THE ISOLATION AND IDENTIFICATION OF CYANIDE-TOLERANT MICROORGANISMS FROM A TRICKLING FILTER

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THE ISOLATION AND IDENTIFICATION OF CYANIDE-TOLERANT MICROORGANISMS FROM A TRICKLING FILTER

by

CHARLES KENNER STEARNS

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ABSTRACT

A scale model trickling filter was put into operation with a cyanide concentration of 10 ppm. The concentration of cyanide was raised to 25 and finally to 56 ppm.

As the microorganisms increased the utilization of cyanide, the production of nitrites was increased while the nitrate concentration remained near a constant level.

Cyanide interfers with the measure of the BOD of the settled sewage in high concentrations. The BOD of the effluent from the filter, though affected by cyanide, did not show as marked an effect as did the BOD of the settled sewage. This may be attributed to the presence of cyanide-tolerant microorganisms and the clarifying action of the filter. It is believed that with the addition of cyanide-tolerant microorganisms to the BOD dilution water, the interference of cyanide on the BOD will be diminished.

Through chemical analysis it was determined that cyanide was converted to nitrate, nitrite, and ammonia.

A medium containing dextrose was more favorable for growth of microorganisms in the presence of cyanide than a medium without dextrose.

Sodium cyanide can be utilized by some microorganisms as a sole source of nitrogen but fail to grow

when used as a carbon source.

The cyanide-tolerant microorganisms were isolated from the trickling filter and identified according to the procedures outlined in Bergey's "Manual of Determinative Bacteriology".

M. Mallmann

INTRODUCTION

Cyanides are by-products of many industrial processes, particularly those associated with the metal industry. Cyanide wastes of varying concentrations result from the purification of gold and silver from their ores, in the manufacture of water gas, and in electroplating processes. These wastes are generally discharged into city sewage systems or directly into streams and lakes.

The cyanides in industrial wastes, when introduced into streams and lakes, may destroy microbial and fish populations. When introduced into sewage plants, the cyanides may affect adversely the biological activity in digesters and activated sludge systems.

Because the cyanides are poisonous, they must be removed or detoxified before being dumped into a water or sewage system. The methods of removing cyanides from industrial wastes include the following: oxidation with chlorine or permanganate, removal of cyanide as the free acid, conversion of cyanide to thiocyanate or cyanhydrin compounds, hydrolysis to ammonia and formate, precipitation of cyanide as the insoluble iron complex, and electrolysis of the waste liquor. The chemical methods involved are both expensive and time consuming, and in addition, require space for the collection of the cyanide-containing wastes. A less expensive method might be the use of a trickling filter system with utilization of the cyanide by microorganisms.

The discovery of a microorganism which will utilize cyanide as an energy source was the ultimate goal of this research.

HISTORICAL REVIEW

The tolerance of a microorganism towards cyanide may depend upon various factors, including concentrations of cyanide, the medium used, pH, growth factors available, and the complex nature of the cell. A simple medium may be used to test the cyanide-tolerance of some micro-organisms although others require special nutrients. The majority of microorganisms found in sewage may be grown on a simple medium without special nutrient constituents or conditions, although a few require anaerobic conditions (3). The literature is lacking in information concerning medium requirements associated with cyanide tolerance.

The general statements involving cyanide-tolerance of microorganisms are scattered and could not be covered in a single paragraph. In order to produce continuity, the microorganisms will be considered individually and discussed in the order of their classification.

Rao and Rao (13) found that both _Nitrobacter_ and _Nitrosomonas_ were inhibited by cyanide. Tam and Clark (16) found that calcium cyanide inhibited nitrification in the soil by _Nitrosomonas_ and _Nitrobacter_.

Pseudomonas can be adapted to cyanide concentrations as high as 200 ppm (8). Cozic (7) found that _Acetobacter xylinum_ was relatively cyanide-tolerant, however no statement of the degree of tolerance was given.

The gram-positive cocci appear to be the most cyanide-tolerant microorganisms although they are not found in abundance in sewage (8). Carpenter (6) found that streptococci tolerated 5,000 ppm of cyanide and Sarcina lutea was cyanide tolerant but to a lesser degree. The activity of virulent cocci was not inhibited by cyanide while the activity of avirulent cocci was decreased (7).

Alcaligenes fecalis was found to be inhibited by low concentrations of cyanide (14).

Burnet found that Eschericheria coli (5) resisted 1,000 ppm of cyanide which agreed with the results obtained by the author. Dessy (9) reported that E. coli was killed in six hours by 100,000 ppm of sodium cyanide. Shigella tolerated low concentrations of cyanide while Salmonella typhosa was completely inhibited (5).

The spores of Bacillus subtilis are more resistant to cyanide than are the vegetative cells. The spores survived five days in 1,000 ppm of cyanide whereas the vegetative cells survived only in concentrations of 100 ppm or less (9).

The remaining microorganisms will be considered as one group and included in this group are molds, fungi, and algae. Among the algae, Nitella is cyanide-tolerant (10) and Chlorella on sugar-free media is not inhibited by cyanide (9). Molds appear to be more resistant to cyanide than any other organism. Molds resist ten percent sodium cyanide for more than 24 hours (16). Soil fungi and Actinomycetes are affected very little by the cyanide.

