FATE OF CYANIDE AND RELATED COMPOUNDS IN
AEROBIC MICROBIAL SYSTEMS—II.
MICROBIAL DEGRADATION

S. F. RAE,* W. G. CHARACKLIS, M. A. KESSICK and C. H. WARD
Environmental Science and Engineering, Rice University,
Houston, Texas 77001, U.S.A.

(Received 10 May 1976; revised 1 December 1976)

Abstract—Cyanide metabolism was studied using starved, acclimated heterogeneous cultures in an
aerated microfermenter containing glucose as substrate. Tests using K14CN indicated that up to
50% of the cyanide was metabolized as evidenced by 14CO2 production. Experiments employed initial
solids concentrations between 483–1563 mg/l and initial glucose concentrations between 100–600 mg/l.
Initial cyanide concentration was 10 mg/l.

INTRODUCTION

The sanitary engineering literature includes numerous
studies of "apparent" cyanide metabolism by hetero-
geneous cultures. However, failure to account for
cyanide loss due to stripping or cyanide reaction in
solution with carbohydrate substrates either as single
substrates or as components of complex media
diminishes the value of such studies. In other cases,
cyanide effects were masked by the toxic effects of
heavy metals or organics such as phenol.

Cyanide destruction by trickling filters has been
studied using both simple cyanides such as NaCN,
HCN, and KCN (Gurnham, 1955; Pettet and Mills,
1954; Porter, 1960; and Brink, 1960), and metal-
cyanide complexes (Gurnham, 1955; Winter, 1962;
and Pettet and Mills, 1954). Cyanide concentrations
above 2 mg/l adversely affected unacclimated filters
initially, but the filter organisms demonstrated rapid
recovery from shock loadings and, once acclimated
to high cyanide concentrations, withstood up to
200 mg/l CN (Gurnham, 1955). Brink (1960) re-
ported that supplementary sewage or substrate was
necessary for successful trickling filter degradation of
cyanide.

Results of activated sludge studies are similar to
those reported for trickling filters. When fed with
sewage, cyanide concentrations up to 50 mg/l have been
tolerated by activated sludge systems (Ludzack, 1960),
but again, the presence of heavy metals or other sub-
strate greatly influenced the tolerance limit. Loss of
cyanide due to volatilization was significant, particu-
larly when cyanide exceeded 60 mg/l (Ludzack, 1960).
Kostenbader (1969) reported on treatment of coke
oven wastes which contained ammonia, phenol, and
thiocyanates in addition to cyanide. Phenol degra-
dation in the systems exceeded 99%, but cyanide and
thiocyanate degradation varied considerably (from
10–99%). Inhibition occurred when the ammonia con-
centration exceeded 2000 mg/l. Catchpole and Cooper
(1972) studied the effects of adding certain "growth
factors" to thiocyanates and cyanide containing
wastes. Several amino acids, p-aminobenzoic acid,
pyruvic acid and D-glucose added to the wastes in
concentrations below 5 mg/l significantly reduced the
retention time required for cyanide and thiocyanate
reduction. When glucose concentrations exceeding
50 mg/l were added to wastes with high cyanide
concentrations up to 200 mg/l and thiocyanate con-
centrations up to 500 mg/l (as NH4CN), a gelatinous
sludge developed which interfered with treatment.
Degradation of organic cyanides has been studied by
Ludzack (1958, 1959). After acclimation, acrylonitrile,
acetonitrile, lacetonitrile, oxacrylonitrile, benzoni-
trile and adiponitrile were successfully assimilated
when mixed with sewage. Effluents contained high
nitrate concentrations.

Murphy and Nesbitt (1964) investigated the effects
of carbon source and sludge wasting on the biological

treatment of potassium cyanide. In general, cyanide
removal was poor using acetate as substrate, and the
system was sensitive to loading changes. Using a syn-
thetic sewage prepared from fish food, however, much
better cyanide removal was obtained. Sodium hydrox-
ide was added to the feed tank to prevent loss of
cyanide due to volatilization. Cyanide seemed to be
converted to nitrate, and failure of the system was
prevented by an increase in effluent ammonia and
nitrite concentrations. A separate study using K14CN
indicated cyanide was being metabolized to 14CO2.
Overall radioactivity recovery for the experiment was
72.5%.

