COO-4502-2

Preliminary Evaluation of the Biological Oxidation of Thiocyanates

Progress Report

for Period November 30, 1977 - February 28, 1978

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DISCLAIMER

Abstract

Acclimated biological cultures were developed from activated sludge taken from coke plant biological wastetreatment facilities. These cultures are capable of degrading potassium thiocyanate from an original concentration of 2000 mg/l SCN to less than 1 mg/l in an aeration period of 9 hours. pH inhibition appears to be significant at values greater than 7.6. Substrate inhibition occurs at concentrations of thiocyanate greater than 500 mg/l. Air stripping of thiocyanate does not appear to occur at neutral pH value. Preliminary assessments indicate that aerobic biological organisms may play a major role in the removal of aqueous thiocyanate as found in the wastewaters from the gasification of certain coals.

Progress Report

Bio-oxidation of Aqueous Cyanogens Typical of

Synthane Gasifier By-product Water

Contract EY-77-5-02-4502.A000

Start Date: 9/1/77

Date of this Report: 4/1/78

Principal Investigator: Dr. Ronald D. Neufeld, Associate

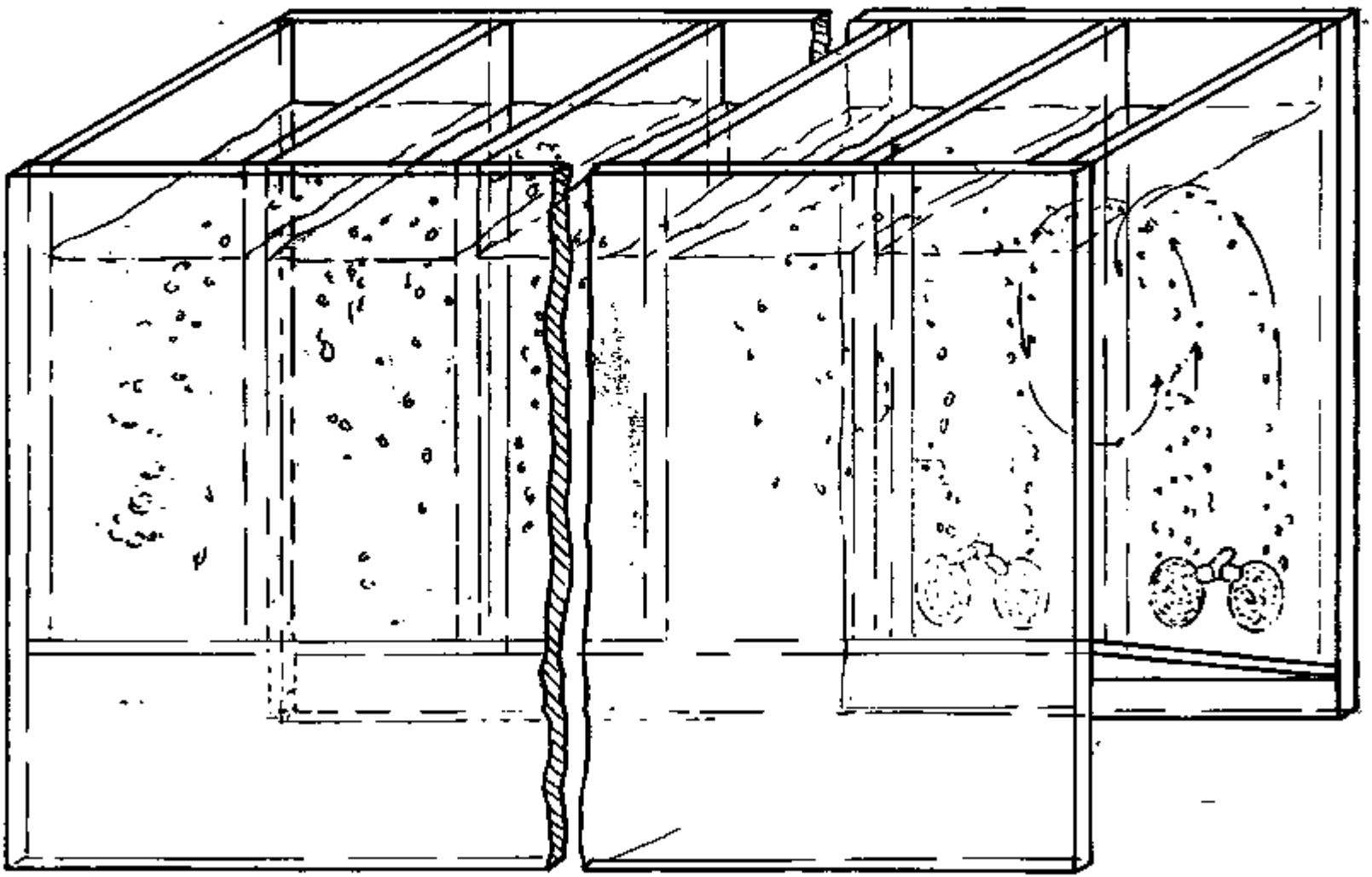
Professor, University of Pittsburgh

All contract requirements regarding the development of thiocyanate degrading biocultures and assembly of bench sized bio-reactors are complete at this time. Sketches of the bioreactor are shown in Figure 1. As a deliverable for this contract, approximately 1 gram of thiocyanate degrading sludge shall be packaged and hand delivered to Mr. W. P. Haynes, the Technical Project Officer of the Pittsburgh Energy Research Center.

The principal investigator has devoted at least 10% of his time to the contract and expects to increase this percentage as time progresses. This level is in excess of the 8% for 6 months time commitment indicated in the contract. A graduate research assistant has been employed for 1/2 time for four months of this project. A technician has been employed for about 50 hours for assistance in the construction of bench scaled reactors.