Literature citing the possibility of sodium cyanide as an energy source for microorganisms could not be found although cyanide reduction was noted in many cases but was not attributed to a direct utilization of the cyanide by microorganisms.

EXPERIMENTAL PROCEDURE

In order to develope a microbial flora that will utilize cyanides as a source of energy, it was necessary to devise a trickling-filter apparatus where the filtrate could be recirculated to build up the microbial population on the filter substrate. A laboratory trickling filter was constructed with proportions similar to those of a pilot plant filter located on the site of the East Lansing Sewage Disposal Plant. The model was 24 inches high with a three inch diameter. The settling tank and feed tank had five and one liter capacities respectively. A roller compressed a length of rubber tubing and forced the liquid from the feed tank to the filter at a rate of 30 milliliters per minute and a recycle ratio of five to one. The rate of flow was controlled by the rubber tubing and by a screw clamp which regulates the flow of the liquid to the pump. Figure 1 is a schematic flow diagram of the system. Figures 2 and 3 are views of the overall system and the pump respectively. The filter substrate consisted of lime glass tubing 12 by 12 millimeters.

The sewage fed to the filter was obtained from the effluent of the primary settling basin in the East Lansing Plant. The domestic sewage, free from industrial wastes, often contained ground garbage which had not settled.

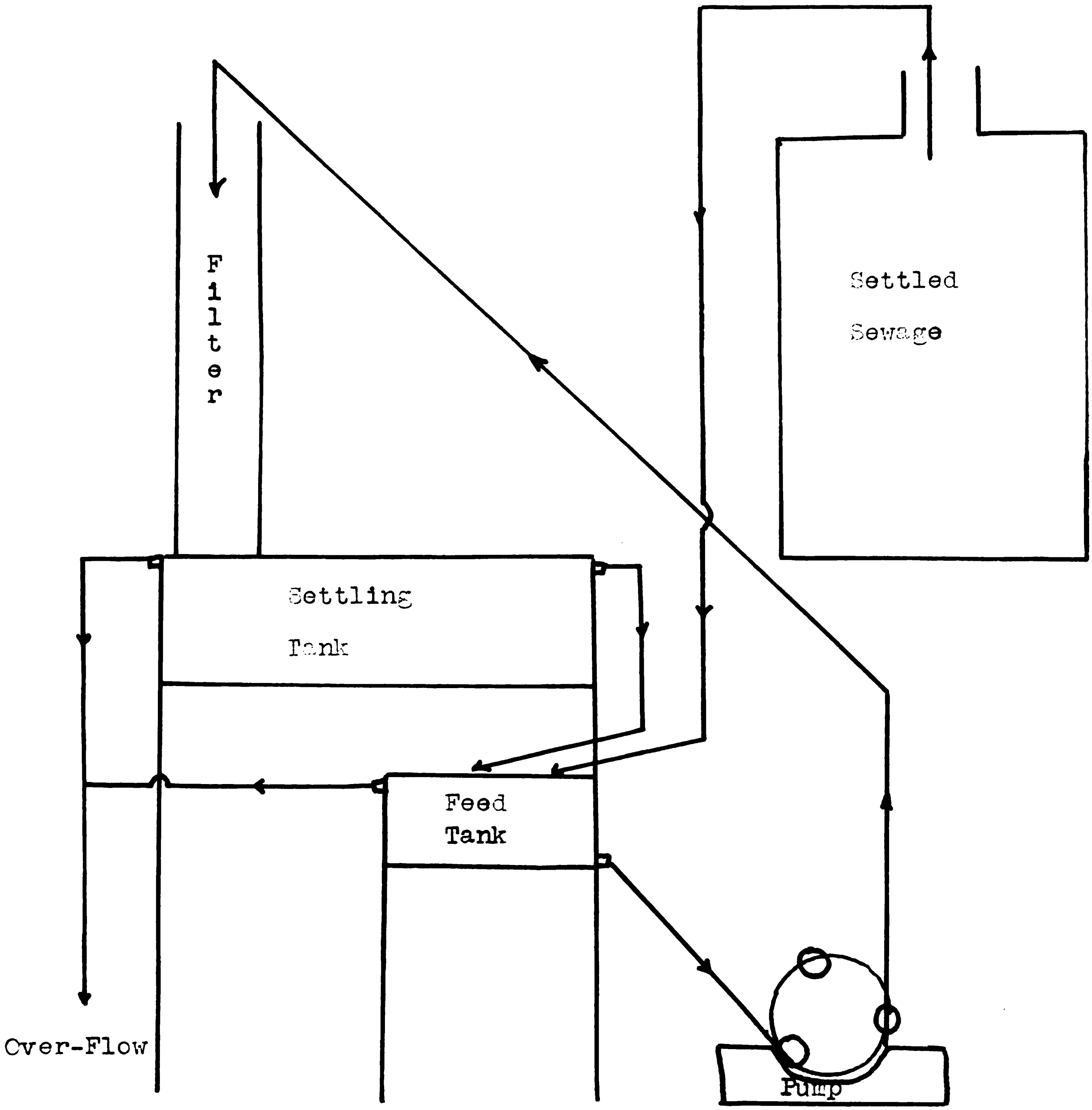


FIGURE 1

SCHEMATIC FLOW DIAGRAM

FIGURE 2

OVER-ALL VIEW

FIGURE 3

THE FILTER

The filter was put into operation with the domestic sewage effluent plus an addition of ten parts per million of sodium cyanide. After a five-week adaption period, the cyanide concentration was raised first to 25 ppm and then to 56 ppm where it was maintained throughout the remainder of the experimentation period.

