A COMPARISON OF BATCH AND CONTINUOUS FLOW KINETIC BEHAVIOR FOR ACRYLONITRILE, 2,4 DINITROPHENOL, AND 1,3 DICHLOROBENZENE

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α = Recycle Rate

C = Monod's Consumption Rate Constant

D = Dilution Rate as $1/t$ = Retention Time

D_m = Maintenance Dilution Rate

d^{-1} = 1/days

F = Flow Rate

hr^{-1} = 1/Hours

[I] = Inhibitor Concentration mg/l

K' + K'' = Saturation Constants

K_e = Eckenfelder's First Order Rate Constant

K_e' = Eckenfelder's Second Order Rate Constant

K_i = Inhibitor Saturation Constant

K_m = McKinney's Rate Constant

l = Volume in Liters

mg = Milligram Mass

S = Substrate Concentration in mg/l

S_0 = Substrate at t = 0

S_e = Effluent substrate or any t where $t \neq 0$

S_R = Substrate Removed

ΔS = Change in S

t = Real Time

U = Specific Substrate Utilization Rate Defined by $\frac{(S_0-S_e)}{Xt}$

X = Biomass as mg/l

X_A = Mean Value of X as mg/l

ΔX = Change in X

μ = Specific Growth Rate

μ_m = Maximum Specific Growth Rate

Y = Yield - Production of Biomass Per Removal of Substrate

CHAPTER I

INTRODUCTION

The microscopic biota present in the biosphere may be the most important agent in equilibrating the environment so all forms of life can coexist. Their foremost and primary function is the degradation of organic materials present to CO_2 and H_2O, thus serving as the apex for the recycling of carbon.

Industrialization and advancement in science and technology has lead to increased usage of both naturally occurring and synthetic organic compounds in processes to achieve desired goods and services. Industrial processes utilizing organic compounds result in the compounds or an altered form (oxidized, reduced, or polymerized) of them discharged as effluent waste waters. Discharged organic substances into a receiving water system may adversely affect the system and those using the system. Effects may be short- and/or long-term in their manifestations. Short-term impacts would be oxygen depletion, aesthetics (taste, color, and odor), and economics (restoration for potable and industrial reuse). Long-term effects would result from continued discharge and/or the presence of stable toxic substances which are acutely or chronically harmful.

The Federal Water Pollution Control Act (P.L. 92-500) enacted by the Environmental Protection Agency (E.P.A.) provided for legal restriction and control of the discharge of chemicals from industrial processes.

In an effort to further delineate the regulations of P.L. 92-500, E.P.A. through extensive research published a priority pollutant list of toxic compounds composed of 129 substances, of which 115 were organic. The basis for appearing on this list was a compound's evidenced or suspected potential health hazard, tenacious endurance in the environment, bio-accumulation tendencies, and in combinations of their tendency for synergism. The list of 115 organic compounds is categorized in three parts according to their acute or chronic health effects (30). The first category consists of those compounds known to be adversely biogenic. The second classified as structurally similar to the first and the third as those showing acute toxicity in bioenties. The importance of the list was to futuristically (1) develop a logistic "cradle to grave" approach for control of these compounds, (2) establish specific guidelines for effluent concentrations, and (3) set standards for the analysis for them.

Of great interest, therefore, is an economical method of removal. This becomes more evident in considering the vast amounts of use and presence in waste waters.

Since the compounds are organic and the environment provides a source of treatment, microorganisms, the most economical method would then be biological oxidation or reduction of them. Research in the past few years has expanded in the compatibility and treatability of priority pollutants using microbiological systems (activated sludge). Two types of studies to achieve this goal are bench scale pilot plant continuous flow systems and bench scale batch systems, to determine kinetic behavior and develop models to predict compatibility and treatability.

The objective and scope for the research presented are to study

three compounds from the list and examine their kinetic behavior in batch systems and compare this behavior to that expressed in continuous systems.

CHAPTER II

LITERATURE REVIEW

The quantitative study of the kinetics and dynamics of activated sludge systems involves knowledge of the mechanism(s) of reaction(s) and reaction rates between microorganisms and the substrate they are subjected to. Kinetics is the study of mass transfer rates occurring for each reaction mechanism to yield and predict an overall reaction rate between the species present (6).

Mechanisms

Recognition and transportation of a substrate to (into) the cell is accomplished by extracellular and/or membrane bound enzymes which are constitutive or induced (56). Induced enzymes are those whose synthesis is stimulated and activity regulated by exposure to a specific substrate (59). Constitutive enzymes are those whose synthesis is independent of need not regulated by inducers or repressors, and function primarily in cellular maintenance functions (56) (57).

Monod (32) (33) proposed the mechanism of induction and repression of protein synthesis in his studies with E. coli in which the bacteria exposed to two substrates effected a dynamic growth curve, and preferential removal of substrates. Gaudy and his associates have investigated this phenomena extensively in both batch and continuous systems. In a once through continuous system, Kómolrit and Gaudy (21)

demonstrated preferential removal of glucose over sorbitol with negligible change in growth rate. Su and Gaudy (53) showed that increasing growth rate increased sequential utilization of substrates. Yu and Gaudy (59) demonstrated that using cells harvested from continuous flow systems placed in a batch reactor achieved sequential removal. However, this tendency decreased as culture age increased.

Once in the cell, the carbon source is inserted into metabolic pathways to be catabolized for energy or serve as a precursor for anabolism in the synthesis of cellular constituents, or both (57). The complexities of the catabolic and anabolic pathways and the interactions between them are beyond the scope of this study. For enlightenment and further information, the reader is referred to the publication by Watson (56).

Many synthetic and naturally occurring organic compounds exhibit inhibition over cellular functions, thus, limiting growth and even affecting death of the organism (15). Such inhibition of metabolic enzymatic processes are one or a combination of three types: (1) competitive, (2) noncompetitive, and (3) uncompetitive, inhibition (35). Competitive inhibition is the result of competition between the inhibitor and preferred substrate for the active site on the enzymes. Uncompetitive inhibition is exhibited when inhibitor complexes with the enzyme substrate complex preventing it from separating and is seen more often in multiple substrate systems. When the inhibitor combines with the enzyme at a site other than the specific site of the substrate, noncompetitive inhibitions occur. All three types of inhibition are discussed in the publication by Morris (35).

Reddy and Kincannon (21) demonstrated competitive growth inhibition

in a batch activated sludge system using phenol modeled by Haldane's equation.

Finally, included in a review of mechanisms is the interactions and relationships between species of microorganisms in a heterogeneous population. Bull and Brown (5) in a review of microbial biochemistry, site work showing the dependence of microbiological communities to achieve degradation of complex organic material such as petroleum wastes and pesticides. The work sited by them and by Hill and Wright (14) demonstrates the existence of functional communities which are mutualistically dependent on its members to achieve degradation of organic compounds by co-metabolism, i.e., enzymatic interaction between organisms.

Kinetics

Assuming growth and growth rate as the sum total of all the mechanisms occurring in a cell provides a mechanism for modeling biological systems. The discussion of kinetic models for modeling growth rates in response to substrate concentration is drawn from Kincannon and Gaudy (18), Fredrickson and Tsuchiya (7), and Gaudy and Gaudy (10), along with the original works of the model developers.

The first model to enjoy success for microbiological systems was that of Monod (32). Monod recognized that growth rates of E. coli subjected to varying concentrations of substrate responded according to a first order monomolecular kinetic relationship, i.e.,

$$\mu = \frac{\mu m S}{K_S + S} \qquad (1)$$

The concepts for the model are substrate is rate limiting for a specific

growth rate and the consumption (C) of the rate-limiting subtrate(s) equals the yield times $\frac{ds}{dt}$.

Teisser (55) proposed that specific growth rate was a function of substrate concentration such that the substrate over K_S was raised to an exponent of maximum growth rate, i.e.,

$$(S) = \mu_m[1 - \exp\ (-\frac{s\ln 2}{K})] \tag{2}$$

resulting in a sinusoidal perturbation (47).

Other models have been proposed by Moser, (7) Shehath and Marr (50), Powell (7), and Jost et al. (17). A review of these models is presented by Fredrickson and Tsuchiya (7).

Adaptation to continuous systems of Monod's model results in Equation (3), or (4)

$$\frac{dc}{dt} = -DC + \frac{\mu_m CS}{K_S + S} \quad \text{or} \tag{3}$$

$$\frac{ds}{dt} = D(S_f - S) - \frac{1}{Y}\frac{\mu_m CS}{K_S + S} \tag{4}$$

where D is the dilution rate or the inverse of the retention time (7).

In using Monod's model in chemostate studies, Herbert found that the apparent yield from the data was different from the theoretical yield (13). This was also observed by Marr et al. (29) and Pirt (41). Herbert assumed that the difference in yields was due to energy required for cell maintenance and the source of this energy came from endogenous respiration. Herbert's addition to Monod's model to compensate for yield is

$$D_m = \mu_m \left[1 - \sqrt{\frac{K_S}{K_S + S_R}} \right] \tag{5}$$

while Marr et al. (29) and Pirt (41) used

$$1/2 \frac{\Delta X}{(\Delta_S)_g} + (\Delta_S)_m \tag{6}$$

The models of Monod and Herbert have become the bases for two of the models used in activated sludge systems to date, i.e, Gaudy's (18) Equation (7) and Lawrence-McCarty's (18) models, Equation (8), i.e.,

$$\frac{ds}{dt} V = FS_i + \alpha FS_e - F(1 + \alpha)S_e - \mu_m \frac{X}{Y_t} \frac{S_e}{K_S + Se} V \tag{7}$$

and

$$\frac{ds}{dt} V = FS_i - FS_e - KX \frac{S_e}{K_X + S_e} V , \tag{8}$$

respectively. The difference between the two being the influence of the recycle rate by Gaudy.

The other major models used in design of activated sludge units are those of Eckefelder (18) and McKinney (26). Eckenfelder (18) assuming first order decreasing rate substrate removal followed discontinuous kinetics and proposed

$$\frac{ds}{dt} V = FS_i - Fs_e - K_e X S_e V \tag{9}$$

McKinney (26) conceptualized a number of constants for mechanisms he felt were occurring in activated sludge systems and suggests that

$$\frac{ds}{dt} V = FS_i - Fs_e - K_m S_e V \tag{10}$$

effluent substrate concentration is dependent on hydraulic detention time with no correlation to the concentration of the microorganisms. A comparison of application for design, of these four models may be found in Kincannon and Gaudy (18) .

Grady and Williams (8) recognizing that influent substrate concentration may have a bearing on effluent quality used a modified form of Monod's equation, i.e.,

$$S = K' S_o D + K'' S_o \tag{11}$$

to try and predict changes in S_e. He emphasized the empirical derivation of the model. Common to all the models for both batch and continuous sytems is the lack of modeling for inhibition. Andrews (1) used Haldane's equation for competitive substrate inhibition, i.e.,

$$\mu(S) = \frac{\mu_m \cdot S}{K_S + S + (S^2//K_i)} \tag{12}$$

Competitive inhibition is exhibited by a change in the denominator while μ_m remains the same. The other two types of inhibition introduced in the section on mechanisms demonstrate different transformations than competitive on a Linweaver-Burk plot. Noncompetitive inhibition results in a change in μ_m but not in K_s, i.e.,

$$\mu = \frac{\mu_m \cdot S}{(1 + \frac{[I]}{K_i})} \tag{13}$$
$$S + K_S$$

Uncompetitive inhibition depicts a change in both K_S and U_m, i.e.,

$$\mu = \frac{\dfrac{\mu_m}{(1 + \frac{[I]}{K_i})}}{\dfrac{S + K_S}{(1 + \frac{I}{K_i})}} \cdot S \qquad (14)$$

The models for inhibition are reviewed by Morris (35).

Yang and Humphrey (58) using both a pure culture batch and continuous system with phenol tested the three models above along with two others and found no statistically significant difference between them.

Neufeld and Valiknac (37) working with waste waters from a coal carbonization process, which contain cyanates and phenols utilized a continuous flow reactor system with them. They concluded the cyanate interfered with the removal of phenol and to maintain the desired levels of both phenol and cyanate in the effluent, the θ_C had to be increased as cyanate concentration increased. They used the modified form of Equations 12, 13, and 14, i.e.,

$$[1 + \sum_{j=0}^{n} I \frac{j+K}{K_{I,j}}] \cdot \qquad (15)$$

Reddy and Kincannon (46) demonstrated competitive growth inhibition using phenol in batch systems and modeled it using Haldane's equation with K_i = 168-182 mg/l. They did not experience any discontinuity in the kinetics as Lowe and Kincannon (22) did with phenol. Lowe had discontinuity develop between 7 and 9 days θ_C.

Compounds

The compounds chosen for the kinetic study were 2,4 dinitrophenol, 1,3 dichlorobenzene, and acrylonitrile. All three appear on the E.P.A. priority pollutant list (43). The early work performed on these compounds was primarily in pure culture work following Koch's procedures (5). Results showing inhibition and toxic effects from this type of research is one reason these compounds appear on the E.P.A. List.

Phenols and substituted benzenes are aryl compounds synthetically derived from diazonium salts and water as outlined in Morrison and Boyd (36). Thus, 2,4 dinitrophenol and 1,3 dichlorobenzene are the nucleophilic substitution of an aryl (benzene) that has reacted with a diazonium salt to produce ortho, para substituted dinitrobenzene and ortho, meta substituted dichlorobenzene, respectively.

Interesting then is the similarities between the metabolic pathways used by microorganisms to degrade and utilize benzenes and phenols. 2,4 Dinitrophenol predominately undergoes a reduction of the nitrogen groups to amines which then are removed by deamination (14). Benzene, in bacteria can be converted to phenol in a reversible reaction which can further be substituted forming hydroquinone (16). Quinones present in the electron transport system of bacteria appear to function as intermediate carriers between the flavoproteins and the cytochromes (56).

Haller (11) reported that the nitro group on 2,4 DNP phenol was cleaved off as nitrate. Raymond and Alexander (45) demonstrated the conversion of para and meta-nitrophenols to nitrohydroquinone in a pure culture, with the release of nitrate. An intermediate formed in the process was nitrocatechol which agrees with the pathway proposed by Stanier (49) for phenols. Hill and Wright (14) propose that nitrophenols

undergo reduction to amines and through subsequent reactions nitrates appear.

Hill (14) shows that benzenes and phenols are transformed to an intermediate catechol which undergoes ortho or meta fission, or are metabolized by a "gentisate" pathway to fumaric and pyruvic acid. The latter agrees with the findings of McKinney et al. (28) in what he terms as β-oxidation.

Halogented benzenes and phenols as reported by Hill (14) undergo four major reactions for removal of the halogen. The four reactions are (1) hydroxyl replacement, (2) halogen migration, (3) reductive dehalogentation, and (4) dehydrodehalogentation. The cyclic ring then is processed as stated above.

2,4 Dinitrophenol is suspect of uncoupling oxidative phosphorylation in bacteria and mammalian tissues (57), with lethal inhibition levels of 50 µg/l (15). For this reason, it has been used as a pesticide, and a precursor for pesticides (14). It is also used in making amyl dyes (36).

Very little work has been published using 2,4 dinitrophenol in bench scale studies with activated sludge and even less for 1,3 dichlorobenzene, until recently. Tabak (54) using a static flask method showed 100% degradation of 2,4 dinitrophenol and only 40% for 1,3 dichlorobenzene after seven days. Medely and Stover (30) determined 2,4 DNP to be a poor economical candidate for complete oxidation by ozone but showed it was more easily degraded after ozonation had occurred. McCartney (26) using 2-nitrophenol as a sole carbon source showed it to be biodegraded, while total removal of the compound involved approximately 13% stripping.

Kincannon and Stover (19) using continuous flow recycle sludge systems achieved better than 95% removal of 2,4 DNP and 1,3 DCB. This was determined by specific G. C. analysis.

A substantial amount of research has been performed on phenols and benzenes using activated sludge. This warrants a brief presentation as a possible predictable tool for the behavior of 2,4 DNP and 1,3 DCB. McKinney et al. (28) using several substituted phenols and benzenes in Warrburg studies showed benzene to be less inhibitory than phenol and decreased O_2 utilization increased with substitution on the cyclic ring. Beltrame et al. (2) (3) (4) show phenol to be inhibitory in batch but not in continuous systems. Graham (9) utilized phenol in a once through and a recycle continuous system which revealed unsteady state kinetics for the once through system and steady state kinetics in the recycle. Pawlowsky and Howell (38) (39) (40) reported that in batch and continuous systems with phenol, two steady states existed, one for wall growth, the other for the completely mixed portion. All the researchers achieved high removal efficiencies with phenols and benzenes of 90 to 100% and yields around 0.80%. However, the discrepancies seem to be mainly in K_S values.

Much of the research performed on benzenes and phenols has been with the use of nonspecific analysis, i.e., total organic carbon, biochemical oxygen demand, (BOD) and chemical oxygen demand (COD). Pitter (42) showed 2,4 dinitrophenol to be 85% degraded by activated sludge at 200 mg/l COD. He also performed this test on several other phenols and substituted benzenes and found the greatest majority biodegradable.

Radhakrishnon and Ray (44) using a pure culture of B. cerus achieved high rates of yield, and μ_m for phenol. They reported μ_m at

0.628 hr^{-1} in a continuous system versus 0.144 hr^{-1} for the batch. The yield of 0.81% was higher in the continuous system than the batch.

Acrylonitrile is a vinyl cyanide which is prevalent in the waste waters from chemical, rubber, plastic, textiles, and steel industries (36). It also has been shown to uncouple oxidative phosphorulation (57). A very toxic and even lethal substance, it has been removed from waste waters by stripping, absorption on soil columns, and biological oxidation (47). Ludzack (15) reports that the mechanism for removal is by hydrolysis to organic acids and ammonia.

Mills and Stack (31) in attempting to acclimate municipal sludge to acrylonitrile could not achieve utilization until it had been hydrolyzed to acrylic acid or sodium acrylate.

CHAPTER III

MATERIALS AND METHODS

To achieve kinetic constants for 2,4, DNP, 1,3 DCB, and acrylonitrile, a completely mixed batch one-step growth curve with an acclimated heterogeneous population was employed. The constants for the continuous flow completely mix, sludge recycle systems, are those developed by Kincannon and Stover (52).

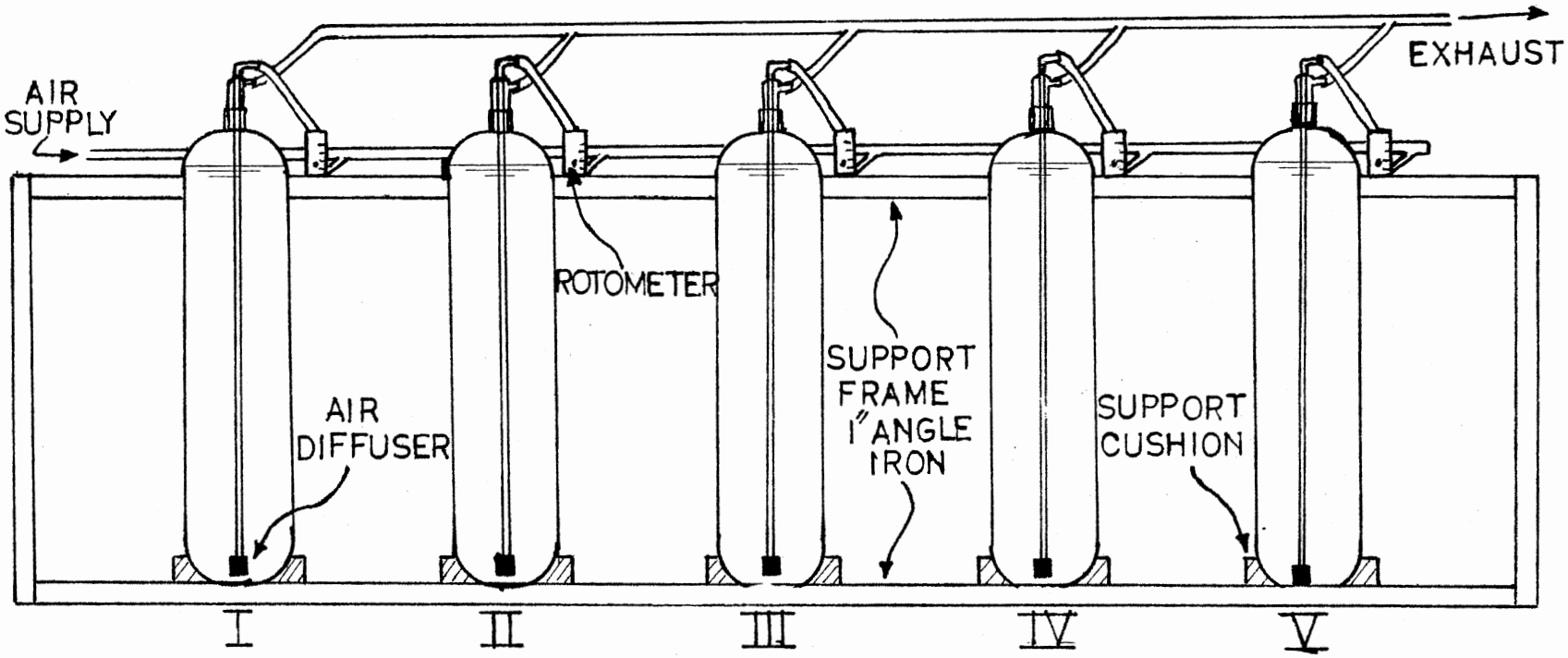
In both experimental approaches, the specific compound was used in conjunction with a "base mix", to simulate a complex industrial waste.

Experimental Units

Reactors used to expose and propagate the activated sludge biomass consisted of five 3.2 liter round bottom graduated glass cylinders (see Figure 1). They were 10 cm in diameter by 105 cm in height. Each had a ground glass cap with an influent airline extending to the bottom terminated by a sintered air diffuser and an effluent airline open to the atmosphere for pressure equalization. All the effluent airlines were connected in parallel to a manifold vented outside the building. Thus, each reactor was in a positive air flow system.

Air flow was filtered over glass wool to remove dust and oil condensates and regulated to each reactor by a rotometer at .5 liter/min. At this flow rate, complete mixing ensued and the dissolved oxygen maintained above 2 mg/l.

Figure 1. Illustration of Biological Reactors and Hardware Used for Study



BIOLOGICAL REACTORS I-V

Temperature was monitored at the onset and completion of each experiment and remained at 22° ± 1° C.

In each of the five reactors, pH was maintained in the range of 6.8 to 7.2 using sodium hydroxide or hydrochloric acid.

Base Mix and Compounds

The base mix consisted of the organic compounds listed in Table I showing their composition, concentration, and proportions. The same ratios were maintained for the entire study for both batch and continuous flow units. Specific compound(s) to be studied were weighed or measured volumetrically and added to the units at the desired concentration. The concentrations were chosen on known adverse effects in biological systems, saturation properties, and experimentation, due to difficulties getting them in solution.

Use of the five reactors thus allowed for a doubling of base mix concentration between each reactor, starting with 50 mg/l in Reactor I ending with 800 mg/l in Reactor V. The quantitative amounts of base mix were based on total organic carbon (TOC). The graduated concentrations from 50 to 800 mg/l TOC of base mix were maintained for the entire study.

The specific compounds used for the investigation 2,4 dinitrophenol, 1,3 dichlorobenzene, and acrylonitrile, were then added to the existing solution of base mix.

2,4 Dinitrophenol, a yellow crystal at standard temperature and pressure with a molecular weight of 184.11 g/m (CRC) (12) was added to the reactors at 25 and 50 mg/l for two experiments. Due to the difficulty of getting it into solution, part of the final three liter volume was

TABLE I

COMPONENTS OF THE BASE MIX

(A) Organic Compounds	Concentration
Ethylene glycol	113 ml/1
Ethyl alcohol	113 ml/1
Acetic acid	113/ml/1
Glutamic acid	113 gms/1
Glucose	113 gms/1
Phenol	22.6 gms/1
(B) Inorganic Compounds	
$(NH_4)_2\ SO_4$	100 gms/1
H_3PO_4	15.7 ml/1
$CaCl_2$	4 gms/1
$MgSO_4 \cdot 7H_2O$	4 gms/1
$FeCl_3 \cdot 6H_2O$	0.2 gms/1

Reactors	(A)	(B)	to 3/1 H_2O
I	3 ml	2 ml	
II	6 ml	4 ml	
III	12 ml	8 ml	
IV	24 ml	16 ml	
V	48 ml	32 ml	

used outside the reactor to dissolve it before addition to the system.

