AEROBIC METABOLISM OF RESIDUAL ORGANICS IN A COMPLETELY CLOSED BATCH OPERATED SYSTEM

By

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DEDICATED TO MY PARENTS

MARGARET AND RUSSEL BLACHLY

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CHAPTER I

INTRODUCTION

One of the most serious problems facing the nation today is the deteriorating quality of its water supplies. With the advent of the industrial revolution came a much higher loading rate of such pollutants as organics, biological nutrients, disease-bearing organisms, and synthetic chemicals. As long as these ever increasing wasteloads were small, the natural self-purification processes of the waterways—dilution, oxidation, biological degradation—could cope with the addition of foreign matter. But as industry grew the ability of the waters to assimilate the material was soon exceeded. This led to a need for systems to remove the water pollutants. The systems developed have generally become known as primary, secondary, and tertiary treatments.

Primary treatment is the process whereby settleable materials are removed. Primary treatment removes 30-40 percent of the organic pollution, measured as biochemical oxygen demand (BOD) or chemical oxygen demand (COD).

Secondary treatment is responsible for the removal of the soluble organic material. There have been many variations of a single concept in the design of secondary treatment facilities. Secondary treatment employs biological processes like trickling filters, biological towers, activated sludge, and rotating biological contactors. The organics

are utilized as food source by microorganisms, mainly bacteria, thus converting the soluble organics to microorganisms. Secondary treatment is capable of removing 95 percent of the organic material.

Tertiary treatment is mainly responsible for the removal of biological nutrients such as phosphates and nitrates. Some organics are also removed during this process.

The subject of this study is the residual organic material which is not removed during secondary treatment. In a well designed and operated wastewater treatment plant the residual organic concentration would be expected to be approximately 5-10 percent of the incoming concentration. Little is known about the nature of this material and why it always persists in a plant effluent and whether it is even residual, i.e., nonbiodegradable. Possibly if the residual material could be held under air for a long enough period the "residual" organic material would eventually be metabolized by a biological population. If this were found to be true then it could be said that the "residual" organics cannot be classified as nonbiodegradable residual as many now believe (1)(2)(3).

The purpose of this study was to determine if in a totally closed aerobic system residual COD would continue to accumulate. To test this possibility batch units were fed daily a synthetic medium consisting of glucose and mineral salts and no liquid or biomass was allowed to exit the system.

CHAPTER II

LITERATURE REVIEW

The nature of the soluble, residual organics entering a stream from a secondary wastewater treatment facility has become of primary concern since the new Federal Water Quality Law, Public Law 92-500, was passed by Congress in October of 1972. The goal of the law is to return the nation's waters to their natural condition, i.e., no discharge of man-made pollutants. The major emphasis has been on construction of new facilities and to upgrade existing facilities with the latest technological advances. By 1985 Public Law 92-500 calls for the elimination of discharge of pollutants into the navigable waters. Specifically the law states, "any plan prepared . . . shall include, a process to control the disposition of all residual waste generated in such areas which could affect water quality."

The primary concern with an organic residual waste entering a river ecosystem is whether the organics will be biodegraded by microorganisms, thus possibly depleting the oxygen concentration to a low enough level, whereby other aquatic organisms will be driven from those waters or possibly killed. If the residual organics can be kept at a minimum (approximately five percent of raw waste) by proper design and operation of the treatment facility then possibly the water quality would not be adversely affected. This would depend on the nature of the residual organics, i.e., whether they are nonbiodegradable or are biodegraded

at such a slow rate that their presence would not adversely affect the water quality.

According to Busch (4), biodegradability in the wastewater field depends on the definition of biodegradable. He states that biodegradability depends on the time interval allowed under specific test conditions. "All organic carbon will ultimately be converted to CO_2 and water by microbial metabolism."

In order for organic matter to be biodegraded there must be present the proper enzymes that are highly specific for the chemical substrate being acted upon. There are nearly 2000 known enzymes, and no single cell or species or organism has all of them. Enzymes within an individual organism may be a natural constituent or may be induced in response to the presence of a substrate chemical (5). An organism may or may not have the capacity to produce a necessary enzyme for available substrate.

Grady and Williams (1) stated that the use of a biodegradable substrate by microorganisms does not insure that all organic matter in a reactor will be biodegradable. As the substrate is oxidized there will be intermediate products released into the system which may possibly not be biodegraded because of an absence of the necessary enzymes for further metabolism.

In order to test efficiency of biological reactors for removal of substrate, Busch (4) proposed the concept of Effluent Refractory Assessment which involves holding a side stream of mixed liquor from the biological reactor under aeration for an additional time and comparing the dissolved organic concentration of the reactor effluent with that from the additional aeration. Unless the dissolved organic

concentration after additional aeration is significantly reduced, performance of the biological reactor is essentially complete. The problem of assessment of biodegradability with this system lies in the nature of the test conditions. The system is a closed system and does not simulate the conditions present in a stream. Some of the same problems exist with this method as exist with the BOD test (6); that is the seed used is the same as that which created the residual. Reddy (7) studied the kinetics of oxygen uptake of effluents from laboratory bench scale extended aeration pilot plants using the open jar technique. He found that upon aeration of the effluent for a period of 120 hours (5 days) there was no appreciable reduction of soluble COD. He concluded that the soluble portion of residual COD can be removed only very slowly and not within the relatively short detention time in the system he studied. He also employed seed taken from the reactor which produced the effluent.

A way of measuring the biodegradable portion of a wastewater was proposed by Hiser and Busch (8) and studied extensively by Gaudy (6). This procedure also measures the residual COD. Since the COD test is a measure of organic matter, biodegradable and nonbiodegradable portions, the difference between the COD of the influent and the COD of the effluent, ΔCOD, is the biodegradable portion of the waste. The portion still remaining is referred to as nonbiodegradable portion. The concept of ΔCOD as a measure of the organic loading on a plant seems to be a good one. However there is much doubt as to the final disposition of the so-called residual in the receiving stream. Again, it would seem that the term nonbiodegradable is relative to time.

There have been attempts to design systems which would totally

oxidize the incoming waste organic material. The extended aeration process differs from other aerobic digestion processes in that the aeration period is much longer (usually 18 to 24 hour) giving the organisms a longer contact time to metabolize the substrate. The biological solids are totally retained in the system by recycle of all cells from the clarifier. This is supposed to allow the biological solids to autooxidize due to endogeneous respiration as well as lysing and release of metabolic products. The release of cell components by lysing makes those products an exogenous substrate for other microorganisms in the reactor. The effluent from such a system does contain a COD like any other biological treatment process. The products exerting the COD in an extended aeration effluent may possibly be of a different nature than those which leave a conventional activated sludge system. In activated sludge which has a relatively short contact time the effluent may possibly contain a higher percentage of intermediate products from the partial oxidation of the incoming waste than does an extended aeration system. The extended aeration effluent would probably contain more products from cell lysing, such as cytoplasmic components, cell walls and extracellular polysaccharides.

Thabaraj and Gaudy (9)(10) studied the effect of initial biological solids concentration and nitrogen supply on metabolic patterns during substrate removal and endogenous metabolism. During the course of the study an analysis of filtrates was made in an effort to gain some insight into the type of metabolic products released during the course of the substrate removal and the endogenous phase. Substrates used in the study were glucose, sucrose, lactose, acetic acid, sodium acetate, propionic acid, glycerol, and sorbitol. It was found that the

extent of elaboration of metabolic intermediates and/or endproducts was primarily dependent upon the type of substrate. Rapid extracellular accumulation of glucose and fructose occurred during the metabolism of sucrose. Lactose was found to be hydrolyzed intracellularly, at least no extracellular monosaccharide units were found. Acetic acid was an extracellular product during the metabolism of glucose, sucrose, glycerol, and sorbitol while glucose systems were also found to contain pyruvic acid. Keto acids were found in the glucose, lactose, acetic acid, sorbitol, and glycerol systems. The sorbitol system was found to contain some fructose. During the endogenous phase all of the systems were found to contain ribose. This suggests that during cell lysing RNA was broken down releasing the 5-carbon carbohydrate. There was an absence of ribose before the endogenous phase.

Kountz and Forney (11) ran a total oxidation continuous flow pilot plant with dry skim milk as a source of organic matter. They concluded that total oxidation was not possible within reasonable times and sizes of treatment systems. There was a residual material equivalent of 20 to 25 percent by weight of the new activated sludge produced. Even though they speculated that total oxidation was not possible, they believed it possible to operate a system with a lower effluent BOD by wasting an amount equivalent to the accumulation. Symons and McKinney (12) reported that operation of a total oxidation treatment process was not possible because of a continual buildup of a biologically inert material. They also reported, upon microscopic examination of the biomass, that the inert fraction was composed of extracellular polysaccharides. This theory says that not only will total oxidation be impossible but also that a system with no sludge

wastage will eventually fail.

To test the biodegradability of extracellular polysaccharides, Obayashi and Gaudy (13) ran batch systems using bacterial heteropolysaccharides as substrate for mixed microbial populations. The bacterial heteropolysaccharides were obtained from pure cultures of five different organisms, grown under conditions which enhanced production of their extracellular polysaccharides. The slimes were separated from the cells, precipitated with acetone, and redissolved in distilled water. Upon feeding the polysaccharides to an acclimated mixed microbial population it was found that after an acclimation period, the polysaccharides were biodegraded at rates comparable to those observed for simple carbohydrates. The extent of degradation in the batch studies varied from 80 to 92 percent. The conclusion was that extracellular polysaccharides were not biologically inert.

There have been reports of success associated with the extended aeration process (14)(15). In a study by Yang and Gaudy (15), it was concluded that total oxidation of biological solids was not inconsistent with sound microbiological and ecological theory. Throughout the three year investigation in which all sludge was retained in the system the biological solids went through cyclic periods of accumulation and deaccumulation. The biochemical efficiency remained high throughout the study.

Thus, it has been shown that the theory of total oxidation is unresolved. While most of the literature shows that it is not possible there have also been reports of success with extended aeration processes. The fact that experience in this laboratory indicated the total oxidizability of cellular components in extended aeration processes led to a

belief that those cellular components which comprise the COD in the effluent might also be metabolized as they proceeded downstream. The present study was designed to gain some insights into this possibility.

