THE EFFECTS OF NITROBENZENE AND 2-CHLOROPHENOL ON THE PERFORMANCE OF A BATCH-OPERATED BIOLOGICAL SYSTEM

By

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Dedicated to my Parents

John I. and Mary A. Chelus

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CHAPTER I

INTRODUCTION

More than a thousand man-made organic compounds enter the nation's municipal biological waste treatment facilities annually. Some emanate from industrial and manufacturing processes and others from domestic uses, but little is known about the effect of these compounds on biological waste treatment efficiency. Some may be harmlessly degraded, while others may upset the microbial population in the waste treatment process. Still others may be only partially degraded or pass through the treatment plant virtually untouched to pollute the receiving stream.

In October,1972, Congress passed a federal water quality law --Public Law 92-500--which calls for the elimination or discharge of pollutants into navigable waters by 1985. It is therefore of utmost importance that compounds which will upset the operation of treatment facilities and possibly cause harm to receiving streams be either pre-treated or totally banned from discharge into waste streams.

The purpose of this study was to determine the effect that two of these potentially dangerous man-made compounds, 2-chlorophenol and nitrobenzene, would have on biological waste treatment efficiency.

CHAPTER II

LITERATURE REVIEW

As the use and re-use of water in our country continues to increase, it will be imperative that wastewater characteristics are controlled so they will be easily assimilated by our waste treatment facilities. Otherwise, harmful nonbiodegradable chemicals will pollute our lakes, rivers and oceans. These recalcitrant substances . . . which persist for extended periods in all environments tested to date, regardless of whether the chemicals are intrinsically nonbiodegradable or not . . . (8) can cause unforeseen health problems and imbalances in our environment.

It is important to evaluate bacterial toxicity as a pollution risk of chemicals in water since it could cause inhibition of self-purification capabilities of the receiving streams and have a damaging effect on activated sludge which would decrease its efficiency (10). Some industrial waste effluents, when discharged into our waterways may affect wild life, reduce drinking water quality, and cause undesirable accumulations (8).

Pitter (9) has classified organic substances into four general categories based on biodegradability and toxicity:

1) nontoxic and biodegradable

2) toxic and degradable

3) nontoxic and nonbiodegradable (refractory)

4) toxic and nonbiodegradable

He also lists four degrees of degradation as primary, partial, acceptable, and total.

There are several conditions, as reported by Alexander (8) which must be present for a substance to be biologically degraded:

1) an organism capable of degrading the compound must exist
2) the microorganism has to be present in the environment
3) the compound must have the proper molecular structure for the microorganism to break bonds
4) if enzymes involved in the initial degradation are inside the cell, the substrate must penetrate the cell
5) if the enzymes needed are not readily available, they must be produced
6) the enviornment must be able to support the microorganism and its enzyme production.

Alexander (8) also lists fifteen mechanisms of recalcitrance.

Attempts have been made to correlate chemical structure with biodegradability, but no consistent pattern has been found (8, 11, 12). It has been found that slight changes in the chemical structure of many small molecules and some polymers greatly change their biological availability (8). High molecular weight materials and tertiary-branched structures appear to be those which do not permit enzyme approach which causes resistance to degradation (12).

Eckenfelder et al. (13) feel that wastewater characteristics from the chemical industry which affect effluent quality from waste treatment plants are the concentration, total dissolved solids content, and biodegradability. Temperature in the aeration basin also has a

measurable effect on treatability.

Pitter (9) summarizes well the factors affecting biodegradability into three categories:

1) physico-chemical factors, solubility, temperature, degree of mixing of the compound in the medium, dissolved oxygen content, and pH
2) biological factors--age and history of the microbial culture, method and time of acclimation, toxicity of compound, and interferences from other substrates
3) chemical factors--molecular size and length, the number and position of substituents in the molecule and stereochemistry.

Different methods have been employed to measure the biodegradability and toxic effects of organic compounds. Batch studies which are used to monitor substrate removal and biomass characteristics are used most frequently for basic research of organic compounds.

Ludzack and Ettinger (19) feel that batch studies are appropriate when used as a guide for toxic wastes, but a pilot plant similar to the proposed design must be run to obtain reliable information when designing for a specific waste. They also conclude that although analytical problems are greater using a mixed feed, the results are more realistic.

Patterson et al. (15) feel that the BOD and COD tests used when monitoring substrate removal are inadequate for the current research needs in the area of toxic measurements. They feel that use of biochemical concepts would increase the accuracy of research, since toxic effects may vary with the food supply and condition of the biomass. The authors prefer the use of oxygen uptake and measurement of cellular

ATP for toxicity determination.

Broecker and Zahn (10) feel that measurement of the substrate removal rate has an advantage over the measurement of respiration in that the test is simple and produces quick results.

Warburg tests have been used to measure bacterial toxicity. The test is used to give an indication of the performance of the toxic substance in regard to self-purification. It has not yet been determined whether the inhibitory values determined in this manner can be applied to receiving streams or activated sludge performance (10).

Tuffey et al. (16) feel that Warburg respirometry is an acceptable method for the measurement of biological activity for high carbonaceous BOD samples where high degrees of accuracy are not required. Hunter and Heukelekian (17) have summarized the advantages and disadvantages of Warburg respirometry in the determination of biodegradability.

Advantages:

1) It can measure biological oxidation directly, which removes any error caused by physical adsorption.
2) One apparatus can be used on numerous compounds, since oxygen is always used during aerobic biodegradation.
3) It is useful when no analytical method is available for the measurement of an organic compound.
4) Replicate samples can be run for use in correlation of the oxygen utilization curve with other data.

Disadvantages:

1) Pure organic compounds must be used because organic additives found in packaged products will also be subject to

biodegradation, which makes the data difficult to interpret.

2) Nitrification may occur in the latter stages of the experiment, which would give higher results and be misleading.

Hunter and Heukelekian (17) also list factors causing variations in data. They include shaking, temperature, minerals, substrate, and the nature and quantity of microorganisms.

Stripping of organic compounds into the atmosphere is an important parameter to examine whenever testing the biodegradability of a compound. Without stripping tests, wrong conclusions as to the biodegradability of a compound could be made. Thiobodeaux and Millican (18) have found that large portions of industrial wastes were stripped into the atmosphere. Gaudy et al. (27) found that the factors affecting stripping rates include temperature, unit airflow rate, and tank geometry.

In 1955, Heukelekian and Rand (19) compiled a summary report on the BOD values of organic compounds tested to date, and in 1960, Ludzack and Ettinger (12) reported on the biodegradability of organic compounds based on chemical structure. The reports were not very conclusive because the research had been done by many researchers using different methodology in their testing. Although very good research has been done since then, the lack of standard testing procedures still exists.

Heidman et al. (20) studied the effect of sodium pentachlorophenol on activated sludge using a batch reactor. It was found that the sodium pentachlorophenol could be present with sufficient acclimation in concentrations up to 250 mg/l without affecting treatment efficiency, but the pentachlorophenol was not used by the biomass and poor settling characteristics were observed. Also, predominance

changes were noticed and shock loading of small concentrations of pentachlorophenol did affect treatment efficiency. The authors concluded that an unacclimated system could tolerate a sodium pentachlorophenol dosage of 20 mg/l without harming treatment efficiency.

In a study done on chlorophenolic wastes (6) under actual field conditions using wastewater from the manufacture of herbicides mixed with municipal sewage, it was found that the chlorophenolic compounds were decomposed rapidly after a sufficient biomass was developed.

Kirsch and Etzel (21) studied the biodegradation of pentachlorophenol (PCP) using both proliferating and non-roliferating cultures in a batch system. Radioscopic analysis of CO_2 done in Warburg flasks revealed that both proliferating and nonproliferating systems could biodegrade PCP. The rate of radioactive CO_2 liberation was highest in the nonproliferating system where PCP was the only carbon source. It was also found that the PCP removal in the nonproliferating system was proportional to the biomass concentration at low cell concentrations.

Alexander (8) in a report on nonbiodegradable and recalcitrant compounds listed various pesticides, polymers, and other organic compounds resistant to biodegradation.

Stracke and Baumann (22) studied the problems associated with treating biologically a toxic industrial waste containing a variety of organic compounds including phenol and substituted benzene compounds which were upsetting a municipal sewage plant. It was found that the industrial waste could be treated separately using an activated sludge process. The activated sludge process had the advantages over a trickling filter of being able to control the biological solids concentration and contact time.

Pitter (9) ran biological degradability tests on 123 compounds by using a batch system. He expressed the rate of degradation in terms of mg/COD removed by a gram of initial dry matter of the inoculum hr^{-1}. Nineteen of the compounds showed zero or low degradability.

Keneko et al. (23) studied the effects of polychlorinated biphenyl (KC-500) on activated sludge by using batch systems and synthetic waste. He found that PCB was not degraded to any appreciable extent, but low concentrations did not affect COD or BOD removal efficiency. Changes in microflora and adolase activity at concentrations as low as 1 mg/l were noticed, and oxygen uptake was increased by addition of PCB.

Reddy (24) used a continuous flow activated sludge pilot plant to study the effects of phenol. He found that COD removal was not appreciably affected at sludge age values in the range of 4.15 to 26.23 days, and good treatment efficiency was obtained for the phenolic waste.

Tabak and Barth (25) in their study of benzidene using an extended aeration pilot plant found that benzidine could be oxidized by the system in continuous concentrations of 1 mg/l, but at higher doses, less complete oxidation occurred and a buildup of intermediates followed. They concluded that the operation of the pilot plant was not affected by the presence of benzidine, but the increase in effluent COD was caused by undegraded benzidine.

Broecker and Zahn (10) studied the effects of 3,5-dichlorophenol on a continuous flow system. Bacterial toxicity limits were found by five different methods. All agreed with the limit of 5 mg/l DCP. The degradation was impaired only at a 25 mg/l concentration. Shock loading exhibited only a minor effect, and it was concluded that an effect on treatment efficiency due to toxic shock loading is less frequent

than feared, so long as the interval between shock loads does not exceed two to three days. It was also found that the consumption rate of sludge suspended in water was inhibited less by DCP than sludge suspended in organically loaded wastewater.

Nitrobenzene

Nitrobenzene is a nitrated aromatic hydrocarbon; the chemical formula is $C_6H_5NO_2$. It has a molecular weight of 123.11, and a density of 1.2037. It is slightly soluble in water (7). Nitrobenzene is produced in tremendous quantities, and 97 percent of it is used as the starting point in the production of aniline which is used in dyes. Nitrobenzene is produced by reacting benzene with nitric acid. The reaction is as follows (1):

$$C_6H_6 + HONO_2 \xrightarrow{H_2SO_4} C_6H_5NO_2 + H_2O$$

benzene — nitric acid — nitrobenzene

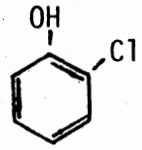
Heukelekian and Rand (19) in a report prepared in 1955 listed nitrobenzene as nonbiodegradable with a BOD of zero. Pitter (9) in 1976 reported that nitrobenzene was highly degradable, with a 98 percent removal rate.

2-Chlorophenol

2-chlorophenol or orthochlorophenol is a chlorinated aromatic hydrocarbon. The chemical formula is ClC_6H_4OH. It has a molecular weight of 128.56, and a density of 1.2410. It is soluble in water (7). The compound is used mainly as an intermediate in organic synthesis, but it can be used as an antiseptic (5). 2-chlorophenol is also a residual byproduct in the manufacture of herbicides, and can also be formed during chlorination of water if phenol is present (4, 6). The compound is manufactured by bubbling chlorine gas through phenol at 50^oC. The chlorine then replaces the hydrogen in the 2 or ortho position. The reaction is as follows (6):

$$C_6H_5OH + Cl_2 \xrightarrow{50^oC} 2ClC_6H_4OH$$

phenol chlorine 2-chlorophenol



Federal government standards for public water supplies limit phenolic compounds in terms of phenol to .001 ppm (3, 4). Phenols can affect the taste of domestic water supplies and also cause disease (3).

In research studies, microorganisms have been able to acclimate to phenolic wastes containing several thousand mg/l of phenol under controlled conditions (26). Phenolic concentrations from 50 to 500

mg/l are generally considered suitable for treatment by biological processes. The concentration that can be treated depends to an extent on other contaminants in the wastewater (2). In studies done on 2-chlorophenol, high rates of biological removal have been found (6, 9, 12).

CHAPTER III

MATERIALS AND METHODS

Experimental Apparatus

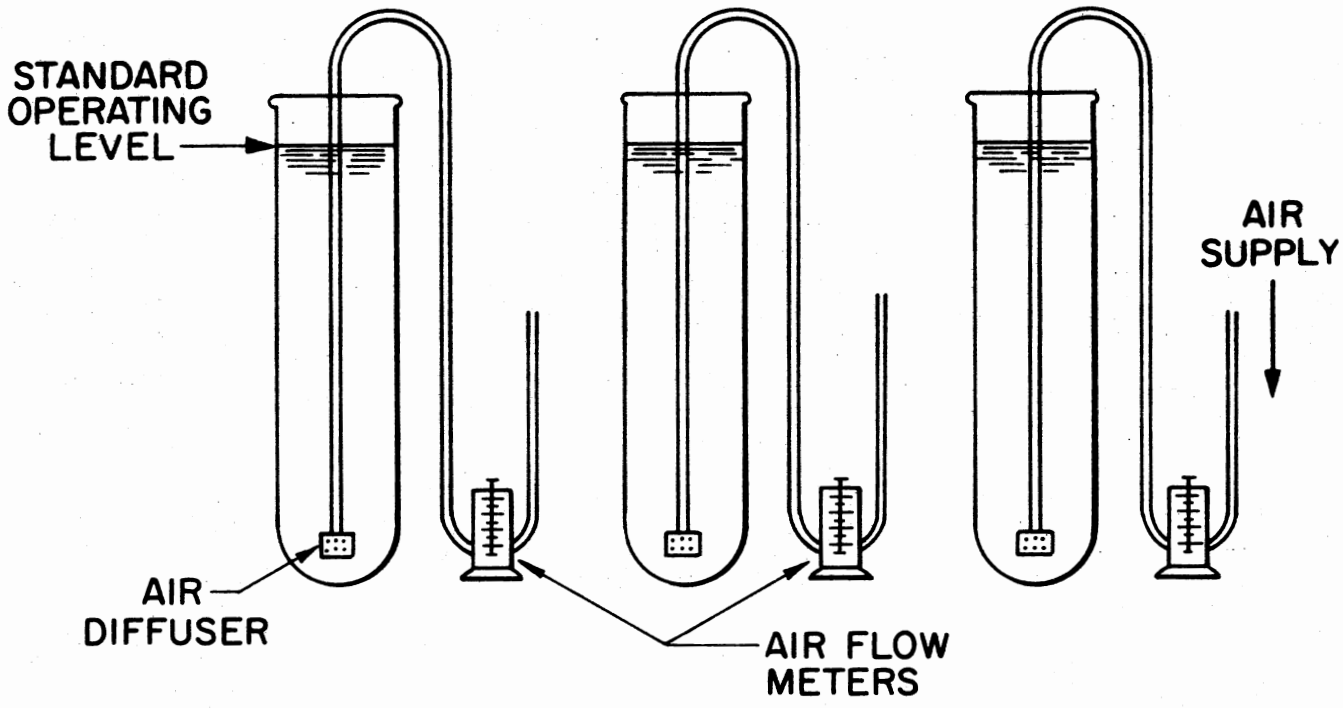
To study the effects of nitrobenzene and 2-chlorophenol on biological systems, three bench scale batch reactors were used. The experimental apparatus used in these studies is shown in Figure 1. Three 3 3/4-inch diameter glass tube reactors were used; each had a reaction liquor volume of 3 liters. One was used as a control, receiving the same feed as the toxic units minus the toxics. Aeration to each reactor was provided by a single carborundum diffuser which was set at an airflow rate of 2 liters/hr $\pm$ 0.5. This flow rate was sufficient to supply adequate mixing and also to keep the unit aerobic. The rate was kept constant by use of an airflow meter. The oxygen concentration was measured periodically; during the study it remained above 6 mg/l. The temperature in the reactors was not controlled. The temperature ranged from 16^{o} to 24^{o}C, i.e., 20 ± 4^{o}C.

Feed Preparation

The daily feed for the batch units consisted of fresh primary effluent sewage from the Stillwater municipal sewage treatment plant. Since the carbon or energy source in the sewage was very low with the total COD of the sewage averaging 137 mg/l, soluble COD averaging

Figure 1. Schematic Diagram of Laboratory Scale Batch Reactors

Unit 1 - Control
Unit 2 - Nitrobenzene
Unit 3 - 2-Chlorophenol



BATCH REACTORS

74 mg/l, total BOD averaging 39 mg/l, and soluble BOD averaging 21 mg/l, the feed was supplemented with glucose. The glucose concentration added to the sewage was designed to increase the chemical oxygen demand by 200 mg/l. The only other nutrient which was deficient in the sewage was nitrogen; therefore 75 mg/l ammonium sulfate $(NH_4)_2SO_4$ was added to the feed. Nitrobenzene and 2-chlorophenol were added to the feed of the toxic units at fixed concentrations of 5, 25, and 50 mg/l in increasing concentrations, as will be described later. Table I is a summary of the constituents of the feed.

