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Kinetic measuremenmt of decomposition of aniline, 2,4-dichlorophenol and 1,2,4-trichlorophenol using a calorimetric method

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ABSTRACT

Kinetic Measurement of Decomposition of Aniline, 2,4-Dichlorophenol and 1,2,4-Trichlorophenol Using a Calorimetric Method

by

Ming-Chin Chang

Biological treatment techniques for detoxification of toxic organic compounds have been studied and applied for many years. There are many research and commercial applications proving that the biological method is more economic and efficient than the other treatment methods.

The traditional biomass measurements are time-consuming tasks. Researchers have intended to develop a simpler, faster, and also accurate method for determining the bioactivity. This study has tried to use the microcalorimetric method to monitor the energy productions from the degradation of aniline, 2,4-Dichlorophenol and 1,2,4-Trichlorobenzene. Thereafter, by utilizing the data obtained from the present study, it was possible to compute kinetic constants such as μ_E, Kc, Ki and N of a substrate inhibition model which in turn can precisely determine the order of biodegradability of these three target compounds. Moreover, these constants can serve as a database of designing the hazardous wastewater treatment plant. The other objective of this study was to understand the effect of toxic substance concentration on cell decay rate expressed by the reduction of cell energy production. This study concluded that when the substrate concentration was below the minimum toxicity level, the order of biodegradability is 2,4-DCP > 1,2,4-TCB > aniline. On the contrary, when the substrate concentration was in excess of its toxic level, the order of toxicity of these three organic pollutants is 1,2,4-TCB > aniline > 2,4-DCP.

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by
Ming-Chin Chang

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Kinetic Measurement of Decomposition of Aniline, 2,4-Dichlorophenol and 1,2,4-Trichlorobenzene Using a Calorimetric Method

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This thesis is dedicated to
my family

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CHAPTER 1
INTRODUCTION

In 1989, Lung-Weng Yao *(1)* studied the biodegradation of Aniline, 2,4- Dichlorophenol, and 1,2,4-Trichlorobenzene by using the microcalorimetric method in her master's thesis. In her study, the biodegradability and kinetic constants have been discussed for "log" phases. In my study, I would like to continue the study of the microcalorimetric method, including discussions of inhibition phenomena of toxic organics and the "endogenous" phases of microbial activities.

The growth of microorganisms for water pollution control is of great interest. Microorganisms use the energy they gain from the substrate for biological growth. During metabolism, the substrate energy carries information to the ATP. At the same time, the charged ATP brings the energy cited to all the organism's cells, which obtain energy in turn for the synthesis of enzymes. In other words, microorganisms carry out inorganic and organic reactions during this energy transfer. Hence, the estimation of the energy released from the microorganisms is, an efficient way of quantifying the utilization of the substrate.

Biological treatment uses microorganisms, mainly bacteria that metabolize organic matter in wastewater to yield energy for synthesis, motility, and respiration. Biological utilization of organic compounds involves a series of enzyme-catalyzed reactions. Simple dissolved organic compounds are readily taken into the cells of

microorganisms and oxidized. When microbial cells come into contact with complex insoluble organics, enzymes are released outside the cells to hydrolyze these materials (proteins and fats) into soluble fractions, enabling their transport through the cell wall for acclimation. Thus, the larger, more complex organic compounds are metabolized at a slower rate. *(2)*

The success of the microbial degradation depends on a biodegradability of organic contaminants, which, using conventional techniques, is a time-consuming task. It usually takes a minimum of three to four weeks to determine the biodegradability of one contaminant at a particular concentration. A specially designed microcalorimeter *(3)* can be used as a rapid tool to assess the biodegradability of a given contaminant within a period of 2-3 days.

The various traditional measurement methods in environmental science and engineering application included,

1. Direct measurement of dry weight
2. Volumetric measurement
3. Measurement of various chemical components
4. Measurement of turbidity
5. Measurement of the number of particles
6. Measurement of the viable population

A member of the National Solid Wastes Management Association, Pirages, S.W., 1988, has mentioned in his keynote address that application of microbial degradation and removal of undesirable constituents in industrial and municipal wastes is a commonly used process for general wastewater treatment and has been for many years. As the awareness of chemical contamination of the environment increases, much research on biological degradation of toxic chemicals has occurred. Within the range of toxic constituent treatment technologies, biological degradation ranks among the most effective. Its management application is enhanced by the potential to apply biological treatment in sequence with other chemical and thermal processes. *(4)*

1.1 The Biodegradation of Hazardous Wastes

Biological treatment is generally used for the removal of organic pollutants from wastewater, and its great popularity for serving this vital function results largely from its low cost and its effectiveness for removing many soluble and colloidal organics. Application of biological treatment to wastewaters containing toxic organic matter requires considerably more care than in the case of nontoxic wastes. A considerable period of time may be required to reestablish an adequate population of microorganisms to treat the wastes.

Halogenated organic compounds, such as the chlorophenolic and chlorobenzoic compounds, tend to be toxic, resistant to the degradation, and bioaccumulate in the

living organisms. Among the chlorophenolic compounds, such as 2,4-dichlorophenol and pentachlorophenol are the most ubiquitous.

Chlorophenolic compounds are the most popular organics employed by wood pulp industries (about 80%). The pulp industries use several kinds of chlorophenolic compounds, such as 2,4-dichlorophenol, para-dichlorophenol and pentachlorophenol, in their bleaching processes and preserving system. The discharge of these wood pulp industries has been detected in public streams, rivers and oceans.

1.2 The purpose of this study

1. Study the feasibility of the microcalorimetric method for the energy output by microbial activities instead of the traditional biomass measurements in order to consume less experimental time.

2. Find the biodegradability order of aniline, 2,4- dichlorophenol, and 1,2,4-Trichlorobenzene.

3. Calculating the biokinetic constants (including μ_E, Kc, Ki, and N) is important for the environmental engineers to make the optimal database for designing the wastewater treatment plants.

4. Discuss the growth of the endogenous phases to compare with the log phases.

1.3 The properties of aniline, 2,4-Dichlorophenol, and 1,2,4-Trichlorobenzene

Figure 1 shows the molecular structures of the 3 different target compounds, and their properties *(5)* list as follows :

1. **Aniline:** $(C_6H_5NH_2)$

   (a) Aniline is recognized as a hazardous substance and a hazardous waste by EPA.

   (b) Aniline is a clear, colorless, oily liquid with a characteristic odor.

   (c) Aniline is widely used as an intermediate in the synthesis of dyestuffs. It is also used in the manufacture of rubber accelerators and antioxidants, pharmaceuticals, marking inks, tetryl, optical whitening agents, photographic developers, resins, varnishes, perfumes, shoe polishes, and many organic chemicals.

   (d) Aniline is a strong acids, and a strong oxidizer.

   (e) The federal standard is 5 ppm (19 mg/m^3) for permissible exposure limits in air. However, there is no criteria set but EPA has suggested an ambient limit in water of 262 $\mu g/L$ based on health effects.

   (f) The routes of entry include inhalation of vapors, percutaneous absorption of liquid and vapor, ingestion, skin and eye contact.

   (g) The points of attack include blood, cardiovascular system, liver, and kidneys.

2. Chlorinated Phenols :

It is well known that the highly toxic polychlorinated dibenzo-p-dioxins may be formed during the chemical synthesis of some chlorophenols and that the amount of contaminant formed is dependent upon the temperature and pressure control of the reaction.

0.37 Evidence has accumulated that the various chlorophenols are formed as intermediate metabolites during the microbiological degradation of the herbicides 2,4-D and 2,4,5-T and pesticides silvex, ronnel, lindane and benzene hexachloride. In view of this, it is clear that chlorinated phenols represent important compounds with regard to potential point source and nonpoint source water contamination.

0.37 Chlorophenols may be produced inadvertently by chlorination reactions which take place during the disinfection of wastewater effluents or drinking water sources. Phenol has been reported to be highly reactive to chlorine in dilute aqueous solutions over a considerable pH range.

3. 2,4-Dichlorophenol ($Cl_2C_6H_3OH$) :

(a) 2,4-D is a hazardous waste and a priority toxic pollutant by EPA.

(b) 2,4-D is a colorless crystalline solid melting at 45°C and boiling at 210°C.

(c) 2,4-DCP is a commercially produced substituted phenol used entirely in the manufacture of industrial and agricultural products. As an intermediate in the chemical industry, 2,4-DCP is utilized as the feedstock for the manufacture of 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,4-D deriva-

tives (germicides, soil sterilants, etc.) and certain methyl compounds used in mothproofing, antiseptics and seed disinfectants. 2,4-DCP is also reacted with benzene sulfonyl chloride to produce miticides or further chlorinated to pentachlorophenol, a wood preservative. It is thus a widely used pesticide intermediate.

(d) There is no standards set for permissible exposure limits in air.

(e) The permissible concentrations in water are : to protect freshwater aquatic life — 2,020 μg/L on an acute toxicity basis and 365 μg/L on a chronic toxicity basis, to protect human health —- 0.3 μg/L based on organoleptic effects and 3,090 μg/L based on toxicity data.

(f) Harmful effects and symptoms: Although a paucity of aquatic toxicity data exists, 2,4-DCP appears to be less toxic than the higher chlorinated phenols. 2,4-DCP's toxicity to certain microorganisms and plant life has been demonstrated and its tumor promoting potential in mice has been reported. In addition, it has been demonstrated that 2,4-DCP can produce objectionable odors when present in water at extremely low levels. These findings, in conjunction with potential 2,4-DCP pollution by waste sources from commercial processes or the inadvertent production of 2,4-DCP due to chlorination of waters containing phenol, lead to the conclusion that 2,4-DCP represents a potential threat to aquatic and terrestial life, including man. 2,4-DCP can irritate tissue and mucous membranes.

4. **1,2,4-Trichlorobenzene** ($C_6H_3Cl_3$)

(a) 1,2,4-TCB is a priority toxic pollutant by EPA.

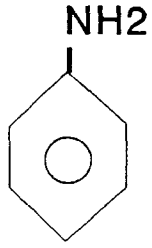
(b) 1,2,4-TCB is a low-melting solid or liquid with a pleasant aroma. It melts at 17°C and boils at 213.5°C.

(c) 1,2,4-TCB is used as a dye carrier (46%), herbicide intermediate (28%), a heat transfer medium, a dielectric fluid in transformers, a degreaser, a lubricant and a potential insecticide against termites.

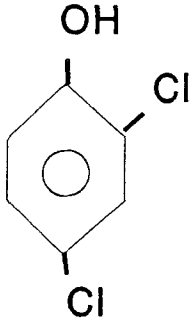
(d) Possible human exposure to trichlorobenzene (TCB) might occur from municipal and industrial wastewater and from surface runoff. Municipal and industrial discharges contained from 0.1 to 500 μg/L. Surface runoff has been found to contain 0.006 to 0.007 μg/L. In the National Organic Reconnaissance Survey conducted by EPA in 1975, TCB was found in drinking water at a level of 0.1 μg/L.

(e) There is no Federal Standard for the permissible exposure limits in air. Moreover, no criterion developed due to insufficient data for permissible concentration in water.

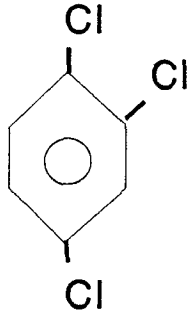
(f) The points of attack include skin, eyes, liver, kidneys, and lungs.



Aniline



2,4-Dichlorophenol



1,2,4-Trichlorobenzene

Figure 1. Molecular structure of target compounds

CHAPTER 2
LITERATURE REVIEW

Many previous reseach papers demonstrated the biodegradations of hazardous wastes and biodegradabilities of halobenzenes and halophenols by various techniques and measurement methods *(6 - 28)*. Some papers focused on kinetic study and searching the kinetic constants *(29 - 40)*. The microbial growth kinetic model can predict the biological activities for wastewater treatment. Therefore, the optimal kinetic model can supply the environmental engineers as one of the design databases. The following lists include the publications which presented biodegradations, biodegradabilities and biokinetics.

2.1 Biodegradation

2.1.1 Biodegradabilty of Chlorophenols and Chlorobenzenes

The biodegradability study has been done by Tabak et al. in 1981 *(6)* using the "static-flask-screening " method including 96 organic compounds. When studying the biodegradability of phenolic compounds and benzenic compounds, they found that chlorinated phenols were significantly biodegradable. However, the phenolic compounds got the higher biodegradability than the benzenic compounds. For example, the initial cultures of 2-CP; 2,4-DCP; 2,4,6-TCP and PCP (pentachlorophenols) yielded 86, 100, 100 and 19 percent removal, respectively. therefore, the biodegradability order was 2,4-DCP = 2,4,6-TCP > 2-CP > PCP. Acclimation to 2-CP was fast, and a removal of 100 percent was obtained with the first sub-

culture. The PCP was gradual acclimation and first subculture yielded 68 percent biodegradation. By the same ways to make comparison for benzenic compounds: the initial cultures of CB (chlorobenzene); 1,2-DCB; 1,3-DCB; 1,4-DCB; 1,2,4-TCB and Hexachlorobenzene yielded 89, 45, 59, 55, 54 and 56 percent removal, respectively. Therefore, the biodegradability order was CB > 1,3-DCB > Hexa-CB > 1,4-DCB > 1,2,4-TCB > 1,2-DCB. The acclimation of CB was fast and a removal of 100 percent was obtained with the first subculture. 1,2-DCB; 1,3-DCB; 1,4-DCB and 1,2,4-TCB were verified as "significant degradation with gradual adaptation followed by a deadaptive process in subsequent substrate. They didn't show much increase with the first subculture. from the above, we can understand that the 2,4-DCP was much biodegradable than 1,2,4-TCB by the 100 and 54 percent removal, respectively.

The EPA supported Pitter *(7)* to study the biological degradability of organic substances by carrying out experiments on the degree and rate of biological degradation of 123 organic compounds with respect to the decrease of organic substance in terms of COD. The organic substances were a sole source of carbon for the microbes of the inoculum, adapted activated sludge being the inoculum. The rate of degradation was expressed in terms of mg of COD removed by a g of the initial dry matter of inoculum by a hour. From Table "Biological degradability of aromatic compounds", aniline; O-chlorophenol; P-chlorophenol and 2,4-dichlorophenol yielded 94.5, 95.6, 96.0 and 98.0 percent COD removal, respectively. The rates of biodegradation (mg COD/g·h) were 19.0, 25.0, 11.0 and 10.5, respectively. Consequently, the biodegrad-

ability of 2,4-DCP was higher than aniline both by the comparisons of COD removal efficiencies and biodegradation rates.

Ingols et al.*(8)* studied the biological activity of halophenols based on BOD tests and ultraviolet adsorption spectra determinations. The results showed many important conclusions. First of all, the di-chlorophenols were degraded less readily than mono-chlorophenols. The maximum degradation obtained from each compound at 100 mg/L: O-CP; M-CP; P-CP; 2,4-DCP and 2,4,6-TCP yielded 100 percent ring degradation completely. 2,5-DCP yielded only 52 percent. However, O-BP (brominephenols); M-BP; P-BP; 2,4-DBP and 2,4,6-TBP yielded 100, 25, 93, 81 and 0 percent ring degradation, respectively. The indolephenols, O-IP; M-IP; P-IP and 2,4,6-TIP yielded 29, 52, 43 and 0 percent ring degradation, respectively. These results demonstrated that chlorophenols were more biodegradable than brominephenols and indolephenols in O- , M- and P- positions. Besides, the biodegradability of 2,4-DCP was higher than 2,5-DCP. 2,4,6-TCP was higher than 2,4,6-TBP; and 2,4,6-TIP.

The effect of chemical structure on microbial decomposition of aromatic herbicides by using the ultraviolet absorption spectrophotometric method. The biodegradations of hazardous wastes and biosegradabilities of halobenzenes and halophenols. has been studied by Alexander and Aleem *(9)*, . The microbial decomposition of chlorophenols in soil suspensions, for example, 2-CP; 3-CP; 4-CP; 2,4-DCP; 2,5-

DCP; 2,4,5-TCP; 2,4,6-TCP; 2,3,4,6-Tetra-CP and PCP yielded the days for complete disappearance 14, 72, 9, 9, 72+, 72+, 5, 72+ and 72+, respectively. (+ after a figure indicates ultraviolet absorption was still present at end of incubation period). However, 2-BP; 3-BP and 4-BP yielded 14, 72+ and 16, respectively. Therefore, the conclusions were that the biodegradability showed 4-CP > 2-CP > 3-CP; 2-BP > 4-BP > 3-BP; 2,4-DCP > 2,5-DCP; 2,4,6-TCP > 2,4,5-TCP and for the chlorophenols, the order was 2,4,6-TCP > 2,4-DCP > 2-CP > 2,4,5-TCP.

2.1.2 Biodegradable Techniques

Bouwer and McCarty *(10)* studied the removal of trace chlorinated organic compounds by activated carbon and fixed-film bacteria. The average removal of organic compounds by GAC column over study period : 1,2-DCB; 1,3-DCB; 1,4-DCB and 1,2,4-TCB yielded 97, 95, 98 and 97 percent removal, respectively. The average removals of organic compounds in BC column after acclimation : CB; 1,2-DCB; 1,3-DCB; 1,4-DCB and 1,2,4-TCB yielded 91, 96, 28, 98 and 90 percent removal, respectively. The influent concentration range were about 10 μg/L. The effluent concentration range were about 0.20-0.90 except 1,3-DCB was 7.1 μg/L. Therefore, biological oxidation of chlorinated benzenes at low concentration to mineralized end products can be an important removal mechanism with GAC columns.

A method used PCP-induced resulting cells of *Flavobacterium* by Steiert and Crawford *(11)* to study the dechlorinated and degradation of chlorinated phenols. The results reported were after 24 hour incubation with resting cells; removal de-

termined by gas-chromatography; chloride release determined with an ion-specific electrode. The dechlorination was standarded by PCP=100. Therefore, the dechlorination yielded from PCP; 2,3,4,5-TetCP; 2,3,4,6-TetCP and 2,3,5,6-TetCP were 100, 27, 100, and 100 percent, respectively. 2,3,4-TCP; 2,3,5-TCP; 2,3,6- TCP; 2,4,5-TCP; 2,4,6-TCP and 3,4,5-TCP yielded 10, 10, 100, 0, 100 and 0 percent dechlorination, respectively. 2,3-DCP; 2,4-DCP; 2,5-DCP; 2,6-DCP; 3,4-DCP and 3,5-DCP yielded 22, 10, 17, 100, 0 and 0 percent dechlorination, respectively. So, the structure effect varied and made no regulations. However, it show the dechloriation order Tetra-chlorophenols > Tri-chlorophenols.

