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Toxic Effects of Selected Industrial Solvents in Batch and Continuous Anaerobic Reactors

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ABSTRACT

Acetone and ethyl acetate were readily degraded in batch reactors containing anaerobic solids from an industrial reactor at concentrations to 1000 mg/L; isoamyl acetate was degraded at concentrations below 200 mg/L. Xylene was noninhibitory in batch cultures at concentrations of 50 mg/L or less. Batch reactors receiving 45 mg/L methylene chloride (MC) experienced a 60% reduction in gas production, and an increase in volatile fatty acids. Semicontinuously-fed reactors dosed with 20 mg/L MC at the start suffered some loss of anaerobic efficiency, but acclimated well with increased gas production after 20 d. A continuously fed sludge blanket reactor with no previous exposure to MC has acclimated to 20 mg/L MC after initial inhibition at 10 mg/L.

Index Entries: Methylene chloride; anaerobic degradation of industrial solvents; sludge blanket reactor; acetone; ethyl acetate.

INTRODUCTION

Toxic substances may reduce reactor efficiency or even cause failure of an anaerobic digestion system. The toxicants may be components of the influent waste stream or byproducts of the metabolic activities (i.e., ammonia and hydrogen sulfide) of the microbes in an anaerobic reactor (*1,2*).

This study focused on the impact of selected organic solvents on the anaerobic activity of biological solids from an industrial reactor. The solids were incubated in a defined carbohydrate/acetate medium with a balance of nutrients that would support active anaerobic activity in order to: (1) observe the effects of various concentrations of methylene chloride (MC), ethyl acetate, isoamyl acetate, xylene, and acetone on the degradation of the medium in batch-fed reactors; and (2) examine the impact of MC in semicontinuously-fed reactors.

For comparison purposes, a sludge blanket reactor containing solids with no previous exposure to MC was also used to examine the impact of MC in such a continuously fed system.

MATERIALS AND METHODS

The batch fed reactors consisted of 100 mL serum bottles capped with butyl rubber stoppers. A defined medium, Table 1, modified from that of Owen et al. (*3*), was used. The serum bottles were seeded with biological solids from a laboratory digester maintained on the defined medium. These solids were originally obtained from an industrial anaerobic digester that had some exposure to the solvents tested in this study. The serum bottles were filled with 60 mL of the medium and biological solids to achieve 1.5–2% solids, using a flushing gas mixture of 70% nitrogen and 30% carbon dioxide in order to maintain anaerobic conditions during setup. The reactors were incubated at 33°C with once-a-day feed until acclimated, as judged by expected gas production (based on the organic loading used) and low volatile fatty acids concentrations. At the start of each batch test, 4 mL of defined nutrient medium with added test substance was added to the bottles. Serum bottles were set up in duplicate unless otherwise indicated in the results section. They were incubated at 33°C, and gas production measured daily or as needed, to determine the effects of a specific test chemical on gas production. Gas production was measured for at least 12 d.

The effects of MC were also examined in semicontinuously-fed serum bottle reactors, using the same biological solids and acclimation procedure as described above. Following acclimation, the serum bottles, set up in duplicate, were fed the defined medium containing specific amounts of MC on a daily draw and feed basis. The typical feeding rate was based on a 15–20 d hydraulic retention time (HRT) and an organic loading of 0.2–0.4 g COD/L/d.

The anaerobic activity of the batch and semicontinuously-fed serum bottle reactors was determined with an emphasis on gas production and/or volatile fatty acids concentration. Gas production was measured daily, using water lubricated glass syringes with graduations that made it possible to obtain consistent gas measurements to the nearest 0.2 mL. The syringes were maintained at the same temperature as the reactors (33°C), and gas measurements were also made at this temperature in order to avoid vol errors caused by temperature changes.

Table 1
Defined Medium

Compound	Amount/L
Sucrose	5.0 g
Acetic Acid	1.2 mL
$NaHCO_3$	6.0 g
$KHCO_3$	2.0 g
NH_4HCO_3	1.0 g
NH_4SO_4	0.2 g
Yeast extract	0.1 g
Trace elements*	13.5 mL
$FeCl_2$*	0.9 mL
Na_2S*	0.9 mL

*Stock solutions (S4, S5, and S6) of Owen (3).

Volatile fatty acids were determined by a procedure used previously (4), using a gas chromatograph with a thermal conductivity detector and a Supelco SP-1000 glass column maintained at 155°C. Calibration was by the use of external fatty acids standards.