CHAPTER III

MATERIALS AND METHODS

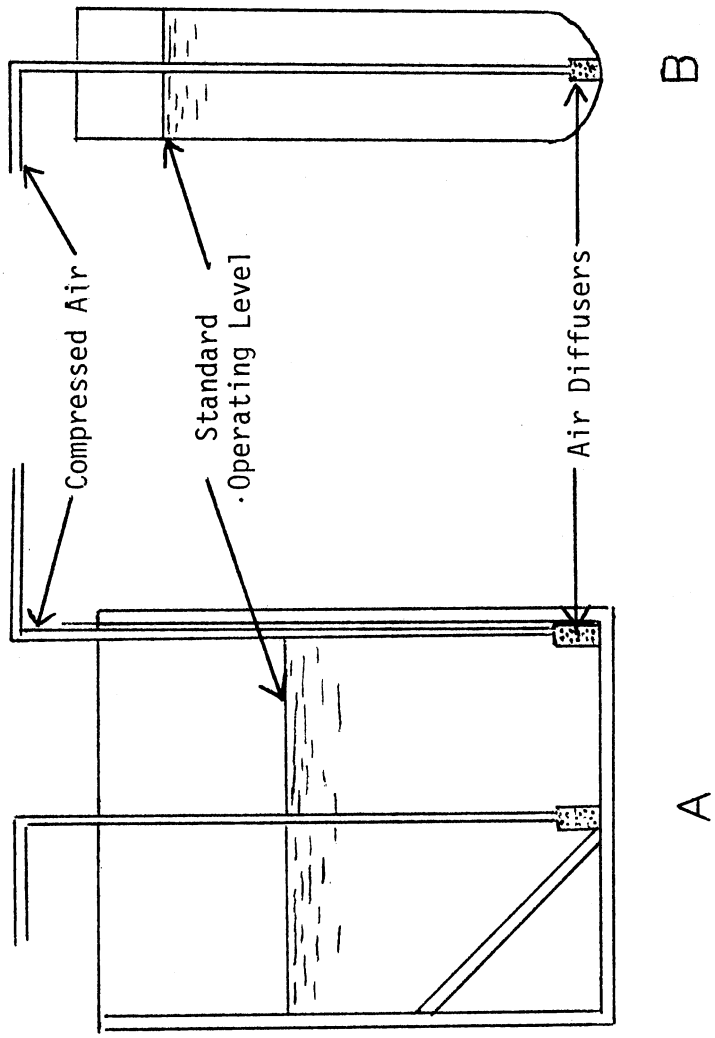
The three biological reactors utilized in this study were operated in such a way as to permit residual COD to accumulate. If it were not metabolized, it was planned to obtain residual organic matter at a higher concentration to facilitate further study of its organic content. In order to get a buildup of the residual COD the unit was operated with no wasting of either sludge or liquid. If the residual organics could be held within the bioreactor it would be expected that they might build up at a rate of 5 percent of the influent COD per day, assuming a removal efficiency of 95 percent. Thus nothing was removed from the unit, except 15 ml taken daily for analysis. To operate a unit in this manner it was necessary to have the daily feed volume less than that lost due to evaporation, thus allowing a constant volume in the biological reactor.

Experimental Apparatus

Unit I

A cross section of Unit I is shown in Figure 1. It consisted of a plexiglass tank. The volume of aeration liquor was 10 liters. The reactor is normally used for continuous flow studies but was converted to batch operation and the baffle separating the aeration tank and

Figure 1. Cross-sections of Units in Study
A - Unit I
B - Unit II and III


Compressed Air
Standard
Operating Level
Air Diffusers
B
A

settling chamber was removed. Compressed air for mixing and oxygenation was admitted through four carborundum diffusers attached to the sidewalls at each corner of the aeration tank. The oxygen concentration was checked periodically. During the study it remained above 4 mg/l. Temperature was not controlled. The temperature in the reactor ranged from 17 to 23, i.e., 20 ± 3°C. Also shown in Figure 1 is a diagram of the reactor used for both Units II and III. The reactor was made of glass. The reaction liquor volume was 3 liters. Aeration was provided by a single carborundum diffuser which kept the unit aerobic and provided adequate mixing.

Composition of Synthetic Waste

The daily synthetic waste used in all three units consisted of glucose as the sole carbon source and other essential inorganic salts, as shown in Table I. The composition of the waste provided for an excess of inorganic salts and assured that the growth limiting factor was glucose.

Procedure

The three units were fed daily. Glucose and the inorganic salts were pipeted into the unit from stock solutions which were stored in a refrigerator to discourage growth. The sides of the units above water line were frequently wiped down to prevent buildup and drying of a solid scum on the reactor walls. The rate of evaporation accounted for more volume than the daily feed stock. Thus it was necessary to maintain the volume constant by addition of a small amount of distilled water daily. The level was brought to 10 liters in Unit I and to

TABLE I

COMPOSITION OF FEED FOR 1000 MG/L
GLUCOSE SUBSTRATE

Glucose	1000 mg/l
$(NH_4)_2SO_4$	500 mg/l
$MgSO_4 \cdot 7H_2O$	100 mg/l
$FeCL_3$	0.5 mg/l
$CaCL_3$	7.5 mg/l
$MnSO_4 \cdot H_2O$	10 mg/l
Phosphate buffer 1.0 M (pH 7.2) (KH_2PO_4, 77 mg/l + K_2HPO_4, 249 gm/l)	5 ml/l
Distilled Water	to volume

three liters in Units II and III.

Feed rate application was different in each of the three units. Units I, II, and III all received a daily dosage of 1000 mg/l glucose but the application rate of the inorganic salts and buffer varied. Units II and III were both brought into operation because of the possibility that high NH_3-N and salt buildups were altering the course of metabolism and species selection in Unit I.

Unit I was fed a daily supply of salts and ammonium sulfate at the rate mentioned previously (Table I). However, because of a fear of salt buildup, on day 144 salts and buffer were eliminated from the daily feed. After day 144 only the 1000 mg/l glucose and 500 mg/l ammonium sulfate were added daily to the unit. On day 364 ammonium sulfate was also eliminated. At this time it was determined that the concentration of ammonia nitrogen (NH_3-N) was well above 2000 mg/l. Unit II received salts, buffer, and ammonium sulfate every other day instead of daily. Eventually, on day 70, salts and buffer were eliminated. This left glucose on a daily basis and ammonium sulfate every two days as the feed. This procedure was carried out until the last day of feeding for Unit II. Unit III was also fed daily 1000 mg/l glucose. Ammonium sulfate was added in the feed only when the concentration of NH_3-N fell below 50 mg/l. Inorganic salts and buffer were added only when ammonium sulfate was added. The NH_3-N level fell below 50 mg/l approximately every 4 to 5 days.

At various times samples (15 ml) were taken from all three units for the determination of MLSS and COD of the filtrate, as well as NH_3-N for Unit III. The concentration of suspended solids was too high to filter directly so the mixed liquor was centrifuged. At less

frequent intervals additional 15 ml samples of mixed liquor were removed, centrifuged and filtered for additional measurements. These samples were stored in glass vials and kept in a freezer for later analysis.

The pH of the mixed liquor was determined frequently and maintained near neutral pH (6.5 to 7.2). During periods of rapid biomass accumulation the pH tended to drop and the addition of NaOH was sometimes needed to adjust to neutral.

Analytical Methods

After first centrifuging the mixed liquor, the biological solids were determined by the membrane filter technique (Millipore Filter Company, Bedford, Massachusetts, HA 0.45 μ). After filtering the supernatant the biomass was removed from the centrifuge tube and placed on the weighed filter paper. The centrifuge tube was then washed out with a small amount of distilled water to remove any cells remaining in the tube. The cell suspension was also filtered with the biomass.

The organic material present in solution was determined by the chemical oxygen demand (COD) test in accordance with Standard Methods (16). A COD test was also run on the mixed liquor at various times. Protein and total organic nitrogen content of the sludge were determined by the biuret and Kjeldahl (16) tests. Ammonia nitrogen (NH_3-N) concentration in the filtrate was determined by a simplified method (17). Carbohydrate concentration was determined by the anthrone test (18). At various times nitrite, nitrate (brucine), and glucose (glucostat) determination were made on the filtrate. Periodically the dissolved oxygen in the mixed liquor was determined with a galvanic cell oxygen

analyzer.

At various times during the investigation special studies and analyses were made. These experimental procedures will be described as the results are presented and discussed.

CHAPTER IV

RESULTS

Operation on the first unit was started on June 6, 1978, and continued through February 8, 1978. The metabolic performance throughout the 613 days of operation is shown in Figures 2, 3, 4 and 5. It is seen that both residual COD and biological solids rose sharply during the first month of operation. During this period of maximum concentration of residual COD was 1570 mg/l on day 32. Thereafter the residual COD dropped almost as sharply as it had risen and attained a value of 324 mg/l at day 66. Over the next 60 days biological solids continued to increase and there was a slight rise in residual COD. After day 110 there was a period of severe oscillation in biological solids concentration but this was not reflected in an oscillation in residual COD. Also shown in Figure 2, 3, and 4 are values of soluble carbohydrate in the reactor filtrate. Carbohydrates were not run on the filtrate during the first month and it is not known if carbohydrates constituted a significant portion of the residual COD during the period of rapid rise. However it is seen that by day 40 carbohydrates constituted less than one-third of the residual COD. As the residual COD fell from day 55 to 65 there was a decrease in the soluble carbohydrate. Carbohydrate concentration leveled at approximately 100 mg/l and through the last 50 days shown in the figure it remained at approximately 200 mg/l.

On day 63 it was noted that the coloration of the mixed liquor

began to change from a light brown to a darker brown. By day 70 the mixed liquor had become extremely dark brown in color and remained this way throughout the remaining 150 days. Periodic examination of dissolved oxygen indicated that values lower than 7.0 were never recorded.

The next 150 days of operation was shown in Figure 3. During this period biological solids concentration in the reactor rose from approximately 35,000 to 50,000 mg/l. Residual COD concentration ranged between 800-900 mg/l until day 185 whereupon there was a rapid rise in residual COD. It attained a value of 2060 mg/l by day 221. Carbohydrate concentration rose from 200 to nearly 300 mg/l during this time. Toward the end of this period of operation there was a very rapid decrease in residual COD (see days 260-274) however the system could not sustain this lower level and residual COD very rapidly rose to 1700 mg/l. From day 280 to 300 there was a fairly steady decrease in residual COD. The decreasing trend in residual COD continued into the next period of operation shown in Figure 4. However by day 305 the residual COD rose to approximately 1650 mg/l and throughout the remaining period of operation oscillated around this value. Carbohydrate content in the filtrate varied between 200 and 300 mg/l. In general biomass concentration ranged between 50,000 and 60,000 mg/l. Throughout the operational period shown in Figures 3, 4 and 5, the system remained the same dark color it had assumed by day 70.

The results for the remainder of this operational period are shown in Figure 5. The biomass concentration rose gradually to approximately 70,000 mg/l and remained at this concentration for approximately the last 100 days of operation. Early in this operational period the

Figure 2. Performance of Unit I During the First 150 Days of Operation.