Steiert et al., in 1987 *(12)* studied the pentachlorophenol (PCP)- degrading *Flavobacterium sp.* for its ability to dechlorinate other chlorinated phenols by using resting cells that had been grown in the presence or absence of PCP. Phenols with chlorine atoms at positions 2 and 6 of the phenol ring were dechlorinated completely by PCP-induced cells. Other chlorinated phenols were not significantly mineralized. Suspensions of cells grown in the presence of 2,4,6-trichlorophenol or 2,3,5,6-tetrachlorophenol did not show a lag period for mineralization of PCP, 2,4,6-trichlorophenol, or 2,3,5,6-tetrachlorophenol, indicating that one enzyme system probably was induced for the biodegradation of all three compounds. Nondegradable chlorophenols were toxic toward the *Flavobacterium sp.*. 2,4-DCP in PCP-induced cell was 10 percent dechlorination, yet in uninduced cells was 17 percent.

2.2 Biokinetics

The importance of biodegradation of toxic organics has presented by Grady 1990, He mentioned that the ability of biological treatment systems to degrade toxic organic compounds is assessed through a review of the literature. While much general information is available, there is little kinetic information that can be used directly by engineers making decisions about process alternatives. Nevertheless, useful kinetic information can be obtained from biodegradation studies in which the toxic compound of interest serves as the sole substrate for microbial growth; a scheme is proposed whereby such information can be used in mathematical models to predict the extent to which the toxic compounds can be removed from complex waste streams. A methodology is also proposed whereby the impacts of the toxic compounds on the biodegradation of the biogenic organic material in the waste streams can be predicted.

While biodegradability information is sufficient for making early feasibility decisions, information about rate of biodegradation is necessary for engineers to compare processes. Furthermore, rate information must be available as intrinsic coefficients to allow its use in models depicting alterative systems. Unfortunately, no data bases contain such information; they only contain qualitative descriptors such as fast, moderate and slow.

The predicting leads to only one conclusion: the potential for biodegradation

in the cleanup of toxic pollutants is bright. Many compounds are known to be biodegradable and the pace of research on biodegradation is the highest it has ever been, suggesting that our knowledge about the biodegradability of xenobiotic chemicals will expand greatly in the future. *(13)*

Saez and Rittmann *(14)* studied the biodegradation kinetics of 4-CP by using batch reactors containing a pure culture of *Pseudomonas putida PpG4*. 4-CP behaved as the only organic substrate and as a cometabolite because of its transformation, possible only with cells previously grown on phenol, did not yield any increase in cell mass. Exponential decay described the time-dependent biomass concentrations reasonably well; the estimated decay coefficient was 0.013/h (0.3/day). The 4-CP-transformation rate was controlled by the 4-CP/biomass (I:X) ratio. For low I:X ratio, the system was uninhibited, complete 4-CP transformation was observed, and the 4-CP-transformation rate was proportional to the biomass-oxidation rate. The link in the rates was observed probably because the electrons consumed during the 4-CP transformation were produced by way of biomass oxidation. The constant of proportionality between the rates of 4-CP transformation and biomass decay corresponds to the cell shunting about 9 % of the electrons provided by biomass oxidation to 4-CP transformation. For high I:X ratio, the system was inhibited by 4-CP itself, the initial 4-CP transformation was slow, it further slowed, and it rapidly a significant fraction of the 4-CP untransformed.

The biodegradation of 2,4-DCP by using the completely-mixed systems and a mixture substrate consisted of 2,4-DCP (20% of total COD) and methanol (MA, 80% of total COD) has been studied by Chudoba et. al. *(15)*. The maximum specific rate of 2,4-DCP removal, q_{max}, related to the total biomass in the cultivation system, increased with the decreasing SRT from $0.0118h^{-1}$ at the SRT of 10.8 d^{-1} up to $0.0783h^{-1}$ at the SRT of 0.6 d. The following values of kinetic constants of microorganic degradation of 2,4-DCP were found (20°C and pH=7.5): K_s(COD)= 0.6 mg/L; q_{max}= $0.233h^{-1}$ (maximum specific substrate removal rate), b= $0.036h^{-1}$ (biomass decay rate constant), μ_{max}= 0.086 h^{-1} (maximum specific growth rate)

In 1982, Beltrame et al. *(29)* studied the biokinetic of mixture culture containing 2,4-DCP and phenol (or glucose) by using the completely mixed continuous flow reactor which operated at steady-state conditions at 20°C. With recycle and controlled wasting of the activated sludge. A carefully adapted sludge was able to degrade DCP, besides the accompanying substrate, with no evidence of substrate inhibition in the concentration range covered (156 mg/L in the influent). The reactivity glucose > phenol > DCP.

Brown, Grady and Tabak *(30)* studied the biodegradation kinetics by using the electrolytic respirometers to evaluate phenol; 4-CP; m-cresol; 2,4-Dimethylphenol (2,4-DMP) and 2,4-Dinitrophenol (2,4-DNP) through use of spreadsheets and non-linear curve-fitting. 4-CP exhibited substrate inhibition kinetics and 2,4-DNP ex-

hibited product inhibition kinetics.

Biodegradation plays an important role in the fate of synthetic organic chemicals in both natural and engineered environments. As a consequence, prediction of the fate of such chemicals requires quantification of their biodegradation rates in the form of intrinstic kinetic parameters that can be used in mass balance equations. kinetics are the Monod (1949) equation for a noninhibitory substrate:

$$q = \frac{\mu}{Y} = \frac{\hat{\mu}}{Y} \cdot \frac{S_s}{K_s + S_s}$$

and the Andrews (1968) equation for a substrate that is inhibitory to its own biodegradation:

$$q = \frac{\mu}{Y} = \frac{\hat{\mu}}{Y} \cdot \frac{S_s}{K_s + S_s + S_s^2/K_i}$$

Determination of the parameter values describing biodegradation has historically been a tedious and labor-intensive undertaking. Recently, however, it has become possible to do it with oxygen uptake data collected automatically from batch reactors using electrolytic respirometry.

A new techniques for the measurement of Monod kinetic coefficients, the microbial yield coefficient and the endogenous decay coefficient has been studied by Speitel and DiGiano *(31)*. Kinetic coefficients and the microbial yield coefficient were determined through measurements of initial rates in short duration batch experiments using a radiolabeled substrate (phenol). The experimental data were

described well at the Monod model. A first order model fits the endogenous decay of radiolabeled cells. The microbial kinetic technique developed in this research may increase the attractiveness of batch experiments because the approach is faster and easier than chemostat studies.

Microbial kinetic coefficients are usually determined from a series of chemostats operated at different dilution rates. Such experiments require a long time to complete and are labor intensive. In this research a new technique was developed. Building upon the work of environmental microbiologists, kinetic coefficients were determined through measurements of initial rates in short duration batch experiments using radiolabeled substrate.

Microbial kinetics were characterized by the Monod model which was fitted to the data to determine the maximum growth rate (μ_m) and the half saturation coefficient (Y) and endogenous decay coefficient (b) were measured.

The measurement of the inhibitory effects studied by Volskay and Grady *(32)* for synthetic organic compounds on the biodegradation of naturally occuring (biogenic) organic matter by using the respirometry. All respiration rates were measured from traces of DO concentration versus time produced by recording the output from a DO meter. The protocol was used on 33 xenobiotic compounds listed in the Resource Conservation and Recovery Act (RCRA) including Chlorobenzene and 1,4-

DCB, PCP and phenol. Observed Monod kinetic parameters for the biodegradation of butyric acid in the presence of the indicated inhobitor: The different concentrations (mg/L) of Chlorobenzne were 0, 120, 135, 150, and 180, respectively. The observations of $\hat{\mu}$ [mg O_2/(L.min)] were 0.258, 0.220, 0.209, 0.175 and 0.000, respectively. The observations of Ks (mg/L butyric acid) were 0.121, 0.230, 0.220, 0.245 and NA, respectively.

The substrate inhibition model has been studied by completely and deeply by Neufeld et. al. *(33)*. The model states the effect of inhibition based on the Monod model ($V = V_o/[1+(K_s/S)]$) as growth phase and developed the inhibition phase as the term $(s/K_i)^n$. The value n presents the inhibition order while the larger the n is, the inhibition effect gets higher.

CHAPTER 3
MICROBIAL GROWTH AND BIOKINETIC MODELING

Microbial growth patten has been proposed for the municipal wastewater treatment for many years. This study is a batch study, therefore this chapter only describes the theory of batch growth of microorganisms. The theoretical growth model for log phase combined two parts, one is the Monod model at low substrate concentrations, the other is the substrate inhibition model (Neufeld, 1980)

3.1 Growth Rate in Batch Reactors

Since there is no flow of substrate or bacteria into or out of the reactor in batch processes, the concentrations of substrate and bacteria vary with time. If a small number of inoculum bacteria are placed into a substrate and nutrient rich liquid, the mass of bacteria as well as the substrate remaining within the liquid vary according to Figure 2 and Figure 3. Initially the bacterial population remains constant during a lag phase during which time the cells become accustomed to the new media. After a lag phase of up to several hours, the organisms begin to grow and multiply during an "cceleration phase". During this second phase, the many intermediates involved in the metabolic reaction chain build up to steady levels. Once the organisms have become accustomed to the media, they multiply very rapidly according to the first order reaction rate,

$$\frac{dX}{dt} = \mu X \qquad (3.1)$$

where X = weight of dry cells/volume

μ = specific growth rate, time^{-1}

Since the integral of Eqation (3.1) is a logarithmic expression, this growth is called the "log phase". During this phase, there is a high food to microorganism ratio with a very high fraction of the cells being viable; cell deaths are not important. Gradually, the food is consumed to a point where there are too many organisms and not enough food left to sustain the rapid growth rate during the log phase. As the food concentration becomes limiting, the death rate is nearly equal to the rate of cell synthesis. Eventually, the food supply will become low enough to cause the death rate to be greater than the synthesis rate and a decreased in the viable cell concentration occurs. During this "endogenous phase" the cells use the stored ATP energy for respiration and motion until the ATP is depleted and the cells die.

The cell wall then ruptures, releasing carbon containing compounds as food for the remaining viable bacteria. During the endogenous phase, bacteria are also eaten by single and multi-celled animals so that in practice the growth curve drops to zero more rapidly after the stationary phase than it would have if death occurred only by "natural causes". *(41)*

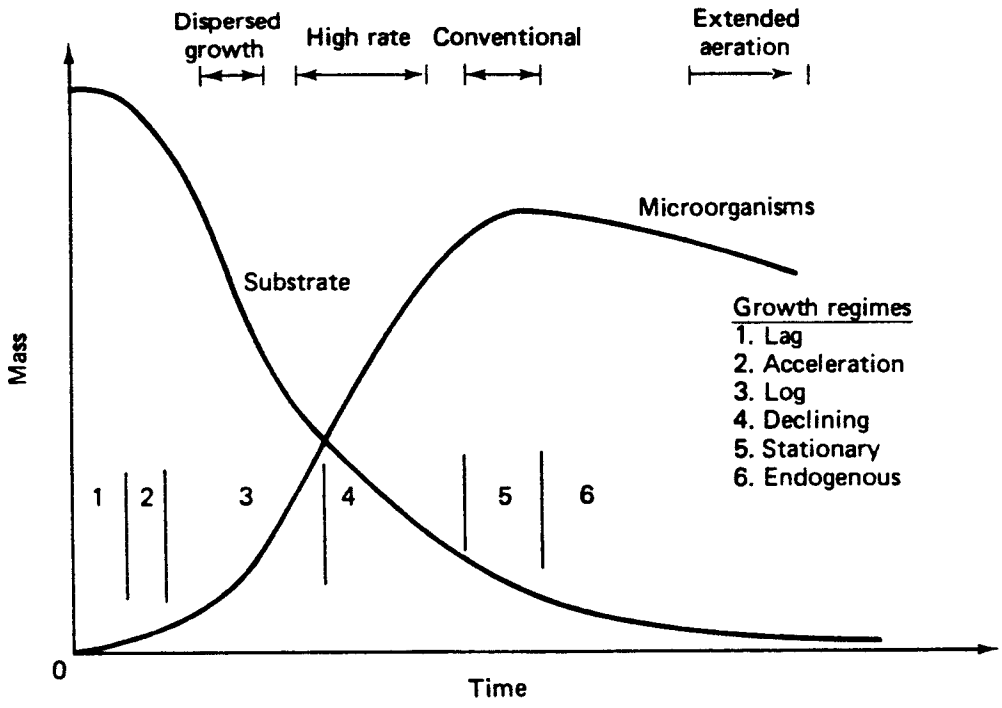


Figure 2 Typical growth curves for a batch reactor.

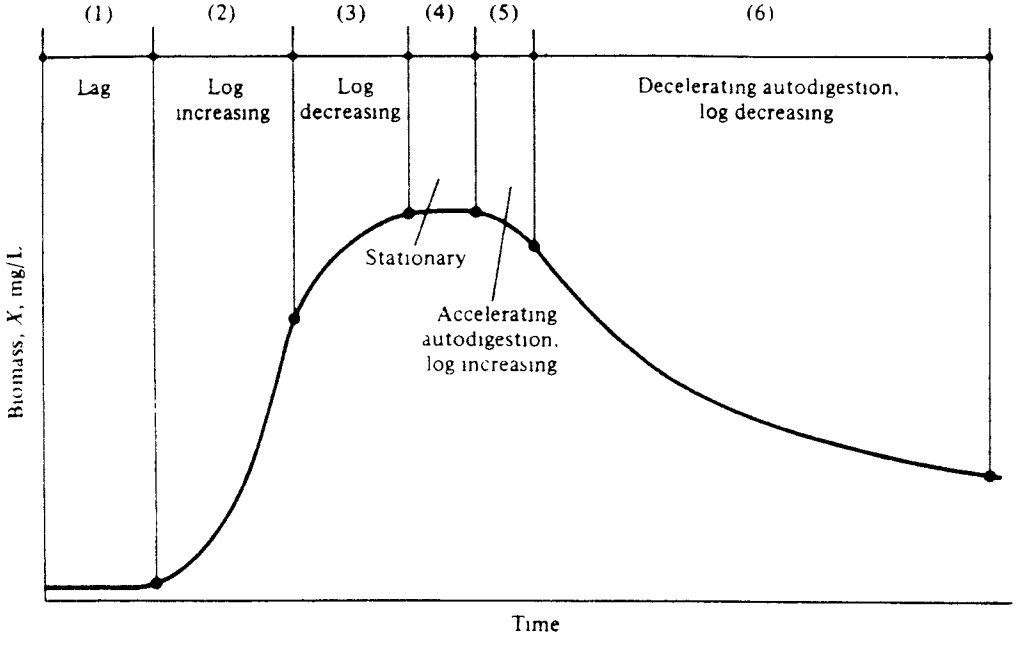


Figure 3 Distinguishable portions of biomass growth and decay curve.

3.2 Log Phase

At the end of the lag phase, the population of microorganisms is well adjusted to its new environment. The cells can then multiply rapidly, and cell mass, or the number of living cells, doubles regularly with time.

There are many reasons that cells do not grow indefinitely, including exhaustion of nutrients, depletion of the dissolved oxygen supply, crowing, growth-induced changes in the chemical environment, and production of toxic substances. It can be learned that in wastewater treatment, the aim is to control the environmental conditions so that the phenomenon limiting growth is exhaustion of the carbon source. *(42)*

3.2.1 Monod Model

Referring to Figure 2 and Figure 3, the rate of change in X, i.e., the slope of the curve, dX/dt, increases in phase 2, passes through an inflection point, and thereafter decreases until the maximum concentration of X is obtained and the population enters the stationary phase in which $(dX/dt) = 0$.

Often in phase 2, the rate of increase in X, (dX/dt), is proportional to the concentration X in the system, and the proportionality factor with the symbol μ as Equation 3.1 shows.

Upon integration, Equation (3.1) may be written as :

$$X_t = X_o e^{\mu t} \qquad (3.2)$$

Equation (3.2) can predict (calculate) the weight of cells at any time from a knowledge of the initial concentration Xo and the specific growth rate μ.

Equation (3.2) can be put in conventional straight line form for easy determination of μ by taking the logarithms of both sides :

$$lnX_t = lnX_o + \mu \cdot t \qquad (3.3)$$

Only when μ is constant can Equation 3.3 plot a straight line; consequently, any straight-line portion of a semilogarithmic plot of growth data marks the extent of exponential growth. Figure 4 is a semilogarithmic plot of growth data. It is clearly seen that there is an exponential phase of growth. The value of the specific growth rate μ can be obtained from the data by solving Equation 3.3 for μ :

$$\mu = \frac{lnX_t - lnX_o}{t} \qquad (3.4)$$

During exponential growth, only a single parameter μ is required to characterize the population. For this reason, the magnitude of the specific growth rate μ is widely used to describe the influence of the cell's environment on its performance. *(43)*

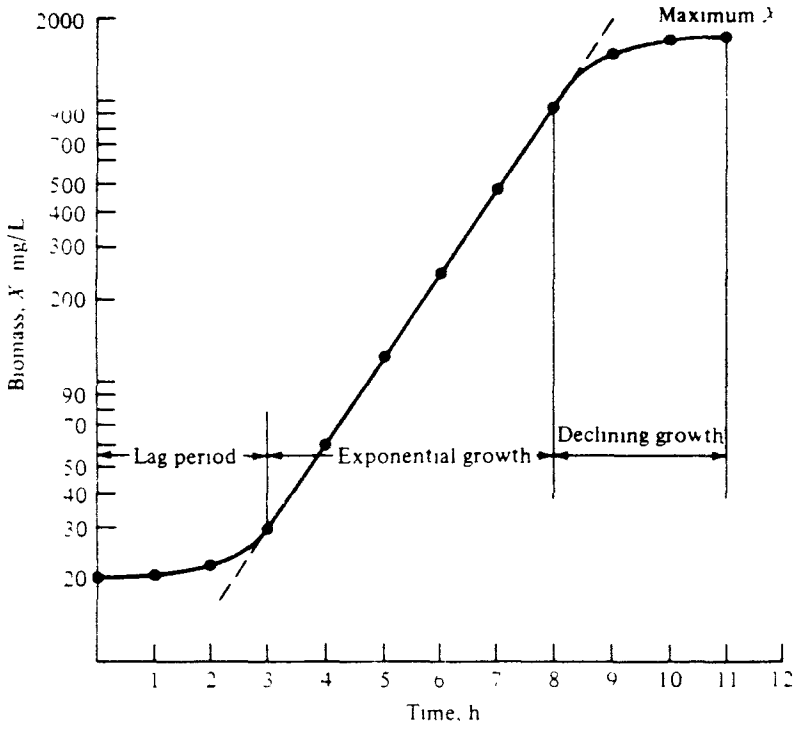


Figure 4 Semilogarithmic plot of a growth curve showing extent of exponential or logarithmic growth

If the concentration of one essential medium constituent is varied while the concentrations of all other medium components are kept constant, the growth rate changes in a hyperbolic fashion, as Figure 5 shows. A functional relationship between the specific growth rate μ and an essential compound's concentration was proposed by Monod in 1942. The standard rate equation for enzyme-catalyzed reactions with a single substrate(Henry in 1902 and Michaelis and Menten in 1913). Monod *(37)* Postulated that the growth of cells by binary fission on a single limiting substrate probably had a single limiting reaction step and therefore behave in a manner analogous to the Michaelis-Menten enzyme kinetics, i.e.

$$\frac{dX}{dt} = \mu_m X \frac{S}{K_s + S} \qquad (3.5)$$

where X = the cell concentrations,

S = the growth limiting substrate concentration,

t = time

μ_m = the maximum growth rate

Since the growth rate of cells, increasing by binary fission, is defined by

$$\mu = \frac{1}{X} \cdot \frac{dX}{dt} \qquad (3.6)$$

Equation 3.6 can be expressed as

$$\mu = \mu_m \frac{S}{K_s + S} \qquad (3.7)$$

Equation 3.7 may be converted as follows :

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{\mu_m}{K_s} \cdot \frac{1}{S} \qquad (3.8)$$

By plotting the $\frac{1}{\mu}$ versus $\frac{1}{S}$ as shown on Figure 6, the values of K_s and μ_m may be estimated by slop and intercept examination. The slop = μ_m/K_s and intercept = $1/\mu_m$.