An 8.5 L continuously-fed sludge blanket reactor (15×50 cm) was subjected to MC addition. The reactor contained biological solids first developed in 1980 and used in work described earlier (5). These solids had no known previous experience with MC. Whereas the reactor has not been in continuous operation during the 10-yr period, it has never been dismantled or reseeded. It has been allowed to go "dormant" from time to time. For this study, it was placed on the defined medium feed for over 6 mo, and was in a highly stable condition, based on gas production, and very low fatty acids, at the time the MC addition was started. The reactor was maintained at a temperature of 35°C with a 3-d HRT and an organic loading of 2.3 g COD/L/d. Feed was by a peristaltic pump programmed to feed every 30 min. Gas from the sludge blanket reactor was continuously measured with a wet test gas meter. Samples of gas and effluent were routinely removed and analyzed for gas composition and fatty acids by gas chromatography.

RESULTS

The work with acetone, ethyl acetate, xylene, and isoamyl acetate was of a qualitative nature, using single serum bottle batch-fed reactors for each test concentration. The results are summarized in Table 2.

Most dosages of acetone, ethyl acetate, and isoamyl acetate resulted in gas producton above that of the control reactors. Because of the preliminary nature of this work, direct measurement of the decay of the solvents was not done, but the increase in gas production correlated with the

Table 2
Summary of Batch Test Results
for the Solvents Acetone Ethyl Acetate, Xylene, and Isoamyl Acetate

Substance (mg/L)	Summary of results (after 20 d)
Acetone	
50 200	No toxicity evident at concentrations of 50 and 200 mg/L. Degradation of acetone contributed to total gas production in excess of that from the controls.
1000 2000	Approx 10% less gas produced during the first 2 d, but after 1 wk, a rapid degradation of acetone occurred, resulting in gas production far in excess of the controls.
Ethyl acetate	
200 500 1000	No evidence of inhibition. Rapid degradation of EA (based on increased gas production beyond that of the control) was evident after 24 h, even at a concentration of 1000 mg/L.
Xylene	
10 50	No inhibition at 10 and 50 mg/L xylene.
200	Gas yield: 80% of control.
1000	Gas yield: 30% of control.
Isoamyl acetate	
10 20 50 200	No evidence of inhibition; IAA degrades well. Increased gas production beyond controls relative to the concentration of isoamyl acetate tested.

amount of test substance added, and hence, provided indirect evidence of the degradation of the solvents to carbon dioxide and methane. More detailed work will be conducted on one or more of these solvents to determine their fate and impact in the anaerobic environment.

Methylene Chloride (MC)

During the first batch study, methylene chloride (MC) appeared to have an inhibitory effect at a concentration as low as 3 mg/L. Another batch study using a concentration of 5 mg/L MC also resulted in a decline in gas production (Table 3), as well as an increase in acetic and propionic acids at day 18. However, by day 25, they were no longer detected in the 5 mg/L MC treated reactors. At concentrations of 45 and 135/mg/L MC, gas production was sharply reduced. These reactors also exhibited elevated levels of acetic and propionic acids. Although not shown in Table 3,

Table 3
Batch Study of Methylene Chloride (MC) Addition
(23 d Cumulative Gas Production)

	Total gas*	Δ gas**	Fatty acids (mg/L) (acetic and propionic)			
			day 18		day 25	
Reactors	(mL)	(mL)	Ac	Pr	Ac	Pr
Controls (fed)	61.5	1.5	ND***	ND	ND	ND
Unfed controls	51	4.0	ND	ND	ND	ND
5 mg/L MC	57	0.5	190	100	ND	ND
15 mg/L MC	43	1.0	240	230	290	40
45 mg/L MC	26	0.5	350	470	500	300
135 mg/L MC	14.5	0.5	370	540	470	550

*Gas produced per bottle; averaged from duplicate reactors.
**ΔGas–gas vol variation between duplicate reactors.
***ND—nondetectable (detection limits were ca 50 mg/L for acetic acid and 30 mg/L for propionic acid).

Table 4
Semicontinuously Fed Reactors Receiving Methylene Chloride
(15 d HRT covering 54–60 d)

	Gas	Acetic and propionic acids (mg/L)					
		day 45		day 52		day 59	
Reactors	% of conc.	Ac	Pr	Ac	Pr	Ac	Pr
Controls	100	40	20	40	20	ND	ND
50 mg/L MC*	100	—	—	—	—	—	—
80 mg/L MC(1)**	61	2100	700	1240	600	340	490
80 mg/L MC(2)**	90	160	70	110	60	75	20

*The gas vols. produced by two 50 mg/L MC treated reactors (1200+mL) were within 1% of each other and fell between the gas vols. produced by duplicate control reactors. The control reactors had final gas vols. (after 58 d) within 2.5% of each other.
**Individual replicates receiving 80 mg/L MC.

longer chain fatty acids, isobutyric, *N*-butyric, and isovaleric acids were also detected in the 45 and 135 MC batch reactors in concentrations ranging from 30 to 70 mg/L.