1,3 Dichlorobenzene, a clear liquid at standard temperature and pressure, has a density of ρ = 1.2884 g/ml (CRC) (12) and was measured volumetrically for distribution into the reactors. Again, to be assured of the compound being in solution, the same procedure was used as that for 2,4 DNP.

Acrylonitrile, a clear pungent liquid at STD temperature and pressure, with a ρ = .806 g/ml (CRC) (12) was measured volumetrically at 50 and 100 mg/l for addition to the systems. Due to the volatile nature of the compound, it was added directly to the reactor cylinders, the cylinders caped, and then vigorously shaken to drive it into solution.

The final set of experiments involved the addition of all three compounds. The lowest quantities for each compound were utilized in an effort to determine the combined effect on the growth curve and sequential removal of the compunds, if any.

Water utilized for the experiments was tap water, since it was being used in the continuous systems. However, due to chlorine residuals and dissolved gasses in tap water, it was aerated for one hour at three liters/min air flow to ensure the absence of these substances.

At all times, an effort was made to minimize deviation from the procedures used for the continuous flow units.

Biomass

Preceding each experimental run for the specific compounds, a seed of 500 ml of activated sludge was taken from the control unit of the continuous flow research program, for acclimation. The seed was then

placed in a 4L reactor and gradually exposed to increasing concentrations of the base mix and compound(s), until a dispersed exponential growth was realized and maintained.

Determination of the state of growth of the systems for acclimation and the kinetic experiments was performed using optical density. Solids (biomass) in solution were measured using a Bausch and Lomb Ser. 0617447 J Spectrophotometer set at 540 nm. The sample was then filtered with a .45 um millipore filter at 15 in. hg and dried as per standard methods (48) for total suspended solid. A plot of absorbance versus dry solids established the standard curve in Figure 2. The curve was randomly verified for the entire project. The curve established had a slope of ~50 mg/l solids/absorbance unit.

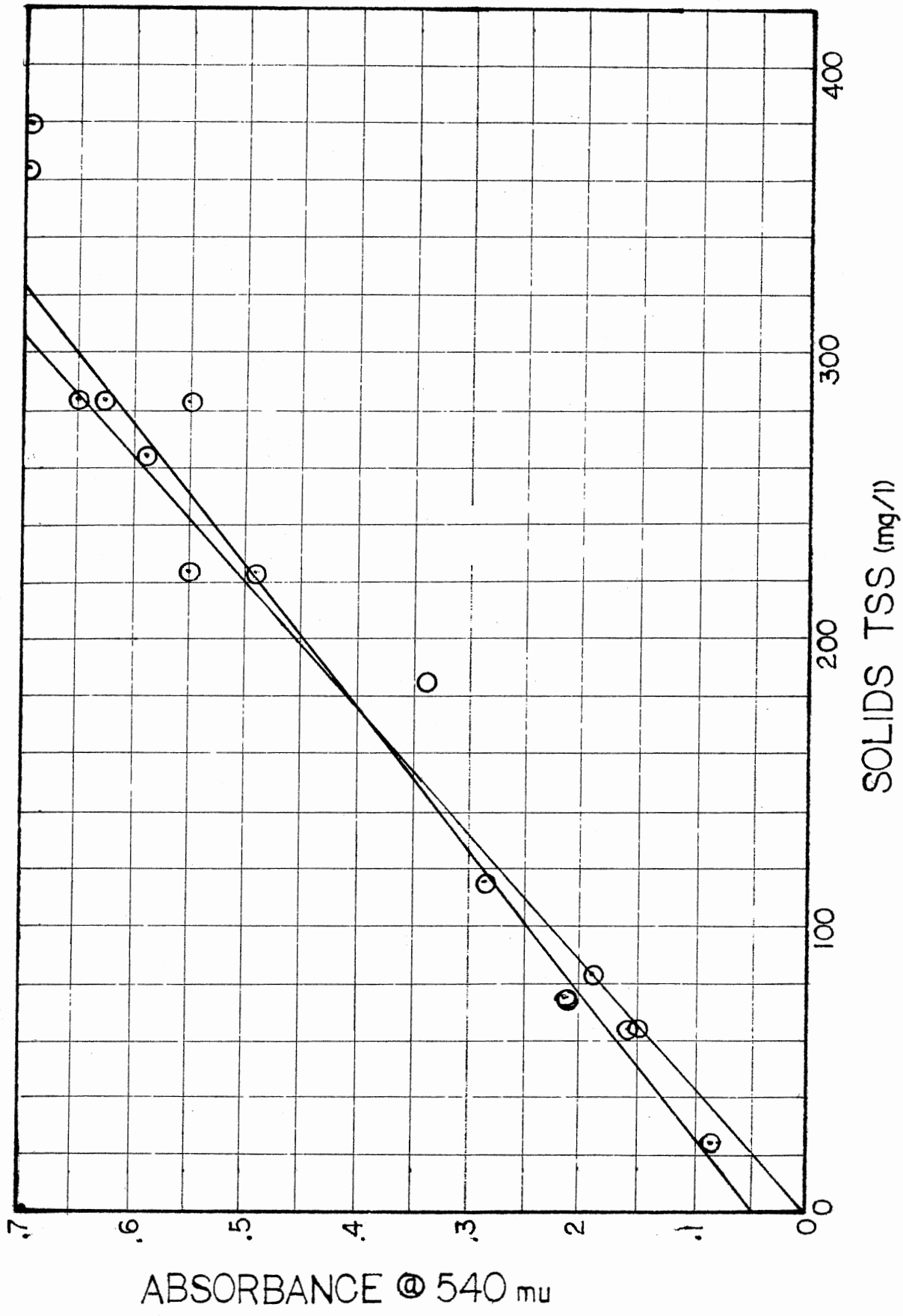
Once acclimated to the compound, the culture became the stock culture for that compound. It was maintained in exponential dispersed growth using optical density measurements by wasting all but 100 ml of the system and resuspending in new substrate. An alliquot of the stock culture became the seed for the experimental runs. The solids at time zero was desired to be at 40-50 mg/l.

Parameters and Analysis

Parameters selected for use in determining kinetic constants were. total organic carbon (TOC), biochemical oxygen demand (BOD), and specific compound analysis by gas chromatography. TOC was accomplished using a Beckman Model 915 TOC analyzer and an Oceanographic International Model 0524B-HR TOC analyzer as per standard methods (57).

BOD, dissolved oxygen, and O_2 uptake employed the use of an Orion

Figure 2. Standard Curve for Adsorbance Vs. Biomass


SOLIDS TSS (mg/l)
400
300
200
100
0
.7
.6
.5
.4
.3
.2
.1
0
ABSORBANCE @ 540 mu

D. O. Probe Model 97-08 and a Beckman Model 76 pH meter. BOD_5 was performed according to standard methods with the modification recommended by Stover (51).

Specific analysis by gas chromatography involved the use of the procedures outlined in the Federal Register #69464, Vol. 44, No. 233 (60), for each compound. 2,4 DNP, an acid extractable, was analyzed using a Hewlett Packard Model 7600 G. C. with flame ionization and a 6' glass column packed with SP 1240-DA. The purge air flow was 300 ml/min, hydrogen, 30 ml/min and nitrogen, 30 ml/min. The G. C. was programmed at 90° C for 2 min isothermol, increased by 8°/min by 200° C and held for 8 min. The compound had a retention time of 12 minutes.

1,3 DCB, a base neutral extractable, was analyzed using F/M Model 810 G. C. with flame ionization and a 8' stainless steel column packed with SP-2250. The gas conditions for the G. C. were purge air 300 ml/min, H_2 30 ml/min, and N_2 30 ml/min. The G. C. was programmed for 150 thermal operation at 100° C. The compound had a retention time of 3.4 min.

Acrylonitrile, a volatile organic acid (VOA), was analyzed using a Tekmar purge and trap coupled with the aforementioned F/M 810 G. C. for flame ionization. The gas setting remained the same. The sample was purged from the purge tube or gas trap by nitrogen at 60 ml/min for 12 min. Then it was driven off at 180° C and passed over a 8' stainless steel column packed with carbowa 1500 for ionization at 100° C isothermal conditions. The compound has a retention time of 4.0 min.

All three compounds were recorded using a Hewlett Packard Model 3380 A Recorder Integrator interfaced with the G. C.'s.

Data Analysis

Absorbance was converted to total suspended solids (X) from the curve (see Figure 2). The specific growth rate (μ) was then calculated using

$$\mu = \frac{\ln X_t - \ln X_o}{t} \tag{16}$$

which describes log growth (10). The specific growth rates for each substrate concentration (S) were plotted on a plot of μ vs. S, Linweaver-Burk double reciprocal plot ($1/\mu$ vs. $1/S$), and Michaelis-Menton plot of S/μ vs. S and the slopes and intercepts were then used to determine μ_m and K_S using linear regression analysis.

If a reduction in μ_m occurred over the base mix with the compound, the data was modeled using models borrowed from enzyme kinetics. The equations presented in the literature review were used if a particular type of inhibition was exerted, i.e.,

competitive Equation (17) $\mu = \dfrac{\mu_m S}{S + K_S\left(1 + \dfrac{[I]}{K_1}\right)}$

noncompetitive Equation (13) $\mu = \dfrac{\dfrac{\mu_m S}{\left(1 + \dfrac{[I]}{K}\right)}}{S + K_S}$

uncompetitive Equation (14) $\mu = \dfrac{\dfrac{\mu_m \cdot S}{\left(1 + \dfrac{[I]}{K_i}\right)}}{S + \dfrac{K_S}{\left(1 + \dfrac{[I]}{K_i}\right)}}$

$$\text{or} \quad \mu = \frac{\mu_m \cdot S}{(K_S + S + \frac{S^2}{K_i})}$$

The model used depended on whether μ_m or K_S was affected (55).

Yields were determined by ΔX vs. ΔS and specific growth rate vs. specific substrate utilization rate U, respectively. Specific substrate utilization rate was calculated using the substrate removed divided by the mean mass time retention time t, i.e.,

$$U = \frac{S_o - S_t}{\frac{X_t + X_o}{2} \cdot t} \tag{18}$$

Eckenfelder's rate constants were delineated plotting U vs. S_e for K_e and the second order constant K_e' by plotting $S \cdot U$ vs. S_e.

CHAPTER IV

RESULTS

The results of the completely mixed dispersed growth batch system experiments will be presented in this chapter along with the kinetic constants achieved in the completely mixed continuous flow pilot plant studies. The constants will be used as the basis for comparing similar and dissimilar kinetic behavior patterns expressed by the two systems.

Kinetic analysis for the batch system study involved determinations of specific growth rates to establish a maximum specific growth rate μ_m with a saturation constant K_S, specific substrate utilization rate U, biomass yield, and Eckenfelder's rate constants K_e and K_e'. The data discussed and utilized for the batch system can be reviewed in the Appendix.

In order to present a kinetic behavior analysis each analytical procedure and resulting constant(s) developed will be presented for all the compounds. With each parameter sectionalized, changes between the compounds may become more apparent and distinguishable. The order of presentation will be as follows: (A) growth curves based on optical density versus time, (B) derivation of maximum specific growth rate (μ_m) and substrate saturation constant (K_S) by (1) μ versus S, (2) Lineweaver-Burke plot of $1/\mu$ versus $1/S$, and (3) Michaelis-Menton plot of S/μ versus S; (C) determination of Eckenfelder's rate constants K_e and K_e'; and (D) biomass yield coefficients.

Growth Curves

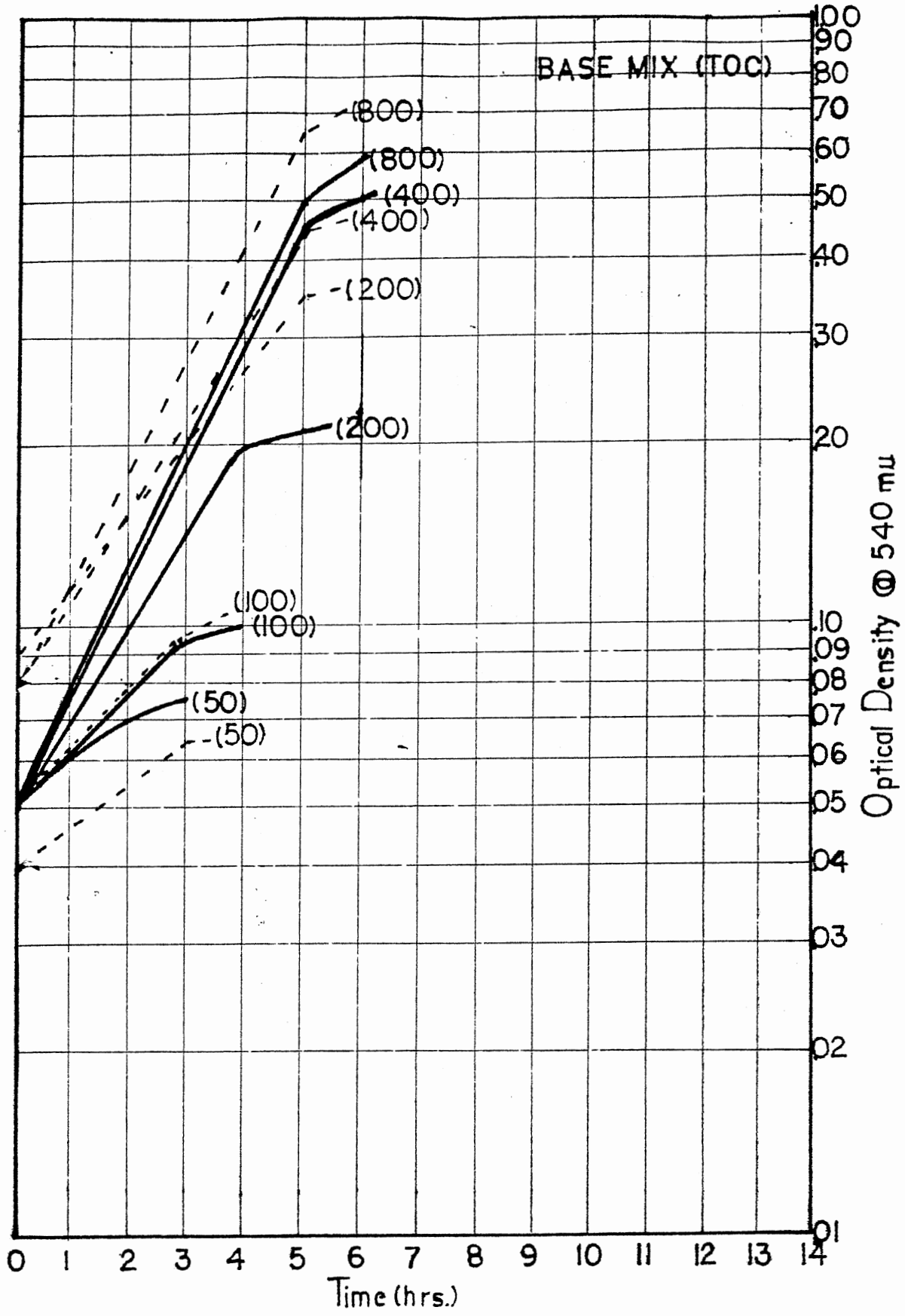
The response of the activated sludge to the base mix was critical to the entire study. Quantitative amounts of base mix remained constant for each reactor at the initial concentrations used. Data for the base mix and the kinetic response achieved from it serves as the reference point for any changes in response from the biomass due to the addition of the specific compounds.

The growth curves presented in the text for the base mix and compounds appear as the increase in biomass, measured by optical density with respect to time. The time frame represented on each graph is that relative to the occurance of the doubling of biomass with lag times deleted. Existence and duration of lag periods to the initiation of growth were consistent for all the experiments at approximately one hour. The only exception to the short lag periods was acrylonitrile. This event will be discussed in the presentation of the growth response to acrylonitrile by the sludge.

Base Mix

Quantitative increases of the base mix in the five reactors from 50 to 800 mg/l TOC resulted in corresponding increases in the slopes of the lines depicting increases in biomass per unit time. This is seen in Figure 3. The specific growth rate μ for a given substrate concentration is determined from the straight line portion of each curve. μ values obtained for substrate applied (in parenthesis) are 0.169 hr^{-1} (79 mg/l), 0.213 hr^{-1} (115 mg/l), 0.347 hr^{-1} (248 mg/l), 0.496 hr^{-1} (469 mg/l), and 0.510 hr^{-1} (835 mg/l) based on TOC. A subsequent experiment with the base mix (dashed lines) achieved μ values of 0.156 hr^{-1} (97 mg/l), 0.250 hr^{-1} (175 mg/l), 0.420 hr^{-1} (310 mg/l), 0.475 hr^{-1} (530 mg/l), and 0.502 hr^{-1} (720 mg/l) TOC.

Figure 3. Growth Curve for the Base Mix


BASE MIX (TOC)
(800)
(800)
(400)
(400)
(200)
(200)
(100)
(100)
(50)
(50)
100
90
80
70
60
50
40
30
20
10
09
08
07
06
05
04
03
02
01
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (hrs.)
Optical Density @ 540 mμ

The values for substrate reported are higher than those printed in the figures for the growth curves. The values in parentheses are actual measured values for TOC. Initial substrate concentrations were higher than the substrate applied because the method of maintaining the stock cultures in the log growth resulted in a residual TOC carry-over. The graphs presented in the text are labled as the theoretical substrate applied, and graphed as actual substrate quantified. Table II presents the measured values for substrate S, specific growth rate, and Se. For a review of the data, see the Appendix.

Acrylonitrile

Incorporation of 50 mg/l acrylonitrile to the corresponding base mix concentrations resulted in the growth curves seen in Figure 4. The specific growth rates obtained reveal an apparent reduction in the specific growth rates compared to those realized with the base mix alone. Acrylonitrile also effected a more pronounced secondary phase of growth than did the base mix.

One hundred mg/l acrylonitrile resulted in a further reduction of the specific growth rates occurring in the primary phase of growth. The ensuing secondary growth phase is steeper as seen in Figure 4. This indicates the system's ability to overcome the effects of the compound, by mechanical or metabolic means.

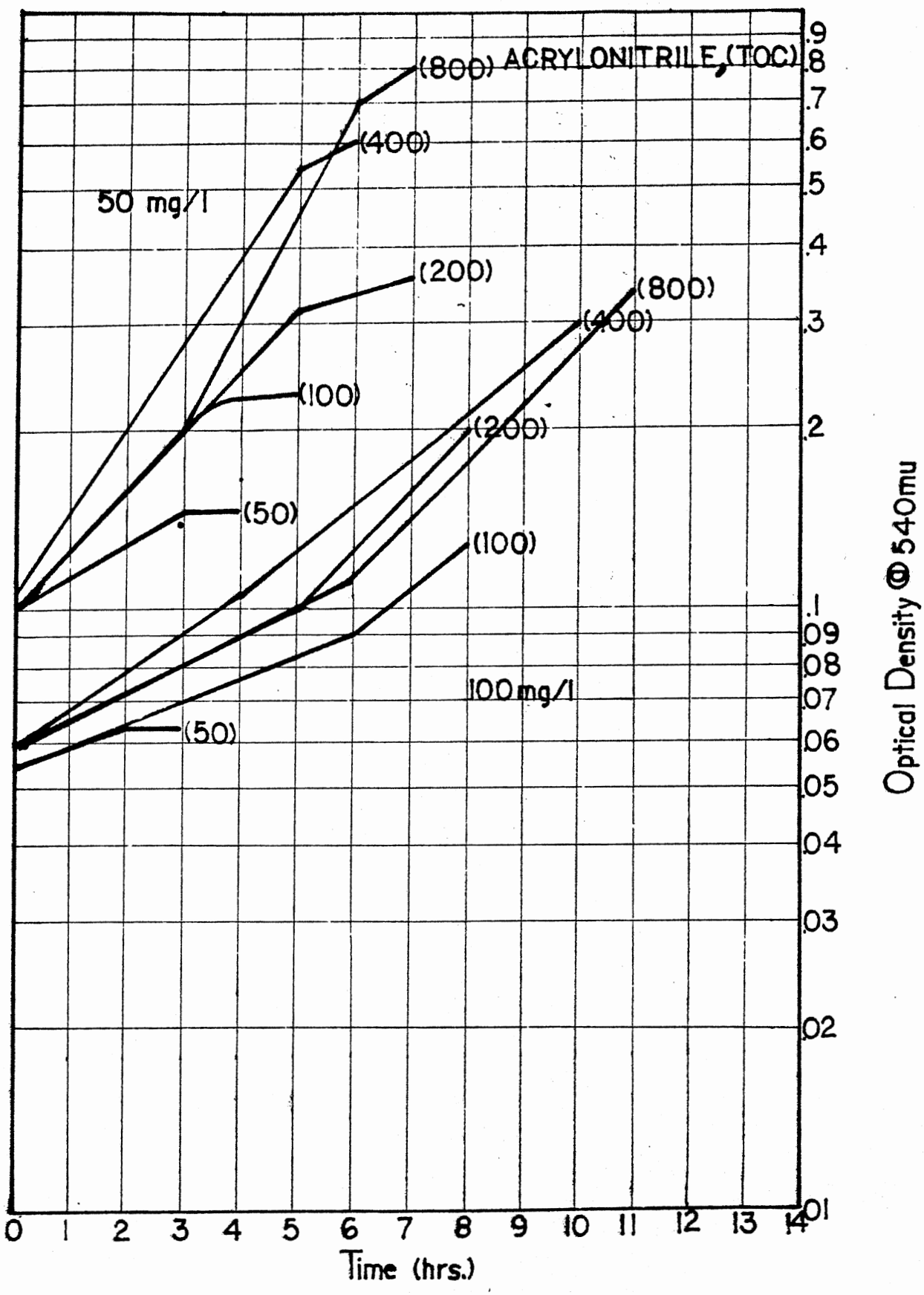
Specific growth rates achieved during the primary response to the compound resulted in values for μ of 0.135 hr^{-1}, 0.231 hr^{-1}, 0.291 hr^{-1}, 0.304 hr^{-1} at 50 mg/l ACN, and 0.068 hr^{-1}, 0.082 hr^{-1}, 0.139 hr^{-1}, 0.132 hr^{-1} and 0.123 hr^{-1} at 100 mg/l ACN. These values show that as the compound remained constant in concentration and the concentration of the base mix increased the values reached a maximum and declined. The

TABLE II

GROWTH RATE μ and S_e From S

Compound	S TOC (mg/l)	S BOD (mg/l)	μ (hr^{-1})	Se TOC (mg/l)	Se BOD (mg/l)
Base Mix	79	170	.168	50	50
	97	174	.156	62	67
	115	297	.213	66	64
	175	252	.250	145	160
	248	630	.347	148	83
	310	389	.420	190	104
	469	1200	.496	205	123
	530	780	.475	250	515
	835	1740	.510	492	240
	720	1433	.502	380	1015
Acrylonitrile	147	234	.135	123	186
	188	330	.213	82	162
(50mg/l)	300	450	.291	94	240
	617	615	.304	194	200
	823	1410	.231	512	300
	198	285	.068	115	198
	265	303	.082	94	120
(100mg/l)	341	435	.139	200	190
	588	670	.132	317	420
	929	1575	.123	788	1440
2,4 Dinitrophenol	250	312	.252	130	96
	450	435	.256	275	235
(25mg/l)	510	1050	.340	300	825
	825	1660	.391	490	1180
	205	156	.138	150	51
	270	375	.187	180	185
(50mg/l)	380	355	.217	220	95
	445	660	.236	225	435
	730	1500	.266	380	1020
1,3 Dichlorobenzene	180	240	.147	130	180
	300	300	.161	130	210
(25mg/l)	350	480	.203	230	420
	490	1140	.220	350	720
	860	1380	.296	540	1200
	180	150	.131	140	90
	325	270	.183	160	105
(50mg/l)	390	420	.213	280	300
	540	1320	.315	600	600
Combined	190		.119	140	
	280		.119	240	
	470		.126	315	
	800		.119	625	
	930		.067	880	

Figure 4. Growth Curve for Acrylonitrile


ACRYLONITRILE, (TOC)
(800)
(400)
50 mg/l
(200)
(100)
(50)
(800)
(400)
(200)
(100)
100 mg/l
(50)
.9
.8
.7
.6
.5
.4
.3
.2
.1
.09
.08
.07
.06
.05
.04
.03
.02
.01
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (hrs.)
Optical Density @ 540mu

values themselves hold no enlightenment as how the combination of ACN and base mix are exerting an effect on the system.

