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Anaerobic Biodegradation of Cyanide under Methanogenic Conditions

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Upflow, anaerobic, fixed-bed, activated charcoal biotreatment columns capable of operating at free cyanide concentrations of >100 mg liter^{-1} with a hydraulic retention time of <48 h were developed. Methanogenesis was maintained under a variety of feed medium conditions which included ethanol, phenol, or methanol as the primary reduced carbon source. Under optimal conditions, >70% of the inflow free cyanide was removed in the first 30% of the column height. Strongly complexed cyanides were resistant to removal. Ammonia was the nitrogen end product of cyanide transformation. In cell material removed from the charcoal columns, [^{14}C]bicarbonate was the major carbon end product of [^{14}C]cyanide transformation.

Reports over one-quarter century old describe anaerobic treatments for cyanide-containing wastes (15–17, 26). However, the widespread observation of cyanide toxicity to methanogens has resulted in minimal utilization of this technology, in spite of the potential for reduced operating costs versus aerobic treatment. Aerobic treatments and associated microbes have received much greater attention (e.g., see references 13, 18, and 32). Adaptation to low concentrations of cyanide can result in successful treatment of organics in anaerobic systems (6, 19, 26, 30, 33), but there has been little evidence for anaerobic biological breakdown of cyanide in most reports (6). In addition, cyanide spontaneously hydrolyzes to formic acid at a rate positively correlated with pH, temperature, and trace metal concentration (6, 9). Therefore, under the right conditions, with a long enough retention time, the cyanide spontaneously disappears, lowering the steady-state concentrations in the reactor. The first modern report of anaerobic biological cyanide transformation was made by Fedorak and Hrudey (6) in 1989 for semicontinuous batch cultures with a 25-day hydraulic retention time (HRT; time for 1 liquid volume replacement). In batch cultures from the same report, most methanogenesis occurred after the free cyanide had been significantly reduced (6). The major product of cyanide transformation could not be identified, although bicarbonate was found as a minor product (6).

Analytical considerations are important in dealing with cyanide. Cyanide complexation is a major source of imprecision in cyanide analysis for many wastes. In the presence of a variety of metals, cyanide forms complexes of varying affinities (1, 9, 27). In stronger complexes, cyanide is not detected by most cyanide assays unless distilled away from the metals (1). Complexed cyanide is also generally less toxic to microorganisms (27). Cyanide in the present report refers to "cyanide amenable to chlorination" unless otherwise indicated. Cyanide amenable to chlorination includes both uncomplexed and weakly complexed cyanide (1, 9).

Coal coking, precious metals mining, and nitrile polymer industries generate over three billion liters of cyanide-containing waste annually (31). Treatment technologies for this waste include both physical/chemical and biological methods (31, 32). Economic considerations make biological technologies especially attractive in wastes with high organic content, in which concentrations of organics and cyanide can be reduced simultaneously by the microbial consortia (31, 32). In attempting to address waste treatment of nitrile production effluents, we have developed an anaerobic, fixed-bed, activated charcoal reactor which successfully reduced free cyanide feed concentrations of ≥100 mg liter^{-1} by 80% or more with at an HRT of <2 days.

MATERIALS AND METHODS

Chemical assays. Gas production was continuously measured by wet gas meter. Flow was measured daily by weighing effluent tanks. Cyanide was analyzed colorimetrically as cyanide amenable to chlorination (1) (detects both free and weakly complexed cyanide). Unless otherwise specified, the term cyanide (CN) refers to this operationally defined material. Fatty acids, ethanol, and phenol were analyzed on a Hewlett-Packard 5880A gas chromatograph with flame ionization detector. Helium gas flow was 20 ml/min. The packed column was 1.8 m by 3-mm inside diameter Supelco stainless steel with 10% SP-1200–1% H_3PO_4 on 80/100 Chromosorb WAW. Temperature programming of 110°C for 0.5 min, 8°C/min to 160°C, and 5-min final time at 160°C was used. Peak identification and quantification were based on external standards. Two types of standards were used: (i) volatile fatty acid standard (10 mM) no. 46975; (Supelco, Bellefonte, Pa.) (ii) 1 and 10 mM mix of ethanol, acetic acid, *n*-butyric acid, isovaleric acid, and phenol. Carbon dioxide and methane were analyzed on a Hewlett-Packard 5880A gas chromatograph with thermal conductivity detection. Helium flow rate was 20 ml/min. The Supelco stainless-steel column (1.52 m by 3-mm inside diameter) was packed with 60/80 Carbosieve G and run isothermally at 110°C. Carbon dioxide and methane were all analyzed in the gas phase, with equilibrium calculations on acidified (pH 1) samples used to estimate liquid concentrations. Ammonia was analyzed colorimetrically as indophenol (29). Sulfide was analyzed colorimetrically, and total cyanide was analyzed as cyanide amenable to chlorination after distillation (1). Total carbohydrate in cell suspensions was analyzed by the phenol-sulfuric acid method (14).