Zintgraf (1968) studied the inhibition by cyanide
of mixed bacterial populations using growth systems
(BOD bottles). Glucose was the primary substrate
and both the initial inoculum and the initial cyanide
concentration was varied. Cyanide was found to be
extremely toxic to unacclimated cultures under the
*Presently with S.I.P. Engineers, Houston, Texas, U.S.A.
test conditions. Each $2.5 \times 10^{-5} \text{M/1 KCN}$ extended the lag phase by 10 h, and KCN concentrations as low as $1.0 \times 10^{-3} \text{M/l}$ produced a noticeable effect on the lag period. Above $2.5 \times 10^{-4} \text{M/1 KCN}$, growth rate also decreased. Using the normal inoculum of bacteria, $5 \times 10^{-4} \text{M/1 KCN}$ appeared to be the toxic limit as manifested by oxygen uptake within 300 h.

Zingtgraf listed three possible explanations for increasing lag periods with increasing KCN concentration:
(A) A few cyanide-resistant bacteria survive and eventually reach sufficient concentration to exert an oxygen demand.
(B) The lag period represents time necessary for the bacteria to overcome the effects of cyanide saturation of active sites in the bacterial cytochrome oxidase systems.
(C) The lag period represents shifts in bacterial populations with increasing cyanide.

In acclimation studies, Zingtgraf studied the effect of cyanide on open aerobic systems, anaerobic systems with nitrate as the electron acceptor, and anaerobic fermentative systems. The fermentative system showed the highest degree of acclimation. Although the aerobic growth system was supposedly acclimated to cyanide, a longer lag period was still obtained prior to glucose removal with KCN present than in the control, and the culture containing KCN left a higher refractory soluble carbon portion.

Howe (1965) also described a growth system (two stage lagoon) which successfully treated cyanide. Simple cyanides have been successfully degraded anaerobically by acclimated sewage, but unsuccessful results were reported for nitriles (Ludzack, 1959).

Howe (1963) described a method of detoxifying cyanide by pretreatment with digester sludge followed by aerobic biological oxidation. Howe hypothesized that a significant amount of cyanide was adsorbed by the sludge and hence, the waste was detoxified.

**EXPERIMENTAL METHODS**

Analytical techniques have been described previously (Raef, et al., 1976).

**Selection and acclimation of a heterogeneous culture**

Preliminary experiments with both *B. pumilus* and *B. megaterium* indicated that these organisms did not readily metabolize glucose under aerobic conditions. Also, maintenance of large quantities of homogeneous cultures was difficult with available facilities. Therefore, heterogeneous sewage organisms were acclimated to cyanide and used in additional experimental work.

A six liter aerated continuous flow reactor (Fäbco-Busch) was initially filled with settled primary effluent from the Belleaire, Texas Sewage Treatment Plant containing a small inoculum of *B. megaterium*. Feed to the unit was maintained at 8.3 ml/min providing a hydraulic detention time of approximately 12 h. Sterilized feed to the reactor consisted of 1000 mg/l glucose, 10 mg/l CN, and inorganic nutrients (Table 1). No solids were wasted other than those in the clarified effluent. After 14 days, the continuous feed was turned off and the reactor was fed batchwise by daily addition of 30 g of dextrose, 24 ml of 2.5 mg/ml (CN⁻) standard cyanide solution, and 600 ml of inorganic nutrient solution. A similar volume was wasted, providing for a sludge age of approximately 8.8 days. One ml of solution containing trace metals was added to the reactor once per week. Batch feeding was continued for the duration of the remaining experiments (three months).

**Experimental procedure**

In addition to adsorption, stripping and reaction with substrate, cyanide metabolism represents another source of cyanide removal. Stripping experiments with $\text{H}^+\text{CN}$ suggested that small amounts of cyanide are metabolized, with cyanide presumably being a primary substrate. Additional tests were conducted with glucose as primary substrate to investigate its effect on cyanide metabolism.