The secondary growth phase in the diauxic growth response at 100 mg/l ACN resulted in specific growth rate values approaching those of the primary phase at the 50 mg/l ACN. Phase II resulted in μ values of 0.184, 0.223, 0.217 and 0.214 hr^{-1} which compares to values of 0.231, 0.291, 0.304, and 0.23 hr^{-1} for Phase I with 50 mg/l ACN.

Off-gas analysis performed on samples collected during operation of the system showed some evidence of acrylontrile stripping from the reactors during the first two hours of the study. Quantitative amounts were 7.8 mg/hr in Reactor II, 5.4 mg/hr in Reactor IV and 3.3 mg/hr in Reactor V. Continued sampling did not reveal any further stripping of the compound. The values presented are found in Table III.

2, 4 Dinitrophenol

Incorporation with the base mix of 2, 4 dinitrophenol (2, 4 DNP) in the systems as potential substrate, produced the growth curves seen in Figure 5. Solid lines depict the response of the activated sludge to 25 mg/l, 2,4 DNP, and the dashed lines are for 50 mg/l, 2,4 DNP. The growth curves again manifest a diphasic response for both concentrations of 2,4 DNP used. Different from the response to acrylonitrile, the μ values achieved with 2,4 DNP are still increasing as the substrate concentration in the reactors increases. The quantitative increase in 2,4 DNP from 25 to 50 mg/l resulted in lowered specific growth rates from 0.252, 0.256, 0.340, and 0.391 hr^{-1} with 25 mg/l to 0.138, 0.187, 0.217, 0.236, and 0.266 with 50 mg/l.

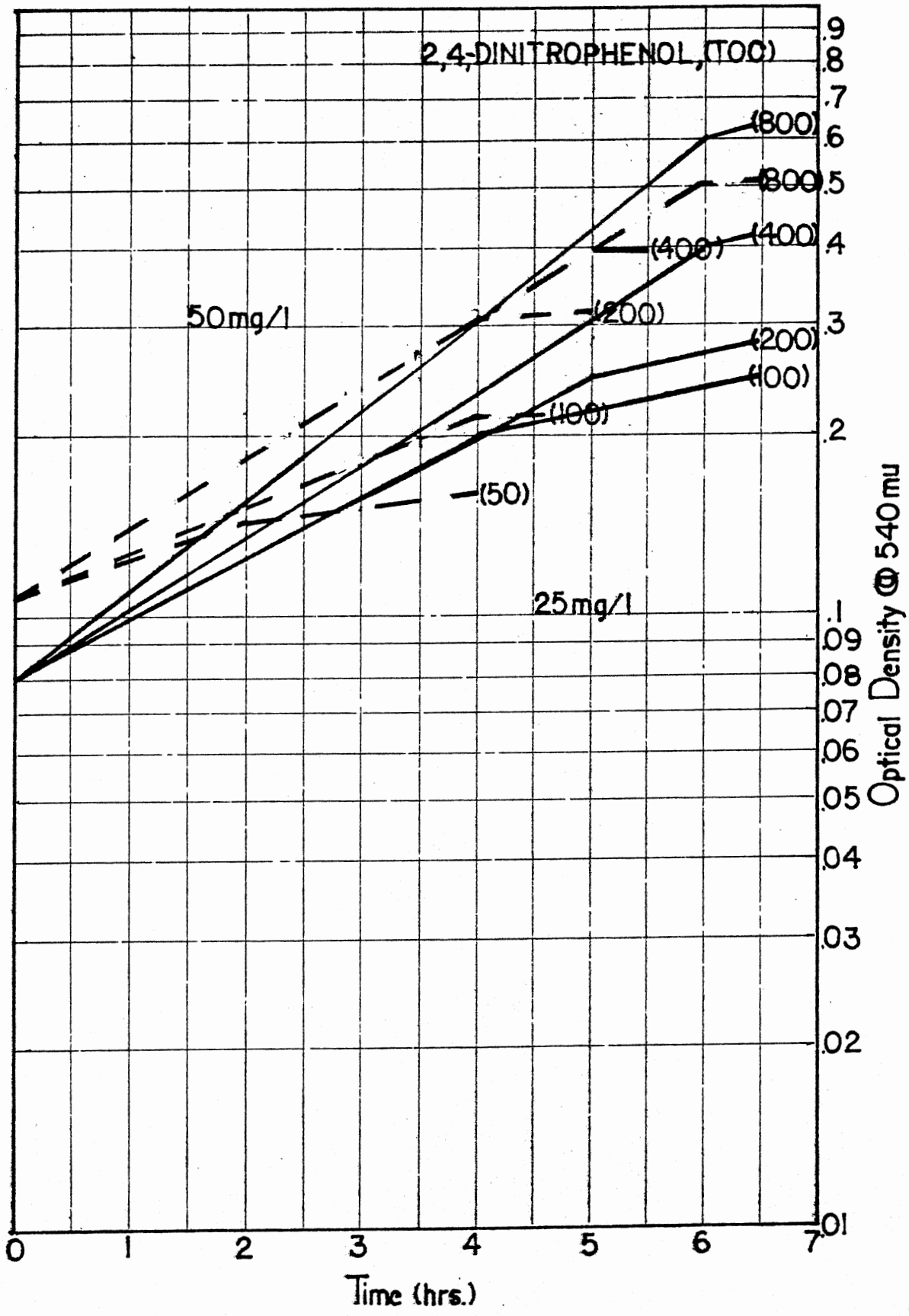
The reduction in specific growth rates by acrylonitrile and

TABLE III

GAS CHROMATOGRAPHY RESULTS FOR THE COMPOUNDS

Compound	Column	C.C.	Reactor	Time (hrs)	Conc (mg/l)	Time (hrs)	Conc (mg/l)	Time (hrs)	Conc (mg/l)
Acrylonitrile	Carbowax 1500	F/M	II	2	7.8 (mg/hr)	4	ND		
Off-Gas			IV	2	5.4 (mg/hr)	4	ND		
(ACN)			V	2	3.3 (mg/hr)	4	ND		
2,4 Dinitrophenol (2,4 DNP)			III	0	55	8	57	24	48.7
	SP1240-DA	HP	V	0	63	8	60	24	50.6
Off-Gas			I-V	0	—	4	ND		
Phenol									
	SP 1240 DA	HP	III	0	10	8	4.7	24	< .5
			V	0	40	8	28	24	< .5
Off-Gas			I-V	0	—	4	ND		
1,3 Dichlorobenzene									
	SP 2250	F/M	I	0	32.6	6	6.6		
			V	0	35.3	6	0.24		
Off-Gas			I-V	0	—	4	ND		
Combined									
			II	0	80	4	7.6	8	6.8
ACN	CARB. 1500	F/M	IV	0	72	4	—	8	3.6
			V	0	86	4	—	8	2.8
Off-Gas			II	0	—	4	9.2	8	ND
			IV	0	—	4	5.1	8	ND
			V	0	—	4	4.3	8	ND
DCB	SP 2250	F/M	II	0	44	8	1.36	38	0.14
			IV	0	47.6	8	0.83	38	0.18
			V	0	46.7	8	1.03	38	0.15
DNP	SP 1240 - DA	HP	II	0	32.2	8	30.0	38	33.0
			IV	0	30.4	8	32.6	38	25.5
			V	0	40.0	8	37.8	38	12.0
Phenol	SP 1240 - DA	HP	II	0	5.4	8	< .5		
			IV	0	22.5	8	< .5		
			V	0	38.4	8	< .5		

Figure 5. Growth Curve for 2,4 Dinitrophenol


2,4-DINITROPHENOL, (TOC)
50mg/l
25mg/l
(800)
(800)
(400)
(400)
(200)
(200)
(100)
(100)
(50)
Optical Density @ 540mu
Time (hrs.)
0
1
2
3
4
5
6
7
.9
.8
.7
.6
.5
.4
.3
.2
.1
.09
.08
.07
.06
.05
.04
.03
.02
.01

2,4 dinitrophenol over those of the base mix showed similar amounts based on equivalent mass of compound. The 50 mg/l concentration of the two compounds had μ values of 0.138, 0.187, 0.217, 0.236 and 0.266 hr^{-1} for 2,4 DNP compared to 0.135, 0.231, 0.291, 0.304, and 0.231 hr^{-1} for ACN, at equivalent base mix concentrations. Unlike acrylonitrile 2,4 dinitrophenol did not have the declining specific growth rates at the higher substrate concentrations.

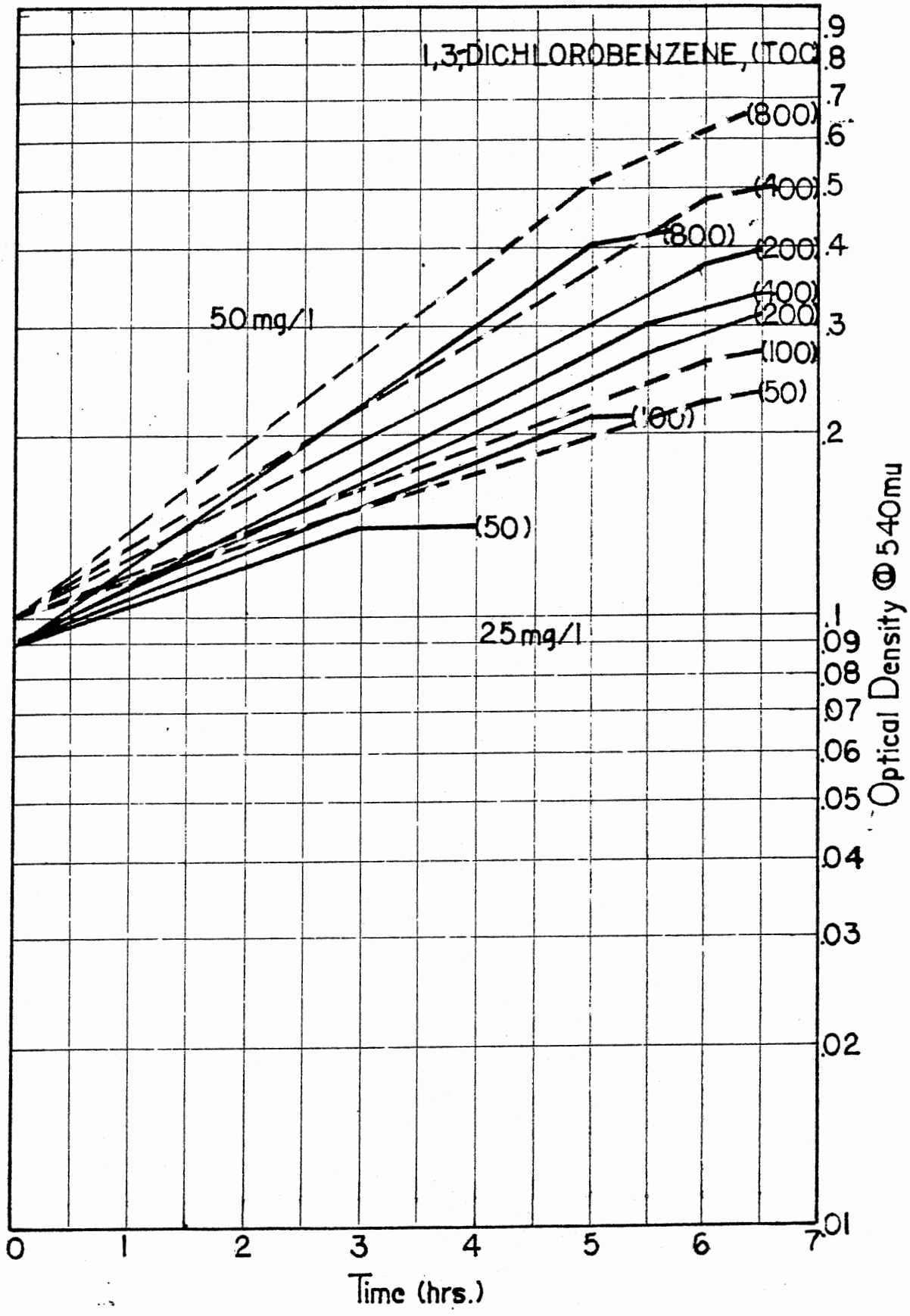
Specific analysis by gas chromatography showed no significant removal of 2,4 DNP during the primary growth phase. Its removal transpired at a very slow rate initiated in the latter stages of the primary growth phase. This occurred at approximately eight hours. Comparatively the phenol present in the base mix was removed ~ 40% by eight hours in Reactor III and V. At 50 mg/l 2, 4 DNP was reduced by 15% in Reactor IV and 20% in Reactor V at 24 hours. This is seen in Table III.

1,3 Dichlorobezene

1,3 Dichlorobenzene introduced into the growth systems as substrate achieved the growth curves seen in Figure 6. The overall observation of the addition of 25 and 50 mg/l 1,3 DCB appears similar to that seen with 2,4 DNP. The significant difference is that the increase from 25 to 50 mg/l 1,3 DCB resulted in a slight increase in specific growth rates. The increase from 25 to 50 mg/l 2,4 DNA had an overall decrease in μ.

Specific growth rates achieved with 25 mg/l 1,3 DCB were similar to those produced by 25 mg/l 2,4 DNP. 1,3 DCB achieved values of 0.147, 0.161, 0.203, 0.220, and 0.296 hr^{-1} compared to 0.220, 0.256, 0.340, and 0.391 hr^{-1} for 25 mg/l DNP. These values, which are presented in Table II, have very little comparative value without a comparison of total substrate applied. This will become apparent in the μ vs. S and $1/\mu$ vs.

Figure 6. Growth Curve for 1,3 Dichlorobenzene


1,3-DICHLOROBENZENE, (TOC)
(800)
(400)
(800)
(200)
(400)
(200)
(100)
(50)
(100)
(50)
50 mg/l
25 mg/l
.9
.8
.7
.6
.5
.4
.3
.2
.1
.09
.08
.07
.06
.05
.04
.03
.02
.01
Optical Density @ 540mu
0
1
2
3
4
5
6
7
Time (hrs.)

1/S plots presented later.

50 mg/l 1,3 DCB resulted in slightly higher values for μ over that achieved with 25 mg/l. These values were still lower than those achieved with the base mix alone. Values for μ in response to 50 mg/l 1,3 DCB were 0.131, 0.183, 0.213, 0.247, and 0.315 hr^{-1}.

Specific analysis for 1,3 dichlorobenzene is found in Table III. At 6 hours, 1,3 DCB had a concentration of 6.59 mg/l in Reactor I, and at 10 hours a concentration of 0.24 mg/l in Reactor V. The initial concentrations of 1,3 DCB were 32 mg/l in I and 35 mg/l in V. Thus a better than 80% removal of the compound was achieved in Reactor I and better than 90% in Reactor V.

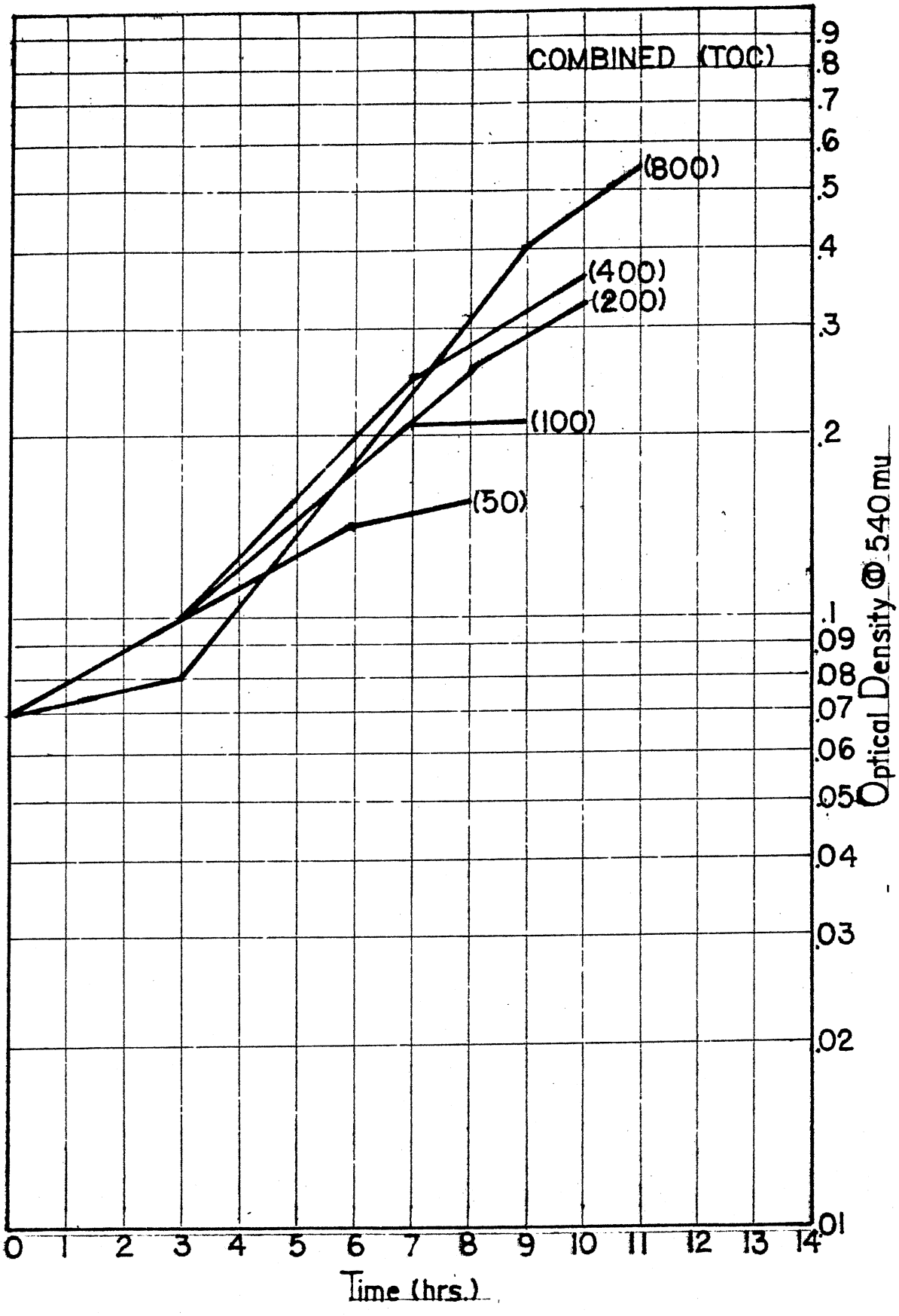
Combined

Addition of all three compounds; acrylonitrile, 2,4 dinitrophenol, and 1,3 dichlorobenzene, at the lower concentrations used in the individual studies, resulted in the growth curves in Figure 7. As can be seen, a triphasic curve was established. The first phase of the growth response to increaesed substrate yielded values for μ of 0.119, 0.119, 0.126, 0.119, and 0.067 hr^{-1}. Phase II resulted in values of 0.150, 0.185, 0.196, 0.239, and 0.282 hr^{-1} Phase III had μ values of 0.033, 0.032, 0.106, 0.118, and 0.181 hr^{-1}.

The primary phase of growth had very little change in μ in response to S. Phase II had higher values resembling the primary phases of 1,3 DCB and 2,4 DNP. Phase III also had lower values possibly due to the availability of substrate at this time in the growth curve.

G. C. analysis on the combined unit depicted again some evidence of stripping of acrylonitrile similar to that seen in the individual study. No stripping of 2,4 DNP or 1,3 DCB was witnessed in any of the studies.

Figure 7. Growth Curve for Combined Study


COMBINED (TOC)
(800)
(400)
(200)
(100)
(50)
.9
.8
.7
.6
.5
.4
.3
.2
.1
.09
.08
.07
.06
.05
.04
.03
.02
.01
Optical Density @ 540mu
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (hrs.)

1,3 Dichlorobenzene and phenol showed evidences of sequential removal in the growth systems. At eight hours, 1,3 DCB had a concentration of 1.4 mg/l in Reactor II, 0.83 mg/l in Reactor IV, and 1.03 mg/l in Reactor V. Comparatively, Phenol at eight hours was 0.12 mg/l (II), 12.8 mg/l (IV), and 35.7 mg/l (V). This suggests that 1,3 dichlorobenzene is more easily utilized by the sludge than phenol.

2,4 Dinitrophenol was reduced by less than 1% in Reactors II, IV, and V by eight hours. At 38 hours, Reactor II had achieved no removal of 2,4 DNP, Reactor IV had a 21% reduction, and Reactor V achieved a 70% reduction. The increased removal of 2,4 DNP as base mix concentration increased suggests that higher substrate concentrations effect increased utilization of the compound.

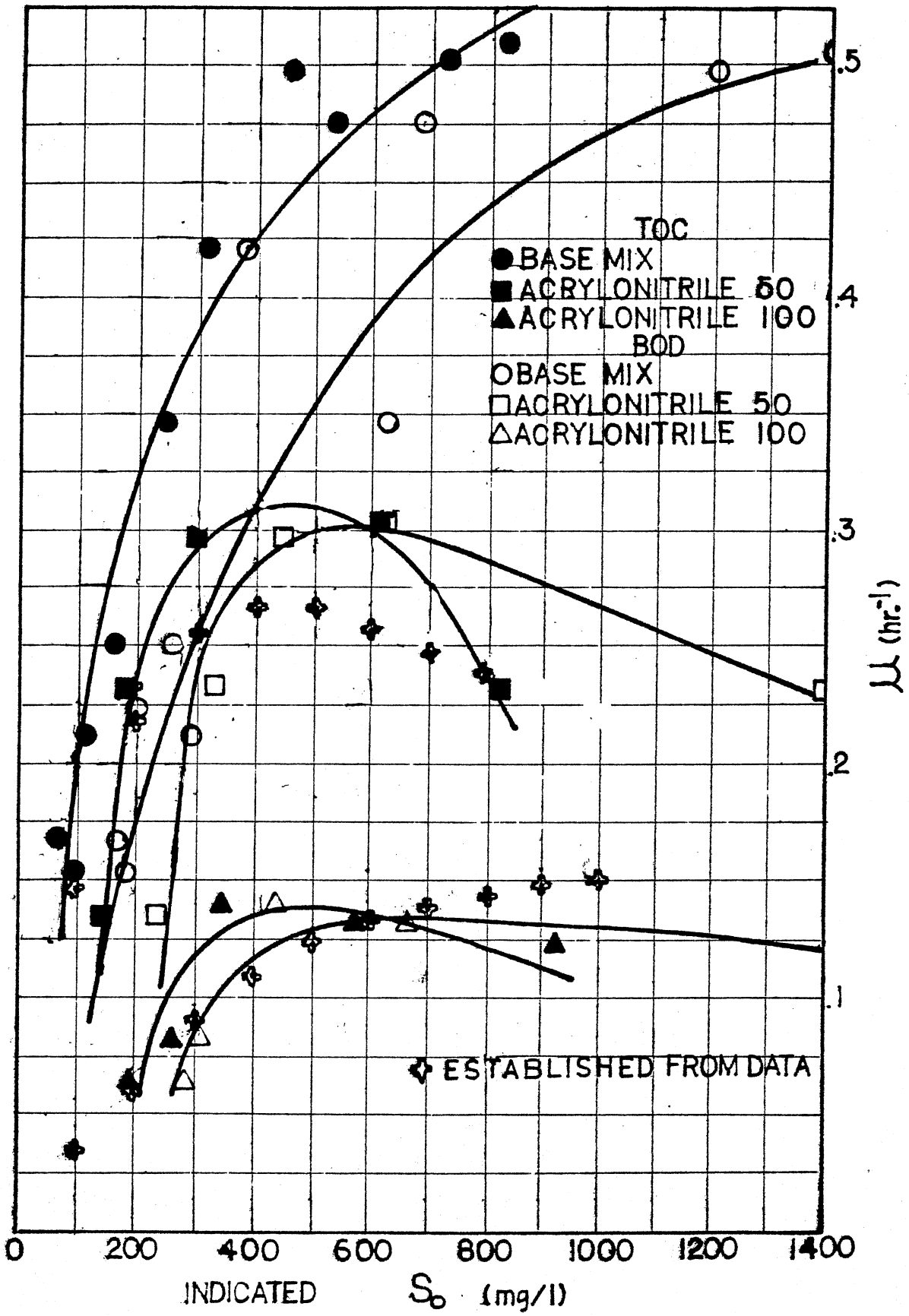
Acrylonitrile at eight hours, well after the primary phase had ended, had residual concentrations of 6.8 mg/l (II), 3.6 mg/l (IV), and 3.8 mg/l (V). Again, as in the individual studies, acrylonitrile involvement appears to be in the primary growth phase.