In order to measure the activity of the microbial population in the filter, various chemical and biological tests were performed. Samples were taken daily: at the outlet of the settling tank (effluent); at a junction after the pump (influent), and from the sewage containing sodium cyanide which had not passed through the filter. These samples were tested in duplicate for biological oxygen demand (BOD), nitrites, and nitrates (phenoldisulfonic method) (1). Nitrates and nitrites were determined colorimetrically employing a Coleman, model 9 Nephlo-Colorimeter. The routine analysis for BOD, nitrites, and nitrates was performed according to the procedure outlined in Standard Methods (1). The cyanide concentrations were determined by distillation of the total cyanide and Liebig titration of the distillate (15). The two common methods of determining the concentrations of cyanide are titration and colorimetry. Titration with silver nitrate with potassium iodide as the indicator was used for the determination of cyanide. The endpoint in low concentrations was rather weak but with experience, the endpoint was more evident. The procedure was taken from a work performed at Yale University (15). Qualitative tests were performed for the presence of ferrocyanide, ferricyanide, and thiocyanate as possible cyanide conversion products. The qualitative tests performed were taken from a qualitative analysis manual (11). The streak-plate method using Nutrient Agar (Difco), Dextrose Agar (Difco), and a modified Bokorney's synthetic agar as growth media were employed as a means of isolating the microorganisms from the filter. The plates were incubated at room temperature. The microorganisms isolated were identified according to descriptions outlined in Bergey's Manual of Determinative Bacteriology (3).

Cyanide-tolerence studies were performed using sodium cyanide in various concentrations as a source of carbon and nitrogen. A medium containing both carbon and a nitrogen source was used as a control.

Those microorganisms which would utilize sodium cyanide as a sole source of nitrogen were then studied to determine the rapidity of cyanide reduction. The microorganisms were repeatedly inoculated into synthetic broth with cyanide as the nitrogen source. Those microorganisms which survived were then inoculated into a number of flasks containing the synthetic medium with cyanide. A distillation every six hours was performed to determine the amount of cyanide utilized by the microorganisms.

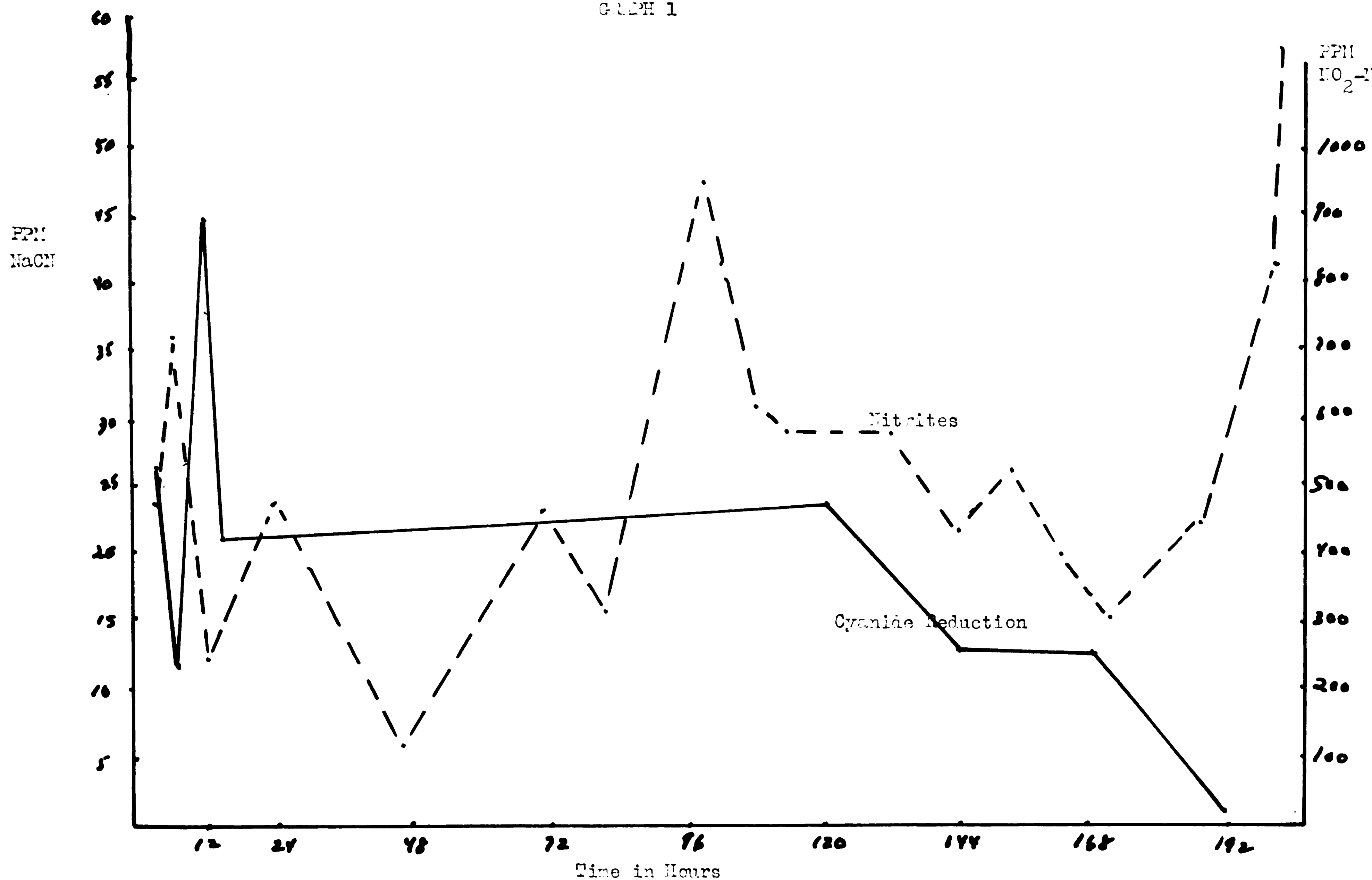
Studies were also made on the effect that different kinds of media would have on the growth of a microorganism in the presence of cyanide.

