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CHAPTER 33

Fate of Cyanide in Aerated Microbial Systems

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Four mechanisms were investigated to determine their relative significance in the removal of cyanide from aerated microbial systems. Cyanide reaction with a common substrate, glucose, was found to be pseudo-first order and pH dependent, with pH 11.0 optimum. The reaction products were biodegradable. Stripping, together with metabolism, was shown to be an important mechanism for the disappearance of cyanide from solution near neutral pH, whereas physical adsorption of cyanide onto biological floc appeared to be relatively insignificant. Heterogeneous flocculant cultures showed some cyanide adsorption, but adsorption by nonflucculant cultures of *Bacillus megaterium* could not be detected. Acclimated heterogeneous cultures metabolized cyanide both as the only carbon source and in the presence of another carbon source, glucose. Glucose was readily metabolized in the presence of up to 10 mg/l KCN.

Introduction

Cyanide removal by biological oxidation has been the subject of considerable research over the past 40 years. Apparent cyanide metabolism has been demonstrated both with heterogeneous (Murphy and Nesbitt 1964) and pure bacterial cultures (Castric and Strobel 1969). Failure to account for stripping, adsorption, and for reaction with substrate could have caused erroneous interpretations of results in these studies. The objective of this research was, therefore, to determine the relative importance of four interrelated mechanisms of cyanide removal: (1) cyanide reaction in solution with substrate; (2) adsorption onto biological floc; (3) stripping; and (4) biological metabolism.

Hydrogen cyanide forms in water whenever soluble cyanide salts are added according to the following equilibrium:

$$HCN \rightleftarrows H^+ + CN^-$$

Thus, the ratio of HCN to cyanide ion is a function of pH. Since the pK for cyanide at 25 C is 9.21 (Izatt et al. 1962), cyanide is largely undissociated below pH 8. Although hydrogen cyanide can hydrolyze or polymerize in aqueous solution, it has been shown that for cyanide concentrations below 650 mg/l and temperatures below 100 C, these reactions are insignificant (Sanchez et al. 1967).

The reaction of cyanide ion (CN^-) with aldoses has received considerable attention in the chemical literature as a means of lengthening the aldose carbon chain. The reaction is dependent on pH, with cyanide ion acting as a nucleophilic reagent to yield a cyanohydrin product which then hydrolyzes to yield α-hydroxy-acids or unsaturated acids. The intermediate cyanohydrins usually are not isolated since they are rapidly hydrolyzed *in situ* (Pigman and Horton 1972). Chemists using this method have used high concentrations of aldoses and cyanide, and little quantitative rate information for the

reaction is available. Such reaction occurring at lower concentrations of substrate and cyanide and at neutral pH could account for a portion of the cyanide removal commonly attributed to biological oxidation. It could also cause errors due to reaction during sample storage. If the reaction products are biodegradable, long lag periods in batch bacterial systems utilizing carbohydrate substrates and containing cyanide may correspond to reaction times necessary to detoxify all or part of the cyanide (Castric and Stobel 1969; Zintgraff et al. 1969).

Adsorption onto biological floc has been proposed as a significant removal mechanism for cyanide, and a patent has been issued describing cyanide removal from waste systems by treatment with anaerobic digester solids, followed by aerobic biooxidation of the wastes (Howe 1964). It is not clear whether other removal mechanisms such as reaction with substrate or air stripping were considered for all or part of the removal. Stripping has been reported to be a significant removal mechanism in aerated biological treatment of cyanide-containing wastes (Ludzack and Schaffer 1960).

Possible aerobic removal of cyanide by biological oxidation is notable considering the toxicity of cyanide toward many biological enzymes and the more common bacterial electron transport systems (Dixon and Webb 1964). Pettet and Mills (1954) showed that cyanide concn of about 4 mg/l (as HCN) are toxic to unacclimated cultures, but that much higher cyanide concn can be tolerated and actually removed from solution by acclimated heterogeneous cultures. Most researchers report that ammonia production accompanied the apparent cyanide removal. Supplemental carbon sources such as glucose or complex media were usually fed to the biological oxidation units along with cyanide.