Methylene chloride was studied in semicontinuously fed serum bottle reactors, using two methods. Table 4 shows data from reactors receiving 50 and 80 mg/L MC in the daily feed. In this study, which covered a period of 60 d, the reactors receiving 50 mg/L MC in the feed produced gas at the same rate as the control reactors. Duplicate reactors receiving 50 mg/L MC over a period of almost 60 d had a total cumulative gas vol of

Table 5
Acetic (Ac) and Propionic (Pr) Acids
in Semicontinuously-Fed Reactors Dosed with MC
at the Start of an Experiment and Receiving MC with Daily Feed (20 d HRT)

	Acetic and propionic acids (mg/L)							
	day 7		day 24		day 21		day 28	
Reactors	Ac	Pr	Ac	Pr	Ac	Pr	Ac	Pr
Controls	ND*	ND	ND	ND	ND	ND	ND	ND
20 mg/L MC	590	170	730	400	120	40	ND	ND
40 mg/L MC	300	50	1760	470	1670	440	300	350
60 mg/L MC	220	50	2200	620	1950	470	1400	500

*ND—not detectable.

over 1200 mL, within 1% of each other. The average gas vol of the 50 mg/L MC reactors was almost identical to the average of the control reactors (< 0.5%). In addition, fatty acids were below the detection limits of the analytical methods used. In the pair of reactors receiving 80 mg/L MC, there was a marked difference from the controls and also between the two replicates used. Reactor #1 receiving 80 mg/L MC had a total gas production only 61% that of the control, whereas the second member of the replicate pair produced gas at 90% that of the control. There was also a marked difference in the concentrations of acetic and propionic acids in the two reactors receiving 80 mg/L MC.

A second type of semicontinuous experiment was conducted, in which duplicate serum bottle reactors were "slug" dosed with three concentrations of MC at 20, 40, and 60 mg/L at the start of the experiment. They then received a daily feed with the defined medum and MC, based on one of the three concentrations. The fatty acids data and gas production results are shown in Table 5 and Fig. 1, respectively. Given the earlier batch test results, it was not surprising to find elevated levels of fatty acids in the reactors early in the study. At day 7 the reactors receiving 20 mg/L MC had the highest concentrations of acetic and propionic acids. By week 2, the 40 and 60 mg/L MC reactors had sharply increased acetic and propionic acids. Gas production also declined significantly in all the MC-fed reactors by the second day of the experiment (*see* Fig. 1). However, beginning about day 9, the 20 mg/L MC reactors began to produce more gas. Recovery is also noted in the decline in acetic and propionic acids by day 21, and a drop to nondetectable levels by day 28 in the 20 mg/L LMC reactors.

In the preliminary work done with MC addition to a sludge blanket reactor, the reactor responded in normal fashion for one week to a 10 mg/L MC addition, but then began to suffer a significant reduction in gas production. By day 13, gas dropped to 50% of normal and acetic and propionic acids had increased from the initial nondetectable levels to 1800

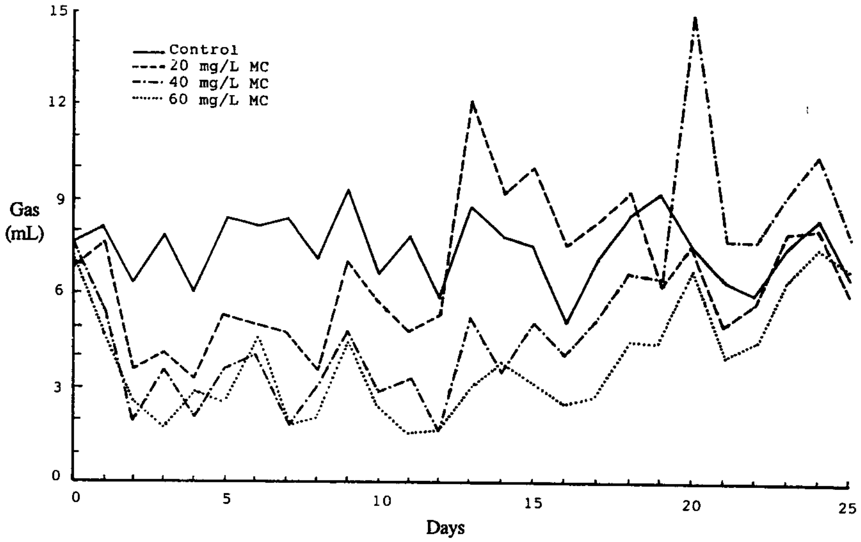

Fig. 1. Gas production in semicontinuously-fed reactors dosed with methylene chloride at the start, and receiving MC with daily feed. Data points are an average of duplicate reactors' gas production measured on a daily basis. Average daily differences in gas production between replicate bottles ranged from 0.20 mL for the controls to 0.5 mL for the 40 mg/L MC reactors, calculated over the course of the experimental period.

and 490 mg/L, respectively. At this point, MC addition was stopped but normal feed continued. Recovery occurred rapidly with a return to normal gas production in 5 d. At this time, the reactor was shut down for a period of almost three weeks because of insufficient technical support. With subsequent startup, the reactor reached normal activity within a week. After several more weeks of operation without MC addition, the reactor was fed 20 mg/L MC in the defined medium. This concentration of MC had no measurable negative effect on reactor performance, based on gas volume, gas composition, and volatile fatty acids concentration. Studies are continuing.