TABLE I

COMPOSITION OF THE FEED

Constituents	Control	Nitrobenzene	2-Chlorophenol
Sewage (Avg. total COD)	137 mg/l	137 mg/l	137 mg/l
Glucose	200 mg/l	200 mg/l	200 mg/l
$(NH_4)_2SO_4$	75 mg/l	75 mg/l	75 mg/l
Nitrobenzene		5, 25, 50 mg/l	
2-Chlorophenol			5, 25, 50 mg/l

Daily Feeding Procedure

The three batch reactors were fed on a daily basis. First, the side walls of the reactors above and below the water line were scraped down to prevent solids buildup. Next, one liter of the mixed liquor

was wasted from each reactor. Then the air diffusers were removed and the remaining two liters were allowed to settle for one hour. After settling for one hour, the second liter of supernatant was wasted from each reactor. The glucose, ammonium sulfate and toxics were then pipetted into each unit from stock solutions, and the units were filled back to the 3-liter mark with sewage.

Sampling

Before and after feeding, samples of 25 ml were taken from all three reactors every other day up to day 112 for the determination of total suspended solids and soluble COD concentrations. From day 112 to the end of the experiment, samples were taken every day. The total suspended solids concentrations were determined using the membrane filter technique (Millipore Filter Company, Bedford, Mass., HA 0.45 μm).

The chemical oxygen demand (COD) test used for the determination of soluble organics and the total suspended solids test followed the procedures set forth in Standard Methods for the Examination of Water and Wastewater (29). pH measurements were also taken at the time of sampling before and after feeding, using an Orion Research Model 701 digital analyser. The dissolved oxygen was measured periodically, using a Weston & Stack dissolved oxygen analyser, Model 330.

Other tests run at periodic intervals were total COD before feeding, soluble TOC before feeding, total COD of supernatant after one hour of settling, and total suspended solids concentrations of the supernatant after one hour of settling. The results of these tests will be discussed later. Before changing toxic concentrations during the experiment, one-liter samples of the mixed liquor, supernatant, and

feed were taken for chemical analysis of the toxic compounds.

24-Hour Batch Studies

Whenever the toxic concentrations in the batch reactors was changed, the units were monitored for 24 hours to observe their performance with respect to substrate removal, biological cell growth, and respiration. Soluble COD and total suspended solids samples were taken at random intervals during the 24-hour period. Oxygen uptake was also monitored, using a GME Lardy Model RWB3 Warburg apparatus.

For the oxygen uptake experiments, 40-ml samples of each batch reactor after feeding were placed in special Warburg flasks. In the center vial of each flask, one ml of 20 percent KOH solution was added. The flasks were connected to the manometers and placed in the water bath of the Warburg apparatus, which was set at $25^{o}C$. Blanks with 40 ml distilled water were also run to adjust for barometric pressure changes.

Stripping Tests

Stripping tests were run on both compounds to determine their strippability. Concentrations designed to have 250, 500, and 1000 mg/l COD values were added to three-liter batch reactors of the same design as the units used for the batch experiments. Tap water was used instead of sewage. The experiments were run for 21 hours, and COD samples were taken at regular intervals throughout the test.

Warburg Experiment

A 26-day Warburg experiment was run to determine the effects of

2-chlorophenol and nitrobenzene on the respiration of unacclimated sludge. A 15-liter batch reactor was run for three weeks to produce a mixed liquor in a steady state. After feeding the batch unit in the same manner as the three small batch reactors were fed, 1-liter samples were inoculated with 5, 25, and 50 mg/l toxics. These were shaken and 40-ml samples were placed in Warburg flasks along with 1 ml of 20 percent KOH in the center vial. The water bath was set at 25^{o}C. A control and blank were also prepared. Readings were taken at short intervals for the first 24 hours and then at longer intervals as the respiration decreased.

CHAPTER IV

RESULTS

Daily Batch Unit Data

The metabolic performance of the control reactor, Unit I, the nitrobenzene reactor, Unit II, and the 2-chlorophenol reactor, Unit III during the 178 days of operation are shown in Figures 2, 3, 4, and 5. Shown in these figures are the MLSS concentrations before and after feeding, the soluble COD before and after feeding, and the toxic concentrations fed to Units II and III.

From day 1 through day 12, no toxics were fed. On day 13, 5 mg/l toxic was added to the two liters of waste fed to Units II and III. This was continued to day 42, when the toxic concentrations were increased to 25 mg/l. On day 81, the toxic concentrations were increased to 50 mg/l, and on day 113, shock loading experiments were started. Toxics were fed in alternating concentrations of 50 and 25 mg/l. On day 127, the concentrations of toxics fed on alternating days were changed to 25 mg/l and 0 mg/l. To see if increasing the time between shock loading of toxics would have any effect, starting on day 137, two days were allowed between feeding of toxics to Units II and III. This time period was increased to three days on day 142 and continued until the end of the experiment on day 158.

On different occasions during the testing, problems were encountered

Figure 2. Performance of Unit I vs the Performance of Unit II From Day 1 to Day 79

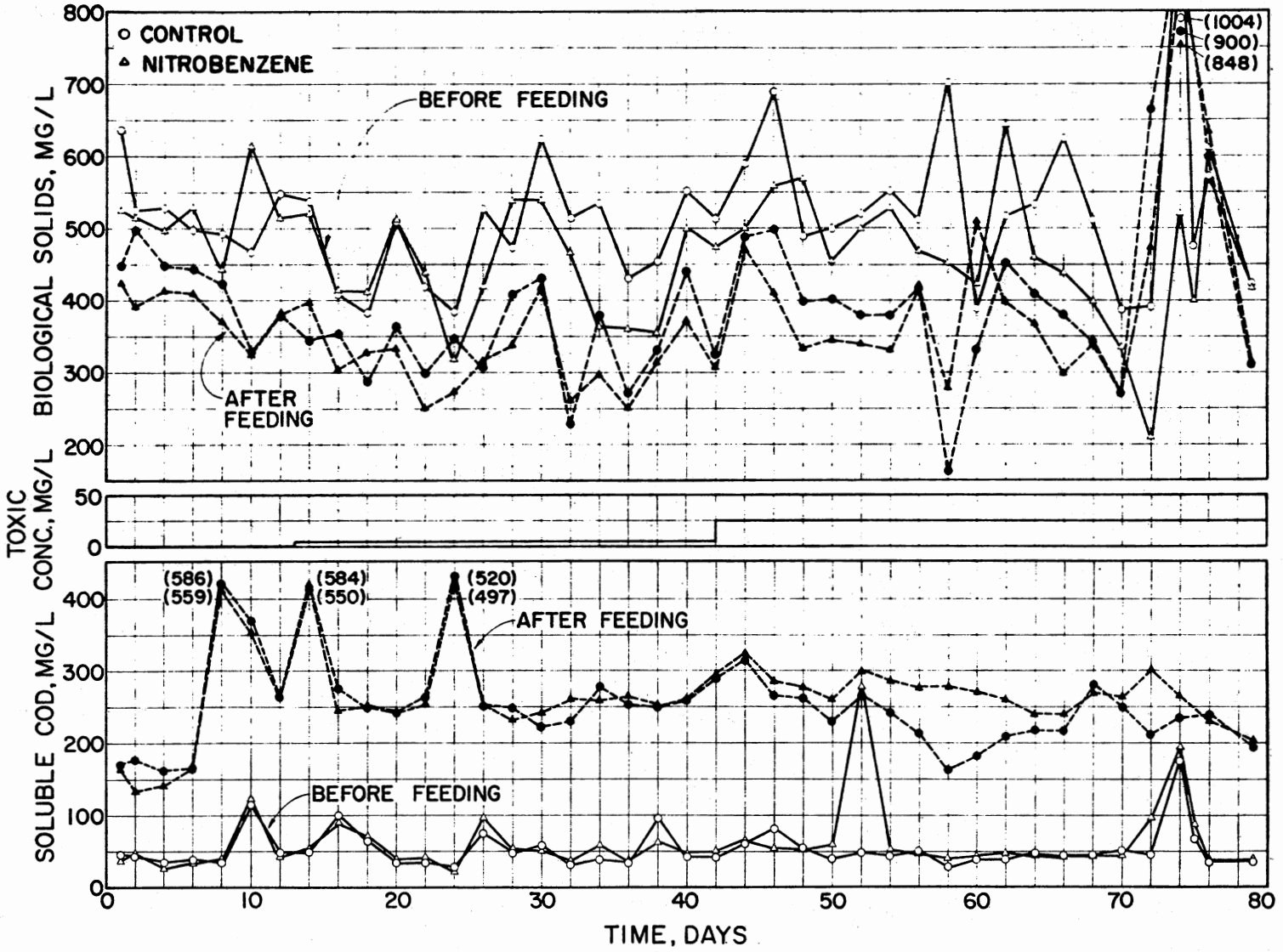

CONTROL
NITROBENZENE
BEFORE FEEDING
AFTER FEEDING
(1004)
(900)
(848)
BIOLOGICAL SOLIDS, MG/L
TOXIC CONC., MG/L
SOLUBLE COD, MG/L
(586)
(559)
(584)
(550)
(520)
(497)
AFTER FEEDING
BEFORE FEEDING
TIME, DAYS

Figure 3. Performance of Unit I vs the Performance of Unit II From Day 79 to Day 158

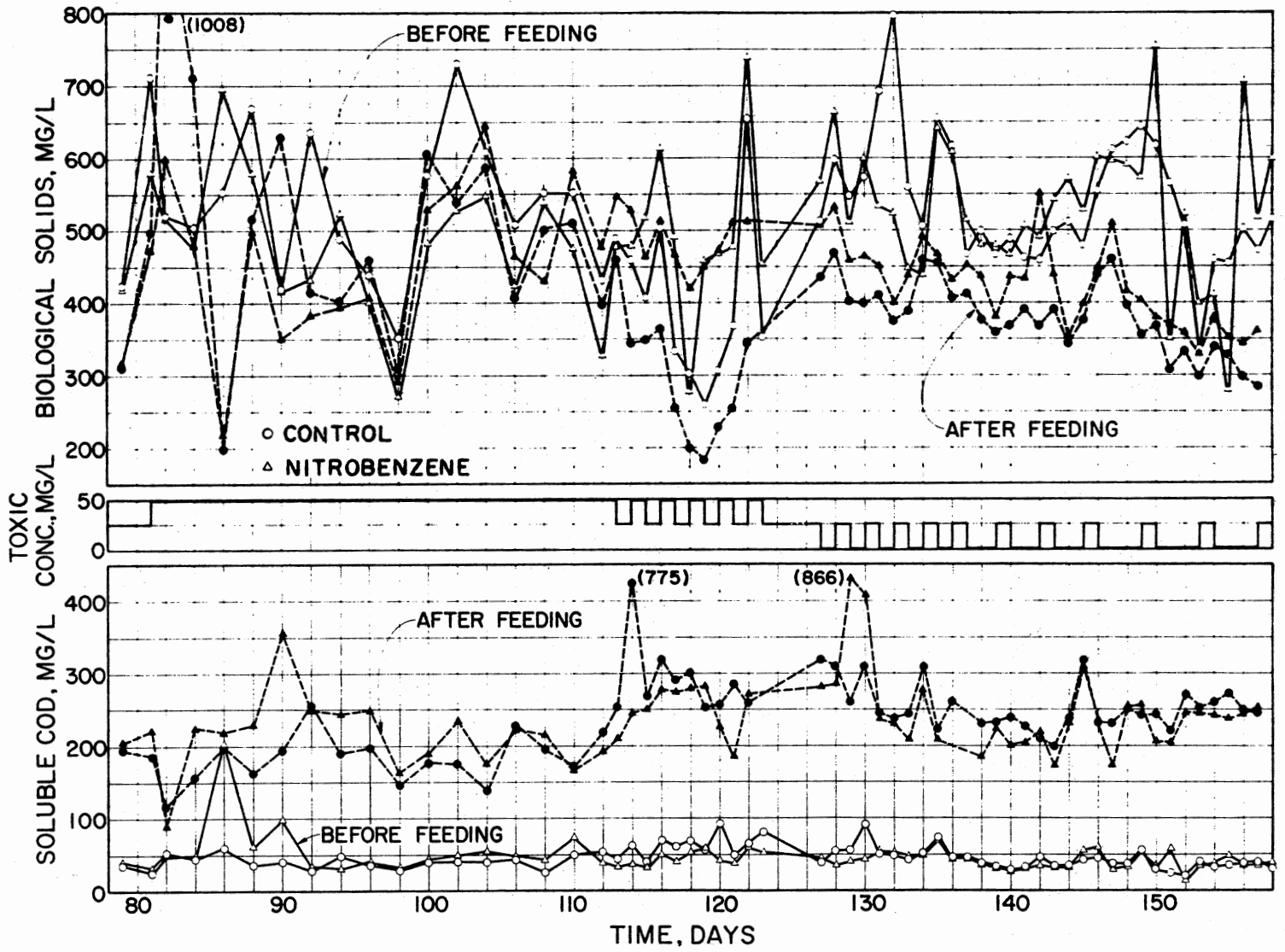

BIOLOGICAL SOLIDS, MG/L
800
700
600
500
400
300
200
(1008)
BEFORE FEEDING
AFTER FEEDING
○ CONTROL
△ NITROBENZENE
TOXIC CONC., MG/L
50
0
SOLUBLE COD, MG/L
400
300
200
100
0
(775)
(866)
AFTER FEEDING
BEFORE FEEDING
80
90
100
110
120
130
140
150
TIME, DAYS

Figure 4. Performance of Unit I vs the Performance of Unit III From Day 1 to Day 79

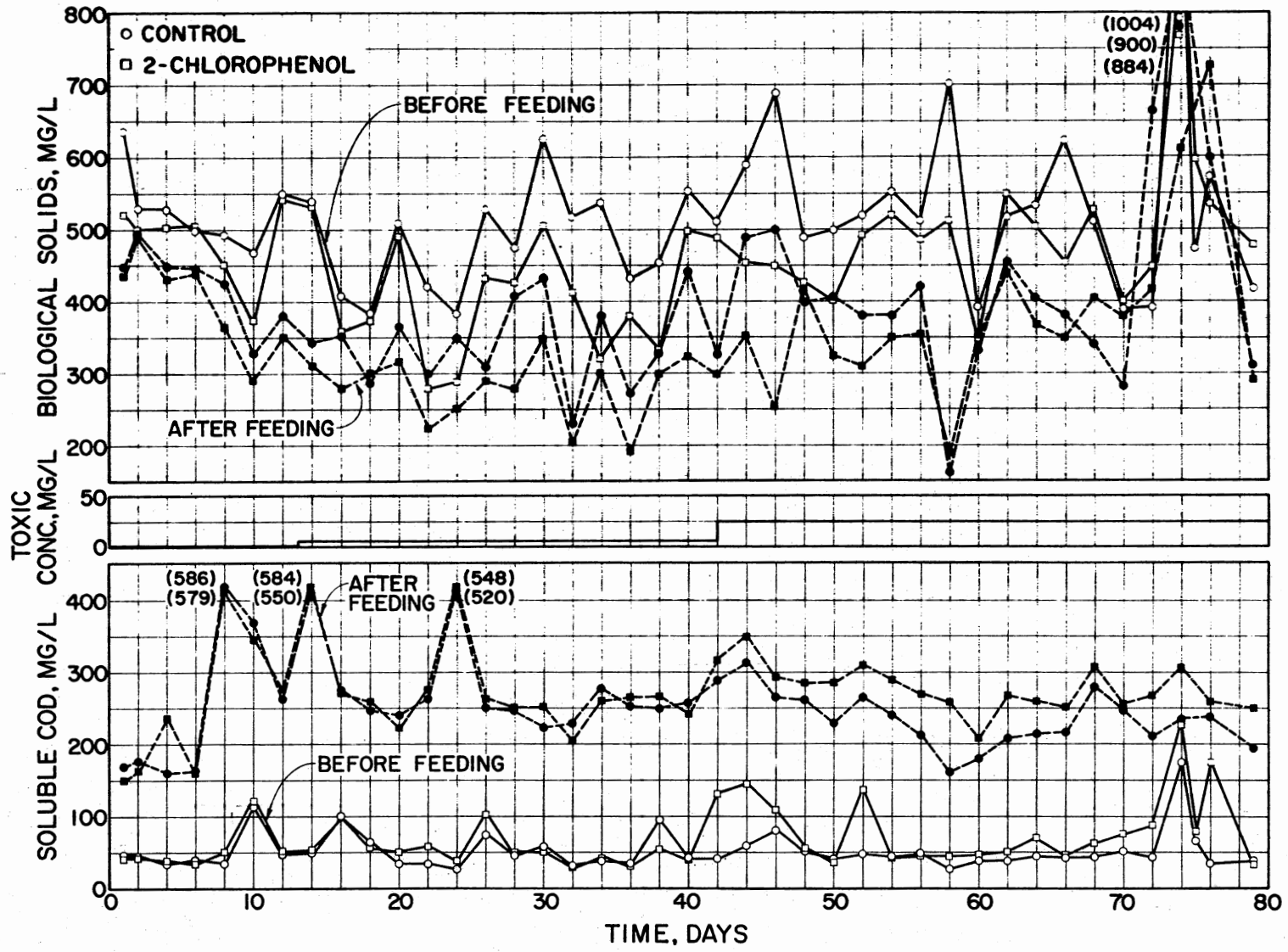
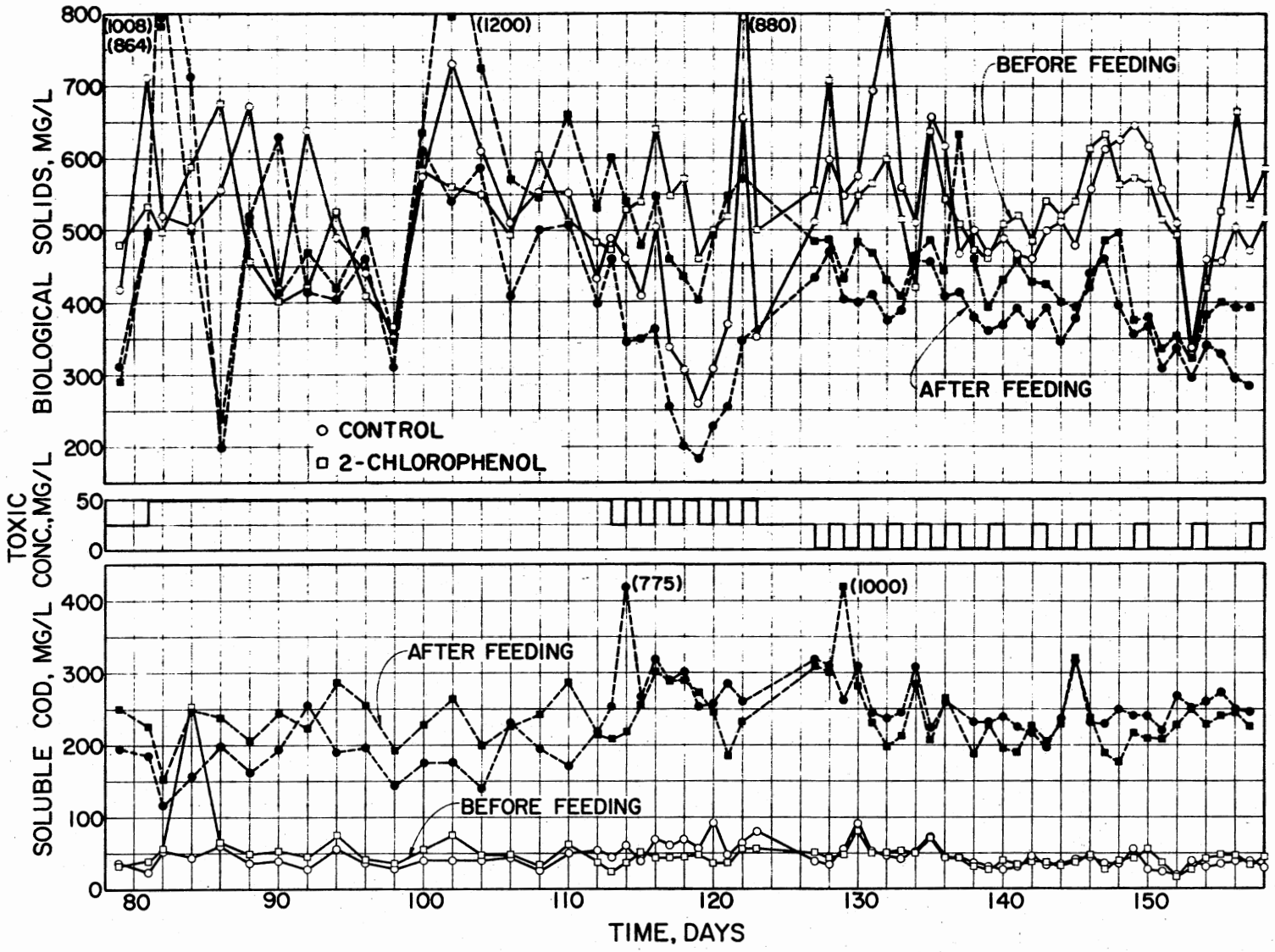