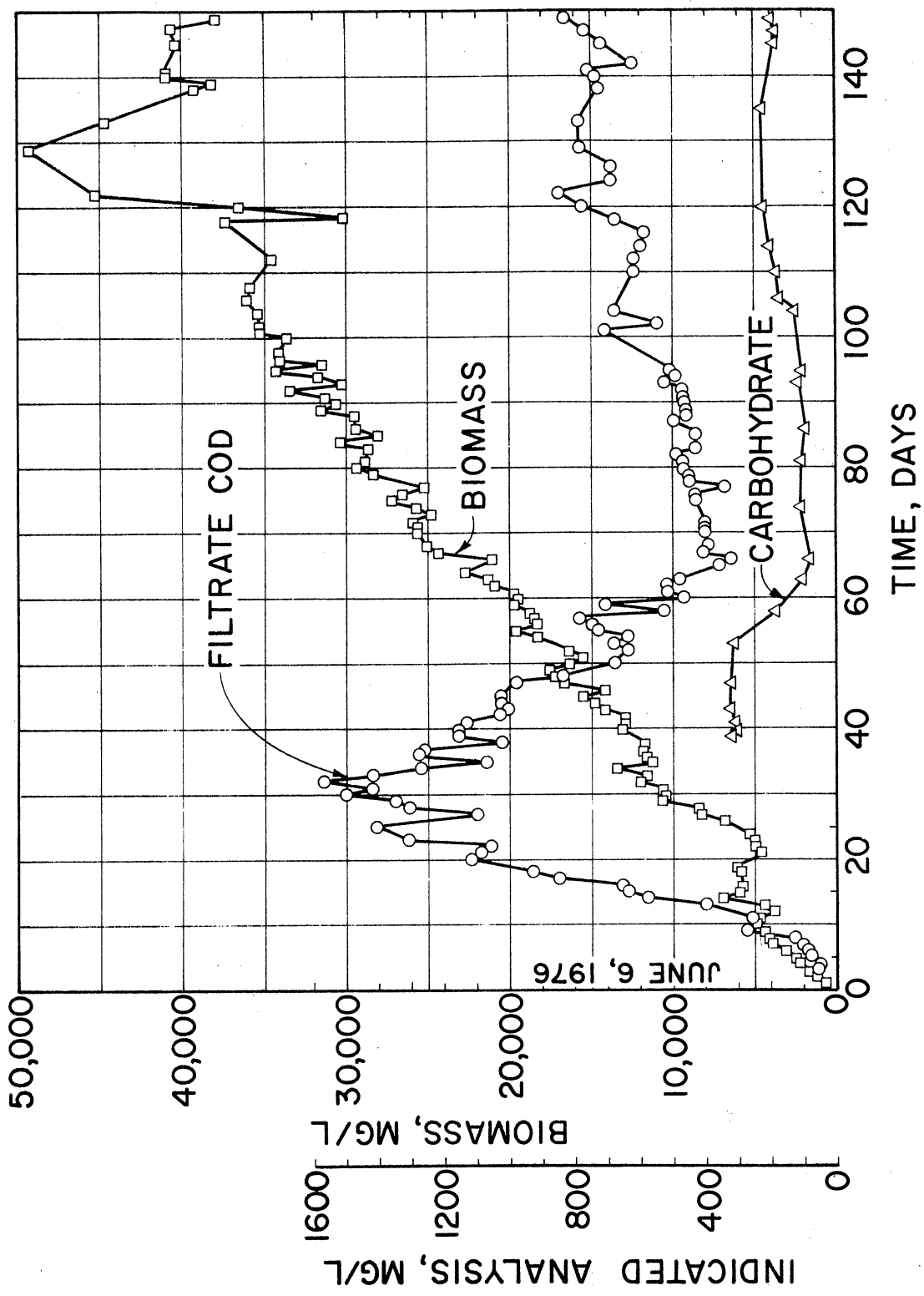

FILTRATE COD
BIOMASS
CARBOHYDRATE
JUNE 6, 1976
TIME, DAYS
INDICATED ANALYSIS, MG/L
BIOMASS, MG/L
0
400
800
1200
1600
0
10,000
20,000
30,000
40,000
50,000
0
20
40
60
80
100
120
140

Figure 3. Performance of Unit I From the 150th Day of Operation to the 300th Day of Operation.

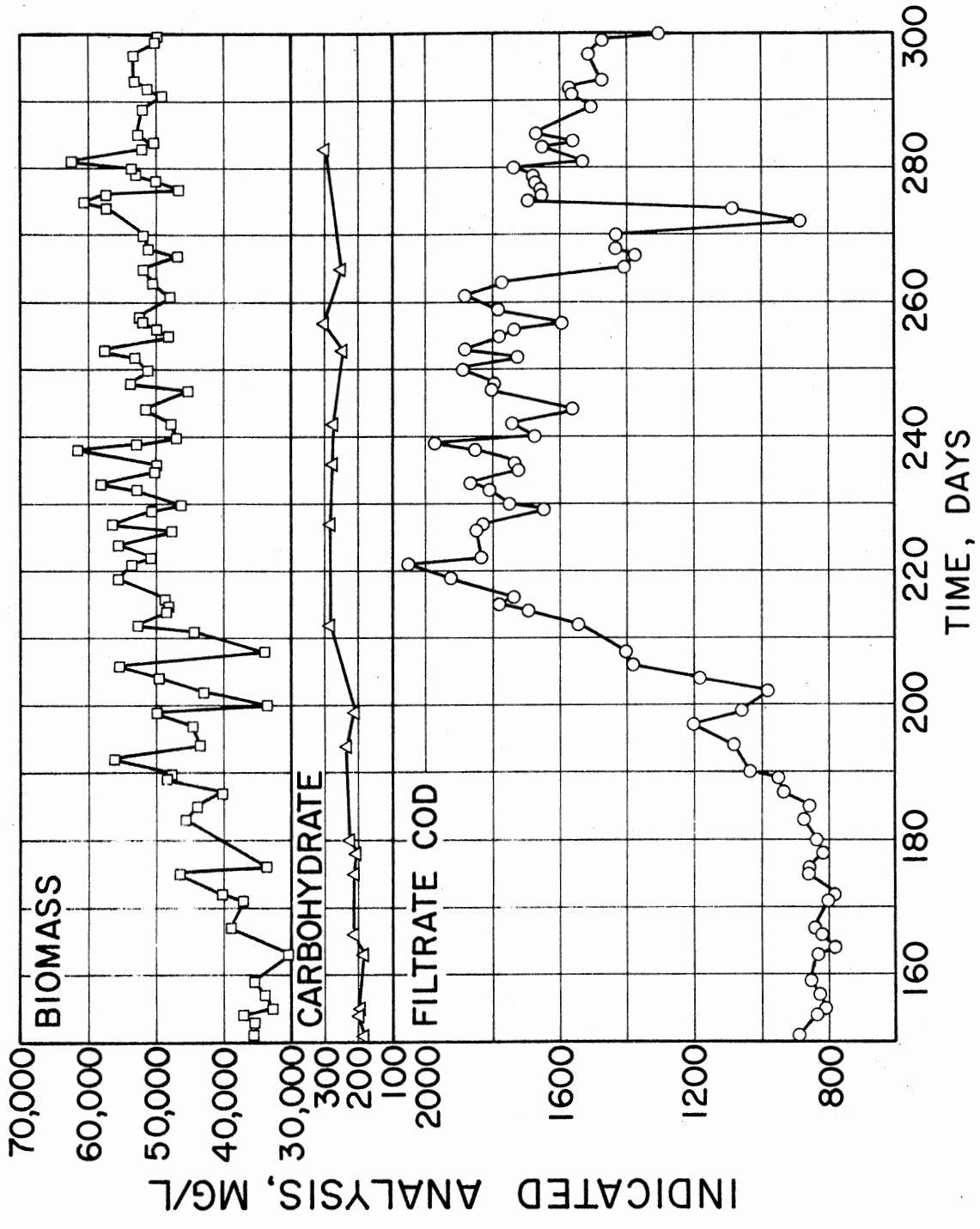

BIOMASS
CARBOHYDRATE
FILTRATE COD
INDICATED ANALYSIS, MG/L
70,000
60,000
50,000
40,000
30,000
300
200
100
2000
1600
1200
800
160
180
200
220
240
260
280
300
TIME, DAYS

Figure 4. Performance of Unit I from the 300th Day of Operation to the 450th Day of Operation.

