EFFECTS OF NITROPHENOLS ON ACETATE UTILIZING METHANOGENIC SYSTEMS

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Abstract

Nitrophenols are widely used for manufacturing explosives, pharmaceuticals, pesticides, pigments, dyes, etc. A literature research shows that there is a lack of quantitative information on the effects of nitrophenols on anaerobic systems. The objective of this research was to study the toxic effects of nitrophenols on acetate enrichment and methanogenic systems. Anaerobic toxicity assays were performed with batch serum bottles. Results showed that among the selected nitrophenols, toxicity decreases in the following order: 2,4- dinitrophenol> 4-nitrophenol>2-nitrophenol>3- nitrophenol. Complete removals of mononitrophenols were seen in serum bottle systems which did not fail due to toxicity. Chemostats (15-day retention time) were used to study toxicity, acclimation, and removal of 4- nitrophenol in continuous systems. New steady-states with high effluent acetate (substrate) concentrations were attained after spiking 5.5,13.5, and 33.0 mgl^{-1} of 4- nitrophenol. A competitive inhibition coefficient model fits well with the experimental data from 4- nitrophenol studies. The competitive inhibition coefficient k_I, for 4-nitrophenol varied between 0.077 and 0.089 mgl^{-1}. Fifty-five to 82% of the 4- nitrophenol was removed in the chemostats; HPLC analysis of effluents showed no intermediate products.

Key words

anaerobic toxicity assay, biodegradation, chemostat, competitive inhibition model, methanogens, mono- and di-nitrophenols, 4-nitrophenols, serum bottles, toxicity

INTRODUCTION

Nitrophenols are among the most important and versatile industrial organic compounds and are widely used in the manufacturing of explosives, pharmaceuticals, pesticides, pigments, dyes, and rubber chemicals. Nitrophenols may be inadvertently produced by microbial hydrolysis of several organophosphorus pesticides, such as parathion (Sethunathan, 1973, Nelson, 1982) or photodegradation of pesticides that contain the nitrophenol moiety (EPA, 1980).They also result from natural processes in the biosphere and are now common pollutants in several ecosystems in developed countries (Blasco and Castillo, 1992). Among the mononitrophenols, 4- nitrophenol is probably the most important in terms of quantities used and potential environmental contaminations. 4- nitrophenol may be produced in the atmosphere through the photochemical reaction between benzene and nitrogen monoxide and has been detected in rainwater in Japan (EPA,1980). Available data indicate that the general public may be exposed to nitrophenols in the atmosphere where several photochemical fog conditions develop (Nojima, 1976). Of the six possible isomers of dinitrophenols, 2,4- dinitrophenol is commercially the most important. It is used as an intermediate in the production of explosives, dyes, wood preservatives, etc. 2-nitrophenol,4-nitrophenol and 2,4- dinitrophenol are listed on the U.S.

Environmental Protection Agency's "Priority Pollutants List". The U.S. EPA recommends restricting their concentrations in natural waters to below 10 ngl^{-1} (Eckenfelder, 1989). The agency has also set pretreatment standards for discharge of nitrophenols applicable to dischargers manufacturing synthetic fibers, thermoplastic resins, thermosetting resins, commodity organic chemicals, bulk organic chemicals and specialty organic chemicals.

Nitrophenols are known to be toxic to microorganisms. The toxicity of a nitrophenol to anaerobic bacteria is thought to be due to the addition of one or more nitro ($-NO_2$) groups to the phenol molecule. Battersby and Wilson (1989) reported 3- nitrophenol, 4- nitrophenol and 2,4-dinitrophenol as inhibitory under studied conditions in a list of anaerobic biodegradability of organic compounds survey based on serum bottle studies. Nandipati (1991) studied the effects of 2-nitrophenol, 4- nitrophenol, and 2,4- dinitrophenol in anaerobic toxicity assays with acetate enrichment, and in an upflow anaerobic column of acetate enrichment culture. He concluded that inhibition was reversible in serum bottles for 2- and 4-nitrophenol spiked at $20mgl^{-1}$ and that 2,4-dinitrophenol caused irreversible inhibition at $20mgl^{-1}$. The literature survey shows that there is a lack of quantitative information on nitrophenol toxicity in anaerobic treatment systems. More information is required on the mechanisms of toxicity by these organic compounds. This research was undertaken to provide such information.