CONTROL
2-CHLOROPHENOL
BEFORE FEEDING
AFTER FEEDING
(1004)
(900)
(884)
BIOLOGICAL SOLIDS, MG/L
TOXIC CONC., MG/L
SOLUBLE COD, MG/L
(586)
(579)
(584)
(550)
AFTER FEEDING
(548)
(520)
BEFORE FEEDING
TIME, DAYS

Figure 5. Performance of Unit I vs the Performance of Unit III From Day 79 to Day 158


BIOLOGICAL SOLIDS, MG/L
800
700
600
500
400
300
200
(1008)
(864)
(1200)
(880)
BEFORE FEEDING
AFTER FEEDING
○ CONTROL
□ 2-CHLOROPHENOL
TOXIC
50
0
SOLUBLE COD, MG/L CONC., MG/L
400
300
200
100
0
(775)
(1000)
AFTER FEEDING
BEFORE FEEDING
80
90
100
110
120
130
140
150
TIME, DAYS

with the sewage used for feed. On these days, foaming accompanied by very high organic loading would upset the balance in the reactors. Sometimes the solids in the reactors would foam out over the top. Foam suppressant was used whenever necessary to help alleviate this problem. The erratic data on days 8, 14, 24, 74, and 84 were definitely caused by the foamy sewage fed to the units. The high after feed solids concentration of the 2-chlorophenol unit on day 102, and the high before feed COD values for both toxic units on day 52 were probably due to experimental error.

On day 100, the control, Unit I, started to change color from a light brown to almost white. At the same time, settling was not as efficient in the unit. On day 114, Unit I after feed COD was 775 mg/l and after feed solids dropped considerably. This may have been caused by cell lysing. The control solids continued to drop, and reached a low on day 119. From that point on, the unit began to recover.

On day 127, the stock solution used for nitrogen supplementation was found to be contaminated.

The nitrobenzene added to Unit II did not cause any noticeable changes to the unit so far as COD and solids concentration are concerned.

The 2-chlorophenol unit, upon addition of 5 mg/l of toxic on day 13, became a darker brown color and within a few days returned to normal. When the 2-chlorophenol concentration was increased to 25 mg/l on day 42 and for approximately one week, the before feed COD values were higher than normal. The brown color again disappeared and the COD values returned to normal. No significant color change or change in COD values was noted when the 2-chlorophenol concentration was increased

to 50 mg/l on day 81 or during the shock loading phase of the experiment.

Table II shows a simple statistical analysis of the daily COD and solids data and also daily pH values. It is interesting to note how closely the three units performed. Both the control unit and the nitrobenzene unit had a before feed COD average value of 46 mg/l and the 2-chlorophenol unit was almost identical with a value of 44 mg/l. This would indicate that the substrate removal in all three units was the same. The after feed COD values were also very similar. The control had an average value of 237 mg/l; the nitrobenzene unit had a value of 221 mg/l, and the 2-chlorophenol unit averaged 214 mg/l. This would indicate that all three units were fed a homogeneous mixture of sewage.

The before feed suspended solids concentrations in the control, nitrobenzene unit, and the 2-chlorophenol unit were 518, 487, and 504 mg/l, respectively, and the after feed suspended solids values were 404, 416, and 429 mg/l. This would indicate that the biomass in all three units was growing at approximately the same rate.

The pH values shown in Table II are very interesting. The after feed or zero hour pH values in all three units was 7.4, but the before feed or 24-hour pH values were all higher. The control increased to 7.8, the nitrobenzene unit increased slightly more--to 8.2--and the 2-chlorophenol unit increased most--to 8.4. This may indicate the formation of different intermediates by the microbial populations and a variation in predominating species.

Table III shows settleability data taken at various times during the experiment. Total COD samples were taken just before wasting from the units. Total COD and settled suspended solids samples of the second

TABLE II

STATISTICAL ANALYSIS OF DAILY BATCH UNIT DATA

	Unit I Control		Unit II Nitrobenzene		Unit III 2-Chlorophenol	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Soluble COD (mg/l) Before feeding	46	±23	46	±27	44	±35
Soluble COD (mg/l) After feeding	237	±87	221	±108	214	±123
Suspended solids (mg/l) Before feeding	518	±109	487	±115	504	±98
Suspended solids (mg/l) After feeding	404	±129	416	±98	429	±141
pH before feeding (24-hr residual)	7.8	±.21	8.2	±.33	8.4	±.25
pH after feeding	7.4	±.21	7.4	±.21	7.4	±.80

TABLE III

SETTLEABILITY DATA

Day	Unit	Total COD Before Settling	Total COD Supernatant After Settling	Suspended Solids After Settling	Soluble COD
42	I	592	46	16	42
	2nd Control	653	-	8	55
	II	568	46	26	51
	III	531	55	46	131
81	I	669	48	17	24
	2nd Control	625	40	-	36
	II	677	56	17	32
	III	669	48	6	40
112	I	660	42	10	54
	2nd Control	620	38	-	46
	II	659	50	12	38
	III	730	78	17	38
113	I	631	86	60	45
	2nd Control	636	53	28	45
	II	578	74	48	33
	III	603	70	48	25
128	I	769	51	8	55
	II	801	39	20	35
	III	737	43	16	43

liter were taken one hour after settling.

Because the control, Unit I, was upset at one period of the study, the data from another researcher's control is also included in Table III. The researcher's control was operated in exactly the same way as the author's control, since both researchers worked together during wasting and feeding. When using both controls as a comparison with the toxic units, it can be seen that the suspended solids and COD values of the supernatant are generally lower for the control, which would indicate poor settling in the toxic units.

Microbial examinations of the control and toxic units were made at different intervals during the 158 days of testing. It was noted that in general there was not much difference although at times it appeared that the toxic units exhibited bacteria of slightly smaller size and in smaller floc particles, and the protozoa at times were fewer in number. No filamentous growth problems were ever encountered.

24-Hour Batch Studies

Along with the daily COD and solids monitoring of the batch reactor, 24-hour studies were run whenever the toxic concentrations were increased and also at the beginning and end of each shock load experiment. Samples for soluble COD and suspended solids were taken at regular intervals over a 24-hour period. Oxygen uptake was also monitored during the 24-hour period, using a Warburg respirometer.

The results of these batch studies are shown in Figures 6 through 19. It can be seen that in Figures 6 through 12, where the control is plotted with the nitrobenzene unit, that in all cases the substrate removal occurred within the first three hours except at the 5 mg/l

Figure 6. Performance of Unit I vs Performance of Unit III During 24-hour Batch Study on Day 13 First Day Unit II was fed 5 mg/l Nitrobenzene

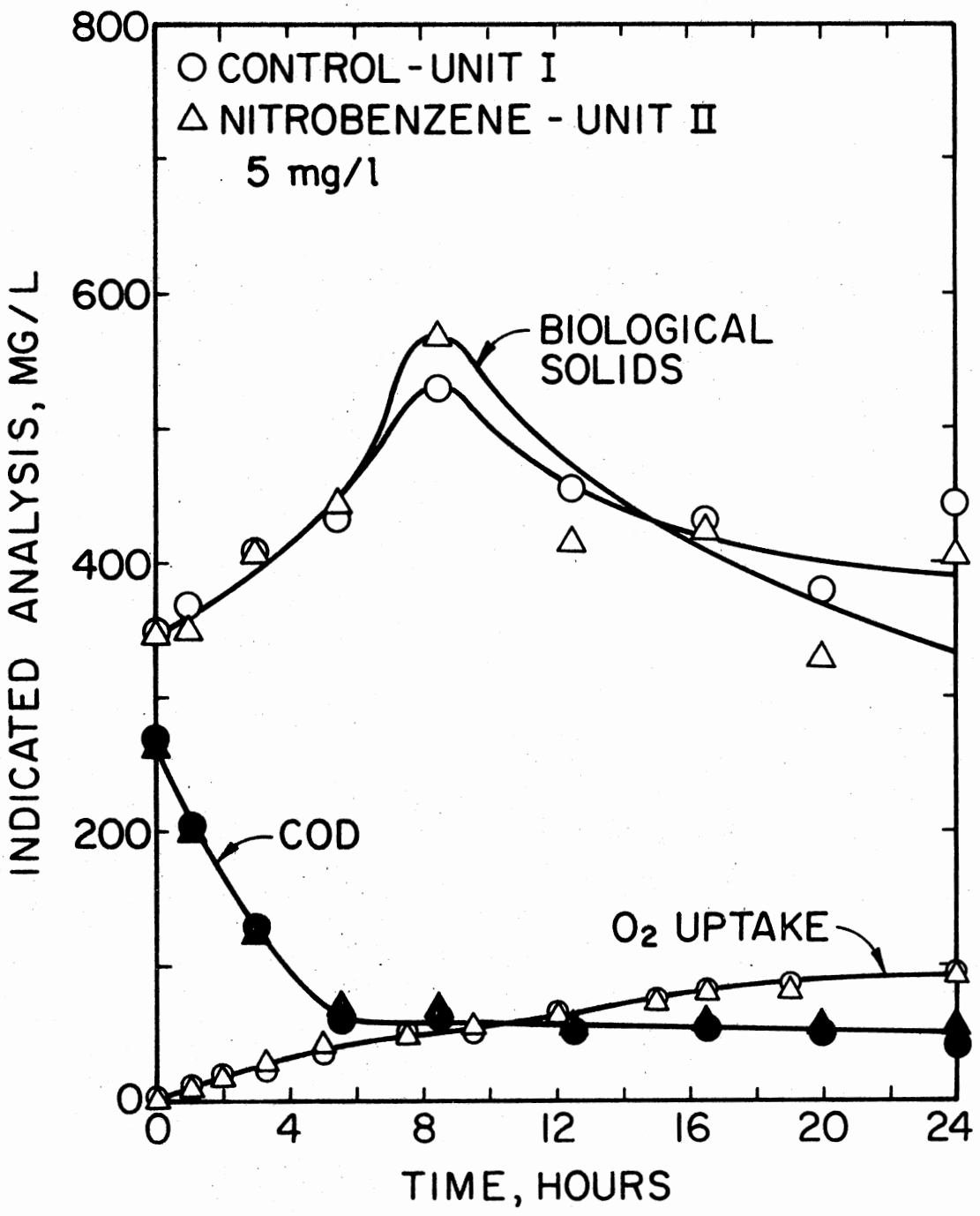

800
600
400
200
0
INDICATED ANALYSIS, MG/L
O CONTROL - UNIT I
Δ NITROBENZENE - UNIT II
5 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
0
4
8
12
16
20
24
TIME, HOURS

Figure 7. Performance of Unit I vs Unit II During 24-hour Batch Study on Day 42 First Day Unit II was fed 25 mg/l Nitrobenzene

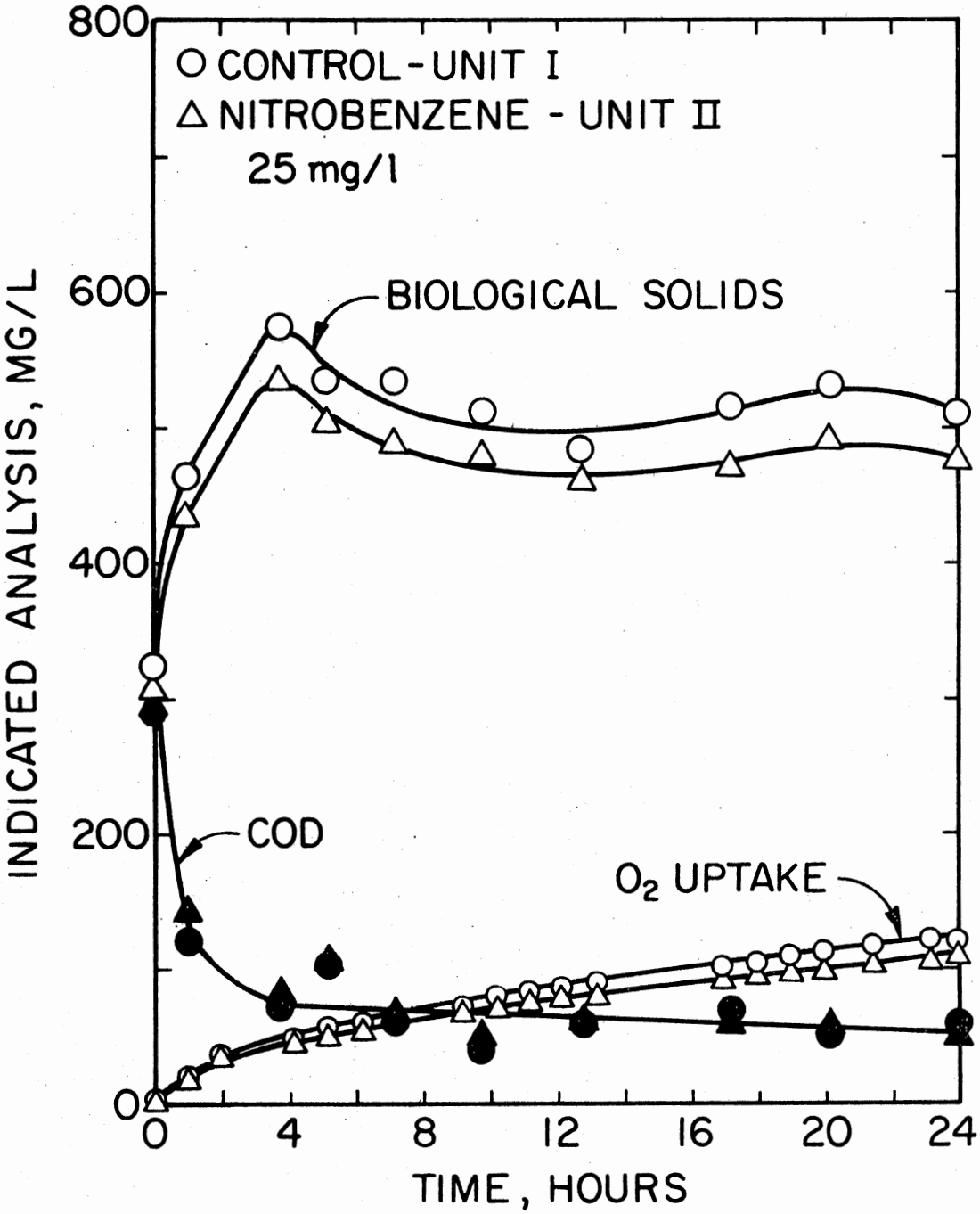

800
600
400
200
0
INDICATED ANALYSIS, MG/L
CONTROL - UNIT I
NITROBENZENE - UNIT II
25 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
0
4
8
12
16
20
24
TIME, HOURS