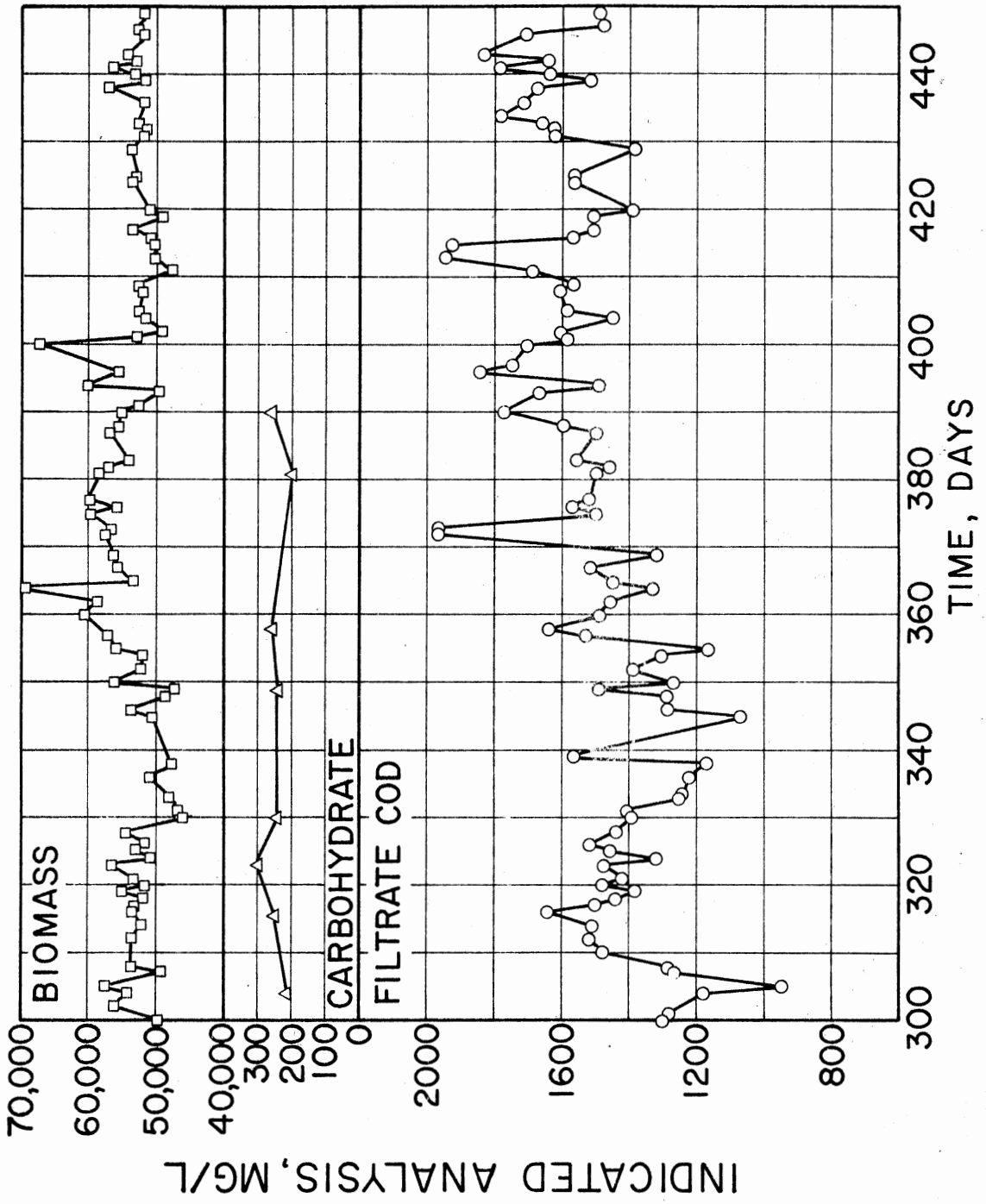
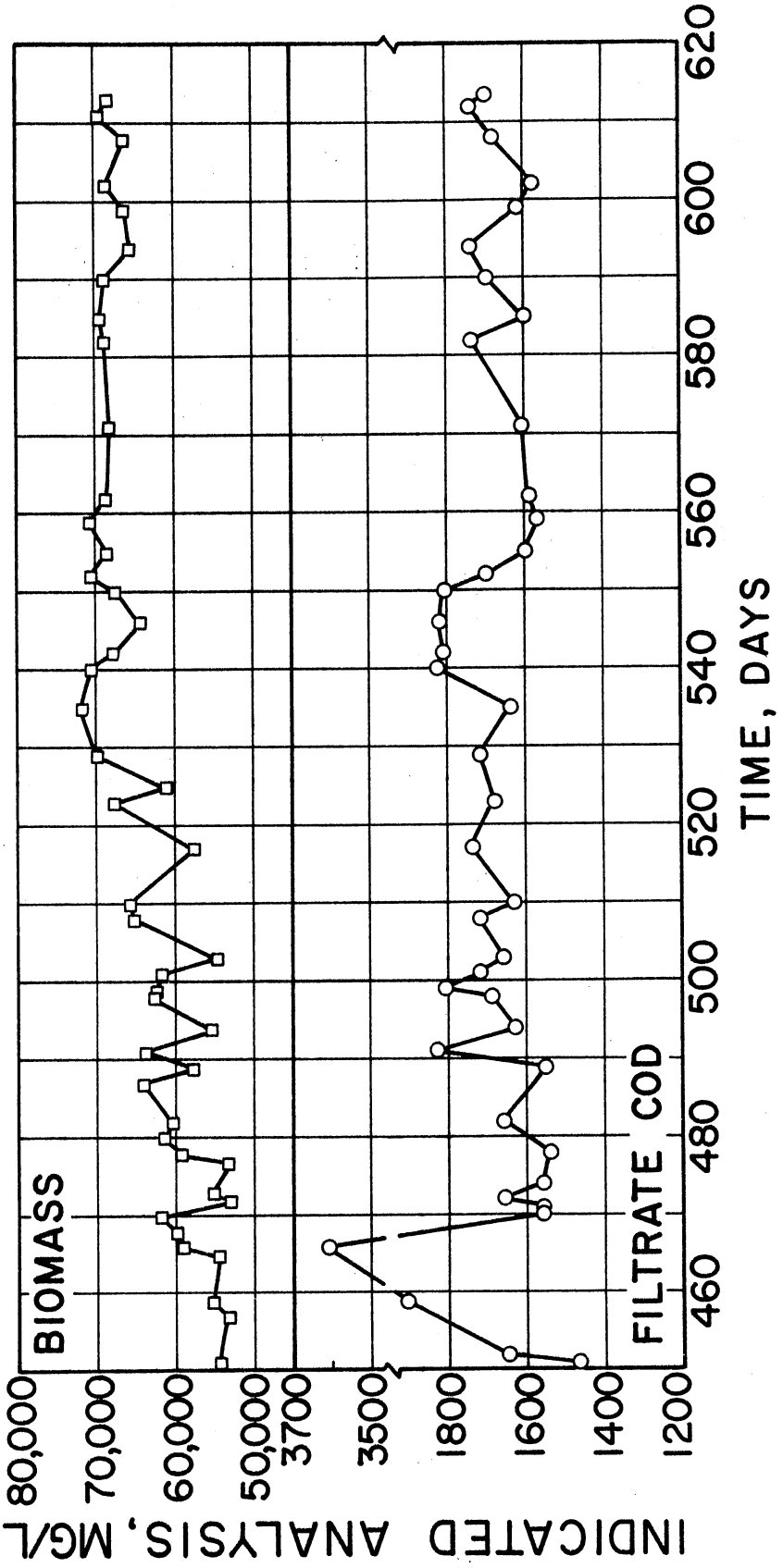

BIOMASS
CARBOHYDRATE
FILTRATE COD
INDICATED ANALYSIS, MG/L
TIME, DAYS
70,000
60,000
50,000
40,000
300
200
100
0
2000
1600
1200
800
300
320
340
360
380
400
420
440

Figure 5. Performance of Unit I from the 450th Day of Operation to the 613th Day of Operation.


INDICATED ANALYSIS, MG/L
80,000
70,000
60,000
50,000
3700
3500
1800
1600
1400
1200
BIOMASS
FILTRATE COD
460
480
500
520
540
560
580
600
620
TIME, DAYS

residual COD showed a very rapid rise. However this occurrence was not due to natural metabolic or ecological factors but to faulty operation of the air supply. The air valves were turned to full open but there was an insufficient degree of agitation and aeration in the reactor. It was determined that the diffusers were severely plugged with biological growth and immediately after replacing them the residual COD returned again to the 1500-1600 mg/l level which existed prior to the malfunction in the air system. Throughout the remainder of the operational period the filtrate COD varied between 1600 and 1800 mg/l.

In accordance with the experimental protocol outlined in the previous chapter it can be seen that the addition, each day, of enough stock solution to make up a feed with the salt concentration shown in Table I provided for a rapid buildup of salts in the reactor. It can be seen from the results shown in Figure 2 that the salts concentration did not hamper the accumulation of biological solids nor did it have a serious affect on the utilization of the 1000 mg/l COD which was fed each day. By day 144 it can be calculated that the salt concentration due to the daily feeding of synthetic waste stock solution was approaching 17,000 mg/l per liter and the concentration of phosphate buffer added was approaching a concentration of 235,000 mg/l. On day 144 a decision was made to omit addition of salts and buffer solution. After this day the only addition of materials in the medium consisted of the 1000 mg/l per day of glucose and the stock solution of 500 mg/l per day of ammonium sulfate. Addition of ammonia nitrogen to the unit was omitted after day 364. At this time the total addition of ammonium sulfate was 182,000 mg/l (NH_3-N = 38,528 mg/l). While a buildup of phosphate salts, inorganic salts in the medium or ammonium sulfate

might be expected to exhibit toxic affects, it is apparent that they did not, since the residual COD did not build up to an extent which would indicate toxicity. Attempts to run colorimetric NH_3-N analyses failed. The pH of the unit remained between 6.5 and 7.3 indicating that there was very little opportunity for ammonia to strip from the unit. Analysis for nitrates indicated there was no nitrification taking place in the reactor. At the pH levels recorded the amount of NH_3-N stripped would not be expected to be great, however at the high nitrogen concentration which was expected to exist in the reactor it is entirely possible that some nitrogen may have been stripped from the unit. Also there may have been other gaseous products from ammonia nitrogen which may have stripped. Early experimental problems with determination of ammonia nitrogen were solved and on day 436 an ammonia concentration of 8700 mg/l was registered in the reactor. The protein content of the cells was not run routinely therefore it is impossible to attempt to calculate a nitrogen balance. However, at the end of the study Kjeldahl nitrogen analysis on the suspended solid indicated a protein content of 10 percent for the organic fraction (75 percent) of the suspended solids. Thus it would appear that a considerable amount of the NH_3-N added to the reactor was stripped.

Periodically samples were analyzed for total organic carbon, TOC. Table II shows filtrate COD values and filtrate TOC values on the days indicated. Also shown in the table is the ratio COD/TOC. These ratios varied from 2.2 to 3.4. The lower values correspond to ratios expected from carbohydrates while the higher values are consistent with those expected from certain of the volatile acids.

On day 436 a sample was taken for analysis of COD of the mixed

TABLE II

$\frac{COD}{TOC}$ RATIOS IN UNIT I

$\frac{COD}{TOC}$ Ratios of Example			
Glucose	=	$\frac{1.06}{.4}$	= 2.65
Propionic acid	=	$\frac{1.51}{0.49}$	= 3.08
Butyric acid	=	$\frac{1.82}{.55}$	= 3.3
Ethanol	=	$\frac{2.09}{.52}$	= 4.02

$\frac{COD}{TOC}$ Ratios in Unit		
Day		
124	$\frac{685}{283}$	= 2.42
133	$\frac{791}{355}$	= 2.23
180	$\frac{840}{310}$	= 2.71
212	$\frac{1546}{480}$	= 3.22
227	$\frac{1832}{545}$	= 3.36
242	$\frac{1745}{570}$	= 3.06
265	$\frac{1410}{510}$	= 2.47
364	$\frac{1331}{395}$	= 3.37

liquor. The results indicated a total COD concentration of 60,600 mg/l. On this day the suspended solids in the unit were 51,900 mg/l. The COD of the filtrate was 1700 mg/l, thus the COD of the cells was approximately 59,000 and the COD of the suspended solids was 1.1 mg/mg.

The ash content of the suspended solids was not routinely run but a few samples taken toward the end of the experiment indicated that the ash content ranged from approximately 20-30 percent.

With the total retention of cells and the possibility of salts or ammonium ion toxicity due to buildup of these constituents there was some concern over the "healthy condition" of the biomass. This concern prompted the running of a batch experiment, the results of which are shown in Figure 6. On day 451 a batch experiment was set up in which the concentration of 500 mg/l cells from the total retention reactor was fed 1000 mg/l glucose. Samples were taken for biological solids concentration, filtrate COD and COD of the mixed liquor. It is seen from Figure 6 that after a lag period of 4 to 5 hours growth and substrate removal proceeded in a diaphisic manner not unlike many systems which have previously been examined in this laboratory for much younger cells suspensions (19)(20). The lower curve shows oxygen uptake calculated as the difference between the initial mixed liquor COD and mixed liquor COD remaining at any time. Material balances for the system are shown in Table III.

It is apparent from this result that after 451 days this biomass exhibited considerable ability to metabolize the feed substrate leaving a rather low residual COD of 50 mg/l.

Unit II was brought into operation on March 15, 1977. This was day 283 of operation of Unit I. The unit was seeded with 100 ml of

Figure 6. Growth Test Utilizing Biomass from Unit I on the 45lst Day of Its Operation.