PRESENTATION AND ANALYSIS OF RESULTS

Graph 1 represents the reaction of the microorganisms to the shock of cyanide as measured by the cyanide reduction and nitrite production when the concentration of sodium cyanide was raised to 56 ppm in the filter. The initial reaction of the microorganisms to the high concentration of cyanide had a stimulatory effect that reduced the cyanide concentration and elevated the nitrite production. In the three-hour period following the addition of cyanide, the cyanide reduction practically ceased with a corresponding drop in the nitrite production. The microorganisms underwent a slow adaption period in the final stages, until the reduction of cyanide was nearly complete and the production of nitrites extremely large. In the final analysis for cyanide and nitrite, the microorganisms reduced the cyanide concentration to one ppm and were producing approximately 5,000 ppm of nitrite measured as nitrite nitrogen. The microorganisms must reduce the cyanide to produce nitrite while the nitrate concentration remains relatively constant. As reported by Pettet and Thomas (12), nitrate production is inhibited by high cyanide concentrations which would account for the build-up of the nitrites in the effluent.

REDUCTION OF CYANIDE AND THE PRODUCTION OF NITRITE

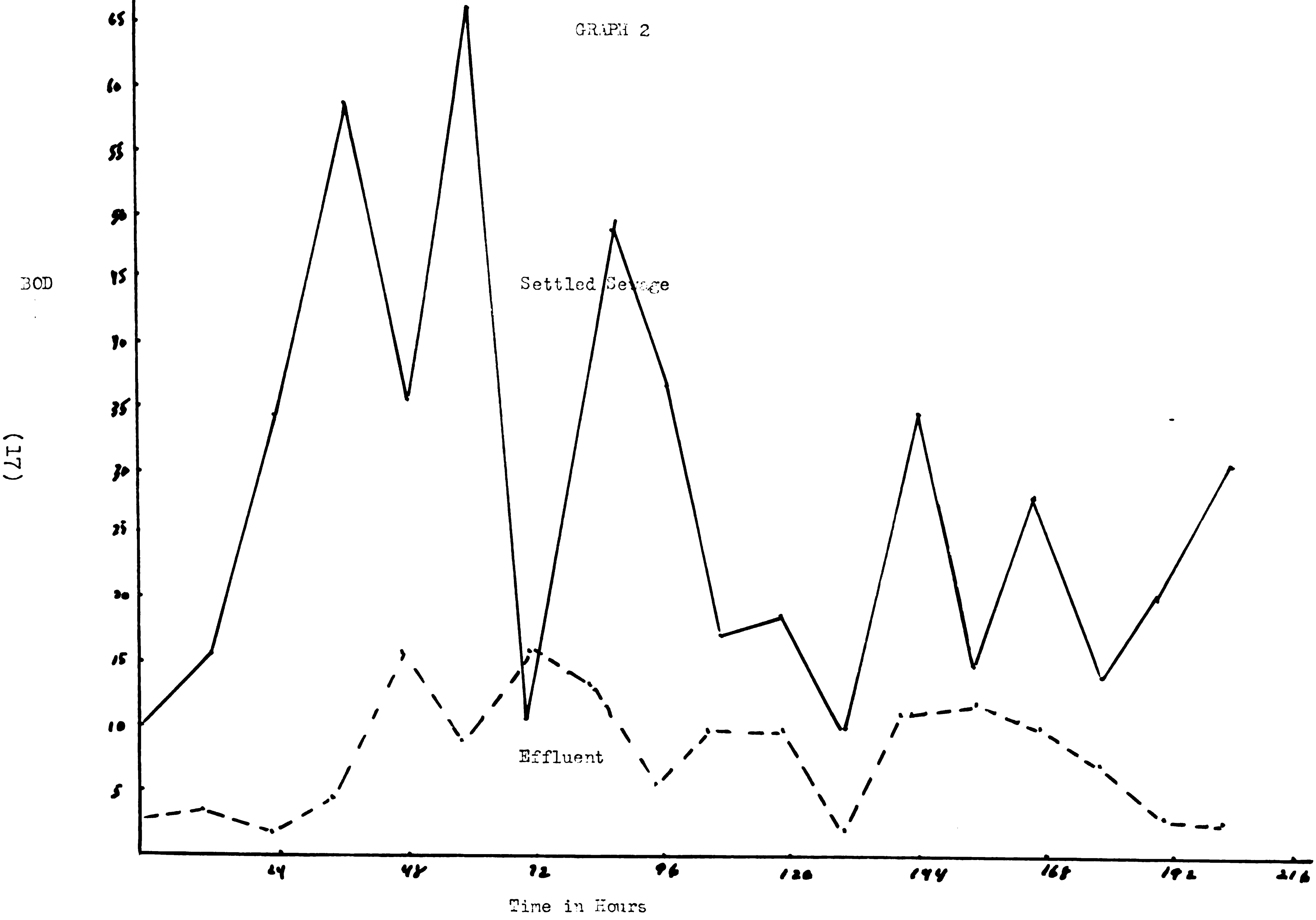
GRAPH 1



The BOD, a measure of microbial activity, is represented in Graph 2, which charts the BOD of sewage containing cyanide and the BOD of the effluent from the filter, both of which were greatly lowered due to the toxicity of the cyanide and the clarification of the sewage passing through the filter. The inhibitory action of the cyanide was evident when the average BOD values obtained from the filter containing cyanide were compared with those of a control filter without cyanide. The average BOD of the raw sewage plus cyanide was 26; the average BOD of the raw sewage without cyanide was 101. The latter value was obtained from the log of the East Lansing Sewage Plant. The microorganisms in the raw sewage were in no way adapted to cyanide; therefore, the addition of cyanide inhibited the activity of the microorganisms and lowered the BOD. The BOD of the effluent from the filter was lowered, not only by the presence of cyanide, but also by the clarification action of the filter substrate, which, coated with a zoogleal mass of microorganisms, acted as the clarifying agent within the filter. The clarification or removal of organic matter from the sewage would tend to lower the BOD. The average BOD of the effluent from the filter containing cyanide was 9.8; a control-filter effluent without cyanide averaged approximately 20, the latter figure having been taken from a similar piece of work performed by D.G.

