactor. A continuous system (14-day SRT) can tolerate a high concentration of formaldehyde in the influent (up to

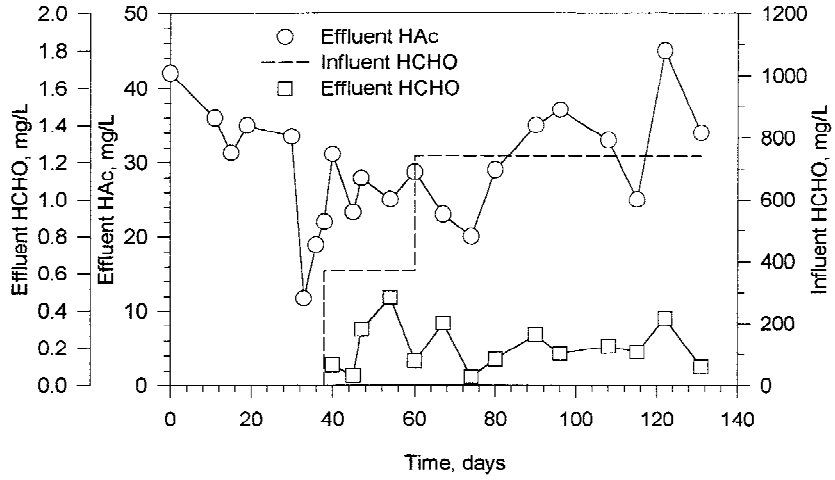

Figure 9. Formaldehyde degradation by the acetate enrichment culture in Chemostat II. SRT or HRT = 14 days, acetate loading rate = 500 mg/L-d.

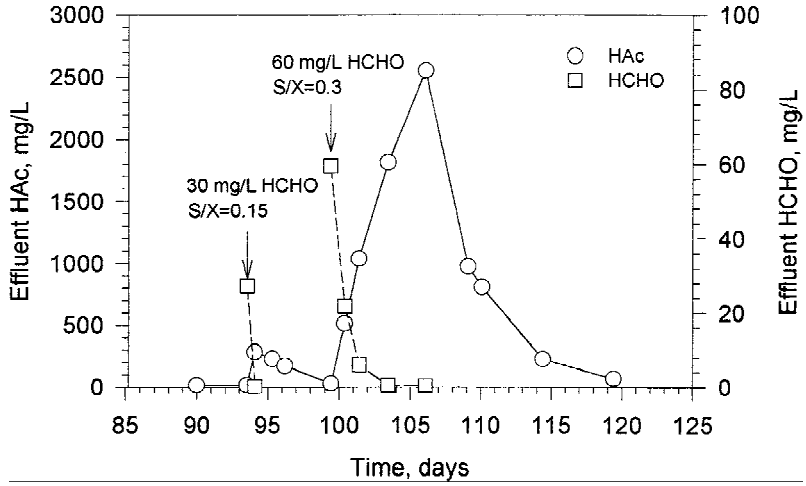

Figure 10. Effect of formaldehyde shock loading on acetate utilization by the unacclimated acetate enrichment culture in Chemostat III. SRT or HRT = 14 days, acetate loading rate = 500 mg/L. VSS = 186 ± 9 and 197 ± 9 mg/L at the time of first and second spike, respectively.

1110 mg/L with a VSS of 444 mg/L, i.e., 167 mg HCHO/g VSS-d) with a 99% formaldehyde removal efficiency.

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