Figure 8. Performance of Unit I vs Unit II During 24-hour Batch Study on Day 81 First Day Unit II was fed 50 mg/l Nitrobenzene

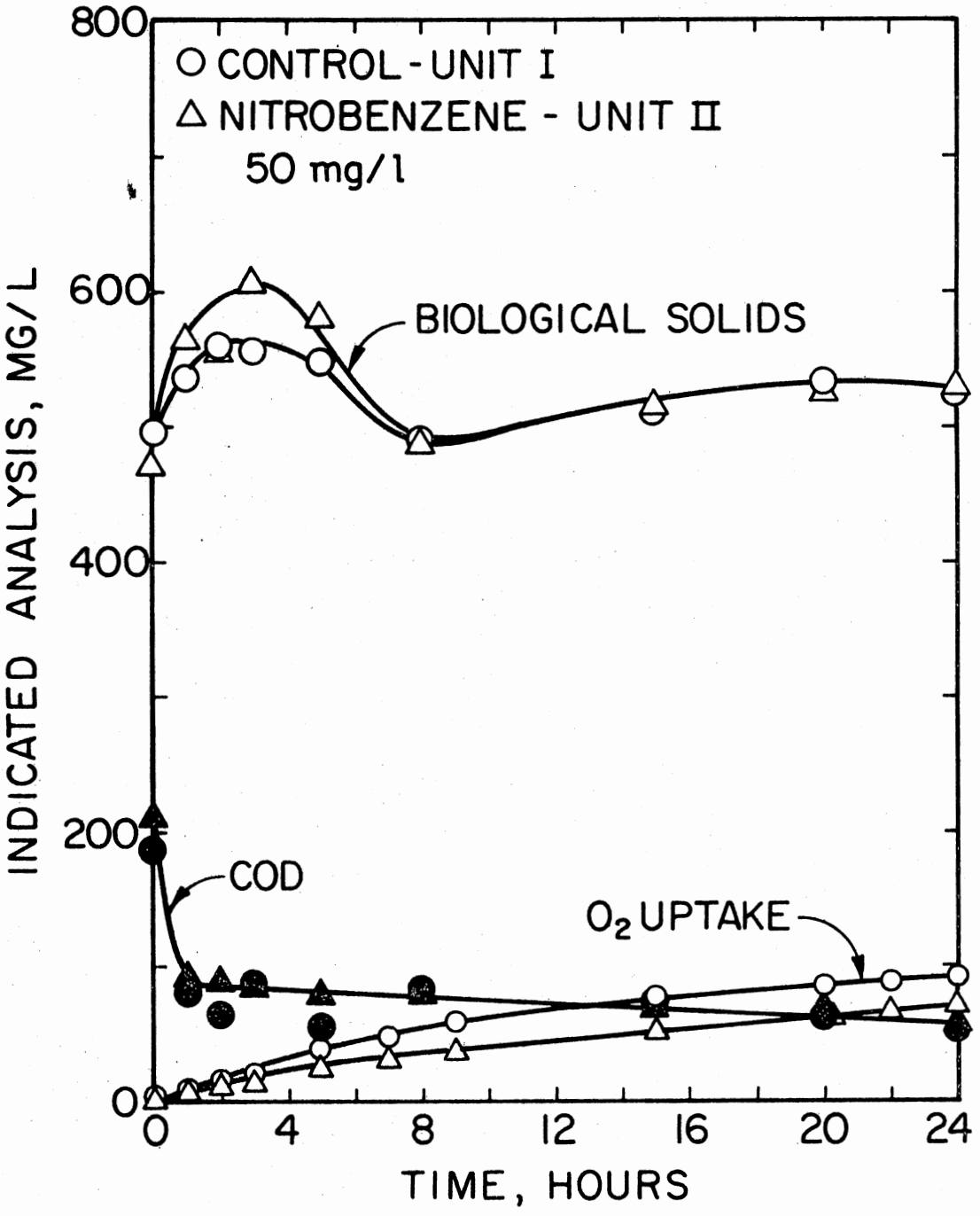

800
600
400
200
0
INDICATED ANALYSIS, MG/L
CONTROL - UNIT I
NITROBENZENE - UNIT II
50 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
0
4
8
12
16
20
24
TIME, HOURS

Figure 9. Performance of Unit I vs Unit II During 24-hour Batch Study on Day 114 at Beginning of Daily Switching of Nitrobenzene Dosage From 50 to 25 mg/l. Nitrobenzene Concentration - 50 mg/l

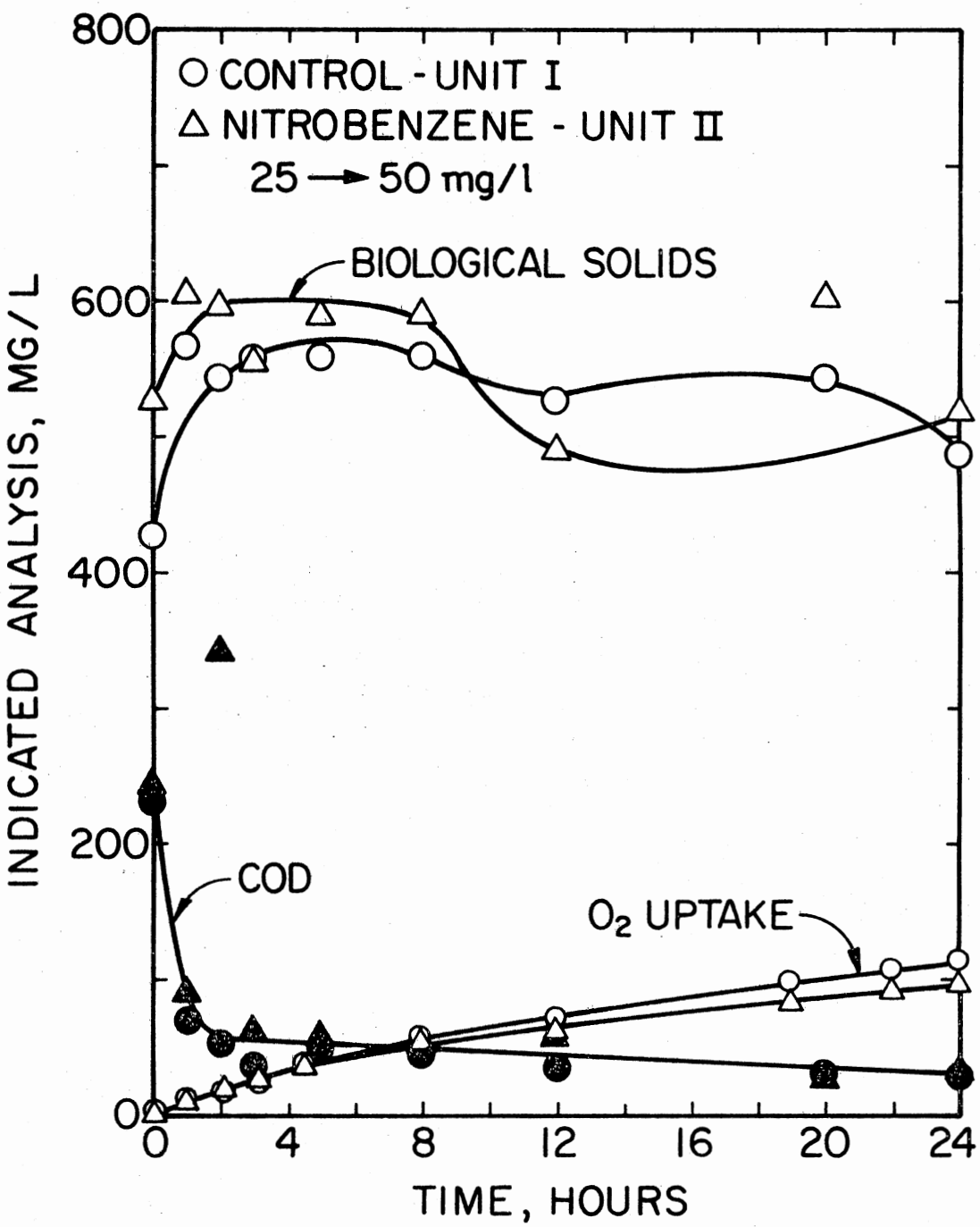

800
600
400
200
0
INDICATED ANALYSIS, MG/L
CONTROL - UNIT I
NITROBENZENE - UNIT II
25 → 50 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
0
4
8
12
16
20
24
TIME, HOURS

Figure 10. Performance of Unit I vs Unit II During 24-hour Batch Study on Day 122 at End of Daily Switching of Nitrobenzene Dosage From 50 to 25 mg/l. Nitrobenzene Concentration - 50 mg/l

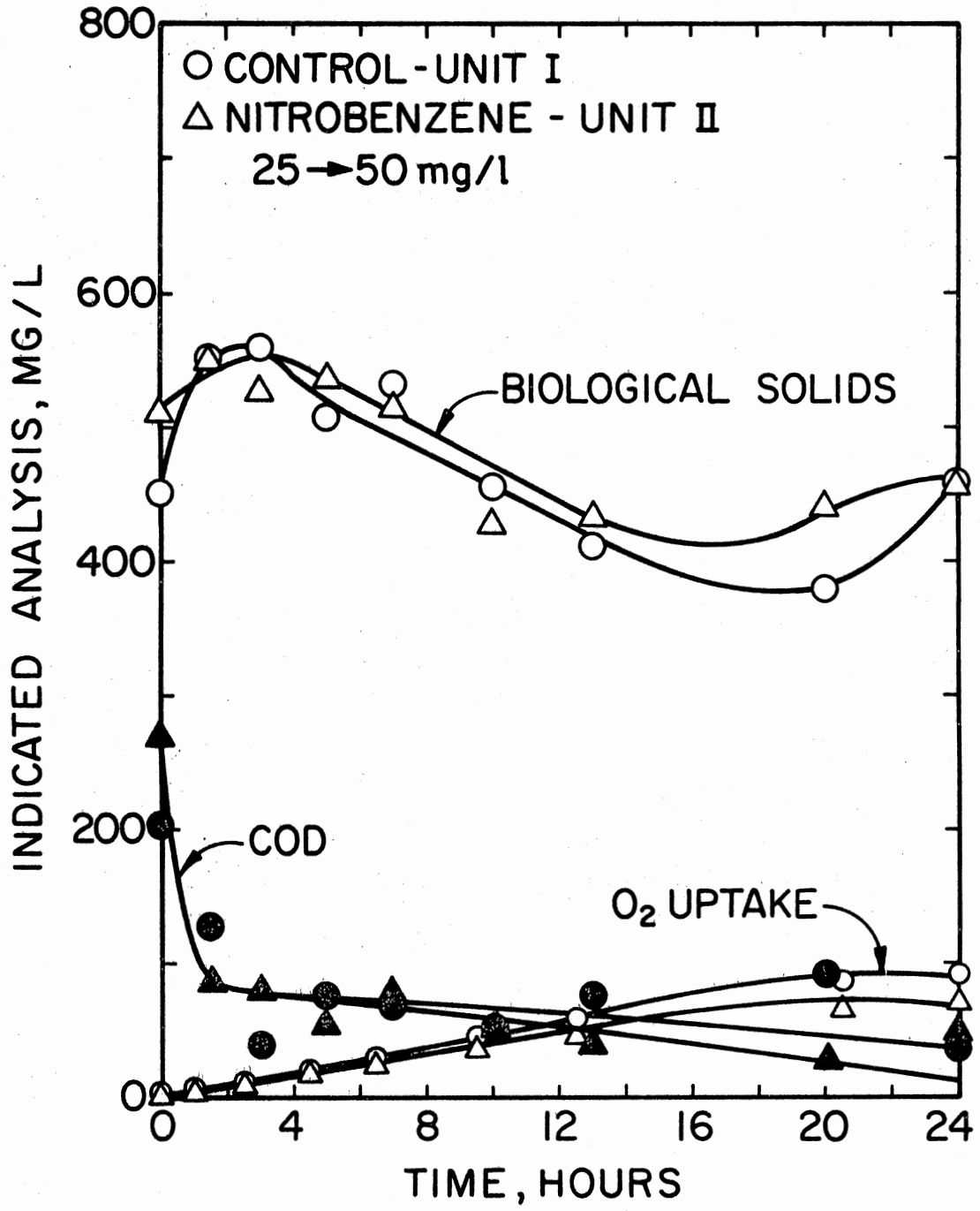

800
600
400
200
0
CONTROL - UNIT I
NITROBENZENE - UNIT II
25 → 50 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
INDICATED ANALYSIS, MG/L
0
4
8
12
16
20
24
TIME, HOURS

Figure 11. Performance of Unit I vs Unit II During 24-hour Batch Study on Day 128 at Beginning of Daily Switching of Nitrobenzene Dosage From 25 to 0 mg/l. Nitrobenzene Concentration - 25 mg/l

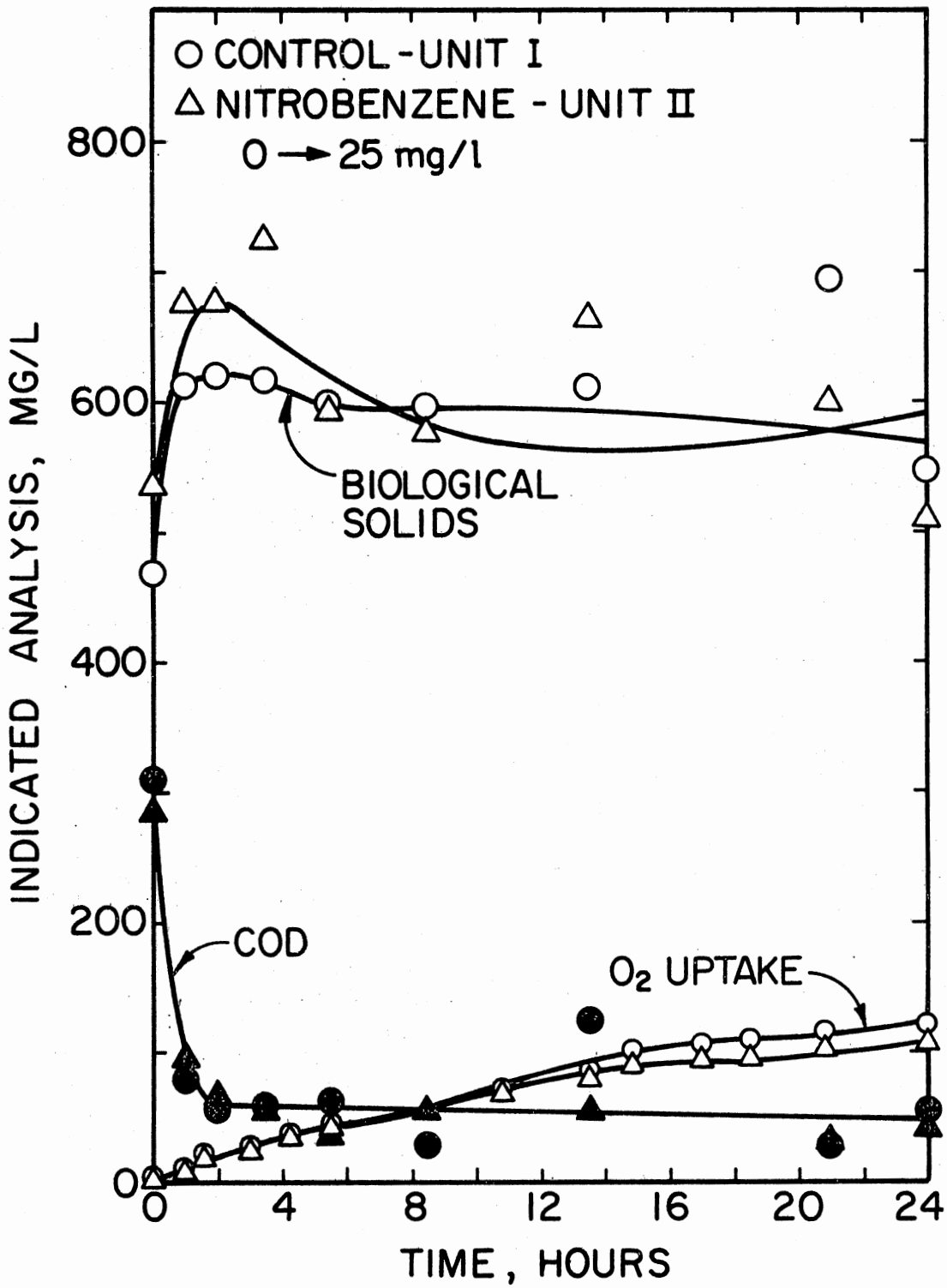
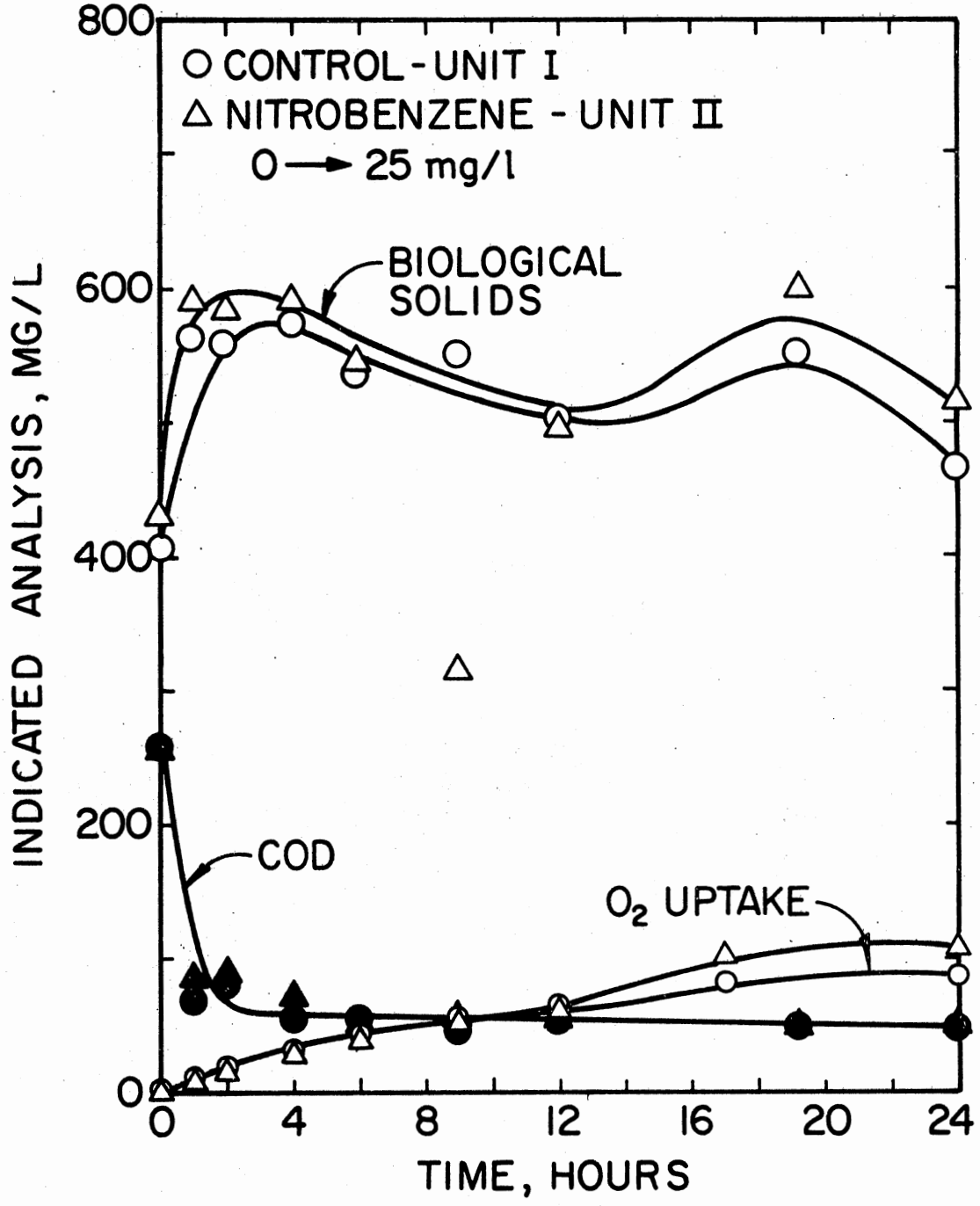