BOD OF SETTLED SEWAGE AND TRICKLING FILTER EFFLUENT

GRAPH 2



mus (8).

According to Standard Methods (1), raw sewage is added to the BOD dilution water as an inoculum of microorganisms. If the raw sewage was substituted by an inoculum from the effluent of the filter, the interference to the BOD by cyanide would be partially eliminated by the presence of cyanide-tolerant microorganisms within that inoculum. The BOD dilution water was seeded with those cyanide-tolerant microorganisms; and, with limited results, the BOD of the raw sewage plus cyanide was similar to the BOD of the raw sewage without cyanide.

The microorganisms reduced or oxidized the cyanide in the filter to other forms which were determined by qualitative tests. The effluent did not contain thiocyanate, ferricyanide, or ferrocyanide but did contain sodium cyanide, nitrates, nitrites, and ammonia. The microorganisms destroyed the cyanide with a production of simple substances rather than complex cyanohydrin compounds.

The microorganisms isolated and identified as cyanide-tolerant are described and named in Appendix I.

The microorganisms which were isolated were tested for the ability of utilizing sodium cyanide as a source of carbon and nitrogen. The microorganisms would not use sodium cyanide as a carbon source. <u>Escherichia coli</u>, *Alcaligenes metalcaligenes*, *Flavobacterium aquatile*, *Achromobacter delicatulum*, *Serratia piscatorum*, and *Achromobacter delmarvae* utilized sodium cyanide as a sole source of nitrogen. Table 1 shows that a synthetic medium containing sodium cyanide in varying concentrations as a nitrogen source produced growth in concentrations of cyanide of 200 ppm before growth was produced in the lower concentrations. This phenomenon may have been due to the higher concentration of cyanide as a nitrogen source. Nutrient broth which was used as a control medium and contained the same concentrations of cyanide as the synthetic medium, did not produce growth in the 200 ppm concentrations of cyanide.

Those microorganisms which grew in repeated transfers of synthetic medium with sodium cyanide as a sole source of nitrogen were then studied to determine the rapidity of cyanide reduction. In 24 hours the cyanide in concentration of 9 ppm was reduced to 1 ppm by *Alcaligenes metalcaligenes*. In 30 hours the cyanide was reduced from 16.6 ppm to 1 ppm by *Achromobacter delicatulum*. *Achromobacter delmarvae* reduced the cyanide from 9 ppm to 2.25 ppm in 24 hours. This reduction is presented in Graph 3.