Nitrogen balances for the systems are based on the

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formula $([NH_4\text{-}N] + [CN\text{-}N])_{in}/([NH_4\text{-}N] + [CN\text{-}N])_{out}$. Carbon balances for the system are based on the formula {([reduced carbon source-C] + $[CO_2\text{-}C]$ + $[CN\text{-}C])_{in}$ × flow rate}/({([reduced carbon source-C] + $[CO_2\text{-}C]$ + [CN-C] + $[CH_4\text{-}C]$ + [acetate-C] + [butyrate-C])$_{liquid\ out}$ × flow rate} + {($[CH_4\text{-}C] + [CO_2\text{-}C])_{gas\ out}$ × gas evolution rate}). These elemental balances were based on weekly samplings done from days 22 through 176.

When indicated, averaged data are cited as untransformed means plus or minus 1 standard deviation, with *n* equal to the number of datum points.

Media. Basal medium, per liter, was 1 ml of trace metals stock solution (stock, per liter = 100 mg of H_3BO_3, 100 mg of $Na_2MoO_4 \cdot 2H_2O$, 160 mg of $MnCl_2 \cdot 4H_2O$, 200 mg of $FeCl_3$, and 2 g of $CoCl_2 \cdot 2H_2O$), 1 ml of vitamin stock solution (stock, per liter = 2 mg of biotin, 2 mg of folic acid, 10 mg of pyridoxine HCl, 5 mg of riboflavin, 5 mg of thiamine-HCl, 5 mg of nicotinic acid, 5 mg of *d*-pantothenic acid, 0.1 mg of vitamin B_{12}, 5 mg of *p*-aminobenzoic acid, and 5 mg of DL-thioctic acid), 60 mg of $CaCl_2 \cdot 2H_2O$, 400 mg of $MgSO_4 \cdot 7H_2O$, 400 mg of KCl, 40 mg of NaH_2PO_4, 16 mg of NH_4Cl, and 2 g of $NaHCO_3$. Cyanide was added as sodium cyanide. Reduced carbon sources were ethanol, phenol, and methanol for the mining industry (M) system and ethanol and phenol for the coke and steel industry (C&S) system. Nominal concentrations for the M system were ethanol-phenol, 45:0 mM, through day 8 increasing in three equal, weekly steps to 45:5.6 mM from days 31 to 87 and then switching to ethanol-methanol, 45:12 mM, through day 94. The composition was then changed in six equal, weekly steps to ethanol-methanol, 18:62 mM, at day 137 which continued through the end of the experiment. Nominal concentrations for the C&S system were ethanol-phenol, 45:0 mM, through day 8 increasing in three equal, weekly steps to 45:5.6 mM on day 31, continuing to day 199. On day 199, concentrations were changed in a single step to 2.1:5.6 mM and remained that way until the end of the experiment. Additional materials were added to bring the media more in line with potential industrial wastes. For the C&S system, NH_4HCO_3 (200 mg of NH_4 liter^{-1}) and iron cyanide (50 mg of CN liter^{-1}; see below) were added. For the M system, iron cyanide (13 mg of Fe liter^{-1}; see below), $CuSO_4$ (42 mg of Cu liter^{-1}), $PbC_4H_6O_4$ (0.5 mg of Pb liter^{-1}), $AlK(SO_4)_2$ (0.5 mg of Al liter^{-1}), $NiCl_2$ (3.9 mg of Ni liter^{-1}), and $ZnCl_2$ (47 mg of Zn liter^{-1}) were added. Iron cyanide complex was added as a 1:1 (CN/CN) mix of potassium ferricyanide and potassium ferrocyanide. Metals other than iron (added as complex) were likely to form complexes of various strengths with free cyanide (9). (Nickel, zinc, and copper complexes are likely to be weak enough to be detectable in the cyanide amenable to chlorination assay used [9].) Summing metal additions, there was the potential for complexing approximately 133 mg of CN (≈50% of free cyanide added) and 2 mg of CN (<5% of free cyanide added) liter^{-1} in the M and C&S systems, respectively. The actual portion of cyanide complexed may have been lower due to a phenomenon such as sulfide mineral precipitation. The feed medium pH was adjusted to 8.0 to 8.6 by the addition of 0.2 to 0.5 ml of concentrated HCl liter^{-1}. Through day 110, distilled water was used as the diluent. After that date, tap water was used.