A pathway for bacterial metabolism of cyanide via an intermediate, β-cyanoalanine, has recently been demonstrated using *Bacillus megaterium* (Castric and Strobel 1969). The β-cyanoalanine is converted by whole cells to asparagine or other protein precursors. It is not clear whether cyanide enters the cell as HCN or as a product of extracellular reaction.

Materials and Methods

Microbial Cultures

A pure culture of *Bacillus megaterium*, ATCC Strain 2300, was obtained, reisolated, and identified according to morphological and physiological criteria (Breed et al. 1957). Heterogeneous sewage cultures from the primary clarifier effluent at the Bellaire, Texas, sewage-treatment plant were either added to a completely mixed flow reactor for cyanide acclimation or grown in batch cultures for use as unacclimated organisms. A 0.1% w/v glucose solution containing 10 mg/l cyanide and inorganic nutrients was fed to the cyanide acclimation reactor at a dilution rate of 0.083/h.

Analytical Methods

Cyanide was determined either by using an Orion Model 94-06 specific ion electrode (Fleet and Storp 1971) or by the modified Liebig titration method (APHA 1971). Soluble organic carbon analyses were carried out with a Beckman Model 915 carbon analyzer. The BOD of glucose and glucose-cyanide reaction products was determined manometrically (Hach Chemical Co.). Standard inorganic nutrients were used with additional NH_4Cl to give a 4 to 1 C/N ratio.

Concentrations of glucose were estimated using the Glucostat method (Worthington Biochemical Corp. 1972). In order to avoid interference with this enzymatic method,

cyanide was stripped from solution at neutral pH. Deionized water was subsequently added to correct for evaporation.

The ^{14}C was determined with a Beckman Model LS-133 scintillation counter equipped with automatic quench compensation. A dioxane cocktail, containing 5 g/l 2,5-diphenyloxazole and 100 g/l naphthalene, was used for both aqueous samples and solids such as whole cells or $Ag^{14}CN$ on filter paper. Radioactive carbon dioxide was separated from radioactive cyanide using the method of Brysk et al. (1969).

All reagents were ACS-certified and all glassware was acid cleaned, rinsed with deionized water, and dried before use. Constant ionic-strength buffer solutions were prepared with inorganic materials (Weast and Selby 1968) and checked against commercial standards (W. H. Curtin Co.). The pH was adjusted where necessary with small additions of acid or base solution.

Experimental Procedures

Glucose was chosen to investigate cyanide-substrate reactions since it is often used in biological investigations and, with other aldoses, is common in raw sewage and complex media. Reaction solutions were made up using fresh glucose and cyanide stock solutions and inorganic buffers. The pH dependence of the rate of the glucose-cyanide reaction was studied at 30 C in sealed glass ampoules to prevent cyanide loss due to volatilization. Samples were titrated with dilute $AgNO_3$ (0.0192N) using a 5-ml Class A burette (APHA 1971). Glucose-cyanide reaction products were produced for biodegradation studies by reacting glucose with excess cyanide in aqueous solution for 10 days at pH 11. No detectable unreacted glucose remained after the excess cyanide had been stripped from solution. Metabolism of glucose and glucose-cyanide reaction products was investigated with manometric measurements of oxygen uptake by acclimated heterogeneous microbial seed. Bottles for manometric measurements were filled with 70 ml of reaction product solution containing 18.1 mg of carbon, 5 ml acclimated seed, and 82 ml of inorganic nutrient dilution water saturated with oxygen. BOD bottles containing the same quantities of carbon (as glucose), inorganic nutrients, and acclimated microbial seed were used as controls. Two additional controls contained only 5 ml of seed and 152 ml of inorganic nutrient dilution water.