CONTROL - UNIT I
NITROBENZENE - UNIT II
0 → 25 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
INDICATED ANALYSIS, MG/L
TIME, HOURS
0
200
400
600
800
0
4
8
12
16
20
24

Figure 12. Performance of Unit I vs Unit II During 24-hour Batch Study on Day 136 at End of Daily Switching of Nitrobenzene Dosage From 25 to 0 mg/l. Nitrobenzene Concentration - 25 mg/l


800
600
400
200
0
INDICATED ANALYSIS, MG/L
CONTROL - UNIT I
NITROBENZENE - UNIT II
0 → 25 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
0
4
8
12
16
20
24
TIME, HOURS

concentration on day 13 (Figure 6), where it took approximately six hours. The substrate was removed equally well in both the control and nitrobenzene units in all of the tests, which would indicate that removal efficiency was not affected by the presence of nitrobenzene. Even though the substrate removal was slower on day 13, it can be seen that this occurred in both the control and the nitrobenzene units, ruling out any effect from nitrobenzene. It may be that a predominance change was occurring in the batch units at this time. Also note that an increase in solids occurs right at the end of the substrate removal phase, or shortly thereafter, in all tests.

There was a slight inhibition of respiration or oxygen uptake in all of the nitrobenzene experiments except at the 5 mg/l concentration (Figure 6). It can be seen that in all other cases there appear to be two phases to the oxygen uptake. In the first phase, the respiration rate is identical in the control and the nitrobenzene units, and in the second phase, the nitrobenzene respiration rate is slower. This may indicate the formation of different intermediates by the microorganisms.

The 2-chlorophenol data vs. the control from the batch studies are plotted in Figures 13 through 19. It can be seen that the substrate removal was affected slightly at the 5 mg/l concentration and at the beginning and end of switching from 25 to 50 mg/l (Figures 14, 16, 17). Respiration was inhibited at all concentrations in the 2-chlorophenol unit, even at the 5 mg/l concentration (Figure 13). The oxygen uptake occurred in two phases, just as it did with the nitrobenzene unit.

Table IV shows materials balances for the 24-hour batch studies at the point of initial substrate removal and at the end of 24 hours. Unit I is the control, Unit II is the nitrobenzene unit, and Unit III

Figure 13. Performance of Unit I vs Performance of Unit III During 24-hour Batch Study on Day 13, First Day Unit III was fed 5 mg/l 2-Chlorophenol

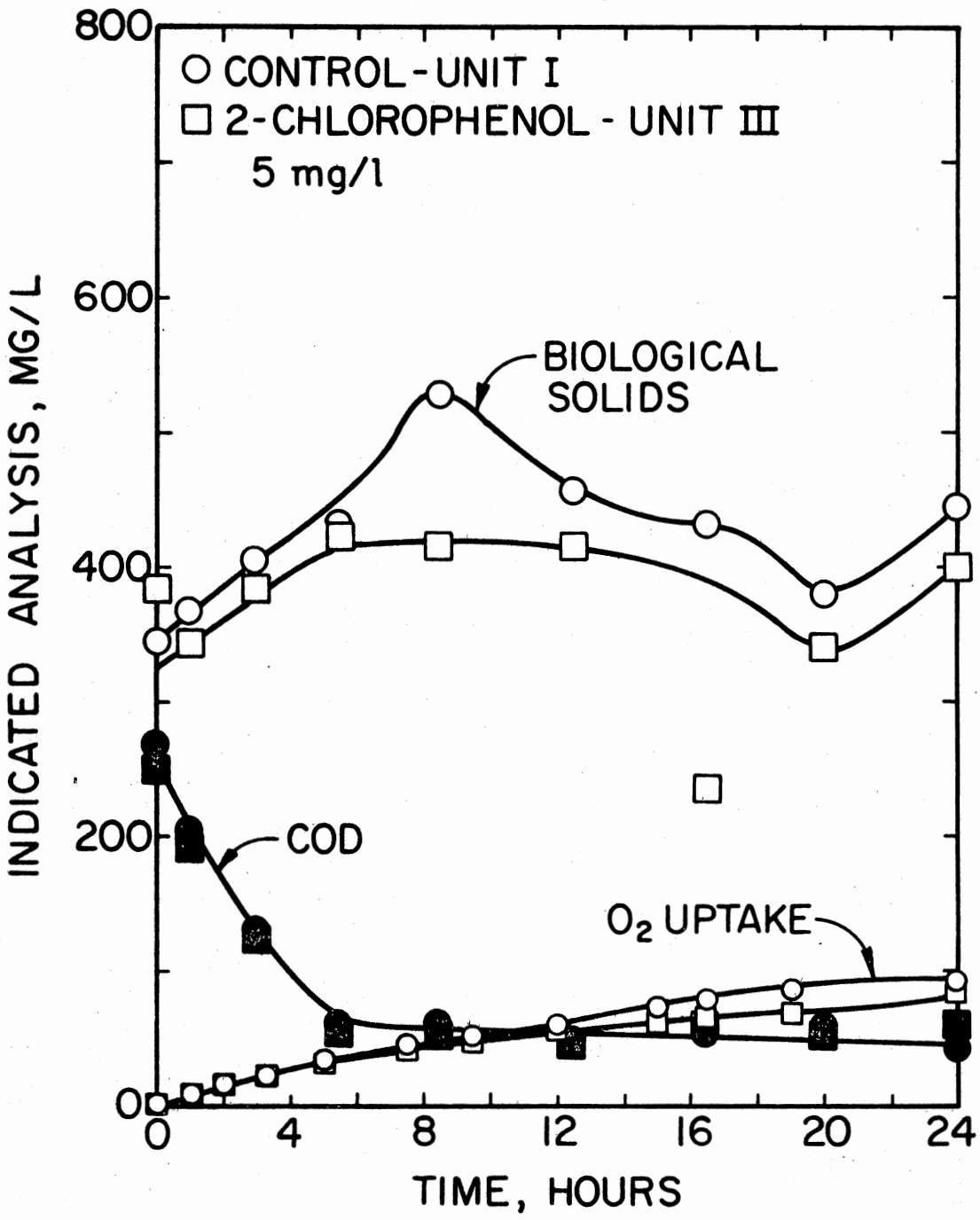

800
600
400
200
0
INDICATED ANALYSIS, MG/L
O CONTROL - UNIT I
□ 2-CHLOROPHENOL - UNIT III
5 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
0
4
8
12
16
20
24
TIME, HOURS

Figure 14. Performance of Unit I vs Performance of Unit III During 24-hour Batch Study on Day 42, First Day Unit III was fed 25 mg/l 2-Chlorophenol

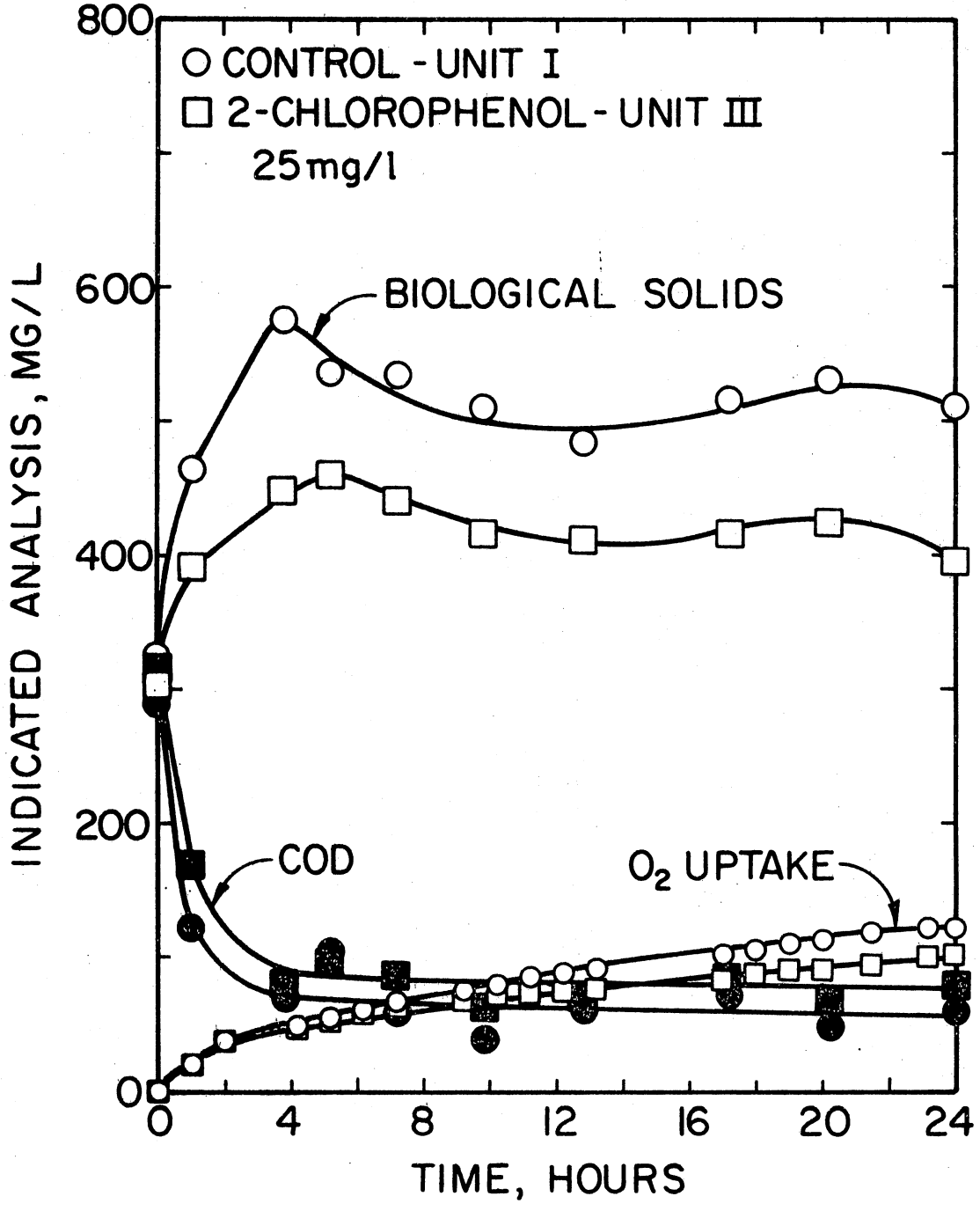

800
600
400
200
0
○ CONTROL - UNIT I
□ 2-CHLOROPHENOL - UNIT III
25 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
INDICATED ANALYSIS, MG/L
0
4
8
12
16
20
24
TIME, HOURS

Figure 15. Performance of Unit I vs Performance of Unit III During 24-hour Batch Study on Day 81, First Day Unit III was fed 50 mg/l 2-Chlorophenol

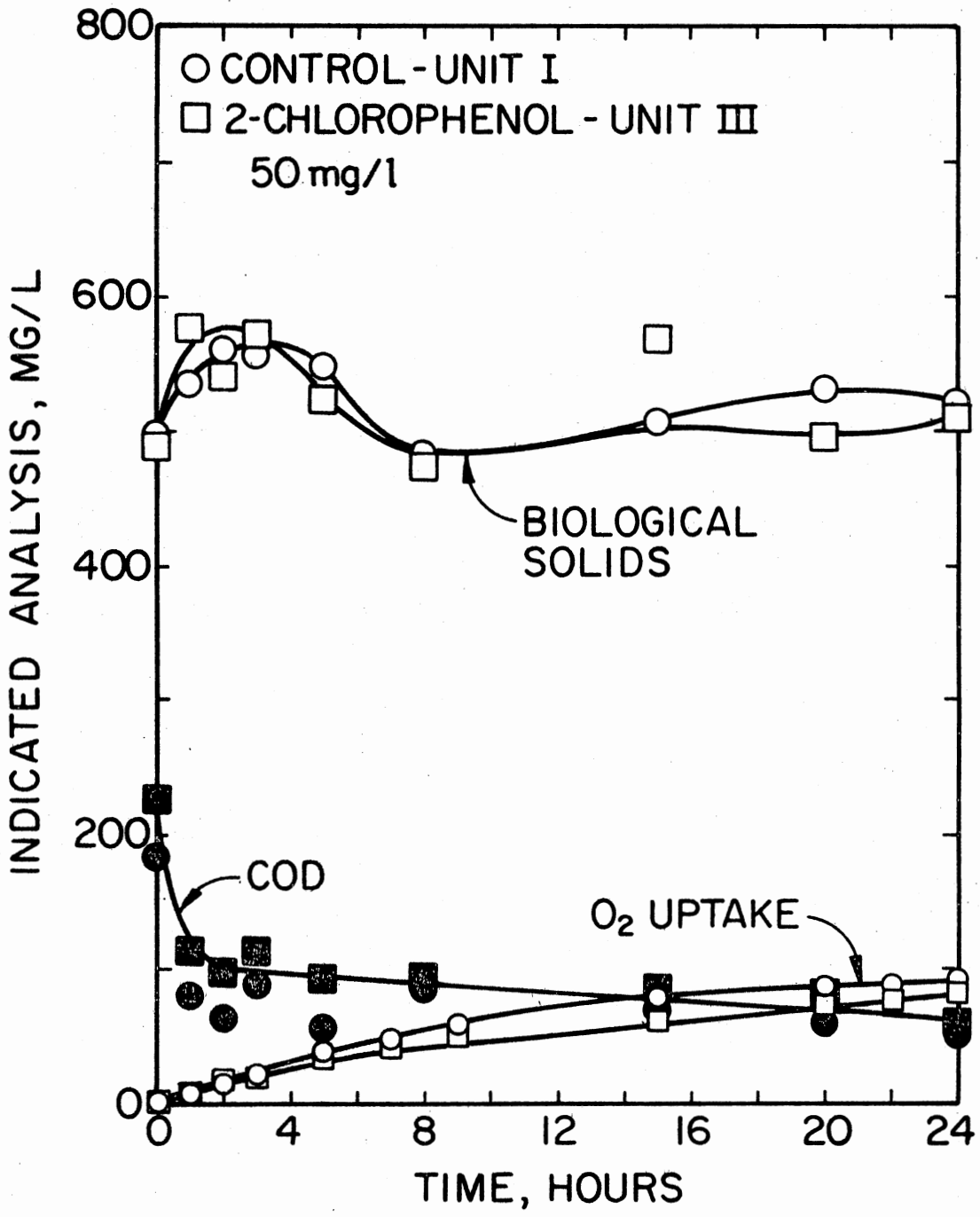

800
600
400
200
0
INDICATED ANALYSIS, MG/L
CONTROL - UNIT I
2-CHLOROPHENOL - UNIT III
50 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
0
4
8
12
16
20
24
TIME, HOURS