Reactor columns. Reactor columns were 5.1-cm inside diameter, 0.5-cm wall, clear polyvinyl chloride tubing. A polyvinyl chloride ball valve was included above the section containing activated carbon to allow separation of the sections without liquid loss. A diffuser of 4-mm glass beads, ≈4 cm in depth, was placed at the base of the carbon packing. A gas separation section approximately 30 cm above the highest sampling port routed evolved gas to the wet gas meters and protected the column from atmospheric oxygen. The carbon packing height was approximately 46 cm, consisting of 600 g of Calgon Filtrasorb 300 granular activated carbon. It had a wet, void volume of ≈66%. An equal unpacked, liquid volume extended 46 cm from the top of the charcoal column to the gas separator. At a nominal flow rate of 40 ml/h, the linear packed volume flow rate was ≈2.54 cm h^{-1}, while the linear unpacked volume flow rate was ≈3.8 cm h^{-1}. HRT to the highest sampling port was approximately 41 h. All effluent samples were collected from this port. To sample along the charcoal column height, 10 no. 4 butyl rubber stoppers were sealed with silicone cement into the polyvinyl chloride tube. These were sampled with a hypodermic needle and syringe. A coil with water circulating at 40°C was used to maintain the temperature in the columns at 35 to 40°C. Systems were started by inoculating 100 ml from a similar column receiving ethanol plus cyanide at the Du Pont Engineering Test Center, Newark, Del., along with a 500-ml inoculum of City of Wilmington, Del., anaerobic digester sludge. For the first 2 weeks, a nitrile mix, 2 ml of adiponitrile, 4 ml of 2-pentenenitrile, and 1 ml of methylglutaronitrile (per liter, final concentration), was added to the basal medium. These additions were used because the original inoculum came from nitrile-containing waste.

Concentrated cell suspensions. Liquid was backflushed from the columns by attaching an evacuated flask (flushed three times with oxygen-free nitrogen) to the base of the column. Vacuum was gently applied to the column with the medium feed shutoff. Up to 400 ml, approximately one-half of the column void volume could be removed without apparent harm to column performance. In an anaerobic bag (Coy Laboratory Products, Ann Arbor, Mich.) with an atmosphere of H_2-CO_2-N_2 (5:10:85), backflushed material was transferred to Sorvall (DuPont, Wilmington, Del.) 35-ml polycarbonate centrifuge tubes with O-ring seals. After centrifugation at 17,000 × *g* for 10 min at 10°C, the supernatant was replaced with enough degased, nitrogen-sparged, unreduced reaction buffer to achieve a 3- to 10-fold net concentration of the pelleted cells. Reaction buffer consisted of the basal medium described above (except sodium bicarbonate) with 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer and 0.0002% resazurin. Depending on the desired treatment, ethanol and sodium bicarbonate were added or not at 45 mM and 2 g liter^{-1}, respectively. With or without added reduced carbon, resazurin reduction occurred within minutes of cell addition and served as a check for healthy cells. Killed treatments were placed in boiling water for 10 min at this stage.

Incubations with the concentrated suspensions were run anaerobically with shaking (300 rpm) at 37°C in the dark in 5-ml serum vials with black rubber, crimped stoppers (Bellco, Vineland, N.J.). Cyanide concentrations were assayed as cyanide amenable to chlorination (described above). Radiolabel experiments, run in reaction buffer with bicarbonate but without ethanol (see above), used New England Nuclear sodium [^{14}C]cyanide, 50.5 mCi mmol, added at approximately 0.5 μCi ml^{-1}. Radiolabel was separated into three fractions: (i) cell associated: sedimented from reaction buffer at 12,000 × *g* for 3 min on a Beckman Microfuge B microcentrifuge; (ii) alkaline barium precipitate: 0.3 ml of the supernatant from spin i was combined with 0.12 ml of 0.1 N NaOH plus 0.03 ml of 40% $BaCl_2$, allowed to react for 5 min at room temperature, and then centrifuged as in spin i; the pellet was collected and washed once in 0.1