FIGURE 1

EXPERIMENTAL APPARATUS



Discussion of Technical Results to Date

Prior research indicates that thiocyanates are produced in an amount of about 0.07 lb per pound moisture ash free coal gasified from the Synthane and Lurgi processes (1), and in amounts of about 2.5 lb per pound moisture ash free coal gasified from the Hygas Process. Concentrations of thiocyanate on the order of 20 to 200 mg/l have been reported from the Synthane gasification of certain coals (2).

At present, several states including Pennsylvania have a "zero discharge" requirement for cyanogens (and cyanides). Thus, cyanogen removal is a requisite for the environmentally acceptable operation of SYNTHANE and other coal gasification processes.

For larger scale coal gasification processes to come on stream by the mid 1980's, wastewater treatment processes must be developed from essentially currently available best treatment alternatives. The traditional approach to thiocyanate (and cyanogen removal) is batch chlorination. This approach, while viable for the metal plating industry, is not applicable to coal conversion processes due to the phenolic nature of gasifier effluents and the inherent tendency to form toxic chlor-phenolic by-products. Chlor-phenolics exhibit tastes, odors, and toxicities to animals in waters at the parts per billion level.

The steel industry has utilized biological processes for the treatment of the phenolic content of coke plant wastewaters. These facilities are not specifically designed for thiocyanate removal and such removal is reported to occur in a somewhat unpredictable fashion. Accordingly while proof of principle data exists in the literature for thiocyanate biodegradation little data exists regarding kinetic and other design parameters necessary for prediction of process applicability and stability for use by SYNTHANE and other coal conversion processes.

The purpose of this initial six month contract is to demonstrate that biological cultures in significant quantity could be developed that are capable of thiocyanate degradation in concentrations typical of SYNTHANE effluents. Previous research in this area (3,4,5) has centered on the biochemistry of such reactions; little is known to date relative to key parameters necessary for the design of an engineered system. In September, 1977, approximately 20 liters of activated sludge were obtained from the Clairton, PA plant of United States Steel Corp. This plant has a coke manufacturing facility and treats its liquid waste biologically. The activated sludge received was amber in color, had significant floating oils, and exhibited strong phenol and cresol odors.

The sludge was brought to the Environmental Engineering Laboratories of the University of Pittsburgh and allowed to aerate for two days for stripping and removal of soluble volatile organic materials and skimming of surface oily materials. Acclimation of the culture to thiocyanate (SCN) was begun by feeding the system a daily diet of 100 mg/l SCN and 100 mg/l dextrose with appropriate levels of phosphate nutrient buffer solution. Dextrose was utilized during this phase because its use is reported to result in shortened times for thiocyanate acclimation (6). This period of acclimation lasted for about 1 month during which time an attempt was made to develop continuous cultures systems with no cell recycle. Organisms capable of growth on the media provided were retained in the continuous reactors while others were washed out. Detention periods of 24 hours resulted in effluent SCN levels of 1 mg/l or less while reactors with detention time of 16 and 21 hours providing poorer quality effluents.

After 4 weeks of culture acclimation and purification, a second phase of acclimation was begun whereby the residual biomass was collected, concentrated, and placed into one 8 liter reactor operated on a batch basis. This reactor was fed 500 mg/l SCN daily with no

dextrose or other supplemental organic carbon sources. The system was fed and aerated for 23 hours. Air was then turned off and organisms were allowed to settle for 1 hour. Samples were taken of solids, pH, NH_3, SCN, and alkalinity as needed. One liter of supernate was wasted each day and replaced with tap water and sufficient potassium thiocyanate so that the SCN concentration at the beginning of the next 24 hourcycle was 500 mg/l. At this point in the experimental program, the 500 mg/l SCN was chosen as the maximum SCN level due to published reports of substrate inhibition occurring at higher levels (7). As shown below SCN degradation at 1000 mg/l were demonstrated as being feasible.

During the course of fill and draw operation, the sludge assumed a light brown color and gave off no odor. A light pin-point floc became evident which is typical of young rapidly growing sludge. Specific thiocyanate removal rates rose from 0.5 lb SCN removed/lb biomass-day to 2.1 lb SCN removed/lb biomass-day during the course of this phase of experimentation. The pH was held at 6.7 to 7.0 and the alkananity was about 300 mg/l as $CaCO_3$. A small increase of about 15-25 mg/l was noted in the NH_3 concentration during the course of aeration. This is consistant with observations that oxidation of SCN leads to CO_2, protoplasm and NH_3. NO_3 may similarly be formed by the biological nitrification of NH_3. Further research is proposed to complete the material balance for nitrogen in the process and to account for possible simultaneous biological nitrification.

A third phase of experimentation was started on October 29 when about 3800 mg of non purified but acclimatedU. S. Steel thiocyanate degrading organisms were placed in a 6 liter batch bio reactor. This system was compared side by side to 2000 mg of the previously developed purified culture for specific rates of thiocyanate degradation. The non-purified culture exhibited a rate of 0.67 mg SCN removed/mg biomass-day. Thus either the purified culture was better acclimated to SCN

or the non purified culture contains biomass or organic inerts that lower the apparent biological activity of the entire macrosystem. In either case, this experimental phase highlights the importance of utilizing a purified and acclimated culture of microorganisms for SCN removal. Microorganism may be developed over time from coke plant wastewater treatment facilities, but a direct utilization of these organisms without an appropriate period of acclimation and purification will lead to unsatisfactory performance.

During the course of experimentation, the pH of each bioreactor was allowed to reach 7.6. At this pH the specific rate of removal dropped to 0.36 and 0.28 mg SCN removed/mg biomass-Day for the purified and non purified cultures respectively indicating inhibition.