Figure 16. Performance of Unit I vs Performance of Unit III During 24-hour Batch Study on Day 114 at Beginning of Daily Switching of 2-Chlorophenol Dosage From 50 mg/l to 25 mg/l. 2-Chlorophenol Concentration 50 mg/l

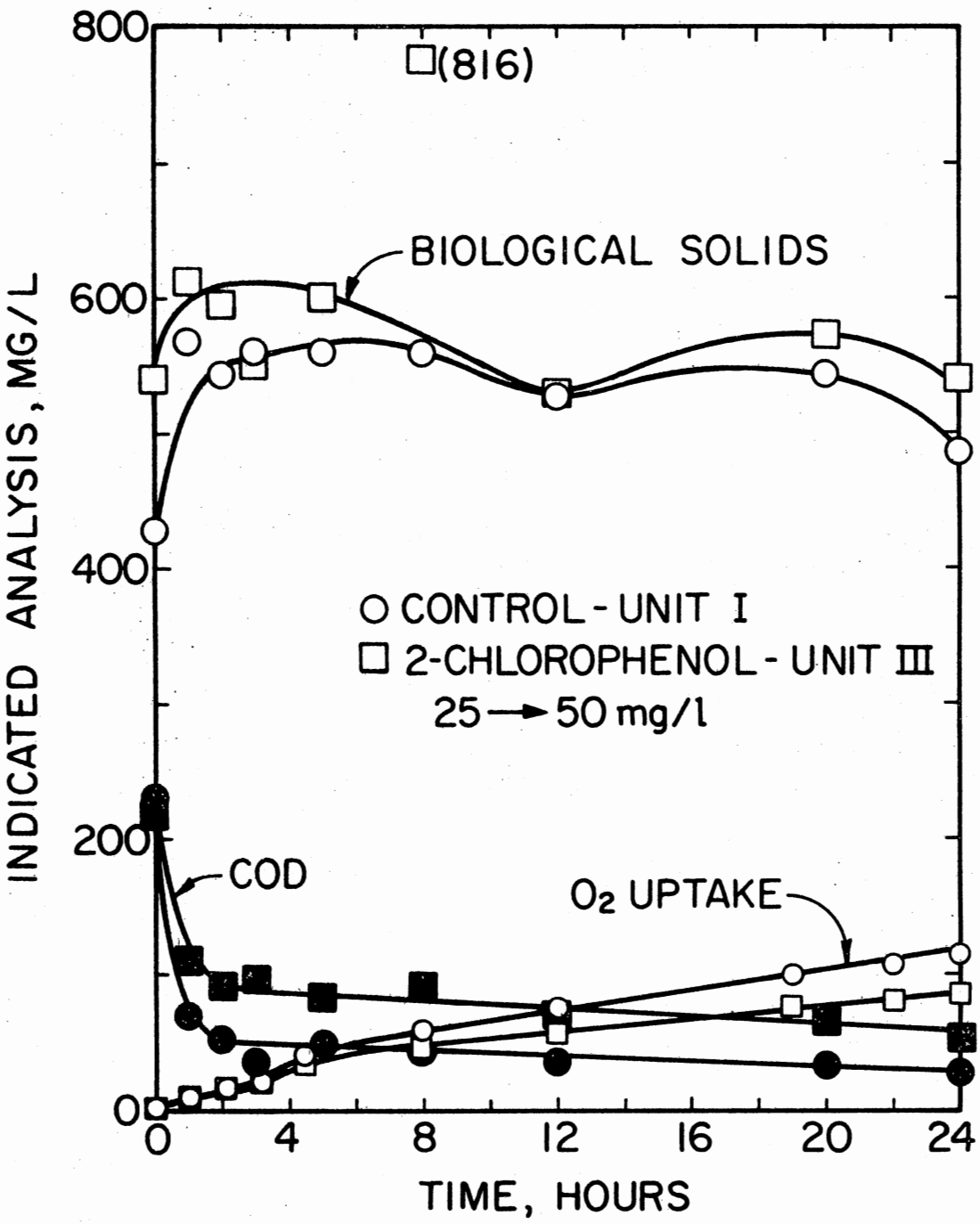

800
600
400
200
0
□(816)
BIOLOGICAL SOLIDS
O CONTROL - UNIT I
□ 2-CHLOROPHENOL - UNIT III
25 → 50 mg/l
COD
O2 UPTAKE
INDICATED ANALYSIS, MG/L
0
4
8
12
16
20
24
TIME, HOURS

Figure 17. Performance of Unit I vs Unit III During 24-hour Batch Study on Day 122 at End of Daily Switching of 2-Chlorophenol Dosage From 50 to 25 mg/l. 2 -Chlorophenol Concentration - 50 mg/l

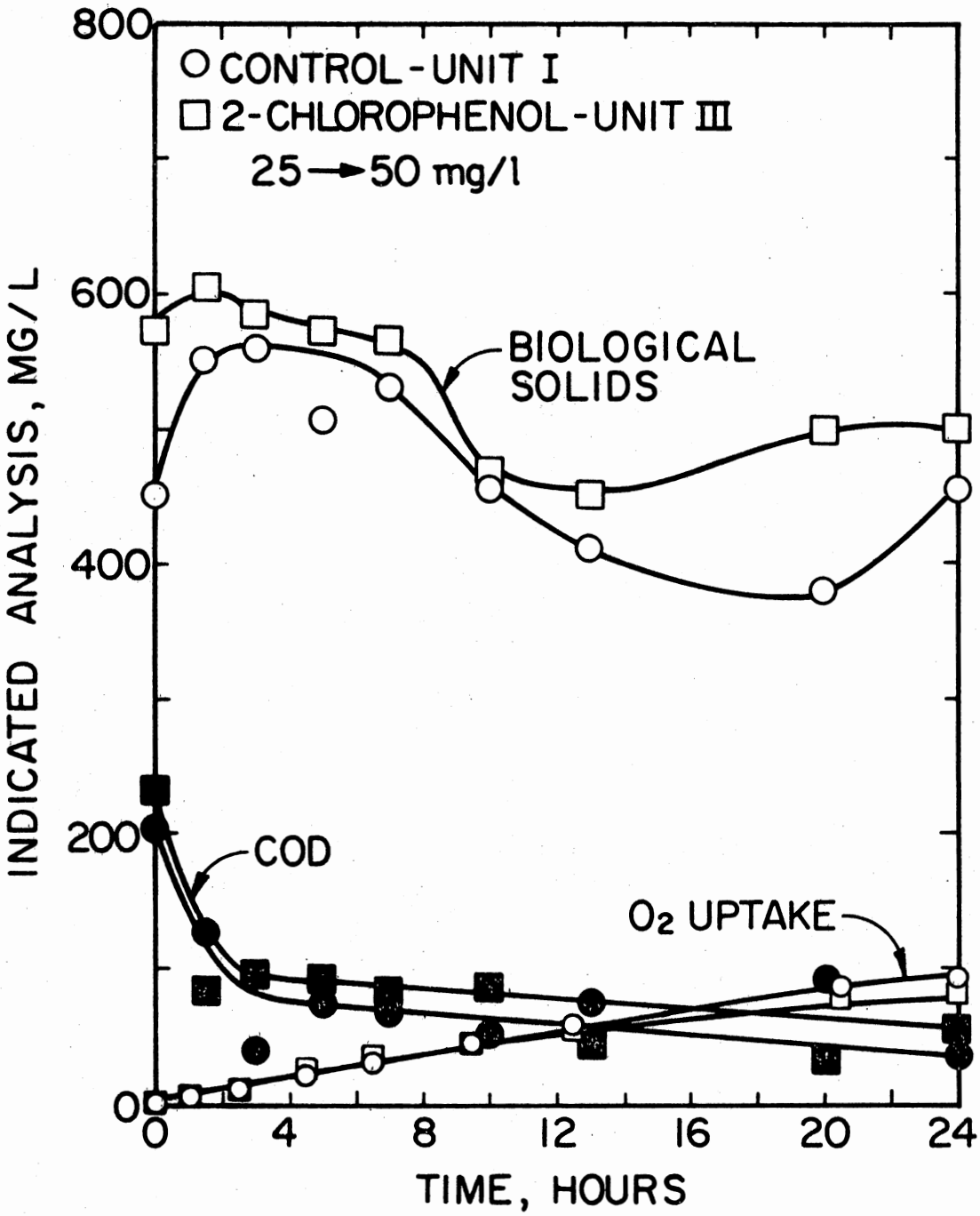

800
600
400
200
0
CONTROL - UNIT I
2-CHLOROPHENOL - UNIT III
25 → 50 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
INDICATED ANALYSIS, MG/L
0
4
8
12
16
20
24
TIME, HOURS

Figure 18. Performance of Unit I vs Unit III During 24-hour Batch Study on Day 128 at Beginning of Daily Switching of 2-Chlorophenol Dosage From 25 to 0 mg/l. 2-Chlorophenol Concentration - 25 mg/l

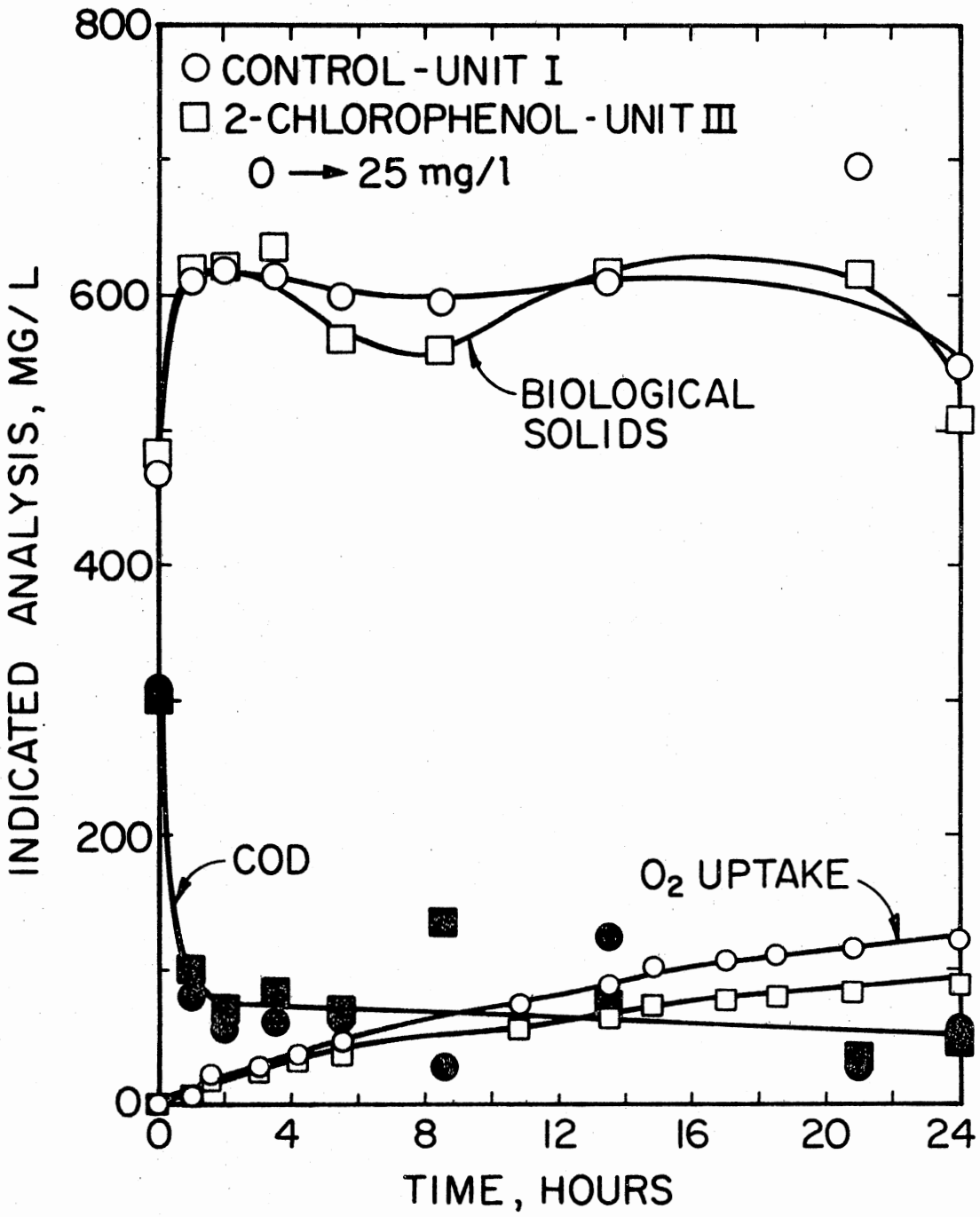

800
600
400
200
0
CONTROL - UNIT I
2-CHLOROPHENOL - UNIT III
0 → 25 mg/l
BIOLOGICAL SOLIDS
COD
O2 UPTAKE
INDICATED ANALYSIS, MG/L
0
4
8
12
16
20
24
TIME, HOURS

Figure 19. Performance of Unit I vs Unit III During 24-hour Batch Study on Day 136 at End of Daily Switching of 2-Chlorophenol Dosage From 25 to 0 mg/l. 2-Chlorophenol Concentration - 25 mg/l

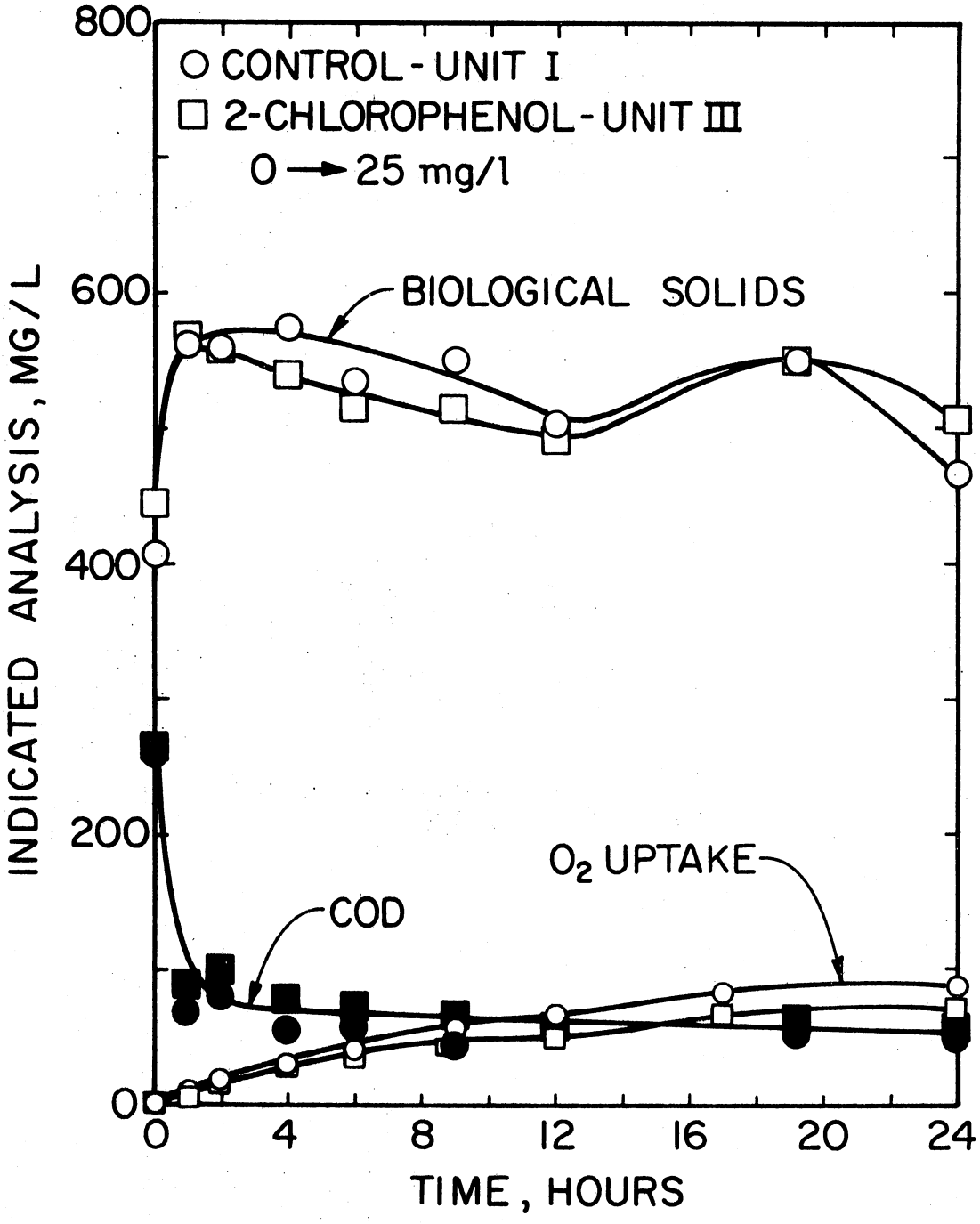

800
600
400
200
0
INDICATED ANALYSIS, MG/L
CONTROL - UNIT I
2-CHLOROPHENOL - UNIT III
0 → 25 mg/l
BIOLOGICAL SOLIDS
O2 UPTAKE
COD
0
4
8
12
16
20
24
TIME, HOURS

