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ABSTRACT

Title of Thesis: The Biodegradation of Phenol and O-chlorophenol using Activated Sludge Bacteria

Jeffrey C. Colish, Master of Science, 1984

Thesis directed by: Dr. Gordon A. Lewandowski
Associate Professor of Chemical Engineering

The biological degradation of phenol up to 500 ppm and o-chlorophenol up to 40 ppm was studied in an aerated 4.0 liter batch reactor using activated sludge bacteria from the Livingston, N.J. wastewater treatment plant. From the concentration versus time data, kinetic rate constants were determined for phenol (@ 100 ppm) and o-chlorophenol (@ 20 ppm and 40 ppm). Air stripping was determined to be an insignificant removal mechanism for the compounds studied.

It was noted that the acclimation times decreased with repeated exposure to a particular concentration of phenol or o-chlorophenol, and that the activated sludge bacteria first had to be acclimated to phenol before they could significantly degrade o-chlorophenol.

The effect of addition of amino acids on the rate of o-chlorophenol degradation was also studied. These were found to decrease the rate of biodegradation of 40 ppm o-chlorophenol.

The Biodegradation of Phenol and O-chlorophenol using Activated Sludge Bacteria

by

Jeffery C. Colish

Thesis submitted to the Faculty of the Graduate school of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
1984

APPROVAL SHEET

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

With increasing numbers of toxic chemical waste dumps being discovered daily and pollution control requirements being made more stringent, an inexpensive and environmentally safe method of disposing of our chemical wastes is needed. A method is also needed to detoxify contaminated groundsites and landfills without resorting to an excavation of the site. Currently acceptable methods of disposal include: sanitary landfills, coagulation, carbon adsorption, photolysis, thermal oxidation, wet oxidation, chemical oxidation, and biological oxidation.

Sanitary landfills, coagulation, or carbon adsorption do not destroy the hazardous waste. They are basically only storage or separation methods.

Photolysis appears to be a promising method that utilizes high energy (UV) light to breakdown halogenated organic compounds. Exner, et al., [1] achieved greater than 99% destruction of tetrachlorodibenzo-p-dioxins (TCDDS) using a photolysis process. This process can be augmented by adding a strong oxidant (ozone or hydrogen peroxide) to breakdown a wide variety of organic compounds [2].

Thermal oxidation (incineration) is probably the most generally accepted method for complete destruction of toxic organic wastes. However, the carbon-halogen bond is not very susceptible to oxidative fracture, and for this reason

incinerator temperatures must be very high (on the order of 1200 C). To achieve these high reactor temperatures requires a lot of energy, and therefore, the treatment cost can be high. [3]

Wet oxidation involves bubbling air or pure oxygen through an aqueous waste stream (maximum 10% organics) at 400-600 C and approximately 1000 psia. The wastes are broken down and heat is generated to run the reactor. This process is cheaper to operate than thermal oxidation because lower reactor temperatures are necessary. However, due to the incomplete oxidation of some wastes, a 99.99% destruction efficiency is difficult to achieve. Miller and Fox [4] presented a bromide and nitrate ion catalyst process which achieves faster destruction rates at much lower temperatures and pressures (165-250 C and 200-1000 psig) than conventional wet oxidation systems.

Chemical oxidation (utilizing hydrogen peroxide, ozone, chlorine, chlorine dioxide, or potassium permanganate) can also be used to break down toxic wastes. However, the end products may not be carbon dioxide and water, but rather oxidized organic intermediates which may still exert a high COD after treatment. Nevertheless, these intermediates are often less toxic and less resistant to biological treatment than the original toxic waste. With respect to phenol, 'chlorine is not considered a satisfactory oxidant because it reacts to form chlorinated phenols which are more toxic and have more objectionable odors and tastes than the original

phenolic compounds." [5]

Finally, biological oxidation can be used to convert the toxic waste to water, carbon dioxide, and (when chlorine is present) HCl. The reactions take place at room temperature due to the presence of effective catalysts (enzymes). This method obviously requires the presence of an organism (or organisms) capable of producing the required enzymes. "It is believed by most microbiologists that all naturally occuring materials and all but a very few synthetic materials are subject to microbial attack." [6] Although a number of investigators have performed batch and continuous-flow tests of degradability, much needs to be learned about the biological mechanisms involved, including reliable kinetic rate constants, conditions that favor degradation, the effect of cosubstrates, the microbial species involved in the degradation, and the products of degradation. Until this information is available, wastewater treatment plants cannot be designed or operated at optimum conditions.

II. BACKGROUND

Many studies have been made of the biodegradation of phenol,chlorinated phenolic compounds and other toxic wastes in shaker-flask, batch and continuous-flow reactors using both pure and mixed microbial populations. The following represents a survey of the literature, which has been broken down according to the compounds studies.

A. Phenol Degradation Studies

Barth and Bunch [7] studied the biodegradation of 104 aromatic compounds at 25 C in a batch reactor (Warburg respirometer) using bacteria acclimated to 300 ppm phenol. A phosphate buffer was used to control the pH of the culture between 6.5-8.5. Among the 104 compounds tested were phenols, benzyl alcohols, heterocyclics, benzoic and other acids, benzaldehydes and benzamides, and substituted benzenes. The phenolic compounds studied included: phenol, catechol, resorcinol, quinol, p-, m-, 2,6-di-, 2,4-di- and 2,4,6-trichlorophenol. Most of the compounds were tested at a concentration of 100 ppm. With the phenolic compounds they observed a decrease in acclimation time with repeated exposure to the compound. They further concluded that "there appeared to be a relationship between molecular structure and resistance to bacterial degradation...the relationship...was apparently affected by the position of a group on the ring,

the type of group, multiples of the same or different substituents, and the size and complexity of the substituent". The results of tests on chlorinated phenols showed that "dichlorophenols were more resistant to oxidation than monochlorophenols."

Walton, et al., [8] reported on work done by the O.H. Material Company involving the use of surface application and underground injection of mutant strains of bacteria to combat a variety of spilled hazardous materials. Using a portable biological treatment system, they were able to degrade 30-40 ppm phenol to 0.1 ppm in approximately 26 days and 200 ppm o-chlorophenol to 40 ppm in approximately 36 days. They also reported an increased resistance to biodegradation with the addition of chlorine to the toxic molecule (i.e. phenol degrades much more rapidly and completely then o-chlorophenol).

Paris and Wolfe [9] tried to determine if correlations between microbial reactivity and properties of the compound could be established. The following phenolic compounds were studied: phenol, p-cresol, p-chlorophenol, p-bromophenol, p-cyanophenol, p-nitrophenol, p-acetylphenol and p-methoxyphenol. Using a single strain of bacteria, Pseudomonas putida strain U, they determined the second order rate constant of phenol degradation, Kb, equal to 7.0 ± 1.3 X 10^{-12} liter/organism-hr.

Kim and Armstrong [10] studied the degradation of phenol (770 ppm) and methanol (1000 ppm) by acclimated activated

sludge in batch tests to determine the effects of temperature, pH, salinity and nutrients on the rate of degradation. For pH adjustment the following solutions were used: sodium carbonate (100 gm/l), hydrochloric acid (1 N) or sodium hydroxide (1 and 6 N). A silver nitrate solution (1000 ppm) was used as an enzyme inhibitor. For phenol they determined the Michaelis-Menton rate constant, Ks = 236 mg/l , a growth yield coefficient of 1.2, and the substrate utilization rate coefficient, K = 0.0724 (hr)-1 (at 28 C, pH
* = 7 and salinity = 0 ppt). In 8 of the 115 phenol tests an initial lag phase was observed, with the average lag phase being 4 hours. They concluded that the "primary factor affecting phenol decomposition rate in natural systems was pH: phenol degradation resulted in considerable decreases in pH so that the buffering capacity of the water was the most important factor."

Beltrame et al., [11] studied the biodegradation of phenol (up to 360 ppm) in a continuous stirred tank reactor with cell recycle. The temperature was kept at 20 C, pH at 7.2 and DO at 7.5 mg/liter. The phenol concentration was determined after filtration, using the 4-amino-antipyrine method. The kinetic parameters were determined by fitting the data to the Monod model: Ks = 245 ± 49 mg/l, k = 0.170 ±
* 0.027 (hr)-1, and Y = 0.45 ± 0.04 mg VSS/mg phenol.

Beltrame et al., [12] studied the biodegradation of a mixture of phenol and 2,4-dichlorophenol (DCP) in a 9:1 carbon ratio. The tests were performed in a 3-liter

continuous stirred tank reactor at 20 C using activated sludge that was first acclimated to phenol. Inorganic nitrogen and phosphorus were added to the feed mixture so that the C:N:P weight ratios were 100:10:2. The pH and DO were usually found to be 7.7 and 6.8 mg/l, respectively. The experiments were performed at constant feed flowrate (v = 0.48 l/hr) and feed concentrations (phenol (162 ppm) and DCP (31.2 ppm)). The hydraulic retention time was also kept constant at 6.25 hr. The concentration of phenol and DCP was determined using UV spectrophotometry at wavelengths of 268 nm and 303 nm, respectively. They reported that the phenol degraded according to a first-order equation with a rate

* constant, K = 6.1 X 10^{-3} l/mghr. The 2,4-dichlorophenol removal rate showed a maximum as a function of substrate concentration, "as is typical of cases of substrate inhibition." For this reason the 2,4-dichlorophenol data was correlated to the Haldane equation, with (k/Ks) = 0.81 X

* 10^{-3} l/mg hr and (k/Ki) = 0.18 mg/l hr.

Holladay, et al., [13] studied the biodegradation of phenolic wastes from coal processing wastewater by acclimated activated sludge. The experiments were performed in stirred-tank (CSTBR), packed-bed (PBBR) and fluidized-bed (FBBR) bioreactors. The initial microorganism population for the 3 bioreactors came from the Bethlehem Steel Corporation. The microorganism population of the FBBR was supplemented with the commercial preparation PHENOBAC. The stirred-tank reactor was operated at 32 C and the packed-bed and

fluidized-bed reactors were operated at room temperature (21 C). Analysis of the activated sludge used in this study revealed the following species: Bacillus, Staphylococcus, Pseudomonas, Citrobacter, Proteus and Escherichia coli. They made a systematic study of the relationship between conversion and degradation rates at different feed rates and concentrations in each of the three reactor types. The concentration of phenolics was determined using the 4-amino-antipyrine method. A typical synthetic phenolic feed for the 3 bioreactors was: 26 liter tap water, 2 drops NALCO 71-D5 antifoam, 26 gm ammonium nitrate, 4.5 gm potassium thiocyanate, 2.6 ml 85% phosphoric acid, phenol (variable), glucose (optional), and 5.4 gm ammonium chloride. The pH of the feed was adjusted to 8.5 with concentrated ammonium hydroxide. The rate of phenol biodegradation for each bioreactor ranged as follows: CSTBR = 0.214-2.67 gm/l-day, PBBR = 0.09-5.09 gm/l-day, and FBBR = 1.90-21.17 gm/l-day.

Lewandowski and Abd-El-Bary [14] studied the biodegradation of shock loadings of phenol and o-chlorophenol by activated sludge bacteria obtained from the Livingston, NJ wastewater treatment plant. The experiments were carried out at room temperature (25 C) in a 4 liter fill-and-draw reactor. Phenol concentrations up to 500 ppm and o-chlorophenol concentrations up to 50 ppm were investigated. Also, the effect of the addition of sucrose (1660 ppm) on the phenol and o-chlorophenol degradation rates was studied. The phenol and o-chlorophenol concentration of the samples was

determined, after centrifusing, by the use of gas chromatography (FID) or UV spectrophotometry. The addition of sucrose had no effect on the degradation rate of phenol, but significantly decreased the degradation rate of o-chlorophenol. First-order kinetic rate constants, determined by fitting the data to the Grau equation, were 0.04 (hr)-1 and 0.002 (hr)-1 respectively, for phenol and o-chlorophenol (without sucrose).

Luthy [15] studied the biological treatment of coal coking and coal gasification wastewaters using activated sludge bacteria in a continuous-flow reactor. The reactors were operated with hydraulic and cell residence times ranging from 1.0-21.6 day and 5-40 days, respectively. Influent phenolic concentrations ranging from 175-1700 mg COD/l were reduced to less than approximately 2 ppm by biological oxidation. The phenolic concentration was measured by the 4-amino-antipyrine colorimetric method. Analysis of the degradation data revealed a first-order removal rate coefficient, K, ranging from 0.002-0.004 liter/mg-day and a cell yield coefficient, Y, ranging from 0.10-0.29 (COD basis). The study concluded that the low yield coefficient "is believed to be a result of inhibitory constituents in the wastewater, and perhaps because of inhibitory compounds formed as a result of biological treatment."

Singer, et al., [16] studied the biological oxidation of coal gasification wastewater obtained from the Chapman-Wilputte gasifier at the Holston Army Ammunition

Plant in Kingsport, Tennessee. Using a 22.5-liter activated sludge CSTR operated with a 10 day hydraulic residence time and 20 day cell residence time, the phenol concentration was reduced from 560 ppm to 1.2 ppm. Also, the COD and TOC of the wastewater were reduced 71% and 68%, respectively. But "despite the effectiveness of the biological treatment in removing phenols, the biologically-treated water is unacceptable for discharge to the aquatic environment or for re-use within the plant, and further treatment is required." The effluent from the activated sludge CSTR was then treated by several physical-chemical methods including: activated carbon adsorption, coagulation, ammonia stripping and ozonation. "The results indicate that a significant portion of the organic carbon consists of high molecular weight material (approximately 70% of the TOC remaining after biological treatment consists of species with molecular weight > 500)...that can be effectively removed by activated carbon and ozonation."

Suidan, M.T., et al., [17] studied the treatment of coke oven wastewater from a steel mill by a contact stablization activated sludge process with powdered activated carbon (PAC) addition followed by denitrification in an anoxic (anerobic) column packed with berl saddles. The coal conversion wastewater had the following characteristics: phenol concentration (250-350 ppm), ammonia concentration (3720-3850 ppm), thiocyanate concentration (800-1000 ppm) and cyanide concentration (800-1000 ppm). The wastewater was treated at

34% full strength and supplemental nutrients were added. The final degradation results were not given in the source consulted.

Baker and Mayfield [18] studied biological and non-biological degradation of phenol, o-, m-, p-, 2,4-di-, 2,6-di-, 3,4-di-, 2,4,6-tri-, 2,4,5-tri-, 3,4,5-tri-, 2,3,4,5-tetra- and pentachlorophenol under aerobic and anaerobic conditions using clay loam soil collected in Waterloo County, Ontario. The biological degradation tests were performed in 25 ml Erlenmeyer flasks containing 10 gm of soil (wet weight) to which 1.0 ml of the phenolic solution at a concentration of 1000 ppm was added. The flasks were sealed with a rubber serum cap and incubated at 23 C. Ethanol (95% solution) was used to extract the phenol and chlorophenols from the soil samples. The phenol or chlorophenol concentration of the extracts was determined using UV spectrophotometry (@ 271 nm for phenol and 275 nm for o-chlorophenol). Their results indicate that phenol, o-, p-, 2,4-di-, 2,6-di- and 2,4,6-trichlorophenol were rapidly degraded by aerobic soil microorganisms. They reported 100% degradation of phenol, o-chlorophenol and 2,6-dichlorophenol in 5.0, 1.5 and 0.75 days, respectively. In contrast, m-, 3,4-di-, 2,4,5-tri- and pentachlorophenol were degraded very slowly under the same conditions. None of the phenolic compounds studied were biodegraded under anaerobic conditions. The non-biological degradation tests indicated that phenol, o-, m-, p- and 2,4-dichlorophenol were rapidly

decomposed in sterile silica sand and sterile soil samples.

A comparison of the phenol degradation studies can be found in Table # 1.

1._Critique_of_Phenol_Studies

Many authors either: (a) did not provide kinetic rate constants, (b) provided constants but did not present the rate expressions, or (c) used a melange of units to express the rate constants. Also, most of the authors report that their results were obtained using "activated sludge" without describing more precisely the nature of the bacterial population, or in many cases even the source. Another problem encountered was that of a language barrier, with so many different people doing research in this area (microbiologists, environmentalists, civil engineers, biochemists, and chemical engineers) and each group having their own special terminology, there exits a very real communication problem.

Another area that few researchers investigated was the problems associated with detecting complete mineralization, or the existance of metabolic intermediates. Gas chromatography, UV spectrophotometry and wet chemistry methods could fail to detect the target compound, if it had been slightly modified. It is therefore necessary to monitor the chemical oxygen demand (COD) of the reactor samples, or in the case of chlorinated compounds, the chloride ion concentration.

One other area that many researchers fail to address is the solubility of the toxic compound and the relationship between solubility and pH. Related to solubility, is the

thermodynamics of the (usually) dilute solutions and the possibility of very rapid air stripping due to large activity coefficients.

As did Barth and Bunch [7], the present study observed a decrease in acclimation (lag) time with repeated exposure to the toxic compound, and also witnessed a decrease in biodegradibility with the addition of chlorine to the molecule.

Walton, et al. [8] also reported a decrease in biodegradibility with the addition of chlorine to the compound.

Kim and Armstrong [10] concluded, and this study also observed, that phenol degradation caused the pH of the reactor to decrease drastically. Therefore, it was necessary to buffer the reactor contents so the pH would remain between 8.0-6.0.

Beltrame, et al. [11] observed that little phenol was air stripped out of the reactor, in the absence of activated sludge, in 24 hours. Tests performed by this study on o-chlorophenol and the calculation of the activity coefficients of phenol and o-chlorophenol support this observation.

Beltrame, et al. [12] determined the phenol and DCP concentration by measuring the UV absorbance at a specific wavelength. Since any modification of either compound by

auto-oxidation or biological transformation could result in a compound that would go undetected by the analytic procedure, one could falsly conclude that complete biological degradation was occuring.

The results of Holladay [13] indicate that the degradation rate for phenol was at least twice that determined by this study. This may be due to the origin of the microbial population (Bethlehem Steel), and also due to the higher operating temperature of the CSTR (32 C as compared to 25 C for the present study).

Lewandowski and Abd-El-Bary [14] also observed the complete degradation of phenol (up to 500 ppm) by activated sludge bacteria. In addition, they observed a decrease in the rate of o-chlorophenol degradation with the addition of a co-substrate. However, the rate of o-chlorophenol degradation was much lower than witnessed by the present study. The difference may be due to the different acclimation procedures used and different supplemental nutrients added.

Baker and Mayfield [18] reported that 2,6-dichlorophenol (2,6-D) degraded faster than both phenol and o-chlorophenol. This is opposite to the trend witnessed by the present study, and also is contrary to the results of other investigators [7],[8],[20].

B._Chlorinated_Phenol_Degradation_Studies

Dence, et al., [19] studied the biological treatability of spent pulp bleaching liquors (chlorophenols) using shaker-flask tests. The flasks were incubated at 28 C on a horizontal shaker for 7 days. The pH of the flasks was 7.0. The concentration of the chlorophenols was determined using gas chromatographic analysis with either thermal conductivity or flame ionization dectectors. Acute toxicity tests were also conducted on the various chlorophenols using Daphnia magna. They found that both, funsi and mixed microbial populations, could effectively eliminate the toxicity of the spent bleaching liquors. Also, they observed an increase in toxicity when a co-substrate, asparagine, was added to the original liquor. Additionally, work was done to determine the amount of chlorophenol degradation by aeration. For 2,4-di and 2,4,6-trichlorophenol, they reported an initial concentration of 20 ppm reduced to 10 ppm in a 1 week test by aeration alone. "In other biodegradation tests, three funsi: Paecilomyces varioti, Penicillium variabile and Trichoderma koningii, were examined for their ability to remove chlorophenolic compounds from a glucose yeast extract peptone liquid medium in a one-week incubation period." The liquid medium had an initial chlorinated phenolic concentration of either 50 ppm or 10 ppm, depending on the toxicity of the compound. For 2,4,6-trichlorophenol an initial concentration of 10 ppm was reduced 33% by Paecilomyces varioti, 100 % by

Penicillium variabile and 48% by Trichoderma koningii. In addition, "spent chlorination liquors were subject to a variety of chemical treatments and the resulting effects on acute toxicity determined. Treatment with elemental chlorine, hypochlorous acid, hypochlorite, ozone and hydrogen peroxide produced increases in the toxicity of the spent liquor. A modest reduction in toxicity accompanied treatment of spent chlorination liquor with chlorine dioxide."

Tabak, et al., [20] studied the biodegradibility of 96 of the 114 organic priority pollutants included on the EPA Consent Decree list to ascertain the extent of microbial degradation and to determine the acclimation period. A static-flask, batch screening procedure "incorporating settled domestic wastewater as microbial inoculum" was used. The incubations were carried out in the dark at 25 C. The investigation involved organic concentrations of 5 and 10 ppm. The substrate concentration was determined through the use of gas chromatography (GC), dissolved organic carbon (DOC), total organic carbon (TOC) and/or chemical oxygen demand (COD) analysis. The following results were determined: with respect to phenolic compounds, "the chlorophenols are more stable to biodegrade than phenol and the resistance to microbial catabolism is greatest amoung the more highly chlorinated phenols."

Haller [21] studied the degradation of 16 substituted aromatic compounds by activated sludge supernatant and soil bacteria in batch shaker-flask tests at 30 C. The compounds

studied included: chlorobenzoates, chlorophenols, nitrobenzoates, nitrophenols, aminobenzoates and an aminophenol. The activated sludge used in this study was taken from the primary settling tank of the Ithaca, NY wastewater treatment plant. The sludge was allowed to settle and the "turbid, grayish supernatant liquid" was decanted for use as microbial inoculum. The substrate concentration was determined using a recording spectrophotometer run in the range 200 to 340 nm. The compounds tested had an initial concentration of 16 ppm. For o-chlorophenol, 19 days was required for 100% degradation using unacclimated activated sludge bacteria. Soil bacteria was unable to degrade 16 ppm o-chlorophenol even after 25 days. The addition of a secondary carbon source (glucose or benzoate) did not affect the length of time neededd for wastewater adaption. When the activated sludge was first adapted to p-chlorophenol or m-chlorobenzoic acid, the subsequent time necessary for 100% degradation of o-chlorophenol was reduced to 10 days.

Ingols, et al., [22] studied the degradation of 20 different halogenated phenolic compounds by an "activated sludge" developed by aerating soil with glucose and peptone in a mineral nutrient medium free of added chloride ions. The activated sludge was acclimated to the halophenol by slowly decreasing the glucose and peptone concentration while maintaining the halophenol level. The tests were conducted in 1.0 liter batch reactors with an initial halophenol concentration of 100 ppm. UV spectrophotometry was used to

measure the substrate concentration and the mercuric nitrate method, from Standard Methods, was used to determine the halide ion concentration. The optimum pH and temperature were determined to be 6.8-7.8 and 27 C, respectively. The results of the degradation experiments revealed 100% degradation of ortho-, meta-, and parachlorophenol in 3, 2 and 3 days, respectively. They also reported no degradation of sodium pentachlorophenol in a 4 day test.

Pitter [23] studied the biodegradability of 123 organic compounds by activated sludge taken from a sewage treatment plant. The sludge was adapted to the test compound for 20 days in a medium which also contained glucose and peptone, before the degradation tests were conducted. The tests were performed using batch reactors, containing 1000-1500 ml of acclimated sludge, that were placed on magnetic stirrers in a dark room at a temperature of 20 C ± 3. The reactors had an initial biomass concentration of 100 mg/l. The substrate concentration was determined by COD analysis. In the degradation experiments the test compound was the sole carbon source and had an initial concentration of 200 ppm COD. The study determined the rate of biodegradation of phenol, o-chlorophenol and p-chlorophenol, which were reported to be 80.0 mg COD/gm initial biomass-hr, 25.0 mg COD/gm initial biomass-hr and 11.0 mg COD/gm initial biomass-hr, respectively.

Wukasch, et al., [24] studied the degradation of pentachlorophenol (PCP) in a continuous reactor system with

consideration given to the amount of PCP lost by adsorption and stripping. They found that less than 0.05% of the PCP feed was lost due to air stripping. The bacteria was acclimated to PCP by initially feeding at 1 ppm and increasing over a 90 day period to 20 ppm. The COD of the feed was increased to 600 ppm by adding dog food extract in order to increase the ratio of suspended biomass to attached biomass. To determine the extent of biodegradation, they used radioactively labled PCP and measured the evolution of radioactive carbon dioxide during a batch test. They stated that the "evolution of labled carbon dioxide provides a most reliable proof of ultimate biodegradation." Their results strongly indicated that the PCP was being degraded to carbon dioxide and energy by the organisms. They determined the kinetic rate constants for PCP degradation using the Monod * equation: (Ks/um) = 593 mg day/l and Kd = 0.05 (day)-1.

Wallin, et al., [25] studied the removal of pentachlorophenol (PCP) from wood preserving process wastewater containing approximately 100 ppm of PCP. The following physical properties were given for PCP: "mildly acidic, boils at 309 C and is soluble in 50 C water at 30 ppm. Oils or emulsions in wastewater can provide a carrier effect, allowing PCP to far exceed its normal solubility in water." They investigated several different treatment technologies: (1) adsorption, (2) biological degradation, (3) chemical oxidation, (4) coagulation, (5) extraction, and (6) pH adjustment. Based on the results of batch tests, they

reported that biological degradation was an ineffective treatment because "bioadsorption rather than biotransformation was found to be the primary removal mechanism. Removal rates continued to diminish as the adsorptive capacity of the biomass was approached." In addition, incineration of the PCP-laden sludge presented a hazardous waste problem because thermal oxidation could release 2,3,7,8-tetrachlorodibenzo-p-dioxin into the atmosphere.

Edgehill and Finn [26] studied the degradation of pentachlorophenol (PCP) using activated sludge. The tests were performed in a 6-liter fill-and-draw reactor, which was maintained at a pH of approximately 7.4. The feed mixture contained 200 ppm glucose and 40-120 ppm PCP. Tests showed an acclimation period of 7 days for the activated sludge while a semi-pure culture of PCP-degrading bacteria showed immediate acclimation. With regard to shock loadings they found that "even with the acclimated sludge, the system was upset for two days by a simple step change from 40 to 120 ppm PCP."

Kirsch and Etzel [27] studied the degradation of pentachlorophenol (PCP) by a culture derived from a soil sample taken from the grounds of a wood products manufacturer who used PCP as a wood preservative. The culture was first acclimated batchwise to phenol and then the phenol concentration was decreased while the PCP concentration was slowly increased until by the third month the PCP

concentration was 30 ppm and the phenol concentration was 0 ppm. The degradation experiments were carried out in shaker-flask and fill-and-draw reactors using radioactive pentachlorophenol. "Ultimate biodegradation of pentachlorophenol was determined as the amount of radioactive carbon dioxide evolved relative to time." They noted three important findings: (1) PCP is readily biodegradable with up to 68% of the PCP being degraded to carbon dioxide within 24 hr. (2) the rate of PCP removal is a function of cell concentration at high cell concentration levels, which may be due to oxygen deprivation. (3) the addition of organic nitrogen to the degradation mixture decreased the rate of PCP removal by a factor of two. "This suggests that PCP is probably not a primary substrate but serves rather as a secondary substrate that does not compete favorable with more easily degraded materials."

Heidman, Kincannon and Gaudy [28] studied the degradation of sodium pentachlorophenol (SPCP) at various concentrations by acclimated and non-acclimated activated sludge. The experiments were performed in 1.5 liter batch sludge units at 22 C. Two types of studies were performed: (1) to determine the effects of long term exposure to SPCP and (2) to determine the effects of shock loadings of SPCP on the activated sludge process. Their results indicated that activated sludge can be acclimated to up to 250 ppm SPCP without a serious decrease in biological treatment efficiency. They note that "prolonged exposure to SPCP

caused large changes in predominating species", and that the biological solids in SPCP acclimated systems would not flocculate or settle during a 1-hr period. The results of the shock loading experiments showed that even small doses of SPCP caused decreases in the system efficiency. "The response of 5, 15, and 30 ppm SPCP indicated successively more deleterious effects."

A comparison of the chlorinated phenol studies can be found in Table # 2.

1._Critique_of_Chlorinated_Phenol_Studies

Dence, et al. [19] reported generally poor biodegradation results, with most chlorophenols tested being degraded only 20-30% in a 1-week test. This may be due to the fact that the liquid medium contained additional carbon and nitrogen sources, which other investigators have indicated as reducing the rate of biodegradation.