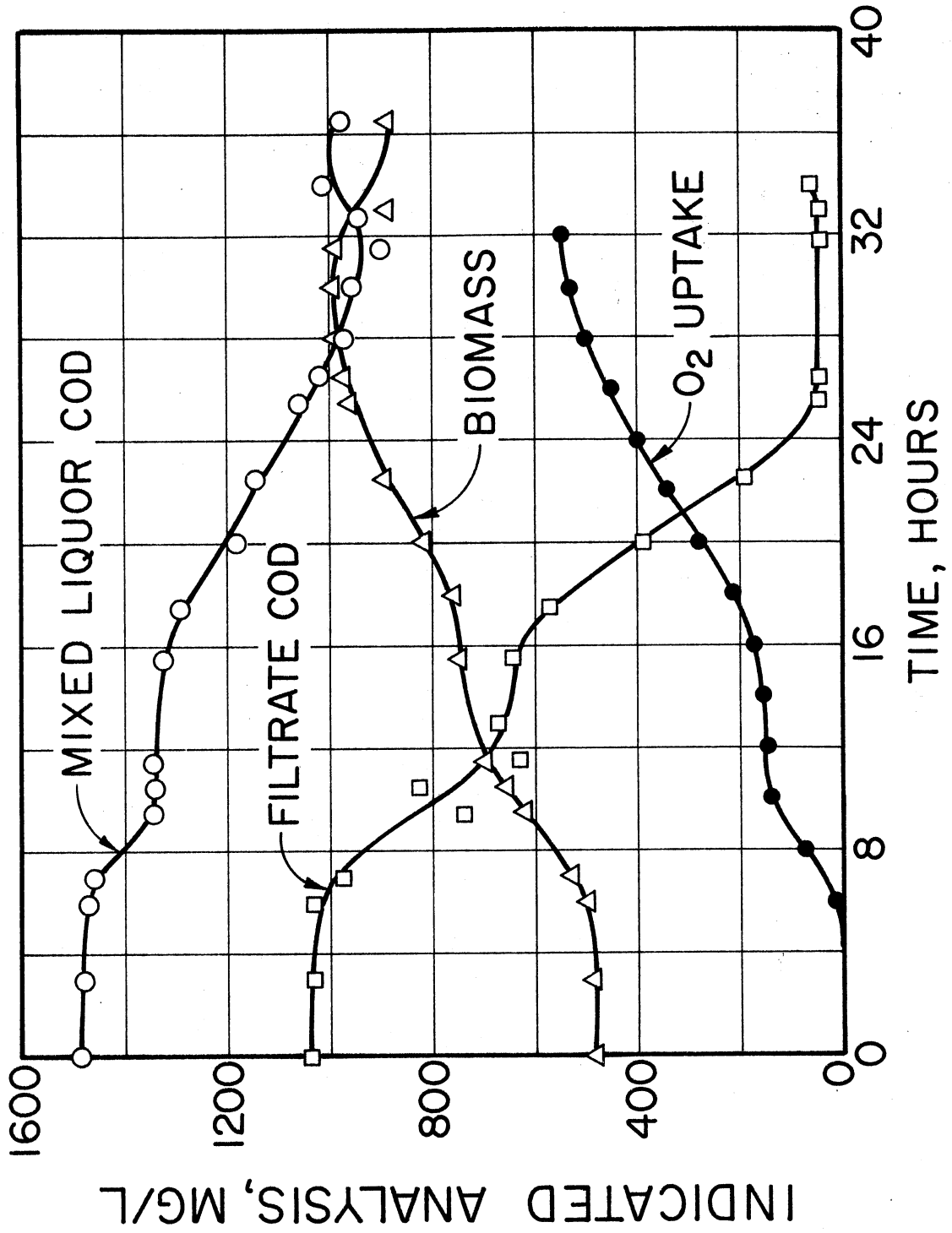

MIXED LIQUOR COD
FILTRATE COD
BIOMASS
O2 UPTAKE
INDICATED ANALYSIS, MG/L
TIME, HOURS
1600
1200
800
400
0
0
8
16
24
32
40

TABLE III

ENERGY BALANCE FOR GROWTH TEST
ON DAY 451

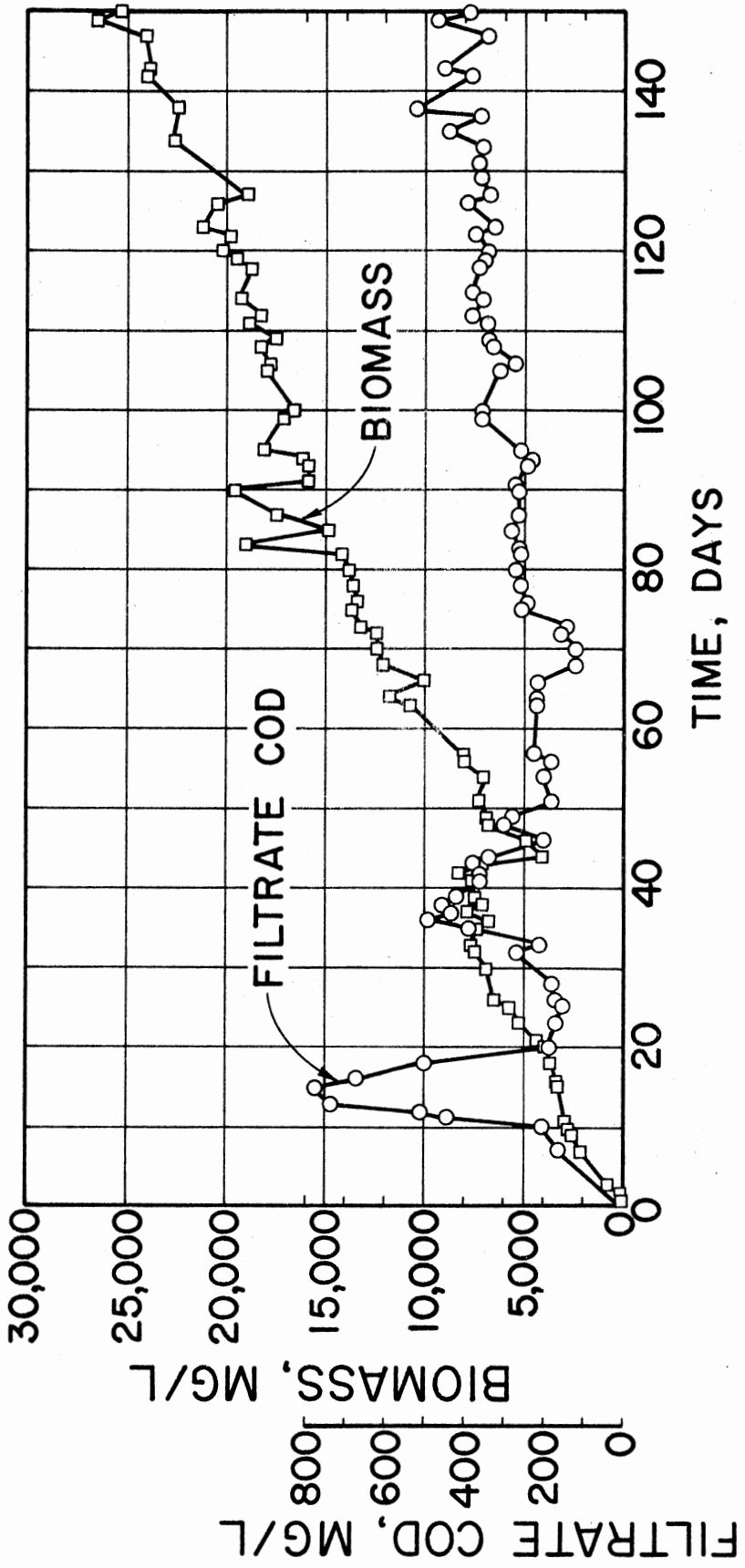
Time hr	COD rem mg/l	Cells mg/l	O_2 Uptake mg/l	COD of cells mg/l	ΔCOD mg/l	ΔCOD mg/l	ΔCOD cells mg/l	Substrate Recovery (percent)
0	1040	485	0	448	0	0	0	
2	1038	485	0	450	2	0	2	
4	1034	488	5	450	6	3	2	117
6	1015	507	18	454	25	22	6	96
8	934	563	75	476	106	78	28	97
10	797	638	136	544	243	153	96	95
12	682	702	150	656	358	217	208	100
14	644	732	156	685	396	247	237	99
16	625	750	178	687	415	265	239	100
18	535	770	217	737	505	285	289	100
20	400	812	270	806	640	327	358	98
22	244	868	340	900	796	383	452	99
24	108	922	400	976	932	437	528	100
26	50	963	457	975	990	478	527	99
28	50	982	502	932	990	497	484	100
30	48	992	532	902	992	507	454	99
32	45	982	548	893	995	497	445	100
34	62	915		924		430		
36		888				403		

mixed liquor obtained from Unit I. Unit II was operated in the same manner as Unit I, i.e., there was no wastage of the soluble and non-soluble portions; however, the daily feed was modified. Because of the buildup of total solids and NH_3-N in Unit I it was felt that there was a possibility of toxicity affecting the biomass and thus the results from Unit I. The modification consisted of feeding salts, ammonium sulfate, and buffer on an every-other-day basis instead of daily. The concentration of these constituents remained the same as previously reported with only their rate of application being modified. Glucose was fed daily at the rate of 1000 mg/l as it was in Unit I.

As indicated in Figure 7, the biological solids increased at a steady rate for the first 33 days of operation, by which time they reached a concentration of 7776 mg/l. The filtrate COD showed an early buildup as was the case in Unit I. On the 15th day of operation the residual COD was approximately 780 mg/l. From day 15 to day 20 there was a sharp decrease, with the COD dropping from 779 mg/l to 378 mg/l at which time the decrease slowed. By day 25 the filtrate COD level had dropped to a value of 304 mg/l. From day 25 to day 33 the residual COD increased steadily to 422 mg/l.

From day 33 to day 51 the unit went through severe oscillations with respect to both filtrate COD and MLSS. During this period of operation there was a build up of foam in the unit. The pH was checked daily and maintained within the range of 6.5 to 7.2. During periods of rapid growth the pH tended to drop faster than during times of "equilibrium" and it was sometimes necessary to adjust to 7 by the addition of NaOH. Evidently, the foam was caused by cells lysing. Steady increase of biomass which had occurred during the first 33 days

Figure 7. Performance of Unit II During the First 150 Days of Operation.


FILTRATE COD, MG/L
0
200
400
600
800
BIOMASS, MG/L
5,000
10,000
15,000
20,000
25,000
30,000
TIME, DAYS
0
20
40
60
80
100
120
140
FILTRATE COD
BIOMASS

of operation ceased with the occurrence of the foaming. Over the next nine days of operation the MLSS tended to remain around the same concentration, approximately 7500 mg/l. This period appeared to be one wherein growth was balanced by autodigestion rate. During this period the filtrate COD rose sharply at first, more than doubling in three days, and then slowly decreased until day 43.