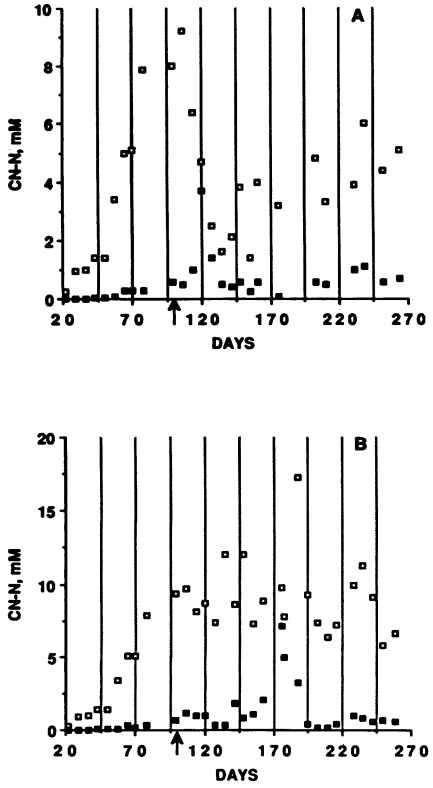

FIG. 1. Cyanide amenable to chlorination: (A) C&S system; (B) M system. Symbols: □, inflow; ■, outflow (41-h HRT). Arrow indicates 99 days, end of startup phase.

N NaOH; (iii) alkaline barium soluble: supernatant from spin ii. Fractions were counted in a Beckman model LS3801 scintillation counter, and counts were quenched-corrected via the H# method (Beckman, Livermore, Calif.).

RESULTS

Material balances/transformations. Based on preliminary experiments, the activated charcoal alone at pH 7.5 had an adsorptive capacity for free cyanide of approximately 5 g. This was exceeded after about 99 days for both columns. At the end of this startup phase, both C&S and M systems were removing cyanide (Fig. 1A and B) and showing reasonable nitrogen mass balances. For the C&S and M systems, respectively, nitrogen balances (N_{in}/N_{out}) fell from highs of 18.9 and 16.1 during startup to averages of 0.94 ± 0.22 (n = 9) and 1.38 ± 0.38 (n = 9) during days 99 through 176. Outflow cyanide concentration remained <20% of inflow concentration, except during two periods of lowered gas evolution. Profiles in both systems (see below) generally showed that free cyanide disappearance was accompanied by a concomitant increase in ammonia. As a percentage of total cyanide, cyanide not amenable to chlorination (i.e., strong complexes) was generally higher in the column effluents than in the inflow. Strongly complexed cyanide as a percentage of total cyanide changed from 19 ± 15.6% (n = 4) in the inflow to 64 ± 14.9% (n = 4) in the effluent in the C&S system. The M system showed a similar trend: 8.2 ± 5.3% (n = 4) in the inflow versus 34 ± 23% (n = 4) in the effluent.

Carbon balances (C_{in}/C_{out}) fell from a maximum during

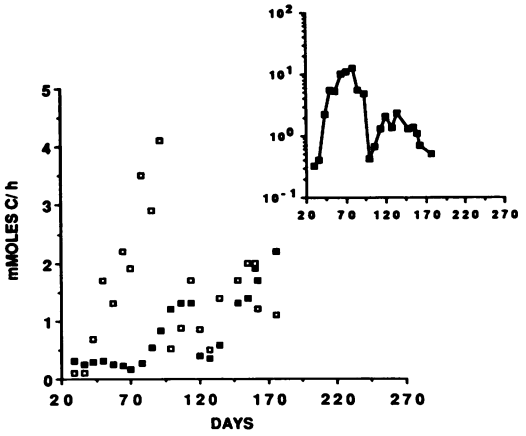

FIG. 2. Effluent carbon fluxes, C&S system. Symbols: ■, fatty acids (acetate and butyrate) in liquid; □, evolved gas (CH_4 plus CO_2). Insert shows ratio, C gas/C liquid (in micromoles).

startup of 6.3 and 7.1 to averages of 1.5 ± 0.51 (n = 11) and 1.16 ± 0.25 (n = 10) during months 4 and 5 for the C&S and M systems, respectively. The high ratio for the C&S system was most likely due to continued adsorption of phenol by the activated charcoal after the startup phase. Gas production started within 20 days of column inoculations (Fig. 2 and 3). Qualitatively, periods with high gaseous methane/dissolved fatty acid ratios correlated with periods of optimal cyanide removal. In both systems, evolved gas had a methane C/carbon dioxide C ratio of 5.8 (rate weighted average) during the post-startup phase (days 99 to 175). Methane accounted for about one-third of the carbon output from both systems during days 99 to 175. The major fatty acids identified were acetate and butyrate in the C&S system and acetate, propionate, and butyrate in the M system. Minor amounts of caproate were also seen.