The results of Tabak, et al. [20] agree with the findings of the present study regarding the biodegradibility of phenol and o-chlorophenol. However, their results for toluene raise the question of air stripping or volatilization, because at the concentration tested toluene would have an activity coefficient greater than 10,000. Salerno [47] studied the biodegradation of toluene in an aerated batch reactor and found that 50 ppm would be stripped to 0 ppm in 2-3 hours.

Haller [21] reported that unacclimated activated sludge required 19 days to completely degrade 16 ppm o-chlorophenol and that preadaption to another aromatic compound reduced the time for complete degradation. However, the rate of o-chlorophenol degradation was much lower than determined by the present study, this may be due to the much lower microbial concentration in the reactors.

Ingols, et al. [22] observed the sludge change color, from light tan to dark brown, with exposure to a halophenol. It was also found that addition of a co-substrate reduced the

o-chlorophenol degradation rate. Both of these findings were confirmed in the present study.

In Pitter's work [23] the rates of phenol and o-chlorophenol degradation appear to be much lower than witnessed in the present study. This may be due to the much lower microorganism concentration (100 mg/l) for Pitter, versus 700-1600 mg/l for the present study.

Wallin, et al. [25] and Edgehill and Finn [26] both reported on experimental work performed at a PCP concentration of approximately 100 ppm. This is far in excess of the solubility of PCP at the temperature of the experiments. Additionally, Wallin, et al. [25] reported that PCP was not biologically degraded, this is in conflict with the results of Wukash, et al [24], Kirsch and Etzel [27] and Heidmann, et al [28].

Kirsch and Etzel [27] found that the addition of an organic nitrogen source reduced the rate of PCP degradation. This is in agreement with the present study which also concluded that the addition of organic nitrogen sources (amino acids) reduced the rate of o-chlorophenol degradation.

C._Chlorinated_Benzoate_Studies

Shamat and Maier [29] studied the ability of activated sludge biomass obtained from the Metropolitan Wastewater treatment plant (St. Paul, Minn.) to completely metabolize mono- and dichlorobenzoic acids and 2,4-dichlorophenoxyacetic acid (2,4-D) in continuous-flow and batch tests. In the continuous-flow tests the concentration of p-chlorobenzoic acid, 2,4-D and o-chlorobenzoic acid were: 50 ppm, 98 ppm and 111 ppm, respectively. The batch experiments were carried out in 2-liter glass bottles placed on a rotary shaker. The bottles had an initial substrate concentration of approximately 50 ppm and an initial biomass concentration of 0.5 mg/l. The tests were performed in the dark at 20 C. During the study the concentration of chlorinated compound was measured using UV absorbance at a specific wavelength for each compound. In addition, the total organic carbon (TOC) concentration, pH, and free chloride ion concentration were monitored. The results of the batch degradation experiments indicated that o- and p-chlorobenzoic acid have a 6-day lag period, while m-chlorobenzoic acid and 2,4-D had 12-day and 10-day lag periods, respectively. "All four substrates showed the same phenomenon, a short lag followed by rapid disappearence once metabolism began. 3,5-dichorobenzoate had a 14-day lag followed by very slow substrate disappearence over a period of over 100 days. The 2,5-dichlorobenzoate had a long 88-day lag followed by a rapid disappearence of all

substrate. Neither 2,4- nor 2,6-dichlorobenzoate was metabolized during this 195-day test." They attributed this lack "to the combined effects of low initial concentration of active biomass and the unavailability of enzyme systems for metabolizing" the chlorinated organic substrates. After the degradation tests, they investigated what species were involved in the degradation and determined that Pseudomonas was the predominate species. The kinetic parameters were determined from the degradation data using Monod kinetics.

Hartmann, et al., [30] studied the degradation of 3-chloro, 4-chloro, and 3,5-dichlorobenzoate by Pseudomonas sp. WR912, which was isolated by continuous enrichment from a mixed population originating from soil samples of the Gottingen area. This is important because several studies have determined that chlorobenzoates are the products of PCB degradation. The degradation experiments were carried out in 250 ml Erlenmeyer flasks incubated at 30 C on a rotary shaker. A phosphate buffer was used to maintain the pH at approximately 7.0. The concentration of the chlorobenzoates and any cometabolic products was monitored using reverse-phase high pressure liquid chromatography (HPLC) in combination with UV spectrophotometry. The pH and chloride ion concentration were measured using specific ion electrodes. The maximum concentration of the 3 chlorobenzoates tested was 20 mM, which is equivalent to 3131 ppm for the monochlorobenzoates and 3820 ppm for the dichlorobenzoate. A long adaptive time (11 months) was

necessary to develope a 3,5-dichlorobenzoate degrading organism. Complete degradation and 100% chloride elimination was reported for 3-chloro, 4-chloro, and 3,5-dichlorobenzoate in 14hr, 11 hr, and 29 hr, respectively. "In the case of 3,5-dichlorobenzoate degradation, liberation of 2 mol of HCl per mol of substrate exceeded the buffer capacity of the medium so that the phosphate concentration had to be doubled." Pseudomonas sp. WR912 was also tested for growth on 120 additional substrates. The following "gave good growth: DL-valine, D-tryptophan, L-phenylalanine, pelargonate, adipate, sebacate, mesotartrate, salicylate, nicotinate, mesconate, citrate, lactate and benzoate. Poor or no growth was observed with carbohydrates."

DiGeronimo, et al., [31] studied the degradation of benzoate, mono-, di- and trichlorobenzoates using a microbial population taken from the primary settling tank of the Ithaca, N.Y. sewage treatment plant. The tests were performed in 2-liter Erlenmeyer flasks that contained 1.0-1.5 liters of activated sludge. The flasks were incubated at 25 C, the dissolved oxygen (DO) and pH were maintained at 6-8 mg/liter and 7.2, respectively. Periodically during the degradation run a 150 ml reactor sample was taken and acidified with 20 ml concentrated HCl. The sample was then extracted at 0 C with three 30 ml portions of diethyl ether. A gas chromatograph equipped with a flame-ionization detector (FID) was used to measure the chlorobenzoate concentration. Before any of the chlorinated benzoates were added to the

flasks, they were neutralized with NaOH. The following compounds were studied at an initial concentration of 100 ppm: benzoate, o-, m-, p-, 2,4-di, 3,4-di and 2,3,6-trichlorobenzoate. It was determined that all the compounds studied except for 2,4-di- and 2,3,6-trichlorobenzoate were biodegradable. The benzoate and o-chlorobenzoate exhibited immediate degradation, while the m-, p- and 3,4-di-chlorobenzoate showed 10, 7 and 3 day lags before the onset of significant biodegradation. They also investigated whether 2,4-di- and 2,3,6-trichlorobenzoate could be metabolized in the presence of glucose or a structurally similar compound (benzoate or m-chlorobenzoate). Their results indicate that the "rate of degradation was not appreciably altered by the addition of glucose" and that "the addition of biodegradable benzoates did not lead to the decomposition of 2,4-di- or 2,3,6-trichlorobenzoates."

A comparison of all the chlorinated benzoic acid studies can be found in Table # 3.

1._Critique_of_Chlorinated_Benzoic_Acid_Studies

Shamat and Maier [29] studied the degradation of 2,4-D at a concentration of 98 ppm, this may be higher than the solubility limit of 2,4-D at 25 C. They reported a one-to-one correspondence between substrate disappearence and chloride release, indicating the complete biodegradation of the chlorinated compounds tested.

Hartmann, et al., [30] witnessed, as did the present study, the acidification of the reactor medium by elimination of the organically bound chlorine as HCl. Hartmann, et al., also achieved a much faster rate of chlorinated benzoic acid degradation then did Shamat and Maier [29] or DiGeronimo [31], the increased rate of biodegradation may be the result of the acclimation procedure used by Hartmann.

The results of DiGeronimo, et al., [31] compare very well with the findings of Shamat and Maier [29] with respect to the biodegradation and lag times of chlorinated benzoic acids.

D._Polycyclic_Degradation_Studies

Saeger and Thompson [32] studied the biodegradability of 32 halogenated diphenylmethanes (DPMs) using a semi-continuous activated sludge (SCAS) procedure, a river die-away (RDA) procedure, and a carbon dioxide evolution procedure. The RDA procedure used Meramec River water as the source of microbial inoculum. The experiments were conducted in 0.95-liter screw-cap bottles, which were stored in the dark at 23 C. The DPM concentration was determined by extracting a 250 ml sample with three 50-ml portions of methylene chloride. The combined extracts were dried over anhydrous sodium sulfate, 5 ml of isooctane added, and the extracts were concentrated in a Kuderna-Danish evaporator. The DPM concentration of the concentrated extracts was measured using either flame-ionization (FID) or electron capture (EC) gas chromatography. It was determined that diphenylmethanes having one unsubstituted phenyl ring generally exhibited high primary degradation rates, while those substituted on both rings were slow to degrade. "On the basis of RDA data, the chlorinated DPMs showed the following order of decreasing biodegradability: 2- > 3,4-

* $\simeq$ 3- $\simeq$ 4- $\simeq$ 2,4- > 2,5- > 2,3,4- $\simeq$ 2,4,5- > 2,6- > 2,3,6- > 2,4,6-.

Su, et al., [33] studied the biodegradation of labeled polychlorinated biphenyls (PCBs) - specifically Aroclor 1242 and 1245 - in shaker-flask tests using microorganisms from

river sediment or activated sludge. The PCB concentration was measured, after extraction with hexane/ether, by gas chromatography with a glass capillary column. For an unreported initial concentration they witnessed a maximum of 26.4% degradation of Aroclor 1242 and 16.0% degradation of Aroclor 1254. Microorganisms capable of degrading PCB are widely distributed in the environment. Six genera were isolated: Acetobacter, Actinobacter, Alcaligens, Klebsiella, Pseudomonas and Escherichia. They also found that PCB degradation was dependent on the degree of chlorine substitution and that chlorinated benzoic acids were the by-products of biological degradation.

Bailey, et al., [34] studied the degradation of orthophenlyphenol (OPP), an antimicrobial distinfectant, a carbon-14 label. The biodegradation was studied in a river die-away experiment, and a simulated wastewater treatment experiment, using both virgin and acclimated muncipal sludge. The tests revealed a parent compound half-life of approximately 1 week in the river die-away study, 24 hr with virgin sludge, and 3 hr with acclimated sludge. In all three experiments, the conversion of carbon-14 to carbon dioxide was found to be 65%, with no extractable intermediates being observed.

Clark, Chian and Griffin [35] studied the degradation of PCBs by 3 mixed-cultures isolated by biphenyl enrichments from a river sediment and two different soil samples. The experiments were carried out in aluminum-foil covered 250-ml

Erlenmeyer flasks. The flasks were shaken at 27 C and had an initial pH of 6.8. The flasks had an initial Aroclor 1242 concentration of 703 ng/ml (0.703 ppm), which was produced by saturating the solution with Aroclor 1242 for 6 months at 25 C. The samples were analyzed using a gas chromatograph or mass spectrometer equipped with a capillary column. The results indicated that the less chlorinated PCB isomers (which were also more water soluble) were degraded at a faster rate. In addition, the cometabolism of PCBs in the presence of sodium acetate was studied and "showed greatly enhanced degradation of the higher-chlorinated isomers as well as other recalcitrant isomers." Horvath [46] described cometabolization as "the process in which a microorganism oxidized a substance without being able to utilize the energy derived from this oxidation to support growth." Alcaligenes odorans and Alcaligenes denitrificans were the most common microorganisms present in the mixed cultures.

Francis, et al., [36] studied the cometabolism of several p,p'-dichlorodiphenyl analogs of DDT in the presence of diphenylethane (DPE) by a Pseudomonas sp. The tests were performed in 250-ml Erlenmeyer flasks and were incubated on a shaker at 28 C. The test compound was studied at a concentration of 100 ppm, while the DPE was present at a concentration of 100-300 ppm. Concentrations were measured using gas chromatography/mass spectrometry. Their results indicated that "bis(p-chlorophenyl)methane and 1,1-bis(p-chlorophenyl)ethane were metabolized in the

presence of DPE to yield p-chlorophenylacetic acid and 2-(p-chlorophenyl)propionic acid, respectively." The other compounds tested weren't found to underso cometabolic degradation with DPE.

A comparison of the polycyclic aromatic studies can be found in Table # 4.

E._General_Degradation__Studies

Rittman and Koybashi [37] performed an extensive review of the literature to determine which species of microorganisms could selectively be used to degrade specific toxic chemicals. For the degradation of phenol they reported that the following species have been found to be effective: Pseudomonas, Vibrio, Spirillum, Flavobacterium, Chromobacter, Bacillus, Nocardia, Chlamydamonas ulvaensis, Phoridium fuveolarum, Scenedesmus basiliensis, Euglena gracilus and Corynebacterium. In addition, they reported that Nocardia and Mycobacterium have been found to be effective in degrading monochlorophenol by cometabolism.

Gaudy, et al., [38] studied the conversion of a nonvolatile compound (glucose @ 5000 ppm) into volatile metabolic intermediates by activated sludge bacteria. The seed came from the municipal sewage treatment plant at Stillwater, Okla. A sealed batch reactor was aerated with compressed air at a flow rate of 4000 cm3/min per liter of reactor volume. The air leaving the batch reactor passed through an adsorption flask containing 100 ml of water to which NaOH had been added to a pH of 10. The results indicated that the maximum loss of the original carbon source was 3%. But Gaudy warned that "since different initial substrates and different microbial populations would undoubtedly produce different, and possibly more volatile, metabolic intermediates, it seems important to be aware that

strippable compounds may be formed during biological treatment."

Rittmann and Kobayashi [39] studied the critical factors involved in the biological degradation of trace organics. The critical factors are: (1) that low concentrations present special problems for biological treatment and (2) the fixed-film (biofilm) organisms are the more effective forms. Very low substrate concentrations present a problem because the rate of cell growth can be less than minimum necessary to sustain the organism. Most substrates have a limiting concentration, Smin, at which the rate of steady-state utilization and biofilm mass decrease to approximately zero. The limitation of Smin can be overcome by uncoupling the rate of substrate utilization from the steady-state growth of the cells. This can be brought about by making the biofilm growth non-steady state, undergoing net decay. Another method of uncoupling trace-substrate utilization from cell growth is to grow and maintain the cells through the utilization of a primary substrate, present in relatively high concentration. These cells are then present to remove the trace-level or secondary substrate. The microorganisms being considered for the removal of trace substrates include: algae, fungi, photosynthetic bacteria and actinomycetes in combination with selected populations of aerobic, facultative and/or anaerobic bacteria.

Alexander, M. [40] explained that microorgisms in sewage, soils, and waters can convert many synthetic organic

chemicals to inorganic products in a process called mineralization. Other toxic organic compounds are transformed only by cometabolism, where the microbial populations are grown on another substrate while performing the transformation of the toxic compound. These processes may lead to environmental detoxification, the formation of new toxicants, or the biosynthesis of persistent products. Some organic molecules are resistant to microbial attack, recalcitrant, for the following reasons: (1) properties of the compound, (2) environmental factors, (3) surface effects, (4) biological evolution, (5) concentrations of the compound, and (6) formation of complexes with resistant polyaromatics. "Absolute recalcitrance is probably a property of synthetic polymers such as polyethylene, polyvinyl chloride" and many other plastics. Also, the size of the polymer may prevent it from penetrating the microorganism's cell wall, and extracellular enzymes which would otherwise reduce the length of the polymer chain may be absent. The surrounding microenvironment also plays an important role in determining biodegradablity. For example, the lack of oxygen has been associated with the resistance of carbohydrates to microbial degradation. "In addition, certain classes of chemicals, when sorbed to surfaces of colloidal materials present in natural ecosystmens, are not readily attacked microbiologically." Recalcitrance can also be caused by biological evolution, which may have left the organism with no enzyme to transform the compound into an intermediate in

an existing biochemical pathway. A low concentration of the toxic compound may also prevent it from being biologically attacked, because the organism isn't able to get enough energy to sustain itself or won't illicit an enzymatic response. "Other hypothesis to account for the resistance of persistant molecules to microbial breakdown included complexing of the normally available substrate with resistant polyaromatics and inaccessibility of the site on the substrate at which the enzyme should function."

Omori and Alexander [41] studied the bacterial dehalogenation of organic compounds by 3 cultures obtained from over 500 soil enrichments. Two of the cultures could grow on 1,9-dichlorononane and the other used 1-chlorooctane as the only carbon source. One of the 1,9-dichlorononane dehalogenating cultures was studied and indentified as a strain of Pseudomonas. The tests were performed in rotary shaker flasks at 30 C. A phosphate buffer was utilized to maintain the pH at 7.0. The 1,9-dichlorononane dehalogenating pseudomonad was also tested for the ability to dehalogenate other compounds. It was determined that this strain could dehalogenate 1,9-dibromonane, 1-chloroheptane, 1-bromoheptane, 1-iodoheptane, 2-bromoheptanoic acid, 7-bromoheptanoic acid, 3-chloropropionic acid and 3-iodopropionic acid.

III. OBJECTIVE

The objective of this study was to determine the kinetic rate constants for the biological degradation of phenol and o-chlorophenol. The effects of the addition of amino acids on the rate of o-chlorophenol degradation was also studied. This was accomplished using activated sludge bacteria from the Livingston wastewater treatment plant in a 4.0 liter batch reactor. This work is part of a larger investigation into the mechanisms of biological detoxification.

IV. APPARATUS

The reactor set-up is shown is Figure # 1. The 4.0 liter batch reactor consists of a 6" diameter clear lucite tube with 1/4 inch thick walls mounted on a 8" square base of 1/4 inch thick lucite. An air filter consisting of a 4" long by 2" diameter plastic pipe stuffed with glass wool was used to keep fine oil droplets from entering the reactor via the air compressor. To keep the liquid in the reactor saturated with oxygen, the filtered air passed through a 1/4 inch Tygon tubing which ended in an aquarium diffuser stone. The air flowrate was measured with a Gilmont rotameter.

V. ANALYTICAL EQUIPMENT

The following analytical equipment was used to perform the experiments for this study.

(1) pH meter: Orion Research model # 701A
pH electrode: Orion Research model # 91-04
ammonia gas electrode: Orion Research model # 95-10

(2) gas chromatograph: Tracor model # 560

operating temperatures: oven = 120 C.
FID = injection port = 250 C.
gas flowrates: N2 = 45 cc/min at STP
H2 = 30 cc/min at STP
AIR = 0.9 SCFH at STP

(3) G.C. column: Supelco - 5' X 1/8" SS
5% SP 2100 on 100/120 Supelcoport

(4) integrator: Hewlett Packard # 3390A

(5) UV spectrophotometer: Perkin Elmer model # 571

(6) centrifuge: DAMON/IEC model # IEC HN-SII

(7) COD Reactor: Hach model # 16500-10

VI. PROCEDURE

A. Air Stripping Experiments

The reactor was cleaned with soap and water and rinsed several times with tap water. Hydrogen peroxide was poured in the cleaned reactor to act as a disinfectant. The reactor was dried with paper towels, covered and set aside. 2.5 liters of deionized water were boiled for 1/2 hours and 2.0 liters poured into the reactor. The reactor was then recovered and the water allowed to cool overnight. The next day enough o-chlorophenol was added to spike the reactor to approximately 20 ppm and the air turned on at a flowrate of 500 ml/min. The air passed through a glass wool filter and a rotameter before entering the reactor. One or two samples per day were taken until the o-chlorophenol concentration fell to zero.

B. Sample Preservation Experiments

One liter of activated sludge from the Livingston treatment plant was poured into a clean glass beaker and spiked with substrate (plus nutrients). The mixture was allowed to aerate approximately 15 minutes, after which six samples were drawn off and treated as follows:

(sp1) take sample, let sludge settle out, decant 10 ml into a vial, add 1/2 ml of 1000 ppm thymol solution, and leave vial unrefrigerated.

(sp2) same as # 1, but refrigerate sample

(sp3) take sample, centrifuge for 10 minutes, decant 10 ml of the supernate into a vial, add 1/2 ml of 1000 ppm thymol solution and refrigerate.

(sp4) same as # 3, but also add 1/2 ml isopropyl alcohol to sample.

(sp5) same as # 3, but also add 1/2 ml concentrated sulfuric acid to sample.

(sp6) same as # 3, but filter sample after centrifuging and before adding the thymol.

After all the samples were prepared, each was injected into the gas chromatograph to determine the initial substrate concentration. The samples were then injected periodically to determine if they were degrading while in storage.

RUN # 1 of the sample preservation experiment used unacclimated (fresh) Livingston sludge to which 20 ppm o-chlorophenol was added.

RUN # 2 - Same as RUN # 1, except the samples were initially 100 ppm phenol and sample sp5 wasn't prepared.

RUN # 3 - Same as RUN # 2, except that sludge acclimated to 500 ppm phenol was used and the samples were initially 100 ppm phenol. Again, sample sp5 was not prepared.

C.U.V. Degradation Experiments

Four samples with an initial o-chlorophenol concentration of 20 ppm were made-up using deionized water. Approximately 15 ml of each sample was placed into a vial

with a snap-on cap, and then each vial was placed in the following locations:

(uv1) in the far left window of laboratory (strong sun, southern-exposure)

(uv2) in the middle window of laboratory (also southern exposure)

(uv3) on lab bench next to the air stripping experiment (diffused light)

(uv4) in the lab desk drawer (dark)

The samples were periodically injected into the G.C. to determine if they were being degraded by U.V. radiation.

D._Experiments_to_Determine_the_Effect_of_pH_on_GC_Results

A series of experiments were performed to determine if the pH of the sample would have any effect on the gas chromatographic analysis of phenol and o-chlorophenol. In each experiment a standard was made-up using phenol (or o-chlorophenol), thymol and deionized water. The standard had a phenol (or o-chlorophenol) and thymol concentration approximately the same as in the degradation runs. The pH of the standard was measured and the standard was injected into the G.C. to determine the initial phenol (or o-chlorophenol) concentration. The standard was then acidified using 0.2-0.3 ml 0.1 N sulfuric acid, the pH measured and injected into the G.C. The pH of the standard was raised to approximately 9.0 using 0.1 N sodium hydroxide in 2 or 3 increments and after

each addition of sodium hydroxide the pH was measured and the standard injected into the G.C.

E._Phenol_and_O-chlorophenol_Degradation_Runs

All experiments were conducted at room temperature (approximately 25 C).

The reactor was filled with 2.0-4.0 liters of activated sludge taken from the aeration tanks of the Livingston, New Jersey wastewater treatment plant, and acclimated batchwise to phenol. The Livingston plant treats mainly domestic wastes, because there is very little industry in the Livingston area. It is assumed that the sludge is not being preadapted to phenolic compounds at the treatment plant. The treatment plant doesn't monitor the concentration of phenol, o-chlorophenol, or any other organics in the influent.

Intially the sludge was spiked to 100 ppm phenol, and the phenol concentration was then allowed to fall to zero. The reactor was then respiked several times to 100 ppm phenol before the phenol concentration was increased to 200 ppm and the whole procedure repeated. The phenol concentration was increased to 500 ppm in 3 additional 100 ppm increments.

The sludge was spiked to the desired phenol concentration using a 10,000 ppm phenol stock solution. In the case of o-chlorophenol experiments, the reactor was spiked using a 2000 or 4000 ppm stock solution. The phenol and o-chlorophenol stock solutions also contained inorganic nitrogen and phosphrous in the form of ammonium carbonate and

ammonium phosphate. The ratio of carbon:nitrogen and nitrogen:phosphrous in the stock solutions was approximately 50:14 and 14:3 [6]. For phenol this worked out to: 10.0 gm phenol, 1.805 gm ammonium phosphate and 6.64 gm ammonium carbonate per liter of tap water. For the 2000 ppm o-chlorophenol stock solution the formula was: 2.0 gm o-chlorophenol, 0.341 gm ammonium phosphate and 1.328 gm ammonium carbonate per liter of tap water.

When the sludge was being acclimated to phenol or o-chlorophenol, the concentration within the reactor was usually checked in the morning and if it was found to be zero the reactor was respiked. The reactors weren't fed over the weekend.

Once the sludge had become acclimated to 500 ppm phenol two phenol degradation runs were performed at 100 ppm (Tables # 19 & 20).

After the phenol degradation runs were performed, the sludge was acclimated to o-chlorophenol at 20 ppm, followed by three degradation runs (Tables # 21, 22 & 23).

After the 20 ppm o-chlorophenol runs were completed, the reactor was acclimated to 40 ppm o-chlorophenol, and again three degradation runs were performed (Tables # 24, 25 & 26).

Two additional runs at 40 ppm o-chlorophenol were also performed with 50 ppm of amino acids added to the reactor. The usual amounts of ammonium carbonate and ammonium phosphate were also added to the reactor. Ten ppm of each of the followins amino acids were added: L-cystein, L-glutamic

acid, L-(+)-lysine, L-arginine and L-(+)-histidine (Tables # 27 & 28).

1._Substrate_Analysis

After spiking the reactor to the desired initial concentration of phenol or o-chlorophenol, 15 ml samples of the reactor fluid were taken every 20 minutes to 1 hour, until the concentration decreased to zero. The samples were centrifuged for 4 minutes at 1500-2000 RPM and analyzed by gas chromatography and/or ultra-violet spectrophotometry. When the analysis was made using UV spectrophotometry, the concentration of phenol or o-chlorophenol was determined by comparing the peak height with calibration curves made with standard solutions. If the analysis was made by gas chromatography, the peak areas were determined using an electronic integrator. Thymol was added to the samples analyzed by gas chromatography as an internal standard, to increase the accuracy of the analytical technique. The accuracy of the G.C. analysis was approximately ± 2.0 ppm.

From the three sample preservation experiments, Table # 11 - 13, it was concluded that the addition of 1/2 ml of isopropyl alcohol to the centrifuged reactor samples, coupled with refriseration at 2 C, would preserve the sample a minimum of 100 hours with no more than 10 % deterioration.

However, during the gas chromatographic analysis of the o-chlorophenol samples, the isopropyl alcohol masked small o-chlorophenol peaks and interfered with the integrator's

peak recognition function. After this was determined, the addition of isopropyl alcohol was discontinued and the samples were centrifuged, thymol was added, and they were stored on ice until injected into the G.C. The maximum length of time the samples remained on ice prior to being injected was approximately 30 minutes, and no significant degradation occurred during this time. Assuming an Arrhenius dependence on temperature, a 20 C reduction in sample temperature should slow the degradation rate by a factor of 4. At 25 C, the maximum rate of o-chlorophenol degradation witnessed by this study was approximately 8.0 mg/l-hr. Therefore, at 5 C the rate should be approximately 2.0 mg/l-hr. If the sample remained on ice for 30 minutes prior to injecting, one could expect a maximum loss of 1.0 mg/l o-chlorophenol, this is within the accuracy of the analytical technique.

An improved sample preservation technique involving the addition of 1000 ppm copper sulfate to the samples, as per Standard Methods [42], was used for all runs performed after 1/83. Other investigators have used mercuric chloride, Hill & Robinson [49], and silver nitrate, Kim and Armstrong [10], as enzymatic inhibitors to stop the degradation of the samples during analysis and storage.

The UV experiments were negative so no special precautions were taken for the 2-8 hour degradation runs (see Table # 14).

Calculations were performed using the literature reported

acidity constant, Ka, of phenol and o-chlorophenol to determine the percentage ionized at the pH range encountered in the degradation experiments, approximately 6.5-7.5 (see Table # 15). It should be noted that at a pH of 7.0, less than 1.0% of the phenol would be ionized, while the calculations for o-chlorophenol showed approximately 7.0% ionization. The ionization experiments on phenol and o-chlorophenol showed that the pH of the degradation samples would not effect the gas chromatographic analysis (see Tables # 16, 17 and 18). Based on the ionization calculations and tests, it is safe to assume that most of the phenol and o-chlorophenol in the degradation experiments exits in the acid form and not as phenoxide salts.

2._Chemical_Oxygen_Demand_(COD)_Determination

Chemical oxygen demand (COD) represents the amount of oxygen consumed in the oxidation of organic and oxidizable inorganic matter in water and wastewater. Chemical oxygen demand analysis was performed on the phenol and o-chlorophenol degradation samples to determine if complete minerialization of the toxic compounds was occuring or if intermediates were being produced. The theoretical chemical oxygen demand of phenol, o-chlorophenol, and thymol can be determined from a balanced equation for the total oxidation of these compounds to carbon dioxide and water:

* Phenol = $C_6H_5OH + 7O_2 \rightarrow 6CO_2 + 3H_2O$

$$\text{o-chlorophenol} = C_6H_4ClOH + 6.75O_2 \rightarrow 6CO_2 + 2.5H_2O$$

$$\text{thymol} = C_{10}H_{13}OH + 13O_2 \rightarrow 10CO_2 + 7H_2O$$

From these balanced chemical equations, the theoretical COD (mg oxygen/mg compound) of phenol, o-chlorophenol and thymol were calculated to be: 2.380, 1.680, and 2.679, respectively.