It is apparent that the Monod equation is probably a great oversimplification. As in other areas of engineering, however, this is a case where a relatively simple equation reasonably expresses interrelationships even though the physical meaning of the model parameter is unknown or perhaps does not exists.

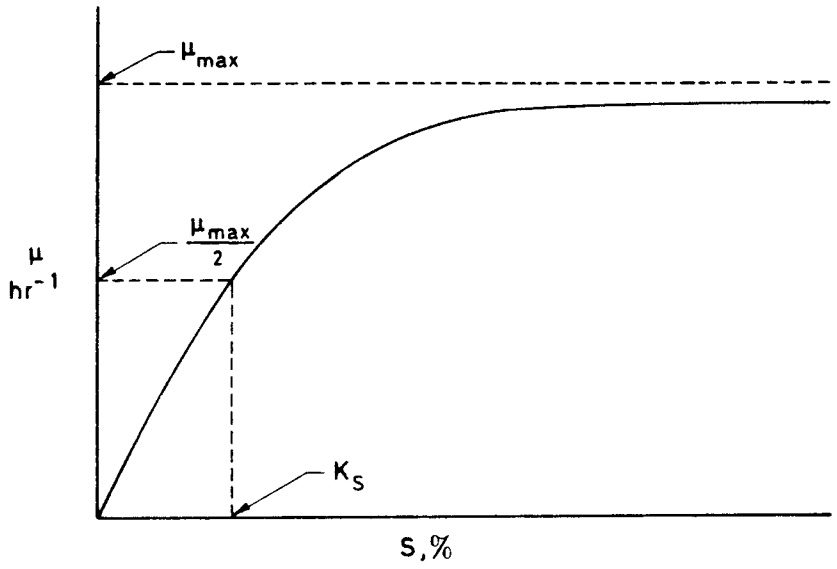


Figure 5 Specific growth rate for biomass at various substrate levels.

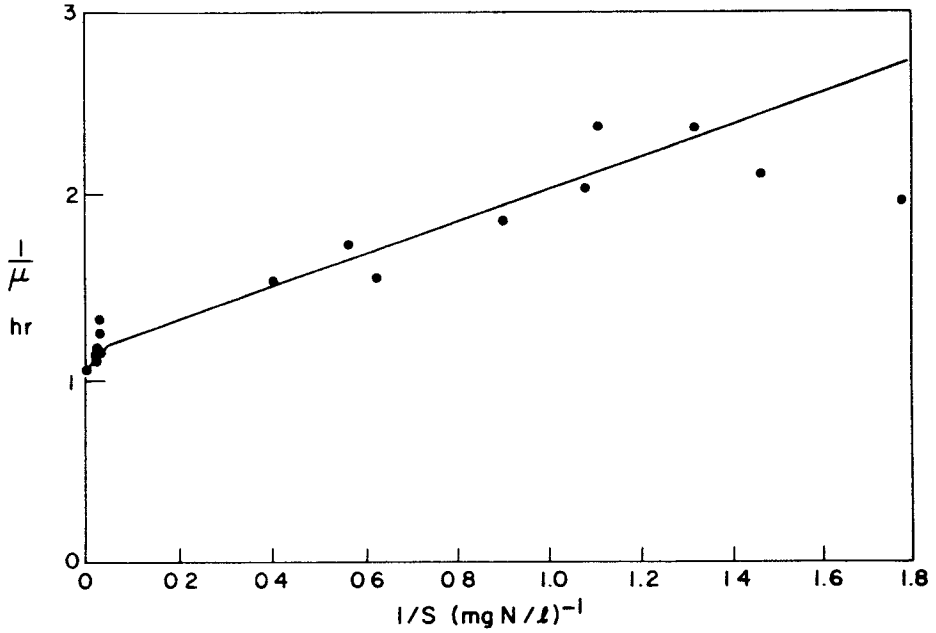


Figure 6 Represtative plot of $1/\mu$ vs. 1/S for the estimation of K_s and μ_m

3.2.2 Inhibition Model

The relationship between microbial growth and substrate utilization can usually be modeled with two basic differential equation :

$$\frac{dX}{dt} = a \cdot \frac{dS}{dt} - bX \qquad (3.9)$$

which describes the relationship between the net rate of growth of microorganisms and the rate of utilization of the growth-limiting substrate, and Equation 3.10 :

$$\frac{dS}{dt} = k \cdot \frac{SX}{K_s + S} \qquad (3.10)$$

Of the Monod form, which relates the rate of substrate utilization to both concentration of microorganisms in the reactor and concentration of the growth-limiting substrate surrounding the organism.

Equation 3.10 predicts that at high substrate concentrations, the specific utilization rate, dS/Xdt, approaches a maximum value. It has been observed that for some substrates such as phenol and cyanides, increased concentrations lead to a maximum in specific growth rate followed by a subsequent decline. This may be described by a biokinetic form for substrate utilization, modified for substrate inhibition as:

$$V = \frac{V_o}{1 + \frac{K_s}{S} + (\frac{S}{K_i})^n} \qquad (3.11)$$

The value of n determines the order of inhibition, while K_i is an inhibition constant.

The approach used in evaluating the parameters Vo, Ks, Ki and n is necessarily one of nonlinear curve fitting. The solution for any set of experimental data can, however, be simplified by an understanding of the influence of these parameters on curve shape.

Equation 3.11 may be converted as follows:

$$\frac{1}{V} = \frac{1}{V_o} + \frac{K_s}{V_o} \cdot \frac{1}{S} + \frac{S^n}{K_i^n \cdot V_o} \qquad (3.12)$$

At low values of S, the term $(S/K_i)^n$ is negligible and the equation is reduced to Monod type as Equation 3.8 and Figure 6.

For comparison, the solid line shown in Figure 7 reflects the influence of the inhibition term $S^n/K_i^n \cdot V_o$ as shown in Equation 3.12

Figure 6 shows a plot of Equation 3.11 with typical but assumed values of K_s, V_o, Ki, and varied values of n. As can be seen by comparison of the generated family of curves with the curve of no inhibition, at levels of S in the neighborhood of or less than the observed maxima, small values of n cause relatively greater deviations from the curve of no inhibition term than do larger values of n. This effect is reversed at levels of S/K_i greater than 1.0, or at substrate levels beyond the intersection of the generated family of curves also shown on Figure 8.

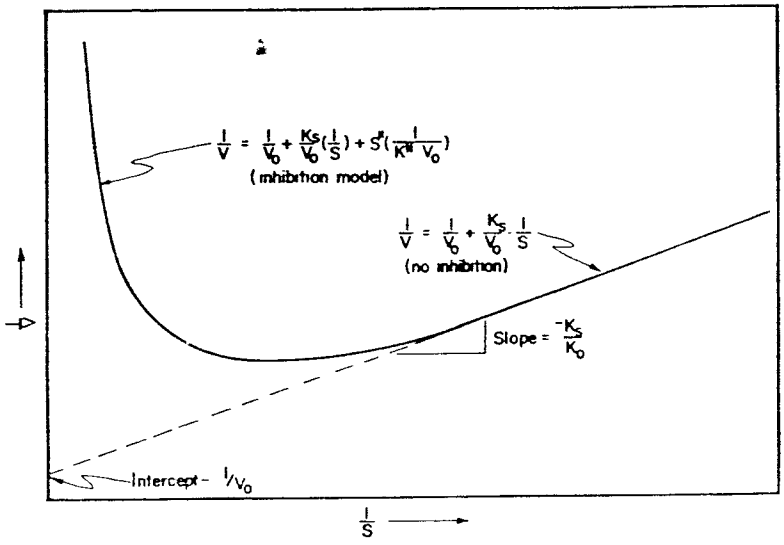


Figure 7 Representative plot of $1/\mu$ vs. $1/S$ for the substrate inhibition model

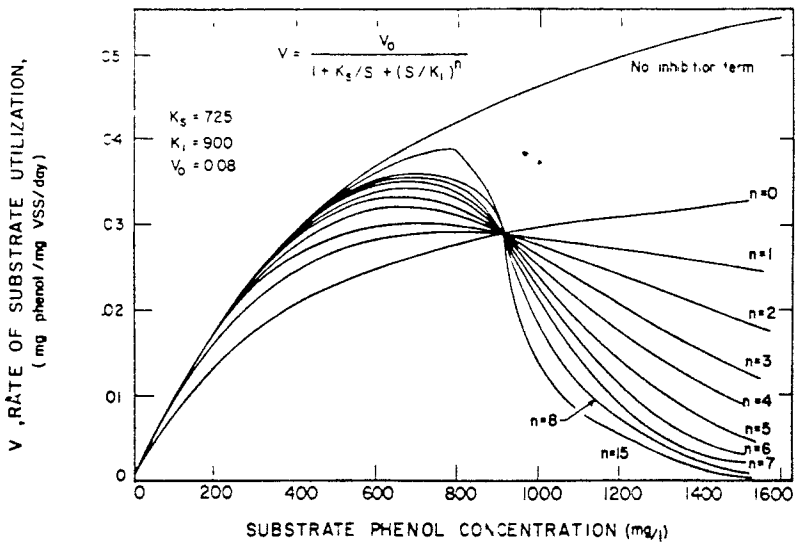


Figure 8 Calculated family of substrate inhibition curves showing influence of parameter n on curve shape

3.3 Endogenous Phase

We should not lose sight of the fate of individual cells when examining the population. In general, the population is (not) homogeneous, and the batch growth curve is a gross overview of a very complex system. For example, during the exponential growth phase, some cells are dividing and giving birth to very young cells at the time others are growing and maturing. The diversity among individual cells becomes increasingly apparent during the stationary and death phase. Some cells are dividing during the stationary phase while others die. Eventually, due to nutrient depletion and toxic-product buildup. However, the population cannot sustain itself, and the endogenous phase begins.

Relatively few studies have been made on the endogenous phase of cell cultures, perhaps because many industrial batch microbiological processes are terminated before the endogenous phase begins. Usually death of the population is assumed to follow an exponential decay

$$N = N_s \cdot e^{-K_d t} \qquad (3.13)$$

where now t denotes the time elapsed since the onset of the death phase. This relationship implies that the number of cells which die at any time is a constant fraction of those living. One physical interpretation of exponential population decay states that there are random lethal events which occur in the culture. When one of these happens to a cell, it dies. *(42)*

Special caution is needed in specifying the way in which X is measured. When

cells are growing (log phase), the correlation between viable cell count and biomass concentration as measures of X is often rather good, but this is generally not so during the autodigestion and endogenous phases. In many systems of natural populations with X based on weight measurement. If the decrease in X is such that half of the biomass concentration is autodigested in a constant time interval, the following equation are applicable:

$$\frac{dX}{dt} = -K_d \cdot t \qquad (3.14)$$

$$X_t = X_o \cdot e^{-K_d t} \qquad (3.15)$$

$$K_d = \frac{lnX_o - lnX_t}{t} \qquad (3.16)$$

The value of K_d can thus be determined in a manner analogous to the determination of μ rather than doubling time t_d, one measures the half time $t_{1/2}$, i.e. the time required for the biomass concentration to decrease by one-half. Equation (3.14) through (3.16) may be recognized as the same as those depicting the kinetics for radioactive decay. While one may be certain that radioactive decay will occur in accordance with these equations, the autodigestion of a biomass may not always follow such a kinetic expression. Again, the environmental technologist can only plot the data to determine whether the system under investigation does follow exponential decay laws, with full knowledge that there is no law saying that it must. *(41)*

CHAPTER 4
EXPERIMENTAL METHOD AND RESULTS

4.1 Experimental Method

The experimental bench-scale microcalorimeter employed in the present study was originally developed and designed by the Department of Biological and Agricultural Engineering at Rutgers University. This experimental apparatus was capable of monitoring the microbial activity within the waste sample by measuring the heat output. The microcalorimeter is a device that offers a convenient and relatively quick way of determining the interaction of the microbiological community with a contaminant.

The microbial activity in terms of heat output for a one gram sample is termed a "thermogram". A normalization test performed on a sample that is the organic medium to sustain the microbilogical community will reflect the endogenous activity of the microorganisms present. The heat output will be proportional to their numerical density. For these tests, dewatered raw sewage sludge was used as the organic medium. To obtain a baseline from which to measure the activity contributed by the microorganisms in the organic medium, a sample of the sludge is tested in the microcalorimeter. The thermogram obtained from the sample in the microcalorimeter provides the information that shows the feasibility of using the microbiological community to degrade an organic contaminant. Measuring the area between the baseline and the normalization thermogram for the sewage sludge provides a quantitative measure of the heat contributed by the sewage sludge.

4.2 Materials, Conditions and Procedures

In this study, the organic compounds employed are aniline, 2,4-dichlorophenol (2,4-DCP) and 1,2,4- trichlorobenzene (1,2,4- TCB) with high purity of 97% to 99%. The sewage sludge used here is a primary dewatered sewage. The sludge was collected from the Camden City Waste Water Treatment Plant, New Jersey. All of the experimental work were performed at fifty degree celsius and under aerobic conditions.

The liquid - liquid extraction method of analytical techniques was used to extract the organic compounds from aqueous leachate samples. The samples were analyzed with Gas Chloromatography (Helett Parchard Model 5730 with dual flame ionization detector).

A predetermined amount of each target compound was put into the sewage in order to test for the biodegradability of the contaminants. A blank sample test was carried out to account for the heat output due to the utilization of the organic medium used to sustain the microbiological community. Also, the heat output supposes to be proportional to the amounts of substrate decomposition and cell synthesis. In these tests, the organic medium mass dewatered raw sludge. One gram of this sewage sludge was placed in the microcalorimetric cell. After a period of time, the heat output was measured by the area of thermogram.

Preparing 0.1%, 0.3%, 0.5%, 0.7% and 1.1% aniline; 0.05%, 0.10%, 0.15% and 0.20% 2,4-dichlorophenol; 0.1%, 1.5%, 3.0% and 3.5% 1,2,4-trichlorobenzene to the sewage sludge separately will detect the input of the toxicant on the microbiological community.

The contaminated sludge samples were placed in the microcalorimetric cell after preparing. The response in terms of heat output was measured as the area under the output curve. Figure 9 shows, for instance, the various concentrations of 1,2,4-RCB with sludge.

In order to measure the bio-activity in one gram of sludge sample without the addition of contaminant test to produce a normalized thermogram (base line). Measurement of the area between the baseline and the normalized thermogram from the microcalorimeter provides a quantitative determination of the heat contributed by the sewage sludge.

4.3 Experimental Results

4.3.1 Experimental Output

The output from thermogram can be seen, for instance, as Figure 9 which shows the various concentrations of 1,2,4-TCB. Then, the printouts were transferred from the area into energy like Figures 10, 11, and 12. Furthermore, for the conveniently computable reasons, these data has been converted into computer file which was based on hourly energy productions as Tables 1, 2 and 3. Therefore, the heat output from various sets of 3 target compounds can be shown as Figures 13, 14 and 15.

If the added compound is a toxicant, the heat output will be less than that observed from the normalized thermogram. Conversely, if the compound can be effectively metabolized, the heat flux will be greater. For comparisons of the energy production between base line and various concentration target compounds with sewage sludge, Figures 16, 17 and 18 show the net energy effects of the aniline, 2,4-DCP and 1,2,4-TCB.