Both systems saw periods of lowered gas evolution: days 114 through 138 for the C&S system (Fig. 2); days 156 through 194 for the M (Fig. 3) system. Poor cyanide removal was associated with these events (Fig. 1A and B). No causative perturbations were clearly linked with these periods. Recovery occurred within a few weeks.

Gas evolution also declined in the C&S system when the ethanol concentration was reduced in the medium on day 199

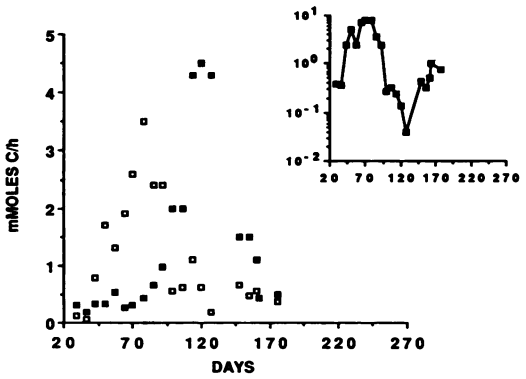

FIG. 3. Effluent carbon fluxes, M system. Symbols: ■, fatty acids (acetate and butyrate) in liquid; □, evolved gas (CH_4 plus CO_2). Insert shows ratio, C gas/C liquid (in micromoles).

TABLE 1. Profiles of pH, ethanol, CN, and NH_4 for C&S column during upset and recovery[a]

HRT (h)	pH				Ethanol (mM)				CN (mM)				NH_4 (mM)			
	Day 111	Day 117	Day 136	Day 167	Day 111	Day 117	Day 136	Day 167	Day 111	Day 117	Day 136	Day 167	Day 111	Day 117	Day 136	Day 167
0	8.66	8.51	8.40	8.7	51.0	48.7	75.3	52.3	6.4	4.7	2.1	7.7	8.1	6.6	11.7	1.8
1	8.43	8.4	8.06	7.88	50.6	49.1	74.4	—[c]	5.9	5.1	2.6	1.5	10.0	6.2	10.8	6.3
3	8.32	8.32	7.91	7.31	51	48.2	68.2	42.6	5.3	5.7	2.4	0.8	9.6	6.6	14.6	6.3
5	8.31	8.32	7.59	6.69	49.6	49.3	66.8	19.8	6.7	5.1	2.5	0.2	9.2	9.0	13.4	6.3
7	8.15	8.2	7.21	6.37	48.4	48.4	45.9	1.6	4.8	5.7	0.9	0.1	10.6	8.2	16.8	6.0
41	6.72	6.48	6.7	6.78	ND[b]	6.4	0.4	0.3	1	3.6	0.4	0.2	15.1	10.6	18.7	6.4

[a] Day 111 was 12/2/88; day 117 was 12/8/88; day 136 was 12/27/88; and day 167 was 1/27/89.
[b] ND, Not detected.
[c] —, no data.

(data not shown). This reduction in gas evolution correlated closely with the reduction in reducing equivalents fed to the column: a drop of ≈75% (42 mM ethanol plus 5.6 mM phenol→2.1 mM ethanol plus 5.6 mM phenol). Cyanide removal was maintained after this alteration in medium composition (Fig. 1A). Phenol was also being removed by the C&S system. On four sampling dates between days 174 and 238, inflow phenol concentrations averaged 5.7 ± 1.8 mM (n = 4), while effluent concentrations averaged 0.33 ± 0.15 mM (n = 4). Since gas evolution only dropped about 75 to 85% in the face of a 20-fold drop in ethanol feed concentration, phenol was serving as a source of reducing equivalents for gas evolution. However, based on a profile on day 231, phenol removal was slower than ethanol removal. Only 17% of inflow phenol was removed by the 7-h HRT position versus ≥80% for ethanol.

Column profiles. Chemical profiles from the C&S system during the failure and recovery periods show that biological activity at the base of the column was first lost and then recovered. Minimum gas evolution occurred during the week ending 12/18/88 (day 127) (Fig. 2). However, profiles show that cyanide metabolism on the column was declining prior to that date (Table 1). Cyanide and ethanol metabolism both were developing again at the base of the column by 12/27/88 (day 136) (Table 1). Gas evolution had also increased substantially by that time (Fig. 2). The profiles suggest that recovery of gas evolution occurred after cyanide removal rates had substantially recovered (Fig. 2, Table 1).