Determination of μ_m and K_S

μ Versus S

Base Mix. Plot of specific growth rate versus substrate applied delineates the curve-linear relationship of μ to S as seen in Figure 8. The growth rate response to substrate increases as substrate concentratio increases until μ reaches a maximum. The plot for the base mix shows no tendency for a maximum specific growth rate as it has no defined inflection point and the curve has not become asymptotic to the X-axis. The plots for TOC and BOD_5 of the base mix have slopes that are similar and appear to be approaching a common maximum value. The

Figure 8. Plot of μ Vs. S for Base Mix and Acrylonitrile


TOC
BASE MIX
ACRYLONITRILE 50
ACRYLONITRILE 100
BOD
BASE MIX
ACRYLONITRILE 50
ACRYLONITRILE 100
ESTABLISHED FROM DATA
.5
.4
.3
.2
.1
μ (hr.⁻¹)
0
200
400
600
800
1000
1200
1400
INDICATED S_o (mg/l)

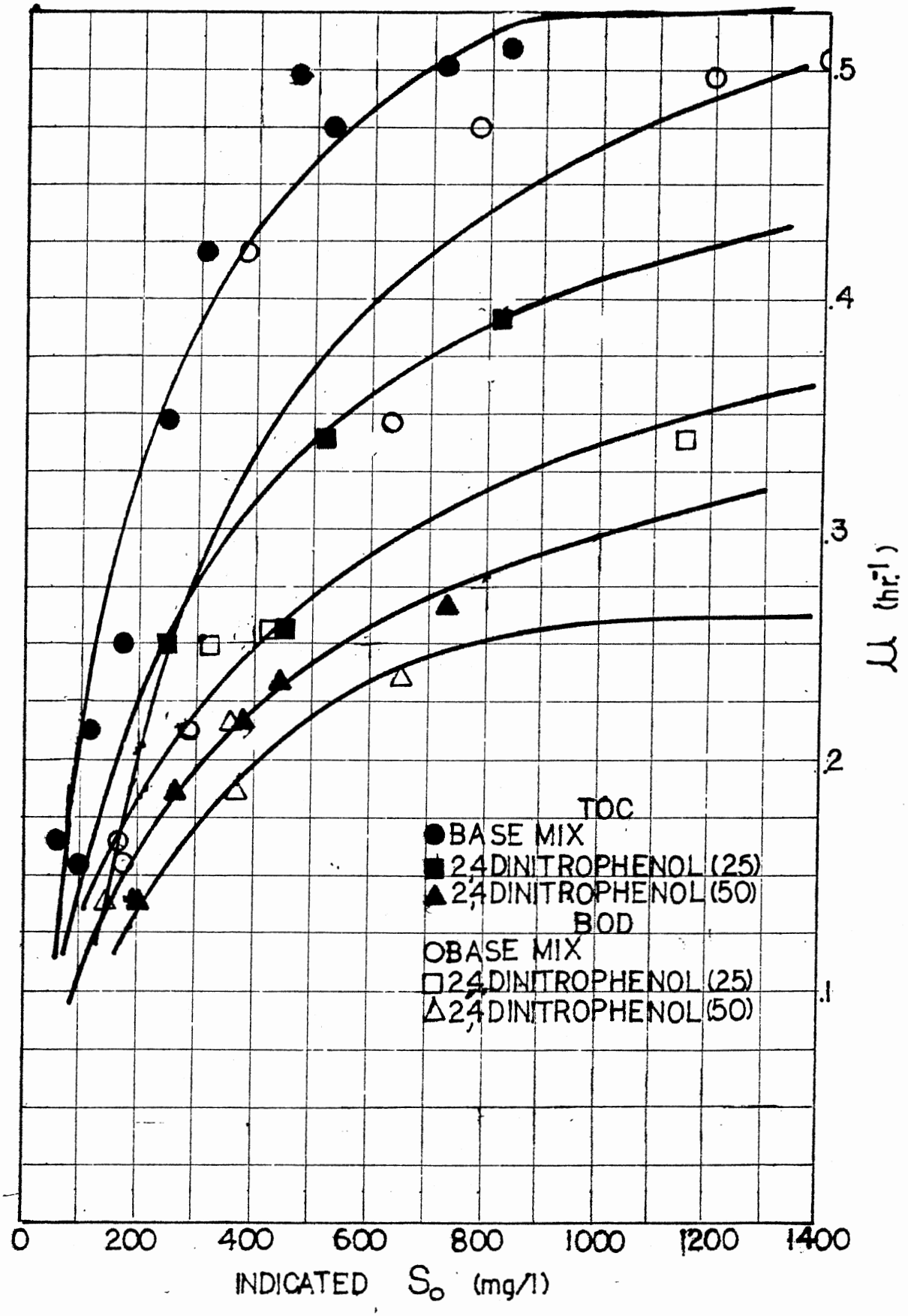
difference between the curves is the relative substrate required to achieve a maximum rate greater for BOD_5 than for TOC.

Acrylonitrile. Curves for the base mix show no evidence of an atypical response in μ to S. Acrylonitrile, as seen in Figure 8, delineates the atypical values presented in the discussion on growth curves. As depicted, the increase in concentration of the base mix in combination with acrylonitrile resulted in higher specific growth rates which reached an apparent maximum and then diminished. This type of behavior is exhibited for both concentrations of ACN used. The difference between the two concentrations is seen in the seemingly quantitative reduction in μ as ACN is quantitatively increased.

If the curves for acrylonitrile or the base mix had become asymptotic to the X-axis, the X-asymptote would delineate a maximum specific growth rate (μ_m) and the substrate concentration at a value of 1/2 μ_m would establish the saturation constant K_S. Since acrylonitrile has exhibited an atypical plot for μ vs. S, the derivation of μ_m and K_S must be accomplished by other means. This will be presented in the $1/\mu$ vs. 1/S section.

2,4 Dinitrophenol. 2,4 DNP resulted in the plot in Figure 9 for μ vs. S. When compared to that of the base mix, the curves show no delineation of a μ_m as does the base mix. Apparent between the curves for 2,4 DNP and the base mix is an effective increase in K_S. Similarly, a quantitative increase in 2,4 DNP from 25 to 50 mg/l shows a further increase in K_S. Evaluation of the curves for the base mix and DNP systems demonstrates the increased relative amount of substrate needed to achieve an equivalent specific growth rate. At a specific growth rate of 0.3 hr^{-1} the base mix alone required 150 mg/l TOC, the base mix plus

Figure 9. Plot of μ Vs., S for 2,4 Dinitrophenol


.5
.4
.3
.2
.1
μ (hr.⁻¹)
TOC
BASE MIX
2,4 DINITROPHENOL (25)
2,4 DINITROPHENOL (50)
BOD
BASE MIX
2,4 DINITROPHENOL (25)
2,4 DINITROPHENOL (50)
0
200
400
600
800
1000
1200
1400
INDICATED S_o (mg/l)

25 mg/l 2,4 DNP required 350 mg/l TOC, and the base mix plus 50 mg/l 2,4 DNP required 675 mg/l TOC.

1,3 Dichlorobenzene. Specific growth rate plotted against substrate applied for 1,3 dichlorobenzene appears in Figure 10. The response for 1,3 DCB appears similar to that of 2,4 DNP. Again, μ_m is not clearly defined by the curve. The change in K_S from that of the base mix with the addition of 25 mg/l (1,3 DCB) is similar to that seen with 25 mg/l 2,4 DNP. 50 mg/l of 1,3 DCB achieved no further change in K_S as was seen with 50 mg/l 2,4 DNP. Both compounds changed the shape of the curves and at this point do not delineate any inhibition or changes in μ_m.

Combined. Figure 11 presents the combined effects of the three compounds and the three phases of growth achieved. The different phases of growth appear to mimick the primary growth phases experienced by the individual compounds. Growth Phase I is the only phase which indicates an inhibited response over that of the individual studies. This assumes that each growth phase is relative to a specific compound.

G. C. analysis from Table III shows that for each growth phase, one compound out of the three was removed and presumably exerting the most effect on μ. Acrylonitrile appears to be involved in the Phase I, 1,3 DCB in Phase II, and 2,4 DNP in Phase III. The corresponding growth curves for each phase show marked agreement in shape with the curves established by the individual studies. As stated earlier, acrylonitrile was removed by 90% in 8 hours, 1,3 dichlorobenzene 95% in 8 hours, and 2,4 DNP 2% in 8 hours.

Figure 10. Plot of μ Vs. S for 1,3 Dichlorobenzene

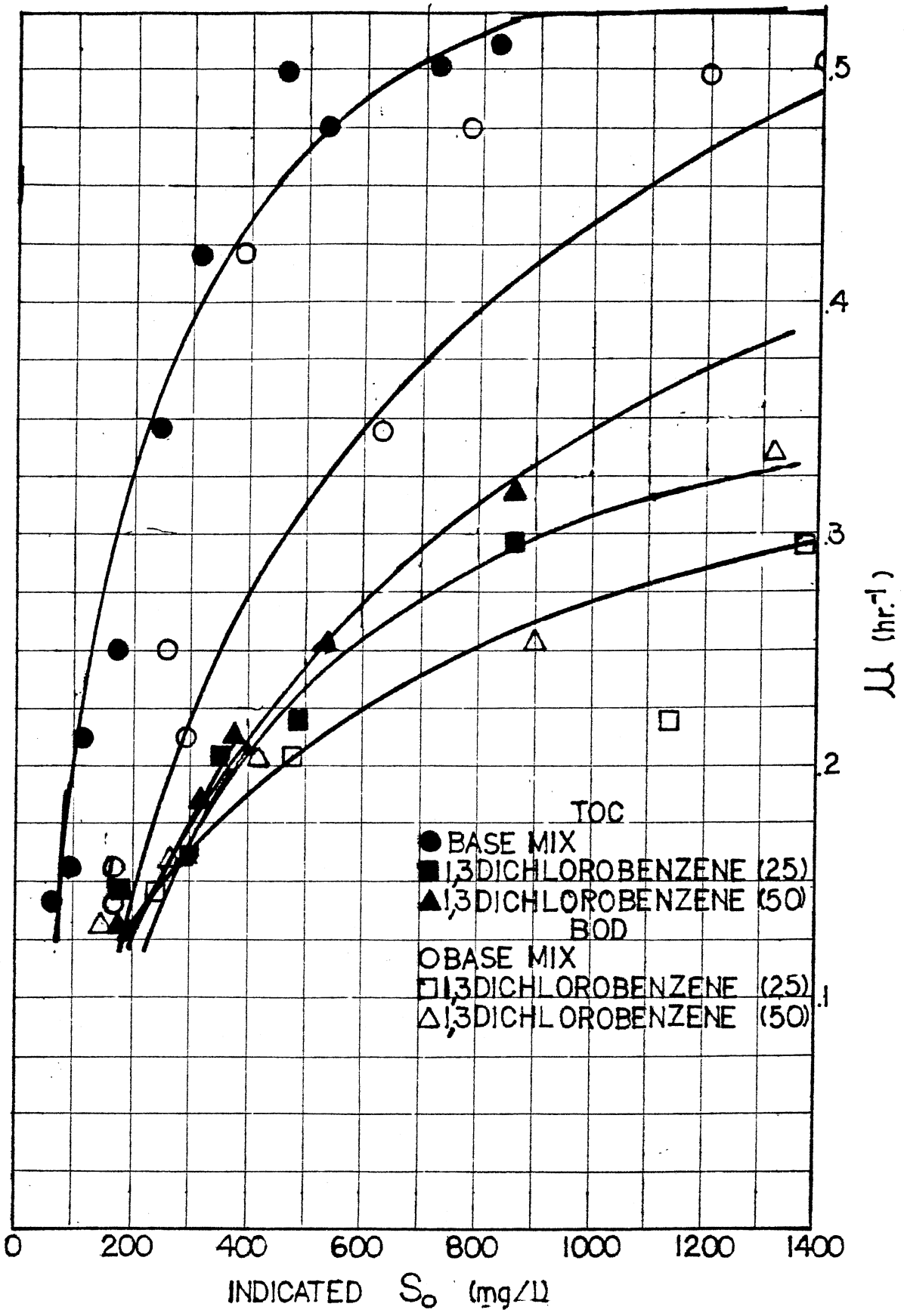
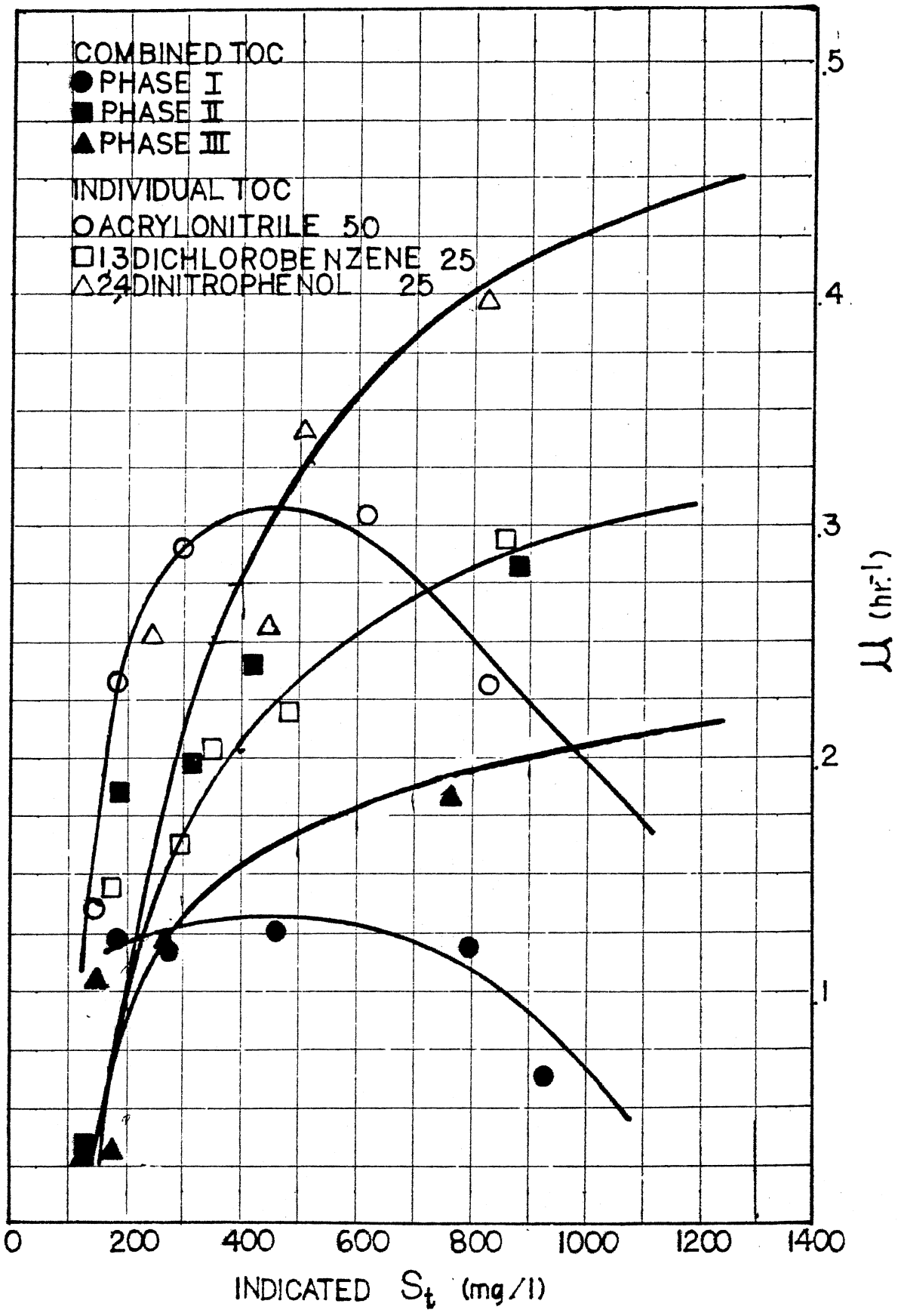

.5
.4
.3
.2
.1
μ (hr.⁻¹)
TOC
BASE MIX
1,3DICHLOROBENZENE (25)
1,3DICHLOROBENZENE (50)
BOD
BASE MIX
1,3DICHLOROBENZENE (25)
1,3DICHLOROBENZENE (50)
0
200
400
600
800
1000
1200
1400
INDICATED S_o (mg/l)

Figure 11. Plot of μ Vs. S_t for Combined Study


COMBINED TOC
PHASE I
PHASE II
PHASE III
INDIVIDUAL TOC
ACRYLONITRILE 50
1,3 DICHLOROBENZENE 25
2,4 DINITROPHENOL 25
.5
.4
.3
.2
.1
μ (hr⁻¹)
0
200
400
600
800
1000
1200
1400
INDICATED S_t (mg/l)

Lineweaver-Burke

Lineweaver-Burke plots are a method of linearizing the data from the μ versus S curves by taking the reciprocal of μ, $(1/\mu)$ plotted against the reciprocal of S, (1/S).

Base Mix. The double reciprocal plot for the base mix is found in Figure 12. The data forms a linear array establishing a line with a $1/\mu$ axis intercept at 1.50 hr and a slope of 346 mg/hr. For this plot, the $1/\mu$-axis intercept has a value of $1/\mu_m$ with the slope of the line equal to K_S/μ_m. The value for μ_m established by the curve is 0.65 hr^{-1} with a K_S of 225 mg/l. BOD_5 data for the base mix exhibits the same μ_m as that for TOC of 0.65 hr^{-1} with a change in K_S to 502 mg/l BOD_5. This would stand to reason since the units for μ_m are hr^{-1} with no reference to mass. Values for μ_m, K_S, and K_i are found in Table IV.

Acrylonitrile. The plot of $1/\mu$ vs. 1/S for ACN also appears in Figure 12. The data does not form a linear function but rather reflects an inverted curve like the one seen in the μ vs. S plots. Evaluation of the data then is dependent on the data points in Figure 8 that occur prior to the bending over of the curve as demonstrated by Reddy and Kincannon (46). Based on the first three points in the 50 mg/l ACN and the first two points in the 100 mg/l ACN experiments, both establish a μ_m or 0.65 hr^{-1}. This is equal to the μ_m of the base mix at 0.65 hr^{-1}.

With μ_m the same, it is also seen that the points establish an increase in K_S from 200 mg/l TOC to 325 mg/l TOC at 50 mg/l ACN and 1755 mg/l TOC at 100 mg/l ACN. The model used to describe this type of relationship is that of Haldane's (46) where

Figure 12. Lineweaver-Burke Plot of $1/\mu$ Vs. $1/S$ for Base Mix and Acrylonitrile

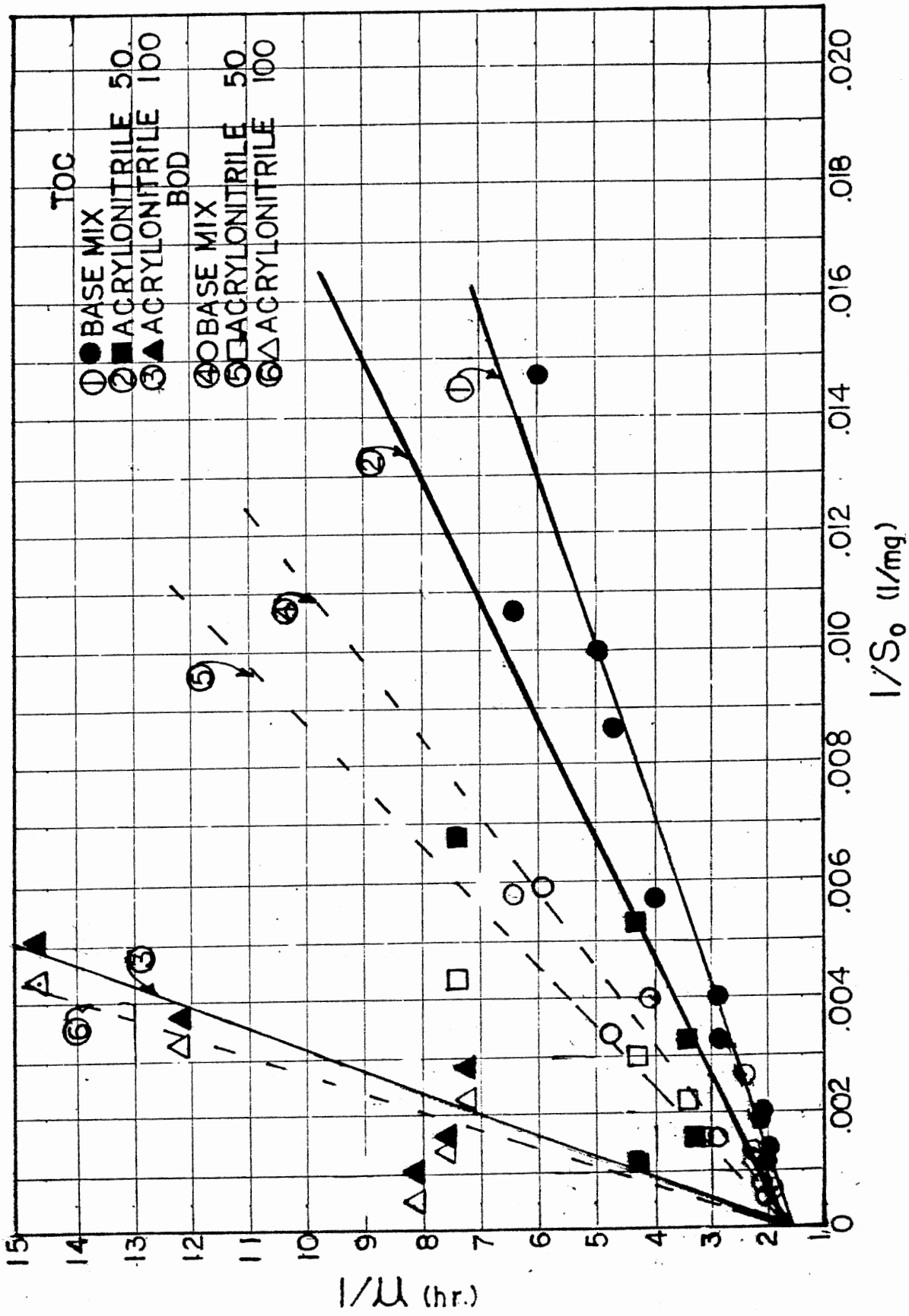

TOC
① ● BASE MIX
② ■ ACRYLONITRILE 50
③ ▲ ACRYLONITRILE 100
BOD
④ ○ BASE MIX
⑤ □ ACRYLONITRILE 50
⑥ △ ACRYLONITRILE 100
1/μ (hr.)
1/S₀ (1/mg.)
0
.002
.004
.006
.008
.010
.012
.014
.016
.018
.020
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

TABLE IV

KINETIC CONSTANTS: μ_m, K_s, K_i

Compound	Conc.	TOC μ_m (hr^{-1})	TOC K_s (mg/l)	TOC K_i (mg/l)	BOD μ_m (hr^{-1})	BOD K_s (mg/l)	BOD K_i (mg/l)
Base Mix							
1/μ vs 1/s		0.65	225		0.65	487	
s/μ vs s		0.65	260		0.65	585	
Acrylonitrile	(50mg/l)						
1/μ vs 1/s		0.65	325	600	0.65	620	600
s/μ vs s		0.65	325	600	0.65	683	600
	(100mg/l)						
1/μ vs 1/s		0.65	1755	600	0.65	2031	600
s/μ vs s		0.65	1755	600	0.65	2000	600
2,4 Dinitrophenol	(25mg/l)						
1/μ vs 1/s		0.50	250	80	0.50	400	80
s/μ vs s		0.51	259	80	0.50	400	80
	(50mg/l)						
1/μ vs 1/s		0.40	356	80	0.40	400	80
s/μ vs s		0.40	374	80	0.40	400	80
1,3 Dichlorobenzene							
1/μ vs 1/s		0.45	450		0.45	439	
s/μ vs s		0,43	430		0.43	473	
Combined							
1/μ vs 1/s							
PHASE I		0.126	30				
PHASE II		0.45	450				
PHASE III		0.50	1400				
s/μ vs s							
PHASE I		0.126	30				
PHASE II		0.45	430				
PHASE III		0.5	850				

$$\mu = \frac{\mu_m \cdot S}{(K_S + S + \frac{S^2}{K_i})} \cdot$$

Using the μ_m and K_S established by the plots with a K_i of 600 mg/l, the relative agreement with the data is seen in Figure 8. The calculated values used are found in Table V.