COMPETITIVE INHIBITION MODEL

The competitive inhibition-coefficient model described by Parkin and Speece (1982) was used in this research to quantify the effects of 4-nitrophenol. Bhattacharya and Parkin (1986) showed that the competitive inhibition coefficient model fits well with formaldehyde toxicity. With competitive inhibition, the effluent substrate concentration gradually increases and reaches a new steady state. The equations for rates of substrate utilization and bacterial growth are:

$$-dS/dt = kSX_a/[k_s(1+T_x/k_I)+S] \quad (1)$$

$$dX_a/dt = YkSX_a/[k_s(1+T_x/k_I)+S]-bX_a \quad (2)$$

where dS/dt is rate of substrate utilization per unit volume ($g\ m^{-3}d^{-1}$), dX_a/dt is rate of bacterial growth ($gm^{-3}d^{-1}$), S is substrate concentration (gm^{-3}), X_a is active biomass concentration ($g\ m^{-3}$), Y is growth yield coefficient (gg^{-1}), b is microbial decay coefficient (d^{-1}), T_x is toxicant concentration (gm^{-3}), k is specific substrate utilization rate ($gg^{-1}\ d^{-1}$), K_s is half-saturation coefficient (gm^{-3}) and k_I is inhibition coefficient (gm^{-3}). The system equations were developed from substrates and biomass balance for a chemostat and are as follows:

$$dS/dt = [(S_o-S)/\theta]-kSX_a/[(k_s*A)+S)] \quad (3)$$

$$dX_a/dt = YkSX_a/[(k_s*A)+S] - X_a(1/\theta+b) \quad (4)$$

where S_o is influent substrate concentration (gm^{-3}), θ is HRT or SRT and A is $1+ (T_x/k_I)$.

OBJECTIVES

The specific objectives of this research were: (i) to determine the toxic effects of nitrophenols (2-nitrophenol, 3- nitrophenol, 4- nitrophenol and 2,4- dinitrophenol) on acetate utilizing methanogens, (ii) to determine the extent of anaerobic biodegradation of nitrophenols, and (iii) to quantify the effects of 4- nitrophenol on methanogens using a competitive inhibition coefficient model.

MATERIALS AND METHODS

Batch serum bottles were used to study the effects of the selected nitrophenols. Chemostats were used to study the effects of 4- nitrophenol more extensively. 4- nitrophenol was selected for this purpose because, as discussed before, this toxicant is the most important one among the selected mononitrophenols. Acetate utilizing methanogens were selected because almost 70% of methane generated in anaerobic sytems is through the pathway with acetate as the key intermediate. These methanogens have shown vulnerability to many toxicants and methane formation from acetate could be the rate limiting step under both non-toxic and toxic conditions (Parkin and Speece, 1982).

Stock culture

The stock acetate enrichment culture was developed from anaerobically digested sludge using acetic acid as the sole source of carbon. The culture was developed in 20-1 plastic carboys in a constant temperature room (35° C). The pH, volatile acids and total alkalinity were measured daily. COD, total suspended solids (TSS) and volatile suspended solids (VSS) were measured every week to check the stability of the enrichment culture.

Anaerobic toxicity assay (ATA)

The ATA was performed using serum bottles (Owen et al., 1979). Serum bottles were seeded anaerobically from the stock culture. Clean serum bottles (150ml) were first filled with tap water which was then displaced with N_2 gas. The bottles were then capped with rubber stoppers to maintain the anerobic conditions. In each prepared bottle, 45ml of acetate enrichment cultures, 3ml of nutrients (Bhattacharya and Parkin, 1986), and 2ml yeast extract (stock concentration of $50gl^{-1}$) were anaerobically transferred with syringes. For each experiment, triplicate controls and test bottles were maintained. A substrate loading rate of 1050 $mgl^{-1}d^{-1}$ was maintained in the serum bottle by adding 50 μ1 of glacial acetic acid per day with a Hamilton microliter syringe. However, to maintain the acetate level at 1050 mgl^{-1} during inhibition, the daily acetic acid feed was calculated follows:

daily feed=

[gas production-(22/50)(previous day's feed)](50/22)

The bottles were stored at 35° C in a constant temperature room. Total gas production was measured daily by a gas displacement device. Steady state attainment was arbitrarily defined as variation of gas production within 10% of the stoichiometric gas production for at least one week. The methane production was estimated based on the following equations:

$$CH_3COOH \longrightarrow H_3COO^- + H^+ \quad (5)$$
$$H^+ + HCO^-_3 \longrightarrow H_2O + CO_2 \quad (6)$$
$$CH_3COO^- + H_2O \longrightarrow HCO^-_3 + CH_4 \quad (7)$$
$$CH_3COOH \longrightarrow CO_2 + CH_4 \quad (8)$$

From stoichiometry, if 1050 mgl^{-1} of acetic acid is added to 50 ml culture, 44 ml of total gas is produced (22 ml CO_2 and 22 ml CH_4). The amount of methane produced was calculated by subtracting 22 ml of carbon dioxide from the total gas production. It was assumed that 22 ml of carbon dioxide was always produced chemically as long as bicarbonate alkalinity was available (equation 6).