DISCUSSION

The focus of this study was to examine the impact of selected, widely used, industrial solvents in batch anaerobic culture, with an emphasis on the effects of MC in batch, as well as semicontinuous and continuously-fed anaerobic reactors. The basis for the work incorporated the use of a readily degradable and defined medium with a balance of nutrients, such that the impact of a particular test substance could be observed under more-or-less ideal conditions.

The batch studies with acetone, ethyl acetate, and isoamyl acetate and xylene were of a preliminary and largely qualitative nature with continuing work expected in the future. Based on increased gas production over that of the control reactors, the first three solvents appeared to be quite degradable in the batch culture. Even at acetone levels of 1000 mg/L or more, this substance degraded readily, although there was some delay in gas production during the first few days. Ethyl acetate degraded readily with no apparent delay, even at a dose of 1000 mg/L. This compares with Chou's (*6*) report of 50% inhibition at a concentration of 11 m*M* (968 mg/L). Acetone and ethyl acetate are among many organic substances described as being "amenable" to anaerobic treatment (*1*), and the results of the preliminary batch study reported here indicates that anaerobic systems may degrade these solvents even at fairly high concentrations.

The literature on the toxic and inhibiting effects of MC under anaerobic conditions is somewhat varied. Bauchop (*7*) reported that 204 mg/L caused 50% inhibition of gas production with rumen microbes, whereas Thiel (*8*) noted the same level of inhibition at a concentration of 94 mg/L MC. In contrast, Stuckey et al. (*9*) reported 50% inhibition at only 14 mg/L MC. Based on the results reported here (Table 3), a 50% inhibiton of gas production would correspond to approx 30 mg/L MC.

Bhattacharya and Parkin (*10*) experienced "system failure" at 44 mg/L, the lowest concentration of MC used in their "slug dose" study. That study is somewhat comparable to one of the semicontinuous studies reported here (Table 5 and Fig. 1), the main difference being that in addition to the initial starting dosages of 20, 40, and 60 mg/L, the MC was also added to the feed on a continuing basis. Whereas the reactors receiving the three concentrations of MC all experienced reduced gas production, acclimation was evident at 20 mg/L and also at 40 mg/L. In both sets of reactors, gas production increased with time (Fig. 1), and acetic and propionic acids declined (Table 5). For example, acetic and propionic acids were nondetectable in the reactors receiving 20 mg/L MC by day 28.

The early studies with MC addition to a sludge blanket system indicated an initial inhibitory response at 10 mg/L MC, which resulted in a 50% decline in gas production after 13 d in a system operated at a 3-d HRT. When MC addition was ended, recovery occurred quickly. A later addition of 20 mg/L MC to the same reactor resulted in no significant decline in gas producton or major change in volatile fatty acids after one month. This indicates rapid acclimation to MC; the work is continuing.

Acclimation was noted in the batch, the semicontinuous, and sludge blanket systems. Acclimation is seen in batch bioassay by an increase in gas production rate with time (*9*), and in semicontinuous reactors, it commonly appears as a rise in daily gas production after an initial inhibitory response, as well as a decline in accumulated volatile fatty acids.

CONCLUSIONS

1. Preliminary work with acetone and ethyl acetate shows that these solvents were readily degradable in batch reactors at concentrations to 1000 mg/L, and with isoamyl acetate degraded at concentrations to 200 mg/L. Xylene was toxic at 200 mg/L but well tolerated at 50 mg/L.
2. MC had a detectable effect on gas production and volatile fatty acids in batch reactors at concentrations as low as 3–5 mg/L.
3. MC was well tolerated in semicontinuously-fed reactors when the MC was added in daily feed at a concentration of 50 mg/L. The difference in volume of gas produced by duplicate 50 mg/L MC treated reactors was less than 0.5% when compared to that of the control reactors after 58 d.
4. MC had an inhibitory impact on the performance of semicontinuously-fed reactors receiving initial slug doses of 20 mg/L MC plus MC in the daily feed, but recovery and acclimation were evident within 20 d.
5. Inhibition of anaerobic activity appeared in a continuously-fed sludge blanket reactor at 10 mg/L MC, but recovery occurred rapidly, and eventual acclimation to 20 mg/L MC was demonstrated.

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