The same microfermentor apparatus used in the stripping experiments was employed (Raef, et al., 1976). Cultures of starved heterogeneous flocculent cultures, acclimated to cyanide, were batch-fed cyanide. $\text{H}^+\text{CN}$ and $\text{CO}_2$ in the offgas were absorbed in washers containing caustic. Reactor sampling was conducted as described in the $\text{C}^+\text{C}$ stripping tests. All CN⁻ samples were drawn into vials containing NaOH. The glucose, solids, and K₄CN samples were collected separately, except for total $\text{C}^+\text{CN}$ samples (KCN + CO₂ + cells) which were collected and transferred to scintillation vials within 10 sec of sampling to avoid loss of HCN. Ten ml glucose samples were stripped of cyanide for 3-5 h and analyzed using Glucostat (Worthington Biochemical Co.). Solids samples were diluted quantitatively so that 20 ml of the diluted sample would filter through double 0.45 μm filters within 10 min. At the completion of the experiment, after all glucose was removed, gas wash solutions and the final reactor solutions were analyzed for $\text{H}^+\text{CN}$, $\text{CO}_2$, and for $\text{C}^+\text{C}$ integrated into cellular material. Solids were separated from solution by both filtration through 0.45 μm filter paper and by centrifugation following 3-4 washes with physiological saline. After removal of biological solids, in the first two experiments, soluble cyanide was separated from carbon dioxide by precipitating the carbon dioxide with barium hydroxide, but the method of Brysk (1969) was used in later experiments since it involved less experimental error and precluded the possibility of HCN adsorbing to BaCO₃ particles.

For each experiment, acclimated organisms were deprived of cyanide 48 h prior to the experiment and the cultures were batch fed glucose and inorganic nutrients in order to build sufficient biomass rapidly. Twenty-four hours prior to the experiment, the culture was batch fed.

Table 1. Feed solution to the continuous acclimation reactor

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>12 g</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>3.38 g as P</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>1.2 g as N</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>0.36 g as Na+</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.12 g as Mg²⁺</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.044 g as Ca²⁺</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>4 mg as Fe³⁺</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>3 mg</td>
</tr>
<tr>
<td>MnSO₄·H₂O</td>
<td>24 mg</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>3 mg</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>3 mg</td>
</tr>
<tr>
<td>CaSO₄·2H₂O</td>
<td>3 mg</td>
</tr>
<tr>
<td>12 l with deionized water</td>
<td></td>
</tr>
</tbody>
</table>

* Solution = 0.5 g/l FeCl₃, 0.001 g/l EDTA pH = 2.0 using concentrated HCl.
Table 2. Comparison of cyanide metabolism experimental results

<table>
<thead>
<tr>
<th>Experiment No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4*</th>
<th>5*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial glucose, mg/l</td>
<td>103</td>
<td>483</td>
<td>455</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Initial biological solids, mg/l</td>
<td>592</td>
<td>606</td>
<td>848</td>
<td>1963</td>
<td>483</td>
</tr>
<tr>
<td>Initial CN⁻, mg/l</td>
<td>10.7</td>
<td>11.4</td>
<td>10.4</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Yield, mg biological solids formed/mg glucose consumed</td>
<td>0.19</td>
<td>0.21</td>
<td>0.22</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cyanide recovery, %</td>
<td>66.4</td>
<td>76.7</td>
<td>23.5</td>
<td>43.6</td>
<td>53.2</td>
</tr>
<tr>
<td>¹⁴C recovery, %</td>
<td>78.1</td>
<td>64.8</td>
<td>49.6</td>
<td>57</td>
<td>65</td>
</tr>
<tr>
<td>Final solution: ¹⁴CN⁻, %</td>
<td>49</td>
<td>90.5</td>
<td>27</td>
<td>4.6</td>
<td>24.2</td>
</tr>
<tr>
<td>¹⁴CO₂, %</td>
<td>49</td>
<td>1.2</td>
<td>39</td>
<td>15.1</td>
<td>0.2</td>
</tr>
<tr>
<td>¹⁴Cells, %</td>
<td>2</td>
<td>8.3</td>
<td>34</td>
<td>6.0</td>
<td>10.5</td>
</tr>
<tr>
<td>Remaining in stripping flask, %</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>74.3</td>
<td>65.1</td>
</tr>
<tr>
<td>Total gas washers ¹⁴CN⁻, %</td>
<td>94</td>
<td>88.4</td>
<td>93.3</td>
<td>68.5</td>
<td>72.3</td>
</tr>
<tr>
<td>¹⁴CO₂, %</td>
<td>6</td>
<td>11.6</td>
<td>6.7</td>
<td>27.8</td>
<td>21.5</td>
</tr>
<tr>
<td>Remaining in stripping flask, %</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Maximum unit reaction rate, mg glucose/mg biol. solids h</td>
<td>0.26</td>
<td>0.09</td>
<td>0.27</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Experiments in gas washers.