Measured amounts of nitrophenols (Aldrich certified) were used as toxicant. When the serum bottles reached steady state, different concentrations of 2- nitrophenol, 3- nitrophenol, 4-nitrophenol and 2,4- dinitrophenol were added to the test bottles. The serum bottles were maintained until the systems failed or there were no effects for several days. The design bicarbonate alkalinity was $5000\ mgl^{-1}$. Hence, pH problems were expected only when the accumulated acetic acid exceeded the alkalinity.

chemostat studies

chemostats were used to study the effects of 4-nitrophenol. The chemostats were 2-1, glass bottles containing 1.5-1 of acetate enrichment culture. Solids retention time/hydraulic retention time (SRT/HRT) was maintained at 15 d, using flow rates of 100 ml d^{-1}. Cassette pumps (Manostat, New York, NY) were used to maintain the continuous feed. The peristaltic pumping action was possible by the squeezing action of rollers on the tubings. Flow rate was proportional to the speed of the rollers and the inside diameter of the tubing, both of which can be varied.

Effects of nitrophenols on methanogens

Each cassette was used to feed one chemostat. The diameter of the tubing (Silicon rubber, 1/16 inch) was kept constantin all flow rates but the shaft speed was controlled to produce the desired flow rates which were recorded two to three times a week to ensuer a constant rate of feed. Chemostats were seeded anaerobically from the stock cultures. Clean glass bottles were first filled with tap water which was then displaced with N_2 gas. Acetate enrichment culture (1.5-1) was transferred to the bottles from the stock by displacing the gas. During the first 3-4 d, the pumps were not started and the systems were maintained in a batch mode by manually feeding acetate and the nutrients. Yeast extract was also added during this time as an extra

Table 1. Summary of analytical techniques

Parameters	Methods
Total volatile acids	Titration (Jenkins et al., 1983)
Bicarbonate alkalinity	Titration (Jenkins et al., 1983)
Volatile suspended solids	Method 423 (Standard Methods)
Total suspended solids	Method 209 C(Standard Methods)
COD	HACH method
Gas composition	Gas chromatography (GC) with TCD
Acetate	Gas chromatography (GC) with FID
Nitrophenols	High performance liquid chromatography

source of minerals and vitamins. After 3-4 d, the systems were switched to the continuous mode. The design bicarbonate alkalinity was 5000 mgl^{-1}.

The chemostats were maintained for at least three retention times(i.e. 45 d) so that steady-state could be reached. Steady-state was arbitrarily defined as variation of acetate, VSS, TSS and percent methane wihin 15% of the average values for at least one week. The base-line (non-toxic) kinetic parameter ($k=3.1\ d^{-1}$, $k_s=10.0\ mg\ 1^{-1}$ and $b=0.01\ d^{-1}$) values were determined from steady-state conditions and Y was thermodynamically calculated as 0.04 (Haghighi-podeh, 1991).

The toxicant (4- nitrophenol) was added to the feed bottle and was also spiked once to the chemostats. This stimulates a combination dose causing a sudden increase in the 4- nitrophenol concentration to the design level. This approach does not give the organisms much opportunity to acclimate. Equations (3) and (4) were developed for combination dose. These equations may be modified to simulate other types of spiking including a gradual increase in concentration of a toxicant in a chemostat by adding it only to the feed bottles (Bhattacharya and Parkin, 1986).

ANALYTICAL TECHNIQUES

Methods used for measuring the various parameters are listed in Table 1. Volatile acids (VA) were measured by gas chromatography or by titration (Jenkins et al., 1983). Analysis by gas chromatography was perfomed with a Shimadzu GC-14A. The gas chromatograph analytical column was 3 mm i.d. × 1.7m glass packed with 50 g of 60/80 mesh carbopack C solid phase washed with carbowax PEG 20M/0.1%H_3PO_4 liquid phase. The column temperature was 120 °C (isothermal) with helium at a pressure of $2kg\ m^{-2}$ as the carrier gas. The detector was a flame ionization detector (FID) and the temperature was set at 200 °C. The samples were prepared by passing through a Whatman 934-AH glass microfilter (0.45μm) to remove bacteria. The samples were then acidifed with formic acid to pH<2 and 5-10μl of samples were injected to GC. Water was injected inbetween samples to eliminate ghost peaks. Titrimetric VA analysis involved titrating with 0.2 NH_2SO_4 to two end points of pH 5.7 and pH 3.7 (Haghighi-podeh, 1991).

Total suspended solids (TSS) and volatile suspended solids (VSS) measured according to Method 209 B in Standard Methods (1985). Glass fiber filters (Whatman 934-AH) were placed at 550C in a muffle furnace for 15 min before filtration to ensure elimination of any volatile matters on the filters.

A Waters HPLC was used to determine nitrophenol concentrations. The instrument had an u.v. detector (Model 428). The column used for detecting nitrophenols was Nova-pak C_{18}. The detection wavelength was 254 nm.The eluent was methanol (40%) and water (60%) mixture. The samples were prepared by extraction using Sep-pak C_{18} cartridges (Waters, Inc.). The cartridges were first activated by passing 1ml of deionized water. Then, the samples were loaded onto cartridges by passing 2 ml sample through the cartridge. The sorbed nitrophenols were then eluted from the cartridge 2 ml of methanol. The standards were prepared from reagent chemicals in distilled water. All the

Table 2. Effects of 2- nitrophenol on methanogenic bacteria in acetate enrichment systems