This level of inhibited activity was maintained in-advertently for a period of about 7 days. Subsequent pH depression to the range 6.8 to 7.0 with H_2SO_4 in each reactor resulted in renewed activity indicated that the alkaline pH level acted as an inhibitor to the system and not as a toxicant.

During the second week in November, the two batch bio reactors were converted to semi-continuous systems with imposed cell wasting rates of 1/20 per day, pH was monitored and held to the range 6.8 – 7.1. Thiocayanate was reduced from 500 mg/l to less than 1 mg in the 23 hour aeration time indicating specific removal rates of 1.14 mg SCN removed/mg biomass-day for the purified culture and 0.653 mg SCN removed/mg biomass-day for the non-purified culture.

For comparative purposes, a rate of 1.14 is translatable to a COD basis as follows as found for the 20 day sludge age system:

$$1.14 \frac{\text{mg SCN removed}}{\text{mg Biomass-Day}} \times \frac{12 \text{ mg Carbon}}{58 \text{ mg SCN}} \times \frac{32 \text{ mg } O_2}{12 \text{ mg Carbon}} = 0.63 \frac{\text{mg COD removed}}{\text{mg Biomass-Day}}$$

Beginning December 1976, the bacteria were fed increasing amounts of potassium thiocyanate to see the effects of higher concentrations on metabolism rates.

Initially, two semicontinuous systems were operated at imposed cell wasting rates of 1/15 and 1/20 per day respectively. Thiocyanate was removed from 1000 mg/l to about 800 mg/l in 24 hours in the 15 day sludge age system and from 1000 mg/l to 600 mg/l in 24 hours in the 20 day sludge age system.

The sludge wasted from these two reactors was collected, concentrated and placed in a separate container. When a sufficient quantity of concentrated sludge was accumulated, another semicontinuous unit was operated at an imposed cell wasting rate of 1/35 per day. 2000 mg/l SCN was fed to this unit and conditions were monitored.

The 3 semicontinuous systems were operated under these conditions for abour 2 months. Each system had become acclimated to the higher concentrations of thiocyanate and were at a point where all thiocyanate added was being removed in 24 hours. Reactor temperatures were maintained at $22°C \pm 2°C$, and pH was maintained at 7.0 ± 0.2.

The sludge at this stage of the experiment was similar in appearance to ordinary activated sludge except that it was lighter in color and gave off no odor. If agitation was stopped, the sludge formed large flocs and settled readily.

A series of batch controlled experiments were conducted to determine cell growth rate, cell yield and ammonia production rate from thiocyanate degradation. The procedure was as follows:

1. Prior to addition of thiocyanate, the pH of each reactor was adjusted to 7.0.

2. Sufficient potassium thiocyanate was added to bring the concentration of thiocyanate to the desired level.

3. At time (t) = 0, and at each succeeding hour, samples were withdrawn and analyzed for SCN, biomass, and NH_3. Samples were withdrawn until the concentration of SCN dropped below 1 mg/l.

Typical data obtained from this experiment is shown in Table 1 and 2 The initial concentration of SCN in this bio-reactor was approximately 2000 mg/l with an initial concentration of biomass measured as total suspended solids (TSS) of 2865 mg/l.

As shown in figure 2, the thiocyanate concentration is reduced to less than 1 mg/l in 9 hours, with a corresponding increase in solids concentration (figure 3). Figures 2 and 3 indicate that growth is in the log phase with no initial lag phase.

The relationship between microbial growth and substrate utilization can usually be modeled by the equation

$$(1)\quad \frac{dx}{dt} = a\frac{ds}{dt} - bx$$

where

$\frac{dx}{dt}$ = net growth rate of microorganisms per unit volume (mass/volume-time)

a = growth yield coefficient (mass/mass)

$\frac{ds}{dt}$ = rate of microbial substrate utilization per unit volume (mass/volume-time)

b = microorganism decay coefficient ($time^{-1}$)

x = microbial mass concentration (mass/volume)

If the bacteria are in the log growth phase, than it may be assumed that decay is insignificant

$$\text{or } b \approx 0$$

and

$$(2)\quad \frac{dx}{dt} = a\frac{ds}{dt}$$

Over a finite time period, this equation becomes

$$(3)\quad \frac{\Delta x}{\Delta t} = a\frac{\Delta s}{\Delta t}$$

and

$$(4)\quad a = \frac{\Delta x/\Delta t}{\Delta s/\Delta t}$$

TABLE 1

TYPICAL MEASURED EXPERIMENTAL DATA - BATCH REACTOR

TIME Δ t	MLSS	[SCN]	$[NH_3]$
0 Hours	2900 2830	2070 2025 2040	243
1	3000 2930	1755 1710	243
2	2850 2840	1575 1500	376
3	2950 2670	1440 1350	376
4	2970 3030	1170 1215 1140	440
5	2920 3130	990 945 900	440
6	3130 2960	720 660 630	458
7	3200 3150	420 418 414	458
8	3160 3050	180 180 174	458

TABLE 2
Batch Bioreactor Data

t (hr)	TSS (Mg/l)	VSS (Mg/l)	VSS_{AVG} (Mg/l)	ΔVSS (Mg/l)	SCN (Mg/l)	SCN_{AVG} (Mg/l)	ΔSCN (Mg/l)	$\frac{\Delta VSS}{\Delta SCN}$	$\frac{\Delta SCN/\Delta t}{\Delta VSS_{AVG}}$	$\frac{VSS}{VSS_0}$
0	2870	2152			1960					1
			2158	12		1870	180	0.067	0.0834	
1	2885	2164			1780					1.01
			2173	18		1690	180	0.10	0.0828	
2	2910	2182			1600					1.014
			2196	28		1500	200	0.14	0.0911	
3	2947	2210			1400					1.03
			2225	27		1290	220	0.123	0.0989	
4	2985	2239			1180					1.04
			2252	26		1060	240	0.108	0.1066	
5	3020	2265			940					1.05
			2280	29		810	260	0.112	0.114	
6	3058	2294			680					1.07
			2308	27		550	280	0.104	0.1127	
7	3095	2321			420					1.08
			2335	28		285	270	0.104	0.1156	
8	3132	2349			150					1.09