The M system over days 189 through 252 showed rapid disappearance of cyanide after recovery from a period of low gas evolution and poor cyanide removal (days 156 through 194) (Fig. 1B). Profile samples collected on days 189, 196, 203, 210, 231, 238, 245, and 252 showed the following mean cyanide concentrations (± standard deviations) at the given HRTs: 0 h, 9.58 ± 3.63; 1 h, 8.50 ± 1.58; 3 h, 1.39 ± 0.67; 5 h, 0.75 ± 0.25; 7 h, 1.64 ± 1.26; 41 h, 0.89 ± 1.01 mM. Detailed profile data show an increase in ammonia at the column base corresponding to cyanide removal (Table 2). Ethanol and methanol were ≥90% removed by the 7-h HRT position (Table 2). Also evident in the profile is the region of high turbidity and cell counts at the column base (Table 2). Microscopic observation showed a variety of cell types, including packets resembling *Methanosarcima*. Large cell clumps were common.

Microbial activities. Suspended cell mass at the column base, the region of most active cyanide removal, averaged 640 ± 250 μg of carbohydrate ml^{-1} (n = 5) in the M column. Cells backflushed and concentrated to approximately this density showed active cyanide removal under anaerobic conditions in the absence of charcoal (visual inspection). In such experiments, reaction buffer composition appeared to have a significant effect on the rate of cyanide disappearance

TABLE 2. M-system profile on 3/14/89 (day 214)

HRT (h)	Cell counts (cells/ml, 10^8)	Turbidity (OD_{660})[a]	pH	Sulfide (mM)	Sulfate (mM)	CN (μM)	NH_4 (μM)	Ethanol (mM)	Methanol (mM)	CO_2 (μl of gas/ml of liquid)[b]	CH_4 (μl of gas/ml of liquid)[b]	Fatty acids[c]
0		0.00	8.28	0.00	2.6	6,600	190	19.71	61.6	940	6	0 (-:-:-)
1	10.00	0.59	7.70	0.95	1.6	4,200	1,856	15.24	48.5	870	37	3.6 (7.8:11.2:1)
3		0.10	7.20	0.40	0.54	768	5,000	2.70	6.7	1,220	48	3.8 (1:1.2)
5	1.40	0.11	7.30	0.49	0.36	660	5,200	1.49	3.4	1,060	76	4.6 (9.5:14.5:1)
7		0.09	7.20	0.47	0.72	492	4,800	1.59	4.28	1,040	44	8.3 (10:22.3:1)
9		0.14	7.23	0.41	0.54	516	5,100	2.37	8.5	1,030	35	10.0 (10.7:20.7:1)
11		0.08	7.24	0.51	0.12	480	5,600	0.89	3.2	990	32	6.9 (9:15:1)
13		0.05	7.20	0.64	0.06	348		0.26	3.8	910	37	9.8 (12:20.3:1)
15	3.50	0.05	7.17	0.83	0.04	408	6,800	ND[d]	ND	990	34	9.4 (1:1.6:-)
17		0.05	7.12	0.91	0.02	336	6,100	0.02	ND	1,020	44	18.0 (42:57:1)
19		0.05	7.09	0.81	0.28	348	6,100	0.02	ND	1,070	48	16.0 (36:63:1)
41	2.20	0.05	7.14	0.80	0.08	456	4,700	0.15	ND	960	50	6.8 (1:3.3:-)

[a] Sample diluted 1:1 with distilled water. Taken in parallel with sample for microscopic count. Not an exact duplicate. OD_{660}, Optical density at 660 nm.
[b] Differences in CH_4/CO_2 ratios here and cited in the text reflect differences in solubility. Text referes to evolved gases; table refers to dissolved gases.
[c] Sum of analyzed volatile fatty acids and their molar ratio (acetate-propionate-butyrate).
[d] ND, Not detected.

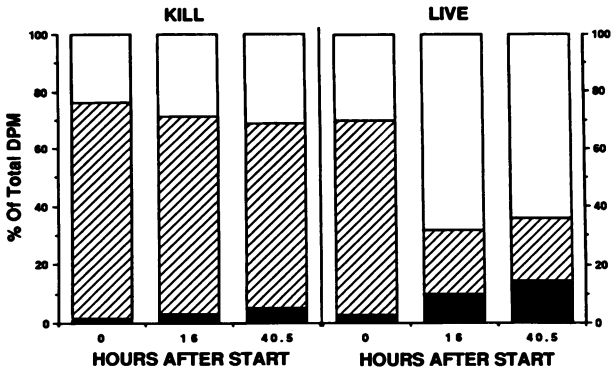

FIG. 4. Change in fractionation pattern versus time of ^{14}CN in concentrated cell suspension. Symbols: □, alkaline barium precipitate; ▨, alkaline barium soluble; ■, cell pellet.