Spiked (PPM)	Final pH	Final VSS*(mgl^{-1})	Recovery (d)	% Removal
Control	7.3	1492		N/A
Control	7.1	1575		N/A
Control	7.0	1460		N/A
1	7.3	1320	--	100
1	7.2	1400	--	100
1	7.3	1340	--	100
10	7.0	1440	--	100
10	7.1	1318	--	100
10	7.0	1434	--	100
20	7.2	1288	3	100
20	7.0	1304	3	100
20	7.0	1226	3	100
30	7.0	1354	5	100
30	6.9	1262	5	100
30	7.0	1112	5	100
40	7.0	1230	7	100
40	6.8	1204	7	100
40	7.0	1062	7	100

*VSS=Volatile Suspended Solids. --=No effects.

standards and samples were filtered using Millex-HV filter (Waters) units with 0.22 μmDurapore membrane filters to remove all particulates prior to injection.

COD was measured by using a HACH kit. Methane and carbon dioxide percentages were determined by gas chromatography using a Shimadzu GC-8 with TCD. A 16 ft×$\frac{1}{8}$ inch stainless steel column, packed with 60/80 chromosorb 102 was used. The column was held at a constant temperature of 60 °C and helium was used as carrier gas. The injector and detector temperatures were set at 160 °C.

RESULTS AND DISCUSSION

Anaerobic toxicity assay

2- Nitrophenol. The effects of 1,10,20,30, and $40mgl^{-1}$ of 2- nitrophenol on the methane production are shown in Fig. 1. With 1 mgl^{-1}, the methane production did not seem to be affected at all, indicating no adverse effects on methanogens. With 10 mgl^{-1}, there was a reduction in methane production compared to the controls for the first 2 days after spiking. With 20 mgl^{-1}, there was inhibitory effect for the first 3 days. With 30 mgl^{-1}, the inhibitory effect continued for 5 days, and with 40 mgl^{-1} there was an inhibitory effect for the first 7 days after spiking. Samples were drawn from serum bottles at the end of experiments and analyzed for 2- nitrophenols using HPLC. The data for 2-nitrophenol are presented in Table 2. 2-nitrophenol was removed completely and was not detected in serum bottles. The final pH at the end of the study was in the normal range. With no significant accumulation of acetate, the design alkalinity was sufficient to resist any pH drops.

3- Nitrophenol. The data from anaerobic toxicity assay with 3- nitrophenol are shown in Fig.2. Twenty milligrams per liter and higher concentrations of 3- nitrophenol affected methane production indicating inhibition of methanogens. With 20 mg/l, there was an inhibitory effect for the first 2 days and with 40 mg/l, the inhibitory effect continued for the first 5 days. The added toxicant did not have any effects on the pH. The data showed that 3- nitrophenol was removed completely (Table 3).

4- Nitrophenol. The effects of 4- nitrophenol on methane generation are shown in Fig. 3. With $1mgl^{-1}$, the methane production was not affected at all, indicating no adverse effects on methanogens.

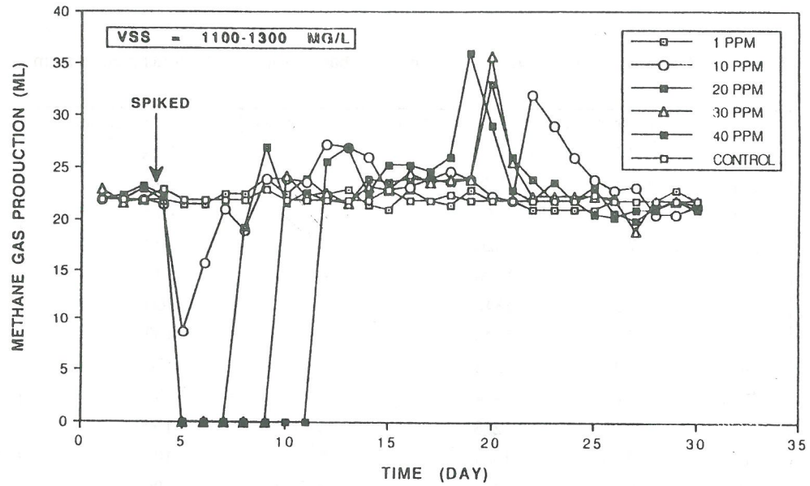

Fig. 1. Effects of 2-nitrophenol on methanogenic bacteria in acetate enrichment systems.