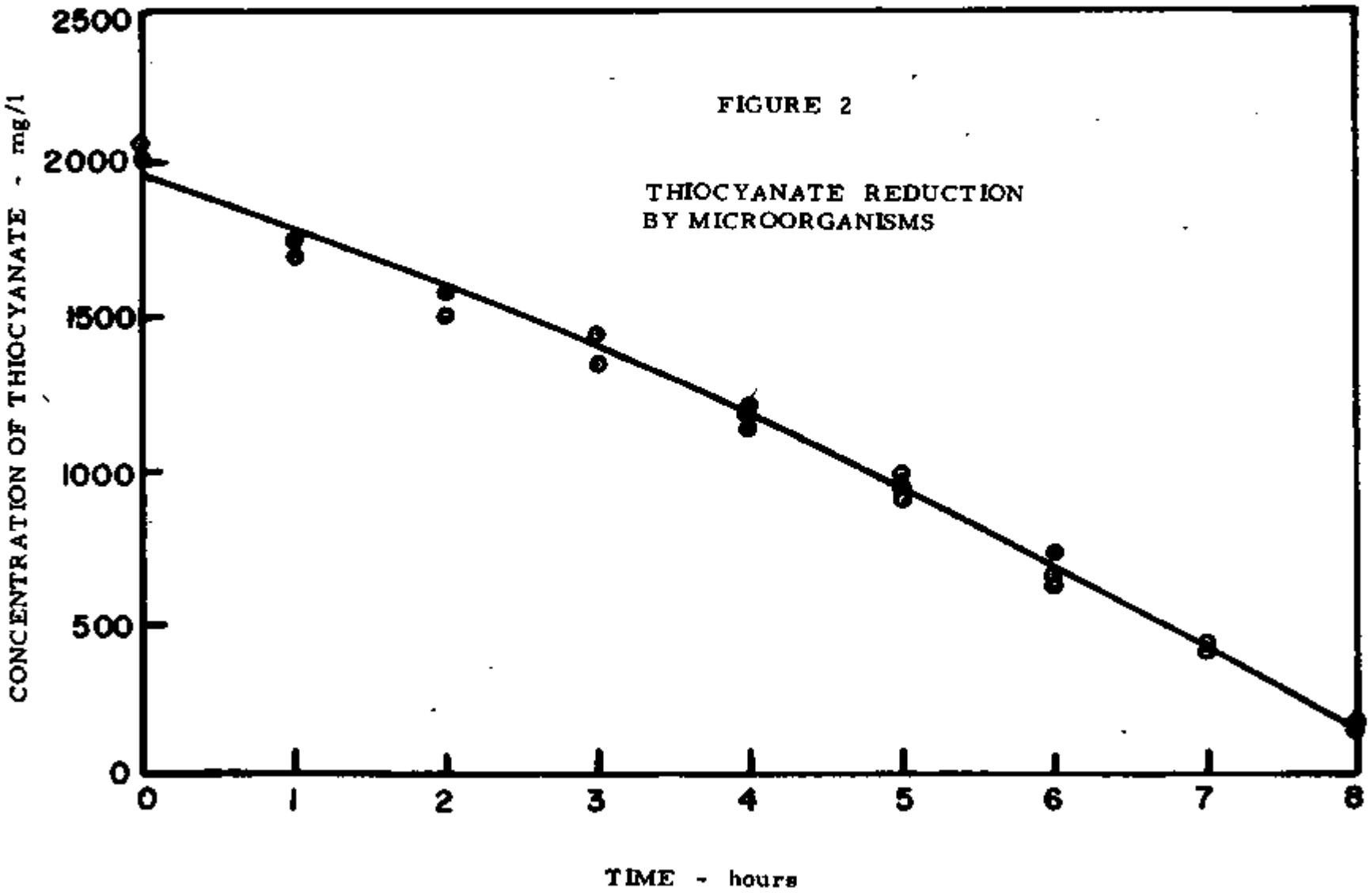
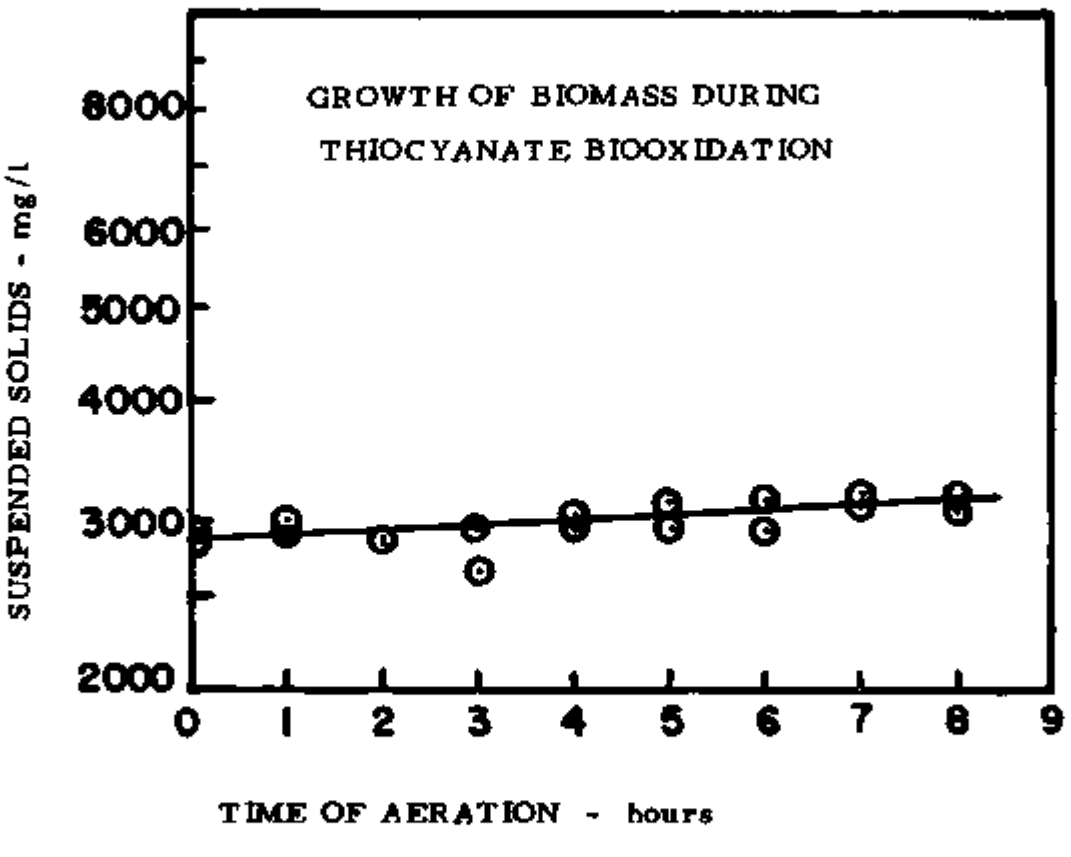