The growth of a microorganism in the presence of sodium cyanide was correlated with the growth medium and the adaptation to cyanide. The media used were glucose

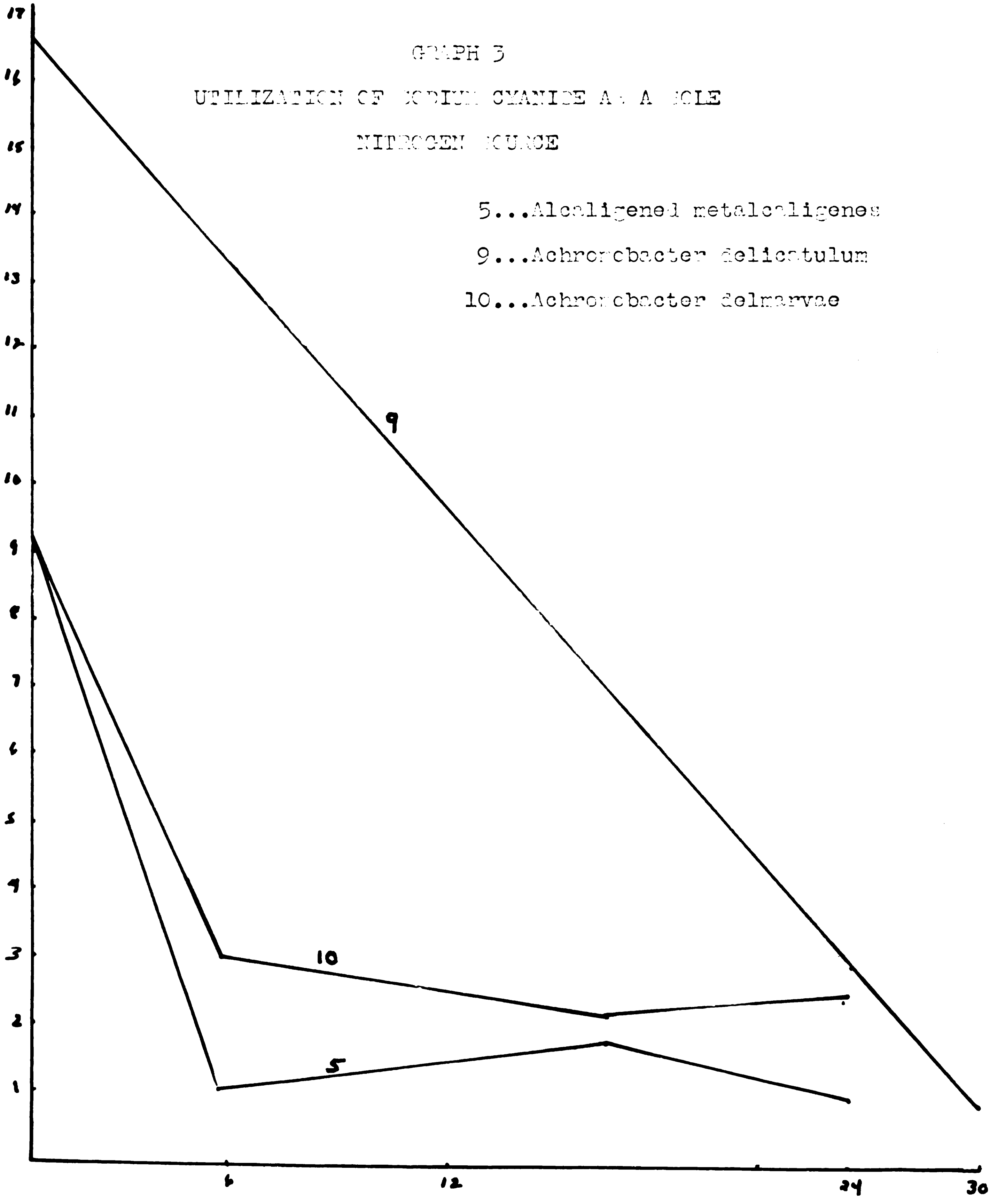

GRAPH 3
UTILIZATION OF SODIUM CYANIDE AS A SOLE
NITROGEN SOURCE
5...Alcaligenes metalcaligenes
9...Achromobacter delicatulum
10...Achromobacter delmarvae
9
10
5
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
6
12
24
30

TABLE 1

THE GROWTH OF MICROORGANISMS IN A MEDIUM CONTAINING SODIUM CYANIDE

AS THE NITROGEN SOURCE

PPM NaCN

Time in days

Organisms	5: 1	2	3	4	5	6	10: 1	2	3	4	5	6
Synthetic Medium plus NaCN												
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	+										
3	-	+										-
4						-						-
5	-	+					-	+				
6	-	-	-	+			-	-	-	-	+	
7	-	-	+				-	-	-	+		
8	+						+					
9	-	-	-	+			-	-	-	-	+	
10				+						+		
Nutrient Broth plus NaCN												
1	-	-	+				-	-	+			
2	+							+				
3	+						+					
4			+						+			
5	+						+					
6	+						+					
7	+						+					
8	+						+					
9		+						+				
10	+						+					