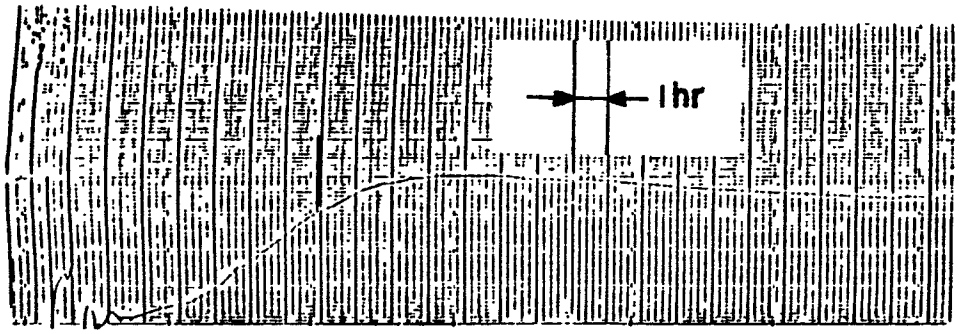


Figure 9(a) Sewage sludge thermogram

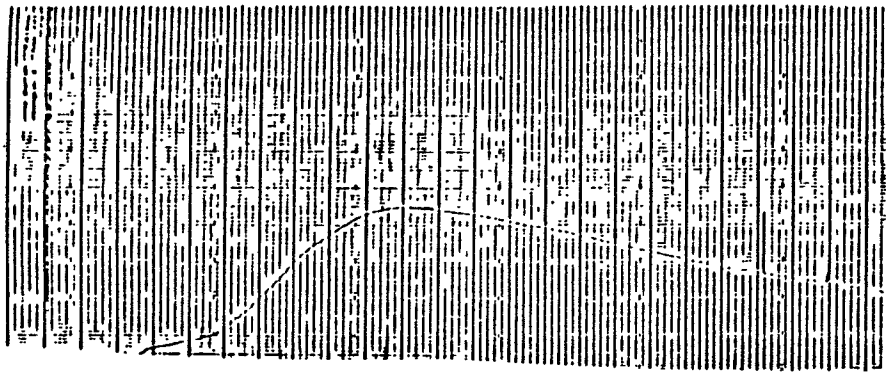


Figure 9(b) 0.01% 1,2,4-TCB and sewage sludge thermogram

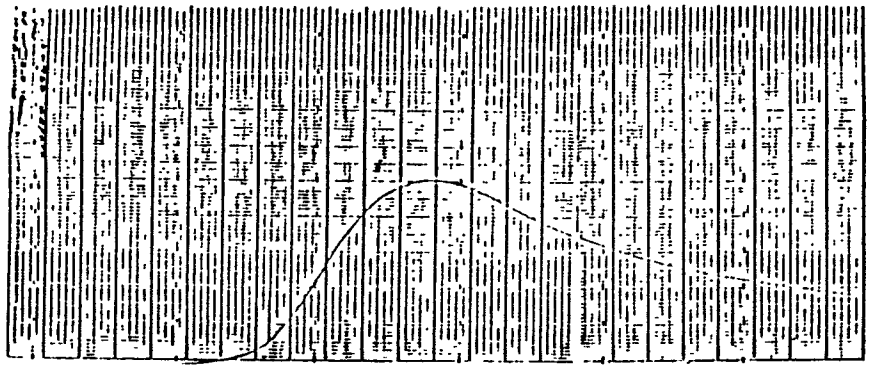


Figure 9(c)0.1% 1,2,4-TCB and sewage sludge thermogram

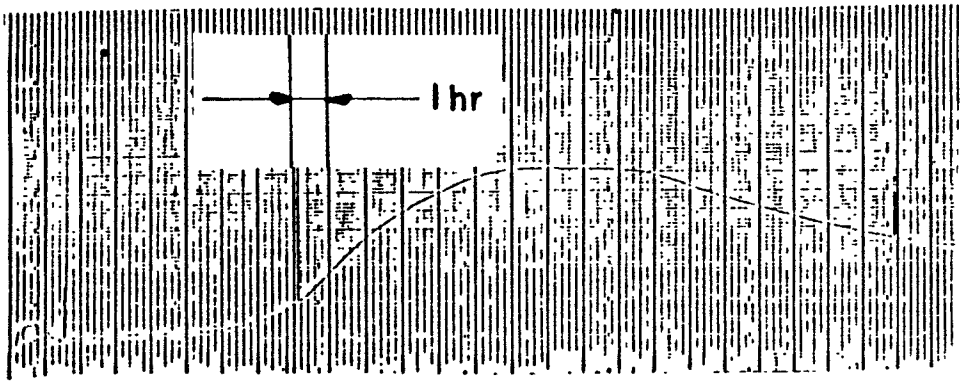


Figure 9(d) 1% 1,2,4-TCB and Sewage sludge thermogram

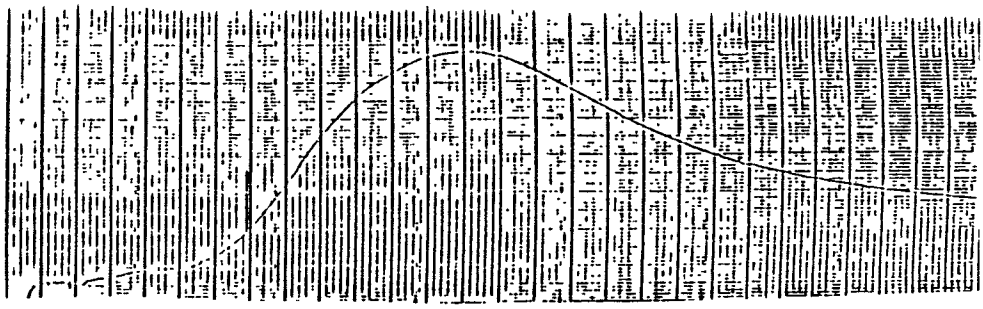


Figure 9(e) 1.5% 1,2,4-TCB and sewage sludge thermogram

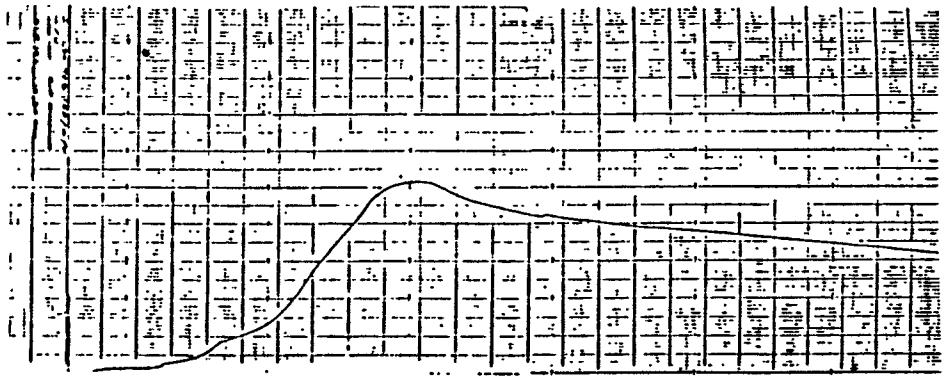


Figure 9(f) 2.0% 1,2,4-TCB and sewage sludge thermogram

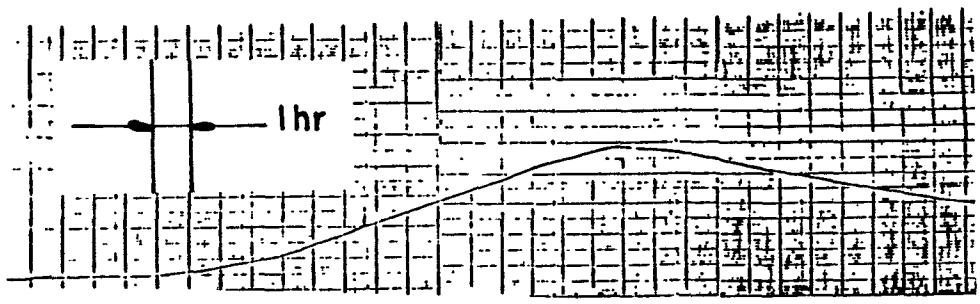


Figure 9(g) 3.0% 1,2,4-TCB and sewage sludge thermogram

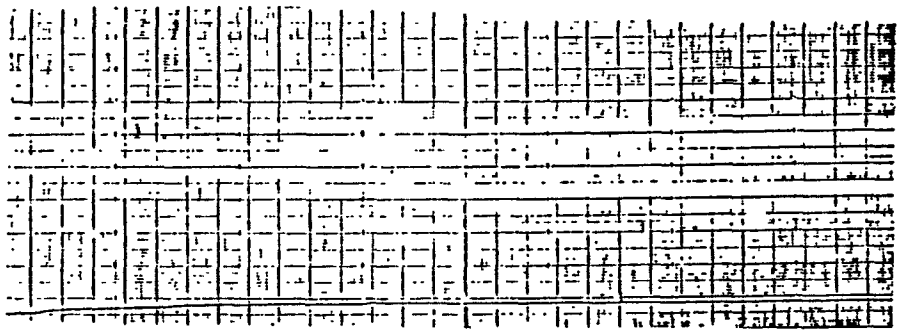


Figure 9(h) 5.0% 1,2,4-TCB and sewage sludge thermogram

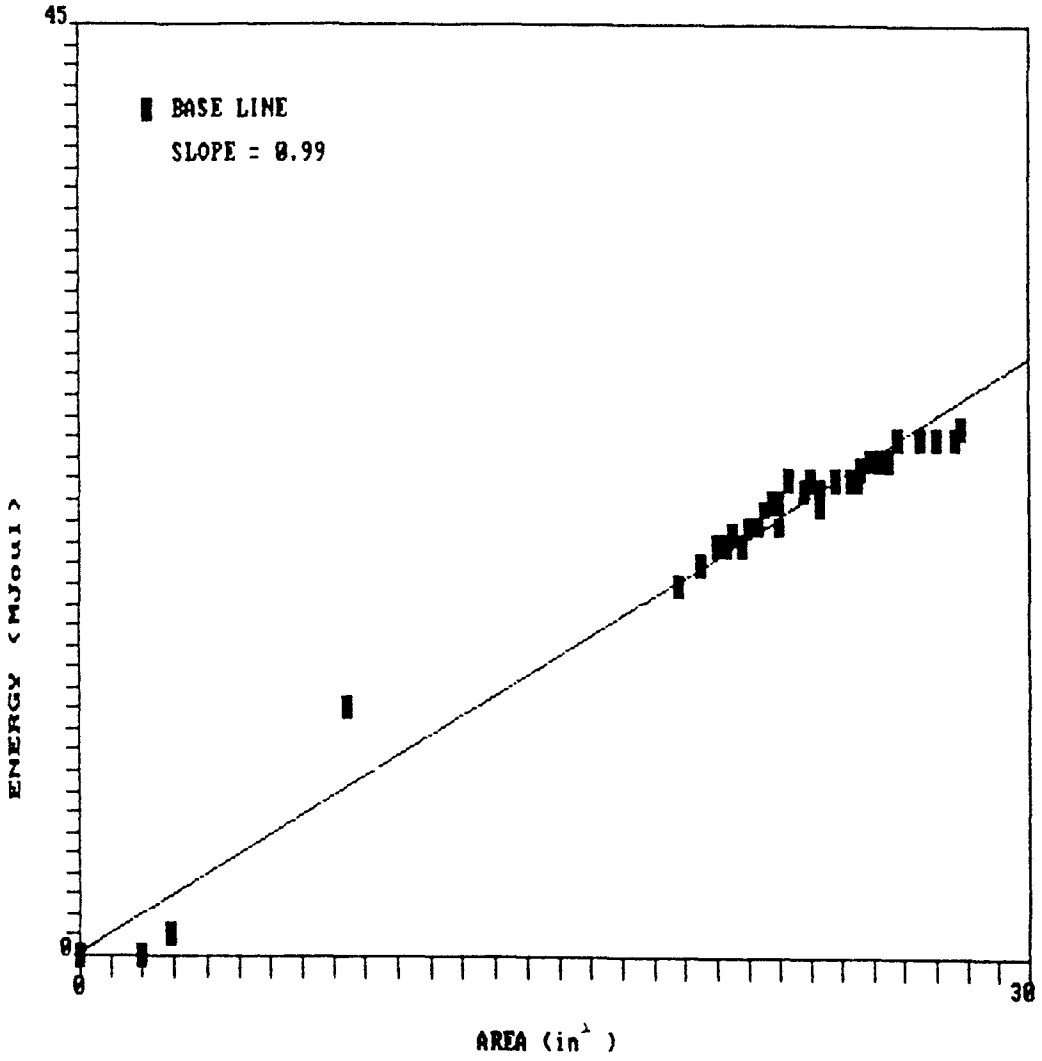


Figure 10 Ratio of energy production rate vs. area at base line of aniline

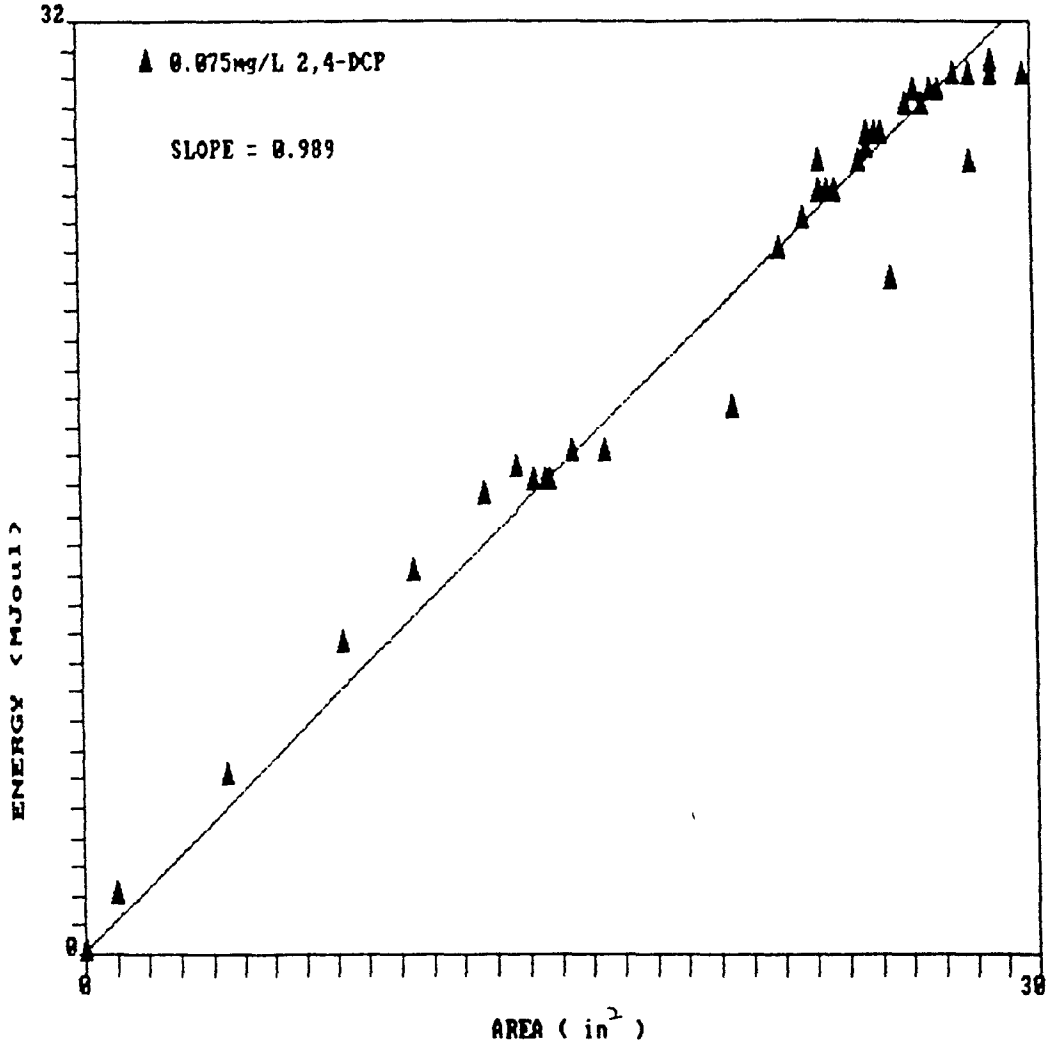


Figure 11 Ratio of energy production rate vs. area at base line of 2,4-DCP

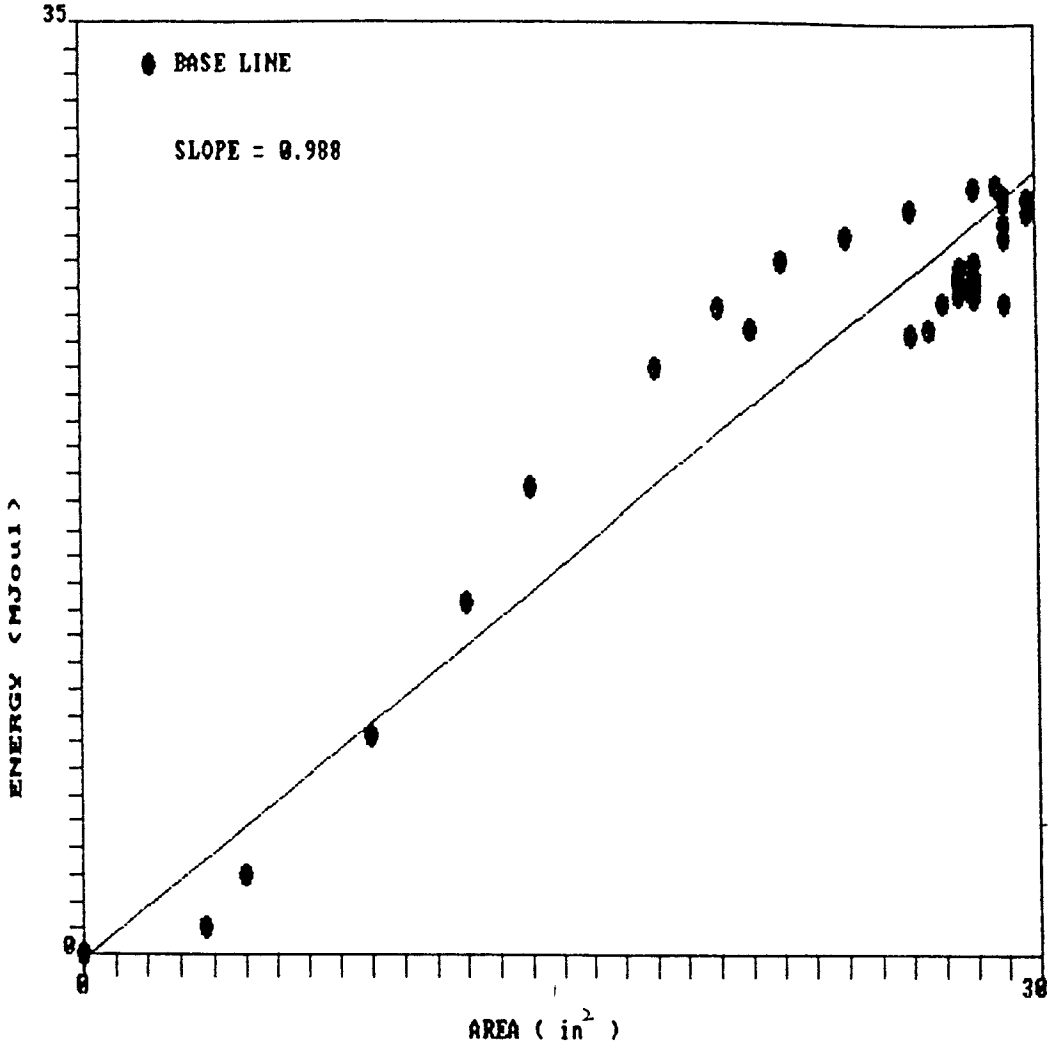


Figure 12 Ratio of energy production rate vs. area at base line of 1,2,4-TCB

Table 1. The Heat Output (MJour) of Aniline at 0.1%, 0.3%, 0.5%, 0.7%, 1.1%, and Base line

Time(hr)	0.1%	0.3%	0.5%	0.7%	1.1%	Base
0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	2.5	0.0	0.0	0.0	0.0
4	13.5	5.0	3.0	0.0	0.0	0.0
5	22.0	14.0	10.0	15.0	0.0	1.0
6	25.5	22.0	17.0	16.0	0.0	12.0
7	28.5	25.0	20.0	16.0	5.0	18.5
8	32.0	29.0	23.0	17.0	8.0	19.0
9	32.5	29.5	28.0	21.0	10.0	20.0
10	31.5	28.0	32.0	22.0	13.5	21.0
11	31.0	27.0	32.5	22.5	14.5	23.0
12	31.0	26.5	32.5	21.0	15.5	25.0
13	31.5	26.0	31.5	21.0	16.0	25.0
14	32.5	26.0	31.0	22.0	17.0	25.5
15	34.0	26.0	32.0	22.5	15.5	25.0
16	34.5	26.5	32.0	25.0	14.0	24.0
17	36.0	27.0	32.0	28.0	13.0	24.0
18	36.5	27.0	32.0	28.5	13.0	24.0
19	37.5	27.3	31.5	28.0	13.0	24.0
20	38.0	27.5	31.5	28.0	13.0	24.0
21	38.0	28.0	31.0	28.0	13.0	23.5
22	38.0	28.0	31.0	28.0	13.0	23.0
23	38.0	28.0	31.0	28.5	13.0	23.0
24	37.5	27.8	31.0	29.0	13.0	23.0
25	38.0	27.5	31.5	30.0	13.0	23.0
26	38.0	27.0	31.5	31.0	13.0	22.5
27	37.5	27.0	31.5	31.0	13.0	21.5
28	38.0	27.0	31.5	31.0	14.0	22.0
29	38.0	27.0	31.0	31.5	14.0	22.0
30	37.5	26.9	31.0	33.0	14.0	22.5