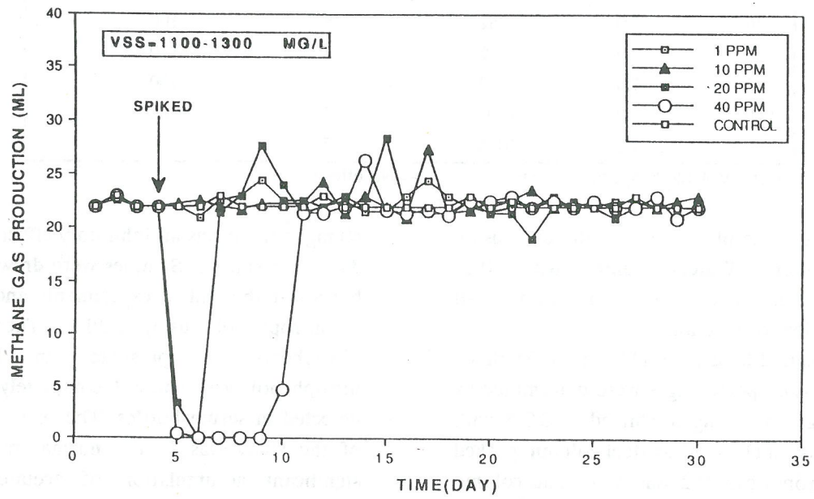

Fig. 2. Effects of 3-nitrophenol on methanogenic bacteria in acetate enrichment systems.

At 5 mg/l, there was a reduction in methane production for the first 2 days. Inhibitory effects were observed for 4-5 days, with 10-20 and 40 mgl^{-1} of 4- nitrophenol. But 80 mgl^{-1} caused irreversible toxic effects and the spiked bottles did not recover. Table 4 shows that 4- nitrophenol up to 40 mgl^{-1} was removed completely and was not detected in the serum bottles. The pH in the serum bottles were normal throughout the experiments.

2,4- Dinitrophenol. The data from the anaerobic toxicity assay with 2,4- dinitrophenol are shown in Fig. 4. The methane production was not affected by 1 and 5 mgl^{-1} of 2,4- dinitrophenol. Ten milligrams per liter of 2,4- dinitrophenol affected gas production for 3 days, indicating reversible inhibition of methanogens. With 15 and 20 mgl^{-1} of 2,4- dinitrophenol, the serum bottles showed failures. Table 5 shows that 1 and 5 mgl^{-1} of 2,4- dinitrophenol were removed completely and were not detected in the samples. But for serum bottles spiked with 10 ppm of 2,4- dinitrophenol, only 72% was removed.

Summary of ATA. The results of the ATA with 2- nitrophenol, 3- nitrophenol, 4- nitrophenol and 2,4- dinitrophenol are summarized in Table 6. Among the nitrophenols studied, 2,4- dinitrophenol had the

Table 3. Effects of 3- nitrophenol on methanogenic bacteria in acetate enrichment systems

Spiked(PPM)	Final pH	Final VSS*(mgl^{-1})	Recovery(d)	% Removal
Control	7.2	1346		N/A
Control	7.1	1287		N/A
Control	7.1	1236		N/A
1	7.2	1400	--	100
1	7.3	1480	--	100
1	7.2	1510	--	100
10	7.2	1268	--	100
10	7.0	1226	--	100
10	6.9	1100	--	100
20	7.0	1430	2	100
20	7.2	1422	2	100
20	7.0	1296	2	100
40	7.1	1196	5	100
40	7.0	1142	5	100
40	7.1	1302	5	100

*VSS=Volatile Suspended Solids. --=No effects.

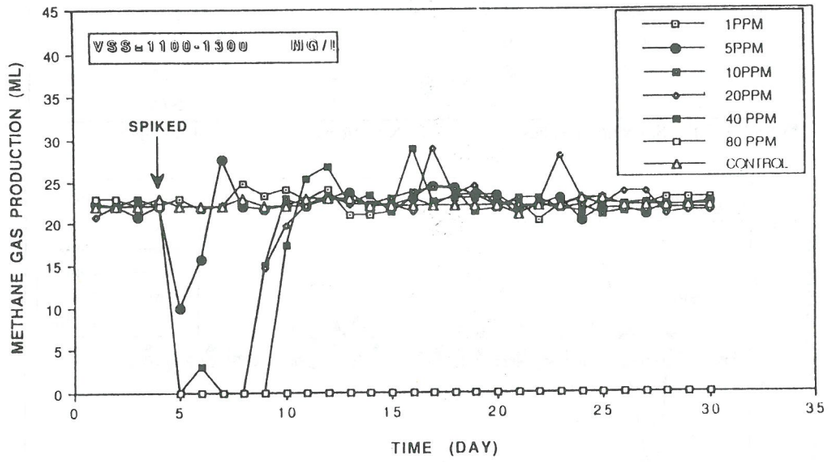

Fig. 3. Effects of 4-nitrophenol on methanogenic bacteria in acetate enrichment systems.

greatest and 3- nitrophenol had the least inhibitory effects. The results showed that for anaerobic treatment systems the nitrophenol toxicity decreased in the following order: 2,4- dinitrophenol> 4- nitrophenol>2- nitrophenol> 3- nitrophenol.