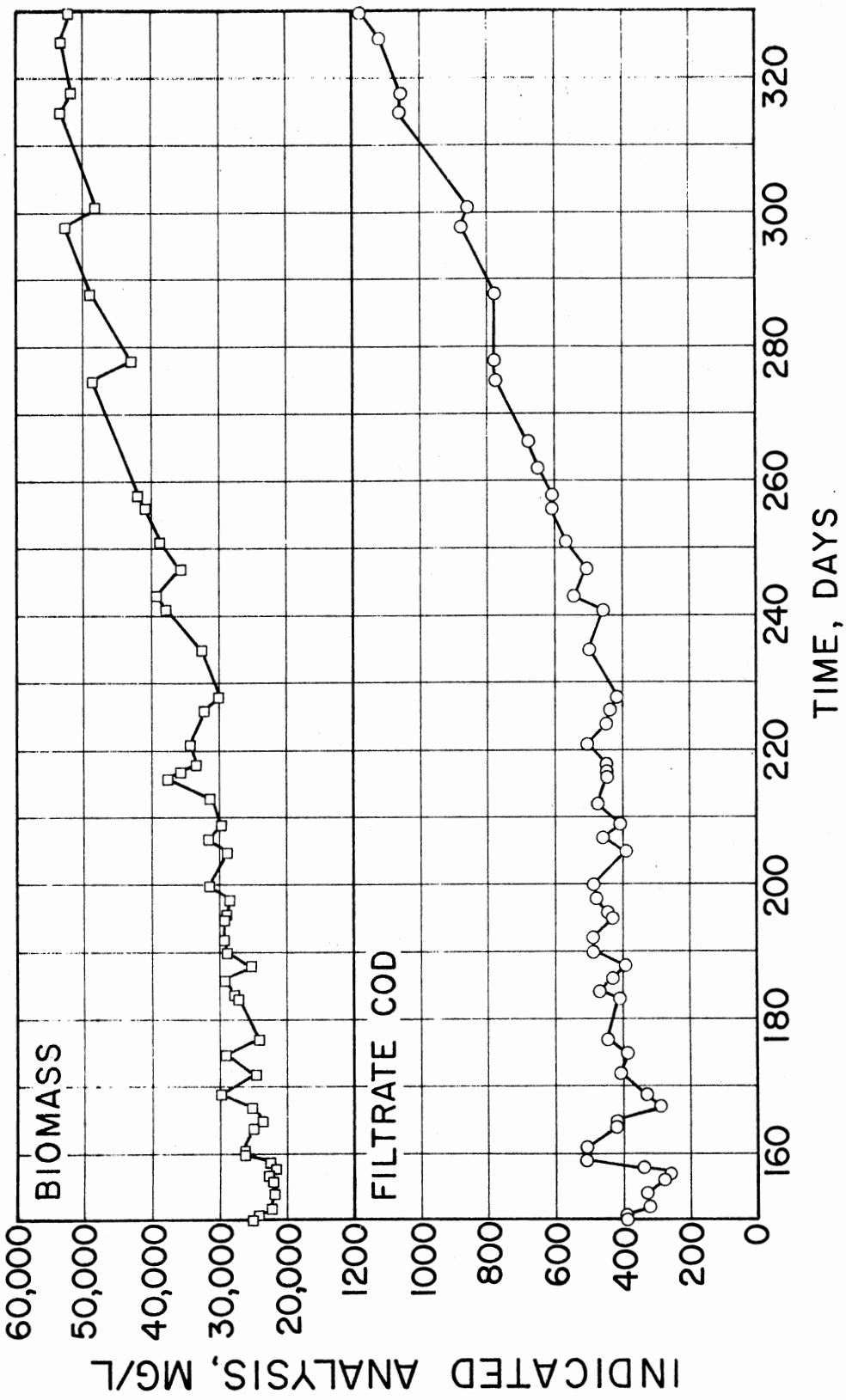
Between samplings on days 43 and 44 Unit II foamed heavily and some of the mixed liquor was lost as the unit overflowed. This occurrence shows in the graph as a decrease in the biomass on day 44. The concentration dropped from 7247 mg/l to 4060 mg/l during this day. It is believed that the foaming was due to lysing of some of the cells in the population but the cause is not discernable. It is known however, that there was no change in the external environmental conditions.

After the loss of biomass on day 44 there began a period of rapid recovery. Foaming stopped. The solids increased by close to 60 percent over the next four days, from 4000 mg/l to approximately 6800 mg/l. During this period of rapid growth the filtrate COD showed some fluctuations but generally was on a decline. By day 51 Unit II began a trend which continued throughout the remaining days of operation shown in Figure 7, i.e., a slow increase of both biomass and residual COD. By day 150 the COD had risen to nearly 400 mg/l.

In Figure 8, it is seen that for the next three months of operation the effluent COD oscillated between 400 and 500 mg/l. At approximately day 250 the effluent COD began a significant rise as did the biomass concentration.

There were no immediately apparent causes for the change in rate of solids accumulation or rise in COD. On day 332 addition of feed was

Figure 8. Performance of Unit II from the 150th Day of Operation to the End of Operation on Day 332.


BIOMASS
FILTRATE COD
INDICATED ANALYSIS, MG/L
60,000
50,000
40,000
30,000
20,000
1200
1000
800
600
400
200
0
160
180
200
220
240
260
280
300
320
TIME, DAYS

terminated (February 11, 1978). The unit is currently undergoing aerobic digestion to determine whether both the solids and the filtrate COD can be reduced.

The end of the period of feeding the total solids concentration of the system was 140,400 mg/l, 60 percent of which registered as ash. The total dissolved solids concentration was 80,000 mg/l, 83 percent of which registered as ash. The ash content of the suspended solids ranged between 25-30 percent.

A third unit, designated here as Unit III, was brought into operation on July 6, 1977. As in the previous two units there was no wastage of either solid or liquid portions of the reaction liquor. Like Unit I, but unlike Unit II, Unit III was seeded with a heterogeneous population of organisms obtained from the Stillwater wastewater treatment facility. The same feed solutions were used as in Units I and II. However the feeding protocol was different.

On the first day the feed concentrations of salts, NH_4SO_4 and buffers were the same as for Table I. Thereafter the filtrate NH_3-N concentration was measured approximately every 2-3 days. Whenever the NH_3-N value fell below 50 mg/l more $(NH_4)_2SO_4$ was added at the standard feed dose of 500 mg/l, which is 106 mg/l of NH_3-N. Also, whenever additional NH_3-N was needed in the unit it was assumed that there may be a need for salts and buffer also, thus they were added along with the $(NH_4)_2SO_4$. The addition of the $(NH_4)_2SO_4$, salts, and buffer took place approximately every 4-5 days. The pH was monitored frequently and during this run it ranged from 6.5 to 7.2.

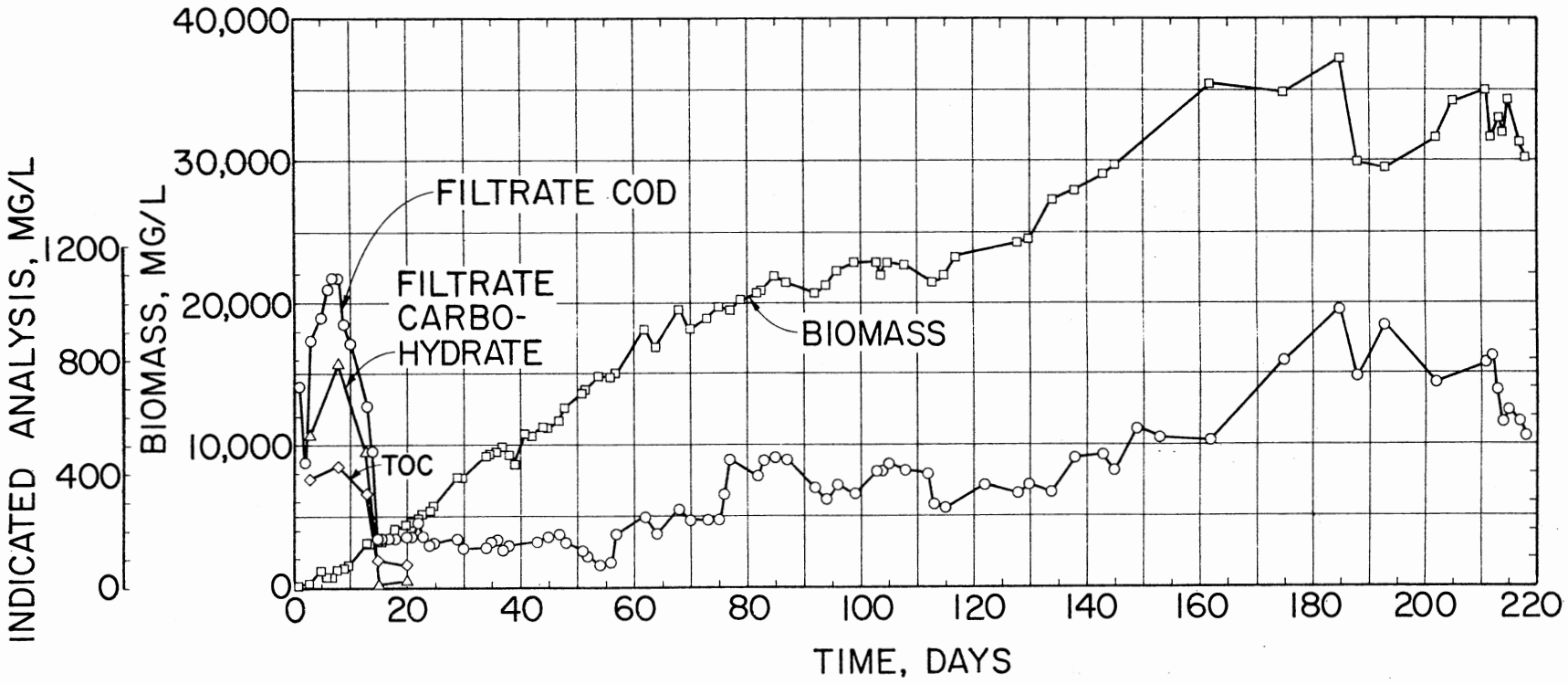
The results for Unit III are shown in Figure 9. It is seen that both biological solids and filtrate COD rose sharply during the first

few days of operation as did the carbohydrate content in the filtrate. The biomass accumulated at a steady rate while residual COD rose sharply during the first week and then dropped the second week just as suddenly. On day 7 the concentration of filtrate COD was 1090 mg/l but by day 15 it had dropped to 170 mg/l. It is seen that over 75 percent of the buildup was due to carbohydrates. After the drop in residual COD by day 15 the percent carbohydrate of the filtrate was less than 10 percent. From day 15 until day 145 the biological solids increased steadily and attained a concentration of 31,500 mg/l. The residual COD during this time went through some oscillations but generally was on a slow upward trend. From day 15 to day 47 the concentration of filtrate COD remained very stable, at approximately 150 mg/l. From day 47 to day 54 there was a slow drop to 80 mg/l followed by a sharp rise back to a level slightly higher than the level before the drop, i.e., approximately 200 mg/l. Until day 147 the filtrate COD shows a series of oscillations with buildups and reductions occurring regularly. Generally the filtrate COD was on the rise, with buildups being greater than the reductions. By day 149 the concentration was 558 mg/l.

The color of the mixed liquor in this unit was caramel, unlike the dark brown color of Units I and II.

After day 150 fewer samples were taken but the general trend of the results remained the same. Biomass continued to climb steadily until day 185 when it reached its highest concentration, 37,900 mg/l. After 185 days of operation the biomass began its first significant drop in concentration. By day 188, just three days later, the biological solids attained a concentration of 30,000 mg/l, a drop of over 20 percent. After two weeks, the biomass had again climbed to

Figure 9. Performance of Unit III During the Entire Time of Its Operation 218 Days.