Table 1. The Heat Output (MJour) of Aniline at 0.1%, 0.3%, 0.5%, 0.7%, 1.1%, and Base line (continued)

Time(hr)	0.1%	0.3%	0.5%	0.7%	1.1%	Base
31	37.0	26.8	30.0	36.0	14.0	22.5
32	36.5	26.7	30.0	35.5	14.0	22.5
33	37.5	26.5	30.0	37.0	14.5	23.0
34	38.5	26.0	30.0	37.5	15.0	22.0
35	40.0	25.9	30.0	37.0	14.0	22.0
36	41.5	26.0	30.0	37.0	14.5	22.0
37	42.0	25.9	30.5	36.5	15.0	22.0
38	40.5	25.9	31.0	37.0	15.0	23.0
39	39.0	25.9	31.0	38.0	15.0	22.0
40	37.0	25.9	31.5	34.0	15.0	22.0
41	35.5	25.9	31.5	33.0	14.0	22.0
42	34.8	25.9	30.8	31.5	14.0	22.0
43	34.4	26.0	30.5	29.0	14.0	21.5
44	33.0	26.0	30.5	27.0	14.0	21.0
45	32.0	26.0	30.0	25.0	14.0	21.0
46	31.0	26.0	29.0	23.0	14.0	20.5
47	30.0	25.9	29.0	22.0	14.0	20.0
48	29.3	25.5	29.0	21.0	14.0	20.0
49	28.0	25.4	28.5	20.0	14.0	19.0
50	27.5	25.0	28.2	19.0	13.5	19.0
51	26.5	25.0	28.0	18.0	13.0	19.0
52	25.8	24.3	28.0	17.0	13.0	18.5
53	25.5	24.3	28.0	16.0	12.5	18.0
54	25.0	24.0	27.5	15.0	12.0	18.0
55	24.5	24.0	27.2	14.0	12.0	17.5
56	24.0	24.0	27.0	14.0	12.0	17.0
57	23.5	23.5	27.0	13.0	12.0	16.5
58	23.0	23.0	26.5	12.0	12.0	16.5
59	22.0	23.0	26.5	11.5	11.5	16.5
60	20.0	23.0	26.5	11.0	11.5	16.5
61	19.0	22.8	26.0	11.0	11.5	15.5

Table 2. The Heat Output (MJour) of 2,4-DCP at 0.05%, 0.1%, 0.15%, 0.2%, and Base line

Time(hr)	0.05%	0.1%	0.15%	0.2%	Base
0	0.0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0	0.0
2	0.0	2.0	0.0	0.0	0.0
3	0.0	6.0	7.0	0.0	0.0
4	0.0	10.5	12.0	0.0	0.0
5	2.0	13.0	11.5	0.0	1.0
6	5.0	15.5	11.0	2.0	12.0
7	9.5	16.5	10.0	4.0	18.5
8	10.5	16.0	9.5	6.0	19.0
9	10.0	16.0	9.0	7.0	20.0
10	9.0	16.0	9.0	8.0	21.0
11	11.0	17.0	10.0	9.0	23.0
12	14.5	17.0	11.5	10.0	25.0
13	17.0	18.5	13.0	11.0	25.0
14	18.0	23.0	15.5	12.0	25.5
15	18.0	27.0	17.0	14.0	25.0
16	18.0	30.0	17.0	14.0	24.0
17	18.0	31.5	16.0	15.0	24.0
18	18.0	31.5	15.0	14.0	24.0
19	17.5	31.5	14.5	14.0	24.0
20	18.0	30.5	14.0	14.0	24.0
21	18.5	30.0	13.0	13.0	23.5
22	19.5	30.0	13.0	13.0	23.0
23	20.0	30.0	12.0	13.0	23.0
24	20.0	30.0	11.5	13.0	23.0
25	20.0	29.5	11.0	13.0	23.0
26	20.0	29.0	11.0	13.0	22.5
27	20.0	29.5	10.5	13.0	21.5
28	20.0	29.5	10.5	12.0	22.0
29	20.0	29.5	10.5	12.0	22.0
30	20.0	29.5	10.5	11.0	22.5

Table 2. The Heat Output (MJour) of 2,4-DCP at 0.05%, 0.1%, 0.15%, 0.2%, and Base line (continued)

Time(hr)	0.05%	0.1%	0.15%	0.2%	Base
31	20.0	29.0	10.5	11.0	22.5
32	20.0	29.0	10.7	10.0	22.5
33	20.5	28.0	11.0	9.0	23.0
34	20.5	28.0	11.0	9.0	22.0
35	20.5	28.0	11.5	9.0	22.0
36	20.0	27.5	11.0	8.0	22.0
37	20.0	27.0	11.0	8.0	22.0
38	20.0	27.0	11.0	7.0	23.0
39	20.0	27.0	10.5	7.0	22.0
40	19.5	26.0	10.5	6.0	22.0
41	19.5	26.0	10.0	5.0	22.0
42	19.5	26.0	10.0	5.0	22.0
43	19.5	26.0	10.0	5.0	21.5
44	19.5	26.0	9.5	5.0	21.0
45	19.0	25.0	9.5	5.0	21.0
46	19.0	24.0	9.2	4.0	20.5
47	19.0	24.0	9.0	4.0	20.0
48	19.0	23.0	9.0	4.0	20.0
49	19.0	22.5	9.0	3.0	19.0
50	19.0	22.0	9.0	3.0	19.0
51	19.0	21.0	9.0	3.0	19.0
52	19.5	21.0	8.5	3.0	18.5
53	19.5	20.5	8.5	3.0	18.0
54	20.0	20.5	8.5	3.0	18.0
55	20.0	20.5	8.0	3.0	17.5
56	20.0	19.5	8.0	3.0	17.0
57	20.0	19.0	8.0	3.0	16.5
58	20.0	19.0	8.0	3.0	16.5
59	20.5	19.0	8.0	2.0	16.5
60	20.5	19.0	7.5	2.0	16.5
61	20.5	18.0	7.5	2.0	15.5

Table 3. The Heat Output (MJour) of 1,2,4-TCB at 0.1%, 1.5%, 3.0%, 3.5%, and Base line

Time(hr)	0.1%	1.5%	3.0%	3.5%	Base
0	0.0	0.0	0.0	0.0	0.0
1	0.0	5.0	0.0	0.0	0.0
2	0.0	5.5	0.0	2.5	3.5
3	0.0	7.0	1.0	2.5	5.0
4	0.0	9.0	3.0	3.5	9.0
5	0.0	10.0	6.0	3.5	12.0
6	3.5	13.0	10.0	3.5	14.0
7	12.0	21.0	14.0	3.5	18.0
8	27.0	33.5	23.0	3.5	20.0
9	41.0	45.0	28.0	4.0	22.0
10	41.0	55.0	31.0	4.0	24.0
11	50.0	62.5	39.0	4.5	26.0
12	48.0	67.0	42.0	4.5	28.0
13	43.0	68.0	41.0	5.0	29.0
14	38.0	63.0	40.0	5.0	29.0
15	34.0	58.0	40.0	6.0	29.0
16	31.0	52.5	37.0	7.0	30.0
17	28.0	47.5	37.0	9.0	30.0
18	25.5	43.0	36.5	11.0	29.5
19	24.0	40.0	35.0	14.0	29.5
20	22.0	37.0	35.0	18.0	29.5
21	20.0	34.5	33.5	22.0	28.5
22	19.0	33.0	33.5	25.0	28.5
23	18.0	32.0	33.0	28.0	28.5
24	17.0	30.0	32.0	32.0	28.0
25	16.0	29.0	32.0	34.5	28.0
26	15.5	28.0	32.0	37.0	28.0
27	15.0	27.5	32.0	38.0	28.5
28	14.5	27.0	31.5	37.0	28.5
29	14.0	26.0	30.0	36.5	28.0
30	13.5	25.0	25.0	34.0	28.5

Table 3. The Heat Output (MJour) of 1,2,4-TCB at 0.1%, 1.5%, 3.0%, 3.5%, and Base line (continued)

Time(hr)	0.1%	1.5%	3.0%	3.5%	Base
31	13.0	24.5	25.0	32.5	28.5
32	13.0	24.0	25.0	31.0	28.5
33	13.0	23.5	25.0	28.0	28.0
34	12.5	23.2	25.0	26.5	28.0
35	12.5	23.0	24.0	25.0	28.5
36	12.0	22.5	24.0	22.5	29.5
37	12.0	21.5	23.0	22.0	28.5
38	12.0	21.0	22.0	19.0	28.5
39	12.0	20.5	22.5	18.0	27.0
40	12.0	20.0	20.0	17.0	27.5
41	12.0	20.0	19.0	17.0	26.0