FIGURE 2
THIOCYANATE REDUCTION
BY MICROORGANISMS
CONCENTRATION OF THIOCYANATE - mg/l
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
7
8
TIME - hours

FIGURE 3


GROWTH OF BIOMASS DURING
THIOCYANATE BIOOXIDATION
SUSPENDED SOLIDS - mg/l
8000
6000
5000
4000
3000
2000
0
1
2
3
4
5
6
7
8
9
TIME OF AERATION - hours

For the data of table 1, a preliminary estimate of "a" is on the order of 0.113 $\frac{\text{Lb VSS produced}}{\text{Lb SCN oxidized}}$

If we consider the growth of bacteria to follow a first order relationship as is the case in the log growth phase, we may write

$$(5)\quad \frac{dx}{dt} = Kx$$

where K is the growth rate (time^{-1})

Rewriting and intergrating

$$(6)\quad \text{Ln } X_2 = \text{Ln } X_1 + Kt$$

A plot of this relationship is shown in figure 3. The slope of this line (k) is 0.011.

The doubling or generation time of these organisms (t_d) may be computed from: $t_d = \frac{\text{Ln}(2)}{K}$ which yields t_d = 63 hrs.

The rate of substrate utilization can be approximated by the following formula outlined by Valiknac (8)

$$V = \hat{V}\frac{S}{K_s + S}$$

where

$V = \frac{\Delta S/\Delta t}{\bar{X}}$ = rate of substrate utilized per unit cell concentration

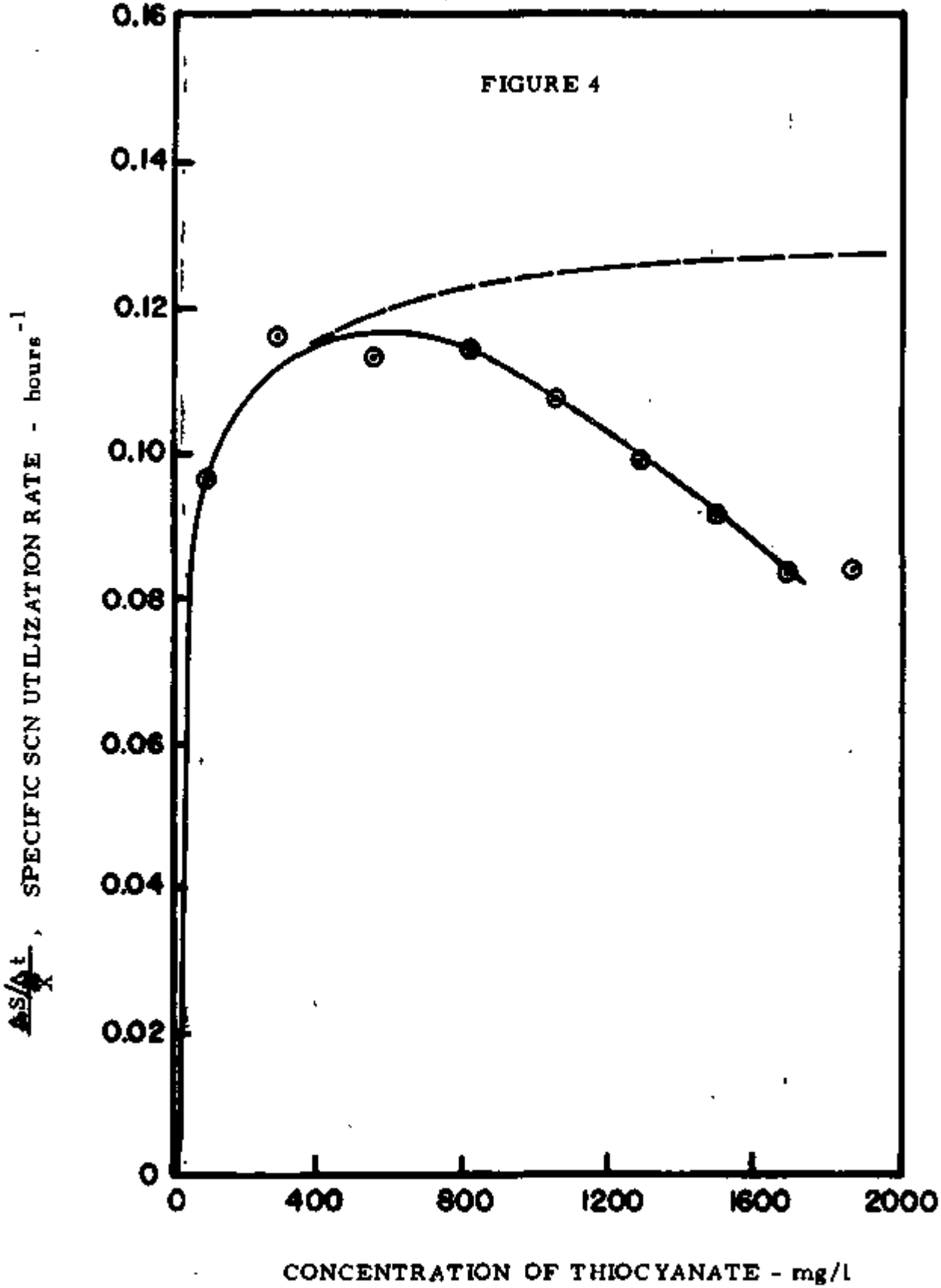
$\hat{V}$ = maximum rate of substrate utilization per unit cell concentration