INDICATED ANALYSIS, MG/L
BIOMASS, MG/L
40,000
30,000
20,000
10,000
0
1200
800
400
0
FILTRATE COD
FILTRATE CARBO-HYDRATE
TOC
BIOMASS
0
20
40
60
80
100
120
140
160
180
200
220
TIME, DAYS

approximately 35,000 mg/l. This was followed by another drop to around 30,000 mg/l on day 218, the last day of the feeding operation. During this period the filtrate COD showed the same general trend shown by the biomass. The concentration increased to close to 1000 mg/l by day 185. This was followed by some large fluctuations with a general downward trend. From day 185 to day 218 the residual COD went from 978 to 531, a 46 percent reduction.

Feeding was terminated on February 11, 1978, and currently the system is undergoing aerobic digestion like Unit II. Ash content of the biomass was not run routinely but early in the operational period (day 24) an ash of 22 percent was recorded. At the end of the operational period the ash content of the suspended solids was 25 percent.

CHAPTER V

DISCUSSION

From the results presented in Figures 2 through 9, it is apparent that the residual COD in solution was of a significant amount but it was much lower than would be expected if the daily residual was in the range 40-50 mg/l which is normally observed in batch experiments to determine ΔCOD or in a continuous flow reactor experiment with 1000 mg/l glucose as feed. It is interesting to compare the COD expected if the residual is truly nonbiodegradable with that which was observed at the end of the experiment in the three reactors.

In Unit I which was run for 613 days the expected residual at the end of this time, if the daily residual was 50 mg/l, would be 30,650 mg/l of COD. On the other hand if the expected residual was 40 mg/l the COD remaining at the end of the experiment would be 24,520 mg/l COD. Comparing these values to the 1697 mg/l COD which was recorded on day 613 leads to a percentage removal of the residual COD of 94.5 and 93 percent respectively. When one considers that during the entire experiment with Unit No. I the total concentration of COD fed was 649,780 mg/l COD the total COD removal was 99.7 percent. In fact, an examination of the filtrate COD from January, 1977 to February, 1978 (see Figures 3, 4, 5, days 220-613) indicates that the unit produced essentially 100 percent removal during this time.

Making the same calculations for the Unit II, the total amount of

residual COD expected would be 16,600 mg/l and 13,280 mg/l for residual COD's of 50 and 40 mg/l respectively. On day 332, the last day of the experiment with this unit the residual COD was 1184 mg/l; this leads to efficiency of removal of residual COD of 93 percent and 91 percent respectively. During this time the total amount of glucose COD fed to the unit was 351,920 mg/l yielding an overall removal efficiency of 99.7 percent.

In Unit III which was run for 218 days the expected residual COD would have been 10,900 mg/l and 8,700 mg/l for residuals of 50 and 40 mg/l respectively. The removal efficiency for the residual COD was 95 percent and 94 percent respectively for anticipated daily residuals of 50 mg/l and 40 mg/l. During the 218 day run a total of 231,000 mg/l was fed leading to an overall removal efficiency of 99.8 percent. It can be seen from the above calculations that the residual COD which is normally observed for a feed of 1000 mg/l glucose cannot be classified as a permanently residual or nonbiodegradable chemical oxygen demand.

It is possible that one may not ascribe the removal of this residual COD to biological action. It does seem possible that some organic material which was strippable could have been produced by the cells and that this represented at least a portion of the residual which was removed. Also it seems possible that some of the residual COD could be adsorbed to the suspended solids (biological solids) in the unit.

To determine if there was strippable material present a large sample of the mixed liquor was taken from Unit No. I after day 613. After centrifuging to remove the suspended solids, the centrifugate was subjected to vigorous aeration to determine if any COD could be stripped. The centrifugate gave essentially a clear liquid and it was

decided to perform the stripping studies without addition of any bacteriacide since they could possibly have an effect on the strippability of organic material present. To aid in discussing the primary results the stripping test is shown in Figure 10 and it is seen that at the end of the experiment, 24 hours, there was no evidence for the presence of strippable materials.

A sample of the centrifugate was also used in studies with activated carbon to determine the extent to which soluble COD could be adsorbed on the carbon surface (see Figure 11). It is seen that extremely high concentrations of activated carbon were required in order to adsorb significant amounts of soluble COD. Bacterial cells would not be expected to have the adsorption efficiency of activated carbon. Therefore it would not appear that the very high amount of residual COD which was removed can be attributed to adsorption on the surfaces of the suspended solids. Autoclaved cells were also used as the adsorptive surfaces. Cells from Unit I were autoclaved at 15 psi, 121°C for one hour. The suspended solids were centrifuged and the supernatant was wasted. The cells were then contacted with 250 mls of a glucose solution of 1000 mg/l, a phenol solution of 1000 mg/l and an acetic acid solution at a 1000 mg/l. COD in solution 15 and 30 minutes after initial contact indicated that no material was adsorbed.

It is well known that glucose cannot be adsorbed on activated carbon or on bacterial cells. Therefore rapid removal of glucose from solution would attest to the healthy condition of the biomass. It was impossible to make studies on rate of COD removal in the unit since the COD was removed so rapidly. In general it was found that even at the end of the study the soluble COD was removed within three or four

Figure 10. Stripping of Soluble Organics in Unit I.

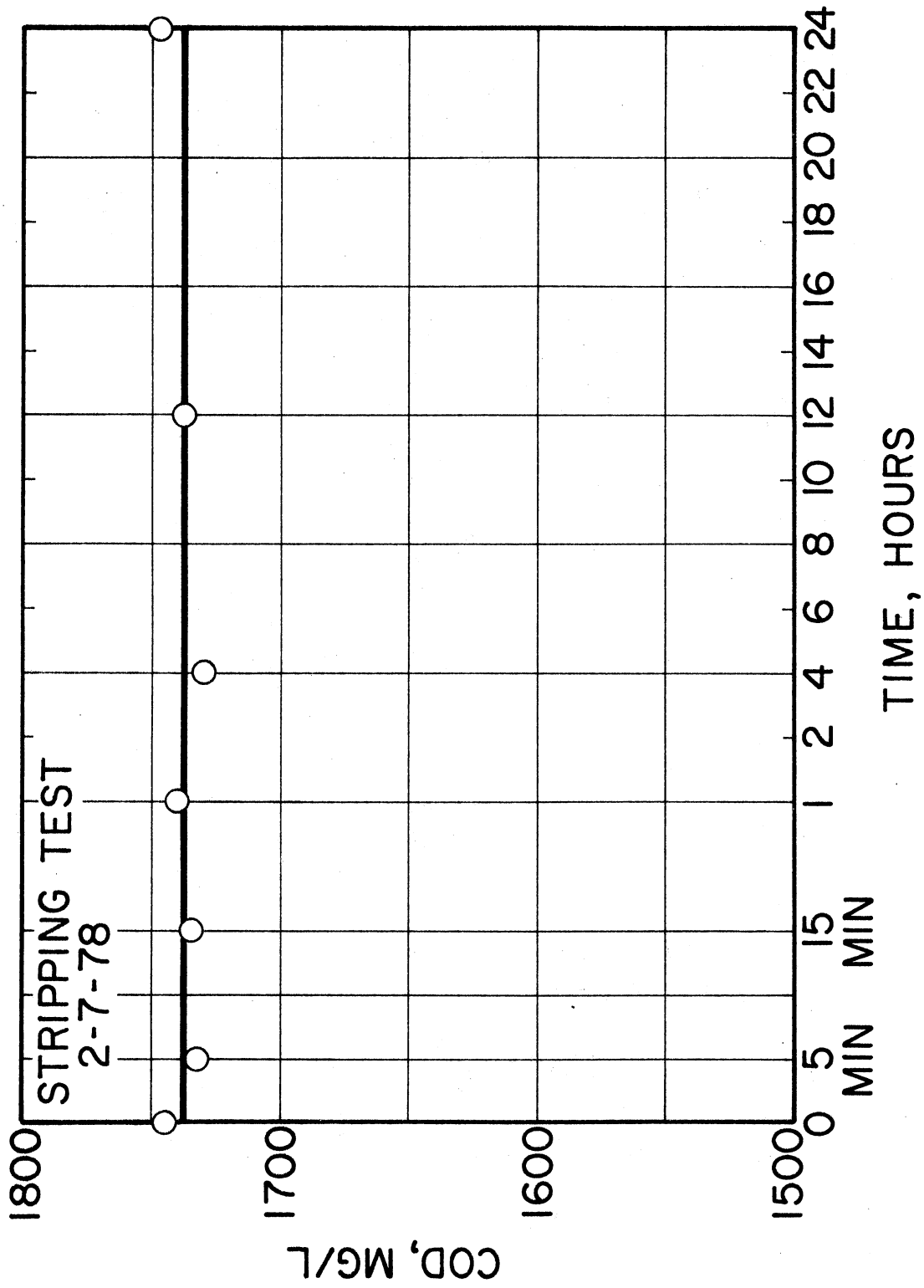
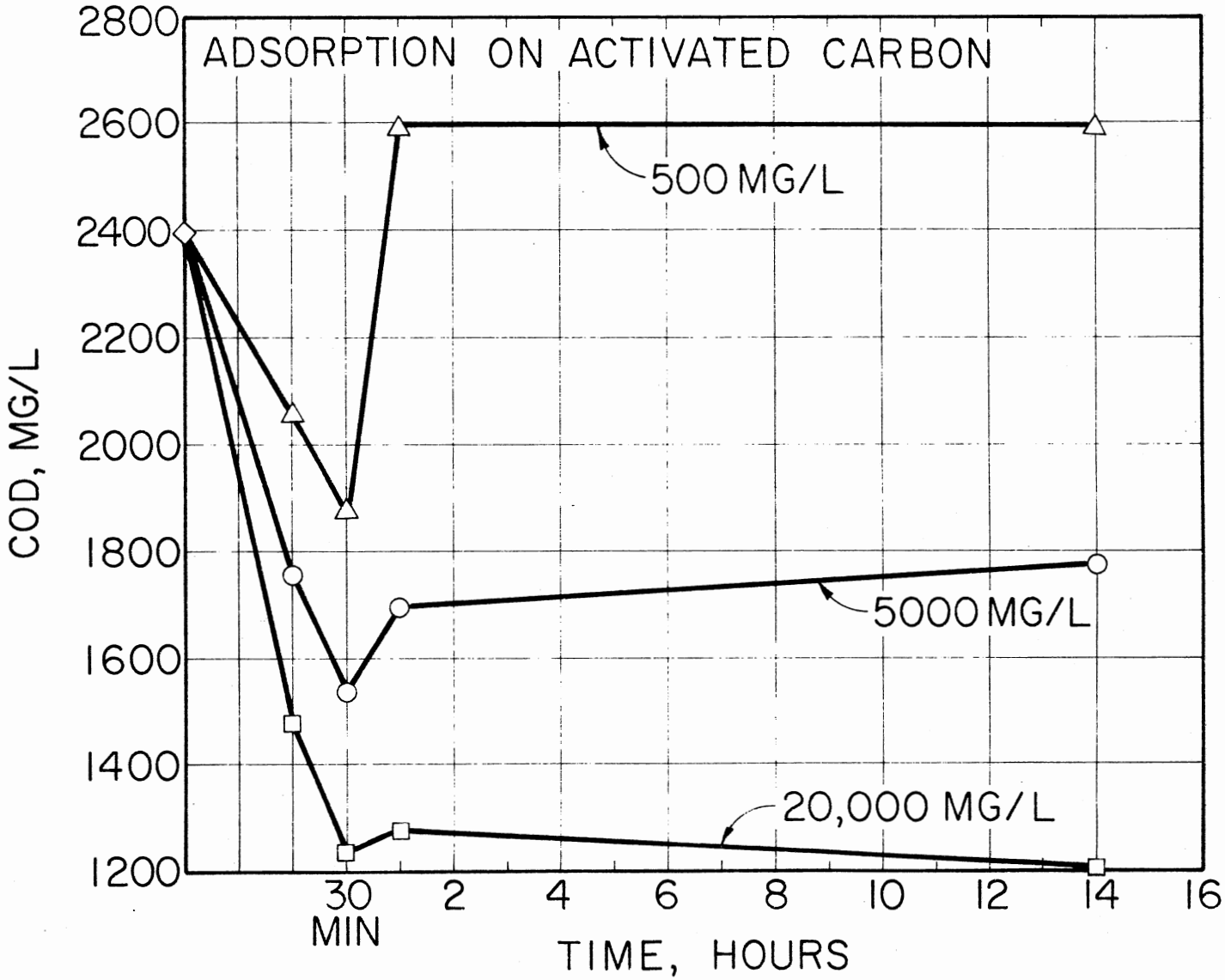