Cyanide adsorption onto biological floc was tested using both nonflocculating organisms, *B. megaterium* ATCC Strain 2300, and heterogeneous flocculant organisms. Unacclimated cells were grown on tryptic soy broth, concentrated by centrifugation, and washed with physiological saline. Concentrated cells were injected by glass syringe through a serological stopper into a 255-ml stirred glass reactor containing a known quantity of cyanide in pH 7.0 phosphate buffer. The reactor was vented to a second syringe which served as a reservoir during injection of solids and removal of samples for cyanide analysis. This reservoir, initially completely filled, decreased in volume as samples were periodically removed. The samples were centrifuged in sealed tubes and the supernatant solutions were analyzed for cyanide.

To test for cyanide stripping, a leak-tight Microferm Fermentor (New Brunswick Scientific Co. Model MMF-07) was used so that temperature, air-flow rate, and mixing could be carefully controlled. Humidified, filtered air was used for stripping. Off-gas cyanide and carbon dioxide were captured in two 250-ml Fisher-Milligan gas traps connected in series, each filled with 200 ml of 0.1 N NaOH. Starved, heterogeneous cultures suspended in deionized water (pH 7.0) were used to detect effects on stripping due to solids.

The microfermentor used in stripping experiments was also used to test the extent of bacterial metabolism of cyanide. Glucose and $K^{14}CN$ solution were added at the start of each experiment to starved, acclimated heterogeneous cultures. The off-gases were absorbed in caustic solution and analyzed for $H^{14}CN$ and $^{14}CO_2$. Final reactor solutions were also analyzed for $H^{14}CN_{(l)}$, $^{14}CO_2$, and cellular ^{14}C-carbon. Reactor cyanide, glucose, and solids concentrations were monitored periodically by withdrawing and analyzing samples.

Results

The reaction between glucose and cyanide is pseudo-first order, with an optimum pH of 11.0 (Fig. 1). The reaction of cyanide with aldoses can thus significantly reduce the amount of free cyanide in solution at pH greater than 8.0. The oxygen-uptake experiments indicate that the glucose-cyanide reaction products are biodegradable (Fig. 2). The lower plateau BOD of the reaction products can be attributed to the higher percentage of oxygen present in the aldonic acid molecules.

No significant adsorption onto nonflocculating cells of *B. megaterium* occurred (Table 1). However, with heterogeneous flocculant suspensions, some rapid uptake of cyanide was apparent which is presumably attributable to adsorption.

Stripping curves for cyanide without solids present (Fig. 3) indicate that virtually 100% cyanide recovery was possible in the apparatus used. With 3300 mg/l of flocculant solids present (Fig. 4), approx. 17% of the cyanide was unaccounted for after 6 h, with

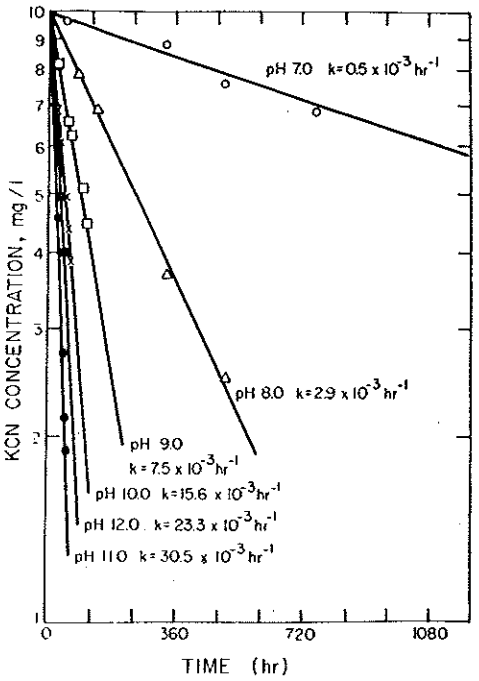

FIG. 1. Cyanide-glucose reaction as a function of pH at 30 C. Initial conditions: glucose 1000 mg/l, KCN 10 mg/l.