Chemostat studies

Figure 5 shows the toxic effects of combination doses of 4- nitrophenol on acetate utilization by methanogens. The chemostats were spiked after reaching steady state. The baseline kinetic constants (k, k_s and b) were determined based on the steady-state data collected before spiking (Haghighi-Podeh, 1991). After spiking the chemostats, the volatile acids started to increase during the first few weeks, and eventually reached new steady state levels. The highest volatile acids accumulation was in the chemostat which was spiked with 33.5 ppm of 4- nitrophenol. Figure 6 shows the 4- nitrophenol removal with time. A new steady state was attained after about 2 weeks with a spike of 5.5ppm. During the first 2 weeks almost all of the 4- nitrophenol in influent were removed by the methanogenic bacteria. The chemostats which

Table 4. Effects of 4- nitrophenol on methanogenic bacteria in acetate enrichment systems

Spiked(PPM)	Final pH	Final VSS*(mgl^{-1})	Recovery (d)	% Removal
Control	7.4	1428		N/A
Control	7.1	1428		N/A
Control	7.0	1390		N/A
1	7.3	1440	--	100
1	7.0	1320	--	100
1	7.1	1420	--	100
5	7.1	1434	--	100
5	7.0	1442	--	100
5	7.3	1374	--	100
10	7.0	1384	4	100
10	7.1	1326	4	100
10	7.0	1362	4	100
20	7.0	1258	4	100
20	6.9	1330	4	100
20	7.1	1332	4	100
40	7.0	1280	5	100
40	7.1	1308	5	100
40	7.3	1274	5	100
80	6.9	1050	F○	N/A
80	7.0	980	F	N/A
80	7.0	1010	F	N/A

*VSS=Volatile Suspended Solids. --=No effects. ○F=Failed

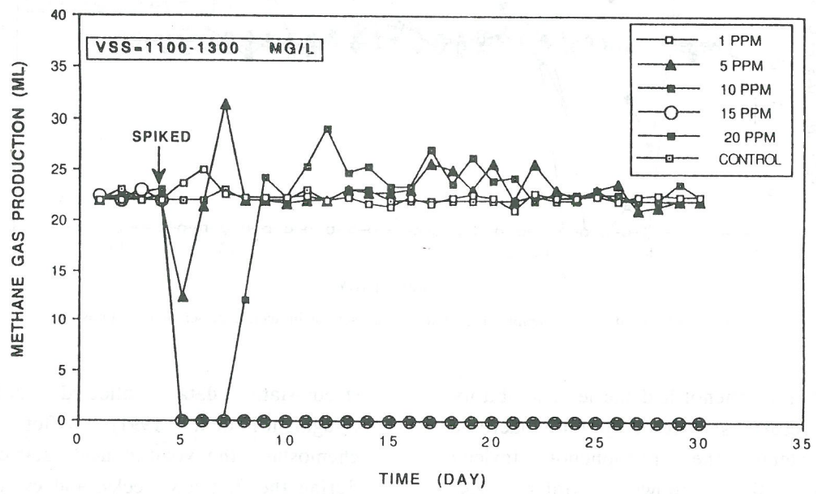

Fig. 4. Effects of 2,4-dinitrophenol on methanogenic bacteria in acetate enrichment systems.

were spiked with 5.5ppm of 4- nitrophenol reached a steady state faster than the chemostats that were spiked with 13.5 and 33.5 ppm.

Figure 6 shows that the new steady state was attained after about 5 weeks with 13.5ppm. During this period one of the chemostats failed because the bicarbonate in the system was lower than the design level of 5000 mgl^{-1}. This caused the pH to drop. Figure 6 also shows that the chemostats that were spiked with 33.5ppm of 4- nitrophenol took a longer time to reach a new steady state. During this period one of the chemostats failed due to low pH because the accumulated acetate exceeded the design bicarbonate alkalinity.

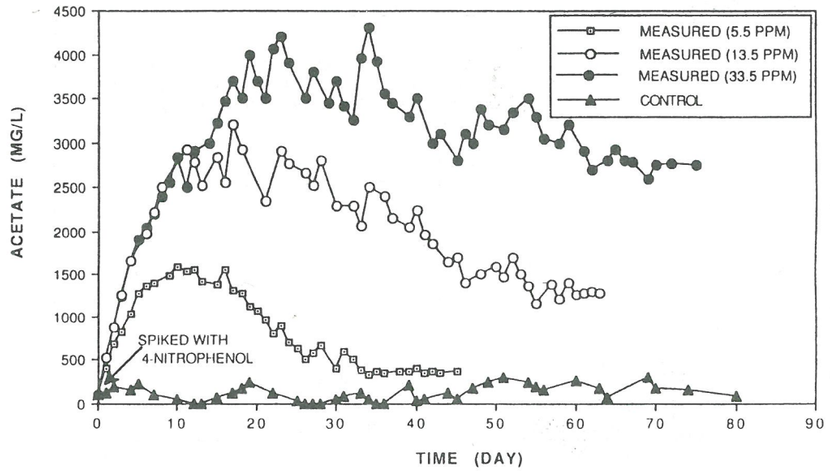

Fig. 5. Effects of combination dose of 4-nitrophenol on acetate enrichment systems.