STRIPPING TEST
2-7-78
COD, MG/L
1800
1700
1600
1500
0
5
15
MIN
MIN
1
2
4
6
8
10
12
14
16
18
20
22
24
TIME, HOURS

Figure 11. Adsorption on Activated Carbon of Soluble Residual Organics from Unit I.


ADSORPTION ON ACTIVATED CARBON
500 MG/L
5000 MG/L
20,000 MG/L
COD, MG/L
2800
2600
2400
2200
2000
1800
1600
1400
1200
30 MIN
2
4
6
8
10
12
14
16
TIME, HOURS

minutes after feeding. The results shown in Figure 6 indicate that even at the relatively low concentration of cells, i.e., approximately 500 mg/l, the biomass could after a fairly short period of acclimation remove substrate rapidly. When one considers that the suspended solids concentration at the end of the run in Unit I was approximately 70,000 mg/l it can readily be appreciated that it was capable of removing the glucose which was fed at a very rapid rate. The endogenous oxygen uptake of the sludge after this period of extended feeding was measured by placing a known weight of sludge in a BOD bottle and recording the rate of loss of DO. The endogenous oxygen uptake in Unit I at the end of the experiment was 2.9 mg O_2 per gram of cells per hour. This is a rather low figure when compared to standard and high rate activated sludge systems but the figure compares favorably with the endogenous oxygen uptake of 1.5 mg O_2 per gram of cells per hour registered by Yang and Gaudy for an extended aeration system (15).

The ability of the sludge to sustain its substrate removal capability as well as its ability to remove COD which had formerly been believed to be residual is rather amazing when one considers the distinct disadvantage under which these microorganisms were working. It will be recalled that the total dissolved solids in the units were extremely high and that in Unit I the NH_3-N concentration was measured at 8,700 mg/l on day 436. Thus inorganic constituents such as these might be expected to exhibit inhibitory effects on many microorganisms. The population was derived from a normal sewage seed and it would not be expected that there would be an abundance of organisms which could withstand such a high total dissolved solids concentration. Thus as time went forward during the experiment the organisms had to acclimate

or the population had to adapt to the high dissolved solids.

During the experimentation it was felt that removal of residual COD might be hampered by salt toxicity. Therefore on day 175 a small portion, 200 ml, of mixed liquor was filtered and the filtrate was dialyzed against distilled water using a cellulosic dialysis membrane with average pore diameter of 0.0048 microns (Arther H. Thomas Company, Philadelphia, Pennsylvania). At this time the filtrate COD was 851 mg/l. After dialyzing for 24 hours the COD remaining in the dialysis tube was 532 mg/l. The TOC before dialyzing was 326 mg/l and after dialyzing for 24 hours 212 mg/l remained in the dialysis tube. It is also interesting to note that the color of the filtrate was yellowish before dialyzing and became colorless after dialyzing. The dialysis test was performed essentially for two reasons. First it was of importance to gain some idea of the nature of the chemical oxygen demand in the residual. It is apparent from the dialysis study that a significant portion of the COD consisted of fairly large molecules. Also it would be expected that the 24 hours of dialysis against distilled water would relieve a considerable amount of the high inorganic salt concentration. Thus if high salt concentration were hampering the removal of the residual COD it was thought that subjecting the dialysate to exposure with various seeds of organisms might permit further reduction in the residual COD. Portions of the dialysate were placed in 250 ml Erlenmeyer flasks and seed from sewage, various biological treatment units, soil and local ponds were added to different flasks containing dialysate. The pH of the dialysate was pH 7. No additional nutrients were added. After prolonged shaking there was no discernible growth in any of the shaker flasks. Such preliminary

experiments naturally do not provide concrete evidence that this residual COD was indeed a final residual but they do provide some evidence that this material is rather difficulty metabolized.

At the time of writing this report Unit I has been terminated and contents discarded. Units II and III are currently aerating without feeding. That is, Units II and III are being subjected to aerobic digestion in an effort to see if the biomass will undergo reduction.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this study was to gain more insight into the nature of the residual COD from biological treatment of a carbon source known not to possess a non-biodegradable portion of COD. A batch fed reactor was operated for 613 days with total retention of both solid and liquid portions within the reactor. Two additional reactors were operated in the same manner for shorter periods of time. It is concluded that 100 percent removal of soluble COD is possible as evidenced by the relatively steady COD_e for day 220-613 in Unit I. Although nothing was wasted from the unit there was not a continued buildup of a biologically inert solids COD, and the system never approached a condition of biochemical failure. In fact, substrate removal efficiency remained extremely high, ± 99.8 percent, in all three units and a growth test after extended operation (415 days) indicated the biomass to be in healthy condition, not unlike one would expect of young cells.

CHAPTER VII

FUTURE WORK

The results of all three studies herein made indicated that if a new system was started, the same results would be obtained. Thus one would be able to retrace the course of this examination and expect essentially the same results. However, if one could make more subsidiary studies as the unit aged and could set up chemical analyses to determine the type of compounds, more definitive conclusions as to why the residual material is not nonbiodegradable could be made. This would be an extremely large task. Also the salts concentrations could be held at a much lower level. Even if detailed chemical analysis are not possible an extended bioassay examination could be maintained. In the current studies seed from local ponds and the treatment plant were employed but in future studies seed should be gathered from a wider sampling of natural populations.

BIBLIOGRAPHY

1. Grady, C.P.L., and Williams, D. R., "Effect of Influent Substrate Concentration on the Kinetics of Natural Microbial Populations in Continuous Culture." Water Research, 9, 171-180 (1975).

2. Busch, A. W., and Myrick, N., "Food Population Euilibria in Bench-Scale, Bio-oxidation Units." Sewage and Industrial Wastes, 32, 949-959 (1960).

3. Washington, D. R., and Symons, J. M., "Volatile Sludge Accumulation in Activated Sludge Systems." Journal Water Pollution Control Federation, 34, 767-789 (1962).

4. Busch, A. W., Aerobic Biological Treatment of Waste Waters. Oligodynamics Press, Houston, Texas (1971).

5. Dugan, Patrick R., "Biochemical Aspects of Water Pollution." Biochemical Ecology of Water Pollution, Plenum Press, New York-London (1972).

6. Gaudy, Anthony F. Jr., "Biochemical Oxygen Demand." Water Pollution Microbiology, Chapter 13, 305-332, John Wiley and Sons (1972).

7. Reddy, M. P., "Studies on BOD Exertion in Open Stirred Reactors." M.S. Thesis, Oklahoma State University, Stillwater, Oklahoma (1975).

8. Hiser, L. L., and Busch, A. W., "An 8-Hour Biological Oxygen Demand Test Using Mass Culture Aeration and COD." Journal Water Pollution Control Federation, 36, 505-516 (1964).

9. Thabaraj, G. J., and Gaudy, A. F. Jr., "Effect of Initial Biological Solids Concentrations and Nitrogen Supply on Metabolic Patterns During Substrate Removal and Endogenous Metabolism." Proceedings, 42nd Annual Conference Water Pollution Control Federation, Dallas, Texas, October (1969); Journal Water Pollution Control Federation, 43, 318-334 (1971).

10. Thabaraj, G. J., "Studies on the Release of Metabolic Intermediates and/or Endproducts During Wastewater Purification of Activated Sludge." Ph.D. Thesis, Oklahoma State University, Stillwater, Oklahoma (1969).

11. Kountz, R. R., and Forney, C., Jr., "Metabolic Energy Balances in a Total Oxidation Activated Sludge System." Sewage and Industrial Wastes, 31, 819-826 (1959).

12. Symons, J. M., and McKinney, R. E., "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge." Sewage and Industrial Wastes, 30, 874-890 (1958).

13. Obayashi, Alan W., and Gaudy, A. F. Jr., "Aerobic Digestion of Extracellular Microbial Polysaccharides." Journal Water Pollution Control Federation, 45, 1584-1594 (1973).

14. Ramanathan, M., Gaudy, A. F. Jr., and Rathaidee, W., "Responses of Extended Aeration Activated Sludge to Quantitative Shock Loads." Proceedings, 19th Oklahoma Industrial Waste Conference, Oklahoma State University, Stillwater, Oklahoma (1968).

15. Yang, P. Y., and Gaudy, A. F. Jr., "Control of Biological Solids Concentration in the Extended Aeration Process." Proceedings, 28th Industrial Waste Conference, Purdue University, Lafayette, Indiana, May 1-3 (1973); Journal Water Pollution Control Federation, 46, No. 3, 543-553 (1974).

16. Standard Methods for the Examination of Water and Wastewater, 14th Edition, American Public Health Association, New York (1975).

17. Ecker, R. E., and Lockhart, W. R., "Specific Effect of Limiting Nutrient on Physiological Events During Culture Growth." Journal of Bacteriology, 82, 511-516 (1961).

18. Ramanathan, M., Gaudy, A. F. Jr., and Cook, E. E., "Selected Analytical Methods for Research in Water Pollution Control." Manual M-2, Center for Water Research in Engineering - Bioenvironmental Engineering, Oklahoma State University, Stillwater, Oklahoma (1968).

19. Gaudy, A. F. Jr., and Gaudy, Elizabeth T., "Mixed Microbial Populations." Advances in Biochemical Engineering, Volume II, 97-143. Springer-Verlay, Heidelberg-New York-Tokyo (1973).

20. Bhatla, M. N., and Gaudy, A. F. Jr., "Sequential Substrate Removal in a Dilute System by Heterogeneous Populations." Applied Microbiology, 13, 345-347 (1965).

VITA[2]

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