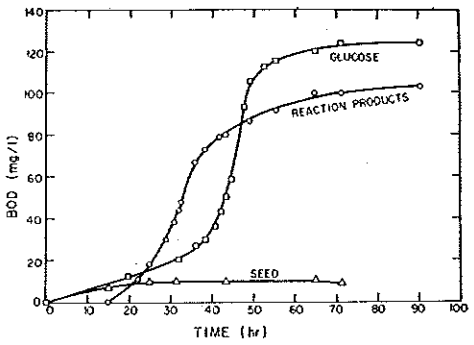

FIG. 2. BOD of glucose and cyanide-glucose reaction products at 20 C, using acclimated seed, 259 mg/l C and C/N, and C/P ratios of 4/1 and 20/1, respectively.

TABLE 1. *Adsorption of cyanide by biological floc*

Contact Time min	Nonflocculant Culture of *Bacillus Megaterium*[a] Remaining KCN mg/l	Flocculant Culture of Heterogeneous Organisms[b] Remaining KCN mg/l
3	20.2	15.6
20	20.7	15.3
40	20.3	14.7
60	20.0	14.3
80	–	14.1

[a] Initial conditions: KCN, 20.0 mg/l; dry solids, 6000 mg/l.

[b] Initial conditions: KCN, 16.0 mg/l; dry solids, 7260 mg/l.

most of the loss occurring within the first hour. These results confirm that rapid initial adsorption is important with flocculant organisms, although the subsequent experiments with $K^{14}CN$ show that metabolism must account for part of the cyanide loss, at least when no other primary carbon source is available (Fig. 5).

Acclimated heterogeneous cultures readily metabolized glucose in the presence of cyanide (Fig. 6). When fed $K^{14}CN$, most of the KCN activity was recovered from the off-gas washers. However, since the pK for CO_2 is 6.3, a large amount of $^{14}CO_2$ remained in solution and was recovered in the reactor samples. Locations of the final activity