K_s = waste concentration at which rate of waste utilization per unit weight of microorganisms is one-half the maximum rate.

Substrate inhibition changes the relationship of substrate concentration as related to specific substrate utilization rate.

Plotting the data of table 1 in this manner produces the plot of figure 4 which indicates substrate inhibition at SCN levels over 500 mg/l. This

FIGURE 4


0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
0
400
800
1200
1600
2000
SPECIFIC SCN UTILIZATION RATE - hours^{-1}
$\frac{\Delta S}{\Delta t} \frac{1}{X}$
CONCENTRATION OF THIOCYANATE - mg/l

agrees with published reports of observed substrate inhibition of SCN concentrations greater than 500 mg/l (7.8).

From figure 4 the maximum removal rate is about 0.12 $\frac{\text{lb SCN}}{\text{lb SS-Hr}}$ from one half the maximum rate and k_s may be estimated to be about 30 mg/l; however, parameters suitable for design have not been obtained at this time.

Overall biomass yield coefficient (a) is approximately 0.157 (SCN basis) and 0.759 (TOC basis).

Ammonia Production – Preliminary Material Balance

Ammonia is produced as a by-product of SCN oxidation. Preliminary results show that when the concentration of ammonia during the bioreaction is plotted versus time (figure 5) it appears that the maximum amount of ammonia produced occurs during the first 4 hours of the test. This is shown more graphically in the semi log plot of figure 6.

Approximately 260 mg/l NH_3 is produced during the biodegradation of 1800 mg/l SCN. Using the growth yield coefficient of 0.113 $\frac{\text{mg VSS}}{\text{mg SCN}}$, approximately 205 mg/l of new cells are produced. Using an average cell nitrogen content of 14%, 3.5 mg/l NH_3 goes to the production of new cells. Theoretically, in the biodegradion of 1800 mg/l SCN, 530 mg/l NH_3 is produced. Considering that 35 mg/l NH_3 goes to cells, 495 mg/l NH_3 should be produced during the bioraction. Accordingly only $\frac{260}{495}$ or 53% of the theoretical amount is accounted for in this experiment, the remainder possibly being oxidized to nitrate or nitrite or being stripped out of solution. Future work shall delineate the pertinent material balance components.