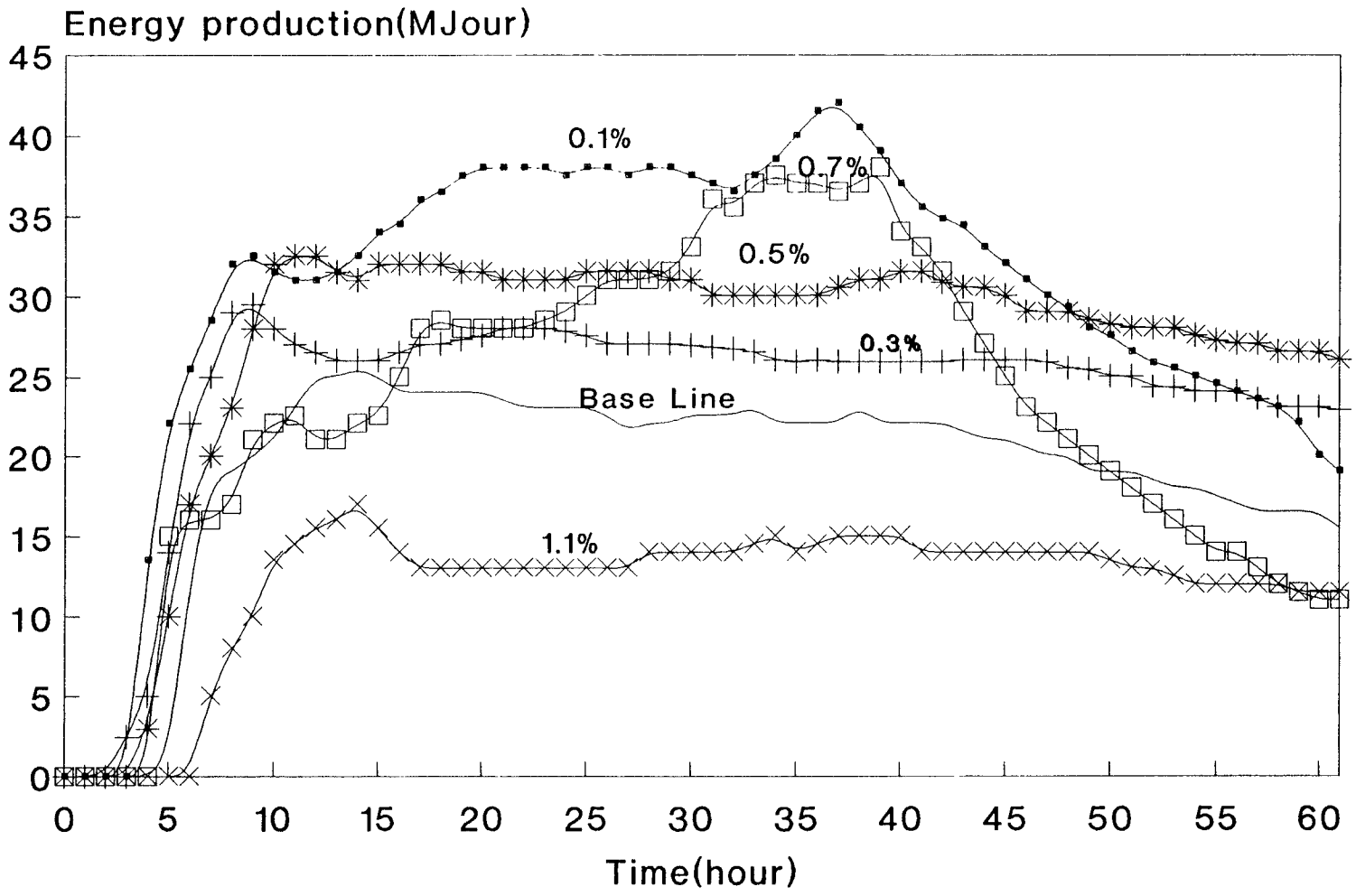


Figure 13 Biodegradation of Aniline at 0.1%, 0.3%, 0.5%, 0.7% and 1.1 %

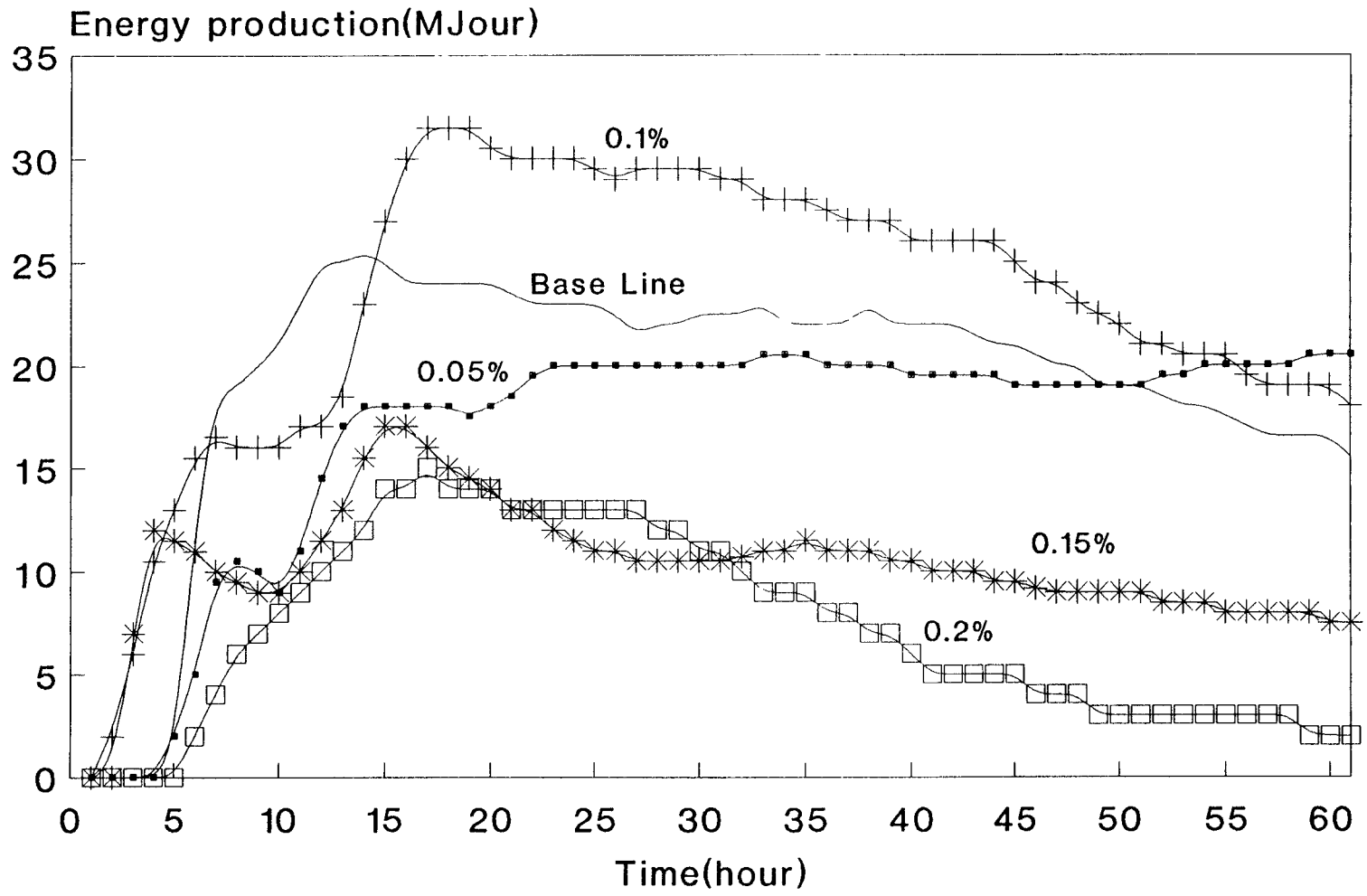


Figure 14 Biodegradation of 2,4-DCP at 0.05%, 0.1%, 0.15% and 0.2%

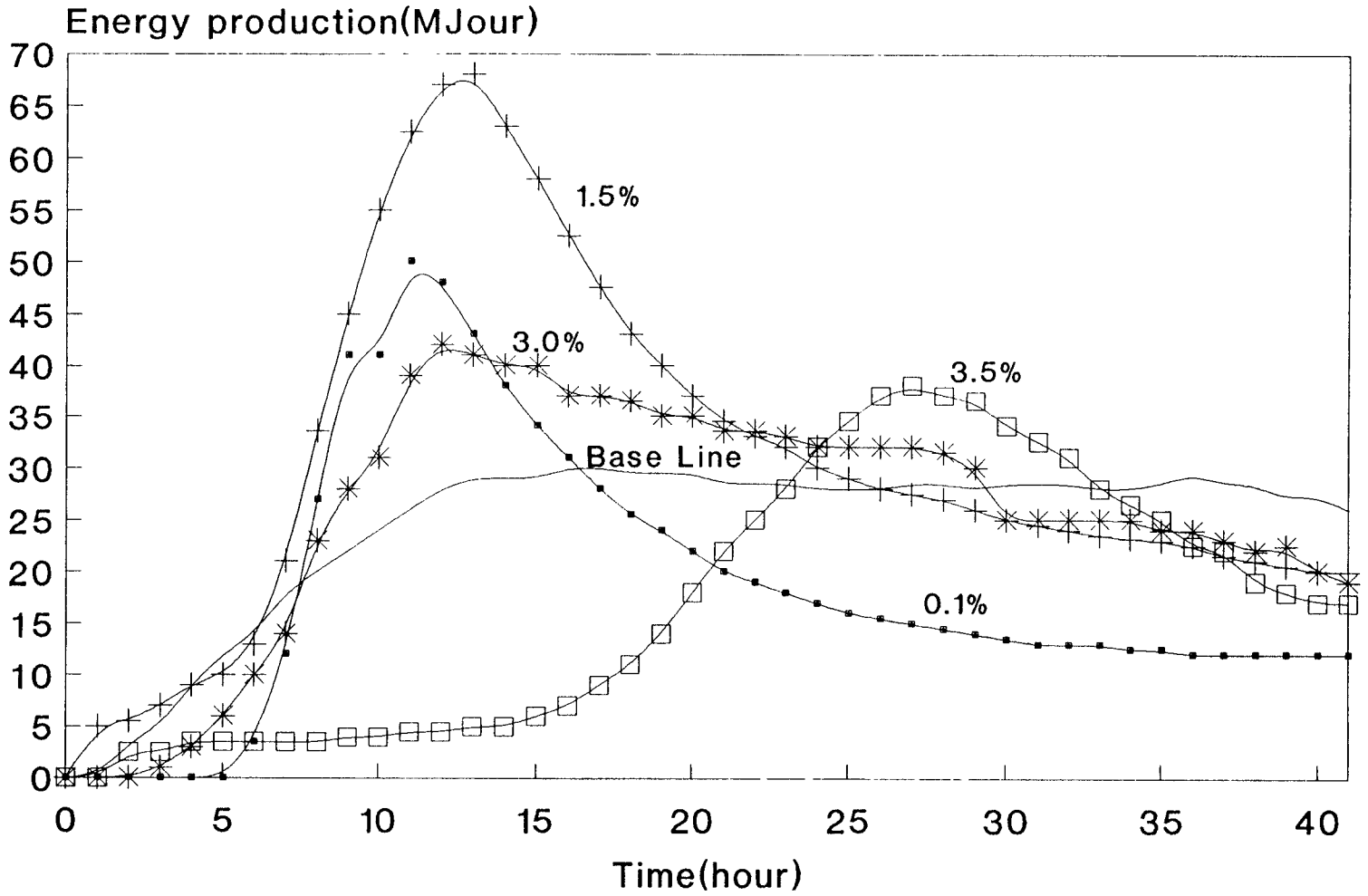


Figure 15 Biodegradation of 1,2,4-TCB at 0.1%, 1.5%, 3.0% and 3.5%

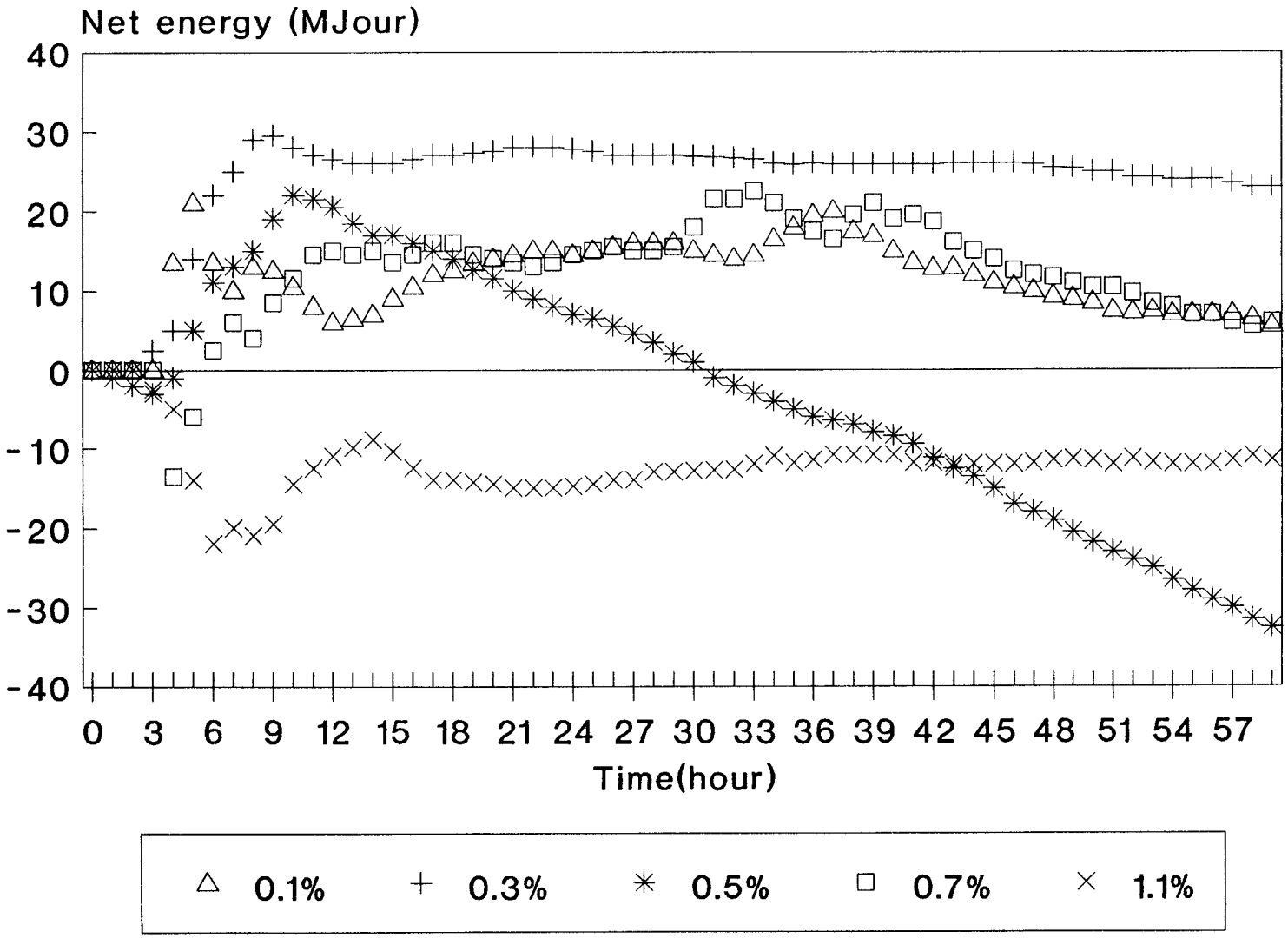


Figure 16 Net energy effect of aniline

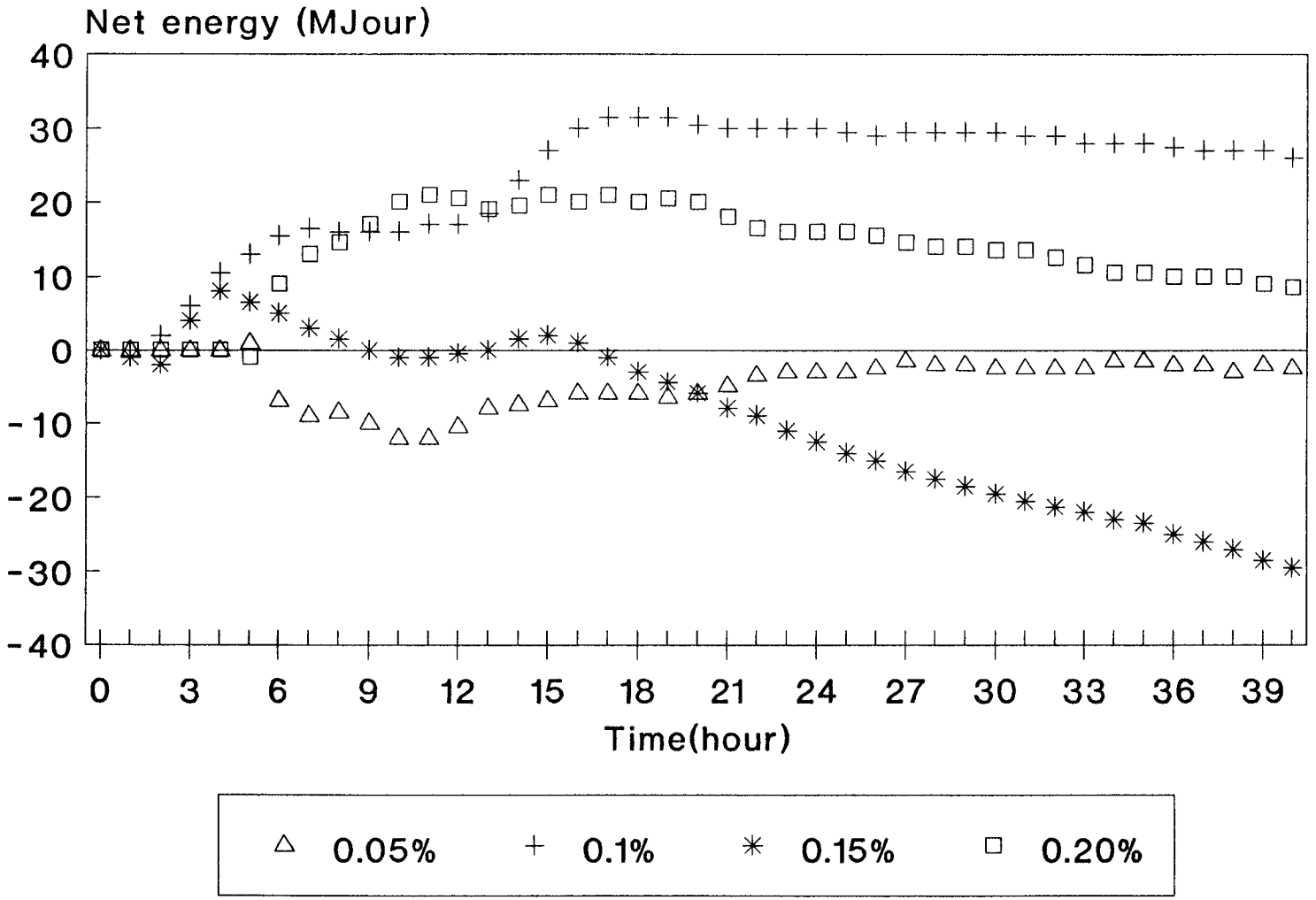


Figure 17 Net energy effect of 2,4-DCP

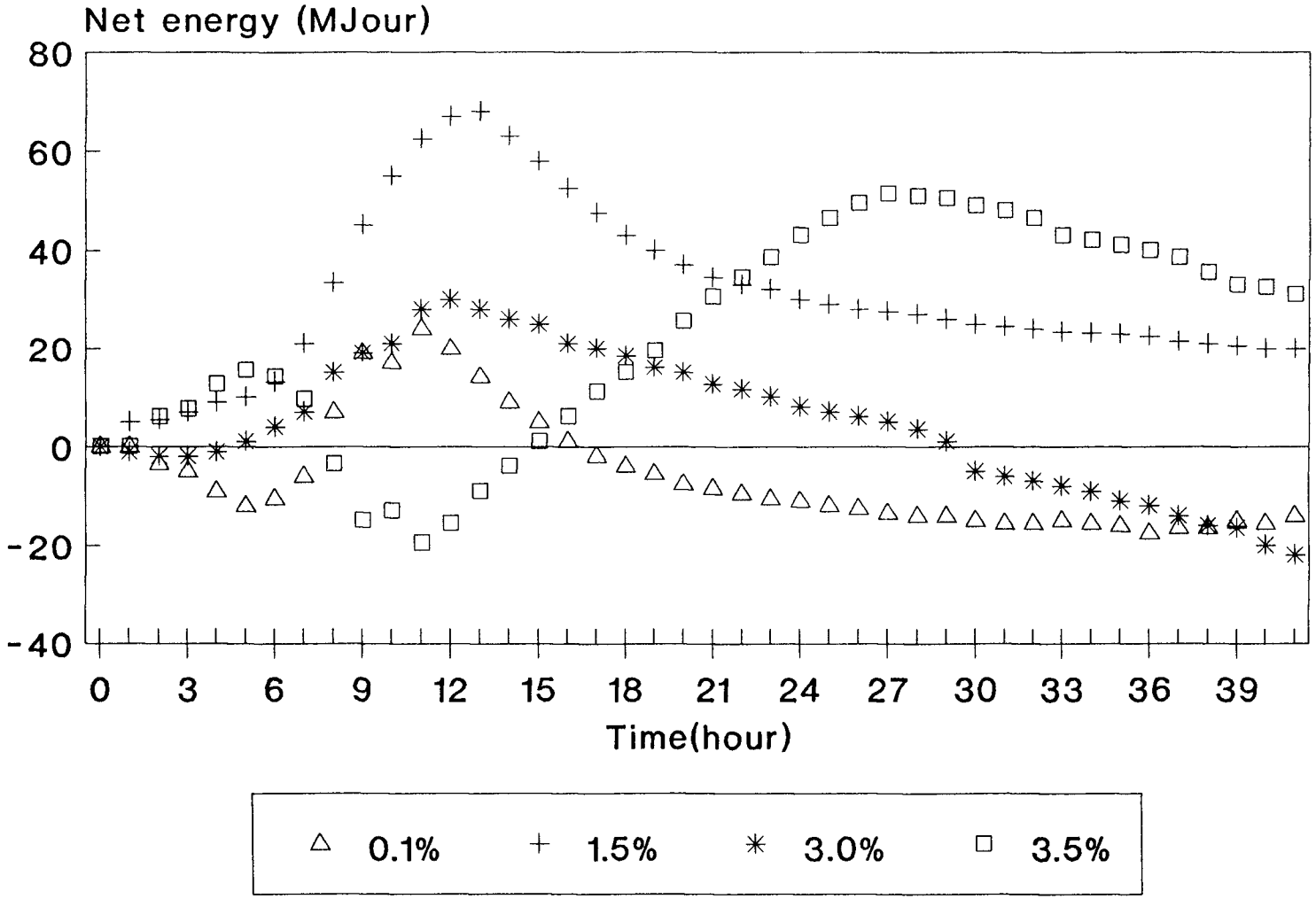


Figure 18 Net energy effect of 1,2,4-TCB

4.3.2 Model Fitting for Log Phase

Suppose the energy productions have been represent the biomass of microoganisms. Then, the Equation 3.1 can be converted as Equation 4.1 :

$$\frac{dE}{dt} = \mu E \qquad (4.1)$$

where E = energy production of sludge, MJour,

μ = specific growth rate, time^{-1}.

From equation 4.1, the Monod model can be converted as :

$$\mu = \mu_E \cdot \frac{C}{K_c + C} \qquad (4.2)$$

where μ_E = the maximum energy output, MJour

C = substrate concentration, %

K_c = the substrate concentration at half of μ_E, %

The substrate inhibition model, then can be transformed into the form as follow :

$$\mu = \frac{\mu_E}{1 + \frac{K_c}{C} + (\frac{C}{K_i})^N} \qquad (4.3)$$

where K_i = the substrate inhibition concentration, %

N = inhibition order

In order to calculate these kinetic constants, the followings indicate the steps. First of all, calculate the specific growth rate (μ) for Aniline, 2,4-DCP and 1,2,4-TCB at different concentrations by Equation 4.1, respectively. Then, plot $\frac{1}{\mu}$ versus

$\frac{1}{C}$ to calculate the μ_E and K_c by the similar method as Equation 3.8 ($\frac{1}{\mu} = \frac{1}{\mu_E} + \frac{\mu_E}{K_c} \cdot \frac{1}{C}$) and Figure 6. After getting μ_E and K_c from Monod type, substitute the constants into Equation 4.3 to compute the K_i and N values by using the Rosenbrock method (nonlinear curve-fitting program). The method can obtain the most optimal μ_E, K_c, K_i, and N by computer program for the minimum error standard deviations. The curve-fitting results are listed as Figures 19, 20, and 21.

4.3.3 Endogenous Phase Results The previous theory (section 3.3) has explained the endogenous patten for microbial activities. In this study, converted the Equation 3.14 through 3.16 as follows :

$$\frac{dE}{dt} = -K'_d \cdot E \qquad (4.4)$$

$$E_t = E_o \cdot e^{-K'_d \cdot t} \qquad (4.5)$$

$$K'_d = \frac{lnE_o - lnE_t}{t} \qquad (4.6)$$

The decay constants (K'_d) can be computed by using Equation 4.6 for Aniline, 2,4-DCP, and 1,2,4-TCB, respectively. Thereafter, plotting the decay rate versus concentration as Figures 22, 23, and 24 can obtain the rate changes.