The procedure used in the present study is a modification of the method presented in Standard Methods [42]. A digestion solution was made-up by adding 7.5 gm potassium dichromate, 10.0 gm silver sulfate and 5.0 gm mercuric sulfate to a 2.5 l bottle of concentrated sulfric acid. (Note: this reagent is extremely corrosive and toxic , care should be exercised during its preparation and use.) A magnetic stirring bar was added to the acid bottle, which was then placed on a magnetic hot plate and heated overnight to dissolve the potassium dichromate and silver sulfate. Once the potassium dichromate and silver sulfate dissolved, the acid bottle was removed from the hot plate and cooled to room temperature. Five ml of the cooled digestion solution was pipetted into a 16 mm X 100 mm screw-top vial, 2.0-5.0 ml of filterred sample added and the cap was screwed on tightly. Several blanks containing 2.0-5.0 ml deionized water were run with each batch of samples. The vials were placed into the Hach COD reactor and heated at 150 C for 2 hours. After heating, the vials were removed from the reactor and cooled to room temperature. The contents of the vial was

transferred to a 250 ml Erlenmeyer flask that contains approximately 50 ml water, rinsing the inside of the vial several with water, and adding the rinsings to the flask. To the flask were added 0.03 gm mercuric sulfate (to reduce chloride ion interference) and 5 drops Ferrion indicator. This solution was then titrated to a bright orange endpoint with 0.0125 N ferrous ammonium sulfate solution (FAS). The 0.0125 FAS solution was made by adding 9.8 gm ferrous ammonium sulfate to approximately 1000 ml deionized water, adding 20 ml concentrated sulfuric acid, cooling the solution to room temperature and finally, diluting to 2.0 l with deionized water. The blanks are titrated in the same manner as the samples. To determine the COD of the sample, the following equation was used:

$$(A - B) \times (N) \times (8000)/C = mg/l\ COD$$

where: A = vol of FAS used to titrate blank

B = vol of FAS used to titrate sample

N = normality of FAS solution

C = volume of sample, ml

The error in the COD analysis was estimated to be ± 10%.

3._Mixed_Liquor_Suspended_Solids_Determination

Samples were taken every 2.0 hours during the degradation experiments. A 10 ml sample of reactor fluid was pipetted into a preweished aluminium dish and dried in an oven at 95 C. The dried samples were then cooled in a

dessicator and reweighed. The MLSS of fresh sludge from the Livingston sewage treatment plant was approximately 2500-2600 mg/l. The error in the MLSS determination was estimated to be ± 50 mg/liter.

4. Hydrogen Ion Concentration (pH) Determination

The pH in the reactor was checked every 1/2 - 2.0 hours using a pH meter. When necessary, sodium carbonate or ammonium carbonate was added to the reactor to maintain the pH in the range of 7.0 - 8.0. To measure the pH, 15 ml of reactor fluid was pipetted directly into a vial. The vial was placed on a magnetic stirrer and a combination pH electrode immersed in the vial. The pH was read after approximately 5 minutes of stirring, when it reached a steady reading. After the pH was measured the fluid was returned to the reactor.

5. Ammonia Determination

During most of the runs, ammonia concentration was not checked regularly. However, for the last few runs an ammonia gas electrode was used, which indicated levels of 140-420 mg/l.

VII. RESULTS

The results of the air stripping experiments (Tables # 6, 7 & 8) show that essentially no o-chlorophenol was stripped out during the 4 to 8 hour biodegradation runs. Therefore, it can be assumed that in the degradation experiments the major mechanism is indeed bioassimilation and not air stripping.

A computer program, AIR, was written to simulate the air stripping experiment (see APPENDIX 1). The results from this computer program appear in Table # 9. From running this program it was determined that the activity coefficient of o-chlorophenol had to be approximately 300-500 to achieve the rate of air stripping witnessed in the air stripping experiments.

From vapor-liquid equilibrium data for the phenol-water system obtained from Gmehling, et al. [43], the infinite dilution activity coefficient of phenol was found to range between 43.85-67.36 for a variety of temperatures and pressures. However, no such data was available for the o-chlorophenol-water system. It was necessary to estimate the infinite dilution activity coefficient of o-chlorophenol to determine whether the loss of o-chlorophenol witnessed in the air stripping experiments was due to volatilization and not some other mechanism. Using a method presented in Reid, et al. [51], the infinite dilution activity coefficients of

phenol and o-chlorophenol were estimated to be 45 and 347, respectively, at 25 C (see Table # 10). Since the estimated activity coefficient for phenol compares very well with the literature values, and due to the similarity between the two phenolic systems, it is assumed that 347 represents a good estimate for the activity coefficient of o-chlorophenol. This checks very well with the experimental results. Since the activity coefficient of phenol is an order of magnitude lower than o-chlorophenol the rate of air stripping is also negligible for phenol.

No tests were performed to determine the amount of phenol or o-chlorophenol adsorbed on the bacterial flocs. However, since the acclimated sludge was exposed to these compounds for a long period prior to a degradation run, it is assumed that all the active surface would be saturated. Therefore, we can safely say that adsorption is not a significant removal mechanism in the degradation experiments.

All the phenol and o-chlorophenol degradation runs were performed using phenol acclimated sludge that was developed from the same initial activated sludge seed taken from the Livingston treatment plant. The degradation runs were performed with a sludge age of 800-2100 hours. Also, no sludge wasting was done at any time during the experimental program.

Detailed concentration verse time data Tables (#19-28) and graphs (Figures # 9-18) are shown for all degradation runs.

The results of the COD analysis indicate complete destruction of phenol without the formation of any metabolic intermediates (see Figure # 19). However, the COD analysis on the o-chlorophenol degradation samples suggest that metabolic intermediates are being formed (see Figure # 20). The equivalent phenol (or o-chlorophenol) concentration was calculated using COD analysis by determining the COD of the degradation sample, subtracting the theoretical COD of the thymol internal standard (approximately 123.2 mg COD/l), and dividing the result by the theoretical COD of phenol (or o-chlorophenol). For a comparison between the substrate and COD removal rates of phenol and o-chlorophenol, see Tables # 29-30 and Figures # 19-20. In both cases there appears to be a residual COD of approximately 20-30 mg/l. This may be due to microorganisms that lysed after copper sulfate was added to the degradation samples. In addition, COD analysis was performed on o-chlorophenol standards (10, 20 and 40 ppm o-chlorophenol), which determined that the COD difference between 10 and 20 ppm could be consistantly and accurately detected.

The virgin activated sludge had an MLSS of about 2500 mg/l. After prolonged exposure to phenol at concentrations of 100 to 500 ppm, the MLSS was reduced to 1100 to 1300 mg/l (see Tables # 19 & 20). After further exposure to 20 ppm o-chlorophenol, the MLSS still ranged from 1400 to 1800 mg/l (see Tables # 21-23). It was only after exposure to 40 ppm o-chlorophenol that the MLSS was drastically reduced to about

750 mg/l (See Tables # 24-26). This would indicate that an o-chlorophenol concentration of 40 ppm is highly toxic to many of the organisms present even in a phenol acclimated sludge. For any given run, the MLSS was essentially constant.

The phenol acclimated sludge had a light tan color, but turned dark brown upon exposure to o-chlorophenol. This phenomenon was also witnessed by Ingols, et al. [22], and further indicates a change in microbial population.

It should be noted that the degradation of o-chlorophenol at a concentration of 40 ppm overcame the buffering capacity of the reactor medium, causing in all 3 runs a reduction of approximately 0.3-0.4 pH units. Because of this it was necessary to add more buffer (ammonium carbonate or sodium carbonate) to the reactor during the course of the run to keep the pH between 7.0-8.0. This reduction in pH is believed to be caused by the production of HCl as a result of the degradation of o-chlorophenol and is an additional indication that complete biodegradation (mineralization) occurred. This was also witnessed by Hartmann, et al. [30].

In the first 20 ppm o-chlorophenol run, the degradation took 8.0 hours instead of 4 to 5 hours as in the second and third runs. The reason for this was insufficient acclimation to o-chlorophenol prior to the first run, as can be seen by the longer lag time in the first run.

The second 40 ppm o-chlorophenol run took 3 hours longer

than the first and third and was believed to be caused by a failure to feed the reactor over the weekend just prior to the run.

The degradation data was regressed to five different kinetic expressions [44] to determine the one that best represented the data and to determine the rate constants. The following five kinetic equations were used: (1) zero-order kinetics, (2) Grau equation, (3) Haldane equation, (4) Gates and Marlar, and (5) Henri equation.

The simplest kinetic equation is the zero-order, which states that the rate of substrate utilization, dS/dt, is a constant and is independent of substrate concentration:

$$\text{rate} = dS/dt = K \qquad (1)$$

To determine the rate constant, K, the substrate concentration is plotted against time on rectangular paper. This should produce a straight line with a slope of K.

The equation for Grau kinetics in a batch reactor is:

$$dS/dt = -KX(S/So) \qquad (2)$$

where: K = rate constant
X = biomass constant
S = substrate conc. at t
So = initial substrate concentration

If X is assumed to be approximately equal to the initial biomass concentration, Xo, during the course of the reaction,

Equation # 2 can be integrated to give:

$$\ln(S/So) = (-KXo/So)t \qquad (3)$$

Plotting (So/S) versus time on semi-log paper should result in a straight line with a slope equal to KXo/So.

Using the Haldane model for substrate inhibition kinetics, the equation for substrate utilization is:

* $$-dS/dt = UmS/(Km + S + S^2/Ki) \qquad (4)$$

where: Um = maximum growth constant

Km = substrate saturation constant

Ki = inhibition constant

S = substrate concentration

* If $S + S^2/Ki \gg Km$, Equation # 4 can be linearized to give:

$$-dS/dt = K/(Ki + S) \qquad (5)$$

where: K = UmKi

The constants, K and Ki, can be determined by: (1) plotting S versus t, (2) fitting S to a second-order polynomial in t, (3) differentiating the polynomial and determing dS/dt at each S, and (4) plotting 1/(-dS/dt) versus S. This should result in a straight line with a slope of 1/K and a y-intercept of Ki/K. It should be noted, that if Ki $\gg$ S, the Haldane equation is zero-order in substrate concentration. Equation # 5 can be rearranged and integrated to give:

$$Ki(So - S) + (So^2 - S^2)/2 = Kt \qquad (6)$$

By knowing Ki and K, one can solve this quadratic equation to determine the substrate concentration at any time. This was the method used in the present study.

If the Michaelis-Menton model is used, the rate of substrate reaction becomes:

$$dS/dt = -KoXS/Y(Km + S) \qquad (7)$$

where: Ko = maximum growth rate constant

X = biomass concentration

S = substrate concentration

Y = biomass synthesis constant

Km = substrate saturation constant

Equation # 7 can be rearanged to give:

$$dS(Km + S)/S = -(KoX/Y)dt \qquad (8)$$

Gates and Marlar developed a method a solve for the kinetic constants [44], which involved intesrating Equation # 8 and rearranging the result:

$$(1/t)ln(S/So) = c[ln(1 + ad)/t] - b \qquad (9)$$

where: a = Y/Xo

b = (Ko/YKm)(Xo + YSo)

c = 1 + (Xo + YSo)/YKm

d = So - S

To solve for the constants: Ko, Km and Y, a trial-and-error

procedure is used where values of a are assumed and 1/tln(S/So) is plotted against ln(1 + ad)/t until the best straisht line is produced. This line has a slope of c and y-intercept of b. The kinetic constants are then calculated as follows:

$$Ko = b/(c - 1)$$

$$Km = (1/a + So)/(c - 1)$$

$$Y = aXo$$

Once the kinetic constants are known, they can be used to determine the substrate or biomass concentration at any time by using the following equations [44]:

$$\ln S = \ln[So + Y(So - S)So/Xo] + (Xo + YSo)/(YKm)\ln[(Xo + Y(So - S))/Xo] - Kot(Xo + YSo)/YKm \quad (10)$$

$$\ln X = Kot + \ln Xo - (YKm/(Xo + YSo))* \ln[(X/Xo)(YSo/(YSo + Xo - X)] \quad (11)$$

Since equations # 10 and 11 are implicit in S and X, they must be solved by trial and error.

If the biomass concentration, X, is assumed to be constant during the course of the reaction, Equation # 7 can be integrated to yield the Henri equation:

$$(1/t)\ln(So/S) = -(So - S)/Kmt + KoXo/YKm \quad (12)$$

By plotting (1/t)ln(So/S) versus (So - S)/t, a straisht line

should result with a slope equal to -1/Km and a y-intercept of KoXo/YKm.

A computer program, REGRESS, was written to fit the degradation data to each of the five kinetic equations and to solve for the kinetic constants. A listing of this program can be found in Appendix 2 and a hierarchical diagram is given in Figure # 21.

After running all the data sets through REGRESS, several important trends were noted (see Tables # 31-34).

A zero-order kinetic equation successfully fitted all sets of data. The problem with a zero-order equation is the fact that it can be extrapolated to negative substrate concentration instead of asymptotically approaching zero as does a first-order equation. The Haldane equation also had this problem. In addition, it was found that the Haldane equation was very sensitive to changes in the initial substrate concentration, So. A 1.0 ppm change in the value of So, could change the value of the kinetic constants, K and Ki, by 100-1000. The Grau kinetic equation gave good fits for the 20 ppm o-chlorophenol and 40 ppm o-chlorophenol + 50 ppm amino acids data. However, it gave very large sums of the squares of the residuals for the 40 ppm o-chlorophenol data. The Henri equation produced negative values for the substrate saturation constant, Km, for most data sets. The method of Gates and Marlar also yielded negative values of Km for most sets of degradation data. Even the few data sets that produced positive Km values had calculated values of the

biomass synthesis constant, Y, that were unrealistically large (69-308 mg biomass/mg substrate). Also, the Gates and Marlar method didn't work on all degradation runs for a particular substrate concentration, even though the data appeared very similar.

The phenol degradation data was best correlated to a zero-order kinetic equation with K ranging from 31.63-61.72 mg/l-hr (see Table # 31). At a concentration of 20 ppm, the o-chlorophenol degradation data was best correlated using the zero-order equation with K ranging from 2.903-5.298 mg/l-hr for all 3 runs (see Table # 32). At an o-chlorophenol concentration of 40 ppm, the degradation data was best correlated using the Haldane equation, with K ranging from
* 454.575-687.609 $(mg/l)^2$/hr and Ki ranging from 51.235-116.32
* mg/l (see Table # 33). The 40 ppm o-chlorophenol + 50 ppm amino acids data was best correlated using the Haldane equation with K ranging from 135.66-220.234 $(mg/l)^2$/hr and Ki ranging from 15.841-22.023 mg/l (see Table # 34). This same effect, the addition of a co-substrate decreasing or having no effect on the degradation rate, has been witnessed by several investigators: Dence, et al. [19], Kirsch and Etzel [27], Haller [21], Lewandowski and Abd-El-Bary [14], and DiGeronimo, et al. [31]. But, other studies have concluded that the addition of a co-substrate increased the rate of degradation or allowed previously non-degradable compounds to be broken down: Edgehill and Finn [26], Clark, Chian, and Griffan [35], Francis, et al., [36] and Rittmann and

Kobayashi [39]. Consequently, more work must be performed in this area to determine the biological mechanisms of co-metabolism and the effects of different co-substrates.

Another possible effect of the amino acids was suggested by Carlson, et al., [45]. L-cystein can be converted to hydrogen peroxide by autooxidation with atmospheric oxygen. The hydrogen peroxide acts as a bactericide and kills some of the organisms in the reactor. As a result the o-chlorophenol concentration decreases at a slower rate due to a reduction in the microbial population.

By comparing the kinetic rate constants for phenol degradation witnessed by the present study (31.6-61.7 mg/l-hr) with other published values, it was determined that the rate was much larger than observed by Pitter [23] which reported phenol degradation at a rate of 3.36 mg/l-hr. The results of the present study compare well with the findings of Holladay, et al. [13] which reported rates of phenol degradation in the range of 0.214-2.67 gm/l-day (8.92-111 mg/l-hr) in a CSTR. The present study also witnessed faster biodegradation of phenol than did [8], [14] and [18].

After comparing the kinetic rate constants for o-chlorophenol degradation witnessed by the present study it was determined that a much faster rate of biodegradation was observed than [21], [22], and [23]. Pitter [23] determined the zero-order rate constant for o-chlorophenol to be 1.49 mg/l-hr, while the present study witnessed rates of degradation ranging from 2.90-7.21 mg/l-hr.

There may be many reasons for these differences with the literature results, not the least of which are the nature of the original microbial population, its acclimation, and subsequent treatment. It is hoped that future studies in this laboratory will shed more light on these matters.

VIII. CONCLUSIONS

1. Activated municipal sludge is capable of completely degrading phenol up to 500 ppm and o-chlorophenol up to 40 ppm, in a 4.0 liter batch reactor. For an assumed zero-order mechanism, the rate constant for phenol (initially 100 ppm) ranged from 31.63-61.72 mg/l-hr. For o-chlorophenol at a concentration of 20 ppm, the rate constant ranged from 2.903-5.298 mg/l-hr. The 40 ppm o-chlorophenol degradation data was best correlated using the Haldane equation with K ranging from 454.575-687.609 $(mg/l)^2$/hr and
* Ki ranging from 51.234-116.32 mg/l.

2. Acclimation times decreased with repeated exposure to a particular concentration of phenol or o-chlorophenol.

3. The activated sludge first had to be acclimated to phenol before it could significantly breakdown o-chlorophenol.

4. For o-chlorophenol at a concentration of 40 ppm, the addition of amino acids increased the acclimation time and decreased the degradation rate of the o-chlorophenol. The rate constants for the Haldane equation fell to: K = 135.66-220.234 $(mg/l)^2$/hr and Ki =
* 15.841-22.023 mg/l.

5. It was determined experimentally that less than 1.0 ppm o-chlorophenol would be lost due to stripping during the course of an 8 hour degradation experiment at an air rate of 500 ml/min. However, this small loss (which is below the detection limit for the GC analysis employed) is larger than predicted by theoretical calculations for the stripping rate. Stripping losses for the phenol runs were insignificant.

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Table # 1 - Phenol Degradation Studies

Ref #	Compound tested	Conc. (ppm)	Reactor type	Kinetic Model	Results
7	phenol	100	batch	-----	100% degradation
8	phenol	30-40	applied to soil	-----	reduced to 0.1 ppm in 26 days
	o-chloro phenol	200	applied to soil	-----	reduced to 40 ppm in 36 days
9	phenol	?	?	2nd order	Kb = 7.0 ± 1.3 X 10^{-12} liter/organism-hr
10	phenol	770	batch	Michaelis Menton	Ks = 236 mg/l Y = 1.2 k = 0.0724 (hr)-1
11	phenol	360	CSTR	Monod	Ks = 245 mg/l Y = 0.45 k = 0.170 (hr)-1
12	phenol	162	CSTR	1st order	k = 6.1 X 10^{-3} l/mg-hr
	DCP	31.2	CSTR	Haldane	k/Ks = .81 X 10^{-3} l/mg-hr k/Ki = .18 mg/l-hr
13	phenol	to 1400	CSTR	-----	k = 2.67 gm/l-day = 111 mg/l-hr
14	phenol	to 500	fill and draw	Grau	k = 0.04 (hr)-1
15	phenol	to 1700	CSTR	1st order	k = .002-.004 l/mg-day (COD basis) Y = .10-.29
16	phenol	560	CSTR	-----	> 99.9% degradation
18	phenol	100	batch	-----	100% degradation in 5 days
	OCP	100	batch	-----	100% degradation in 1.5 days
	2,6-DCP	100	batch	-----	100% degradation in 0.75 days

Table # 2 - Chlorinated Phenol Degradation Studies

Ref #	Compounds tested	Conc. tested (ppm)	Reactor type	Results
19	chlorophenols	50 or 10	batch	most were degraded 20%-30% in 1 week
20	chlorophenols and phenol	5 & 10	batch	chlorophenols are more resistent to biodegradation than phenol
21	o-chlorophenol	16	batch	100% degradation in 19 days using unacclimated bacteria
22	o-chlorophenol	100	batch	100% degradation in 3 days using acclimated bacteria
23	o-chlorophenol	200 COD (119 ppm)	batch	K = 25.0 mg COD/gm initial biomass-hr = 1.49 mg/l-hr
	phenol	200 COD (84 ppm)	batch	K = 80.0 mg COD/gm initial biomass-hr = 3.36mg/l-hr
* 24	PCP	20	CSTR	Ks/um = 593 ug-day/l Kd = 0.05 (day)-1
25	PCP	100	batch	biological treatment was ineffective
26	PCP	40-120	fill and draw	PCP can be degraded
27	PCP	30	batch, fill and draw	68% PCP degraded to carbon dioxide in 24 hr
28	SPCP	to 250	batch	sludge can be acclimated to degrade PCP

Table # 3 - Chlorinated Benzoate Degradation Studies

Ref #	Compounds tested	Conc. tested (ppm)	Reactor type	Kinetic Model	Results
29	o-chlorobenzoate	50	batch	Monod	Um = 1.0 (day)-1 Ks = 2.4 mg/l Y = 0.22
	m-chlorobenzoate	50	batch	Monod	Um = 0.6 (day)-1 Ks = 2.0 mg/l Y = 0.14
	p-chlorobenzoate	50	batch	Monod	Um = 1.2 (day)-1 Ks = 1.1 mg/l Y = 0.25
	2,4-D	50	batch	Monod	Um = 2.3 (day)-1 Ks = 5.4 mg/l Y = 0.14
30	m-chlorobenzoate	3131	batch	-----	100% degradation in 14 hr
	p-chloro benzoate	3131	batch	-----	100% degradation in 11 hr
	3,5-dichloro benzoate	3820	batch	-----	100% degradation in 29 hr
31	o-chlorobenzoate	100	batch	-----	100% degradation in 6 days
	m-chloro benzoate	100	batch	-----	100% degradation in 12 days
	p-chloro benzoate	100	batch	-----	100% degradation in 9 days

Table # 4 - Polycyclic Degradation Studies

Ref #	Compounds tested	Conc. tested (ppm)	Reactor type	Results
32	DPM	100 ppb	batch	> 95% degradation in 5 days
33	Aroclor 1242	?	batch	26.4% degradation
	Aroclor 1254	?	batch	16.0% degradation
34	o-phenylphenol	?	batch	65% degradation to carbon dioxide
35	Aroclor 1242	0.703	batch	microorganisms can be isolated from soil or sediment that can degrade PCBs
36	DDT analogs	100	batch	bis(p-chlorophenyl)methane and 1,1bis(p-chlorophenyl)ethane were degraded in the presence of DPE

Table # 5 - Physical Properties of Phenolic Compounds

Compound	MW	MP (C)	BP (C)	Solubility in H2O at 25 C (ppm)	Ka X 10 10
phenol	94.11 a	41 b	182 c	93000 c	1.1 c
o-chloro phenol	128.56 a	9 b	173 c	28000 c	77.0 c
m-chloro phenol	"	33 b	214 c	26000	16.0 c
p-chloro phenol	"	43 b	220 c	27000 c	6.3 c
2,4-di chloro phenol	163.00 a	45 a	210 c	4500 c	large c
2,6-di chloro phenol	"	68-9 a	219-20 a		
2,4,6-tri cloro phenol	197.45 a	69.5 a	246 c	900 c	very large c
penta chloro phenol	266.34 a	174 a	309 c	30 c $	very large c
thymol	150.22 a	52 a	233 a		

$ = at 50 C.

a = [50] CRC Handbook, 61st Edition

b = [48] Morrison and Boyd

c = [25] B.K. Wallin, et al.

Table # 6 - Results of First Air Stripping Experiment

Date	Time	# Hr. from Start	o-chlorophenol conc. (ppm)
6/23	1:00 p.m.	0.0	17.266
6/23	3:00 p.m.	2.0	18.732
6/24	10:00 a.m.	21.0	17.573
6/24	5:30 p.m.	28.5	18.880
6/25	1:30 p.m.	48.5	14.805
6/25	4:30 p.m.	51.5	14.125
6/28	11:00 a.m.	118.0	13.132
6/28	3:30 p.m.	122.5	14.319
6/29	2:00 p.m.	145.0	12.508
6/29	6:00 p.m.	149.0	11.044
6/30	2:00 p.m.	169.0	11.841
6/30	5:00 p.m.	172.0	11.265
7/1	4:00 p.m.	195.0	5.206
7/1	8:00 p.m.	199.0	3.972
7/6	9:00 a.m.	308.0	0.0

Table # 7 - Results of Second Air Stripping Experiment

Date	Time	# Hr. from Start	o-chlorophenol conc. (ppm)
7/12	2:00 p.m.	0.0	22.624
7/12	2:45 p.m.	0.75	22.605
7/13	2:00 p.m.	24.0	19.882
7/14	3:00 p.m.	49.0	17.624
7/15	2:30 p.m.	72.5	20.891
7/16	3:30 p.m.	97.5	17.575
7/19	7:30 a.m.	161.5	0.0
7/21	10:30 a.m.	212.5	0.0

Table # 8 - Results of Third Air Stripping Experiment

Date	Time	# Hr. from Start	o-chlorophenol conc. (ppm)
7/22	5:30 p.m.	0.0	18.005
7/23	2:00 p.m.	20.5	19.594
7/26	11:00 a.m.	89.5	12.884
7/27	2:00 p.m.	116.5	11.226
7/28	8:00 a.m.	134.5	9.148
7/29	12:30 p.m.	163.0	8.896
8/2	8:00 a.m.	254.5	1.746

Table # 9 - Results of Air Stripping Simulation Program

Temperature = 26.0 C

Pressure = 760.0 mm Hg

Reactor Volume = 2.0 liters

Intial o-chlorophenol concentration = 20 ppm

Air flowrate = 500 ml/min = 1.339 mole/hr

Air leaving the reactor is assumed 100% saturated

	o-chlorophenol concentration (ppm)					
time (hrs)	$ = 1	$ = 10	$ = 100	$ = 300	$ = 500	$ = 750
0.0	20.0	20.0	20.0	20.0	20.0	20.0
20.0	20.152	20.066	19.593	17.470	15.869	14.064
50.0	20.384	20.165	18.993	14.233	11.177	8.249
100.0	20.783	20.336	18.018	10.057	6.171	3.340
200.0	21.633	20.692	16.160	4.911	1.810	0.516
300.0	22.557	21.070	14.423	2.323	0.503	0.073

Note: $ = activity coefficient

Table # 10 - Estimated Infinite Dilution Acitivity Coefficients for Phenol and O-chlorophenol

	Infinite Dilution Activity Coefficients	
temperature C	phenol	o-chlorophenol
0.0	29.2	211.0
25.0	44.5	347.4
50.0	63.6	529.6

Table # 11 - Results of First Preservation Experiment

Date	Time	# Hr. from Start	Sample #	o-chlorophenol conc. (ppm)
6/29	5:00 p.m.	0.0	sp1	31.270
			sp2	28.407
			sp3	28.169
			sp4	22.995
			sp5	*
			sp6	**
6/30	4:30 p.m.	23.5	sp1	30.516
			sp6	13.907
7/1	8:30 a.m.	51.5	sp1	29.507
7/6	11:00 a.m.	162.0	sp1	31.328
			sp2	26.975
			sp4	21.628
			sp6	22.833

* - concentrated H2SO4 destroys column packing

** - didn't run sample

Table # 12 - Results of Second Preservation Experiment

Date	Time	# Hr. from Start	Sample #	Phenol conc. (ppm)
7/16	6:00 p.m.	0.0	sp1	102.710
			sp2	99.508
			sp3	45.865 *
			sp4	91.768
			sp6	96.085
7/19	9:30 a.m.	63.5	sp1	97.656
			sp2	85.316
			sp3	46.664
			sp4	86.407
			sp6	88.012
7/21	9:30 a.m.	111.5	sp1	0.0
			sp2	84.014
			sp3	44.648
			sp4	87.972
			sp6	86.660
7/22	3:00 p.m.	141.0	sp2	77.931
			sp3	40.731
			sp4	91.062
			sp6	91.832

Table # 12 - Continued

Date	Time	# Hr. from Start	Sample #	Phenol conc. (ppm)
7/26	10:00 a.m.	232.0	sp2	12.810
			sp3	25.016
			sp4	100.090
			sp6	56.210
7/27	4:00 p.m.	262.0	sp2	0.0
			sp3	14.715
			sp4	92.460
			sp6	38.290
7/28	10:30 a.m.	280.5	sp2	0.0
			sp3	4.837
			sp4	82.290
			sp6	23.426
8/2	9:30 a.m.	399.5	sp3	0.0
			sp4	86.238
			sp6	0.0

* - added 1.0 ml thymol

Table # 13 - Results of Third Preservation Experiment

Date	Time	# Hr. from Start	Sample #	Phenol conc. (ppm)
8/6	9:00 a.m.	0.0	sp1	108.933
			sp2	108.057
			sp3	106.607
			sp4	115.400
			sp6	109.037
8/11	10:00 a.m.	119.0	sp1	66.389
			sp2	53.770
			sp3	55.629
			sp4	114.737
			sp6	107.413
8/12	3:00 p.m.	148.0	sp1	0.0
			sp2	22.591
			sp3	32.002
			sp4	102.273
			sp6	98.494
8/13	4:00 p.m.	173.0	sp2	0.0
			sp3	0.0
			sp6	105.440
8/17	4:00 p.m.	269.0	sp2	0.0
			sp3	0.0
			sp4	152.720
			sp6	131.345

Table # 14 - Results of U.V. Degradation Experiment

Date	Time	# Hr. from Start	Sample #	o-chlorophenol conc. (ppm)
8/4	9:00 a.m.	0.0	uv1	24.757
			uv2	23.892
			uv3	22.501
			uv4	24.146
8/5	6:00 p.m.	36.0	uv1	27.468
			uv2	26.667
			uv3	22.734
			uv4	21.904
8/11	12:00 noon	174.0	uv1	21.036
			uv2	26.776
			uv3	15.392
			uv4	13.306
8/13	2:00 p.m.	224.0	uv1	27.031
			uv2	26.669
			uv3	13.252
			uv4	12.115
8/17	3:00 p.m.	321.0	uv1	24.812
			uv2	24.332
			uv3	9.681
			uv4	8.546

Table # 15 - Ionization Calculations Using Ka

		C - amount of compound not ionized (ppm)		
compound	Co	pH = 6	pH = 7	pH = 8
phenol	100 ppm	99.99	99.89	98.91
o-chloro phenol	40 ppm	39.6	37.0	22.6

$$C = Co - Co[(Ka/H+)/(1 + Ka/H+)]$$

where: Co = initial concentration
Ka = acidity constant
H+ = log(-pH)

Table # 16 - Results of Phenol Ionization Test

Phenol Standard: 50.391 ppm, pH = 7.3

pH	Phenol Concentration (ppm)
3.5	50.196
7.5	50.003
8.7	49.362
9.4	49.634

Note: Phenol standard contains thymol at a concentration of 50 ppm as an internal standard.