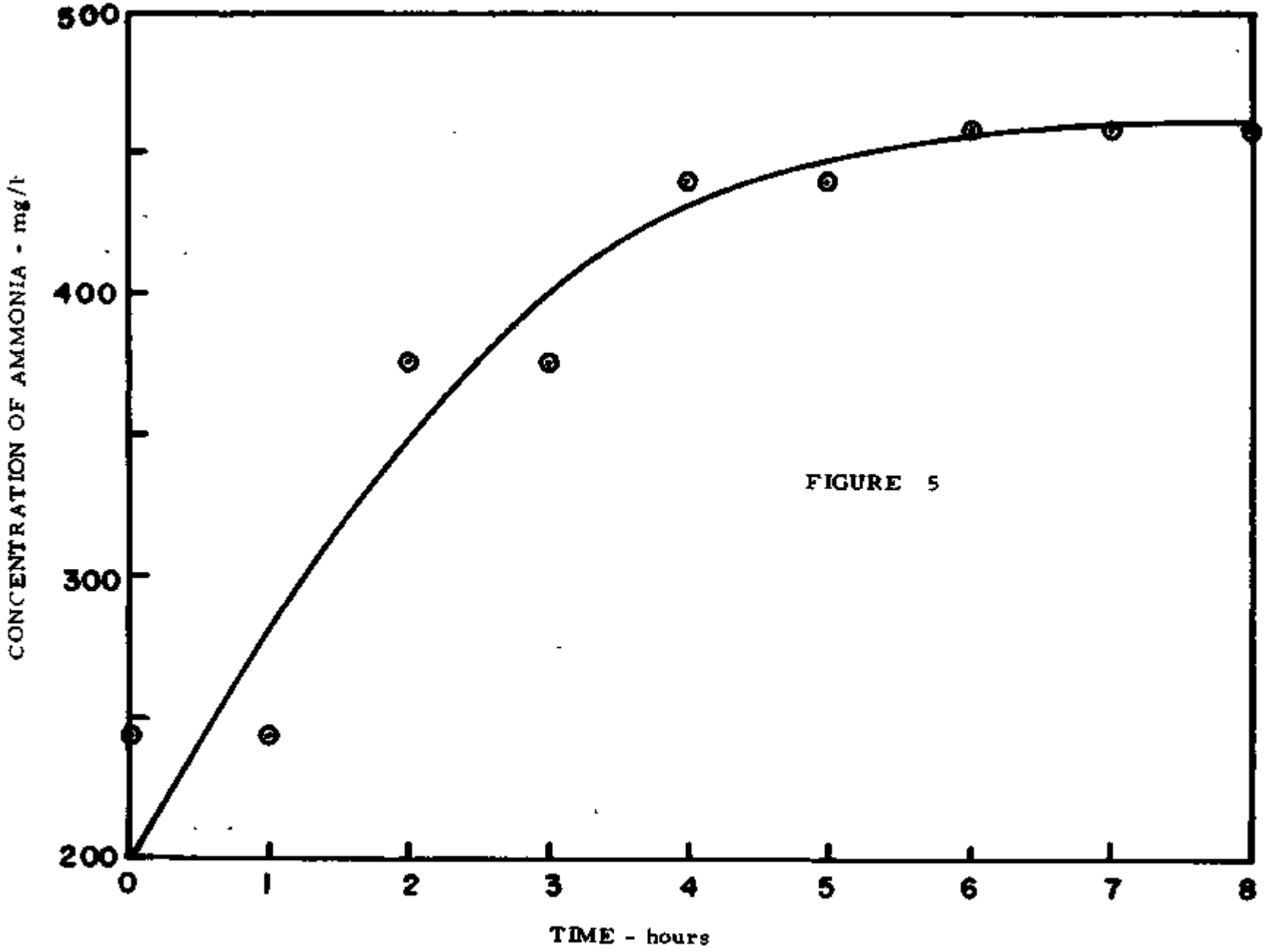
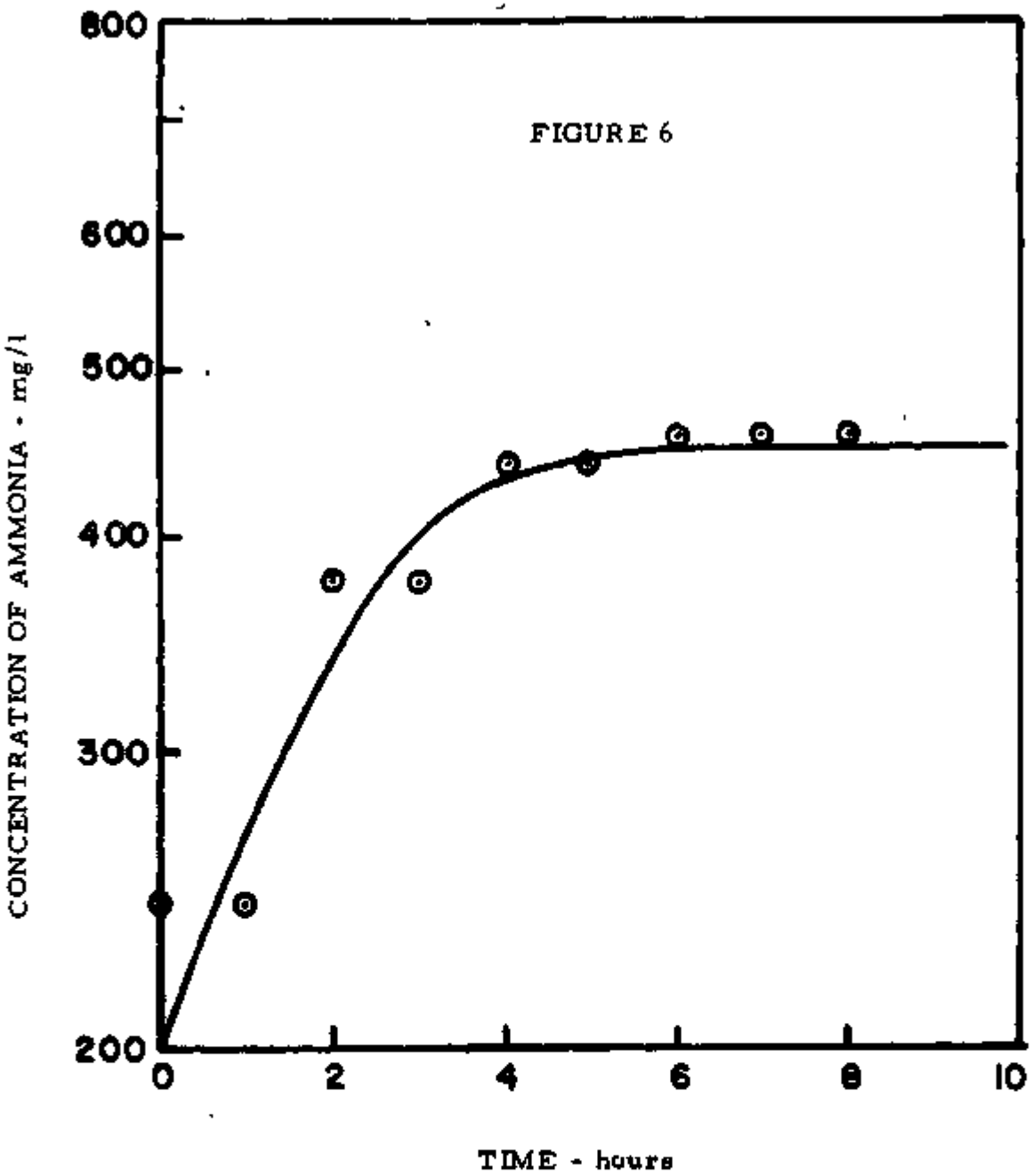