in the live treatments. The rate of cyanide loss in the treatment with ethanol and bicarbonate added (1.13 ± 0.025 mg of CN liter^{-1} h^{-1}; $n = 3$) was significantly (t test; df = 2; $\alpha = 0.05$) different from the treatments with either bicarbonate (1.30 ± 0.01 mg of CN liter^{-1} h^{-1}; $n = 3$) or bicarbonate and ethanol (1.32 ± 0.046 mg of CN liter^{-1} h^{-1}; $n = 3$) removed. The rate of loss in the kill control was 0.31 ± 0.19 mg of CN liter^{-1} h^{-1} ($n = 3$), significantly lower than any of the live treatments (t test; df = 2; $\alpha = 0.025$).

Transformation of radiolabeled cyanide into bicarbonate went substantially faster in the presence of live cells (Fig. 4). Over 75% of the radiolabeled cyanide (soluble counts) was transformed in 16 h in the presence of live cells. Little transformation occurred in the presence of boiled cells. However, a significant portion of the radiolabel was not converted to bicarbonate, even with an extended incubation (Fig. 4) in the live treatment. In the radiolabel experiment, for the live treatment, cyanide (amenable to chlorination) went from 116 ± 18.8 to 7.3 ± 10.0 mg liter^{-1} in 16 h, equaling a loss rate of 6.8 ± 0.7 mg of CN liter^{-1} h^{-1} ($n = 3$). For the killed treatment, comparable data were 99.2 ± 8.3 to 84.3 ± 6.6 mg liter^{-1} in 16 h, equaling a loss rate of 0.93 ± 0.88 mg of CN liter^{-1} h^{-1} ($n = 3$). The lesser relative transformation in the radiolabeled cyanide versus the cyanide amenable to chlorination indicates that the alkaline-barium soluble material remaining at 40.5 h was either strongly complexed cyanide (undetectable in the assay) or some noncyanide product.

DISCUSSION

Although adaptation to CN concentrations of a few milligrams per liter has been described, with few exceptions, free cyanide has been considered highly toxic to anaerobic systems, especially methanogenesis (e.g., see references 5, 7, and 33). However, Fedorak and Hrudey (6) recently reported anaerobic degradation of cyanide at up to 30 mg liter^{-1} as total CN. In addition, Howe (15–17) patented a process for cyanide removal based on a tank-type, sequenced, anaerobic-aerobic digester. Inflow concentrations to the anaerobic system as high as 670 mg liter^{-1} as CN are claimed in the patent, but steady-state concentrations were ≤365 mg liter^{-1} (16, 17). A few other anaerobic waste treatment systems able to remain active in the presence of low concentrations of cyanide have also been reported (19, 30). The mechanisms of adaptation to and degradation of cyanide in anaerobic systems are not well understood (6).

In contrast to the small body of anaerobic literature, there are numerous reports of aerobic microorganisms and treatment systems able to degrade cyanide. Successful degradation generally requires free cyanide concentrations of 50 mg liter^{-1} or less (e.g., see references 20, 21, 27, and 32; for reviews, see references 13 and 19), but cyanide degradation in pure and mixed cultures at concentrations of up to 2,600 mg of free cyanide liter^{-1} have been reported (22, 28, 32). A variety of enzymatic pathways for cyanide degradation have also been described from aerobic organisms, including hydrolytic pathways leading to formamide (8) or formate plus ammonia (12, 21, 32) and the direct formation of bicarbonate plus ammonia via cyanide oxidation by what is described as a dioxygenase (12, 27).

The anaerobic systems described here showed good, long-term removal of cyanide in the presence of a variety of feed media. These systems retained metabolic activity at cyanide concentrations of up to about 300 mg liter^{-1}, which appears to be higher than reported previously for anaerobic microbes under controlled conditions (5, 7, 33). The charcoal included in the system may play an important role in the success of the columns. It is a surface rapidly colonized by microbes (34), preventing washout of the cells under less than optimal growth conditions. The appearance of methane in the presence of high cyanide levels seen in the M system (Table 2) also appears to be unusual. Fedorak and Hrudey (6) noted that in their systems methanogenesis recovered only after cyanide fell to below 5 mg of CN liter^{-1}. Development of microsites favorable for methanogenesis on the charcoal surface may be responsible for this observation. The charcoal may also serve to buffer the system from excursions in cyanide concentrations. When operating optimally, only charcoal at the column base is exposed to high cyanide concentrations. The upper portions may retain some adsorptive capacity, allowing a site of reduced free cyanide levels for microbial recovery. The observations of recovery of activity from the top down in the C&S system suggests that such a mechanism may be working.