TABLE IV

MATERIALS BALANCES FOR 24-HOUR BATCH STUDIES AT INITIAL SUBSTRATE REMOVAL AND AT END OF TEST

Fig.	Test Day	Unit No.	Time hrs.	X_{IS}	COD_{IS}	COD_W	TOTAL	X_{FS}	COD_{FS}	COD_R	O_2 Uptake	TOTAL	Materials Recovery Percent
	12	I	0	344	488	269	757				0		
			6					432	613	58	40	711	94
			24					444	630	42	93	765	101
6		II	0	344	488	265	753				0		
&			6					444	630	70	45	745	99
13			24					406	577	54	95	726	96
		III	0	384	545	251	796				0		
			6					424	602	56	38	696	87
			24					400	568	58	82	708	89
	41	I	0	496	704	185	889				0		
			5					548	778	56	55	889	100
7			24					520	824	52	121	997	112
&		II	0	472	670	212	882				0		
14			5					580	824	81	49	954	108
			24					530	753	54	109	916	109
		III	0	492	699	226	925				0		
			5					524	744	93	51	888	96
			24					510	724	56	101	881	95
	81	I	0	496	704	185	889				0		
			3					556	790	89	23	901	101
8			24					520	738	52	92	882	99
&		II	0	472	670	212	882				0		
15			3					608	863	85	14	962	109
			24					530	753	54	69	876	99
		III	0	492	699	226	925				0		
			3					571	812	113	19	944	102
			24					510	724	56	81	861	93
	114	I	0	428	608	230	840				0		
			3					560	795	36	23	854	102
9			24					488	693	28	115	834	100
&		II	0	528	750	246	996				0		
16			3					556	790	64	25	879	88
			24					520	738	32	97	868	87
		III	0	540	767	217	984				0		
			3					552	784	96	21	901	92
			24					540	767	52	84	903	92
	122	I	0	344	488	260	748				0		
			5					404	574	76	21	671	90
10			24					352	500	80	93	673	90
&		II	0	510	724	270	990				0		
17			5					536	761	56	19	836	84
			24					460	655	55	75	785	80
		III	0	572	812	232	1044				0		
			5					572	812	92	26	930	89
			24					500	710	56	80	846	81
	128	I	0	468	665	309	947				0		
			2					620	880	56	22	959	98
			24					548	778	56	131	965	99
11		II	0	536	761	285	1046				0		
&			2					676	960	68	20	1047	100
18			24					512	728	40	118	886	85
		III	0	484	687	301	988				0		
			2					620	881	72	18	972	98
			29					508	722	48	95	865	87
	136	I	0	408	579	260	839				0		
			2					560	795	81	20	896	107
12			24					468	665	48	87	800	95
&		II	0	432	613	260	873				0		
19			2					584	829	89	19	937	107
			24					516	730	52	107	891	102
		III	0	444	630	264	894				0		
			2					560	795	102	16	913	102
			24					508	721	56	72	849	95

COD_{IS} + COD_W = COD_{FS} + COD_e + O_2 Uptake

Initial Solids; Soluble Waste; Final Solids; Soluble Effluent; Accumulated O_2 Uptake

is the 2-chlorophenol unit. It can be seen that in most cases, the percent recovery is very high. An exception would be the results obtained on day 122. The percent recovery data indicate that all of the materials were accounted for and the results of the experiments can be considered reliable.

Oxygen Update

The data obtained from a long-term oxygen uptake experiment are shown in Figures 20, 21, 22, and 24. Forty-ml samples obtained from a batch reactor which was run for three weeks to ensure steady state conditions were inoculated with varying concentrations of toxics and placed in flasks on a Warburg respirometer. The initial suspended solids concentration before inoculation of the mixed liquor was 538 mg/l, and the initial soluble COD was 242 mg/l. Figures 20 and 21 show the performance during the first 24 hours, and Figures 22 and 23 show the performance of the same samples over a period of 26 days. In Figures 20 and 21 it can be seen that the respiration rate was slower for all of the samples containing toxics except for the 5 mg/l nitrobenzene sample. But in Figures 22 and 23 it can be seen that the accumulated oxygen uptake surpassed the control in the 5- and 50-mg/l samples of 2-chlorophenol and in the 25 mg/l sample of nitrobenzene after a few days.

Stripping Tests

Figures 24 and 25 show the results of stripping tests run in 3-liter batch reactors using tap water and toxic concentrations of 250, 500, and 1000 mg/l theoretical COD. It can be seen that the

Figure 20. Accumulated Oxygen Uptake Performance of Unacclimated Mixed Liquor With Varying Concentrations of Nitrobenzene vs a Control During the First 24 Hours of Experiment

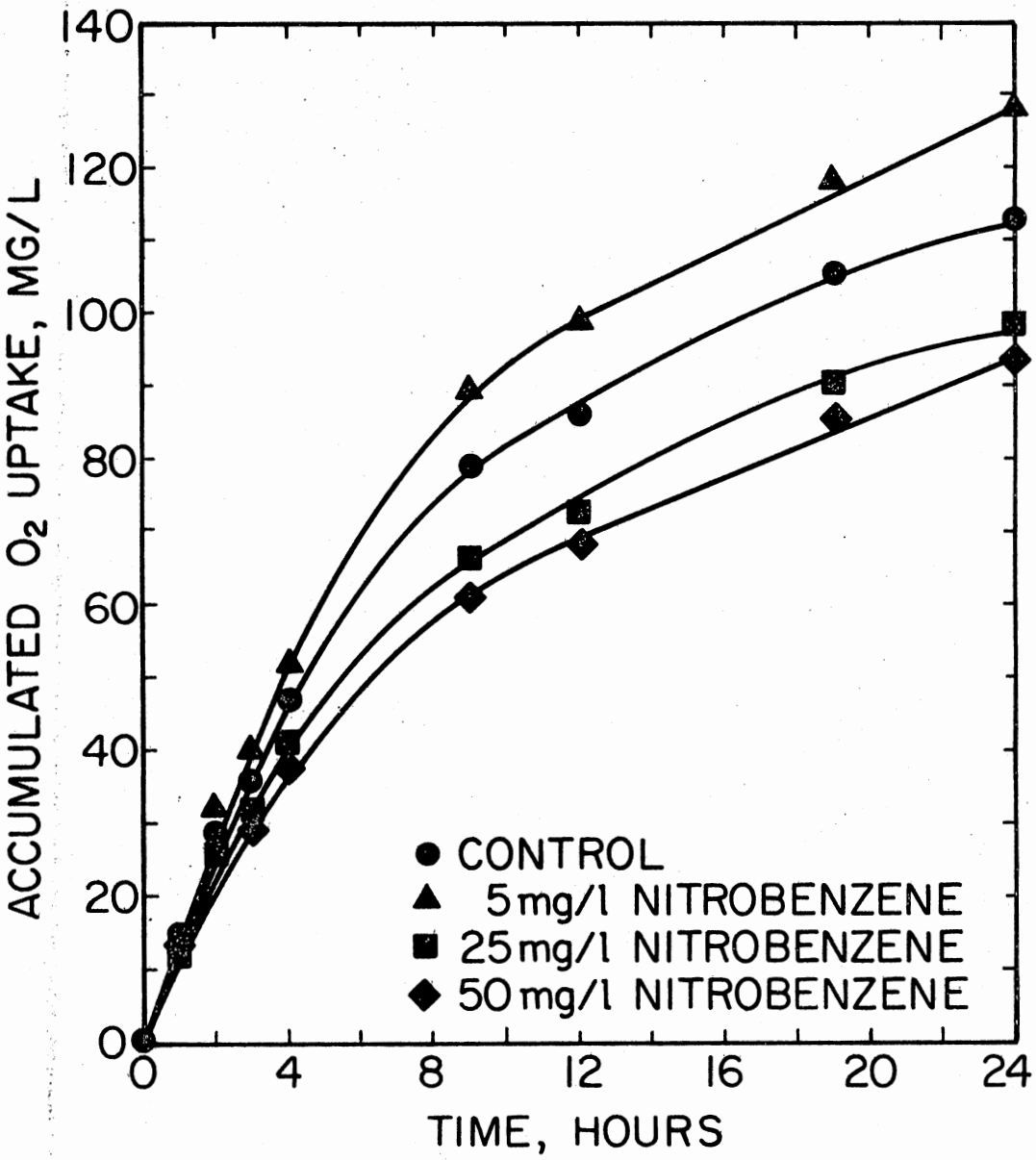

ACCUMULATED O_2 UPTAKE, MG/L
140
120
100
80
60
40
20
0
0
4
8
12
16
20
24
TIME, HOURS
CONTROL
5 mg/l NITROBENZENE
25 mg/l NITROBENZENE
50 mg/l NITROBENZENE

Figure 21. Accumulated Oxygen Uptake Performance of an Unacclimated Mixed Liquor With Varying Concentrations of 2-Chlorophenol vs a Control During the First 24 Hours of Experiment

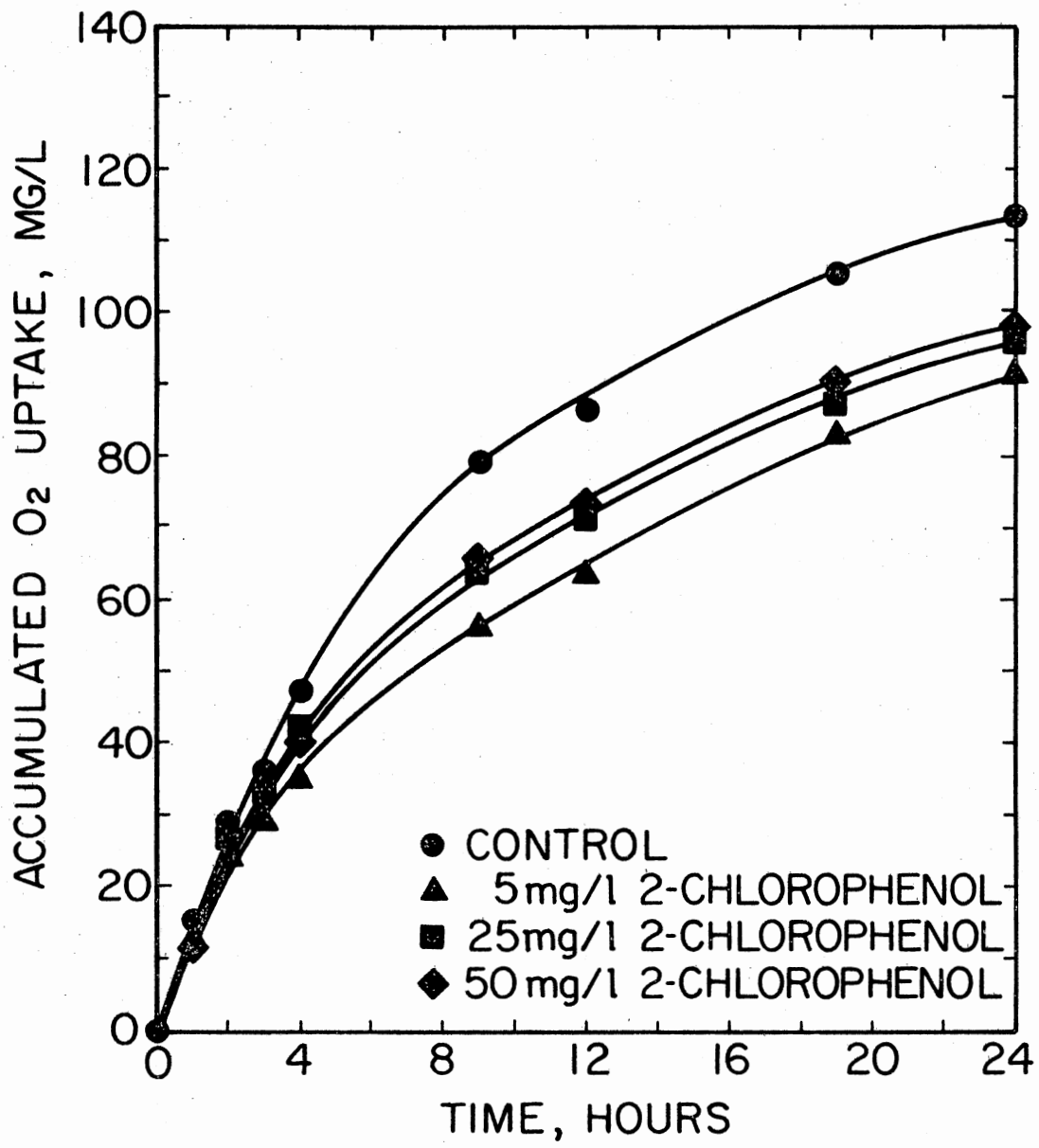

ACCUMULATED O2 UPTAKE, MG/L
140
120
100
80
60
40
20
0
0
4
8
12
16
20
24
TIME, HOURS
CONTROL
5mg/l 2-CHLOROPHENOL
25mg/l 2-CHLOROPHENOL
50 mg/l 2-CHLOROPHENOL

Figure 22. Accumulated Oxygen Uptake Performance of Unacclimated Mixed Liquor With Varying Concentrations of Nitrobenzene vs a Control During the 26 Days of the Experiment

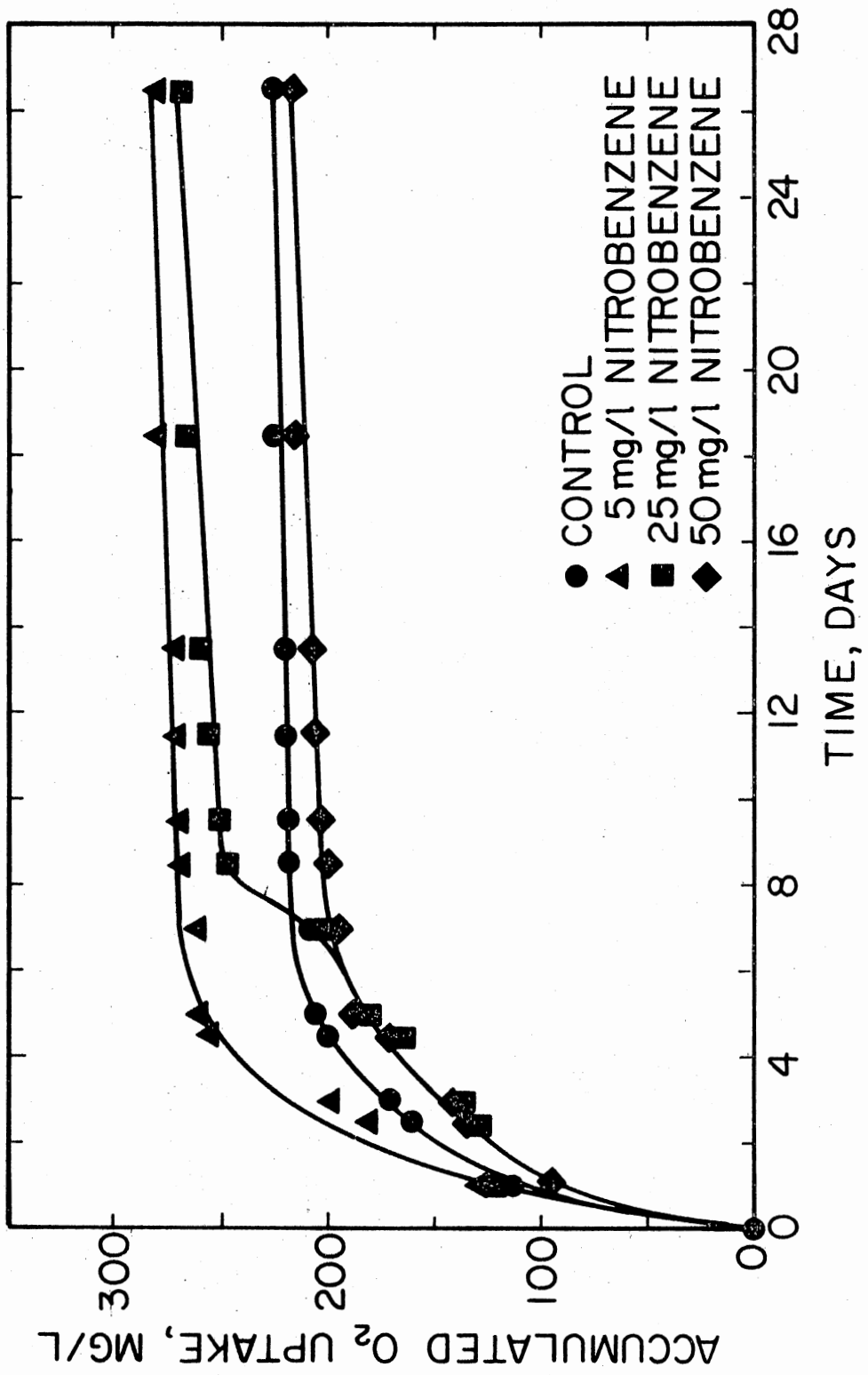

ACCUMULATED O2 UPTAKE, MG/L
300
200
100
0
0
4
8
12
16
20
24
28
TIME, DAYS
CONTROL
5 mg/l NITROBENZENE
25 mg/l NITROBENZENE
50 mg/l NITROBENZENE

Figure 23. Accumulated Oxygen Uptake Performance of an Unacclimated Mixed Liquor With Varying Concentrations of 2-Chlorophenol vs a Control During the 26 Days of the Experiment