Table # 17 - Results of O-chlorophenol Ionization Test

O-chlorophenol Standard: 47.626 ppm, pH = 7.2

pH	O-chlorophenol Concentration (ppm)
3.5	46.220
7.3	47.745
8.6	48.695
9.4	45.315

Note: O-chlorophenol standard contains thymol at a concentration of 51.400 ppm as an internal standard.

Table # 18 - Results of O-chlorophenol Ionization Test

O-chlorophenol Standard: 40.130 ppm, pH = 7.2

pH	O-chlorophenol Concentration (ppm)
3.9	40.166
8.7	37.201
9.3	39.060

Note: o-chlorophenol standard does not contain any thymol.

Table # 19 - Results of First 100 ppm Phenol Run

Time sample was taken	# Hr. from Start	phenol conc. (ppm)	pH	MLSS (ms/l)
8:30 a.m.	0.0	90.749		
9:30 a.m.	1.0	51.673		
10:30 a.m.	2.0	5.320	7.3	1080
11:30 a.m.	3.0	0.0		
1:00 p.m.	5.0	0.0	7.5	
2:30 p.m.	6.0	0.0		

Note: No isopropyl alcohol was added to the samples. The samples were stored on ice as soon as they were taken and remained until they were injected into the G.C.

Table # 20 - Results of Second 100 ppm Phenol Run

Time sample was taken	# Hr. from Start	Phenol conc. (ppm)	pH	MLSS (mg/l)
8:20 a.m.	0.0	133.687	6.9	
8:40 a.m.	0.333	121.007		
9:00 a.m.	0.667	103.234	6.9	
9:20 a.m.	1.0	91.888		
9:40 a.m.	1.333	43.417	6.9	1100
10:00 a.m.	1.667	31.503		
10:20 a.m.	2.0	15.270	6.8	1090
10:40 a.m.	2.333	0.0		
11:00 a.m.	2.667	0.0	6.8	1220
11:40 a.m.	3.333		6.9	1280

Note: No isopropyl alcohol was added to the samples. The samples were stored on ice as soon as they were taken and remained until they were injected into the G.C.

Table # 21 - Results of First 20 ppm O-chlorophenol Run

Time sample was taken	# Hr. from Start	o-chlorophenol conc. (ppm)	pH	MLSS (mg/l)
7:50 a.m.	---	-----	6.0 *	-----
8:00 a.m.	0.0	19.584	7.0	1780
9:00 a.m.	1.0	17.922		
10:00 a.m.	2.0	18.192		
11:00 a.m.	3.0	13.758	7.0	1780
12:00 noon	4.0	11.853		
1:00 p.m.	5.0	7.523		
2:00 p.m.	6.0	6.042		1860
3:00 p.m.	7.0	2.319		
4:00 p.m.	8.0	0.0		
5:00 p.m.	9.0	0.0	7.0	1840

Note: Isopropyl alcohol was added to all samples.

* - added NH4CO3 to raise pH

Table # 22 - Results of Second 20 ppm O-chlorophenol Run

Time sample was taken	# Hr. from Start	o-chlorophenol conc. (ppm)	pH	MLSS (mg/l)
11:20 a.m.	---	-----	6.0 *	-----
11:30 a.m.	0.0	19.121	7.0	1540
12:30 p.m.	1.0	15.922	7.2	
1:30 p.m.	2.0	10.561	7.4	
2:30 p.m.	3.0	4.582		1650
3:30 p.m.	4.0	0.915	7.5	
4:30 p.m.	5.0	0.0		

Notes: No isopropyl alcohol was added to the 3:30 and 4:30 samples, because it was found that the addition of isopropyl alcohol masks small peaks. All other samples contain isopropanol.

* - added NH4CO3 to raise pH

Table # 23 - Results of Third 20 ppm O-chlorophenol Run

Time sample was taken	# Hr. from Start	o-chlorophenol con. (ppm)	pH	MLSS (mg/l)
10:00 a.m.		19.810	6.9	
11:00 a.m.	1.0	17.409	6.8 *	1360
12:00 noon	2.0	9.754	6.9	
1:00 p.m.	3.0	4.049	6.8 *	1430
2:00 p.m.	4.0	0.0	7.0	
3:00 p.m.	5.0	0.0	7.0	

Note: No isopropyl alcohol was added to the samples. The samples were stored on ice as soon as they were taken and remained until they were injected into the G.C.

* - add NH4CO3 to reactor to increase pH

Table # 24 - Results of First 40 ppm O-chlorophenol Run

Time sample was taken	# Hr. from Start	o-chlorophenol conc. (ppm)	pH	MLSS (mg/l.)
8:30 a.m.	0.0	36.927	7.8	690
9:00 a.m.	0.5	37.568		
9:30 a.m.	1.0	38.144	7.7	
10:00 a.m.	1.5	36.131		
10:30 a.m.	2.0	33.300		
11:00 a.m.	2.5	30.978	7.6	720
11:30 a.m.	3.0	28.965		
12:30 p.m.	4.0	23.106	7.5	
1:00 p.m.	4.5	20.916		
1:30 p.m.	5.0	14.635	7.4	730
2:00 p.m.	5.5	11.547		
2:30 p.m.	6.0	7.678		
3:00 p.m.	6.5	2.461	7.3	740
3:30 p.m.	7.0	0.385	7.4	
4:00 p.m.	7.5	0.0		820

Note: No isopropyl alcohol was added to the samples. The samples were stored on ice as soon as they were taken and remained until they were injected into the G.C.

Table # 25 - Results of Second 40 ppm O-chlorophenol Run

Time sample was taken	# Hr. from Start	o-chlorophenol conc. (ppm)	pH	MLSS (mg/l)
9:00 a.m.	0.0	39.764	7.33	730
9:30 a.m.	0.5	43.189	7.34	
10:00 a.m.	1.0	37.345	7.37	
10:30 a.m.	1.5	37.795	7.39	
11:00 a.m.	2.0	37.840	7.34	780
11:30 a.m.	2.5	36.375	7.30	
12:30 p.m.	3.5	35.582	7.30	
1:00 p.m.	4.0	34.108	7.31	730
1:30 p.m.	4.5	32.016	7.32	
2:00 p.m.	5.0	28.447	7.19	
2:30 p.m.	5.5	27.311	7.14	
3:00 p.m.	6.0	24.139	7.11	740
3:30 p.m.	6.5	21.793	7.09	
4:00 p.m.	7.0	19.779	7.05	
4:30 p.m.	7.5	17.066	7.03	
5:00 p.m.	8.0	14.474	6.98 *	830
5:30 p.m.	8.5	11.968	7.21	
6:00 p.m.	9.0	10.338	7.19	
6:30 p.m.	9.5	7.365		
7:30 p.m.	10.5	0.750	7.34	780
9:00 p.m.	12.0	0.0		

Note: No isopropyl alcohol was added to the samples. The samples were stored on ice as soon as they were taken and remained until they were injected into the G.C.

* - added NH4CO3 to raise pH

Table # 26 - Results of Third 40 ppm O-chlorophenol Run

Time sample was taken	# Hr. from Start	o-chlorophenol conc. (ppm)	pH	MLSS (mg/l)
12:00 noon	0.0	39.665	7.34	790
12:30 p.m.	0.5	36.011	7.34	
1:00 p.m.	1.0	37.724	7.33	
1:30 p.m.	1.5	37.165	7.26	
2:00 p.m.	2.0	39.422	7.22	730
2:30 p.m.	2.5	34.920	7.19	
3:00 p.m.	3.0	29.626	7.12	
3:30 p.m.	3.5	27.539	7.10	
4:00 p.m.	4.0	23.711	7.08	
4:30 p.m.	4.5	19.209	7.04	720
5:00 p.m.	5.0	13.895	6.96	
5:30 p.m.	5.5	11.804	6.97	
6:00 p.m.	6.0	8.317	6.95 *	830
6:30 p.m.	6.5	4.337	7.12	
7:00 p.m.	7.0	1.146	7.18	
7:30 p.m.	7.5	0.0	7.24	840

Note: No isopropyl alcohol was added to the samples. The samples were stored on ice as soon as they were taken and remained until they were injected into the G.C.

* - added buffer to raise pH

Table # 27 - First Run Amino Acids (40 ppm o-chlorophenol)

Time sample was taken	# Hr. from Start	o-chlorophenol conc. (ppm)	pH	MLSS (mg/l)
11:30 a.m.	0.0	35.605	7.0 *	690
12:00 noon	0.5	37.089	7.20	
12:30 p.m.	1.0	37.712	7.30	
1:00 p.m.	1.5	37.311	7.32	
1:30 p.m.	2.0	34.468	7.35	580
2:00 p.m.	2.5	30.421	7.33	
2:30 p.m.	3.0	30.695	7.33	
3:00 p.m.	3.5	28.780	7.34	620
3:30 p.m.	4.0	26.168	7.31	
4:00 p.m.	4.5	22.960	7.32	

10 ppm L-cystein

10 ppm L-glutamic acid

10 ppm L-(+)-lysine

10 ppm L-arginine

10 ppm L-(+)-histidine

Note: No isopropyl alcohol was added to the samples. The samples were stored on ice as soon as they were taken and remained until they were injected into the G.C.

* - added buffer to raise pH

Table # 28 - Second Run Amino Acids (40 ppm o-chlorophenol)

Time sample was taken	# Hr. from Start	o-chlorophenol conc. (ppm)	pH	MLSS (ms/l)
9:30 a.m.	0.0	36.866	7.88 *	610
10:00 a.m.	0.5	44.581	7.95	
10:30 a.m.	1.0	40.696	8.03	
11:00 a.m.	1.5	39.139	8.11	
11:30 a.m.	2.0	38.629	8.14	
12:00 noon	2.5	38.121	8.14	580
12:30 p.m.	3.0	no samples		
1:00 p.m.	3.5	34.884	8.20	
1:30 p.m.	4.0	34.165	8.14	
2:00 p.m.	4.5	32.477	8.22	
2:30 p.m.	5.0	28.462	8.25	600
3:00 p.m.	5.5	27.708	8.20	
3:30 p.m.	6.0	27.268	8.25	
4:00 p.m.	6.5	26.126	8.14	
4:30 p.m.	7.0	23.791	8.20	670
5:00 p.m.	7.5	22.694	8.21	

10 ppm L-cystein
10 ppm L-glutamic acid
10 ppm L-(+)-lysine
10 ppm L-arginine
10 ppm L-(+)-histidine

Note: No isopropyl alcohol was added to the samples. The samples were stored on ice as soon as they were taken and remained until they were injected into the G.C.

* - added buffer to raise pH

Table # 29 - Comparison of Phenol and COD Removal Rates

Time sample was taken	# Hr. from Start	Phenol conc. by GC (ppm)	Phenol conc. by COD (ppm)
2:15 p.m.	0.0	120.063	135.2
2:30 p.m.	0.25	95.808	97.4
2:45 p.m.	0.50	75.174	91.1
3:00 p.m.	0.75	51.831	80.6
3:15 p.m.	1.0	27.591	47.0
3:30 p.m.	1.25	3.191	31.2
3:45 p.m.	1.50	0.837	35.4
4:00 p.m.	1.75	0.0	38.6
4:15 p.m.	2.0	0.0	28.1
4:30 p.m.	2.25	0.0	28.1

Table # 30 - Comparison of O-chlorophenol and COD Removal Rates

Time sample was taken	# Hr. from Start	O-chlorophenol conc. by GC (ppm)	O-chlorophenol conc. by COD (ppm)
11:15 a.m.	0.0	22.298	46.3
11:30 a.m.	0.25	16.110	51.4
11:45 a.m.	0.50	12.216	46.3
12:00 noon	0.75	8.017	51.7
12:15 p.m.	1.0	4.914	53.8
12:30 p.m.	1.25	1.984	39.4
12:45 p.m.	1.50	1.348	42.3
1:00 p.m.	1.75	0.439	40.8
1:15 p.m.	2.0	0.018	45.5

Table # 29 - Results of Phenol Degradation Data Regression

Comp.	Run #	Kinetic Eq.	Lag Time	Corr.	DY**2/NP	Kinetic Constants
Phenol 100 ppm	1	zero order	0.0	-0.963	131.8	K = 31.63 mg/l-hr
Phenol 100 ppm	2	zero order	0.0	-0.986	96.21	K = 61.72 mg/l-hr
"	"	Haldane	0.0	0.981	93.14	K = 254120.5 mg/l Ki = 4050.27 mg/l

The phenol degradation was best correlated to a zero-order kinetic equation with K ranging from 31.63-61.72 mg/l-hr.

Table # 30 - Results of 20 ppm O-chlorophenol Degradation Data Regression

Comp.	Run #	Kinetic Eq.	Lag Time	Corr.	DY**2/NP	Kinetic Constants
O-chloro phenol 20 ppm	1	zero order	1.0	-0.996	0.623	K = 2.903 mg/l-hr
"	"	Grau	1.75	0.956	3.231	K = 0.00416 (hr)-1
"	"	Haldane	0.5	0.976	0.722	K = 126.11 mg/l Ki = 37.33 mg/l
"	"	Gates & Marlar	1.5	-0.609	1.038	Km = 397.80 mg/l Ko = 6.735 (hr)-1 Y = 177.99 mg/mg
O-chloro phenol	2	zero order	0.0	-0.984	1.722	K = 4.188 mg/l-hr
"	"	Grau	0.75	0.961	4.612	K = 0.0117 (hr)-1
"	"	Henri	1.0	-0.966	0.030	Km = 5.970 mg/l KoXo/Y = 11.919
"	"	Gates & Marlar	0.25	-0.972	0.060	Km = 18.948 mg/l Ko = 1.3286 (hr)-1 Y = 307.99 mg/mg
O-chloro phenol 20 ppm	3	zero order	0.0	-0.990	2.122	K = 5.298 mg/l-hr
"	"	Grau	0.75	0.993	1.499	K = 0.0106 (hr)-1
"	"	Henri	0.75	-0.929	0.525	Km = 14.397 mg/l KoXo/Y = 16.804
"	"	Haldane	0.0	0.988	1.842	K = 786.45 mg/l Ki = 138.63 mg/l
"	"	Gates & Marlar	0.5	-0.538	0.606	Km = 21.89 mg/l Ko = 1.070 (hr)-1 Y = 135.99 mg/mg

At a concentration of 20 ppm, the o-chlorophenol degradation data was best correlated using the zero-order equation with K ranging from 2.903-5.298 mg/l-hr for all 3 runs.

Table # 31 - Results of 40 ppm O-chlorophenol Degradation Data Regression

Comp.	Run #	Kinetic Eq.	Lag Time	Corr.	DY**2/NP	Kinetic Constants
O-chloro phenol 40 ppm	1	zero order	1.5	-0.994	1.995	K = 6.665 mg/l-hr
"	"	Grau	1.5	0.845	100.3	K = 0.0384 (hr)-1
"	"	Haldane	1.25	0.984	1.948	K = 687.61 mg/l Ki = 88.67 mg/l
"	"	Gates & Marlar	1.00	-0.691	2.957	Km = 21.914 mg/l Ko = 0.5780 (hr)-1 Y = 68.99 mg/mg
O-chloro phenol 40 ppm	2	zero order	2.0	-0.992	4.308	K = 4.379 mg/l-hr
"	"	Grau	2.0	0.827	112.56	K = 0.0188 (hr)-1
"	"	Haldane	1.75	0.981	3.278	K = 579.51 mg/l Ki = 116.32 mg/l
O-chloro phenol 40 ppm	3	zero order	1.50	-0.996	3.648	K = 7.218 mg/l-hr
"	"	Grau	2.0	0.914	58.54	K = 0.0320 (hr)-1
"	"	Henri	2.0	-0.453	2.329	Km = 11.990 mg/l KoXo/Y = 12.710
"	"	Haldane	0.75	0.956	7.319	K = 454.575 mg/l Ki = 51.235 mg/l

At an o-chlorophenol concentration of 40 ppm, the degradation data was best correlated using the Haldane equation, with K ranging from 454.575-687.609 and Ki ranging from 51.235-116.32 mg/l.

Table # 32 - Results of Amino Acids + O-chlorophenol Degradation Data Regression

Comp.	Run #	Kinetic Eq.	Lag Time	Corr.	DY**2/NP	Kinetic Constants
Amino Acids + O-chloro phenol	1	zero order	1.0	-0.983	1.247	K = 4.185 mg/l-hr
"	"	Grau	1.0	0.973	1.654	K = 0.0072 (hr)-1
"	"	Haldane	1.0	0.977	0.919	K = 220.234 mg/l Ki = 22.023 mg/l
Amino acids + O-chloro phenol	2	zero order	1.5	-0.992	0.528	K = 3.044 mg/l-hr
"	"	Grau	2.0	0.984	0.515	K = 0.00698 (hr)-1
"	"	Haldane	1.0	0.978	0.665	K = 135.66 mg/l Ki = 15.841 mg/l

The 40 ppm o-chlorophenol + 50 ppm amino acids data was best correlatd using the Haldane equation with K ranging from 135.66-220.234 and Ki ranging from 15.841-22.023 mg/l.