Table 5. Effects of 2,4-dinitrophenol on methanogenic bacteria in acetate enrichment

Spiked(PPM)	Final pH	Final VSS*(mgl^{-1})	Recovery (d)	% Removal
Control	7.3	1428		N/A
Control	7.4	1250		N/A
Control	7.0	1310		N/A
1	7.1	1480	--	100
1	7.0	1430	--	100
1	7.0	1400	--	100
5	7.1	1216	--	100
5	7.1	1276	--	100
5	7.0	1484	--	100
10	7.0	1256	3	72.8
10	6.9	1288	3	70.7
10	7.0	1270	3	71.0
15	6.9	992	F○	N/A
15	7.0	844	F	N/A
15	7.0	878	F	N/A
20	6.8	1004	F	N/A
20	7.0	1160	F	N/A
20	7.0	1233	F	N/A

*VSS=Volatile Suspended Solids. --=No effects. ○F=Failed

Even though about 82% of added 4- nitrophenol was removed from 5.5mgl^{-1}, only about 55% was removed when the 4- nitrophenol spike was 33mgl^{-1} (Fig. 6). The HPLC results showed no formation of aminophenols or any other detectable intermediate compounds. Previous batch studies have indicated that in anaerobic filters, the 4- nitrophenol can be removed but byproducts such as aminophenol could be formed (Bhattacharya and Nandipati, 1991).

Since the chemostat was not at steady state, 4-nitrophenol concentration in the chemostat varied with time (Fig. 6). Since T_x in equations (3) and (4) is not the influent 4- nitrophenol concentration, the measured 4- nitrophenol concentrations in the chemostats were simulated by cubic polynomial equations (Fig. 6) and were substituted into equations (3) and (4) for T_x. The values of k_I were obtained from equations (3) and (4) by nonlinear

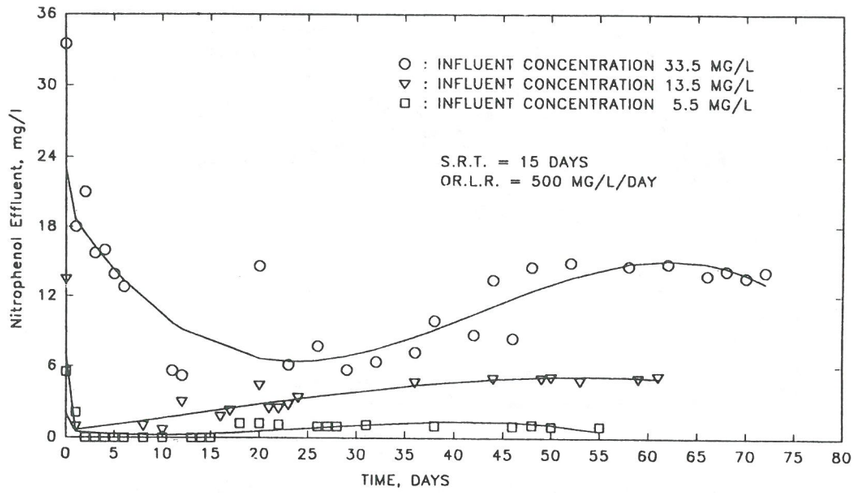

Fig. 6. Measured in 4-nitrophenol in acetate enrichment systems.

Table 6. Effects of nitrophenols on methanogenic bacteria in serum bottles (ATA)

Compound(mgl^{-1})	pH*	VSS*	Recovery (d)	% Removal
2-Nitrophenol				
1	7.3	1353	0	100
10	7.0	1397	0	100
20	7.1	1273	3	100
30	7.0	1243	5	100
40	6.9	1165	7	100
3- Nitrophenol				
1	7.2	1463	0	100
10	7.0	1198	0	100
20	7.1	1383	2	100
40	7.1	1213	5	100
4- Nitrophenol				
1	7.1	1393	0	100
5	7.1	1417	0	100
10	7.0	1357	4	100
20	7.0	1307	4	100
40	7.1	1287	5	100
80	7.0	1013	Failed	N/A
2,4-Dinitrophenol				
1	7.0	1437	0	100
5	7.1	1325	0	100
10	7.0	1271	3	72
15	7.0	905	Failed	N/A
20	6.9	1135	Failed	N/A

*Average at the end of experiments.

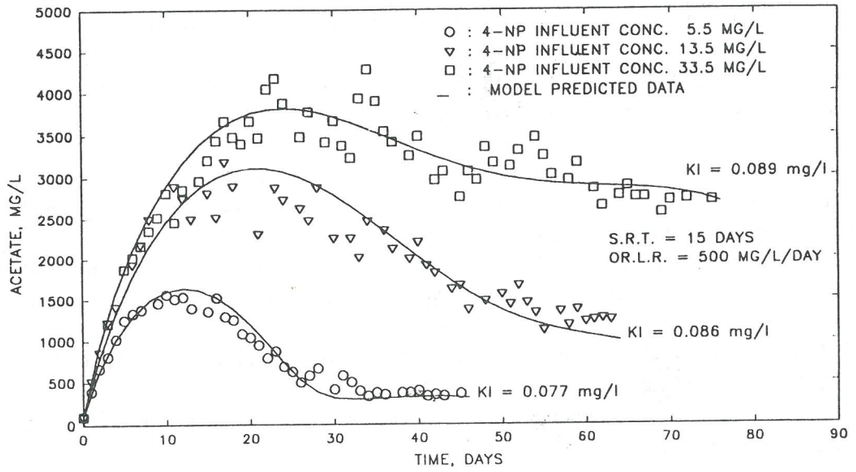

Fig. 7. Comparison of predicted and observed data with combination addition of 4-nitrophenol to acetate enrichment systems.