Table 3. Distribution of ¹⁴C at the termination of the cyanide metabolism experiments*

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Initial biological solids (mg/l)</th>
<th>¹⁴CN (%)</th>
<th>¹⁴CO₂ (%)</th>
<th>¹⁴Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>592</td>
<td>62.8</td>
<td>36.0</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>606</td>
<td>89.6</td>
<td>9.7</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>848</td>
<td>75.7</td>
<td>15.2</td>
<td>9.1</td>
</tr>
<tr>
<td>4</td>
<td>1963</td>
<td>74.5†</td>
<td>23.5</td>
<td>2.1</td>
</tr>
<tr>
<td>5</td>
<td>483</td>
<td>84.9†</td>
<td>8.8</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* Assumes 100% ¹⁴C recovery.
† Assumes some CN in gas washer either polymerized or formed organic compounds when cyanide was added.

10 mg/l cyanide with no glucose to starve the culture. After centrifuging and washing in physiological saline to remove other nutrients, the cells were resuspended in 51 of deionized water buffered at pH 7.0 and containing inorganic nutrients. Standardized K¹⁴CN solution was added to yield 10 mg/l CN⁻. Required glucose was added as a 10% solution, the reactor was sealed, and the gas washer siphon and air flow were started. All experiments were conducted at a mixing speed of 700 rev/min, and at an air flow rate of 2 cc/min at STP. Sampling procedures were the same as those used in earlier stripping experiments. Temperature was adjusted to 30°C prior to adding K¹⁴CN and glucose. When all glucose was consumed, the experiment was terminated (due to stripping requirements to remove cyanide from glucose samples, experiments were terminated approximately three hours after the glucose was consumed). Gas washers were changed periodically to ensure that all HCN and CO₂ would be recovered. In two simultaneous experiments, 500 ml glass gas washers with fritted glass diffusers and magnetic stirring bars were used as reactors. After initial samples, the reactors were not sampled for 12 h at which time the experiments were terminated. Since no reactor or gas washer sampling occurred during the experiments, the experiments were free of sampling loss. These reactors, shown in Fig. 1, were initially fed 10 mg/l CN⁻ and 600 mg/l glucose.

![Fig. 1. Typical gas washer reactor used in the second set of cyanide metabolism experiments.](image_url)
and contained two different biological solids concentrations. Air flow was controlled using identical Matheson NBS Model 602 flow meters.

RESULTS

Results of experiments run in the microfermentor are given in Figs. 2-10. Unfortunately, $^{14}$C other than in the $^{14}$CN or $^{14}$CO$_2$ form was not analyzed in the first three experiments and thus appears as CN$^-$. One experiment (not reported) was terminated due to formation of a viscous biological polymer which interfered with the cyanide test. This phenomenon may have occurred in experiment No. 3 which resulted in apparent rapid loss of cyanide. Adhesion to the cells by the polymer would have resulted in higher cellular $^{14}$C reacted with cyanide.

Acclimated heterogeneous cultures readily metabolized glucose in the presence of cyanide. Maximum yield, based on glucose, averaged approximately 0.2, and unit reaction rates were as high as 0.27 mg MLSS/mg glucose-h. When fed K$^{14}$CN, most of the $^{14}$C activity was recovered from the off-gas washers as $^{14}$CN$^-$. Since the pK for CO$_2$ is 6.3, $^{14}$CO$_2$ did not strip from solution as readily as H$^{14}$CN. Recovery of $^{14}$C ranged from 50 to 78%, probably due to incomplete recovery when separating HCN and CO$_2$ from the soluble reactor contents and the caustic gas washers. Significant $^{14}$C remained in the acidified distillation flask for separation into H$^{14}$CN and $^{14}$CO$_2$ fractions by acid stripping. The $^{14}$C residual may represent cyanide reactions that took place either biologically or extracellularly during the time between sampling and analysis while preserved in caustic, resulting in formation of acid stable compounds. Another possibility is that polymerization of CN$^-$ under basic conditions occurred. The $^{14}$C fraction separated with the cellular material was either present as adsorbed, or as $^{14}$C incorporated into cellular material due to metabolism.

SUMMARY DISCUSSION

The relative importance of four interrelated mechanisms for cyanide removal in aerated biological systems was investigated. In an aerated system at neutral pH with both cyanide and glucose present, the

---

![Fig. 2. Cyanide metabolism experiment No. 1—glucose, CN, and biological solids.](image)

![Fig. 3. Cyanide metabolism experiment No. 1—$^{14}$C.](image)
Fig. 4. Cyanide metabolism experiment No. 1—Cyanide recovery.