FIGURE #1 - Diagram of Reactor System

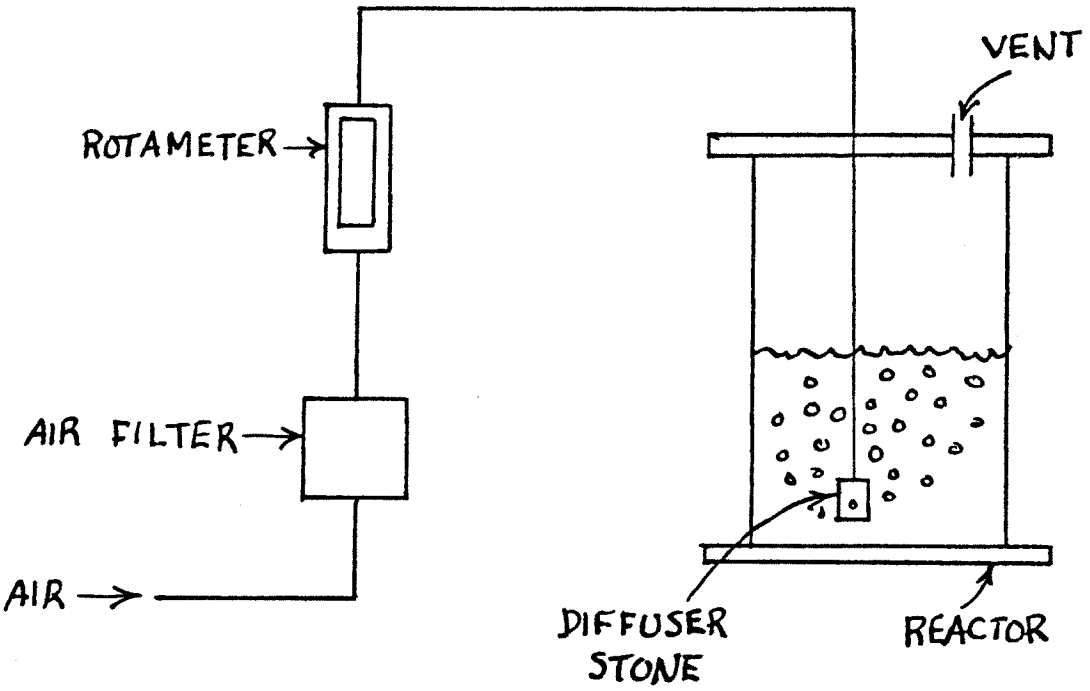


Figure #2 - Results of First Air Stripping Experiment

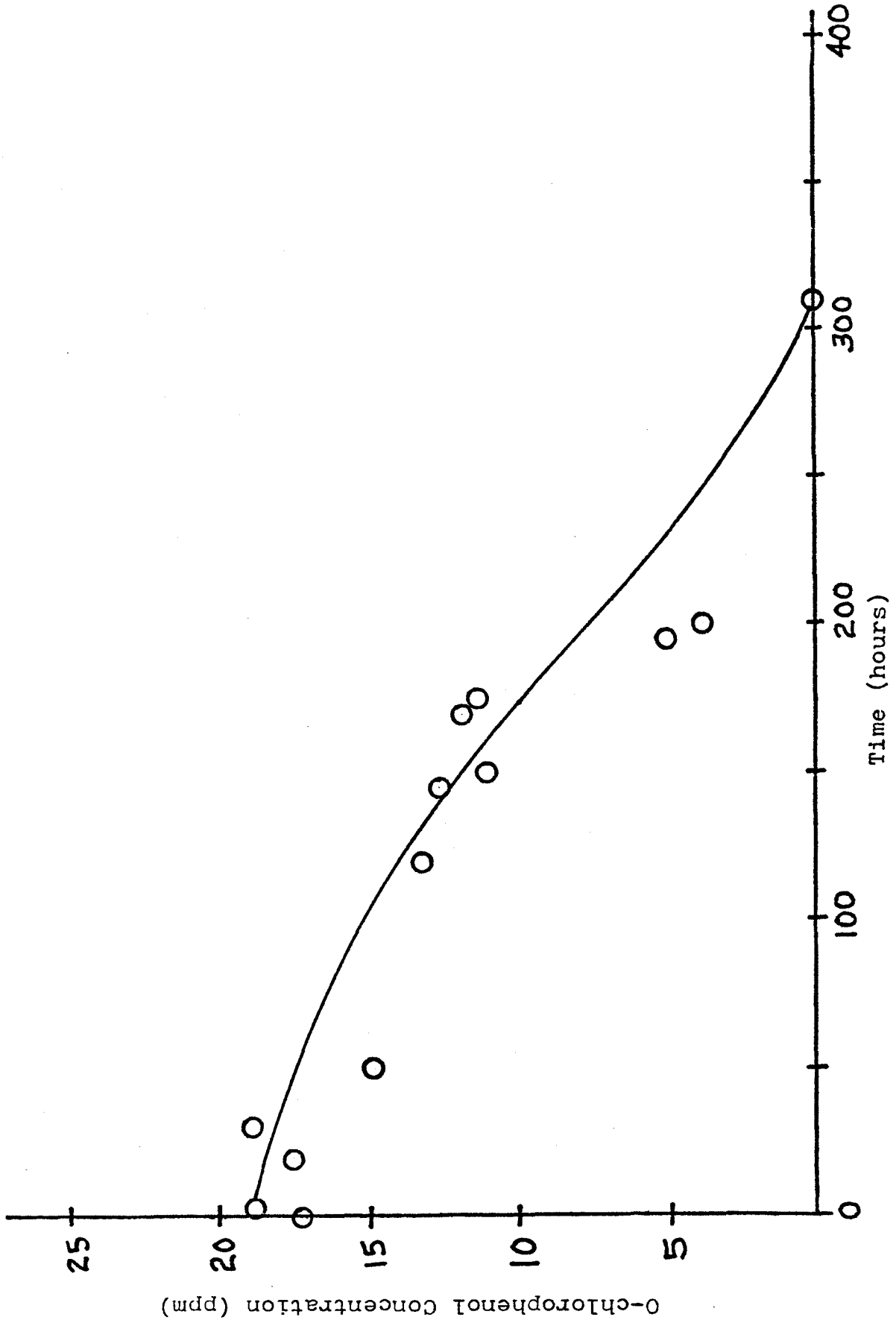


Figure # 3 - Results of Second Air Stripping Experiment

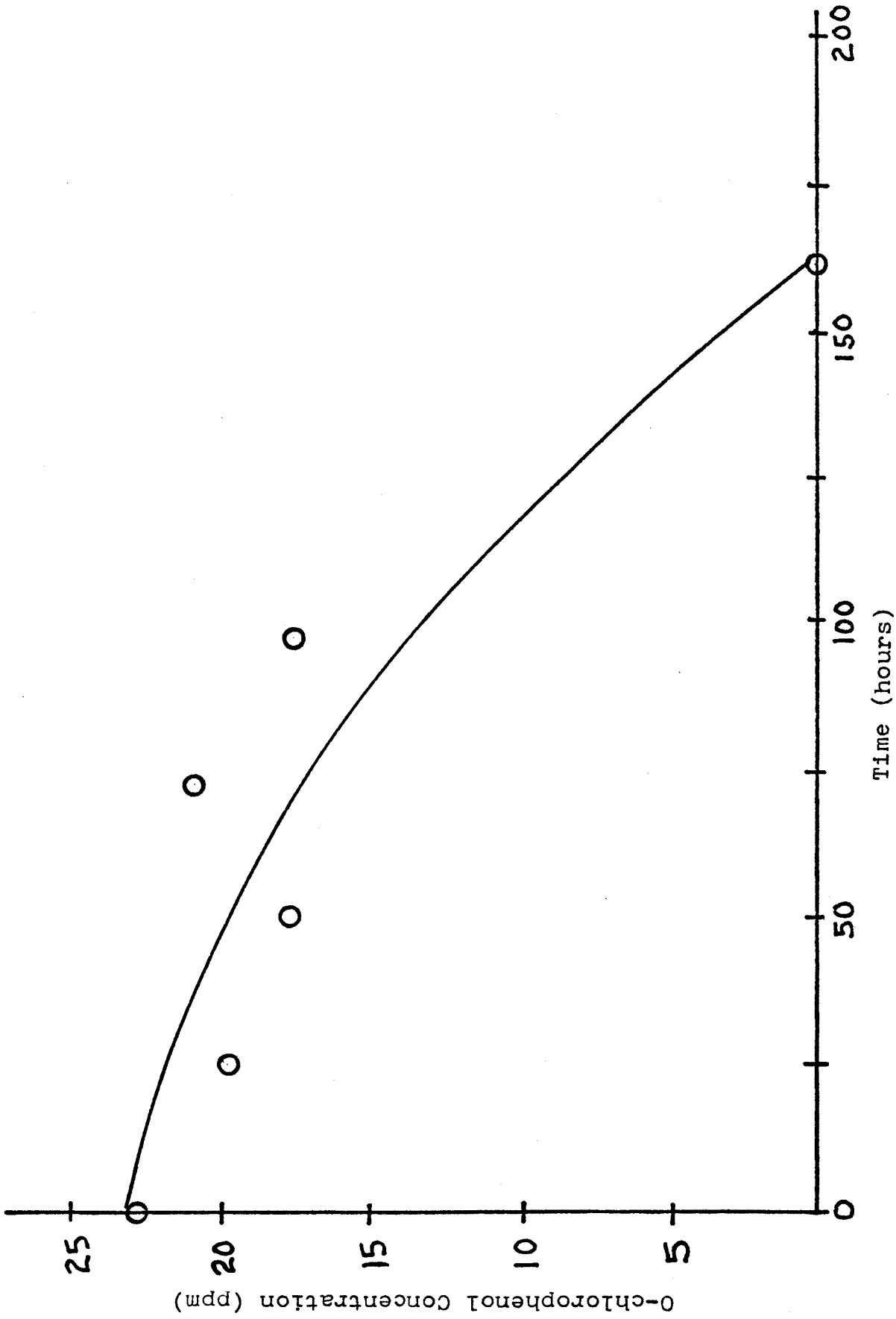


Figure # 4 - Results of Third Air Stripping Experiment

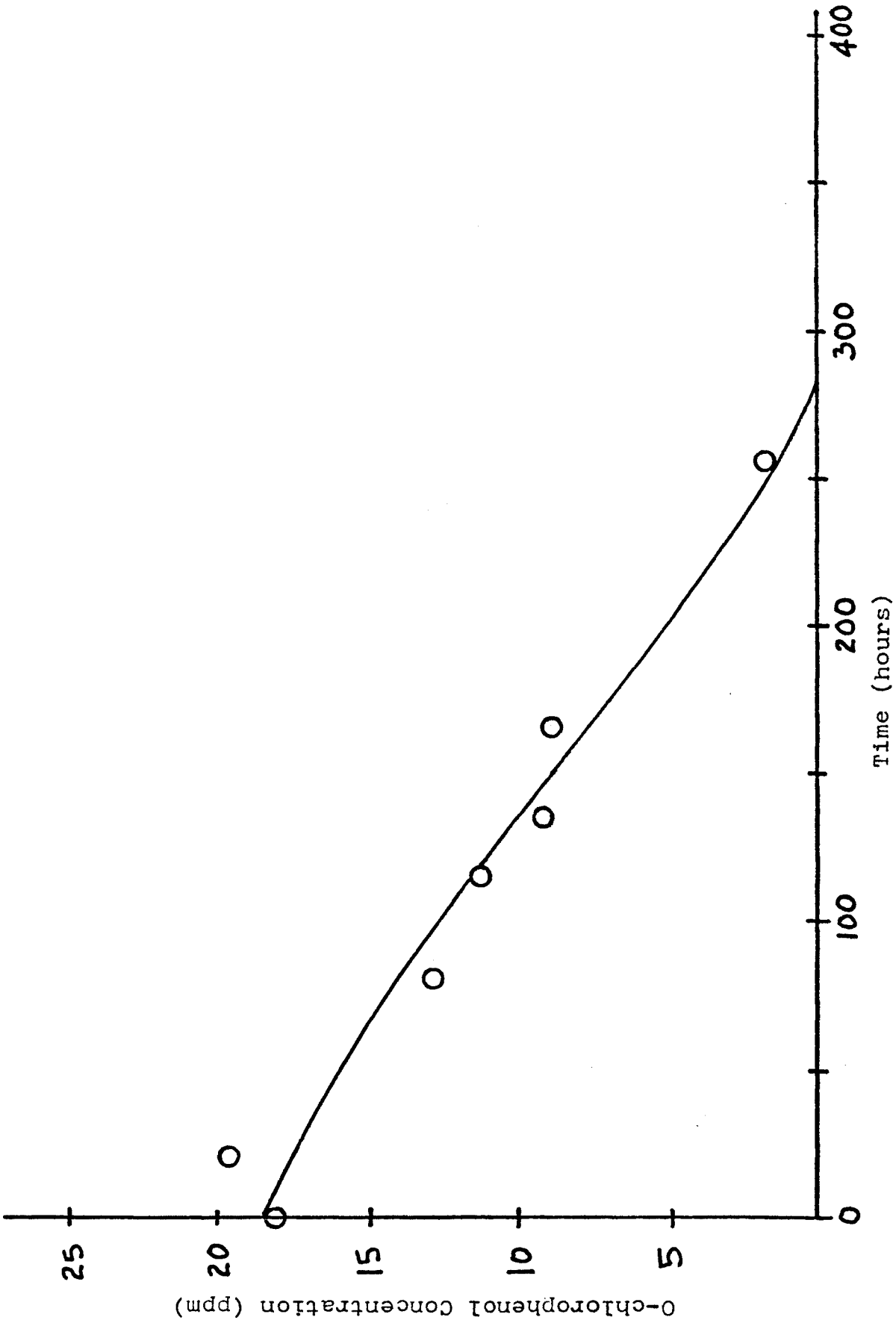


Figure # 5- Results of First Perservation Experiment

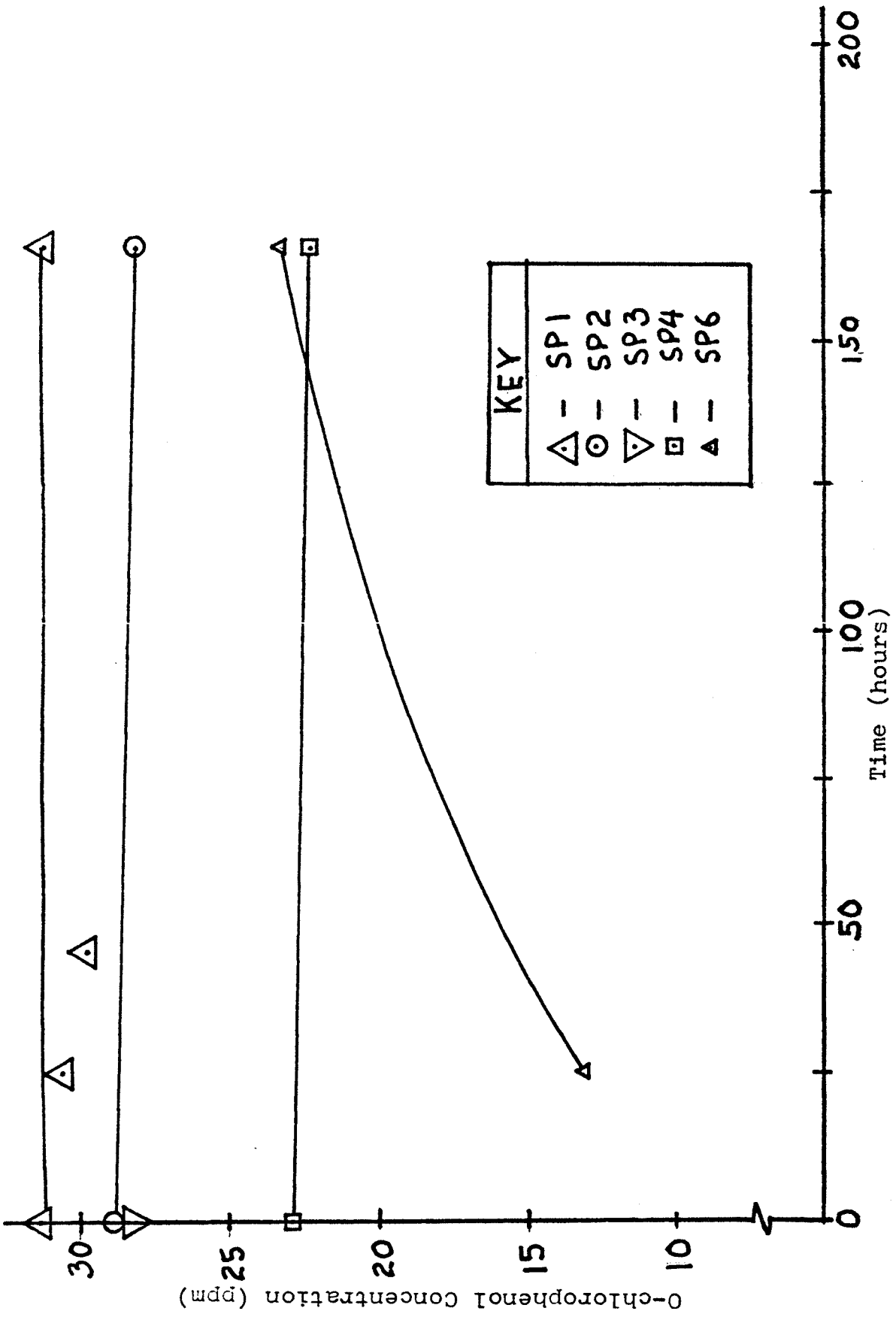


Figure # 6 - Results of Second Perservation Experiment

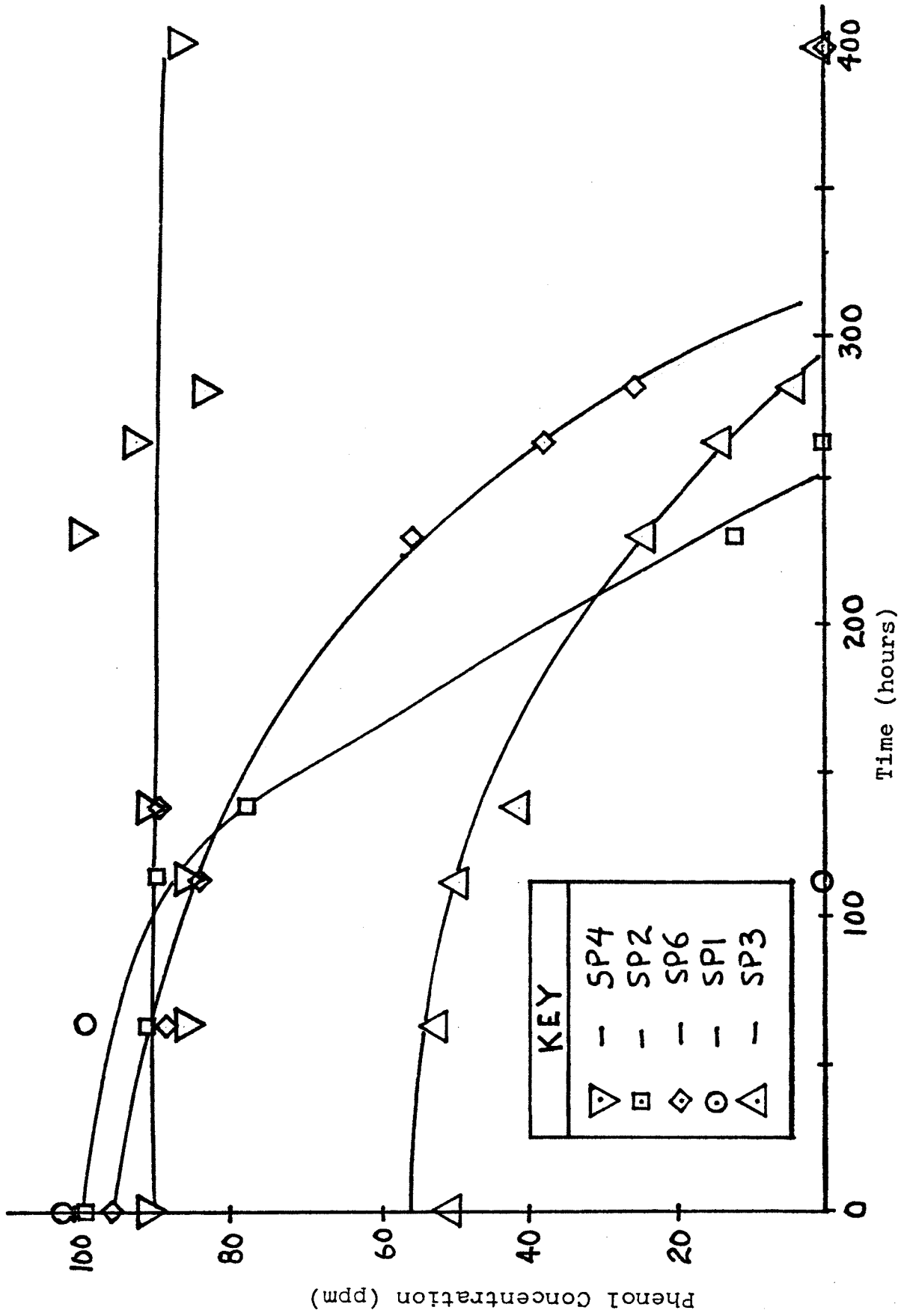


Figure #7 - Results of Third Perservation Experiment.