regression using the Levenberg-Marquardt algorithm with the known values of k,k_s, Y and b. The best fit k_I values were 0.077, 0.086, and 0.089 mgl^{-1} for 4- nitrophenol

influent concentrations of 5.5, 13.5, and 33.5mgl^{-1}, respectively (Fig. 7). This shows that the effects of 4- nitrophenol on methanogens are similar to competitive inhibition. This range of k_I values (i.e. 0.077-0.089 mgl^{-1}) compares very well with the k_I value of 0.07 mgl^{-1} reported by Wang et al. (1991) for competitive inhibition caused by 4- nitrophenol in anaerobic systems.

CONCLUSIONS

Among the nitrophenols studied in this research, the toxicity to acetate-utilizing methanogens decreased in the following order: 2,4- dinitrophenol >4- nitrophenol>2- nitrophenol>3- nitrophenol. The mononitrophenols (2- nitrophenol, 3- nitrophenol and 4- nitrophenol) were completely removed in serum bottles up to 40 mgl^{-1}. 2,4- Dinitrophenol was completely removed up to 5 mgl^{-1}. The competitive inhibition coefficient model adequately described the fate of acetate enrichment systems exposed to 4- nitrophenol. New steady states with higher effluent substrate concentrations were attained after 4- nitrophenol addition (combination dose). The estimated inhibition coefficient, k_I for 4- nitrophenol in acetate systems varied between 0.077 and 0.089 mgl^{-1}.

REFERENCES

Battersby, N.S., and Wilson, V. (1989) "Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge". *Appl. environ. Microbiol.* **55**,433-449.

Blasco, R., and Castillo, F.(1992) "Light-dependent degradation of nitrophenols by the phototrophic bacterium : *Rhodobacter capsulatus*" EIFI. *Appl. Environ. Microbiol.***58**, 690-695.

Bhattacharya, S.K., and Parkin, G.F., (1986) "Toxic substances in methane fermentation systems: fate and effect on process kinetics". *Final Report to* NSF. Grant NO. CEE-83-00987, Drexel University, Philadelphia, PA.

Bhattacharya, S.K., and Nandipati, R. (1991) "Combined anaerobic-aerobic treatment of hazardous leachate". *Proc. HMC-South, April, Houston,* TX.

Environmental Protection Agency, (1980) "Ambient Water Quality Criteria for Nitrophenols". EPA-440/s-80-063.

Eckenfelder, W.W., (1989) Industrial Water Pollution Control". McGraw-Hill, New York.

Jenkins, S. R., Morgan, J.M., and Sawyer, C.L. (1983) "Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration". J. *Wat. Pollut. Control Fed.*,**55**,448-453.

Haghighi-Podeh, M.R., (1991) "Fate and toxic effects of cobalt, cadmium, and nitrophenols on anaerobic treatment systems". Doctoral dissertation, Civil and Environmental Engineering Department, Tulane University, New Orleans, LA.

Nandipati, R.M., (1991) "Effects and fate of nitrophenols in combined anaerobic-aerobic treatment systems". M.S. Thesis, Civil and Environmental Engineering Department, Tulane University, New Orleans, LA.

Nelson, L. M., (1982) "Biologically-induced hydrolysis of parathion in soil: isolation of hydrolyzing bacteria". *Soil Biol. Biochem.,* **14**, 219-222.

Nojima, K., (1976) Studies of photochemistry of aromatic hydrocarbons III.*Chemosphere*, **5**, 25-30.

Owen, W. F., Stuckey, D.C., Young, L. Y. and McCarty, P.L., (1979) "Bioassay for monitoring biochemical methane potential and anaerobic toxicity". *Wat. Res.*,**13**, 485-492.

Parkin, G.F., and Speece, R.E., (1982) "Modeling toxicity in methane fermentation systems". *J. Environ. Engng. Div.,* ASCE, **108**, 515-531.

Sethunathan, N., (1973) "Degradation of parathion in flooded acid soil". *J. Agric. Food Chem.*,**21**, 602-604.

Standard Methods for Exmination of Water and Wastewater (1985) 15th Ed., American Public Health Association, Washington, DC.

Wang, Y. T., Gabbard, H. D., and Pai, P. C., (1991) "Inhibition of acetate methanogenesis by phenols". J. Environ. Eng. *Div.,* ASCE, **117**, 487-500.