Fig. 5. Cyanide metabolism experiment No. 2—glucose, CN, and biological solids.

Fig. 6. Cyanide metabolism experiment No. 2—$^{14}$C.
Fig. 7. Cyanide metabolism experiment No. 2—Cyanide recovery.

Fig. 8. Cyanide metabolism experiment No. 3—glucose, CN, and biological solids.

Fig. 9. Cyanide metabolism experiment No. 3—$^{14}$C.
relative importance for cyanide acclimated heterogeneous cultures appears to be: (1) stripping; (2) biological metabolism; (3) adsorption onto biological floc, and; (4) chemical reaction in solution with substrate. At higher pH, chemical reactions in solution become significant.

The reaction of cyanide with glucose is pseudo-first order and pH dependent, with an optimum pH near 11.0. The reaction is sufficiently rapid above pH 8 to cause serious experimental error in cyanide determinations if samples containing both cyanide and glucose (or other aldoses) are stored at high pH. The cyanide-glucose reaction products were found to be biodegradable, suggesting the possibility of combining cyanide-containing wastes at high pH with wastes containing large amounts of aldoses in a pretreatment step prior to biological oxidation. In this manner, cyanide-containing wastes could be biologically destroyed in aerated systems without stripping HCN into the atmosphere.

Combined effects of the cyanide-glucose reaction and increased biological polymer formation (resulting in increased cyanide adsorption and bacterial resistance to cyanide) may have caused the “induced” metabolism of cyanide in an activated sludge plant fed small amounts of glucose (Cate and Cooper, 1972). In the experiments of Murphy and Nesbitt (1964), cyanide was added to soluble fish meal substrate which probably contained significant aldoses, and the solution was stored at high pH prior to being fed to the continuous reactors. This probably explains the high biological reaction rates and low HCN stripping experienced. Complex media such as tryptic soy broth also contains aldoses, and biological experiments involving cyanide should consider effects of cyanide-substrate reactions.

The cyanide-glucose reaction is not considered important in the BOD experiments of Zintgraff (1968) providing neutral pH was maintained.

Adsorption effects can be important as a cyanide removal mechanism. Thus, the benefits of mixing digester solids with cyanide-containing wastes prior to aerobic biological treatment (Howe, 1963), can probably be attributed to adsorption and/or stripping effects in the aerated system and chemical reactions in solution. Surface properties of biological floc influence the degree of adsorption, possibly due to more surface sites in larger polysaccharide matrices. Adsorption to dispersed, pure culture of bacteria was insignificant. Stripping of cyanide is significant under the neutral conditions of biological treatment and is not appreciably affected by the presence of biological solids.

Acclimated heterogeneous bacteria metabolize cyanide as evidenced by $^{14}CO_2$ production in cultures fed $K^4CN$. The $^{14}C$ recovered as $^{14}CO_2$ and in cells greatly exceeds background levels predicted from exchange between carbon atoms and from consideration of the HCN/CO$_2$ separation efficiency. It is unclear whether $^{14}C$ not recovered as $H^4CN$ or $^{14}CO_2$ by acid stripping represents an acid-stable chemical reaction or metabolic product or represents inefficiency of the stripping system.

**SUMMARY CONCLUSIONS**

1. Significant reactions between cyanide and aldoses such as glucose can occur above pH 8, indicating that alkaline storage of cyanide samples containing aldoses can result in errors in analysis of cyanide. The reaction with glucose is pseudo-first order, with an optimum pH of 11.0. The reaction products are biodegradable.

2. In an aerated biological system demonstrating cyanide removal, both cyanide stripping and cyanide metabolism are important removal mechanisms. Adsorption on biological floc is of lesser importance, but the extracellular composition of bacterial cells does influence the degree of cyanide adsorption.

3. In instances where a plant discharging a cyanide-containing waste is in close proximity to a plant discharging an aldose carbohydrate waste, the possibility exists to combine the two waste streams at high pH in a pretreatment step prior to biological ox-
dation. Pretreatment will detoxify the waste and eliminate HCN as a potential air pollutant during aerobic biological treatment.

Acknowledgments—The senior author gratefully acknowledges financial support from the U.S. Environmental Protection Agency (Training Grant No. T900175) and the National Science Foundation (ENG 74-1957) during this research.

REFERENCES