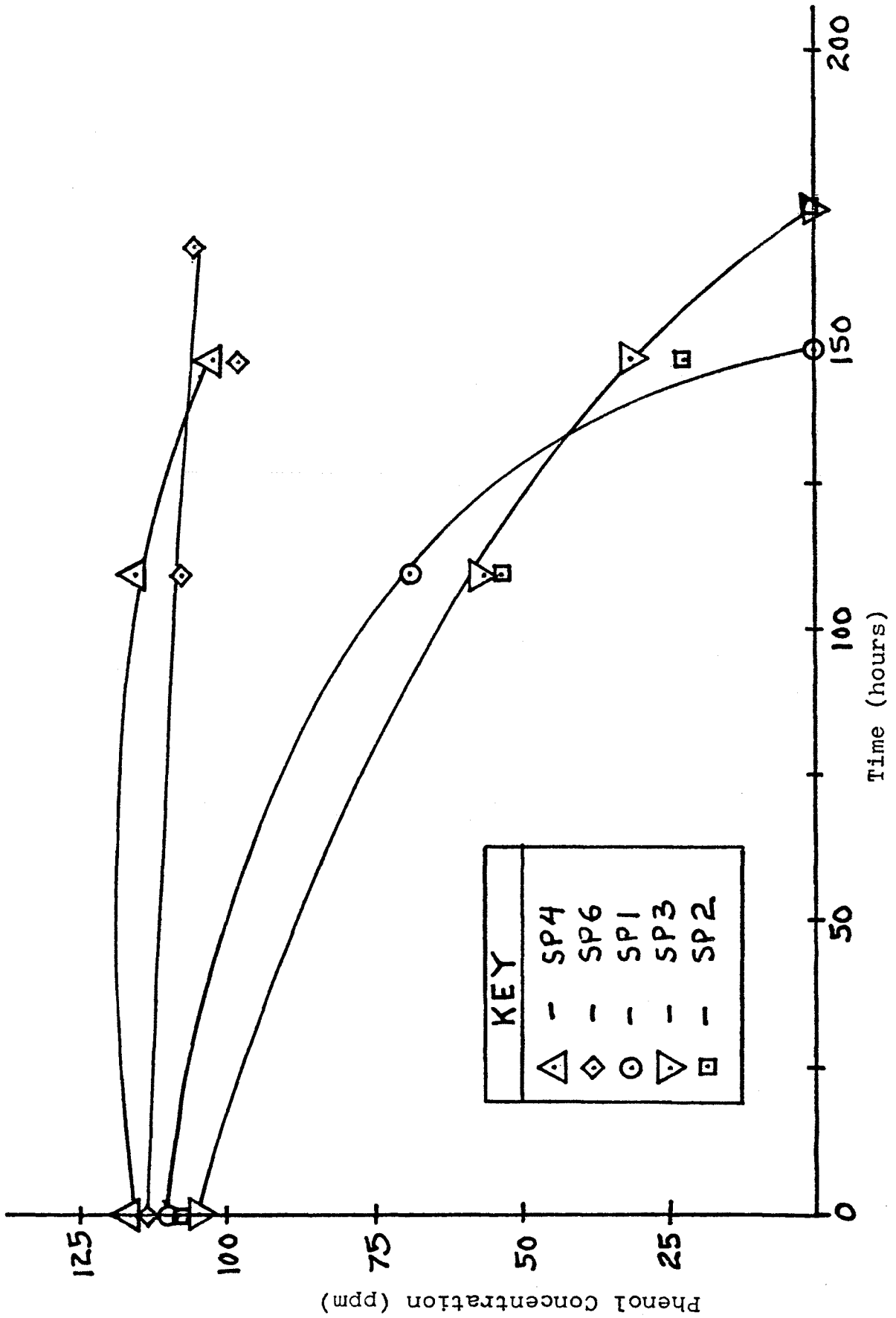


Figure # 8 - Results of UV Degradation Experiment

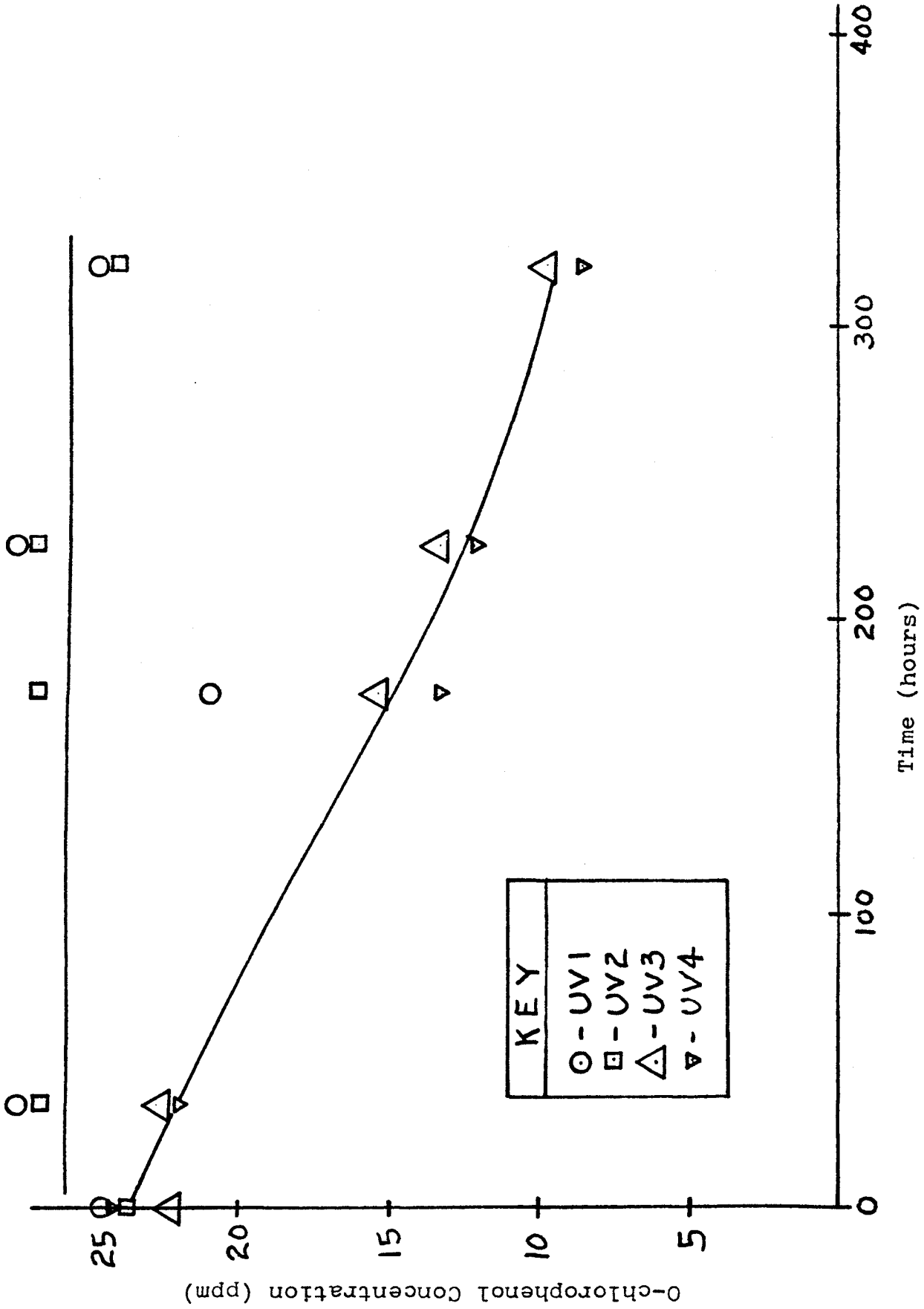


Figure # 9 - Results of First 100 ppm Phenol Run

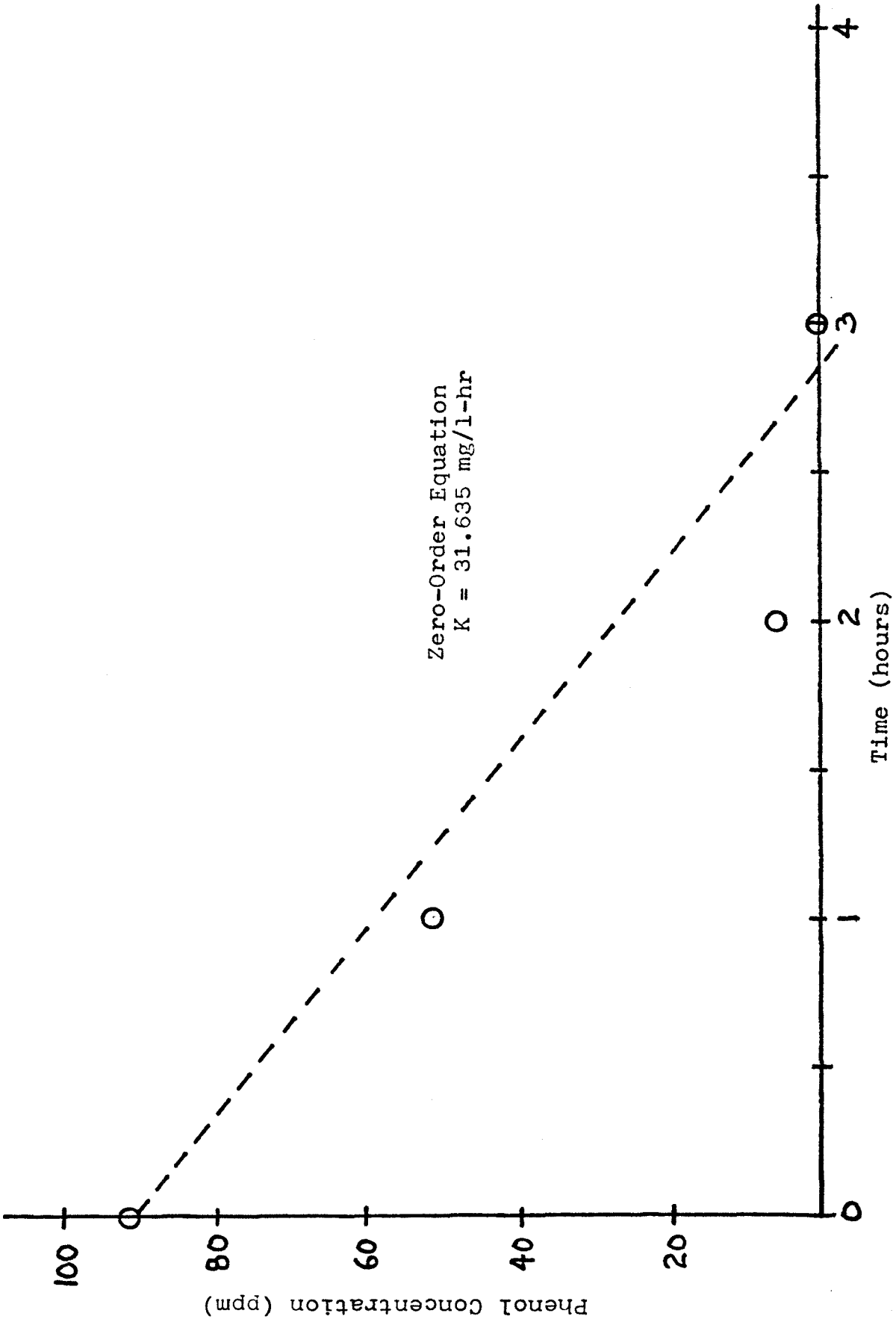


Figure #10- Results of Second 100 ppm Phenol Run

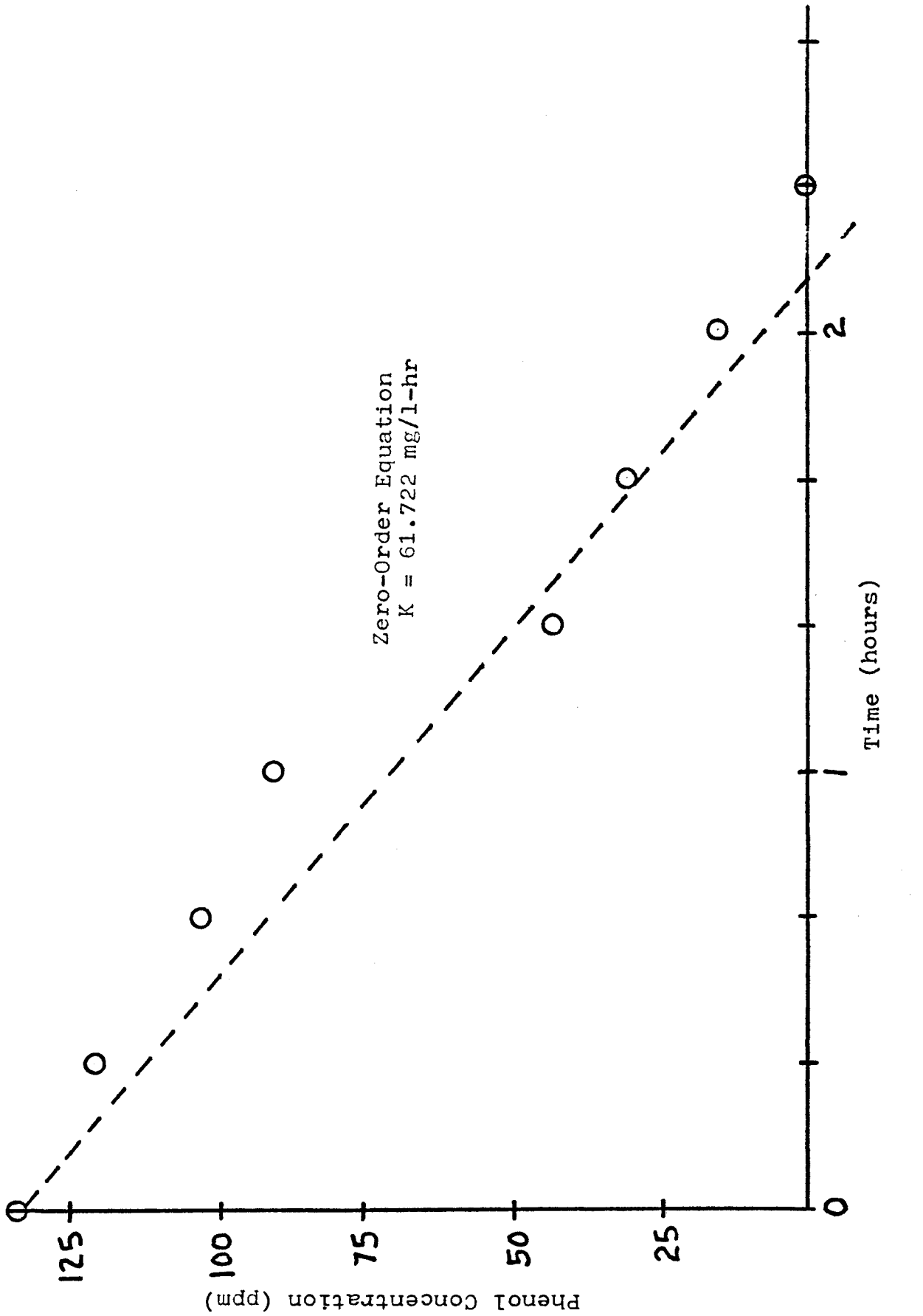


Figure #11- Results of First 20 ppm O-chlorophenol Run

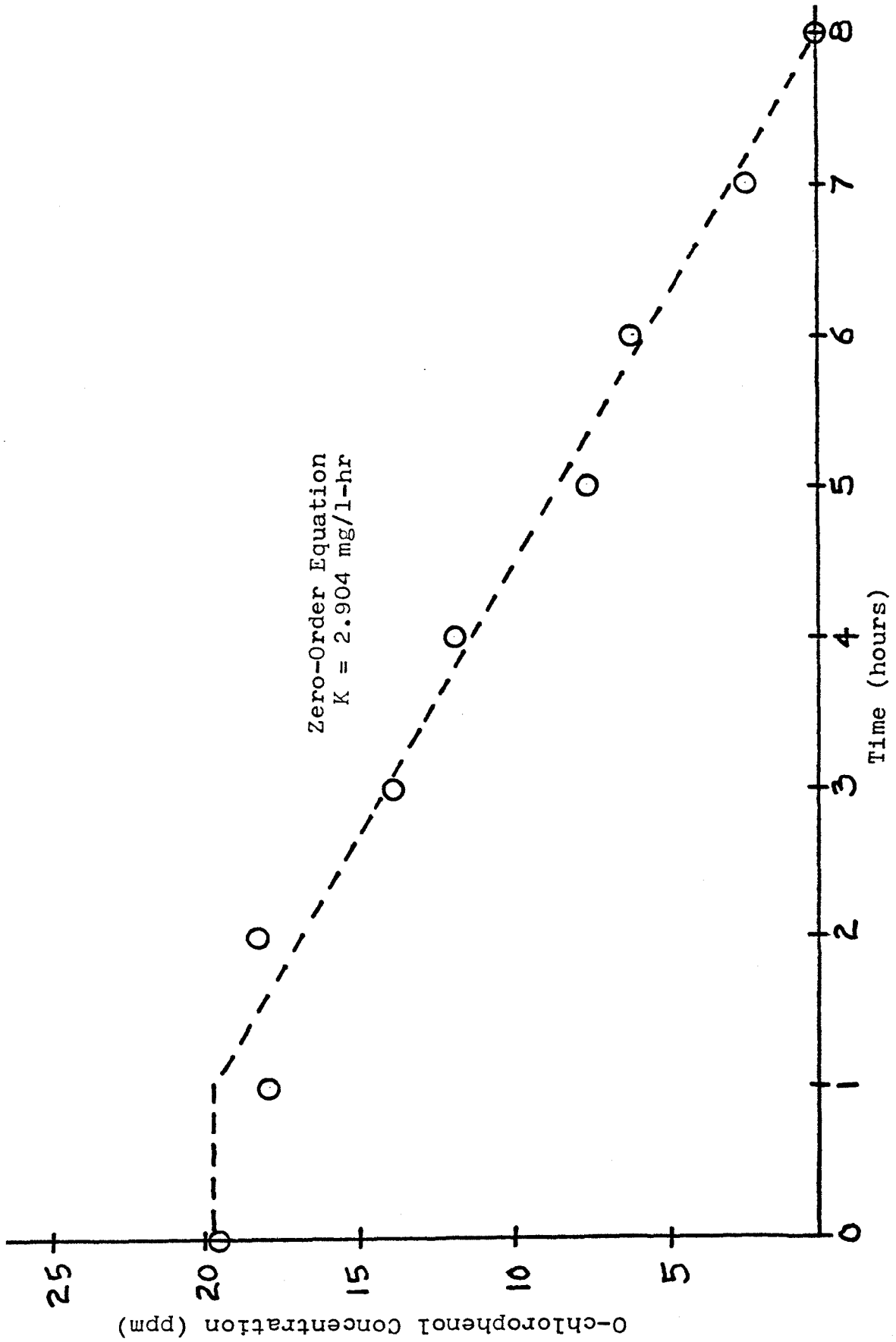


Figure #12 - Results of Second 20 ppm O-chlorophenol Run

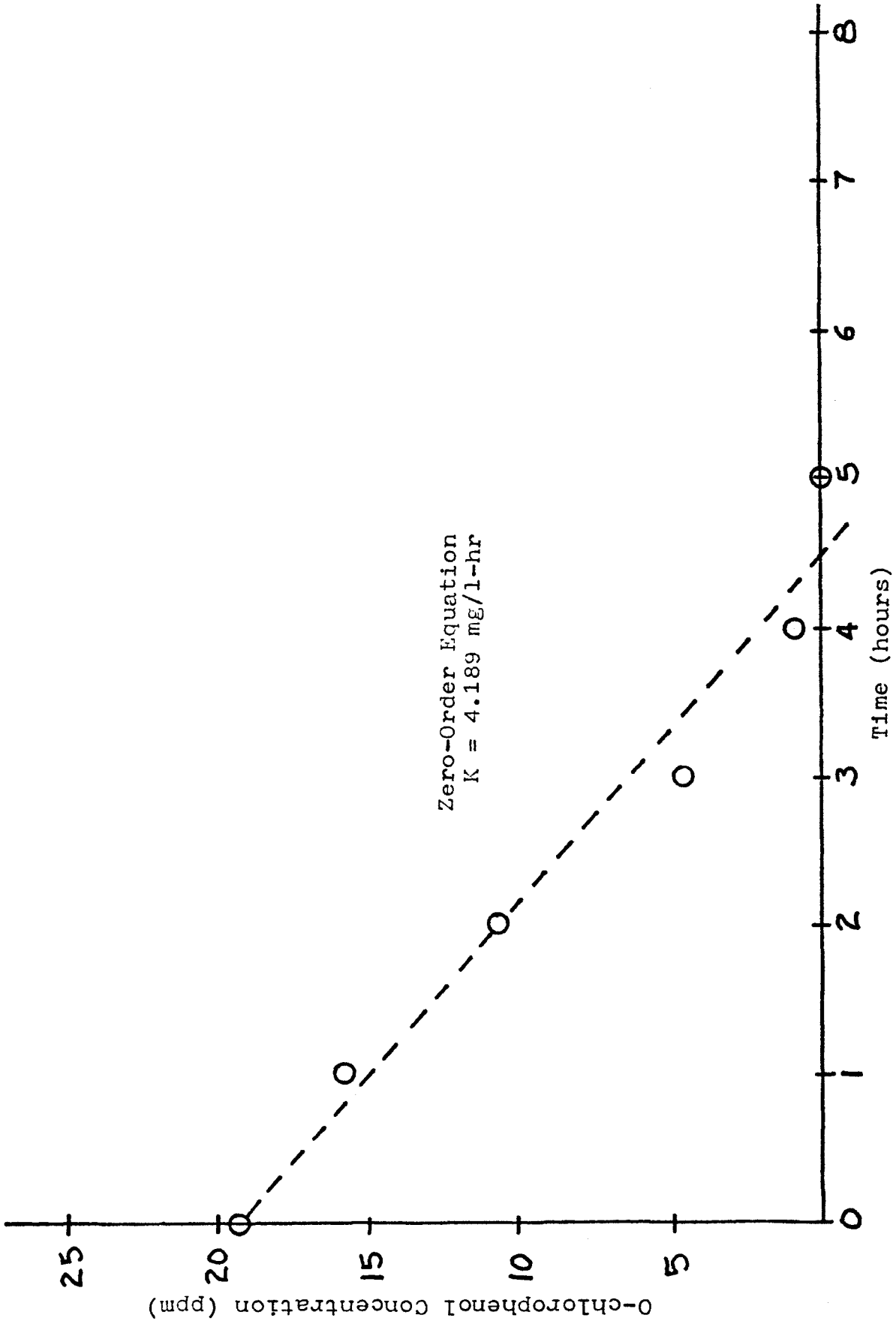


Figure # 13- Results of Third 20 ppm O-chlorophenol Run

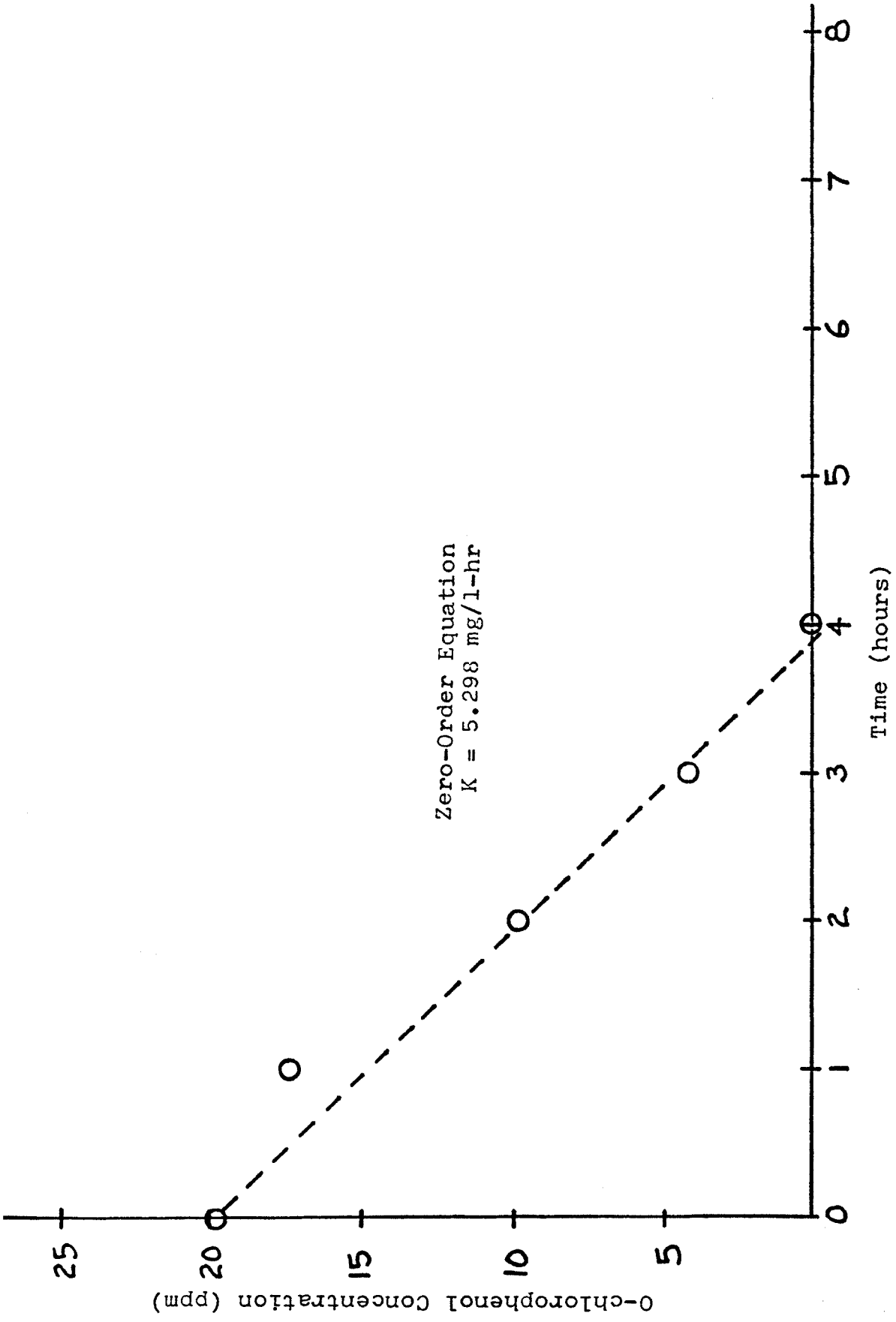


Figure #14- Results of First 40 ppm O-chlorophenol Run

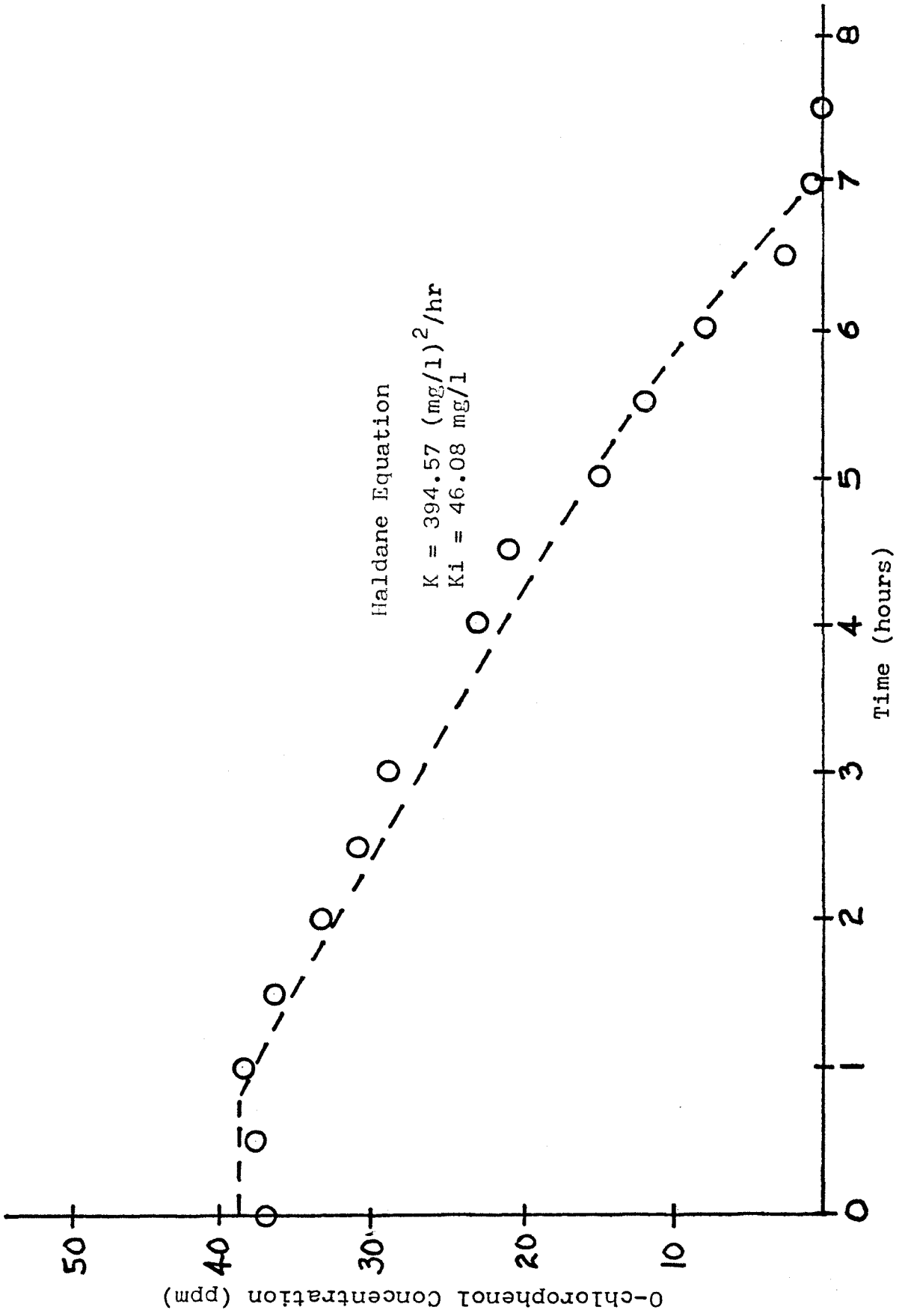


Figure #15 Results of Second 40 ppm O-chlorophenol Run

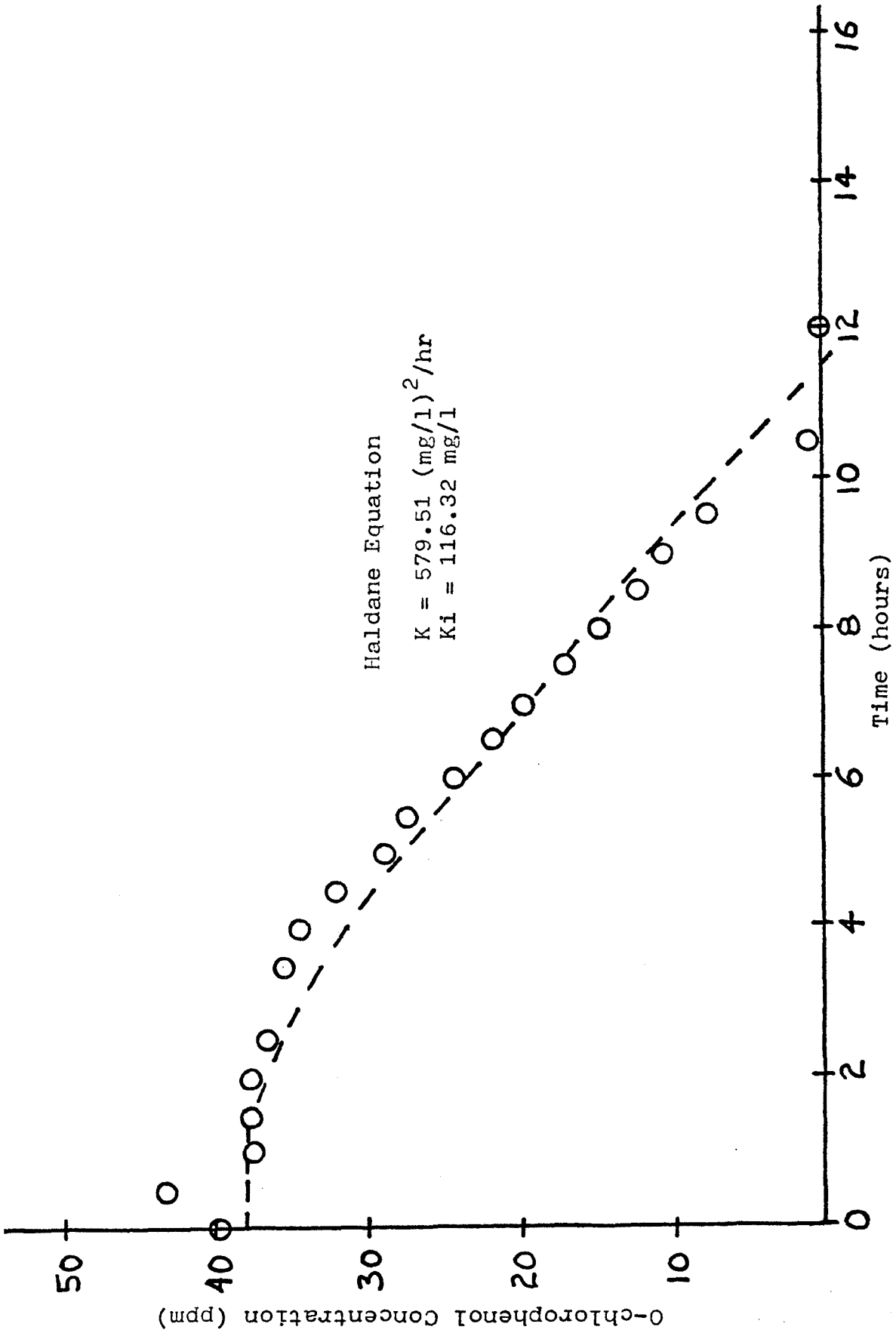


Figure #16- Results of Third 40 ppm O-chlorophenol Run

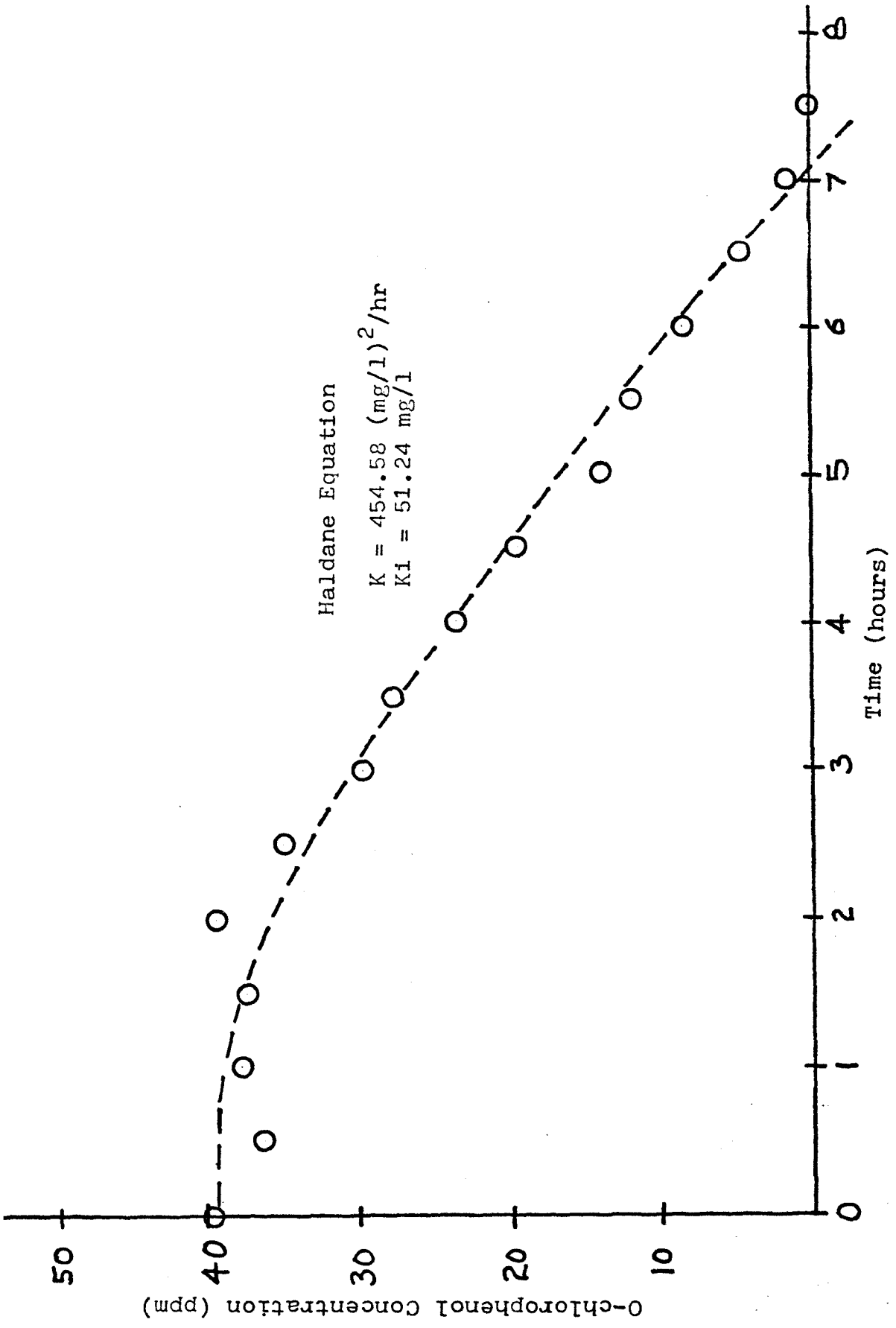


Figure #17- Results of First Amino Acids Run

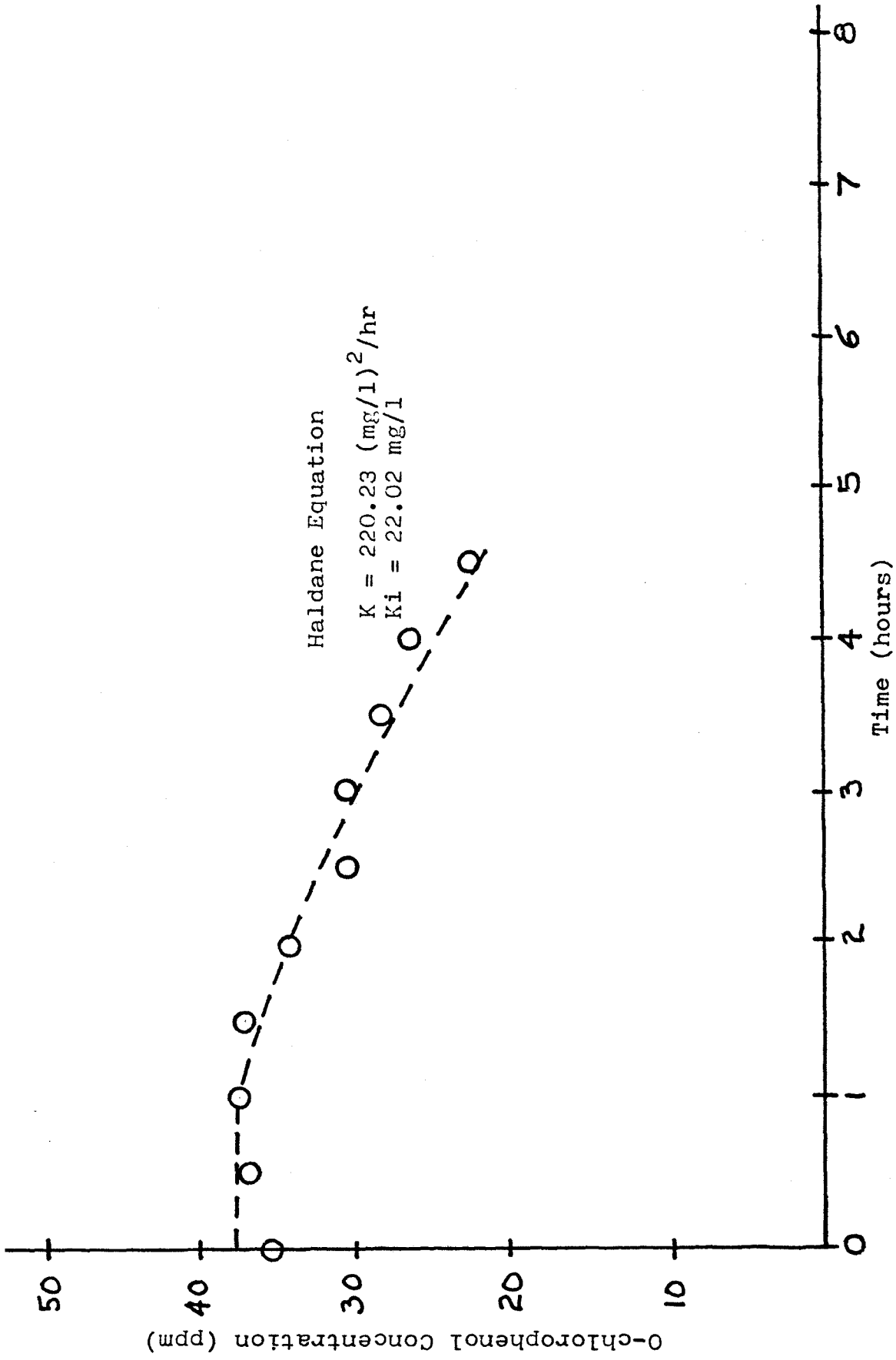


Figure #18 - Results of Second Amino Acids Run

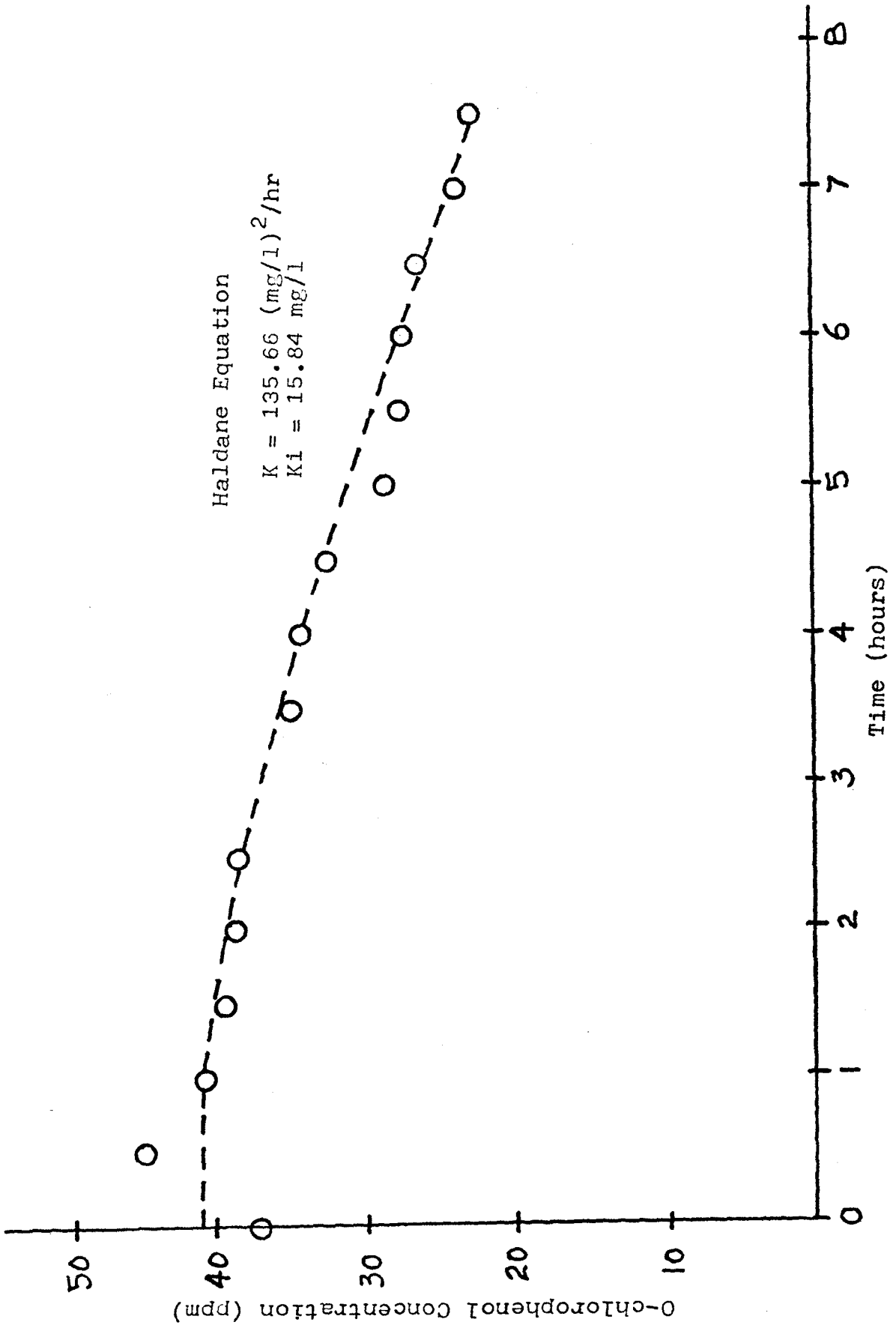


Figure #19- Comparison of Phenol and COD Removal Rates

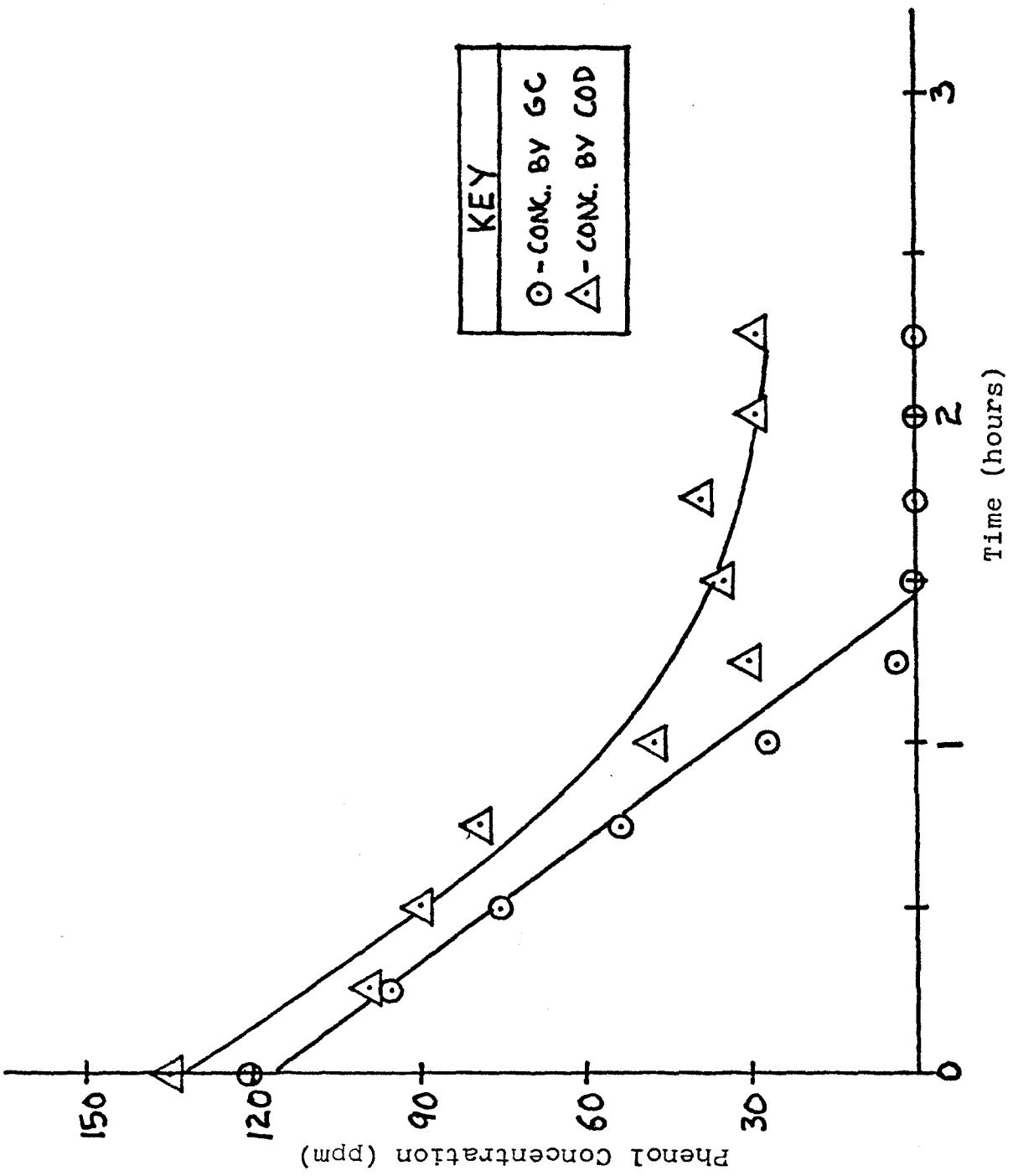
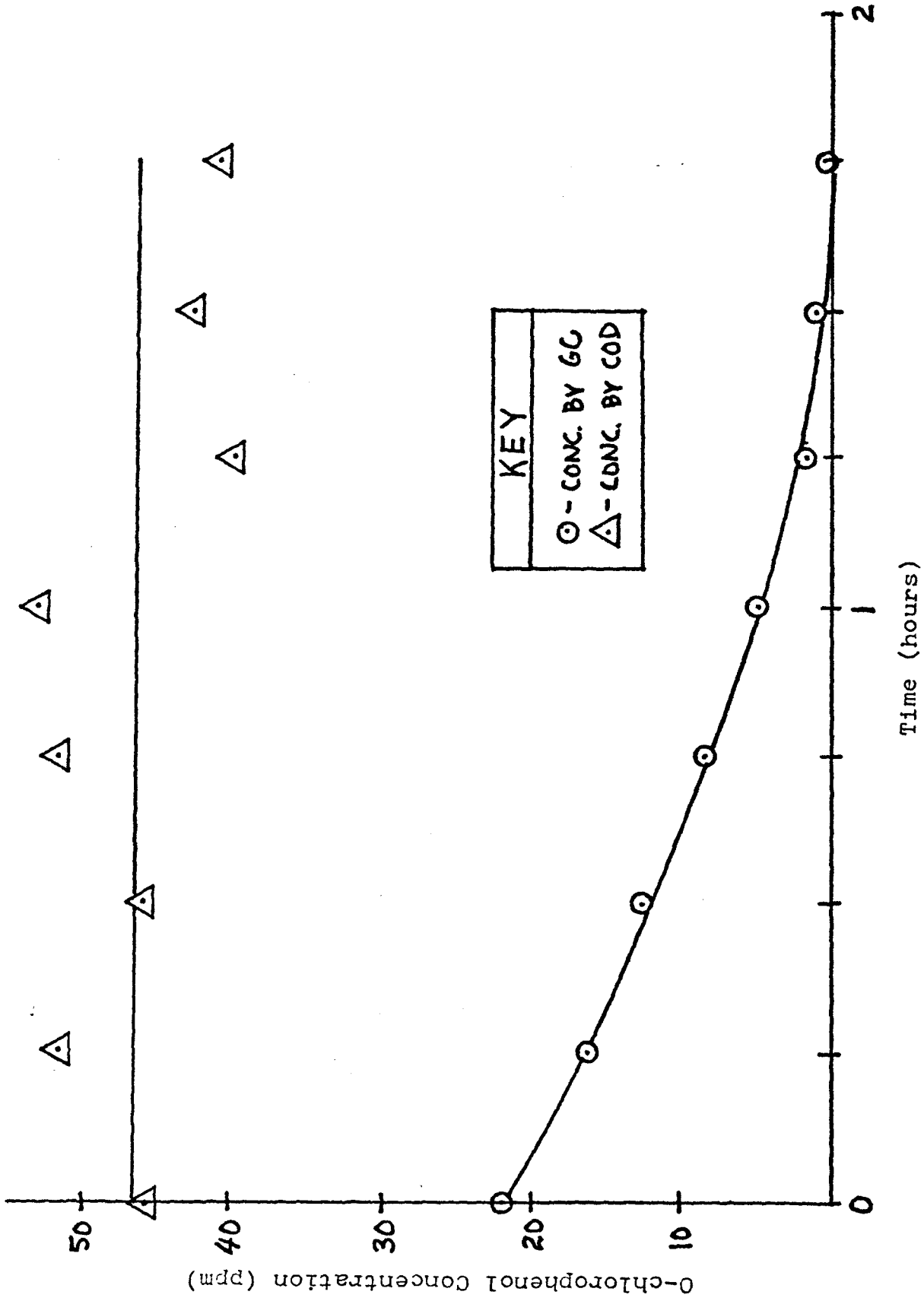


Figure #20 - Comparison of O-chlorophenol and COD Removal Rates



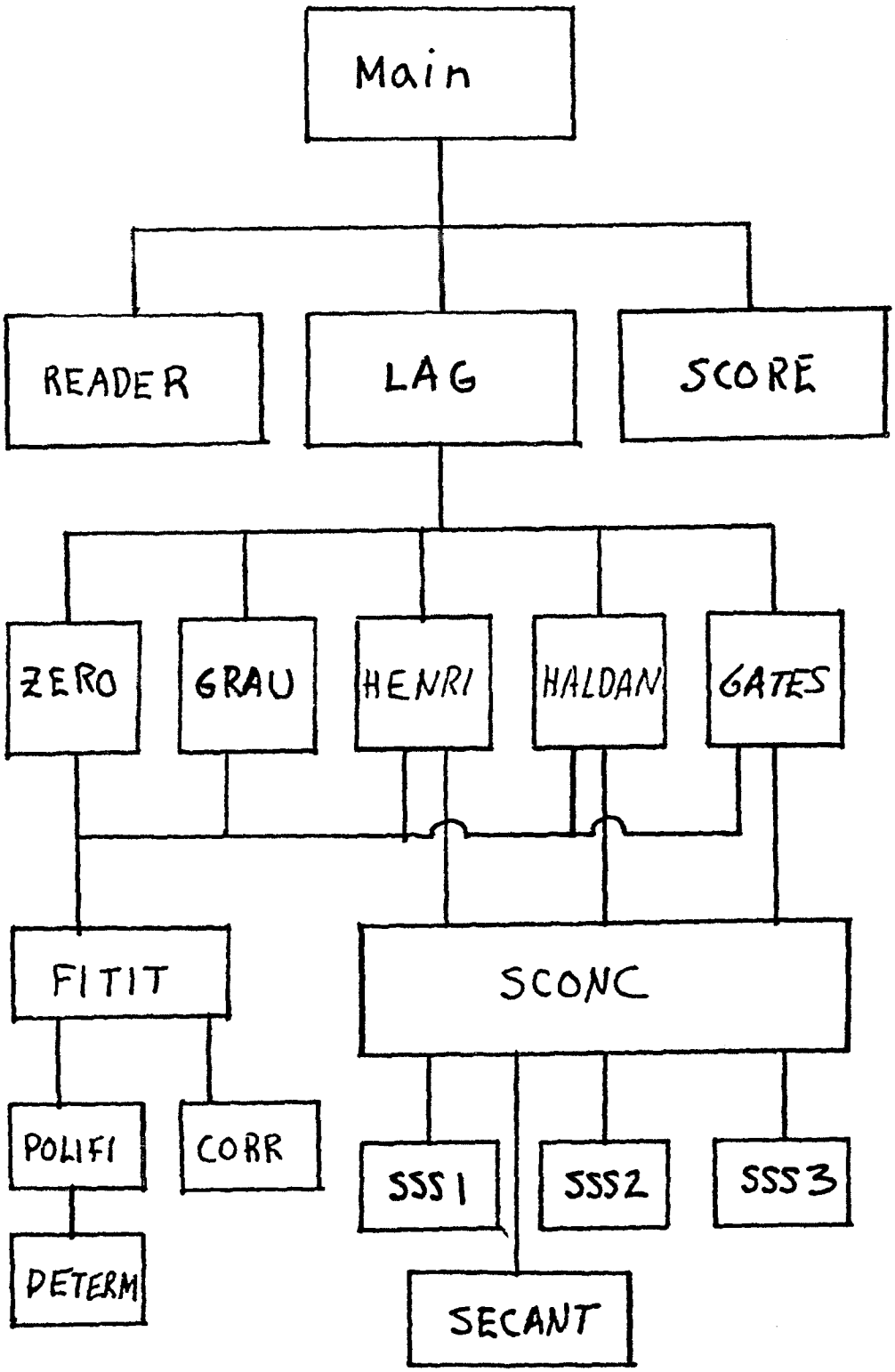
APPENDIX 1. COMPUTER PROGRAMS

```
C     ****************************************
C     *                                      *
C     *   PROGRAM AIR                        *
C     *                                      *
C     ****************************************
C
C     written by:  Jeffrey C. Colish       7/1/82
C
C     PURPOSE:  to simulate the air stripping experiments
C
C     DATA INPUT:
C           NCASE = number of cases
C           NC = number of compounds
C           T = temperature, C
C           PT = total pressure, mm Hg
C           A(I), B(I), C(I) = Antoine coefficients for I
C           MW(I) = molecular weigth of I
C           GM(I) = number of grams of I in the reactor
C           AIRFLO = air flowrate, mole/hr
C           NHOUR = length of simulation, hr
C           GAM(I) = activity coefficent of I
C           PHI(I) = fugacity coefficent of I
C           SATR(I) = % saturation of I in air leaving reactor
C
C
C
      DOUBLE PRECISION A(10),B(10),C(10),Y(10),X(10),VP(10),
     %SUM,GM(10),MW(10),MOL(10),GAM(10),PHI(10),SATR(10),GMM(10)
      NCRD=1
      NPRT=2
C
C       Input data
C
      READ(NCRD,*)NCASE
      READ(NCRD,*)NC
      READ(NCRD,*)T,PT
      DO 100 I=1,NC
      READ(NCRD,*)A(I),B(I),C(I),MW(I)
      READ(NCRD,*)GM(I)
      GMM(I)=GM(I)
      VP(I)=10.0**(A(I) - (B(I)/(T + C(I))))
100   CONTINUE
      READ(NCRD,*)AIRFLO
      READ(NCRD,*)NHOUR
      DO 25 L=1,NCASE
      DO 150 I=1,NC
      READ(NCRD,*)GAM(I),PHI(I),SATR(I)
150   CONTINUE
C
C       Output data
C
      WRITE(NPRT,9978)
      WRITE(NPRT,9980)VP(1)
```

```
      WRITE(NPRT,9981)VP(2)
      WRITE(NPRT,9982)GAM(1)
      WRITE(NPRT,9983)GAM(2)
      WRITE(NPRT,9984)PHI(1)
      WRITE(NPRT,9985)PHI(2)
      WRITE(NPRT,9986)T,PT
      WRITE(NPRT,9987)AIRFLO