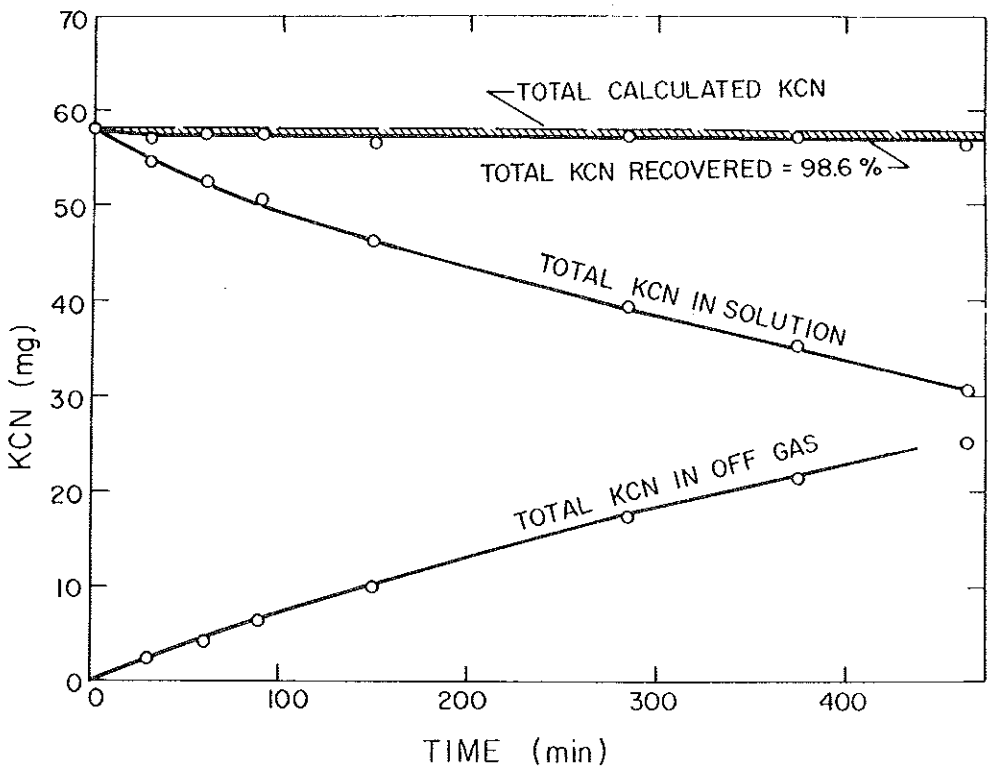

FIG. 3. Cyanide recovery in stripping apparatus with no solids present, at pH 7.0 and 30 C. Stirring rate 700 rpm, air-flow rate 2000 cc/min, and liquid vol of 6 liters.

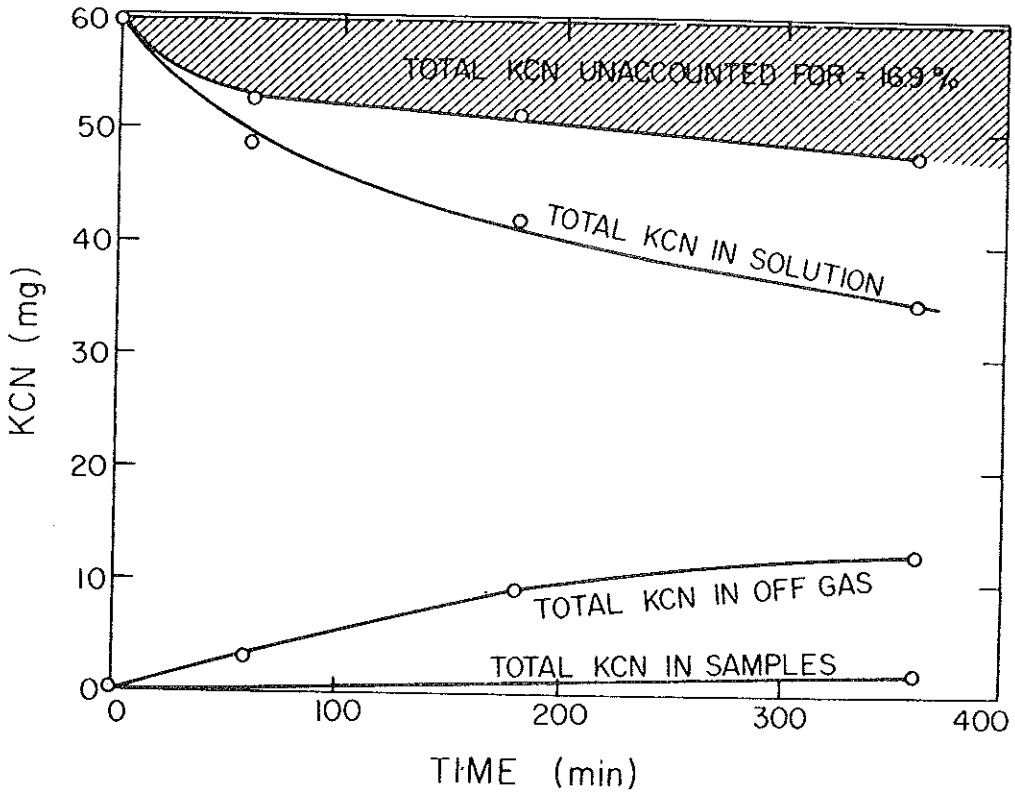

FIG. 4. Cyanide stripping with 3300 mg/l solids present. pH 7.0, 30 C, stirring rate 700 rpm, air-flow rate 2000 cc/min, and liquid vol of 6 liters.