Again, as seen with the base mix, the BOD_5 data establishes the same μ_m as that of TOC with a change in K_S. K_S increased from 500 mg/l BOD_5 for the base mix to 620 mg/l BOD_5 for 50 mg/l ACN and 2031 mg/l BOD_5 for 100 mg/l ACN.

2,4 Dinitrophenol. The $1/\mu$ vs. $1/S$ plot for 2,4 DNP, Figure 13, resulted in a different response than acrylonitrile. Both the 25 and 50 mg/l 2,4 DNP concentrations effected changes in the $1/\mu$-axis intercept and the slope of the line. The μ_m for 25 mg/l, 2,4 DNP, is 0.5 hr^{-1} with a K_S of 250 mg/l TOC. 50 mg/l of 2,4 DNP resulted in a μ_m of 0.4 hr^{-1} with a K_S of 356 mg/l TOC.

Kinetic models available to model changes in μ_m and K_S are those used in enzyme kinetics. In enzyme systems, changes in μ_m and K_S are evaluated based upon three basic types of response and labeled as competitive, uncompetitive, and non-competitive inhibition. The terminology is applied to specific-enzyme substrate-inhibitor interactions. In dealing with complex cellular systems, the same terminology is not necessarily adequate. The mixture of two waste waters could effect a change in the metabolic activities in the system reflected by a change in μ_m and K_S (5).

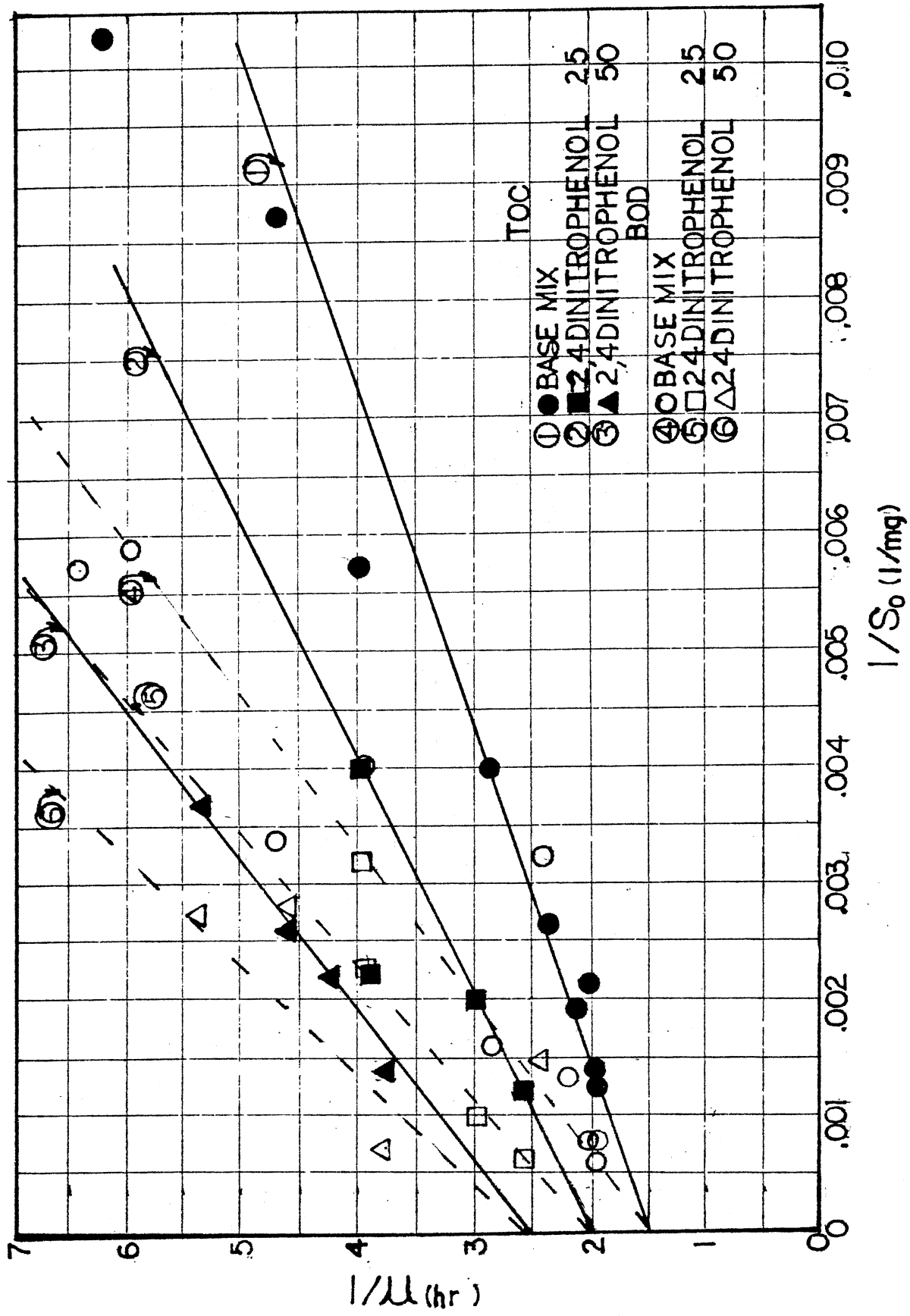
Evaluating the change in $1/\mu$-axis intercept as seen in Figure 13 depicts a change in μ_m. This is equal to $(1 + \frac{[I]}{K_i})/\mu_m$ (35). Solving

TABLE V

CALCULATED VALUES FOR MODEL

Compound	μ (hr^{-1})	Given S (mg/l)	Equation
Acrylonitrile	.147	100	
	.220	200	
μ_m = 0.65(hr^{-1})	.252	300	
K_s = 325(mg/l)	.252	400	
K_i = 600(mg/l)	.262	500	
	.262	600	
	.256	700	
	.247	800	$\mu = \frac{\mu_m \cdot S}{(K_s + S + S/K_i)}$
	.035	100	
	.064	200	
μ_m = 0.65(hr^{-1})	.088	300	
K_s = 1755(mg/l)	.107	400	
K_i = 600(mg/l)	.122	500	
	.132	600	
	.139	700	
	.144	800	

Figure 13. Lineweaver-Burke Plot of $1/\mu$ Vs. $1/S$ for 2,4 Dinitrophenol


TOC
①●BASE MIX
②■2,4DINITROPHENOL 25
③▲2,4DINITROPHENOL 50
BOD
④○BASE MIX
⑤□2,4DINITROPHENOL 25
⑥△2,4DINITROPHENOL 50
1/Lt (hr)
1/So (1/mg)
0
1
2
3
4
5
6
7
.001
.002
.003
.004
.005
.006
.007
.008
.009
.010

for K_i in both cases, gives a K_i of 80 mg/l.

BOD_5 achieved agreement with the μ_m obtained for both experiments with subsequent changes in K_S from 487 mg/l to 429 mg/l at 25 mg/l 2,4 DNP and 567 mg/l BOD_5 at 50 mg/l 2,4 DNP.

The TOC data realized a reduction in μ_m with an increase in K_S. This is not addressed by the models presented in the literature, i.e., Equations (12, (13), and (14).

1,3 Dichlorobenzene. 1,3 Dichlorobenzene produced a different response to substrate than 2,4 dinitrophenol and acrylonitrile as seen in Figure 14. The quantitative increase in 1,3 dichlorobenzene from 25 to 50 mg/l effected an apparent increase in the specific growth rates but, as can be seen in Figure 14, μ_m and K_S are common to both concentrations used. μ_m is determined to be 0.45 hr^{-1} with K_S of 450 mg/l. Again, BOD_5 had the same μ_m of 0.45 hr^{-1} with a K_S of 439 mg/l.

Combined. The combined study plotted on the double reciprocal plot in Figure 15 shows the relationship of the three growth phases compared to the individual studies. Assuming each of the three growth phases was resultant from the effect of an individual compound, the $1/\mu$-axis intercepts and slopes should mimick those seen with the individual studies.

Phase I of the growth curve was the only phase affected as far as a change in μ_m over that of the individual studies. μ_m for Phase I is 0.125 hr^{-1}. Looking at the μ vs. S (Figure 8) curve it is seen that a similar curve to that for acrylonitrile was established. Phase II and Phase III both show agreement with the μ_m from the individual studies, Phase II had no change in K_S, while Phase III saw an increase from 250 mg/l to 1400 mg/l TOC.

Figure 14. Lineweaver-Burke Plot of $1/\mu$ Vs. $1/S$ for 1,3 Dichlorobenzene

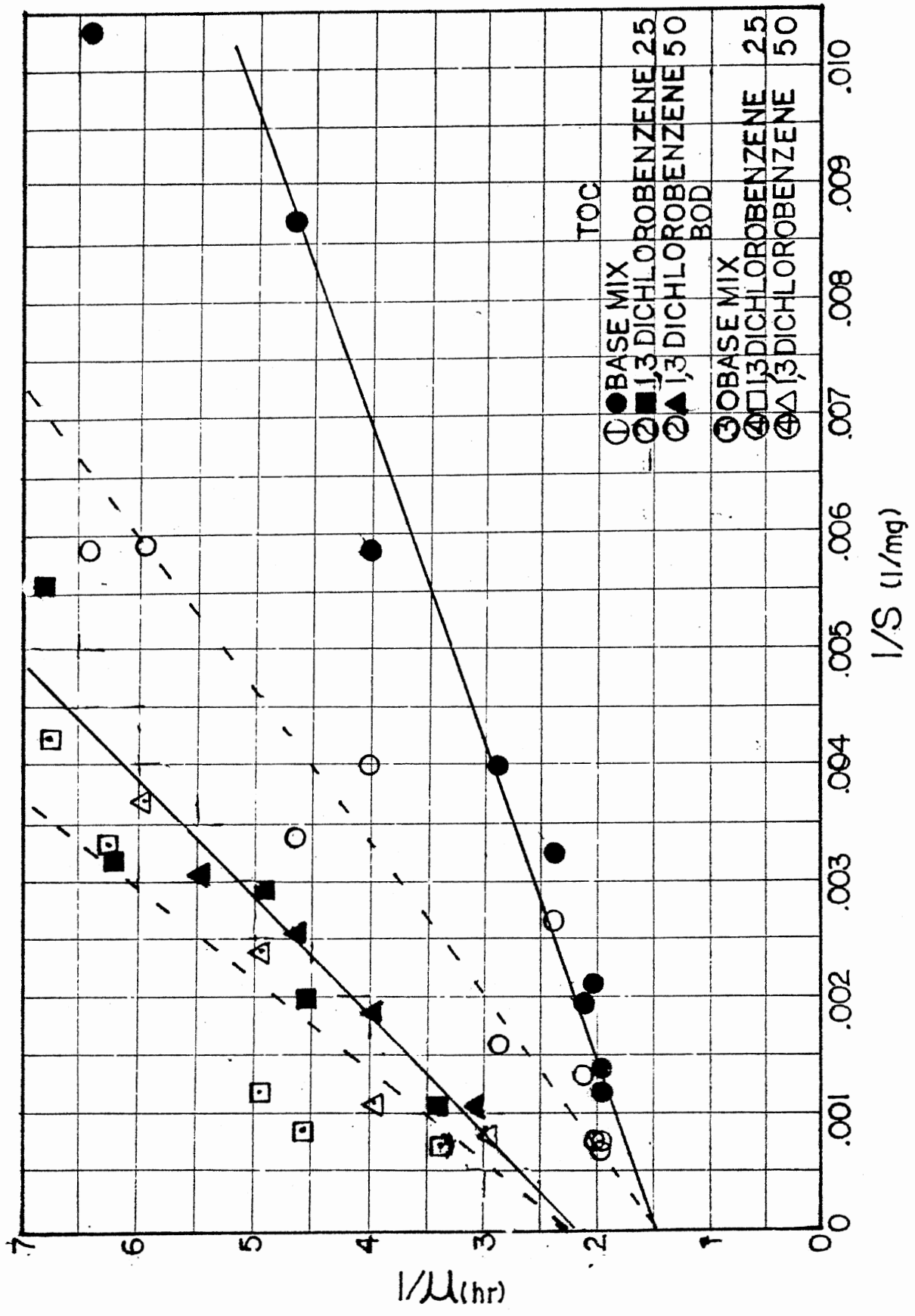
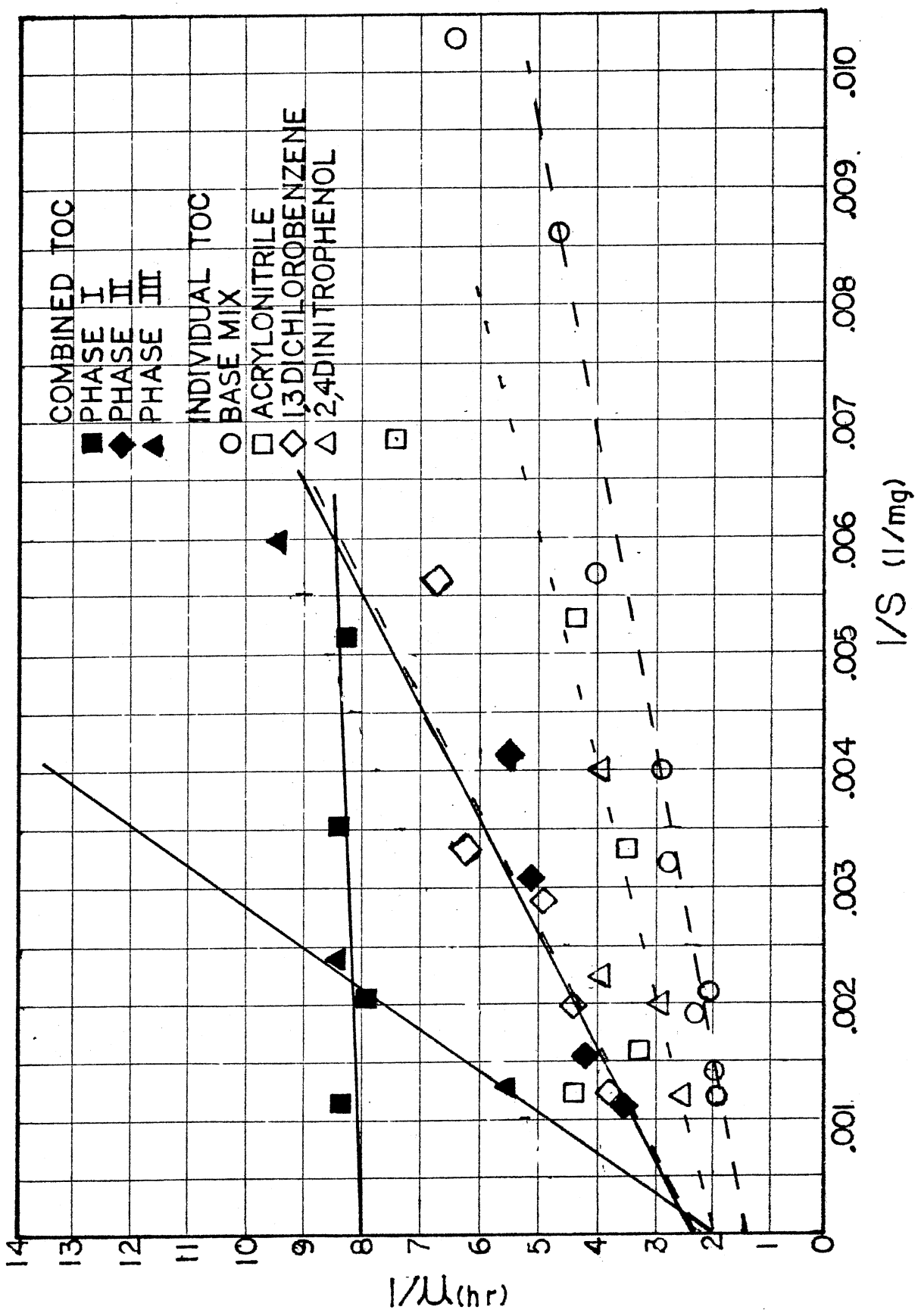

TOC
①●BASE MIX
②■1,3 DICHLOROBENZENE 25
②▲1,3 DICHLOROBENZENE 50
BOD
③○BASE MIX
④□1,3DICHLOROBENZENE 25
④△1,3DICHLOROBENZENE 50
1/μ(hr)
1/S (l/mg)
0
1
2
3
4
5
6
7
.001
.002
.003
.004
.005
.006
.007
.008
.009
.010

Figure 15. Lineweaver-Burke Plot of $1/\mu$ Vs. $1/S$ for Combined Study


COMBINED TOC
PHASE I
PHASE II
PHASE III
INDIVIDUAL TOC
BASE MIX
ACRYLONITRILE
1,3 DICHLOROBENZENE
2,4 DINITROPHENOL
1/S (1/mg)
1/μ (hr)
.001
.002
.003
.004
.005
.006
.007
.008
.009
.010
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Michaelis-Menton

Base Mix. Evalation of the specific growth rates as a function of substrate by the S/μ vs. S plot gives a value for μ_m as the slope of the line established and the S/μ axis intercept has a value of K_S/μ_m.

The base mix data based on this method is seen in Figure 16. μ_m is 0.65 hr^{-1} with K_S of 260 mg/l TOC which is in agreement with 0.65 hr^{-1} and K_S of 225 mg/l from Lineweaver-Burke. As with the Lineweaver-Burke plots, we see that BOD_5 achieves the same μ_m as TOC but with a higher K_S value. K_S for BOD_5 is 585 mg/l from the S/μ vs. S curve compared to 487 mg/l from the 1/μ vs. 1/S plot.

Acrylonitrile. Acrylonitrile, Figure 16, establishes the same non-linear plot as was seen in the Lineweaver-Burke plot. Analyzing the same two points that were used before, the value for μ_m is 0.65 hr^{-1} with a K_S of 325 which agrees with the values from the 1/μ vs. 1/S plot. BOD_5 established a μ_m of 0.65 hr^{-1} with an increase in K_S to 683 mg/l which compares to 620 mg/l from Lineweaver-Burke as seen in Table IV.

Both the Lineweaver-Burke and Michaelis-Menton plots of the data for acrylontrile fail to establish a linear relationship for the data. Haldane's model (46) for the data appears to address the changes in μ as presented earlier.

2,4 Dinitrophenol. 2,4 Dinitrophenol plotted on S/μ vs. S is seen in Figure 17. The data shows addition of the compound changed the slope and intercepts relating to the reduction in overall reaction rate as seen with the Lineweaver-Burke plot.

Values for μ_m and K_S from this plot are 0.51 hr^{-1} for μ_m with 259 mg/l for K_S at 25 mg/l 2,4 DNP. With 50 mg/l, 2,4 DNP, μ_m was reduced

Figure 16. Michaelis-Menton Plot of S/μ Vs. S for Acrylonitrile

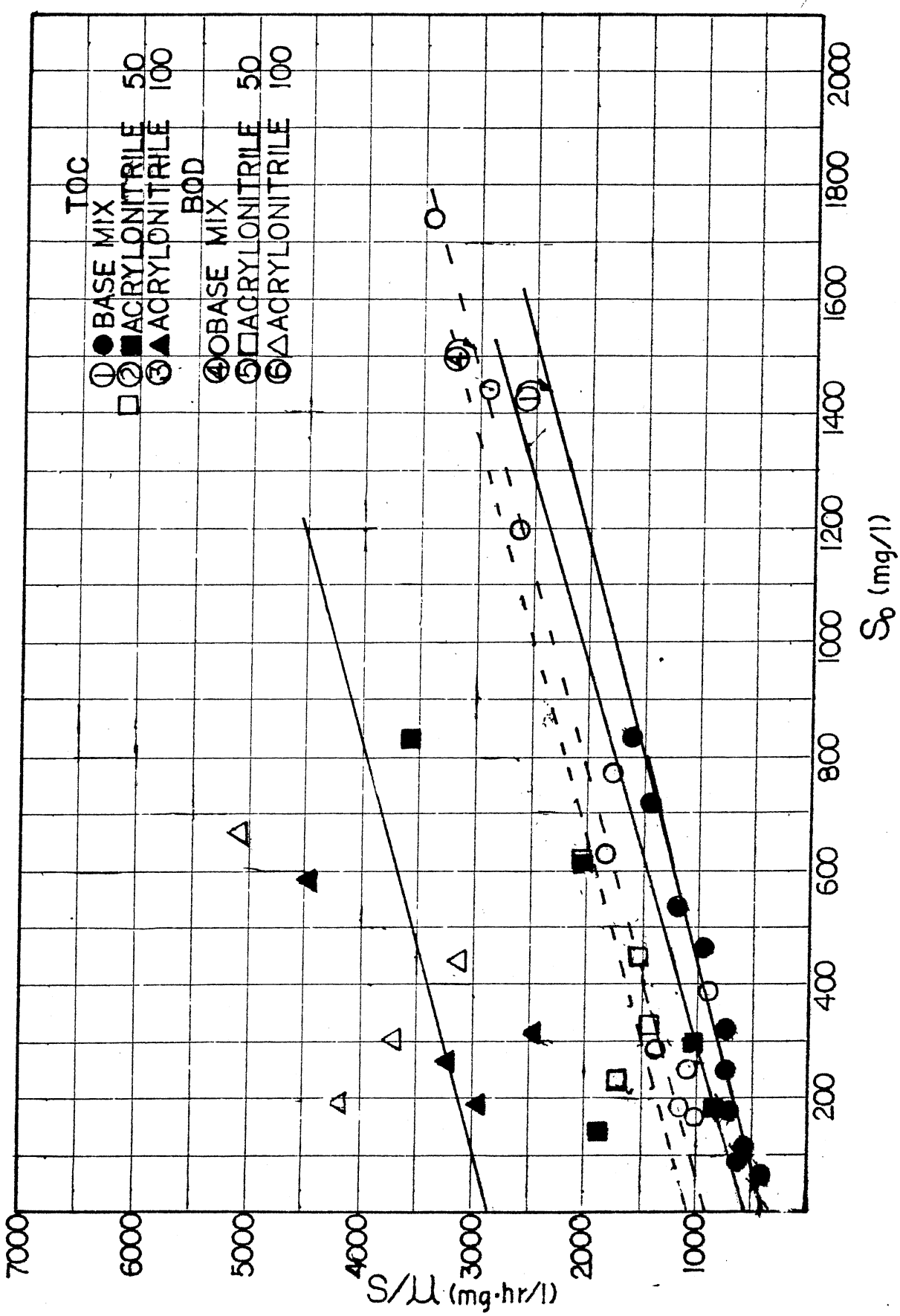
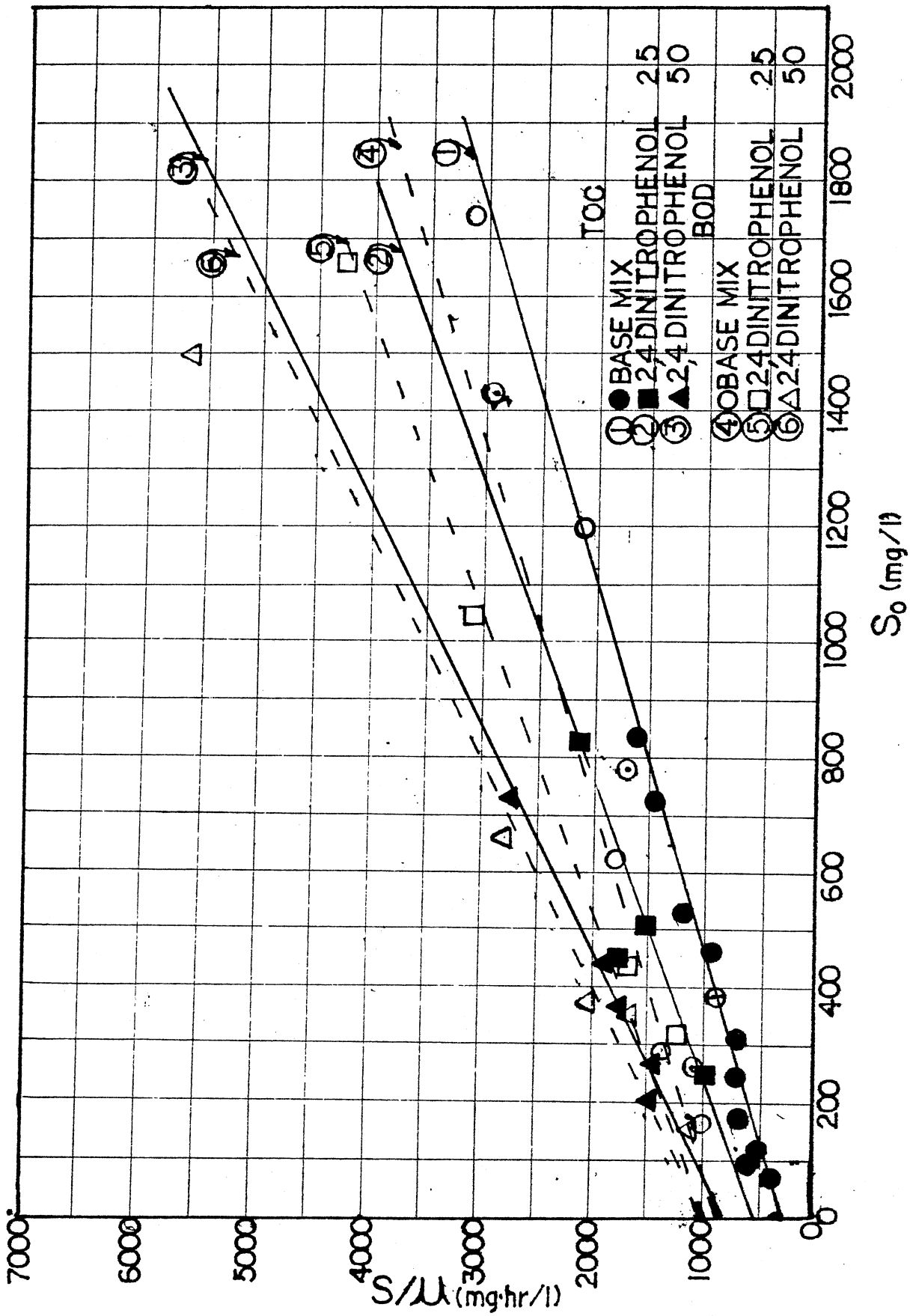

TOC
① ● BASE MIX
② ■ ACRYLONITRILE 50
③ ▲ ACRYLONITRILE 100
BOD
④ ○ BASE MIX
⑤ □ ACRYLONITRILE 50
⑥ △ ACRYLONITRILE 100
S/μ (mg·hr/l)
7000
6000
5000
4000
3000
2000
1000
200
400
600
800
1000
1200
1400
1600
1800
2000
S₀ (mg/l)

Figure 17. Michaelis-Menton Plot of S/μ Vs. S for 2,4 Dinitrophenol


TOC
BOD
①●BASE MIX
②■2,4 DINITROPHENOL 2.5
③▲2,4 DINITROPHENOL 5.0
④○BASE MIX
⑤□2,4 DINITROPHENOL 2.5
⑥△2,4 DINITROPHENOL 5.0
S/μ (mg·hr/l)
So (mg/l)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
1000
2000
3000
4000
5000
6000
7000

to 0.40 hr^{-1} while K_S increased to 374 mg/l TOC. Again, the change in slope and intercept follows the same relationship seen with Lineweaver-Burke and develops a K_i of 80 mg/l.