      WRITE(NPRT,9988)SATR(1)
      WRITE(NPRT,9989)SATR(2)
      WRITE(NPRT,9991)
C
C        Begin simulation
C
      TIME=0.0
      PPM=GMM(2)/GMM(1)*1.0E06
      WRITE(NPRT,9990)TIME,PPM,GMM(1)
      NN=NHOUR/10
      DO 50 J=1,NN
      DO 75 K=1,10
      SUM=0.0
      DO 200 I=1,NC
      MOL(I)=GMM(I)/MW(I)
      SUM=SUM + MOL(I)
200   CONTINUE
      DO 300 I=1,NC
      X(I)=MOL(I)/SUM
300   CONTINUE
      DO 400 I=1,NC
      Y(I)=X(I)*VP(I)*GAM(I)*SATR(I)/(PHI(I)*PT*100.0)
      GMM(I)=GMM(I)-Y(I)*MW(I)*AIRFLO
400   CONTINUE
      TIME=TIME+1.0
      PPM=GMM(2)/GMM(1)*1.0E06
75    CONTINUE
      WRITE(NPRT,9990)TIME,PPM,GMM(1)
50    CONTINUE
25    CONTINUE
9978  FORMAT('1',5X,'SIMULATION OF AIR STRIPPING EXPERIMENT')
9980  FORMAT('0',5X,'VAPOR PRESS OF WATER = ',F8.3)
9981  FORMAT('0',5X,'VAPOR PRESS OF O-PHENOL = ',F8.3)
9982  FORMAT('0',5X,'ACTIVITY COEFF OF WATER = ',F8.3)
9983  FORMAT('0',5X,'ACTIVITY COEFF OF O-PHENOL = ',F8.3)
9984  FORMAT('0',5X,'FUGACITY COEFF OF WATER = ',F8.3)
9985  FORMAT('0',5X,'FUGACITY COEFF OF O-PHENOL = ',F8.3)
9986  FORMAT('0',5X,'TEMP = ',F8.3,5X,'TOTAL PRESS = ',F8.3)
9987  FORMAT('0',5X,'AIR FLOWRATE = ',F8.3,'  MOLES/HR')
9988  FORMAT(' ',5X,'THE AIR IS ',F8.3,' % SATURATED WITH WATER')
9989  FORMAT(' ',5X,'THE AIR IS ',F8.3,' % SATURATE WITH
     %O-CHLOROPHENOL')
9990  FORMAT(' ',5X,F7.3,6X,F8.3,9X,F8.2)
9991  FORMAT('0',8X,'TIME',6X,'CONC O-PHENOL',5X,'ML WATER')
      STOP
      END
```

```
C     ***********************************
C     *                                 *
C     *  Program REGRESS                *
C     *                                 *
C     ***********************************
C
C
C     Purpose:  to fit the substrate versus time data
C               to the following kinetic equations
C               and determine the rate constants:
C
C              (1) Zero-order rate equation
C
C              (2) Grau Kinetics (first-order kinetics)
C
C              (3) Eq. # 4-23 in Sundstrum & Klei
C
C              (4) Henri equation (first-order)
C
C              (5) Haldane equation (substrate inhibition)
C
C              (6) Gates & Marlar equation (first-order)
C
C
C        Note:  The program does not destroy the input data
C
C
C     Example of data input to program (free format)
C
C     1st Card:  number of points
C     2nd Card:  first data point, (time, substrate conc.)
C                (continue for # of points)
C                Note: time is in hours
C                Note: substrate conc. is in PPM
C                Note: last data point should have
C                      a substrate conc. of 0.0 ppm
C     3rd Card:  initial substrate conc., So (PPM)
C     4th Card:  initial biomass conc., Xo (mg/l)
C     5th Card:  number of different lag times
C                to perform data regression
C     6th Card:  first lag time (hours)
C                (continue for # of different lag times)
C
      DIMENSION Y(50),X(50),TITLE(40),A(10),TLAG(10)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      CALL READER(NP,X,Y,TITLE,SO,AXO,TLAG,LOOP)
      CALL LAG(NP,X,Y,TITLE,SO,AXO,TLAG,LOOP)
      CALL SCORE(LOOP,TLAG,TITLE)
      STOP
      END
```