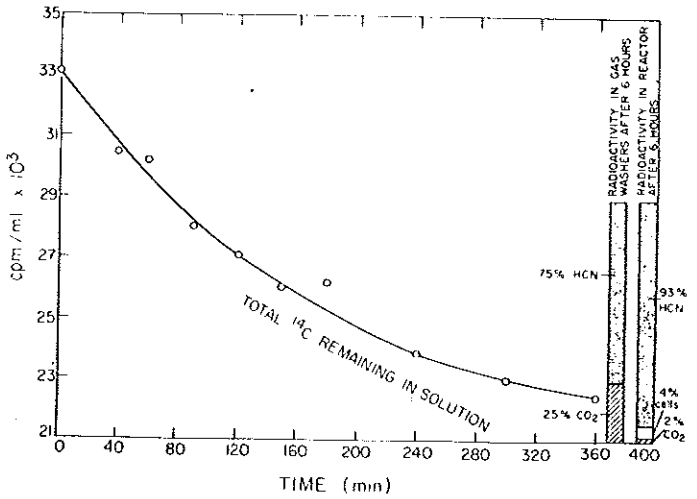

FIG. 5. Distribution of ^{14}C during stripping in the presence of 3130 mg/l solids. pH 7.0, 30 C, stirring rate 700 rpm, air-flow rate 2000 cc/min, and liquid vol of 6 liters.

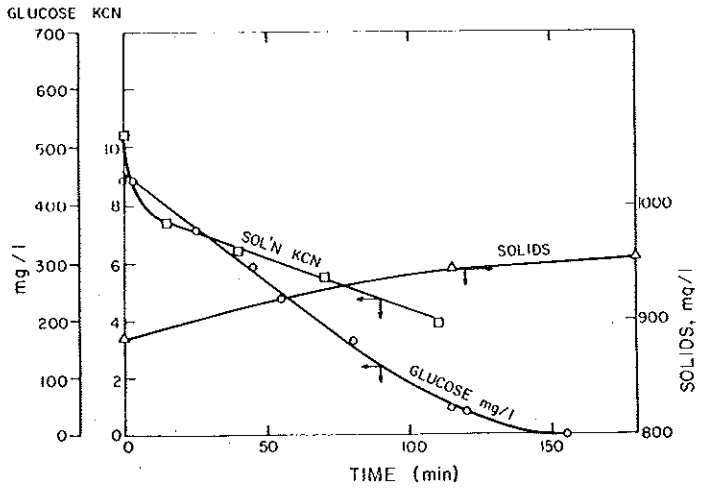

FIG. 6. Glucose metabolism in the presence of cyanide. Aerated batch reactor, 30 C and stirring rate 700 rpm.

TABLE 2. *Metabolism of $K^{14}CN$ by aerated batch cultures of heterogeneous bacteria*

Solids Concentration mg/l	% of Recovered Activity		
	$K^{14}CN$	$^{14}CO_2$	^{14}Cells
835	64	26	10
1963	41	56	3

immediately after depletion of glucose are shown in Table 2. Recovery of radioactivity ranged from 87 to 95%. Stripping and metabolism appear to account for approx. 90% of total cyanide removal. The ^{14}C in the cells was either present in cyanide, adsorbed so tightly that washing of the cells did not remove it, or represents ^{14}C incorporated into cellular material due to metabolism.

Discussion

Since cyanide reacts rapidly with aldoses such as glucose at pH above 8.0, alkaline storage of samples containing both can lead to serious analytical errors. The reaction could also account for cyanide removal in prolonged bacterial growth experiments, particularly if pH was not controlled at or below neutral. Since the reaction products are biodegradable, disappearance of CN could be partially due to chemical reactions followed by biodegradation. Experiments utilizing cyanide concentrations near the toxicity threshold particularly could be affected.

Physical adsorption of cyanide onto biological floc probably accounts for only a small portion of total cyanide removal in aerated microbial systems. Since adsorption was undetected in the nonflocculating suspensions, it is inferred also that adsorption occurs only onto the mesh-like polysaccharide matrix characteristic of the flocculant organisms.

Production of significant amounts of $^{14}CO_2$ by cultures fed $K^{14}CN$ indicates that bacterial metabolism of cyanide does take place, both with and without the presence of another primary carbon source. Stripping of HCN is significant in aerated systems and should be considered as a primary removal mechanism.

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