Organisms	25: 1	2	3	4	5	6	50: 1	2	3	4	5	6
Synthetic Medium plus NaCN												
1	-	-	-	-	-	-	-	-	-	-	-	-
2												
3						-						-
4						-						-
5	-	-	-	+			-	-	-	+		
6	-	-	-	-	+		-	-	-	+		
7						-	-	-	-	+		
8						+					+	
9	-	-	-	-	+		-	-	-	+		
10						-				+		
Nutrient Broth plus NaCN												
1	-	-	-	-	-	+	-	-	-	-	-	-
2		+										+
3		+							+			
4						+						-
5					+							-
6		+						+				
7	+						+					
8	+						+					
9		+							+			
10		+							+			

Organisms	100: 1	2	3	4	5	6	200: 1	2	3	4	5	6
Synthetic Medium plus NaCN												
1	-	-	-	-	-	-	-	-	-	-	-	+
2												
3						-						-
4						-						-
5	-	-	-	+								-
6	-	-	-	+			-	-	+			
7	-	-	+				-	-	+			
8	+							+				
9	-	-	-	+			-	-	+			
10			+					+				
Nutrient Broth plus NaCN												
1	-	-	-	-	-	-	-	-	-	-	-	-
2						-						-
3						-						-
4						-						-
5						-						-
6						+						-
7						+						-
8						+						-
9						+						-
10						-						-

TABLE 2

THE AFFECT OF MEDIUM AND CYANIDE-TOLERANCE

Glucose Broth

PPM NaCN	125	250	375	500
Org.				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	-	-	-	-

Tryptose Broth

Org.	125	250	375	500
1	+	+	+	+
2	+	+	+	+
3	+	+	+	-
4	-	-	-	-

Glucose Broth

Org.	500	550	600	650
1	-	-	-	-
2	-	-	-	-
3	+	+	-	-

Tryptose Broth

Org.	500	550	600	650
1	All Negative			
2				
3				

TABLE 3

CYANIDE ADAPTATION

Glucose

PPM NaCN	500	550	600	650
Org.				
1	+	-	-	-
2	+	+	+	+
3	+	+	+	+

Tryptose

PPM NaCN	500	550	600	650
1	-	-	-	-
2	+	+	+	+
3	+	+	+	+

	Nutrient Broth								Glucose Broth							
PPM NaCN	100		200		300		400		100		200		300		400	
Org.	8	9	8	9	8	9	8	9	8	9	8	9	8	9	8	9
Time Days																
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-

TABLE 4

CYANIDE-TOLERANCE OF MICROORGANISMS

Glucose Broth

PPM NaCN	100			200			300		
Time	1	2	3	1	2	3	1	2	3
Org.									
5	-	-	-	-	-	-	-	-	-
6	-	+	+	-	-	+	-	-	-
7	-	+	+	-	-	+	-	-	-
10	-	+	+	-	-	+	-	+	-
11	-	+	-	-	-	-	-	-	-
12	-	-	+	-	-	+	-	-	+
13	-	+	+	-	-	-	-	-	-
14	-	-	+	-	-	+	-	-	+
15	-	-	+	-	-	+	-	-	-
16	-	+	+	-	+	+	-	-	-

TABLE 5

BOTCHNEY'S SYNTHETIC MEDIUM

KH_2PO_4	2 Grams
$MgSO_4$	.32 Grams
$CaCl_2$	.08 Grams
$(NH_4)_2SO_4$	1 Gram
Sucrose	30 Grams
Water	1000 Milliliters

MODIFIED BOTCHNEY'S SYNTHETIC MEDIUM

KH_2PO_4	2 Grams
K_2HPO_4	2 Grams
$MgSO_4$	0.2 Grams
$CaCl_2$	0.01 Grams
$FeSO_4$	0.0005 Grams
$(NH_4)_2SO_4$	1 Gram
Glucose	15.0 Grams
Water	1000.0 Milliliters

broth, tryptose broth, and nutrient broth. The microorganisms grew best in glucose broth with tryptose broth the second best medium and nutrient broth last. With the knowledge that tryptose broth and nutrient broth contain no sugar as a carbohydrate source, the glucose in glucose broth may aid in the protection of the microorganisms from the inhibitory effects of cyanide. Tables 2 and 3 support the above statements. The same tables also present data showing the adaptation of a microorganism to high concentrations of sodium cyanide. Over a period of three months, the microorganisms were transfered to higher and higher concentrations of sodium cyanide. Flavobacterium aquatile was eventually adapted to 550 ppm of cyanide. After a period of three months rest, this adaptation to the high concentration of cyanide was lost (See table 1, Microorganism 3).

CONCLUSIONS

(1) The reduction of cyanide is accompanied by the production of nitrites.

(2) Nitrates maintained a constant level and appeared to have a definite correlation with cyanide in high concentrations.

(3) The production of nitrites is correlated with the adaptation of the microorganisms to the cyanide concentration in the filter. The microorganisms will not produce nitrites in high quantities until this adaptation has occured.

(4) Cyanide in concentrations of 10 to 56 ppm interferes with the BOD of the raw sewage and of the effluent.

(5) The addition of cyanide-tolerant microorganisms to the BOD water decreased the interference of cyanide on the BOD.

(6) Qualitative tests seem to verify the belief that cyanide is converted to nitrites, nitrates, and ammonia.

(7) The presence of glucose in combination with sodium cyanide in a medium is more favorable for the growth of a microorganism than a nutrient medium without glucose.