BOD_5 for both experiments established the same μ_m but a higher K_S than the base mix of 400 mg/l BOD_5.

1,3 Dichlorobenzene. Figure 18 shows the determination of μ_m and K_S for 1,3 DCB using the S/μ vs. S plot. As with the Lineweaver-Burke plot, both concentrations tend to achieve a common μ_m and K_S of 0.43 h^{-1} with a K_S of 430 mg/l. This compares to a μ_m of 0.45 hr^{-1} and K_s of 405 mg/l from the $1/\mu$ vs. 1/S as seen in Table IV.

BOD_5 achieved a μ_m of 0.43 hr^{-1} with a K_S of 473 mg/l which compares to 0.45 hr^{-1} and K_S 475 mg/l from Lineweaver-Burke plot.

Combined. The Michaelis-Menton plot for the three phases of growth resulting from the combined study are presented in Figure 19. The outcome of the plot shows a change in Phase I with no changes in Phase II or III as far as μ_m. K_S increased in Phase III while remaining constant for Phase II, as seen in Table IV.

Determination of Eckenfelder's Rate Constants Ke and Ke'

Ke

Data plotted for the base mix and the three compounds to establish Ke based on TOC is seen in Figure 20. All the data for this plot show some degree of scatter, with not one compound having any more than another. Scatter would be more understandable for acrylonitrile than the other studies since stripping from the reactors appears to be

Figure 18. Michaeli-Menton Plot of S/μ Vs. S for 1,3 Dichlorobenzene

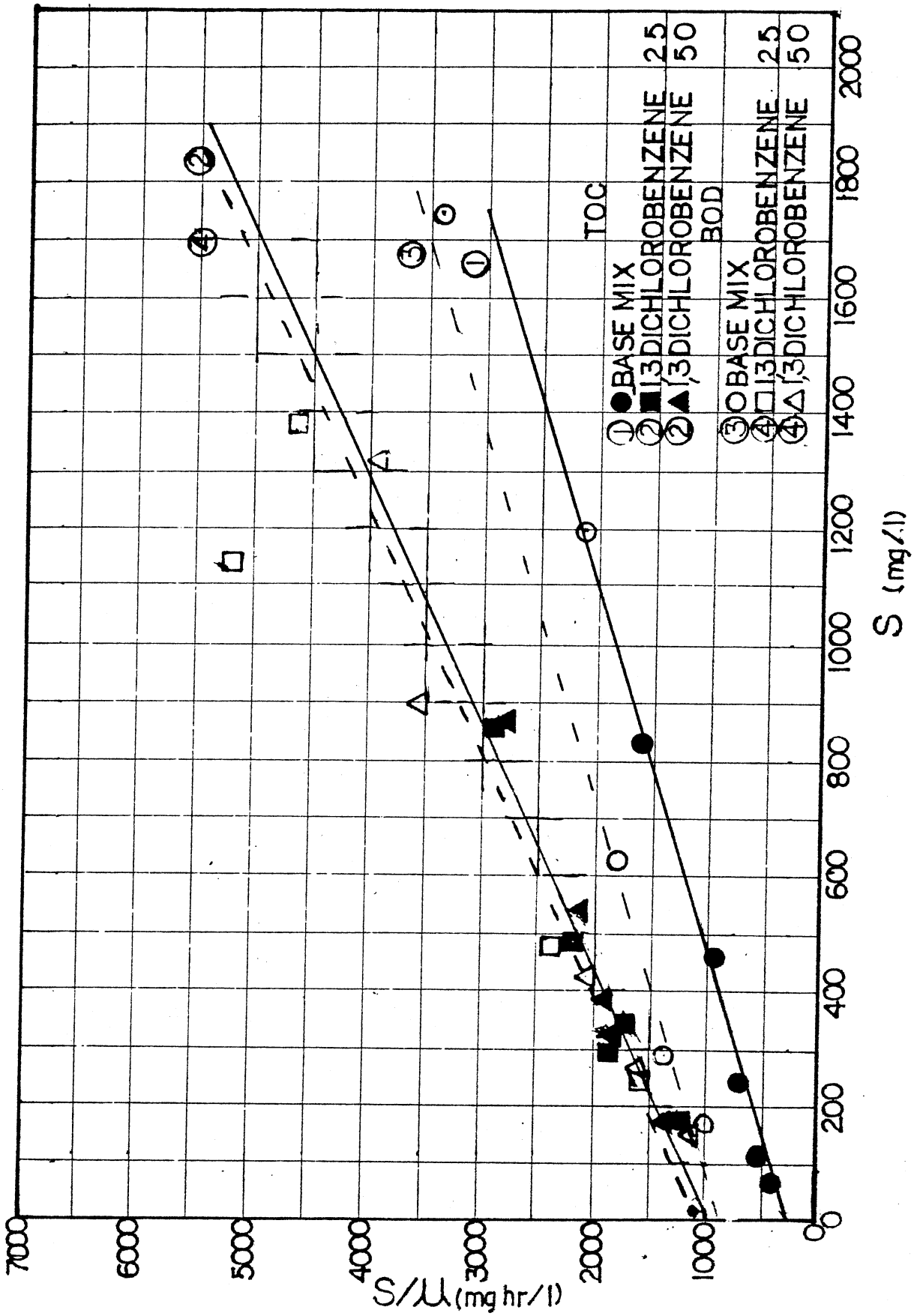

TOC
BOD
①●BASE MIX
②■1,3DICHLOROBENZENE 2.5
②▲1,3DICHLOROBENZENE 50
③○BASE MIX
④□1,3DICHLOROBENZENE 2.5
④△1,3DICHLOROBENZENE 50
S (mg/l)
S/μ (mg hr/l)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
1000
2000
3000
4000
5000
6000
7000

Figure 19. Michaelis-Menton Plot of S/μ Vs. S for Combined Study

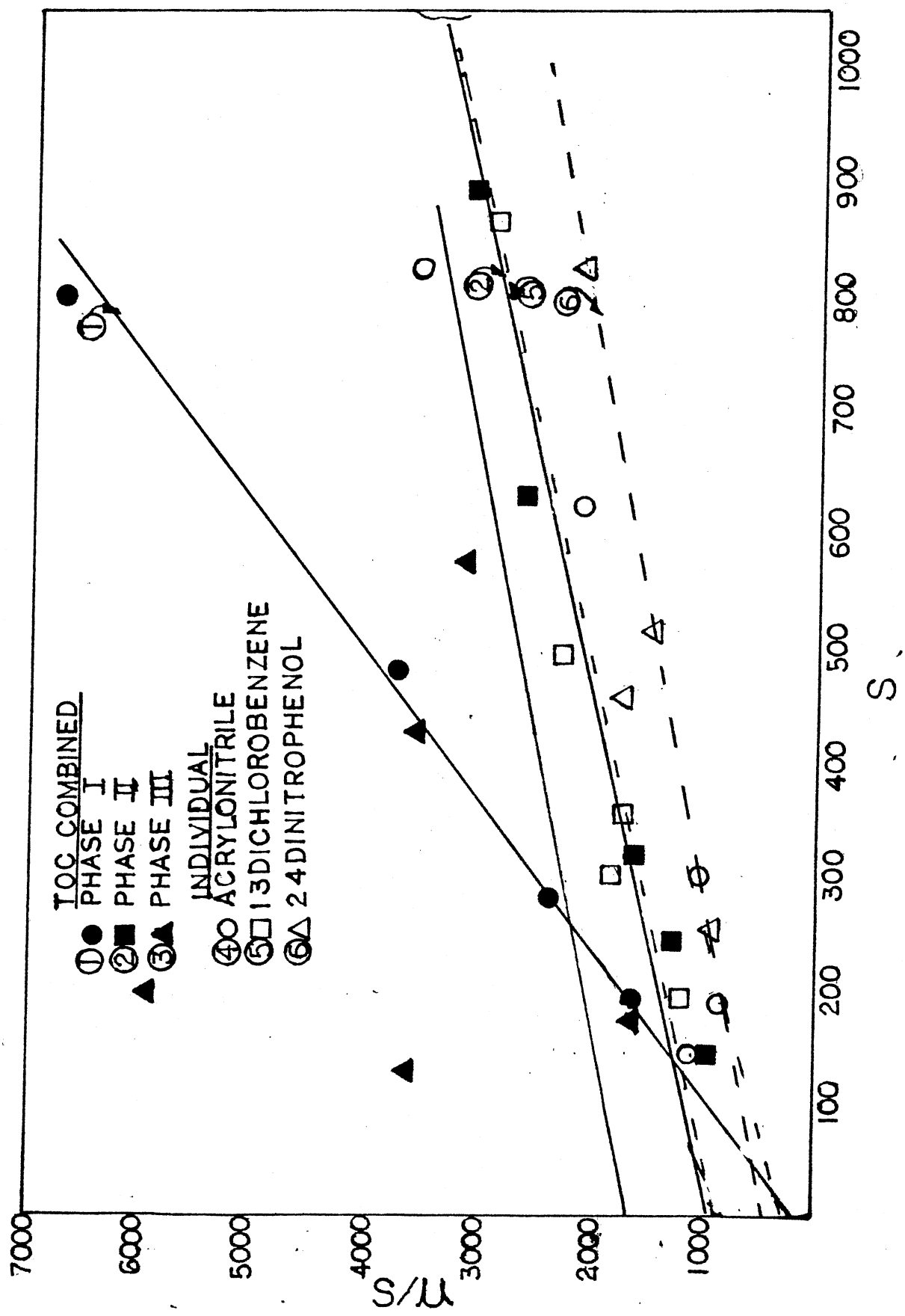
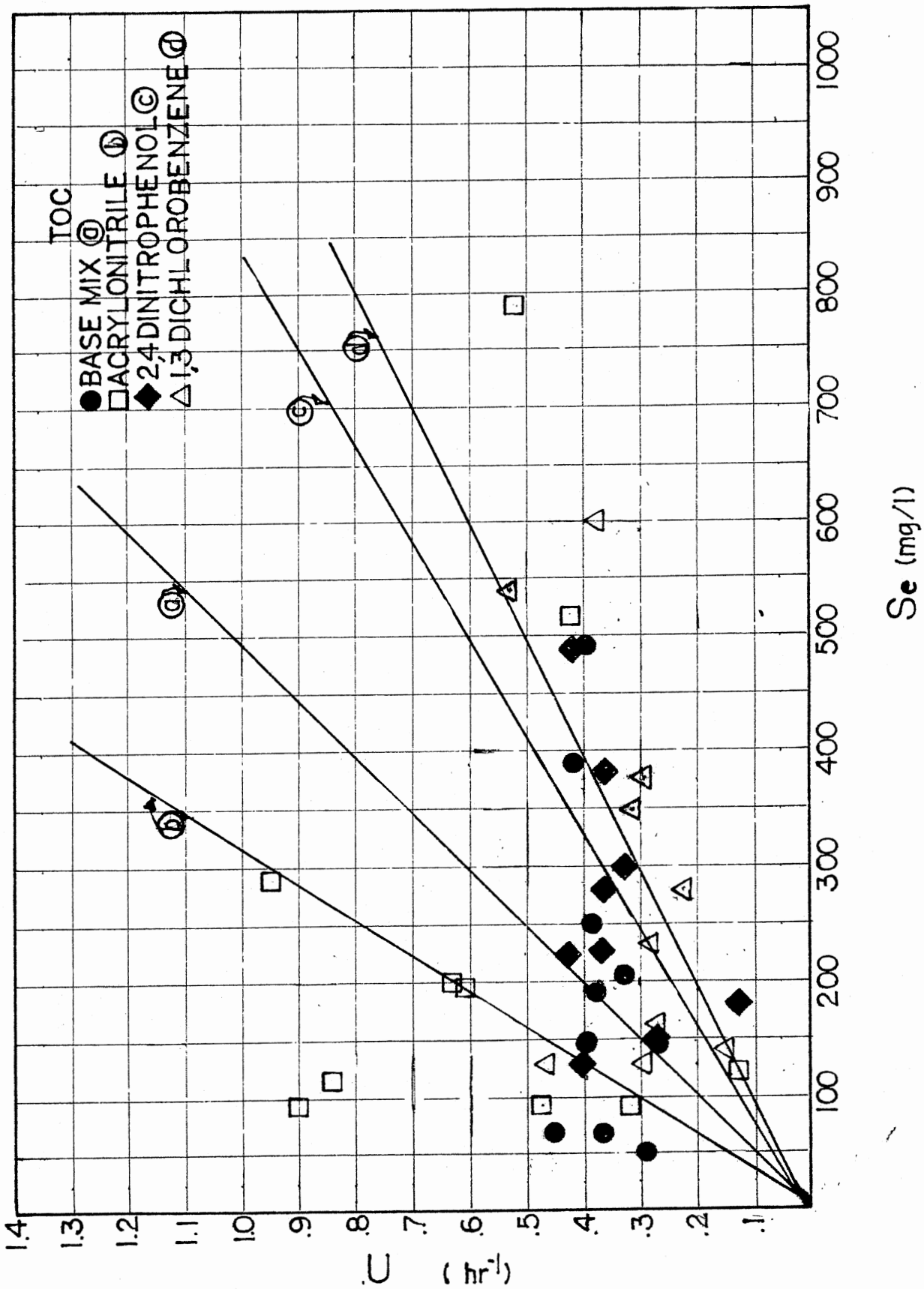

TOC COMBINED
PHASE I
PHASE II
PHASE III
INDIVIDUAL
ACRYLONITRILE
13DICHLOROBENZENE
24DINITROPHENOL
S/μ
S
7000
6000
5000
4000
3000
2000
1000
100
200
300
400
500
600
700
800
900
1000

Figure 20. Plot of U Vs. S_e to Determine K_e for Compounds Depicted Based on TOC


TOC
●BASE MIX ⑧
□ACRYLONITRILE ⑥
◆2,4 DINITROPHENOL ⑥
△1,3 DICHLOROBENZENE ⑦
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
U (hr⁻¹)
100
200
300
400
500
600
700
800
900
1000
Se (mg/l)

occurring none of the other compounds showed any evidence of stripping. Values for Ke and Ke' are found in Table VI.

Ke determined for the base mix is 0.002 $(mg/l \cdot hr)^{-1}$ from Figure 20. This value was higher than all the other values achieved except for the 100 mg/l experiment for acrylonitrile which was 0.0031 $(mg/l \cdot hr)^{-1}$. This quantity was higher than that for the 50 mg/l ACN study even though the inhibition in specific growth rates for the 100 mg/l study were extremely reduced. This tends to suggest involvement of several removal mechanisms in the Ke value for acrylonitrile.

2,4 Dinitrophenol achieved a Ke value of 0.0012 $(mg/l \cdot hr)^{-1}$ compared to a similar value of 0.001 $(mg/l \cdot hr)^{-1}$ for 1,3 dichlorobenzene. Both values were lower than that achieved for the 100 mg/l acrylonitrile study which as stated earlier showed a very reduced growth rate.

Ke determined using BOD_5 for the studies performed are found in Figure 21. Overall, the BOD_5 data showed less scatter than did the TOC. Values achieved were 0.015 $(mg/l \cdot hr)^{-1}$ for the base mix, 0.0045 $(mg/l \cdot hr)^{-1}$ for acrylonitrile, 0.003 $(mg/l \cdot hr)^{-1}$ for 2,4 dinitrohpenol and 0.0021 $(mg/l \cdot hr)^{-1}$ for 1,3 dichlorobenzene. Acrylonitrile was still higher than 2,4 DNP and 1,3 DCB.

Ke'

Data plotted to establish Ke' is found in Figure 22 and, as can be seen, shows better correlation than that for Ke, although some scatter is still evident. Ke' for the base mix TOC was 0.68 hr^{-1} compared to 1.0 hr^{-1} for acrylonitrile, 0.73 hr^{-1} for 2,4 dinitrophenol, and 0.46 hr^{-1} for 1,3 dichlorobenzene.

BOD_5 used as a parameter for Ke' resulted in the plot in Figure 23.

TABLE VI

KINETIC CONSTANTS: K_e, K'_e, Y

Compound	K_e $(mg/l \cdot hr)^{-1}$	K'_e (hr^{-1})	Y(dX/dS) (mg/mg)	Y(μ/U) (hr/hr)
TOC				
Base Mix	.0020	.68	.83	.82
Acrylonitrile	.0031	1.00	.49	.50
2,4 Dinitrophenol	.0012	.73	.63	.64
1,3 Dichlorobenzene	.0011	.46	.71	.75
BOD				
Base Mix	0015	8.00	.38	.30
Acrylonitrile (50mg/1)	.0045	1.50	.30	.45
(100mg/1)	.0045	1.50	.11	.11
2,4 Dinitrophenol	.003	1.8	.45	.45
1,3 Dichlorobenzene	.0021	1.2	.57	.57

Figure 21. Plot of U Vs. S_e to Determine K_e for Compounds Depicted Based on BOD_5

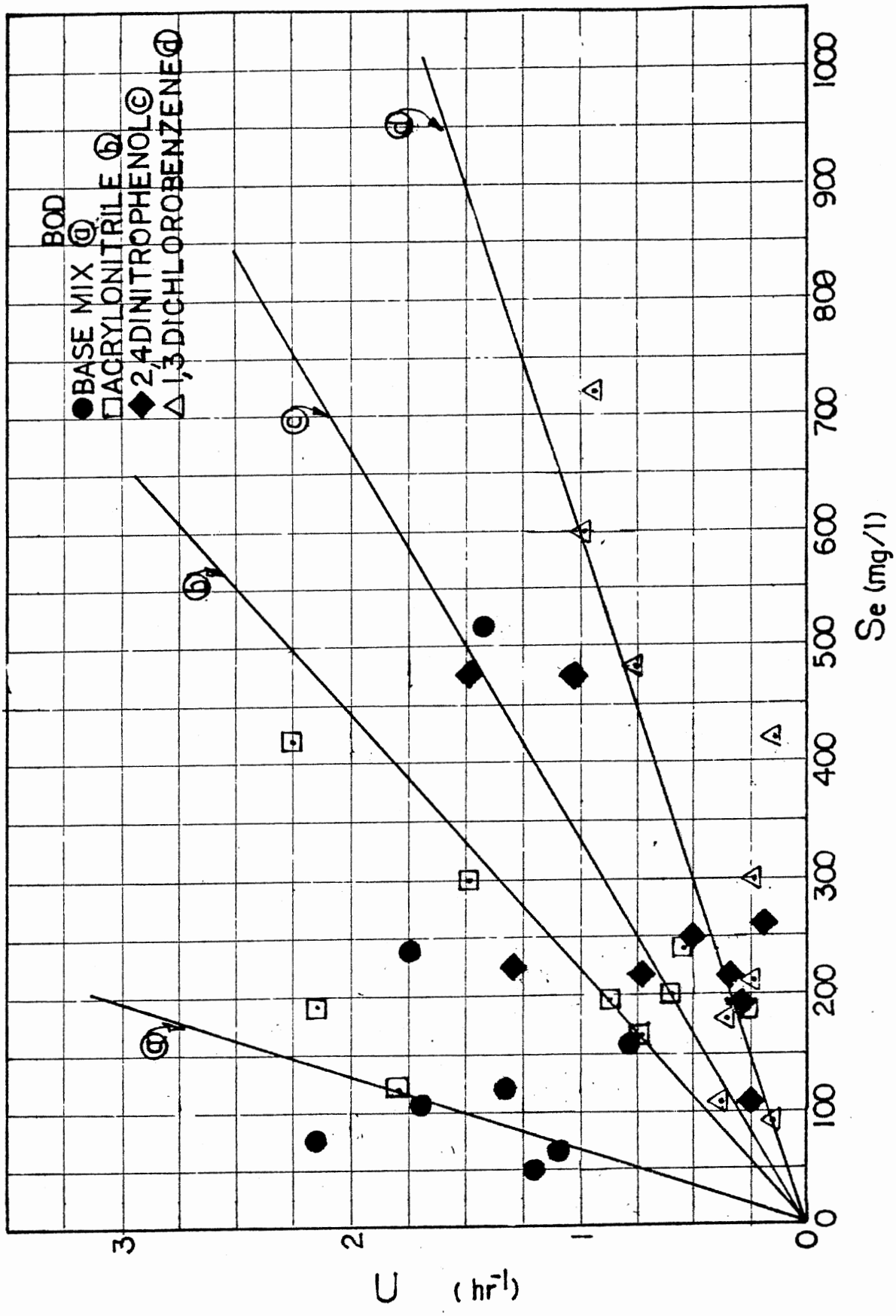

BOD
●BASE MIX ⑤
□ACRYLONITRILE ⑥
◆2,4DINITROPHENOL⑥
△1,3DICHLOROBENZENE⑥
U (hr⁻¹)
Se (mg/l)
0
100
200
300
400
500
600
700
800
900
1000
1
2
3

Figure 22. Plot of S • U Vs. S_e to Determine Ke' for Compounds Depicted Based on TOC