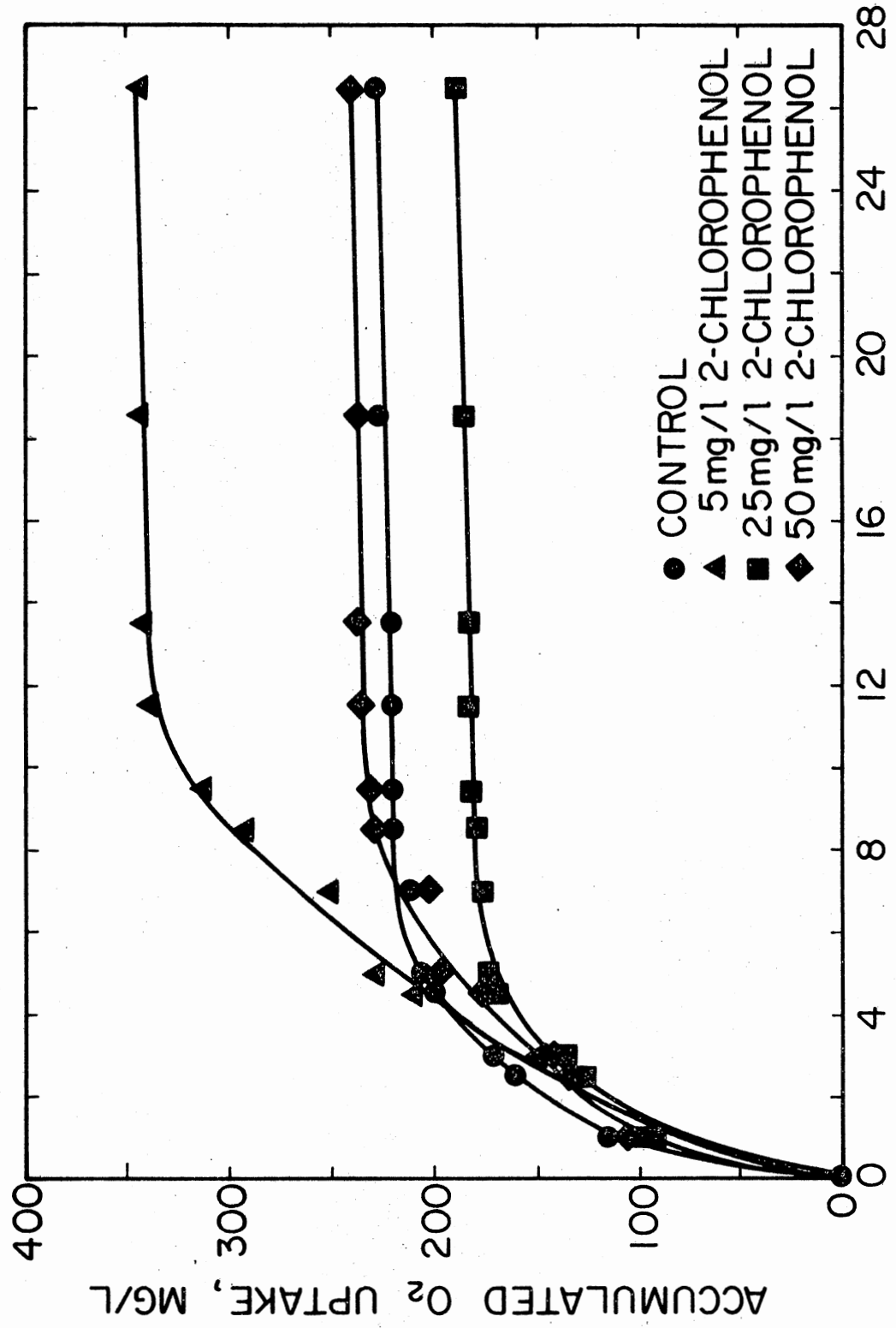

ACCUMULATED O2 UPTAKE, MG/L
400
300
200
100
0
0
4
8
12
16
20
24
28
TIME, DAYS
CONTROL
5mg/l 2-CHLOROPHENOL
25mg/l 2-CHLOROPHENOL
50mg/l 2-CHLOROPHENOL

Figure 24. Stripping Test of Nitrobenzene at Concentrations of 250, 500, and 1000 mg/l

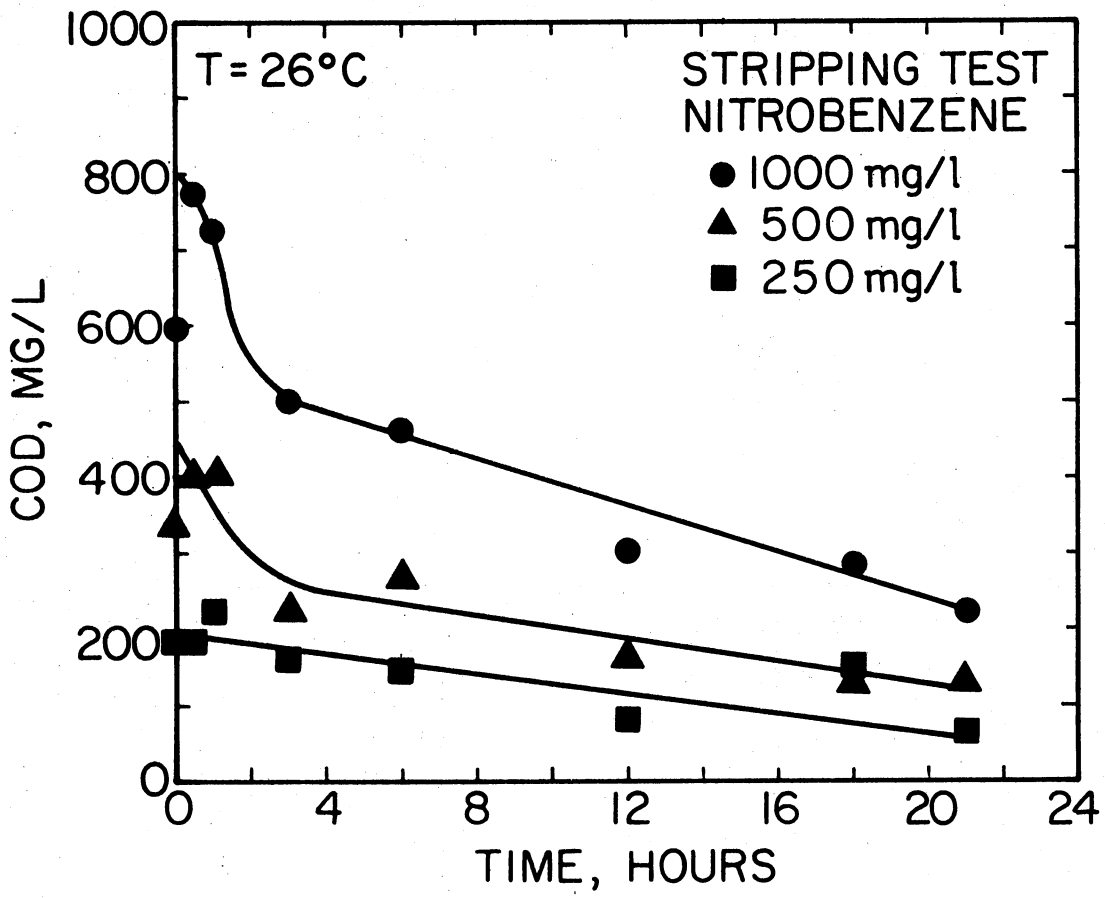
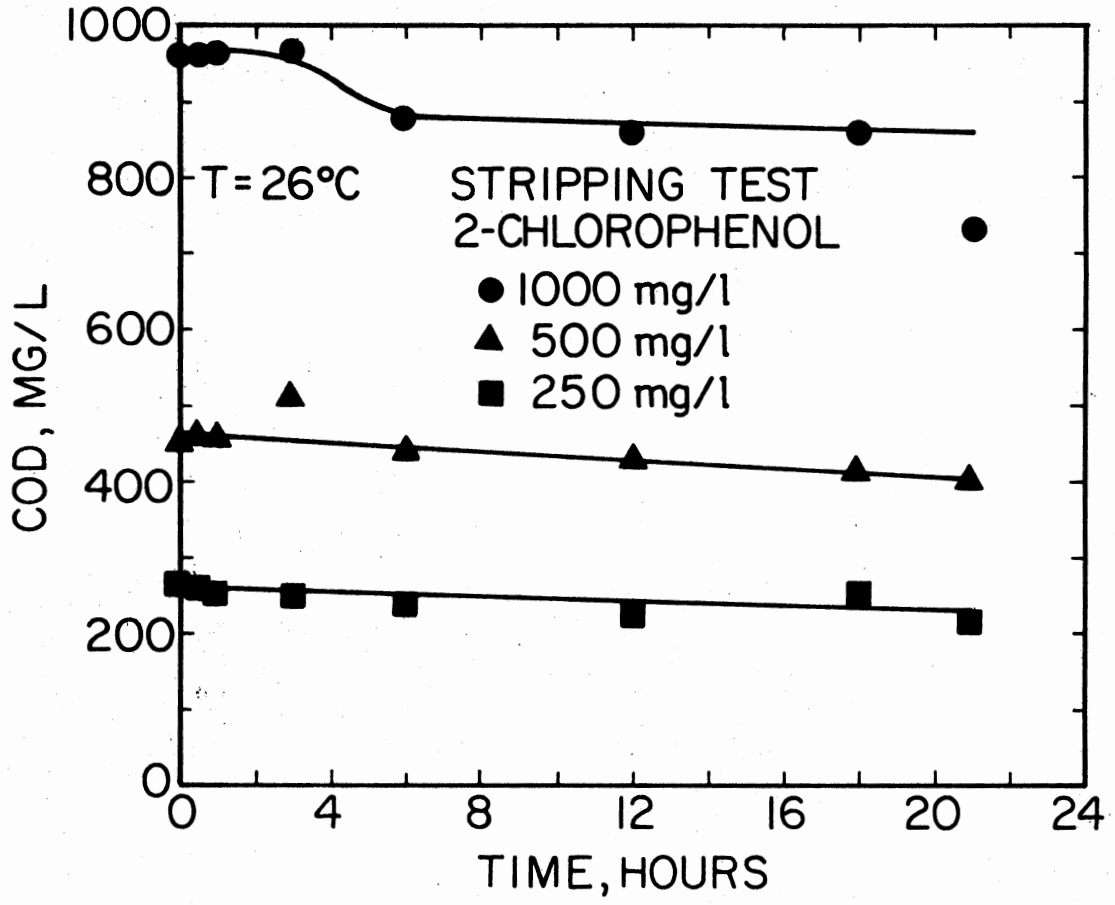

T = 26°C
STRIPPING TEST
NITROBENZENE
● 1000 mg/l
▲ 500 mg/l
■ 250 mg/l
COD, MG/L
1000
800
600
400
200
0
0
4
8
12
16
20
24
TIME, HOURS

Figure 25. Stripping Test of 2-Chlorophenol at Concentrations of 250, 500, and 1000 mg/l


1000
800
600
400
200
0
COD, MG/L
T=26°C
STRIPPING TEST
2-CHLOROPHENOL
● 1000 mg/l
▲ 500 mg/l
■ 250 mg/l
0
4
8
12
16
20
24
TIME, HOURS

2-chlorophenol exhibited very little stripping at any concentration, and that nitrobenzene stripped approximately 50 percent at all concentrations. The experiments were duplicated with very good results.

CHAPTER V

DISCUSSION

The purpose of this investigation was to establish whether or not 2-chlorophenol and nitrobenzene would have an inhibitory effect on the performance of a biological system. Batch units were operated at toxic concentrations of 5, 25, and 50 mg/l, and were also exposed to various shock loads as is shown in Figures 2 through 5.

It is apparent from the before feed COD values in Table II that biological purification efficiency was not inhibited by either toxic when compared to the control.

The rate of substrate removal in the nitrobenzene unit was not inhibited at any concentration or during shock loading experiments, as is shown in Figures 6 through 12.

On the other hand, the substrate removal rate in the 2-chlorophenol unit was inhibited slightly at the 25 mg/l concentration and during switching from 25 to 50 mg/l, as is shown in Figures 13 through 19. This would indicate that the 2-chlorophenol did have some effect on the microbial population, but not enough to affect the overall removal efficiency.

It is also interesting to note that there was a color change in the 2-chlorophenol unit upon addition of 5 mg/l and 25 mg/l of the toxic which eventually disappeared, and no color change was observed later in the experiment. It could be theorized from this observation that the

biomass in the 2-chlorophenol unit went through an acclimation period. Color changes were noted by Heidman et al. (20) during experiments with sodium pentachlorophenol, and the researchers concluded that the change was a result of a shift in predominating species.

The high substrate removal efficiency obtained during these experiments for both nitrobenzene and 2-chlorophenol is supported by the work of Pitter (9) who found during batch experiments that 95.6 percent of 2-chlorophenol and 98 percent of nitrobenzene was biologically degraded at a concentration of 200 mg/l COD.

Biological solids concentrations in both toxic units closely followed those found in the control, as can be seen in Table II, and the solids in the toxic units performed in the same manner as the control during the 24-hour batch studies, shown in Figures 6 through 19. But the settleability in both units seemed to have been slightly impaired. This can be seen in Table III. Although the difference between the control and toxic units was slight, it is worth mentioning. This impaired settleability may have resulted from a predominance change in both the 2-chlorophenol and nitrobenzene units.

The concept of a predominance change could also be supported by the difference in pH values between the control and toxic units, as seen in Table II. With a variation in microbial populations due to the toxic compounds, different intermediates and end products may have been formed which produced variations in pH.

During the 24-hour Warburg respirometry experiments shown in Figures 6 through 19, the respiration rate in both toxic units was inhibited when compared with the control. In addition to this, there appears to have been a two-phase oxygen uptake in most cases. In the

first phase, the respiration rate in the toxic units and the control are the same and, in the second phase, the control respiration rate is greater. It is not possible to determine the reason for the variation in respiration. It may indicate sequential substrate removal, where the glucose which was supplemented in the feed is utilized first, followed by the sewage and the toxics. The variation in respiration may again indicate a predominance change as did the difference in pH and settleability. If the toxic units lacked sufficient protozoa, or if different types of protozoa existed, the second phase of respiration might have been inhibited. There may also have been a complexing of the toxics with the sewage and glucose which created different compounds to be acted on by the microorganisms.

In the extended Warburg experiment shown in Figures 20 through 23, it can be seen that although respiration was inhibited in the toxic units, unacclimated microorganisms were able to adapt to the new substrate up through the 50 mg/l that was tested. As can be seen in Figure 20, the oxygen uptake for nitrobenzene was inhibited slightly at the 25 and 50 mg/l concentrations and at the 5 mg/l concentration the oxygen uptake was actually greater than the control. In Figure 20, the oxygen uptake of the 2-chlorophenol was inhibited at all concentrations. In Figures 21 and 22 it can be seen that the 25 mg/l nitrobenzene sample and the 5 and 50 mg/l 2-chlorophenol samples surpassed the control after a few days.

It is not known whether any appreciable amount of either toxic was stripped from the batch units during the experiments. Stripping tests were conducted and are shown in Figures 24 and 25.

It was found that very little stripping occured during the

2-chlorophenol stripping experiment, even at an initial concentration of 1000 mg/l COD. It could therefore be assumed that little was stripped during the batch experiment. On the other hand, over 50 percent of the nitrobenzene was stripped during the stripping experiments at all three initial COD concentrations of 250, 500, and 1000 mg/l; the highest concentration of nitrobenzene ever used in the feed was 50 mg/l. At this low concentration it is likely that little was lost due to stripping since nitrobenzene is slightly soluble in water, and the pH, respiration, and settleability were affected. Also, the materials balances in Table III show very good recovery values.

There is one important aspect which must also be considered when deciding on the treatability of the two compounds in question. The average initial solids level in all three units during the course of the experiments was approximately 400 mg/l. In an actual activated sludge plant, the average solids concentration would be much higher with a low value of approximately 1000 mg/l MLSS. This would probably cause a definite improvement on sludge characteristics and removal efficiency. Heukelekian and Hunter (17) consider cell concentration a major variable to be considered when determining biodegradability. Kirsch and Etzel (21) found that the rate of oxidation of pentachlorophenol was related directly to the microbial cell concentration. With the low solids concentration used in these experiments it would be safe to say that both toxics are highly degradable.

CHAPTER VI

CONCLUSIONS

Batch-fed biological reactors using domestic sewage and glucose as substrate were inoculated with various concentrations of nitrobenzene and 2-chlorophenol and compared with a control. The effects of the toxics were monitored. This study has led to the following conclusions:

1. Substrate removal efficiency was not inhibited by the toxic compounds at concentrations of 5, 25, and 50 mg/l.

2. The rate of substrate removal was slightly inhibited in the 2-chlorophenol unit.

3. Respiration rates decreased in both the nitrobenzene and 2-chlorophenol units.

4. pH values were higher in the toxic units.

5. Stripping tests indicated that 2-chlorophenol is slightly strippable and nitrobenzene is moderately strippable.

6. Shock loadings did not affect substrate removal efficiency.

7. Settleability was affected slightly by both toxics.

CHAPTER VII

SUGGESTIONS FOR FUTURE WORK

The author found that the domestic sewage used in the batch experiments had large variations in its daily composition and concentration. This variation made it very difficult to analyze the data obtained. In future work, it is recommended that a totally synthetic waste along with the toxic or the toxic alone be used as substrate, such as Pitter (9) has done. This would greatly decrease the variables in the experiment.

With a uniform initial substrate concentration and composition, complexing of the toxic would be minimized and data obtained at different times by different researchers could be compared much more easily. The batch experiments should be used only as a guide to establish the toxicity and biodegradability of the compound. When a specific waste containing this compound is encountered in the field, a continuous flow pilot plant could then be run to establish design and treatment parameters.

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