(8) Sodium cyanide may be utilized as a sole source of nitrogen by some of the isolated microorganisms, but not as a source of carbon.

(9) The microorganisms can be adapted to sodium cyanide in high concentrations, but the adaptation to the high concentrations can be lost in a period of three months or less.

SUGGESTIONS FOR FURTHER WORK

(1) Determine the physiological activities involving the cyanide-tolerant microorganisms and sodium cyanide.

(2) The effect of cyanide upon the enzyme systems of the cyanide-tolerant microorganisms.

(3) Determine the decomposition products produced by cyanide-tolerant microorganisms grown in sodium cyanide.

(4) Genetic studies on the adaptability of any microorganism to sodium cyanide.

(5) Seeding of the BOD water with cyanide-tolerant microorganisms which might decrease the interference of sodium cyanide on the BOD.

(6) Adaptation of the filter to sodium cyanide as a sole source of carbon and nitrogen.

(7) Development of a nitrate test in the presence of carbohydrates.

(8) Study the respiration of cyanide-tolerant microorganisms in the presence of varying concentrations of cyanide using the Warburg technique.

(9) Development of a simple test for cyanide in the presence of interfering ions.

(10) The possibility of adapting a mold to sodium cyanide to be used in the filter as the destructive agent.

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APPENDIX I

IDENTIFICATION OF CYANIDE-TOLERANT MICROORGANISMS

Microorganism 1

Morphology

Mycelium--- Conidia in chains, branching, small elongated cells

Colonies--- White on Nutrient Agar, potato, and starch agar.

Flakey white on Nutrient Broth

Physiology

Aerobic

Reduces nitrates to nitrites

Peptonizes litmus milk

Actively proteolytic, liquifies gelatin

Catalase positive

Isolated on synthetic agar in the presence of 200 ppm of sodium cyanide.

Characteristics fit best those of *Streptomyces albus*, Bergey's (3) page 954.

Microorganism 2,12, 13, and 16

Morphology

Rods occuring singly

Motile

Gram-negative

Physiology

Aerobic

Nitrates reduced to nitrites

Litmus milk acid

Gelatin not liquified

Lactose fermented to acid and gas

Glucose fermented to acid and gas

EMB produced characteristic colonies

Isolated from the effluent of the filter containing upto 5 ppm of cyanide.

Characteristics fit best those of Escherichia coli, Bergey's (3) page 445.

Microorganism 3

Morphology

Rods, long and occuring singly

Motile

Gram-negative

Yellow pigment not water soluble

Physiology

Aerobic

Nitrates not reduced to nitrites

Litmus milk alkaline

Gelatin liquified

No acid or gas from lactose or glucose

Isolated from the effluent of the filter containing five ppm of sodium cyanide.

Characteristics best fit those of _Flavobacterium aquatile_, Bergey's (3) page 428.

Microorganism 4

Morphology

Rods, small, slender, occurring singly

Non-motile

Gram-negative

Red pigment not soluble in water or alcohol

Physiology

Aerobic

Nitrites produced from nitrates

Litmus milk alkaline

No gas from carbohydrate medium

Isolated from raw sewage containing 50 ppm of cyanide. Characteristics fit best those of _Bacillus mycoides corallinus_, Bergey's (3) page 644

Microorganism 5

Morphology

Rods, occurring singly

Non-motile

Gram-negative

Physiology

Aerobic

Nitrites not produced from nitrates

Litmus milk unchanged or alkaline

No action on carbohydrate medium

Gelatin not liquified

Believed to be *Alcaligenes metalcaligenes*, Bergey's (3) page 414.

Microorganism 6, 7, and 9

Morphology

Rods, occurring singly

Motile

Gram-negative

Physiology

Aerobic

Nitrites produced from nitrates

Litmus milk acid and coagulated

Acid produced in glucose and lactose

Gelatin liquified

Achromobacter delicatulum, Bergey's (3) page 419.

Microorganism 8

Morphology

Rods, occuring singly

Actively motile

Gram-negative

Red pigment soluble in water and alcohol

Physiology

Aerobic

Nitrites produced from nitrates

Litmus milk acid and curdled

Acid and gas in glucose

Gelatin liquified

Serratia piscatorum, Bergey's (3) page 483.

Microorganism 10

Morphology

Rods, short, occurring singly

Non-motile

Gram-negative

Physiology

Aerobic

Nitrites produced from nitrates

Litmus milk reduced, acid

Acid from glucose and lactose

Gelatin not liquified

Achromobacter delmarvae, Bergey's (3) page 422.

Microorganism 11

Morphology

Spheres, occurring in packets of four and more

Gram stain varible

Yellow pigment not water soluble

Physiology

Aerobic

Nitrites not produced from nitrates

Litmus milk alkaline

Gelatin slowly liquified

Sarcina flava, Bergey's (3), page 288

Microorganism 14

Morphology

Rod, occurring singly

Motile

Gram-negative

Yellow pigment not water soluble

Physiology

Aerobic

Nitrites not produced from nitrates

Litmus milk weak acid

Acid from glucose and lactose

Gelatin not liquified

Characteristics fit best those of Pseudomonas perlurida, Bergey's (3), page 174.