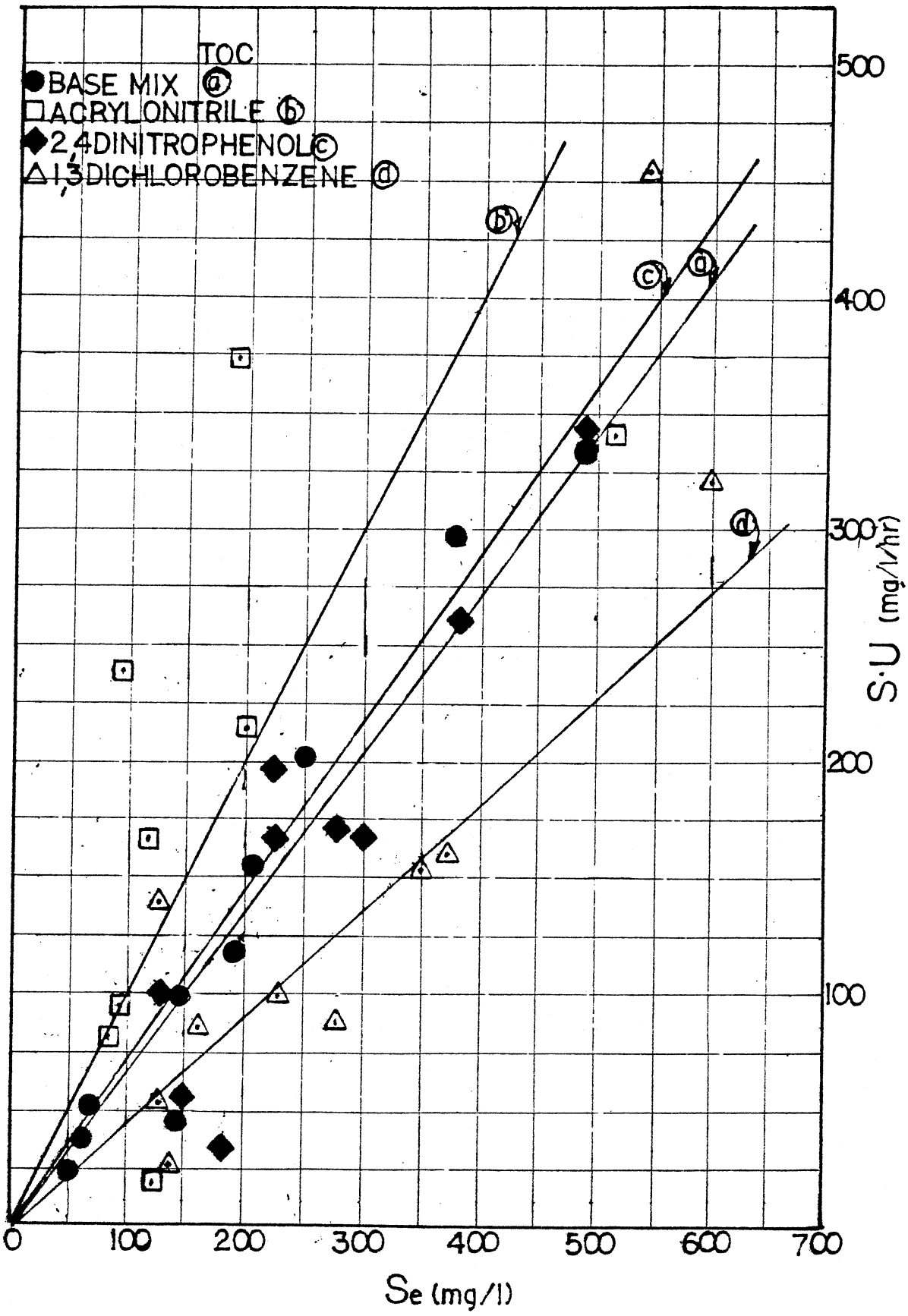
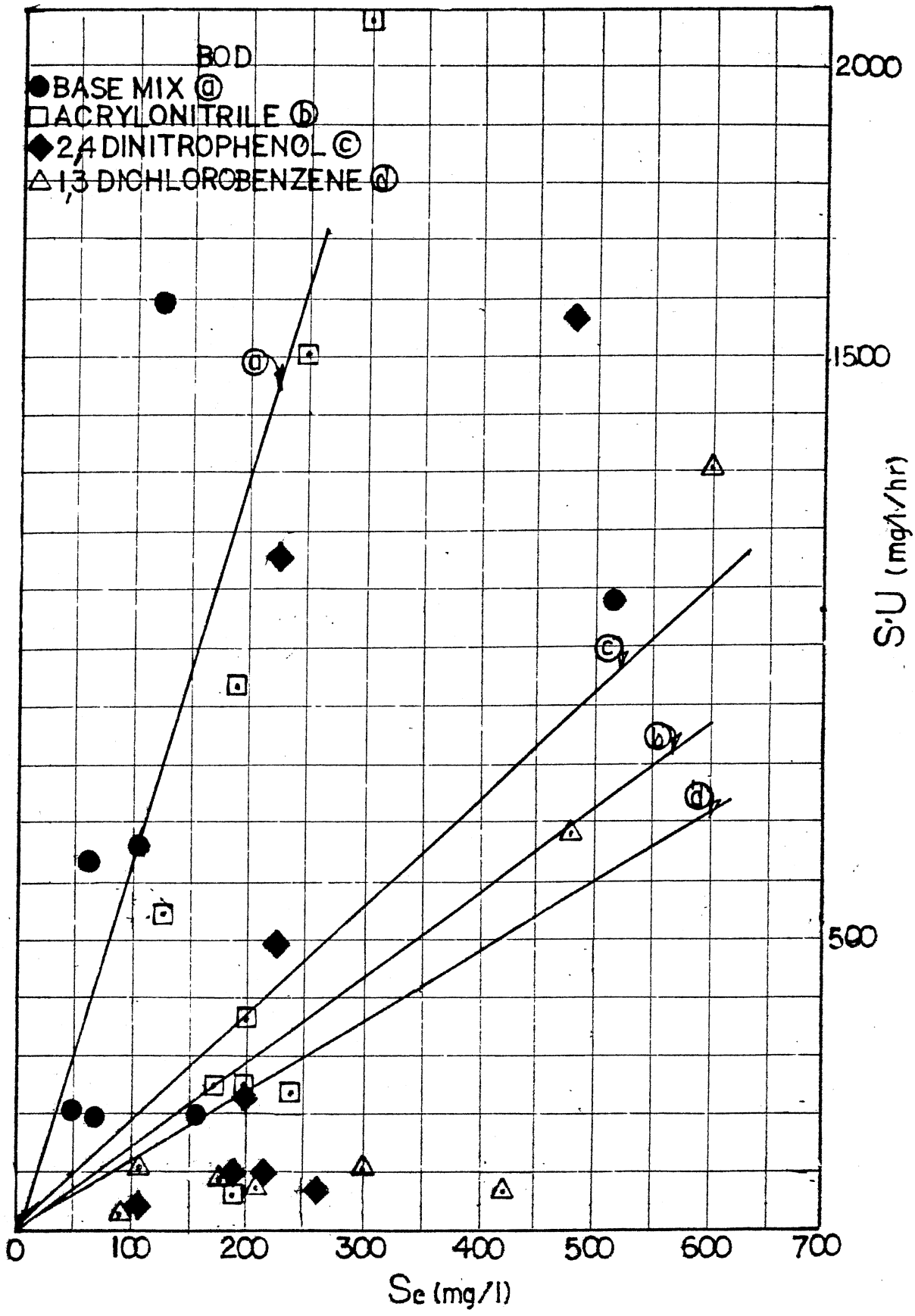

TOC
BASE MIX (a)
ACRYLONITRILE (b)
2,4DINITROPHENOL (c)
1,3DICHLOROBENZENE (d)
0
100
200
300
400
500
600
700
100
200
300
400
500
S·U (mg/l/hr)
Se (mg/l)

Figure 23. Plot of S . U Vs. S_e to Determine K_e' for Compounds Depicted Based on BOD_5


BOD
BASE MIX @
ACRYLONITRILE ⓑ
2,4 DINITROPHENOL ©
1,3 DICHLOROBENZENE @
2000
1500
500
S·U (mg/l/hr)
0
100
200
300
400
500
600
700
Se (mg/l)

Data for the base mix showed very good correlation while the other compounds still evidence some scatter. Values for Ke' determined by the plot are 8.0 hr^{-1} for the base mix, 1.5 hr^{-1} for acrylonitrile, 1.8 hr^{-1} for 2,4 dinitrophenol and 1.12 hr^{-1} for 1,3 dichlorobenzene.

Determination of Yield

Y Using ΔX Vs. ΔS

The yield values for mass produced versus substrate removed are presented in Figure 24 based on TOC analysis. The data for the determination of yield did not experience as much scatter as seen for Ke and Ke'. The values achieved from the plot of X as ΔX, vs. S_R or ΔS, are 0.83 mg/mg TOC for the base mix. Acrylonitrile at 50 mg/1 achieved a value of 0.49 mg/mg which dropped to 0.17 mg/mg at 100 mg/1 compound. 2,4 Dinitrophenol resulted in a yield of 0.63 mg/mg while 1,3 dichlorobenzene showed a value of 0.71 mg/mg TOC. Yield values may be found in Table VI. Yield based on BOD_5, Figure 25, resulted in considerably lower values than those seen with TOC. Yield for base mix was 0.38 mg/mg BOD_5. Acrylonitrile again had a value higher at 50 mg/1 of compound with 0.30 mg/mg BOD_5, than the 100 mg/1 study which achieved 0.11 mg/mg BOD_5. 2,4 Dinitrophenol and 1,3 dichlorobenzene achieved higher values than acrylonitrile of 0.45 mg/mg BOD_5 and 0.57 mg/mg BOD_5, respectively.

Y Using μ Vs. $\frac{(S_o-S)}{Xt}$

As a further evaluation for yield of biomass versus substrate

Figure 24. Observed Yield Plot of X Vs. S_R for Compounds Depicted Based on TOC

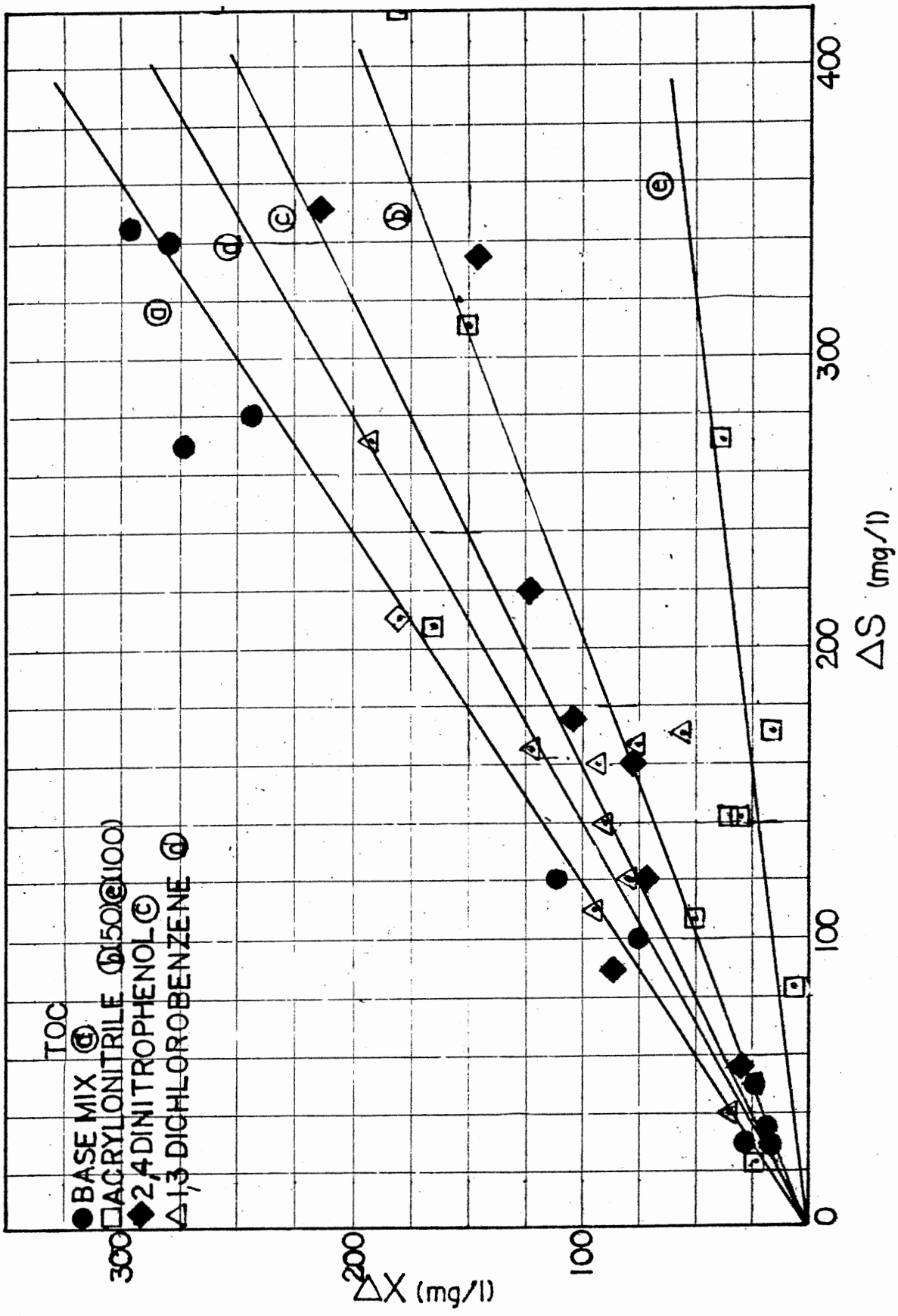
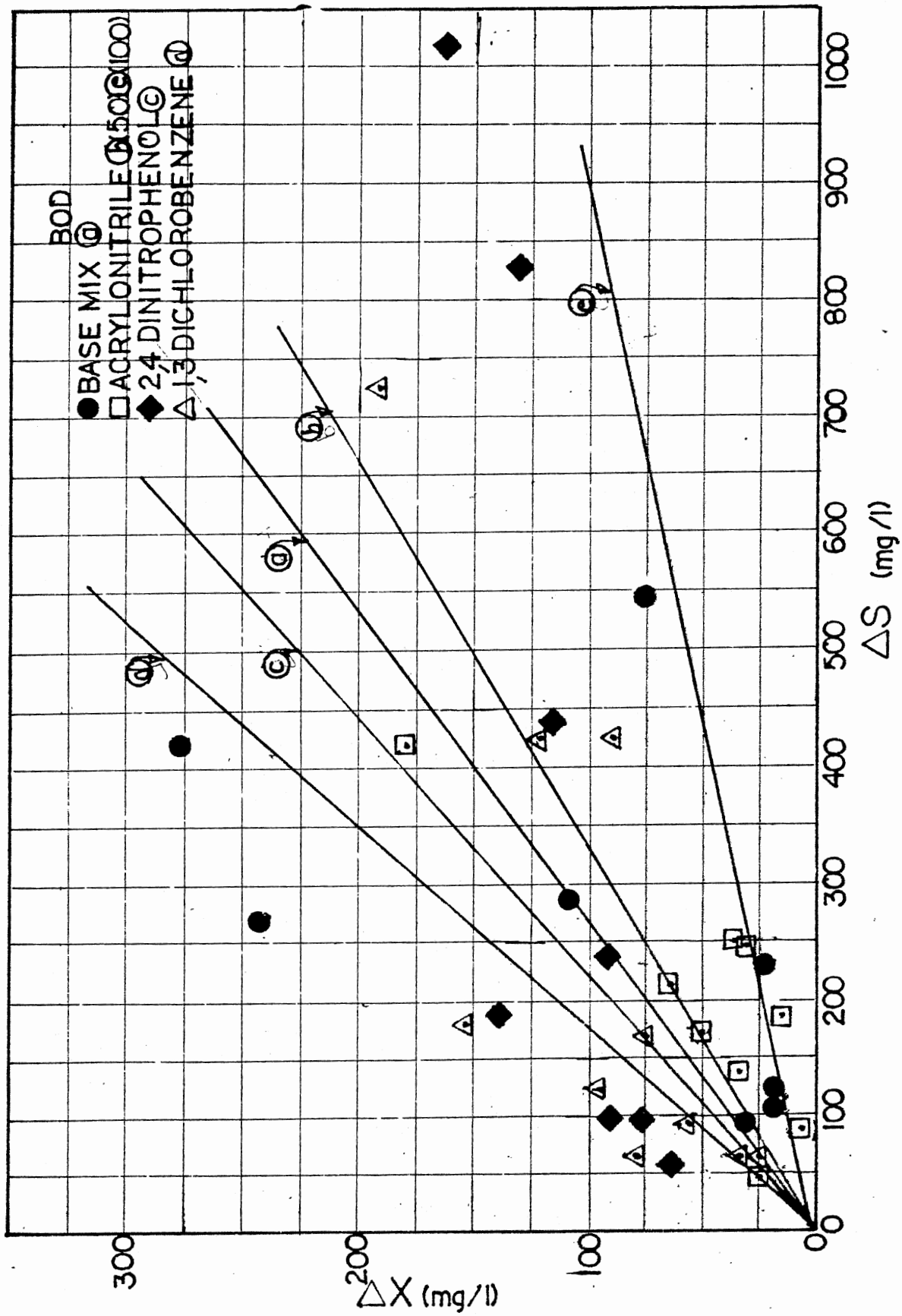

TOC
●BASE MIX ⓐ
□ACRYLONITRILE ⓑ150 ⓒ100
◆2,4 DINITROPHENOL ⓒ
△1,3 DICHLOROBENZENE ⓓ
300
200
100
ΔX (mg/l)
0
100
200
300
400
ΔS (mg/l)
ⓐ
ⓑ
ⓒ
ⓓ
ⓔ

Figure 25. Observed Yield From X Vs. S_R for Compounds Depicted Based on BOD_5


BOD
BASE MIX ⓐ
ACRYLONITRILE ⓑ
2,4 DINITROPHENOL ⓒ
1,3 DICHLOROBENZENE ⓓ
ΔX (mg/l)
ΔS (mg/l)
0
100
200
300
400
500
600
700
800
900
1000

removed, specific growth rate was plotted against specific substrate removal rate for TOC (Figure 26) and BOD_5 (Figure 27).

The data plotted for all the studies as seen in Figure 26 showed some scatter. The values resulting from the plot are 0.82 for the base mix, 0.50 for acrylonitrile at 50 mg/l, 0.16 for acrylonitrile at 100 mg/l, 0.64 for 2,4 dinitrophenol and 0.75 for 1,3 dichlorobenzene. These values agree with those from the ΔX vs. ΔS plot as seen in Table VI, BOD_5 resulted in the plot presented in Figure 27. Quantities obtained were 0.30 BOD_5 for the base mix, 0.45 for acrylonitrile at 50 mg/l, 0.11 for acrylonitrile at 100 mg/l, 0.45 for 2,4 dinitropenol and 0.57 for 1,3 dichlorobenzene. Like TOC, BOD_5 showed agreement with those obtained from the ΔX vs. ΔS plots, as seen in Table VI.

Comparison of Kinetic Behavior

As stated in the objective of the thesis, the completely mixed, dispersed batch system experiments were performed in order to obtain kinetic constants to compare to the constants achieved from the completely mixed internal recycle system. The objective of the comparison is to examine similarities or dissimilarities between the two systems. The values used for the comparison found in Table VII are the constants developed in the previous sections of the text and those obtained from the continuous flow pilot plant studies (52).

In comparing kinetic behavior between two distinct systems, the systems themselves warrant some description. In the dispersed batch system, the activated sludges were exposed to an excess of substrate for the major portion of time in which the primary growth phase ensued. In comparison, the continuous flow internal recycle systems exposed the

Figure 26. Specific Growth Rate μ Vs. Specific Substrate Utilitzation Rate U for Compounds Depicted Based on TOC

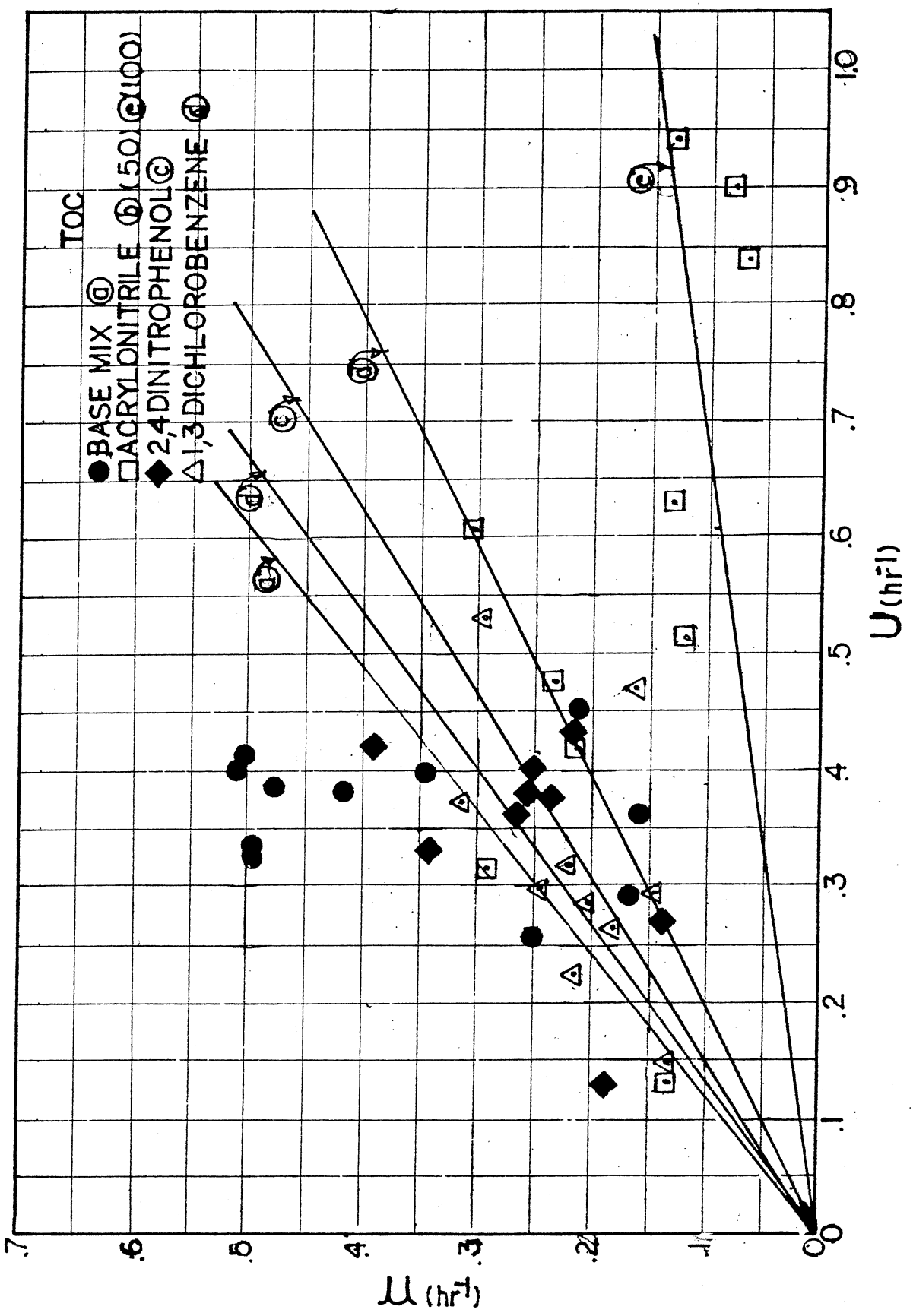

TOC
●BASE MIX ⓓ
□ACRYLONITRILE ⓓ(50)ⓒ(100)
◆2,4DINITROPHENOLⓒ
△1,3DICHLOROBENZENE ⓓ
μ (hr⁻¹)
U(hr⁻¹)
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
1
2
3
4
5
6
7

Figure 27. Specific Growth Rate μ Vs. Specific Substrate Utilization Rate U for Compounds Depicted Based on BOD_5