Although cyanide may react abiologically in aqueous medium at 37°C (9, 15, 25), the mechanism for removal in the present study depended on active microbes. We observed a strong correlation between microbial activity and cyanide removal, implying an important role for biological activity. The observed pattern of decline and then recovery in cyanide removal fits a model of loss and then recovery of microbial transformation of cyanide. These observations are inconsistent with simple charcoal adsorption. Boiled kill controls showed significantly lower rates of cyanide transformation. Rapid transformation of cyanide in the absence of ethanol in the medium and the observation of bicarbonate as a reaction product are inconsistent with known reactions involving cyanide and ethanol fermentation products. However, these observations are consistent with known enzymatic mechanisms (8, 23).

Although Fedorak and Hrudey (6) observed anaerobic cyanide degradation, they were not able to identify the major end product clearly. Bicarbonate was identified as only a minor product. Our observations are the first to suggest that bicarbonate is an important end product for anaerobic cyanide degradation. Bicarbonate end product and the correlation of cyanide disappearance with ammonia appearance are consistent with a number of the hydrolytic pathways known from aerobic organisms (8, 23). Other potential enzymatic mechanisms for cyanide degradation appear less likely.

Insufficient sulfur availability and the observation of bicarbonate as a major end product argue against involvement of rhodanese or nitrogenase, enzymatic candidates capable of metabolizing cyanide which are known to be present in many anaerobes (13). The similar chemical structures of cyanide and carbon monoxide raise the intriguing possibility that carbon monoxide dehydrogenase (2) may play a role in anaerobic cyanide metabolism. However, recent reports on carbon monoxide dehydrogenase from *Rhodospirillum rubrum* suggest that this is an unlikely mechanism (3, 4).

A search for the organism(s) responsible for cyanide metabolism was not successful. A variety of enrichment cultures from the columns, including anaerobic carbon monoxide metabolizers (10), ethanol-metabolizing sulfate reducers (24), and *Clostridium klyveri* (11), all yielded positive cultures in the presence of up to 50 mg of free cyanide liter^{-1} (data not shown). However, no enrichment could be clearly shown to have cyanide-degrading activity. In addition, cyanide hydrolysis to formate is a thermodynamically favorable reaction ($HCN + 2H_2O \rightarrow HCOO^- + NH_4^+$, $\Delta G^0 = -15.6$ kcal [ca. -0.07 kJ]/mol), but enrichments for organisms capable of using this pathway for growth were negative. The potential for formic acid as an end product of cyanide metabolism suggests that formate metabolism (e.g., by methanogens) may be important in maintaining thermodynamically favorable conditions for cyanide removal. The occurrence of methane in the region of rapid cyanide transformation at the base of the column (Table 2) and our observations of methanogenesis in concentrated cell suspensions in the presence of >20 mg of cyanide liter^{-1} (data not shown) suggest that methanogenesis could play a role in removal of the end products of cyanide hydrolysis.

The utility of anaerobic treatment systems for cyanide remains generally unappreciated. In spite of the early work of Howe (15–17), anaerobic systems have generally not been used for waste containing high levels of cyanide. The kinetics of disappearance seen in the current systems imply that cyanide treatment systems with hydraulic retention times of <24 h may be possible for wastes with as much as 130 mg of free cyanide liter^{-1}. In addition, the use of a solid support (especially activated charcoal) allows cyanide degradation with simultaneous generation of methane, an improvement over tank reactor systems in which methanogenesis often occurs only after cyanide levels have been reduced, leading to longer retention times. A disadvantage of the described system is the failure to remove cyanide to very low levels. Effluent concentrations of cyanide amenable to chlorination rarely fell to below 1 mg of CN liter^{-1}, a concentration too high to release to surface waters (31). In addition, ammonia, generally an undesirable product, is the major form of effluent nitrogen. Strong cyanide complexes were also not completely removed. Thus, the most promising application for an anaerobic system may be as a pretreatment step for an aerobic system. Such pretreatment is likely to improve aerobic system efficiency by substantially lowering inflow cyanide concentrations, while at the same time removing a portion of the biological oxygen demand. A better understanding of the microbiology of anaerobic cyanide degradation could lead to improved biological treatment systems for aqueous cyanide wastes.

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