500
400
300
200
0
1
2
3
4
5
6
7
8
CONCENTRATION OF AMMONIA - mg/l
TIME - hours

FIGURE 5

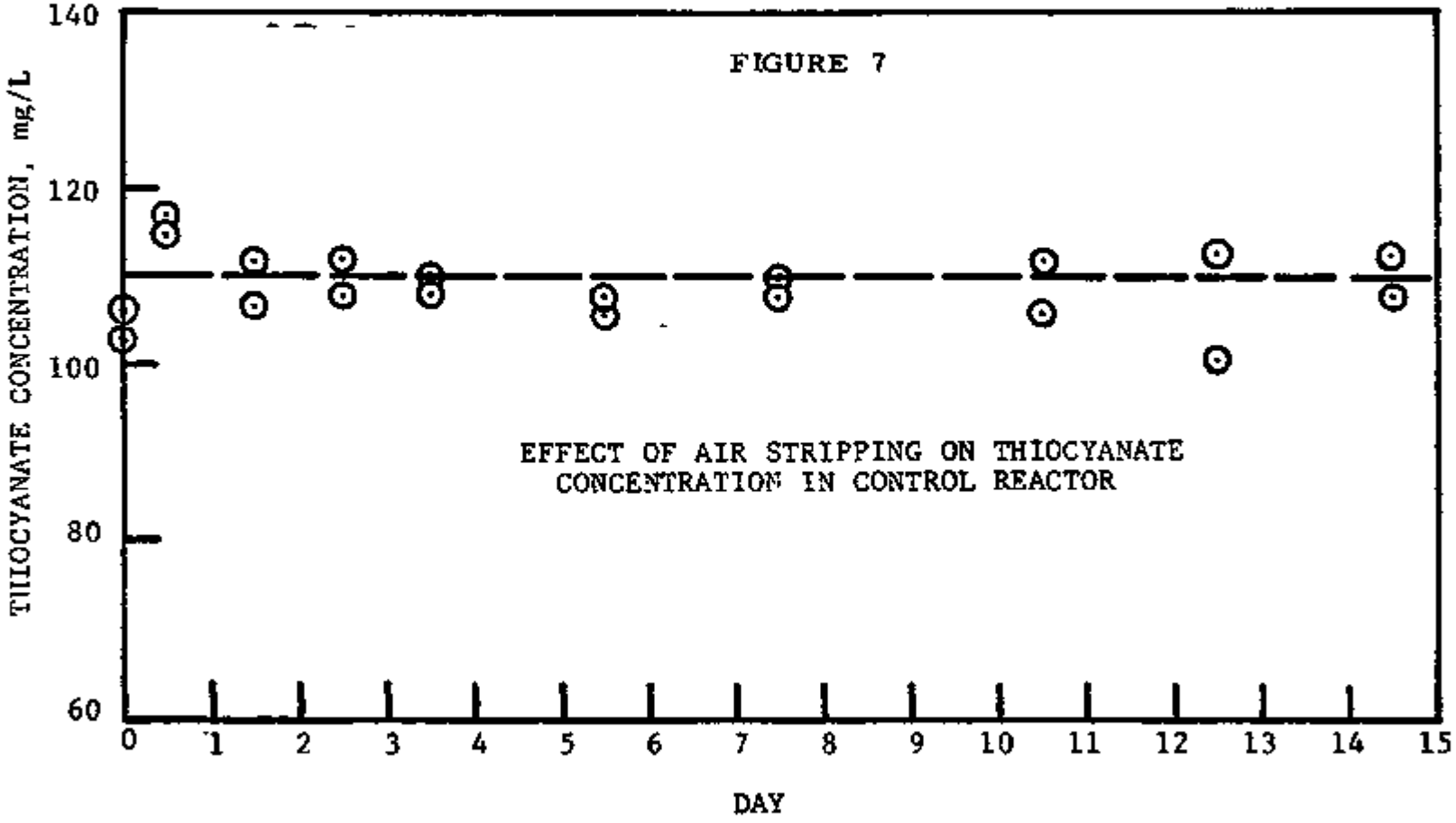
FIGURE 6


800
600
500
400
300
200
0
2
4
6
8
10
CONCENTRATION OF AMMONIA - mg/l
TIME - hours

Thiocyanate Stripping

Prior to beginning the batch biological experiment a control reactor with no biota containing approximately 110 mg/l of thiocyanate in a liquid volume of 2 liters at neutral pH was aerated for a 2 week period in order to determine if thiocyanate would be stripped from solution. The control reactor contained only thiocyanate with no chemical nutrients. The liquid volume was brought to 2 liters daily to account for evaporation losses prior to sampling. Results are shown in figure 7 from which it may be concluded that air stripping of thiocyanate does not occur and that any reductions in thiocyanate are due to microbial degradation (5).

FIGURE 7



EFFECT OF AIR STRIPPING ON THIOCYANATE CONCENTRATION IN CONTROL REACTOR

Conclusions

1 - Mixed cultures have been developed from coke plant biological wastetreatment facilities that are capable of degrading thiocyanate from levels of 2000 mg/l to 1 mg/l in a 9 hour aeration period.

2 - Thiocyanate biodegradation appears to be inhibited at pH levels of 7.8 and higher. Maximum biodegradation rates were observed in the pH range of 6.7 to 7.2.

3 - Ammonia appears to be a by product of SCN biodegradation. A definitive nitrogen balance however, has not as yet been done.

4 - Organisms taken directly from a coke plant biological wastewater treatment facility should be acclimated and purified prior to use to assure satisfactory performance.

5 - Substrate inhibition is evidenced at thiocyanate concentrations greater than 500 mg/l.

6 - Thiocyanate is not air stripped from abiotic reactors at neutral pH; hence all SCN removal is due to biological degradation.

7 - Cell yield is about 0.16 lb. $\frac{\text{cells produced}}{\text{lb thiocyanate}}$ oxidized

8 - The maximum observed cell growth rate is 0.011 hr^{-1}

9 - The maximum rate of substrate utilization is approximately 0.125 $\frac{\text{lb SCN}}{\text{lb VSS-hr}}$

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