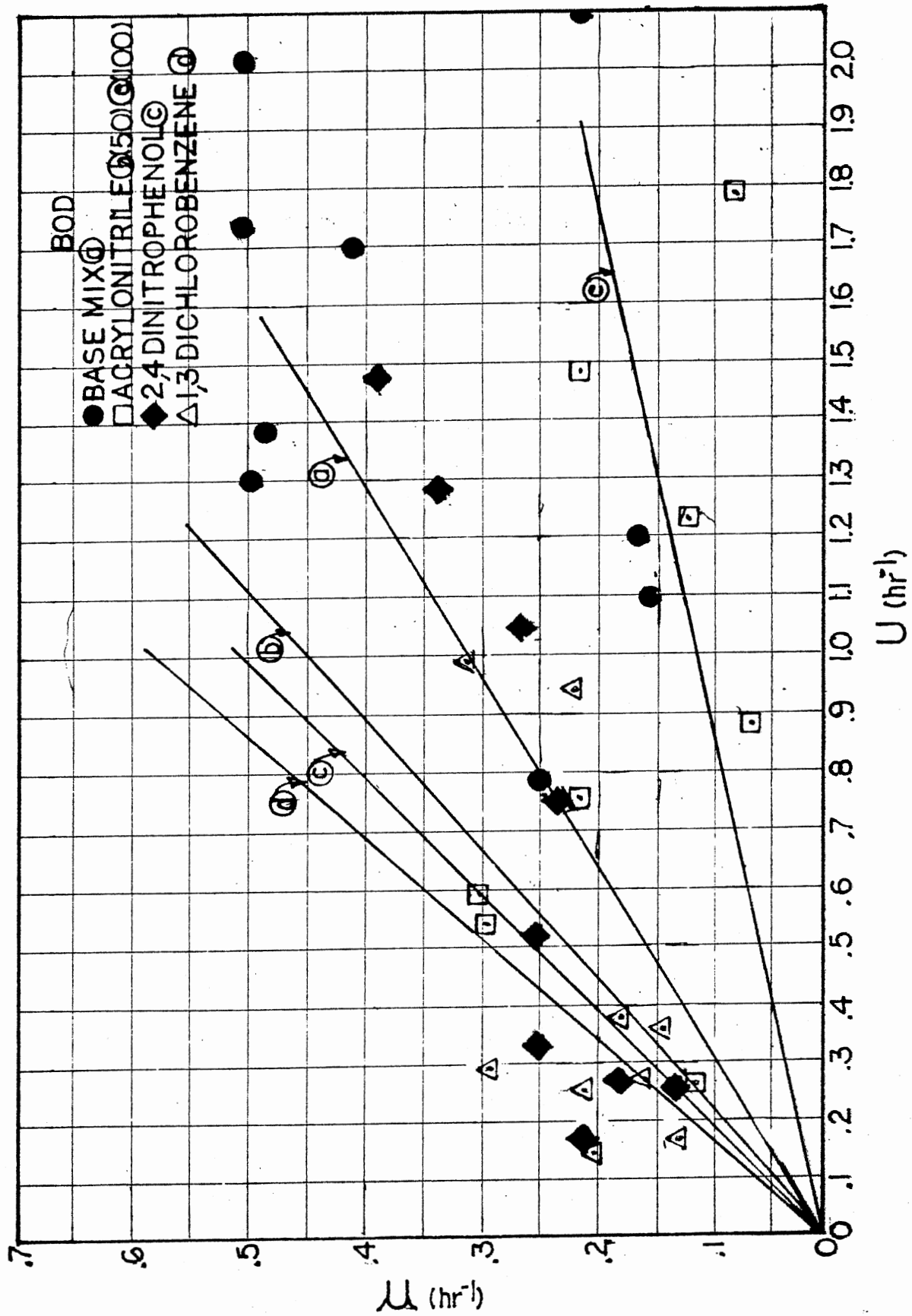

BOD
●BASE MIX①
□ACRYLONITRILE②(150)③(100)
◆2,4 DINITROPHENOL④
△1,3 DICHLOROBENZENE ⑤
μ (hr⁻¹)
U (hr⁻¹)
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
.1
.2
.3
.4
.5
.6
.7

TABLE VII

KINETIC CONSTANTS BATCH AND CONTINUOUS FLOW

Compound	μ_m $(hr.)^{-1}$	(d^{-1})	K_s (mg/l)	K_i (mg/l)	K_e $(mg/l \cdot hr)^{-1}$	$(mg/l \cdot d)^{-1}$	K_d $(hr.)^{-1}$	(d^{-1})	Y (mg/mg)
TOC BATCH									
Base Mix	.65	15.6	225		.002	.048	.68	16.3	.83
Acrylonitrile	.65	15.6	325	600	.003	.072	1.00	24.0	.50
2,4 Dinitrophenol	.50	12.0	250	80	.0012	.029	.73	17.5	.64
1,3 Dichlorobenzene	.45	10.8	450		.0011	.026	.46	11.0	.75
BOD BATCH									
Base Mix	.65	15.6	500		.015	.36	8.0	192	.38
Acrylonitrile	.65	15.6	650	600	.0045	.108	1.5	36	.30
2,4 Dinitrophenol	.50	12.0	400	80	.0030	.072	1.8	43	.45
1,3 Dichlorobenzene	.45	10.8			.0021	.050	1.2	28	.57
TOC CONTINUOUS FLOW									
Base Mix		3.2	110			.03		4.1	.81
Acrylonitrile		3.8	167			.02		3.8	.96
2,4 Dinitrophenol		1.6	32			.03		5.6	.96
1,3 Dichlorobenzene		1.7	16			.08		11.5	.97
BOD CONTINUOUS FLOW									
Base Mix		.90	1.4			.26		82	.62
Acrylonitrile		2.90	26			.73		22	.73
2,4 Dinitrophenol		5.90	29			.88		56	.88
1,3 Dichlorobenzene		1.20	2.9			.65		73	.65

activated sludge to a substrate at constant concentrations on a continuous basis at steady state conditions. In such systems and dependent on the wastage from the reactor basin, the activated sludge is maintained in excess to the substrate.

μ_m and K_S

The importance of μ_m and K_S is basic to kinetic modeling or activated sludge systems whether batch or continuous systems when using Monod's model (32)(10). It allows for a predictive tool in modeling behavior of activated sludge systems on a monomolecular basis.

The behavior of the three compounds in the batch system demonstrated a different response from that of the base mix. Acrylonitrile achieved a μ_m or 0.65 hr^{-1} the same as the base mix. Also seen with ACN is a dynamic relationship with the base mix as modeled by Haldane's equation (46).

2,4 DNP and 1,3 DCB both achieved reductions in μ_m over that achieved with the base mix. In the batch system, 2,4 DNP reponded with quantitative decreases in μ_m from 0.65 hr^{-1} to 0.50 hr^{-1} to 0.40 hr^{-1} with increases in compound. 1,3 DCB resulted in a single value reduction in μ_m of 0.45 hr^{-1} independent of compound concentration.

In the continuous flow system the base mix and three compounds achieved a similar pattern in values for μ_m relative to that of the base mix. Acrylonitrile achieved a μ_m of 3.8 d^{-1} close to that of the base mix at 3.2 d^{-1}. There was no inhibition seen in the continuous system for acrylonitrile. 2,4 DNP and 1,3 DCB both effected reductions in μ_m with values of 1.6 d^{-1} for 2,4 DNP and 1.7 d^{-1} for 1,3 DCB.

The changes in both systems address changes in metabolic response

of the activated sludge to the compounds. Such a change in response could be attributed to the biodegradability of the compound or a change in predominant species in the heterogeneous population (5).

A comparison of the values for μ_m and the K_S between the batch and continuous flow system shows the batch constants were several times higher compared to the continuous flow constants. This is seen in Table VII. The batch compared to the continuous flow achieved values for μ_m of 15.6 d^{-1} versus 3.2 d^{-1} for the base mix, 15.6 d^{-1} versus 3.8 d^{-1} for acrylonitrile, 12.0 d^{-1} versus 1.6 d^{-1} for 2,4 DNP and 10.8 d^{-1} versus 1.7 d^{-1} for 1,3 dichlorobenzene.

The use of μ_m derived from each system has totally different objectives. The objective of the μ_m for the batch is to obtain a maximum growth rate in the presence of excess substrate not concerned with the state of S_e. In comparison, μ_m for the continuous flow system has an objective of establishing a maximum specific growth rate under steady state conditions to achieve an effluent quality, S_e.

Ke and Ke'

Ke. Ke, Eckenfelder's first order removal rate constant based on TOC resulted in mixed pattern between the two systems. The base mix and acrylonitrile achieved values higher than the continuous flow system while 2,4 dinitrophenol showed agreement in value and 1,3 dichlorobenzene was lower. The base mix achieved a value of 0.048 $(mg/l \cdot d)^{-1}$ for the batch compared to 0.03 $(mg/l \cdot d)^{-1}$ for the continuous flow system. Acrylonitrile with problems of scatter and possible effect of stripping on Ke resulted in 0.072 $(mg/l \cdot d)^{-1}$ at 50 mg/l for the batch compared to 0.02 $(mg/l \cdot d)^{-1}$ for the continuous flow. 2,4

Dinitrophenol achieved a value from the batch of 0.029 $(mg/l \cdot d)^{-1}$ which compared to 0.03 $(mg/l \cdot d)^{-1}$ for the continuous system. The only value from the batch system that was lower than that achieved in the continuous flow was for 1,3 dichlorobenzene with 0.026 (mg/l . d)-1 compared to 0.08 $(mg/l \cdot d)^{-1}$.

Ke based on BOD_5 resulted in all the values except for the base mix, for the continuous flow being higher than the batch unit. The base mix batch gave a Ke of 0.36 $(mg/l \cdot d)^{-1}$ compared to 0.26 $(mg/l \cdot d)^{-1}$ for the continuous flow. For the three pollutants Ke for the continuous vs. batch was 0.73 $(mg/l \cdot d)^{-1}$ vs. 0.108 $(mg/l \cdot d)^{-1}$ for acrylonitrile, 0.88 $(mg/l \cdot d)^{-1}$ vs. 0.072 $(mg/l \cdot d)^{-1}$ for 2,4 dinitrophenol, and 0.65 $(mg/l \cdot d)^{-1}$ vs. 0.050 $(mg/l \cdot d)^{-1}$ for 1,3 dichlorobenzene.

Ke'. Ke' determined from TOC achieved similar results to that of Ke. All the studies performed in the batch system achieved higher values than the continuous flow except 1,3 dichlorobenzene. The values from the batch compared to the continuous flow are: 16.3 d^{-1} vs. 4.1 d^{-1} for the base mix, 24.0 d^{-1} versus 3.8 d^{-1} for acrylonitrile, 17.5 d^{-1} versus 5.6 d^{-1} for 2,4 dinitrophenol and 11.0 d^{-1} versus 11.5 d^{-1} for 1,3 dichlorobenzene.

BOD_5 used to determine Ke' had a little different pattern than Ke as the base mix was higher at 192 d^{-1} vs. 82 d^{-1} from the continuous flow. Acrylonitrile resulted in relative agreement between the batch and continuous flow, with 36 d^{-1} vs. 22 d^{-1}. 2,4 Dinitrophenol and 1,3 dichlorobenzene were both lower in the batch than the continuous flow with 43 d^{-1} vs. 56 d^{-1} and 29 d^{-1} vs. 73 d^{-1}, respectively.

The yield values achieved from the batch system were lower than

those achieved with the continuous flow system except for the base mix for both TOC and BOD5, as seen in Table VI. In comparison, the yield based on TOC for the compounds between the batch and continuous flow were, 0.82 mg/mg vs. 0.81 mg/mg for the base mix, 0.50 mg/mg vs. 0.96 mg/mg for acrylonitrile, 0.64 mg/mg vs. 0.96 mg/mg for 2,4 dinitrophenol, and 0.75 mg/mg vs. 0.97 mg/mg for 1,3 dichlorobenzene.

Finally, the batch system constants were developed for the primary phases of growth resulting from each compound. The removal of substrate relative to the occurrance of the growth phases studies averaged approximately a 50% reduction in substrate compared to the continuous flow systems which averaged better than 90% removal of substrate. The key importance of Ke and Ke' is their use in Eckenfelder's design equation as a factor in the quantity K_eS_eXv. Realizing this, would demonstrate that even though the numbers for Ke and Ke' show some relative agreement, that K_eS_eXV from the batch would not be assured of being equal to K_eS_eXV from the continuous flow for design purposes.

Discussion

The dynamics involved in a "simple" dispersed batch system with a heterogenous population and a complex substrate for specific modeling is in need of continued investigation as many questions are still unanswered. When a system is placed in a continuous mode, this further complicates the mechanics involved as the system becomes one of "constant molecular motion", in more than one dimension.

The three compounds studied, acrylonitrile, 2,4 dinitrophenol, and 1,3 dichlorobenzene in conjuction with a synthetic industrial waste base mix, exhibited kinetic behavior consistent with proposed metabolic

mechanisms in the literature (15)(50)(59). The batch and continuous flow systems both experienced changes in kinetic constants in reference to the base mix from the compounds.

In the completely mixed dispersed batch studies, three methods were utilized to determined μ_m and K_S, a plot of μ vs. S, $1/\mu$ vs. 1/S, and S/μ vs. S. All three methods showed agreement for values of $\mu_m + K_S$ obtained, however, in the case of acrylonitrile that had inhibition occurring which could be modeled by Haldane's relationship the μ_m and K_S values could not be determined readily with $1/\mu$ vs. 1/S and S/μ vs. S plots.

Of the three compounds studied in the batch unit system, acrylonitrile was the only compound which at the concentration's used exhibited inhibition. 2,4 Dinitrophenol and 1,3 dichlorobenzene both effected a reduction in the specific growth rates and μ_m. This may be due to the decreased ease of biodegradation of the compunds. The continuous flow systems experience the same type of behavior with 2,4 DNP and 1,3 dichlorobenzene with the differences in their μ_m to that of the base mix approaching a constant in both systems, i.e, μ_m base mix/μ_m 2,4 DNP for the continuous flows 2 vs. a μ_m/μ_m or 1.3 in the batch while 1,3 DCB had a ratio of 1.4 for the batch and 1.9 for the continuous flow system. Acrylonitrile achieved a μ_m for each system which showed agreement with that of the base mix, except that the continuos flow system showed no evidence of inhibition.

The modeling of inhibition and/or a reduction in μ_m in batch and continuous sytems is lacking for models which address the results. The kinetic models used for inhibition for enzyme kinetics carry labels and terminology that are not adequate for whole cell systems in which

multiple enzyme systems are present and under the direct control of the cell. The importance to the environmental engineer for modeling reduction and inhibition from waste waters is to be able to design and predict the operation of a waste water treatment plant which is receiveing toxic wastes or a mixture of wastes which effect changes in μ_m and K_S. With such a model, the operation of a continuous flow waste treatment plant could be changed as the proportional amounts of the materials in the waste water changed.

A very important observation made between the two systems studied was the consistency of the activated sludge in the reproducable response to substrate. With the complex heterogeneous populations present and the dynamics involved in such populations, it is encouraging that the response is reproducable. The ability of these populations to achieve biodegradation of compounds that are not easily degraded in pure culture or limited cultures systems, holds mechanistic possibilities for exploitation for the enhancement of our environment.

Synthesis of byproducts occurring in the batch system was quite evident as a soluble color developed in all of the experiments except the one for acrylonitrile. The color was a light green appearing at about 4 hours progressing to a dark green at 8 hours and finally a brown as 12 hours. The intensity of the color was proportional to the base mix concentration in the systems. The production of metabolic products is of primary concern to environmental engineers since it effects the quantity $S_i - S_e$ for use in mass balances. If the products are a direct function of metabolism then the effluent quality designed for must take into consideration the presence and existence of such products and be adjusted accordingly. Synthesis products may be one explanation for the

scatter seen in the data plotted for Ke, Ke' and Y. This could also offer a possible explanation for the specific substrate utilization rate being lower than the specific growth rate at the higher concentrations used in the study. This is seen in the Appendix.

Another problem experienced in the study was the evidenced stripping of acrylonitrile. This was not seen in the continuous flow systems. Stripping presents a real problem to contend with in creating mass balances for systems. Stripping coupled with the probability of the existence of synthesis products would change the mass balance to include these factors.

It is quite obvious that the kinetic constants developed from a dispersed batch system should not be applied to the design of continuous flow systems. The intent of the approach in the text by the author was not to develop design criteria for continuous flow biological systems from batch systems, but rather to determine the kinetic behavior exhibited by the two systems as an aide in understanding and prediction of behavior in the design of continuous flow systems.

CHAPTER V

CONCLUSIONS

1. Acrylonitrile; 1,3 dichlorobenzene; and 2,4 dinitrophenol are biodegradable in batch systems with heterogeneous populations.

2. Inhibition occurred with acrylonitrile while 2,4 DNP and 1,3 DCB effected a reduction in μ_m over that of the base mix.

3. The existence of more than one type of reduction in μ_m occurred with each compound compared to that predicted by the models.

4. All the compounds are compatible with activated sludge from a municipal waste treatment system.

5. 2,4 dinitrophenol and 1,3 dichlorobenzene result in a mechanistically similar response by the microorganisms at equal concentrations.

6. The three compounds studies were preferentially removed on an order of (a) acrylonitrile, (b) 1,3 dichlorobenzene, and (c) 2,4 dinitrophenol when in combination in the batch units.

7. Synthesis products occurred in the experiments as evidenced by soluable color changes.

CHAPTER VI

SUGGESTIONS FOR FURTHER RESEARCH

1. Investigate the intercell enzymatic relationship in heterogeneous populations, to further exploit their use.

2. Investigate inhibition mechanisms involved in complex wastes to develop a predictable model.

3. Investigate synthesis products in heterogeneous populations by gas chromatography.

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APPENDIX

DATA

	Theo. TOC (mg/l)	Poll. Conc. (mg/l)	S (mg/l)	1/S (l/mg)	μ (hr⁻¹)	1/μ (hr.)	S/μ (mg/l)(hr/l)	Se (mg/l)	Sr (mg/l)	Xp (mg/l)	X (mg/l)	t (hr.)	(So-S)/X (hr.)	So(So-S)/X (mg/l/hr.)	μ II (hr⁻¹)	1/μ II (hr.)	S/μ II (mghr/l)	Se (mg/l)	μ III (hr⁻¹)	1/μ III (hr.)	S/μ III (mghr/l)	S BOD (mg/l)	1/S (l/mg)	S/μ (mghr/l)	Se (mg/l)	Sr (mg/l)	So-S/X (hr.)	So(So-S)/X (mg/l/hr.)	X (mg/l)	t (hr.)
Base Mix	50		79	.0147	.168	5.95	470	50	29	16	33	3	.291	23								170	.0059	1012	50	120	1.20	204.8	33	3
	50		97	.0103	.156	6.41	622	62	35	15	32	3	.360	35								174	.0057	1115	67	107	1.10	192	32	3
	100		115	.0087	.213	4.69	539	66	49	22	36	3	.451	52								297	.0034	1393	64	233	2.15	637	36	3
	100		175	.0057	.250	4.00	700	145	30	28	39	3	.256	45								352	.0040	1008	160	92	.796	198	39	3
	200		248	.0040	.347	2.88	716	148	100	75	63	4	.397	98								630	.0016	1814	83	547	3.80	2393	48	3
	200		310	.0032	.420	2.38	738	190	120	109	79	4	.380	118								389	.0026	926	104	285	1.70	660	56	3
	400		469	.0021	.496	2.02	947	205	266	274	162	5	.326	153								1200	.0008	2419	123	1077	1.33	1596	162	5
	400		530	.0019	.475	2.11	1118	250	280	244	147	5	.381	202								780	.0013	1646	515	265	1.38	1077	64	3
	800		835	.0012	.510	1.96	1637	492	343	295	173	5	.397	331								1740	.0006	3410	240	1500	1.73	3017	173	5
	800		720	.0014	.502	1.99	1434	380	340	290	165	5	.417	297								1433	.0007	2852	1015	418	2.02	2894	69	3
Acrylonitrile	100	50	147	.0068	.135	7.40	1088	123	24	25	62	3	.129	19								234	.0043	1732	186	48	.258	60	62	3
	150	50	188	.0053	.231	4.33	814	82	106	50	75	3	.427	187								330	.0030	1429	162	168	.747	246	75	3
(50mg/l)	250	50	300	.0033	.291	3.44	1032	96	206	164	132	5	.312	96								450	.0022	1548	240	210	.530	239	132	3
	450	50	617	.0016	.304	3.29	2030	194	423	179	140	5	.506	373								615	.0016	2023	200	415	.539	365	140	5
	850	50	823	.0012	.231	4.33	3564	512	311	150	125	6	.415	341	.239	4.2	2142					1410	.0007	6105	300	1110	1.480	2087	125	6
	150	100	198	.0051	.086	11.71	2931	115	83	7	33	3	.538	156								285	.0035	4192	196	87	.880	250	33	3
	200	100	265	.0038	.082	12.20	3233	96	171	15	38	5	.900	239	.184	5.4	510					303	.0033	3697	120	183	1.79	544	34	3
(100mg/l)	300	100	346	.0029	.139	7.19	2459	200	141	30	45	5	.627	214	.233	4.5	898					436	.0023	3135	190	245	2.15	937	38	3
	500	100	588	.0017	.132	7.58	4457	317	271	36	48	6	.941	553	.217	4.6	1458					670	.0015	5079	420	250	2.25	1509	37	3
	900	100	929	.0011	.123	8.13	7553	788	141	33	46	6	.511	475	.214	4.7	3680					1575	.0006	12805	1440	135	1.22	1915	37	3
2,4 Dinitrophenol	75																					312	.0039	1236	216	96	.32	100	75	4
	125	25	250	.0040	.252	3.97	993	130	120	70	75	4	.400	100	.082	12.07	1569					435	.0023	1697	200	235	.511	222	92	5
(25mg/l)	225	25	450	.0022	.256	3.91	1760	275	175	104	92	5	.380	171	.057	17.6	4829					1050	.0010	3098	225	825	1.28	1055	129	5
	425	25	510	.0020	.340	2.94	1499	300	210	179	129	5	.326	166	.048	20.5	6150					1660	.0006	4250	480	1140	1.47	2433	161	5
	825	25	825	.0012	.391	2.56	2112	490	335	243	161	5	.416	343																	
	100	50	305	.0049	.138	7.25	1481	150	55	28	69	3	.566	55	.067	15.0	2243					156	.0064	1129	105	51	.246	38	69	3
	150	50	270	.0037	.187	5.35	1445	180	90	85	140	5	.129	35	.095	10.3	1888					375	.0027	2006	190	186	.264	99	140	5
(50mg/l)	250	50	380	.0026	.217	4.61	1752	220	160	76	93	4	.430	198	.062	16.0	3518					355	.0028	1633	260	95	.174	62	109	5
	450	50	445	.0022	.236	4.24	1887	225	220	124	117	5	.376	168	.074	13.4	3015					660	.0015	2739	225	435	.74	491	117	5
	850	50	730	.0014	.266	3.76	2745	380	350	216	163	6	.358	261	.074	13.4	5112					1500	.0007	5640	480	1320	1.04	1564	163	6
1,3 Dichlorobenzene	75	25	180	.0056	.147	6.85	1224	130	50	25	57	3	.292	53								210	.0042	1632	180	60	.351	84	57	3
	125	25	300	.0033	.161	6.21	1863	130	170	56	73	5	.466	140								300	.0033	1863	210	90	.247	74	73	5
(25mg/l)	225	25	350	.0029	.203	4.93	1726	230	120	79	85	5	.282	99	.090	11.1	2553					480	.0021	2366	420	60	.140	67	86	5
	425	25	490	.0020	.220	4.55	2230	350	140	90	90	5	.311	152	.105	9.5	3325					1140	.0009	5187	720	420	.933	1064	90	5
	825	25	860	.0017	.296	3.38	2907	540	320	153	121	5	.529	454	.033	30.3	16364					1380	.0007	4664	1200	180	.298	411	121	5
	100	50	180	.0056	.131	7.63	1373	180	60	34	67	4	.149	27	.044	22.7	909					150	.0067	1145	90	60	.164	25	73	5
	150	50	325	.0031	.183	5.46	1775	160	165	75	87	5	.264	86	.047	21.3	3405					270	.0037	1474	1185	165	.379	102	87	5
(50mg/l)	250	50	390	.0026	.213	4.69	1819	280	110	95	98	5	.224	88	.053	18.9	5289					420	.0024	1970	300	120	.245	103	98	5
	450	50	540	.0019	.247	4.05	2187	375	165	122	111	5	.297	140	.054	18.5	6945					900	.0011	3645	480	420	.757	681	111	5
	850	50	870	.0011	.315	3.17	2758	650	270	160	146	5	.370	172	.122	9.0	5382					1320	.0008	4184	600	720	.986	1320	146	5
Combined	250	100	190	.0053	.119	8.41	1598	140	54						.150	6.56	3637	120	.533	30.3	3637									
	350	100	280	.0036	.119	8.40	2352	240	60						.185	5.40	5984	190	.332	31.3	5984									
	400	100	470	.0021	.126	7.94	3732	315	155						.196	5.10	1579	168	.106	9.4	1579									
	600	100	800	.0013	.119	8.40	6720	625	175						.239	4.18	2613	420	.118	8.5	3557									
	1000	100	935	.0011	.067	14.93	13885	880	50						.282	3.55	3124	570	.181	5.5	3146									

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