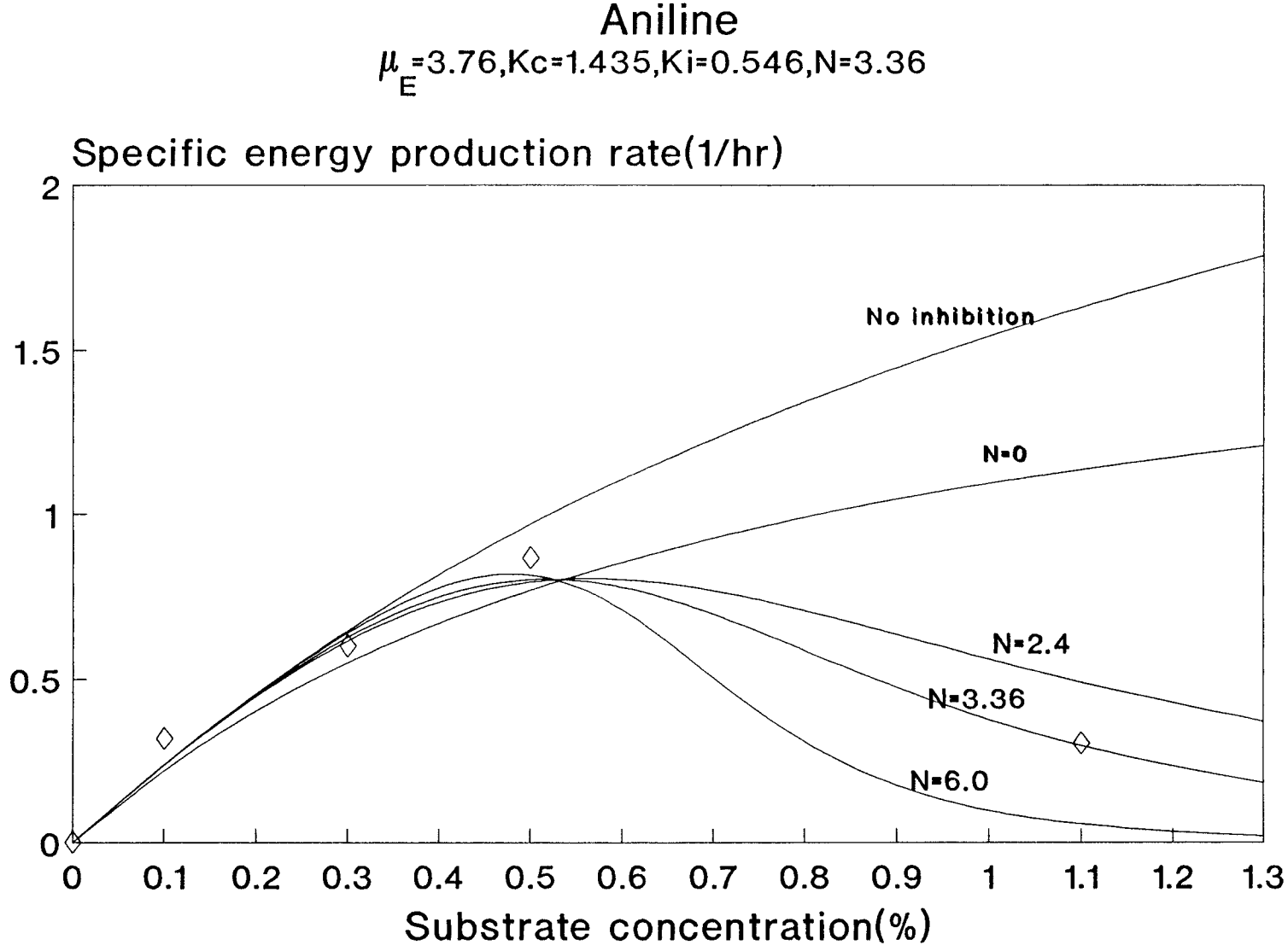


Figure 19 Substrate inhibition curve of aniline

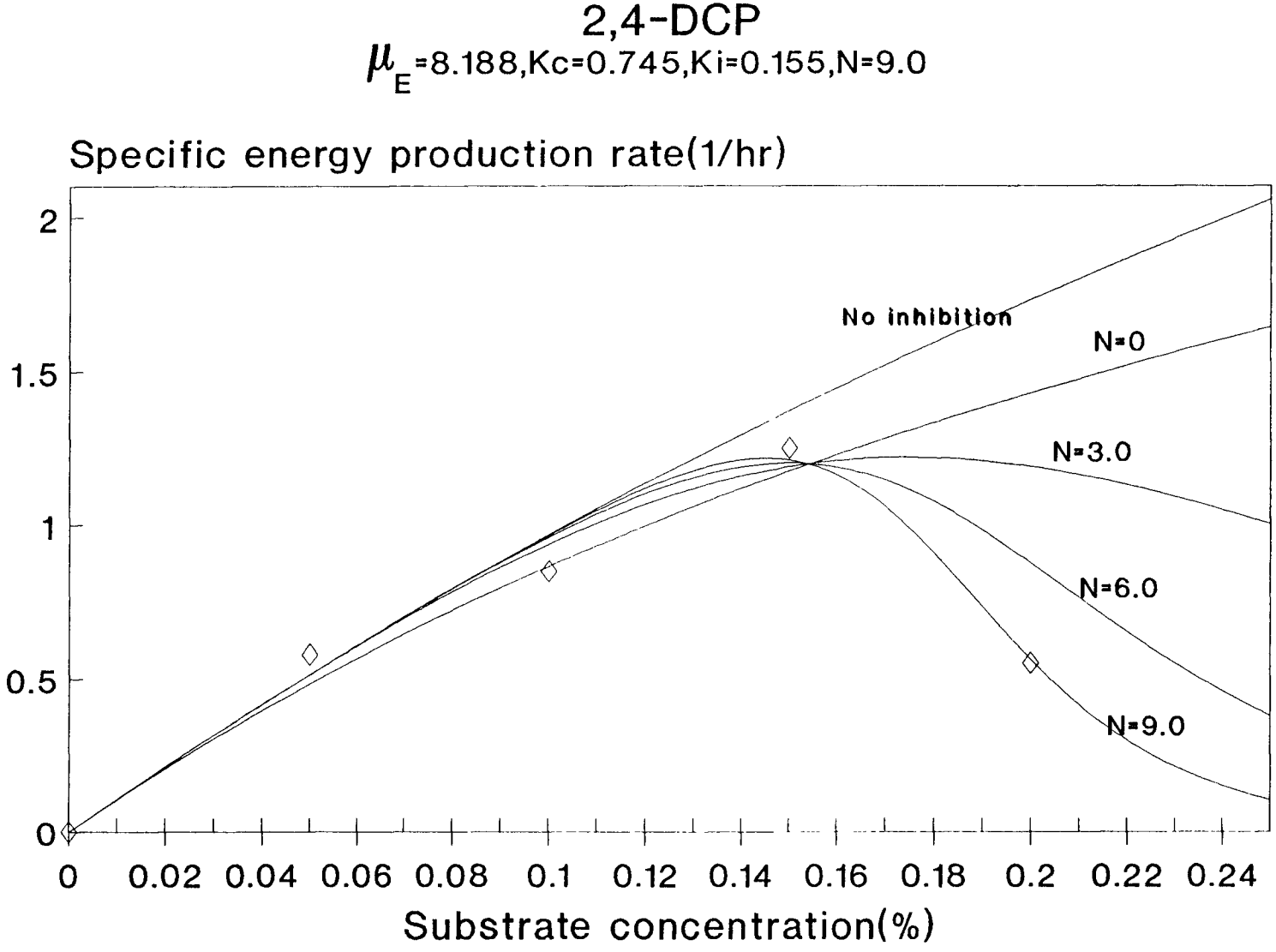


Figure 20 Substrate inhibition curve of 2,4-DCP

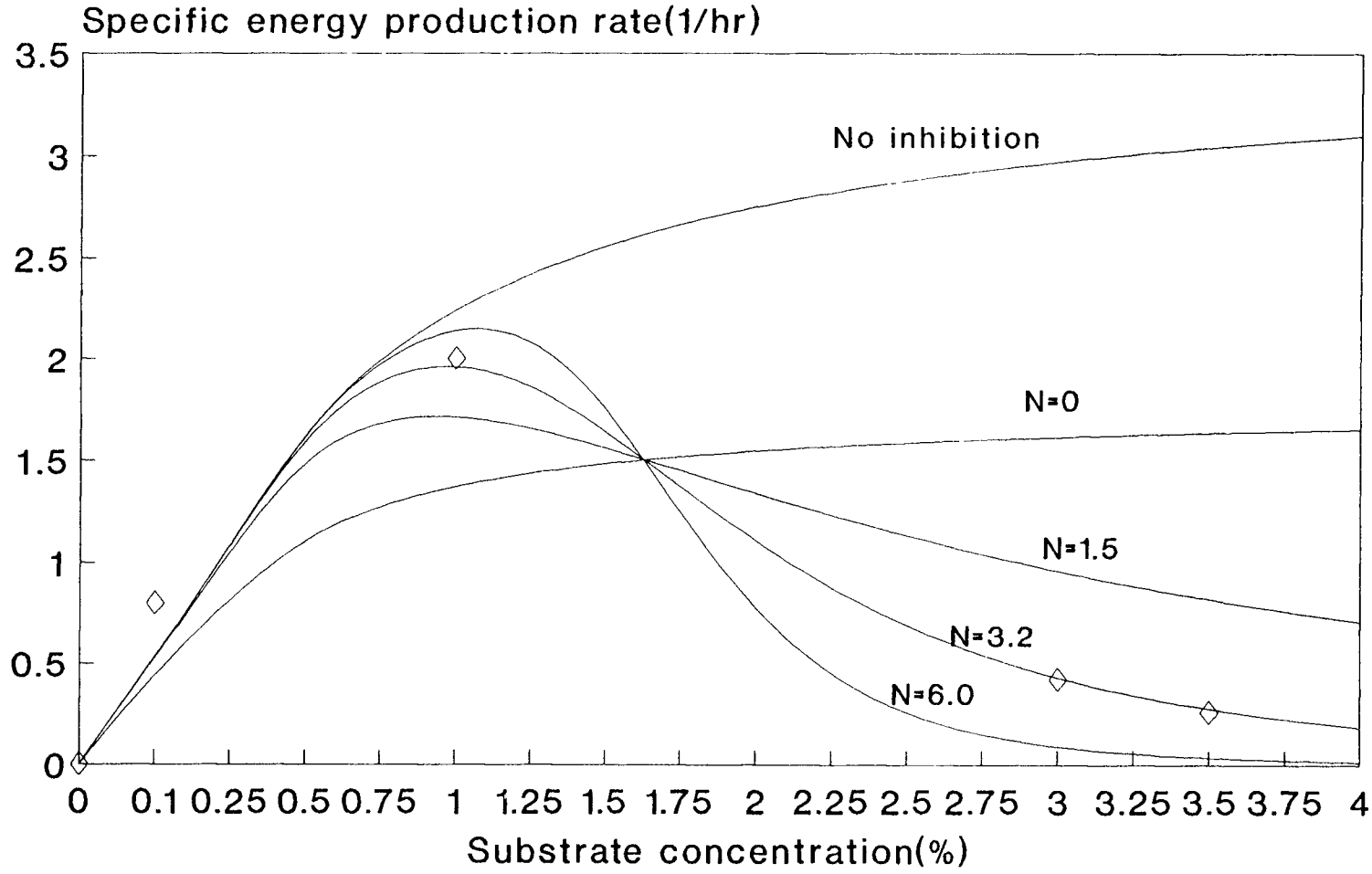


Figure 21 Substrate inhibition curve of 1,2,4-TCB

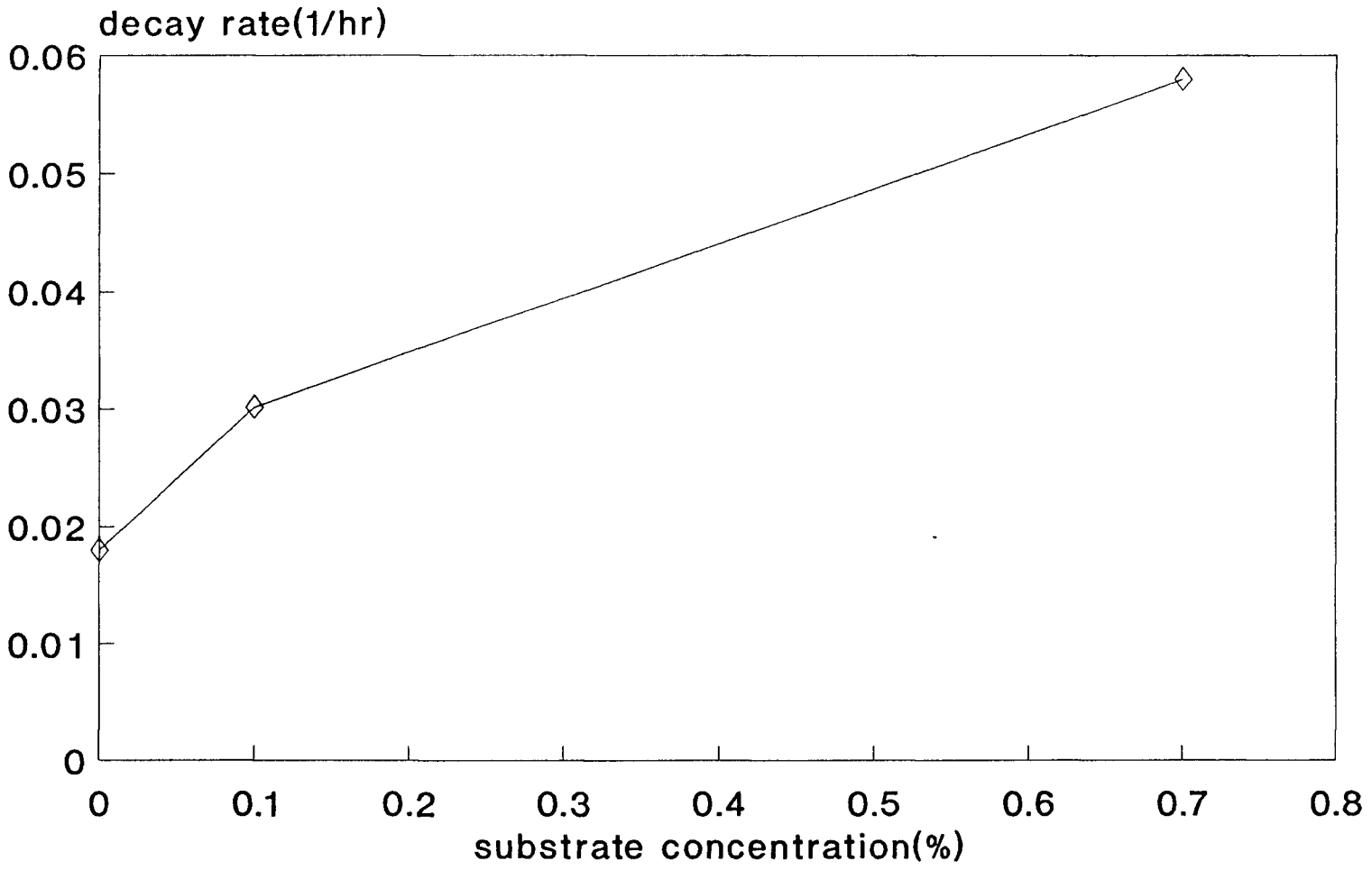


Figure 22 Endogenous decay rate vs. substrate concentration of aniline

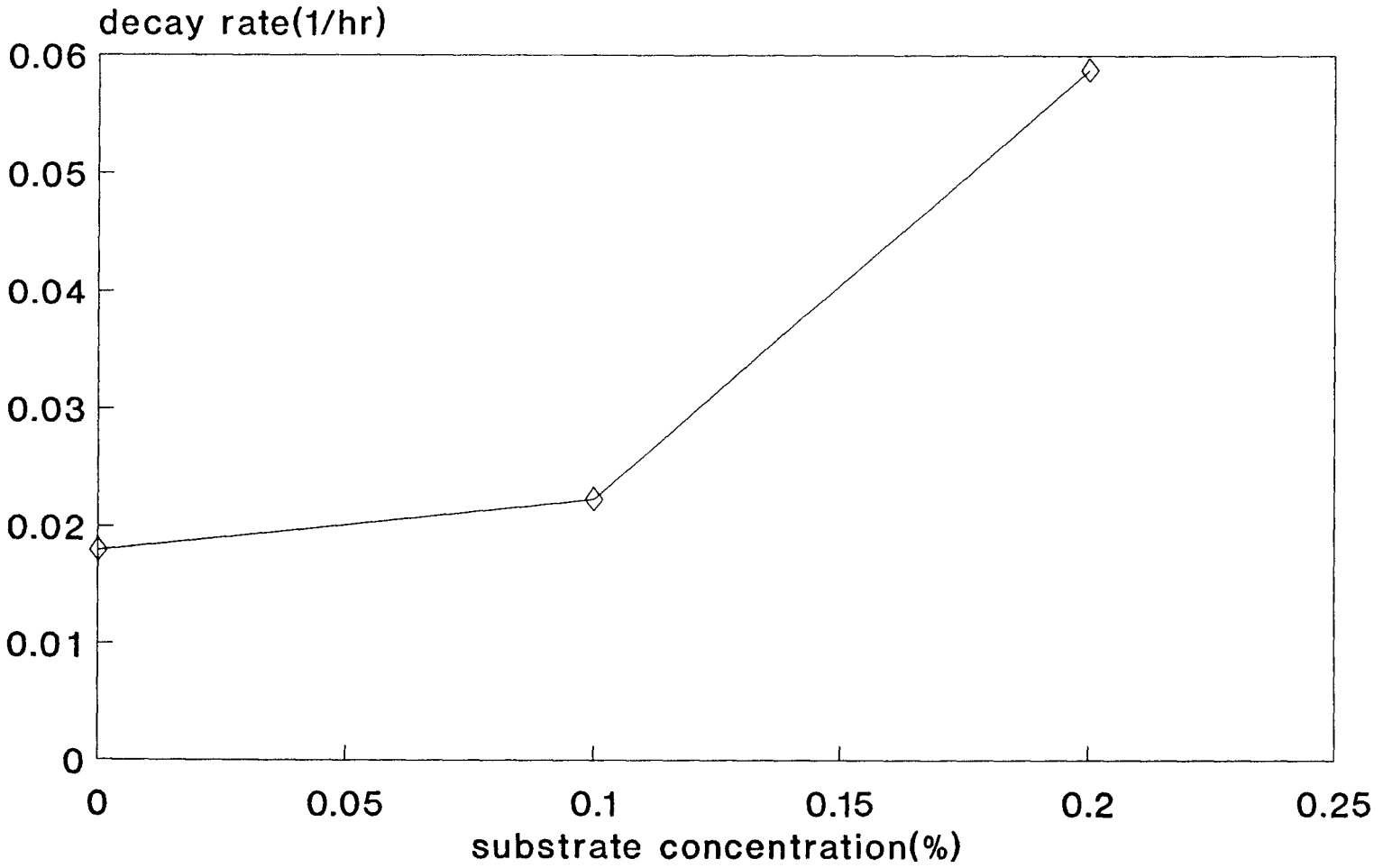


Figure 23 Endogenous decay rate vs. substrate concentration of 2,4-DCP

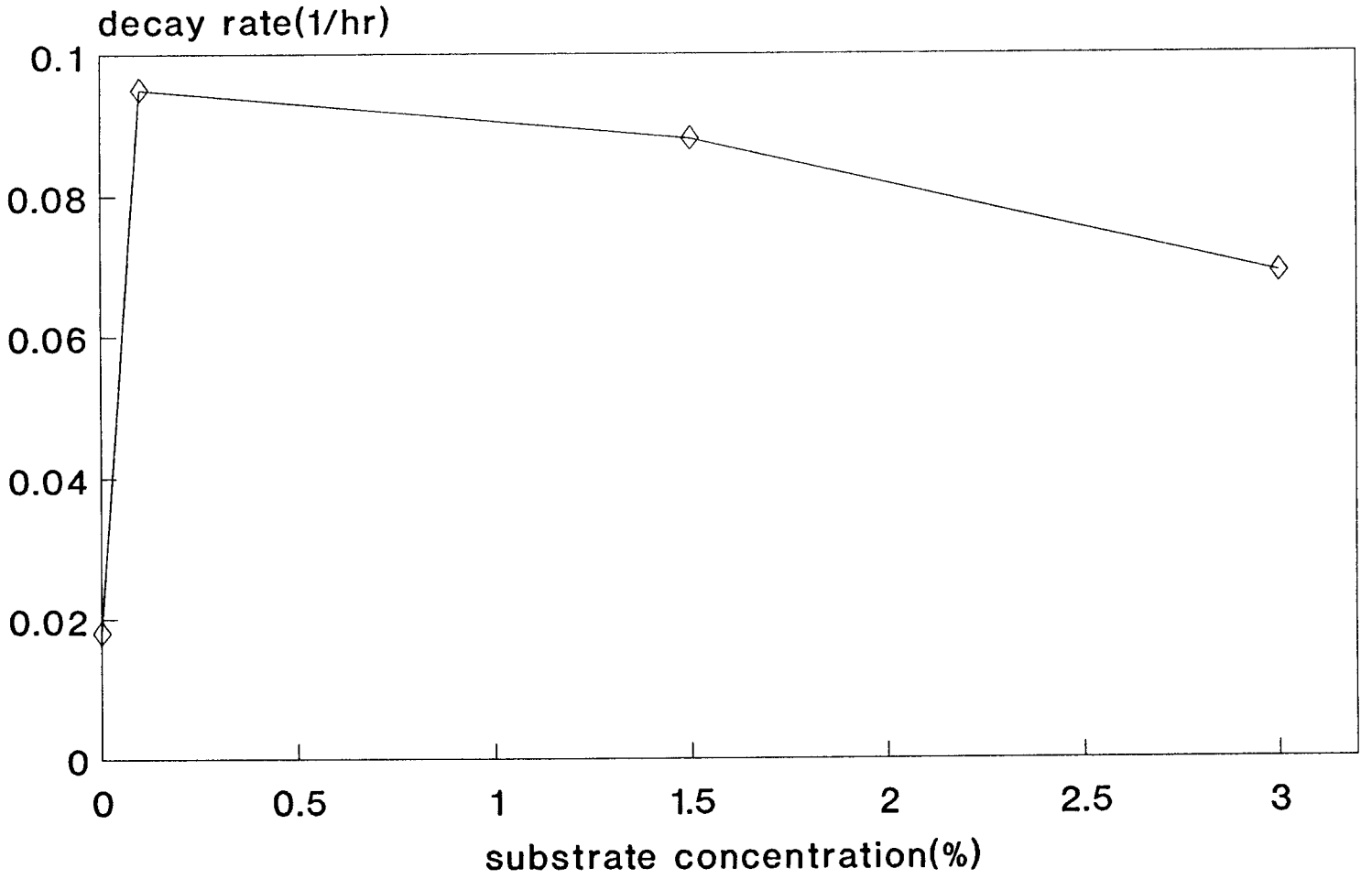


Figure 24 Endogenous decay rate vs. substrate concentration of 1,2,4-TCB

CHAPTER 5
DISCUSSION AND CONCLUSIONS

5.1 Discussion

Since this study was primarily planned to investigate the concentration effect of aniline, 2,4-DCP, and 1,2,4-TCB on their biodegradability, a thermogram was employed. The thermogram can be directly used to measure the heat output generated from microbial activities in one gram sludge culture. It is normally expected that heat output varies significantly depending on the substance contained in the bacterial culture. When an excessive amount of toxic chemical is added, the total heat output will drop significantly due to its inhibitory effect on cell growth. More importantly, the degree of bio-inhibition is possible to be determined by simply measuring the difference in heat production from the process of biodegradation with or without the interference of toxicant involved.

5.1.1 Log Phase

The heat output curves are shown as Figure 13, 14, and 15. Figure 13 shows the results of biodegradation of aniline at 0, 0.1%, 0.3%, 0.5%, 0.7% and 1.1% . It is clearly indicated that the energy production versus time curve is very similar to the typical batch culture cell generation curve, which consists of lag phase, log phase, stationary phase, and endogenous phase, except that the lines of 0.3%, 0.5% and 1.1% didn't approach the endogenous phases. The curve of heat output vs. time of 1.1% is below the base line(no feeding the toxicants). That means that the

concentration of 1.1% inhibited the growth of microorganisms. When feeding the higher concentration of toxicant in the culture, the microorganisms need more time to adapt to the new substrate. That means the lag phase increased by gradually concentrated substrate. If the substrate comes out as a inhibitor to the culture, more lag phase time will be required. Figure 13 also revealed that the total energy heat output and the rate of energy production during the log growth phase is highly dependent upon the initial concentration of toxicant involved. The target substrate, aniline, becomes toxic to microbes as its maximum degradable level is passed. It seems likely that the higher the concentration of toxic contaminant , the higher the biological inhibition effect.

From Figure 14, most of the lines were under the base line, except the 0.1% line. The 0.05% and 0.15% lines did not approach the endogenous phases. The stationary phases of the 0.1% and 0.2% lines showed that they did not match the theoretical exponential curve very well. They decreased gradually the energy productions right after reaching the maximum energy. It means the stationary phases were very short. The endogenous phases took place in a short time.

From Figure 15, we see that all the four energy vs. time curves showed that they decreased right after the maximum energy had passed. That matches the theoretical inhibition theories and curves. Furthermore, the endogenous phases were all shown the pretty exponential curves except the 3.0% line. There is a significant long

lag phase for the 3.5% line. The stationary phases are all very short, and endogenous phases start almost right after the log phase. It implies that the microorganisms grew up to the maximum mass, then they died immediately.

All investigation presently demonstrated revealed that the addition of toxicant to the bacterial culture could possibly result in a negative heat output during each growth phase. It was observed from the present studies that the greater the degree of substrate the longer the lag time occurred. This meant that the microorganisms always needed a longer time for producing the required extracellular enzyme in degrading the toxic chemical at higher concentration . In other words, these degrading bacteria absolutely required more acclimation time for adopting to the external living environment.

The inhibition effect of aniline, 2,4-DCP, and 1,2,4-TCB on their biodegradability is shown as Figures 19, 20, and 21. Besides, the computations list as Table 4.

Table 4. The Computations of μ_E, K_c, K_i and N

Compounds	μ_E	K_c	K_i	N
	hr^{-1}	%	%	
Aniline	3.76	1.435	0.546	3.36
2,4-DCP	8.188	0.745	0.155	9.0
1,2,4-TCB	3.537	0.57	1.63	3.2

At a low substrate concentration, the term $(\frac{C}{K_i})^N$ of the inhibition model (Equation 4.3) equal to zero or no inhibitory effect occurred. Therefore, based on the $\frac{\mu_E}{K_c}$ term, the biodegradability order of aniline, 2,4-DCP, and 1,2,4-TCB can be expressed as :

$$2,4-\text{DCP} > 1,2,4-\text{TCB} > \text{aniline}$$

According to the decreasing $\frac{\mu_E}{K_c}$ order, the $\frac{\mu_E}{K_c}$ is equal to 10.99, 6.21, and 2.62 respectively. However, at high substrate concentration, the term $\frac{\mu_E}{(\frac{C}{K_i})^N}$ is apparently important beyond the $\frac{\mu_E}{K_c}$ term. In the same way as above, the $\frac{\mu_E}{(\frac{C}{K_i})^N}$ has been computed for each compound which caused the inhibitory effect. The inhibitory effect result is as follows :

$$1,2,4-\text{TCB} > \text{aniline} > 2,4-\text{DCP}$$

Because the values are equal to 16.889, 0.492, and 4.225×10^{-7}. Furthermore, we can recognize the biodegradability order $2,4-\mathrm{DCP} > \mathrm{aniline} > 1,2,4-\mathrm{TCB}$. There is a comparison curve for the inhibitory effect shown as Figure 25 among the aniline, 2,4-DCP, and 1,2,4-TCB. From the figure, the slopes also show

$$2,4-\mathrm{DCP} > 1,2,4-\mathrm{TCB} > \mathrm{aniline}$$

at no inhibition concentration. However, the decreasing rate shows apparently

$$2,4-\mathrm{DCP} > \mathrm{aniline} > 1,2,4-\mathrm{TCB}$$

The results are slightly different from the former one. It means that the growth rate is strictly affected by substrate concentration, especially for the hazardous inhibitory compounds.

From Figure 25, activated sludge can only endure 2,4-DCP at the low concentration (up to 0.2%), aniline can be tolerable at the moderate concentration (up to 1.1%), and the highest concentration for 1,2,4-TCB for up to 3.5%. That means microorganisms can be acclimated faster when feeding 2,4-DCP media, then getting inhibitory effect also quickly. When the higher toxic compounds (like aniline and 1,2,4-TCB) are fed into the media , the microorganisms require a longer time to be acclimated in the new environment. After adapting new media, the microorganisms can use the toxic compounds as a carbon source to make growth and mature. While the nutrients are being exhausted, the inhibitory effect happens gradually by the increasing substrate concentrations. Therefore, the highly toxic compounds can be

fed into microorganisms higher concentration.

Comparing the biodegradability of target compounds of this study with the previous studies, the results showed the same order. Tabak et al. (6) found that chlorophenols were significantly biodegradable. Moreover, the chlorophenols could obtain the higher biodegradability than the chlorobenzenes. Pitter (7) investigated the biodegradations of 123 organic compounds including aniline and 2,4-DCP. The test result of biodegradability which he had gained was 2,4-DCP higher than aniline. Tabak et al. used the "static-flask-screening" method to measure the biodegradation removal efficiencies. The comeouts showed that 2,4-DCP obtained 100 percent removal; however, 1,2,4-TCB obtained 54 percent removal. Therefore, the biodegradability order was 2,4-DCP > 1,2,4-TCB. Pitter examined the biodegradation with respect to the decrease of organic substance in term of COD. Aniline gained 94.5 percent COD removal; however, 2,4-DCP gained 98.0 percent removal. As a result, the biodegradability order was clearly 2,4-DCP > aniline.

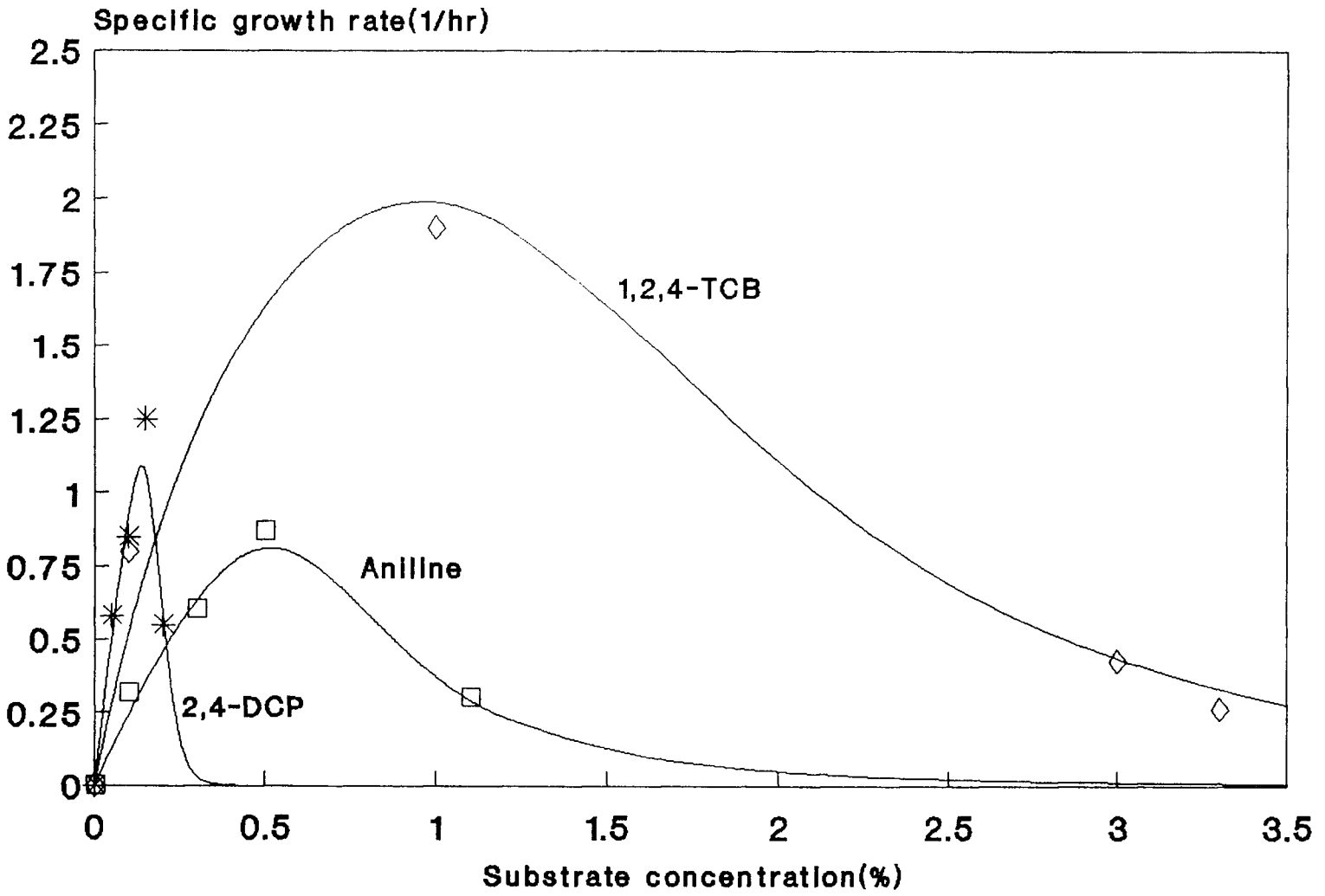


Figure 25 Comparison of inhibitory effect of target compounds

5.1.2 Endogenous Phase

Aniline (0.1-1.1 %), 0.3; 0.5; 1.1 % did not approach their endogenous phases after 61 experimental hours. The base line; 0.1%; 0.7% lines show the significant Endogenous Decay Rates (K_d). Besides, when substrate concentration is high, then the decay rate is high. When at 0.1%, microorganisms adapt to the environment very quickly, the compound is offered as the nutrient (carbon source) to the culture. Then a quick stationary phase and an endogenous phase occurred when the nutrient had been used completely. When at 0.3% and 0.5%, microorganisms require a longer time to adapt to the environment. However, while the microorganisms can be acclimated, they use the substrate as a food source. Moreover, they remained a longer time in the log and stationary phase than the low concentration (0.1%) did. Therefore, in our experimental time, the endogenous phase has not occurred at 0.3% and 0.5%. That might mean that the concentration can cause the microorganisms to grow for more than two and half days. 0.7% is enough to inhibit the growth. Therefore, the death rate is higher than the result of 0.1%. If we had enough time to continue the experiment, we might find the final death rate for the two concentration conditions.

2,4-DCP (0.1-0.2%), got the similar situation to Aniline, the 0.1% and 0.2 % lines obtained the significant endogenous decay rate.

1,2,4-TCB (0.1-3.5%), 3.0% lines didn't approach the endogenous phase

after 41 experimental hours. When substrate concentration is increased, the endogenous decay rate is decreased. While the substrate fed to the microorganisms, the inhibitory effect occurs, except that the very low concentration can is used as the nutrient. After the low concentration nutrient being used, the inhibitor affected the growth of microorganisms at the same time. The more concentration of substrate that is offered, the more nutrient after it is acclimated. Therefore, the higher the concentration of substrate becomes the lower the death rate.

There is a comparison figure shown as Figure 26. The aniline and 2,4-DCP lines get increasing tendency; however, 1,2,4-TCB gets the reverse result. This may show that the most inhibitory effect of 1,2,4-TCB decreases the endogenous decay rate gradually. However, the higher substrate concentration of aniline and 2,4-DCP gets the faster decay rates.

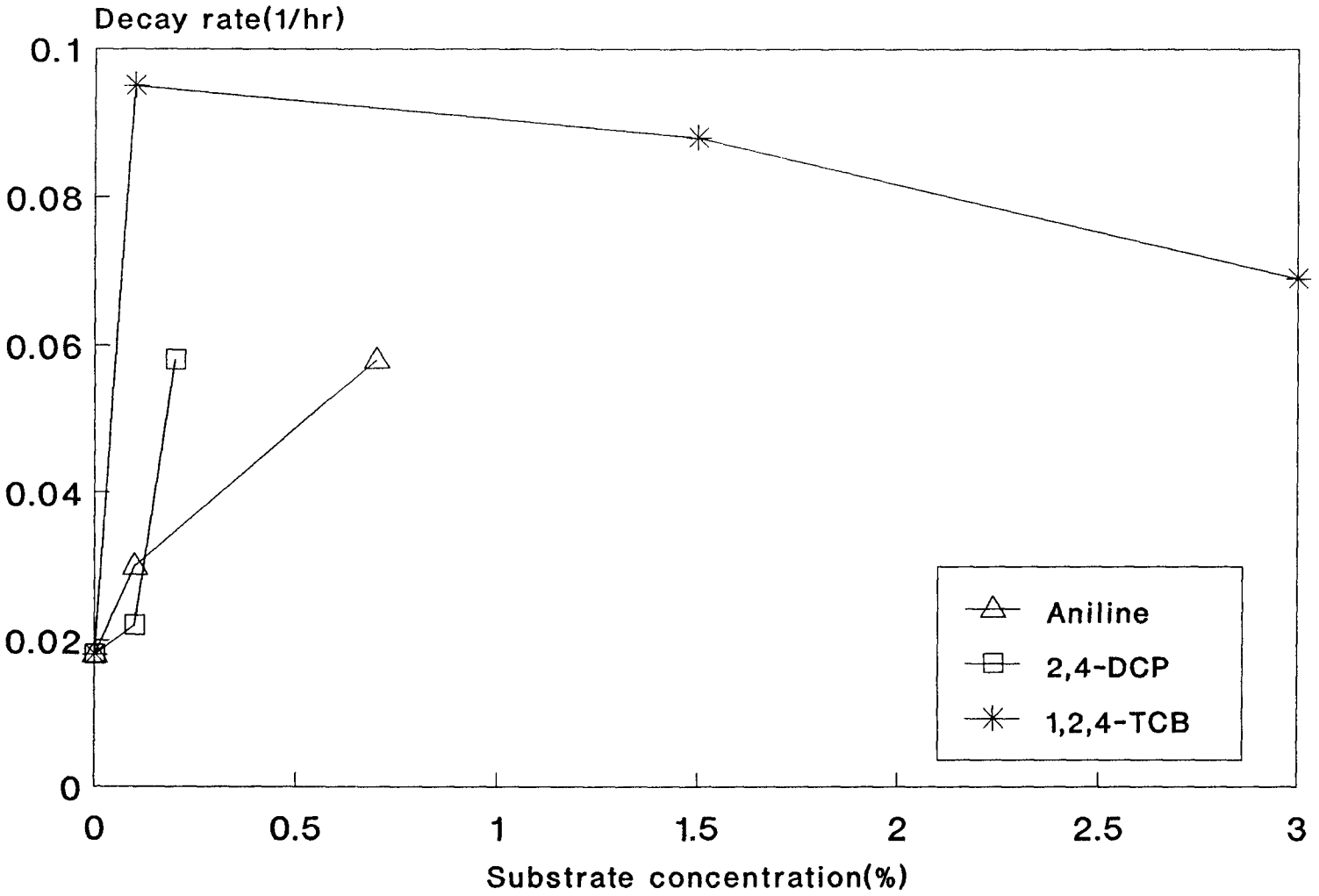


Figure 26 Comparison of endogenous decay rate vs. concentration

5.2 Conclusions

The present study evidently shows that the metabolic activities of microorganisms in degrading the toxic contaminants such as aniline, 2,4-DCP and 1,2,4-TCB can be precisely measured by the direct determination of energy heat output. A thermogram describes the heat output as a result of bioassimilation of toxic organics and it resembles a normal cell growth curve that consisting of lag phase, log phase, stationary phase, and endogenous phase.

The rate of heat energy generation during the period of log phase could be mathematically expressed by a first-order reaction Monod kinetic model; however, when the concentration of toxicant, aniline, 2,4-DCP or 1,2,4-TCB in this case, exceeded the maximum tolerable level, the inhibitory effect on its biodegradation occurred. Substrate inhibition simply followed Equation 4.3

The constants of μ_E (the maximum rate of heat energy production), K_c (saturation constant), K_i (constant of inhibition) and N (degree of inhibition) were determined to be of 3.76 hr^{-1}, 1.435%, 0.546% and 3.36 for aniline; 8.188 hr^{-1}, 0.745%, 0.155%, and 9.0 for 2,4-DCP; and 3.537 hr^{-1}, 0.57%, 1.63% and 3.2 for 1,2,4-TCB.

The biodegradability of these compounds presently investigated followed the order of $2,4-\text{DCP} > 1,2,4-\text{TCB} >$ aniline when substrate concentration was be-

low the inhibitory level. However, the order is $2,4-\text{DCP} > \text{aniline} > 1,2,4-\text{TCB}$ when the substrate concentration exceeded the minimum toxicity level. This study has demonstrated that the chemical nature and property of any toxic compound can greatly influence its biodegradability and toxicity.

No research has been done to study the result of toxic concentration effect on growth in the endogenous phase. This study has successfully achieved the endogenous decay rate increased as both aniline and 2,4-DCP concentrations also increased. However, the endogenous decay rate in 1,2,4-TCB case was not concentration-dependent.

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