Hieracherical Diagram of REGRESS



```
      SUBROUTINE READER(NP,X,Y,TITLE,SO,AXO,TLAG,LOOP)
C
C
C     written by:  Jeffrey C. Colish      4/1/83
C
C     Purpose:  input data for regression program
C
C     variable listing:
C     NP - # OF POINTS
C     X(I) - INDEPENDENT VARIABLE = time
C     Y(I) - DEPENDENT VARAIABLE = substrate concentration
C     AXO = initial MLSS of reactor
C     MAXORD = maximum order of polynomial to
C              the data will be regressed
C     TLAG = length of time lag
C     LOOP = # of loops to perform lag calculations
C
C
      DIMENSION Y(50),X(50),TITLE(40),TLAG(10)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      READ(1,9004)(TITLE(I),I=1,40)
      WRITE(2,9005)(TITLE(I),I=1,40)
      READ(1,*)NP
      WRITE(2,9000)NP
      WRITE(2,9001)
      DO 10 I=1,NP
      READ(1,*)X(I),Y(I)
      WRITE(2,9006)X(I),Y(I)
10    CONTINUE
      READ(1,*)SO
      WRITE(2,9007)SO
      READ(1,*)AXO
      WRITE(2,9010)AXO
      READ(1,*)LOOP
      WRITE(2,9013)LOOP
      DO 20 I=1,LOOP
      READ(1,*)TLAG(I)
      WRITE(2,9015)TLAG(I)
20    CONTINUE
      DO 100 I=1,20
      DO 200 J=1,LOOP
C
C     initialize all positions in NSCORE to 0
C
      NSCORE(I,J)=0
200   CONTINUE
100   CONTINUE
      RETURN
9004  FORMAT(40A2)
9005  FORMAT('1',5X,40A2)
9000  FORMAT('/',5X,'NUMBER OF PTS = ',I3)
9001  FORMAT('0',11X,'X(I)',10X,'Y(I)')
9006  FORMAT('/',5X,F12.5,5X,F12.5)
```

```
9007  FORMAT('0',5X,'Intial substrate conc. = ',F12.5,' mg/l')
9010  FORMAT('0',5X,'Xo = ',F12.5,' mg MLSS/l')
9013  FORMAT('0',5X,'LOOP = ',I3)
9015  FORMAT('0',5X,'Will regress data with ',F12.5,' hours lag')
      END
```

```
      SUBROUTINE LAG(NP,X,Y,TITLE,SO,AXO,TLAG,LOOP)
C
C     written by:  Jeffrey C. Colish        4/1/83
C
C     Purpose:  to regress the degradation data using
C               a maximum of 6 different TLAGs
C
      DIMENSION Y(50),X(50),TITLE(40),TLAG(10)
      DIMENSION XX(50),YY(50),A(10)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      NCOL=0
      DO 1000 J=1,LOOP
      NCOL=NCOL + 1
      IF(TLAG(J) .LT. 0.0  .OR.  LOOP .LT. 1)RETURN
      DO 1500 I=1,NP
C
C     put substrate conc. versus time data in dummy
C     arrays, so that the input data is not destroyed
C
      XX(I)=X(I)
      YY(I)=Y(I)
1500  CONTINUE
      DO 2000 I=1,NP
C
C     substract lag time from time data to determine
C     the falling rate period
C
2000  XX(I)=XX(I) - TLAG(J)
      NEWNP=0
      DO 3000 I=1,NP
C
C     if time is less than zero, discard point
C
      IF(XX(I) .LT. 0.0) GO TO 3000
      NEWNP=NEWNP + 1
      XX(NEWNP)=XX(I)
      YY(NEWNP)=YY(I)
3000  CONTINUE
      TIME=TLAG(J)
C
C     check if the 1st value in XX-array is equal to 0
C     if it isn't, add this point:  (0.0,So)
C
      IF(XX(1) .EQ. 0.0)GO TO 5000
      NNP=NEWNP
      NEWNP=NEWNP + 1
      DO 4000 I =1,NNP
      L=NEWNP - I
      K=L + 1
      XX(K)=XX(L)
      YY(K)=YY(L)
      XX(L)=0.0
      YY(L)=SO
```

```
4000  CONTINUE
5000  CONTINUE
C
C
C     check if the 1st value in YY-array is equal to So
C     if it isn't, change 1st value to So
C
      IF(YY(1) .NE. SO) YY(1)=SO
C
C
      CALL ZERO(NEWNP,XX,YY,TITLE,SO,AXO,TIME)
      CALL GRAU(NEWNP,XX,YY,TITLE,SO,AXO,TIME)
      CALL LOW(NEWNP,XX,YY,TITLE,SO,AXO,TIME)
      CALL HENRI(NEWNP,XX,YY,TITLE,SO,AXO,TIME)
      CALL HALDAN(NEWNP,XX,YY,TITLE,SO,AXO,TIME)
      CALL GATES(NEWNP,XX,YY,TITLE,SO,AXO,TIME)
1000  CONTINUE
      RETURN
      END
```

```
      SUBROUTINE ZERO(NP,X,Y,TITLE,SO,AXO,TIME)
C
C     written by:  Jeffrey C. Colish       4/1/83
C
C     Purpose:  to regress the concentration versus time data
C               according to a zero-order kinetic expression
C
      DIMENSION Y(50),X(50),TITLE(40),A(10)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      WRITE(2,9006)(TITLE(I),I=1,40)
      WRITE(2,9007)
      WRITE(2,9001)TIME
      METH=1
      NFLAG=1
      CALL FITIT(NP,X,Y,1,A)
      WRITE(2,9975)
      AK= -A(2)
      TMAX=SO/AK
      IF(AK .GT. 0.0)NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 10
      WRITE(2,9008)AK
      WRITE(2,9985)TMAX
      WRITE(2,9980)AXO
      WRITE(2,50)
      WRITE(2,100)
      SUM=0.0
      DO 1000 I=1,NP
      S3=SO - AK*X(I)
      DY=S3 - Y(I)
      SUM=SUM + DY**2
1000  WRITE(2,9015)X(I),Y(I),S3,DY
      SUM=SUM/NP
      IF(SUM .LE. 5.0)NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 100
      WRITE(2,9971)SUM
      RETURN
9006  FORMAT('1',5X,40A2)
9007  FORMAT('/',5X,'DATA REGRESSED TO A ZERO ORDER EQ.')
9001  FORMAT('/',5X,'REGRESSION PERFORMED WITH ',G12.5,
     %' HOURS LAG TIME')
9975  FORMAT('0',5X,'Kinetic Rate Constants')
9008  FORMAT('0',5X,'K = ',F12.5,' mg/l-hr')
9985  FORMAT('/',5X,'Tmax = ',F12.5,' hrs')
9980  FORMAT('/',5X,'Xo = ',F12.5,' mg MLSS/l')
50    FORMAT('0',5X,'Use Calc Rate Const to deter
     %v Time')
100   FORMAT('0',9X,'XEXP',8X,'YEXP',8X,'YCAL',8X,'DY')
9015  FORMAT('/',5X,4G12.5)
9971  FORMAT('0',5X,'THE SUM OF DY SQUARED DIVIDED BY THE'
     %' # OF PTS = ',G12.5)
      END
```

```
      SUBROUTINE GRAU(NP,X,Y,TITLE,SO,AXO,TIME)
C
C     written by:  Jeffrey C. Colish     6/1/83
C
C     Purpose:  to regress the concentration versus
C               time data according to the Grau
C               Equation.
C
C     Assumptions:  Kd = 0.0
C                   Xo = X = constant during run
C
      DIMENSION Y(50),X(50),TITLE(40),A(10)
      DIMENSION XX(50),YY(50)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      WRITE(2,9006)(TITLE(I),I=1,40)
      WRITE(2,9765)
      WRITE(2,9001)TIME
      METH=2
      IF(NP .LE. 4)RETURN
      NPM2=NP - 2
      DO 1000 I=1,NPM2
      J=I + 1
      XX(I)=X(J)
1000  YY(I)=ALOG(SO/Y(J))
      NFLAG=1
      CALL FITIT(NPM2,XX,YY,1,A)
      AKPRIM=A(2)*SO/AXO
      IF(AKPRIM .GT. 0.0)NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 10
      WRITE(2,9975)
      WRITE(2,9978)AKPRIM
      WRITE(2,9980)AXO
      WRITE(2,50)
      WRITE(2,100)
      SUM=0.0
      DO 2000 I=1,NP
      S3=SO*EXP(-AKPRIM*AXO*X(I)/SO)
      DY=S3 - Y(I)
      SUM=SUM + DY**2
2000  WRITE(2,9015)X(I),Y(I),S3,DY
      SUM=SUM/NP
      IF(SUM .LE. 5.0)NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 100
      WRITE(2,9971)SUM
      RETURN
9006  FORMAT('1',5X,40A2)
9765  FORMAT('0',5X,'DATA REGRESSED USING GRAU EQUATION')
9001  FORMAT('/',5X,'REGRESSION PERFORMED WITH ',G12.5,
     %' HOURS LAG TIME')
9975  FORMAT('0',5X,'Kinetic Rate Constants')
9978  FORMAT('/',5X,'K = ',F12.5,' hr-1')
9980  FORMAT('/',5X,'Xo = ',F12.5,' mg MLSS/l')
50    FORMAT('0',5X,'Use Calc Rate Const to deter
     %v Time')
100   FORMAT('0',9X,'XEXP',8X,'YEXP',8X,'YCAL',8X,'DY')
```

```
9015  FORMAT('/',5X,4G12.5)
9971  FORMAT('0',5X,'THE SUM OF DY SQUARED DIVIDED BY THE'
     %' # OF PTS = ',G12.5)
      END
```

```
      SUBROUTINE LOW(NP,X,Y,TITLE,SO,AXO,TIME)
C
C     written by:  Jeffrey C. Colish     6/1/83
C
C     Purpose:  to regress the concentration versus
C               time data according to eq. # 4-23 in
C               Sundstrom & Klei.
C
C     Assumptions:  Kd = 0.0
C                   Xo = X = constant during run
C                   So << Km
C
      DIMENSION Y(50),X(50),TITLE(40),A(10)
      DIMENSION XX(50),YY(50)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      WRITE(2,9006)(TITLE(I),I=1,40)
      WRITE(2,9765)
      WRITE(2,9001)TIME
      METH=3
      IF(NP .LE. 4)RETURN
      NPM2=NP - 2
      DO 1000 I=1,NPM2
      J=I + 1
      XX(I)=X(J)
1000  YY(I)=ALOG(SO/Y(J))
      NFLAG=1
      CALL FITIT(NPM2,XX,YY,1,A)
      AKPRIM=A(2)/AXO
      IF(AKPRIM .GT. 0.0)NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 10
      WRITE(2,9975)
      WRITE(2,9978)AKPRIM
      WRITE(2,9980)AXO
      WRITE(2,50)
      WRITE(2,100)
      SUM=0.0
      DO 2000 I=1,NP
      S3=SO/EXP(AKPRIM*AXO*X(I))
      DY=S3 - Y(I)
      SUM=SUM + DY**2
2000  WRITE(2,9015)X(I),Y(I),S3,DY
      SUM=SUM/NP
      IF(SUM .LE. 5.0)NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 100
      WRITE(2,9971)SUM
      RETURN
9006  FORMAT('1',5X,40A2)
9765  FORMAT('0',5X,'DATA REGRESSED USING EQ. # 4-23 ')
9001  FORMAT('/',5X,'REGRESSION PERFORMED WITH ',G12.5,
     %' HOURS LAG TIME')
9975  FORMAT('0',5X,'Kinetic Rate Constants')
9978  FORMAT('/',5X,'K = ',F12.5,' hr-1')
9980  FORMAT('/',5X,'Xo = ',F12.5,' mg MLSS/l')
50    FORMAT('0',5X,'Use Calc Rate Const to deter
     %v Time')
```

```
100   FORMAT('0',9X,'XEXP',8X,'YEXP',8X,'YCAL',8X,'DY')
9015  FORMAT('/',5X,4G12.5)
9971  FORMAT('0',5X,'THE SUM OF DY SQUARED DIVIDED BY THE'
     %' # OF PTS = ',G12.5)
      END
```

```
      SUBROUTINE HENRI(NP,X,Y,TITLE,SO,AXO,TIME)
C
C     written by:  Jeffrey C. Colish     4/1/83
C
C     Purpose:  to regress the concentration versus
C               time data according to the Henri
C               Equation.
C
C     Assumptions:  Kd = 0.0
C                   Xo = X = constant during run
C                   So is approx. equal to Km
C
      EXTERNAL SSS2
      DIMENSION Y(50),X(50),TITLE(40),A(10)
      DIMENSION XX(50),YY(50)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      WRITE(2,9006)(TITLE(I),I=1,40)
      WRITE(2,9765)
      WRITE(2,9001)TIME
      METH=4
      IF(NP .LE. 4)RETURN
      NPM2=NP - 2
      DO 1000 I=1,NPM2
      J=I + 1
      XX(I)=(SO - Y(J))/X(J)
      YY(I)=(ALOG(Y(1)/Y(J)))/X(J)
1000  CONTINUE
      NFLAG=1
      CALL FITIT(NPM2,XX,YY,1,A)
      AKM=(-1.0/A(2))
      AKO=AKM*A(1)
      WRITE(2,9975)
      WRITE(2,9978)AKM
      WRITE(2,9979)AKO
      WRITE(2,9980)AXO
      IF(AKM .LT. 0.0)GO TO 2000
      CALL SCONC(NP,X,Y,SO,AXO,AKM,AKO,0.0,SSS2)
2000  RETURN
9006  FORMAT('1',5X,40A2)
9765  FORMAT('0',5X,'DATA REGRESSED USING HENRI EQUATION')
9001  FORMAT('/',5X,'REGRESSION PERFORMED WITH ',G12.5,
     %' HOURS LAG TIME')
9975  FORMAT('0',5X,'Kinetic Rate Constants')
9978  FORMAT('/',5X,'Km = ',F12.5,' mg/l')
9979  FORMAT('/',5X,'KoXo/Y = ',F12.5,' 1/hr-l-mg biomass')
9980  FORMAT('/',5X,'Xo = ',F12.5,' mg MLSS/l')
      END
```

```
      SUBROUTINE HALDAN(NP,X,Y,TITLE,SO,AXO,TIME)
C
C     written by:  Jeffrey C. Colish     6/1/83
C
C     Purpose:  to regress the concentration versus
C               time data according to the Haldane
C               Equation.
C
C     Assumptions:  Kd = 0.0
C                   Xo = X = constant during run
C                   S >> Ks
C
      EXTERNAL SSS3
      DIMENSION Y(50),X(50),TITLE(40),A(10)
      DIMENSION XX(50),YY(50)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      WRITE(2,9006)(TITLE(I),I=1,40)
      WRITE(2,9765)
      WRITE(2,9001)TIME
      METH=5
      IF(NP .LE. 3)RETURN
      NFLAG=0
      CALL FITIT(NP,X,Y,2,A)
      DO 1000 I = 1,NP
      SLOPE = A(2) + 2*A(3)*X(I)
      YY(I) = -1.0/SLOPE
      XX(I) = Y(I)
1000  CONTINUE
      NFLAG=1
      WRITE(2,9610)
      CALL FITIT(NP,XX,YY,1,A)
      AK=(+1.0/A(2))
      AKI=AK*A(1)
      TMAX=(AKI*SO + (SO**2)/2.0)/AK
      WRITE(2,9975)
      WRITE(2,9978)AK
      WRITE(2,9979)AKI
      WRITE(2,9985)TMAX
      WRITE(2,9980)AXO
      IF(AK .LT. 0.0  .OR. AKI .LT. 0.0)GO TO 2000
      CALL SCONC(NP,X,Y,SO,AXO,AK,AKI,0.0,SSS3)
2000  RETURN
9006  FORMAT('1',5X,40A2)
9765  FORMAT('0',5X,'DATA REGRESSED USING HALDANE EQUATION')
9001  FORMAT('/',5X,'REGRESSION PERFORMED WITH ',G12.5,
     %' HOURS LAG TIME')
9610  FORMAT('0',5X,'Plot -dt/ds versus substrate conc.')
9975  FORMAT('0',5X,'Kinetic Rate Constants')
9978  FORMAT('/',5X,'K = ',F12.5,' mg/l')
9979  FORMAT('/',5X,'Ki = ',F12.5,' mg/l')
9980  FORMAT('/',5X,'Xo = ',F12.5,' mg MLSS/l')
9985  FORMAT('/',5X,'Tmax = ',F12.5,' hrs')
      END
```

```
      FUNCTION SSS3(Z1,Z2,Z3,Z4,Z5,Z6,Z7)
C
C     written by:  Jeffrey C. Colish      6/1/83
C
C     purpose:  SSS3 = equation for substrate conc.
C                      using Haldane equation
C
      DUM1 = (Z1**2 - Z7**2)/(2*Z4)
      DUM2 = (-Z3*Z2/Z4)
      SSS3 = DUM1 + Z1 + DUM2 - Z7
      RETURN
      END
```

```
      SUBROUTINE GATES(NP,X,Y,TITLE,SO,AXO,TIME)
C
C     written by:  Jeffrey C. Colish       4/1/83
C
C     Purpose:  to regress the concentration versus time
C               data using the method of Gates & Marlar.
C
C     Assumptions:  Kd = 0.0
C                   So is approx. equal to Km
C
      DIMENSION Y(50),X(50),TITLE(40),A(10)
      DIMENSION XX(50),YY(50)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      EXTERNAL SSS1
      DO 1000 K=1,10
      METH=METH+1
      WRITE(2,9006)(TITLE(I),I=1,40)
      WRITE(2,9001)TIME
      WRITE(2,9766)
C     AA=FLOAT(K)/10.0
      AA=FLOAT(K)/100.0
      WRITE(2,9676)AA
      IF(NP .LE. 4)RETURN
      NPM2=NP - 2
      DO 2000 I=1,NPM2
      J=I + 1
      DUM1=1.0 + AA*(Y(1) - Y(J))
      IF(DUM1 .GT. 0.0)GO TO 2500
      WRITE(2,9010)
      GO TO 3000
2500  XX(I)=ALOG(DUM1)/X(J)
      YY(I)=ALOG(SO/Y(J))/X(J)
2000  CONTINUE
      NFLAG=1
      CALL FITIT(NPM2,XX,YY,1,A)
      WRITE(2,9975)
      AKM=(1.0/AA + SO)/(-A(2) - 1.0)
      AKO=A(1)/(-A(2) - 1.0)
      WRITE(2,9978)AKM
      WRITE(2,9979)AKO
      AY=AA*AXO
      WRITE(2,9980)AXO
      WRITE(2,9981)AY
      IF(AKM .LT. 0.0)GO TO 1000
      CALL SCONC(NP,X,Y,SO,AXO,AKM,AKO,AY,SSS1)
1000  CONTINUE
3000  RETURN
9766  FORMAT('0',5X,'DATA REGRESSED USING GATES & MARLAR EQ.')
9006  FORMAT('1',5X,40A2)
9001  FORMAT('/',5X,'REGRESSION PERFORMED WITH ',G12.5,
     %' HOURS LAG TIME')
9676  FORMAT('/',5X,'AA = ',G12.6)
9010  FORMAT('/',5X,'ERROR:  Ln(X), WHERE X < 0.0')
```

```
9975  FORMAT('0',5X,'Kinetic Rate Constants')
9978  FORMAT('/',5X,'Km = ',F12.5,' mg/l')
9979  FORMAT('/',5X,'Ko = ',F12.5,' hr -1')
9980  FORMAT('/',5X,'Xo = ',F12.5,' mg MLSS/l')
9981  FORMAT('/',5X,'Y = ',F12.5,' mg biomass/mg substrate')
      END
```

```
      SUBROUTINE SCONC(NP,X,Y,SO,AXO,AKM,AKO,AY,SSS)
C
C     written by:  Jeffrey C. Colish       4/1/83
C
C     Purpose:  to use the calculated kinetic rate
C               constants to determine the substrate
C               concentration versus time.
C
      DIMENSION X(50),Y(50)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 10
      WRITE(2,50)
      WRITE(2,100)
      S3=Y(1)
      SUM=0.0
      DO 1000 I=1,NP
      INTR=0
      S1=S3
      FN1=SSS(SO,X(I),AKM,AKO,AXO,AY,S1)
      S2=0.9*S1
2000  FN2=SSS(SO,X(I),AKM,AKO,AXO,AY,S2)
      IF(INTR .GE. 10)GO TO 3000
      INTR=INTR + 1
      CALL SECANT(S1,S2,FN1,FN2,S3)
      DIFF=ABS(S3 - Y(I))
      IF(DIFF .LT. 0.001)GO TO 3000
      S1=S2
      FN1=FN2
      S2=S3
      GO TO 2000
3000  DY=S3 - Y(I)
      SUM=SUM + DY**2
      WRITE(2,200)X(I),Y(I),S3,DY,INTR
1000  CONTINUE
      SUM=SUM/NP
      IF(SUM .LE. 5.0)NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 100
      WRITE(2,9971)SUM
      RETURN
50    FORMAT('0',5X,'Use Calc Rate Const to deter
     %v Time')
100   FORMAT('0',9X,'XEXP',8X,'YEXP',8X,'YCAL',8X,'DY',8X,'INTR')
200   FORMAT('/',5X,4G12.5,3X,I2)
9971  FORMAT('0',5X,'THE SUM OF DY SQUARED DIVIDED BY THE'
     %' # OF PTS = ',G12.5)
      END
```

```
      FUNCTION SSS1(Z1,Z2,Z3,Z4,Z5,Z6,Z7)
C
C     Written by:  Jeffrey C. Colish          4/1/83
C
C     Purpose:  SSS1 = equation for substrate conc. verse
C                      time using MONOD kinetics
C
      DUM1=(Z1 + Z6*(Z1 - Z7)*Z1/Z5)
      DUM2=((Z5 + Z6*Z1)/(Z6*Z3))
      DUM3=(Z5 + Z6*(Z1 - Z7))/Z5
      DUM4=Z4*Z2*(Z5 + Z6*Z1)/(Z6*Z3)
      IF(DUM1 .LT. 0.0 .OR. DUM3 .LT. 0.0)GO TO 1000
      SSS1=EXP( ALOG(DUM1) + DUM2*ALOG(DUM3) - DUM4) - Z7
      GO TO 2000
1000  CONTINUE
      WRITE(2,9900)
9900  FORMAT('0',5X,'ERROR: Ln(X), where X< 0.0')
      SSS1=Z7*.9
2000  RETURN
      END
```

```
      SUBROUTINE SECANT(X1,X2,F1,F2,X3)
      IF(ABS(F1 - F2) .GT. 1.E-08)GO TO 900
      X3=X2
      GO TO 1000
900   X3=X2 - F2*(X2-X1)/(F2-F1)
1000  CONTINUE
      RETURN
      END
```

```
      FUNCTION SSS2(Z1,Z2,Z3,Z4,Z5,Z6,Z7)
C
C     written by:  Jeffrey C. Colish      4/1/83
C
C     purpose:  SSS2 = the equation for substrate conc.
C                      using the Henri Equation
C
      DUM1=(Z7 - Z1)/Z3
      DUM2=Z4*Z2/Z3
      DUM3=EXP(DUM1 + DUM2)
      SSS2=Z1/DUM3 - Z7
      RETURN
      END
```

```
      SUBROUTINE FITIT(NP,X,Y,MAXORD,A)
C
C       THIS PROGRAM FITS A POLYNOMIAL OF ORDER 6
C
        DIMENSION SIGMAY(50),X(50),Y(50),A(50),DELTAY(50),YCAL(50)
     %,TITLE(40)
        COMMON METH,NCOL,NSCORE(20,10),NFLAG
C       IF(NP.LE.3) GO TO 99
C
        DO 2 I=1,NP
        SIGMAY(I)=0.
2       CONTINUE
        NCODE=0
C       MAXORD=NP/2
C       IF(NP.LE.4) MAXORD=2
C       IF(MAXORD.GT.6) MAXORD=6
        NNK=MAXORD
        DO 3 K=1,NNK
       IF(NFLAG .EQ. 0  .AND. K .EQ. 1) GO TO 3
        K1=K+1
C
        CALL POLIFI(X,Y,SIGMAY,NP,K1,0,A,CHISQR)
       WRITE(2,600)K
600    FORMAT(/,7X,'POLYNOMIAL FITTED IS OF THE DEGREE =',I3)
       WRITE(2,100)
100    FORMAT('0',9X,'XEXP',8X,'YEXP',8X,'YCAL',8X,'DY')
C
        ERROR=0.0
        DO 4 J=1,NP
        SUM=A(1)
        DO 5 I=2,K1
        SUM=SUM+A(I)*X(J)**(I-1)
5       CONTINUE
        YCAL(J)=SUM
        DELTAY(J)=YCAL(J)-Y(J)
       WRITE(2,200)X(J),Y(J),YCAL(J),DELTAY(J)
200    FORMAT('/',5X,4G12.5)
4       ERROR=ERROR+DELTAY(J)**2
        ERROR=ERROR/NP
       WRITE(2,500)
500    FORMAT(/,5X,'POLYNOMIAL CONSTANTS')
       DO 20 I=1,K1
       WRITE(2,250)I,A(I)
250    FORMAT(/,5X,'A(',I2,')=',G12.5)
20     CONTINUE
       IF(K .EQ. 1)CALL CORR(X,Y,NP,K,A,R)
       WRITE(2,300)ERROR
300    FORMAT(/,5X,'SUM OF DELTAY SQUARED DIVIDED BY THE'
     %' # OF POINTS =',G12.5)
3       CONTINUE
99      RETURN
        END
```

```
      SUBROUTINE POLIFI(X,Y,SIGMAY,NPTS,NTERMS,MODE,A,CHISQR)
C
C     EXTRACTED FROM: BEVINGTON,P. R., "DATA REDUCTION AND
C     ERROR ANALYSIS FOR THE PHYSICAL SCIENCES",
C     MCGRAW HILL, 1969.
C
C     SUBROUTINE POLIFIT PURPOSE
C
C     MAKE A LEAST-SQUARES FIT TO DATA WITH A POLYNOMIAL CURVE
C     Y = A(1) + A(2)*X + A(3)*X**2 + A(4)*X**3 + ...
C
C     DESCRIPTION OF PARAMETERS
C     X    -ARRAY OF DATA POINTS FOR INDEPENDENT VARIABLE
C     Y    -ARRAY OF DATA POINTS FOR DEPENDENT VARIABLE
C     SIGMAY - ARRAY OF STANDARD DEVIATIONS FOR Y DATA POINTS
C     NPTS   -NUMBER OF PAIRS OF DATA POINTS
C     NTERMS -NUMBER OF COEFFICIENTS(DEGREE OF POLYNOMIAL + 1)
C     MODE   -DETERMINANTS METHOD OF WEIGHTING LEAST-SQUARES FIT
C               +1 (INSTRUMENTAL) WEIGHT(I)=1./SIGMAY(I)**2
C                0 (NO WEIGHTING) WEIGHT =1.
C               -1 (STATISTICAL) WEIGHT(I) = 1./Y(I)
C     A  - ARRAY OF COEFFICIENTS OF POLYNOMIAL
C     CHISQR - REDUCED CHI SQUARE FOR FIT
C
C      SUBROUTINES AND FUNCTION SUBPROGRAMS REQUIRED
C       DELTERM (ARRAY,NORDER)
C       EVALUATES THE DETERMINANTS OF A SYMMETRIC
C       TWO-DIMENSIONAL MATRIX OF NORDER
C
      DOUBLE PRECISION SUMX,SUMY,XTERM,YTERM,ARRAY,CHISQ
      DIMENSION X(50), Y(50), SIGMAY(50), A(50)
      DIMENSION SUMX(50),SUMY(50),ARRAY(8,8)
C
C     ACCUMULATE WEIGHTING SUMS
C
11    NMAX = 2*NTERMS - 1
      DO 13 N=1, NMAX
13    SUMX(N) = 0.
      DO 15 J=1, NTERMS
15    SUMY(J)= 0.
      CHISQ =0.
21    DO 50 I=1, NPTS
      XI=X(I)
      YI= Y(I)
31    IF (MODE) 32,37,39
32    IF(YI) 35,37,33
33    WEIGHT = 1./YI
      GO TO 41
35    WEIGHT = 1./(-YI)
      GO TO 41
37    WEIGHT = 1.
      GO TO 41
39    WEIGHT = 1. / SIGMAY(I)**2
```

```
41      XTERM=WEIGHT
        DO 44 N=1,NMAX
        SUMX(N) = SUMX(N) + XTERM
44      XTERM = XTERM * XI
45      YTERM = WEIGHT*YI
        DO 48 N=1, NTERMS
        SUMY(N)=SUMY(N) + YTERM
48      YTERM = YTERM *XI
49      CHISQ = CHISQ + WEIGHT*YI**2
50      CONTINUE
C
C       CONSTRUCT MATRICES AND CALCULATE COEFFICIENTS
C
51      DO 54 J=1, NTERMS
        DO 54 K=1, NTERMS
        N = J + K - 1
54      ARRAY(J,K) = SUMX(N)
        DELTA = DETERM (ARRAY,NTERMS)
        IF(DELTA) 61,57,61
57      CHISQR = 0.
        DO 59 J=1, NTERMS
59      A(J) = 0.
        GO TO 80
61      DO 70 L=1, NTERMS
62      DO 66 J=1, NTERMS
        DO 65 K=1,NTERMS
        N = J+K-1
65      ARRAY(J,K)=SUMX(N)
66      ARRAY(J,L)=SUMY(J)
70      A(L)=DETERM(ARRAY,NTERMS)/DELTA
C
C       CALCULATES CHI SQUARE
C
71      DO 75 J=1, NTERMS
        CHISQ = CHISQ - 2.*A(J)*SUMY(J)
        DO 75 K=1, NTERMS
        N=J+K-1
75      CHISQ=CHISQ+A(J)*A(K)*SUMX(N)
76      FREE=NPTS-NTERMS
77      CHISQR=CHISQ/FREE
       WRITE(2,100)CHISQR
100    FORMAT('/',5X,'CHISQR =',G12.5/)
80      RETURN
        END
```

```
      FUNCTION DETERM(ARRAY,NORDER)
C
C     EXTRACTED FROM: BEVINGTON,P. R., "DATA REDUCTION AND
C     ERROR ANALYSIS FOR THE PHYSICAL SCIEINCES",
C     MCGRAW-HILL, 1969.
C
C     FUNCTION DETERM
C
C     PURPOSE
C     CALCULATES THE DETERMINANT OF A SQUARE MATRIX
C
C     DET = DETERM(ARRAY,NORDER)
C
C     DESCRIPTION OF PARAMETERS
C       ARRAY  -MATRIX
C       NORDER -ORDER OF DETERMINANT (DEGREE OF MATRIX)
C
C     SUBROUTINE AND FUNCTION SUBPROGRAMS REQUIRED
C     NONE
C
C     COMMENTS
C     THIS SUBPROGRAM DESTROYS THE INPUT MATRIX ARRAY
C
      DOUBLE PRECISION ARRAY,SAVE
      DIMENSION ARRAY(8,8)
10    DETERM =1.
11    DO 50 K=1, NORDER
C
C      INTERCHANGE COLUMNS IF DIAGNOL ELEMENT IS ZERO
C
      IF(ARRAY(K,K)) 41,21,41
21    DO 23 J=K, NORDER
      IF(ARRAY(K,J)) 31,23,31
23    CONTINUE
      DETERM = 0.
      GO TO 60
31    DO 34 I=K, NRODER
      SAVE = ARRAY(I,J)
      ARRAY(I,J)=ARRAY(I,K)
34    ARRAY(I,K)=SAVE
      DETERM = -DETERM
C
C     SUBTRACT ROW K FROM LOWER ROWS TO GET DIAGONAL MATRIX
C
41    DETERM = DETERM*ARRAY(K,K)
      IF(K - NORDER) 43,50,50
43    K1=K+1
      DO 46 I=K1, NORDER
      DO 46 J=K1,NORDER
46    ARRAY(I,J)=ARRAY(I,J)-ARRAY(I,K)*ARRAY(K,J)/ARRAY(K,K)
50    CONTINUE
60    RETURN
      END
```

```
      SUBROUTINE CORR(X,Y,NP,K,A,R)
C
C     written by:  Jeffrey C. Colish     4/1/83
C
C     purpose:  to calculate the correlation coefficient
C               of a linear plot
C
      DIMENSION X(50),Y(50),A(10)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      SUMX=0.0
      SUMX2=0.0
      SUMY=0.0
      SUMY2=0.0
      DO 1000 I=1,NP
      SUMX=SUMX + X(I)
      SUMX2=SUMX2 + X(I)**2
      SUMY=SUMY + Y(I)
      SUMY2=SUMY2 + Y(I)**2
1000  CONTINUE
      RR=SUMX2 - (SUMX**2)/NP
      RR=RR/(SUMY2 - (SUMY**2)/NP)
      R=A(2)*SQRT(RR)
      IF(ABS(R) .GT. 0.95)NSCORE(METH,NCOL)=NSCORE(METH,NCOL) + 1
      WRITE(2,375)R
375   FORMAT('0',5X,'THE CORRELATION COEFFICIENT = ',G12.5)
      RETURN
      END
```

```
      SUBROUTINE SCORE(LOOP,TLAG,TITLE)
C
C     written by:  Jeffrey C. Colish          6/1/83
C
C     purpose:  to provide a one page summary of
C               the results of the data regression
C
      DIMENSION Y(50),X(50),TITLE(40),A(10),TLAG(10)
      COMMON METH,NCOL,NSCORE(20,10),NFLAG
      WRITE(2,8980)
      WRITE(2,8981)(TITLE(I),I=1,40)
      WRITE(2,8982)
      WRITE(2,8984)
      WRITE(2,8986)
      WRITE(2,8988)
      WRITE(2,8989)
      WRITE(2,8990)
      WRITE(2,8992)
      WRITE(2,9000)
      WRITE(2,9002)
      WRITE(2,9004)
      WRITE(2,9002)
      WRITE(2,9000)
      WRITE(2,9006)(TLAG(I),I=1,LOOP)
      WRITE(2,9000)
      DO 1000 I=1,15
      WRITE(2,9008)I,(NSCORE(I,J),J=1,LOOP)
      WRITE(2,9000)
1000  CONTINUE
      WRITE(2,9500)
      WRITE(2,9502)
      WRITE(2,9504)
      WRITE(2,9506)
      WRITE(2,9508)
      RETURN
8980  FORMAT('1',15X,'Regression Scorecard')
8981  FORMAT('/',5X,40A2)
8982  FORMAT('0',15X,'METH # 1 = Zero-order equation')
8984  FORMAT('/',15X,'METH # 2 = Grau kinetic equation')
8986  FORMAT('/',15X,'METH # 3 = Equation # 4-23')
8988  FORMAT('/',15X,'METH # 4 = Henri equation')
8989  FORMAT('/',15X,'METH # 5 = Haldane equation')
8990  FORMAT('/',15X,'METH # 6 - 15 = Gates & Marler Method')
8992  FORMAT('/',30X,'with A= 0.1 - 1.0')
9000  FORMAT('/',10X,57('-'))
9002  FORMAT('/',10X,8(' ',7X))
9004  FORMAT('/',10X,'          ',17X,'# OF HR OF LAG',16X,' ')
9006  FORMAT('/',10X,'  Meth #',7(' ',F6.3,1X))
9008  FORMAT('/',10X,8('    ',I3,2X))
9500  FORMAT('0',10X,'Explanation of Regression Scorecard Codes')
9502  FORMAT('/',10X,'1 in units column = ABS(R) > 0.95')
9504  FORMAT('/',10X,'1 in tens column = rate constants have the
     % correct sign')
```

```
9506  FORMAT('/',10X,'1 in hundreds column = sum DY**2/NP < 5.0')
9508  FORMAT('/',10X,'A score of 0 = data failed to meet any
     % of the "good fit" criterior')
      END
```

```
**********************************
*                                *
* Sample Input to REGRESS        *
*                                *
**********************************


TABLE # 20 - RESULTS OF 2ND 100 PPM PHENOL RUN
8
0.0,133.687
0.333,121.007
0.667,103.234
1.0,91.888
1.333,43.417
1.667,31.503
2.0,15.270
2.333,0.0
133.687
1100.0
2
0.0
0.333
```

```
**********************************
*                                *
*  Sample Output from REGRESS    *
*                                *
**********************************
```

TABLE # 20 - RESULTS OF 2ND 100 PPM PHENOL RUN

NUMBER OF PTS = 8

X(I)	Y(I)
0.00000	133.68700
0.33300	121.00700
0.66700	103.23399
1.00000	91.88800
1.33300	43.41701
1.66700	31.50301
2.00000	15.27000
2.33300	0.00000

Intial substrate conc. = 133.68700 mg/l

Xo = 1100.00000 mg MLSS/l

LOOP = 2

Will regress data with 0.00000 hours lag

Will regress data with 0.33300 hours lag

TABLE # 20 - RESULTS OF 2ND 100 PPM PHENOL RUN
DATA REGRESSED TO A ZERO ORDER EQ.
REGRESSION PERFORMED WITH 0.00000 HOURS LAG TIME
CHISQR = 83.135
POLYNOMIAL FITTED IS OF THE DEGREE = 1

XEXP	YEXP	YCAL	DY
0.00000	133.69	139.51	5.8197
0.33300	121.01	118.95	-2.0536
0.66700	103.23	98.338	-4.8956
1.0000	91.888	77.785	-14.103
1.3330	43.417	57.232	13.815
1.6670	31.503	36.617	5.1138
2.0000	15.270	16.063	0.79345
2.3330	0.00000	-4.4899	-4.4899

POLYNOMIAL CONSTANTS
A(1)= 139.51
A(2)= -61.722
THE CORRELATION COEFFICIENT = -0.98626
SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 62.341
Kinetic Rate Constants
K = 61.72162 mg/l-hr
Tmax = 2.16597 hrs
Xo = 1100.00000 mg MLSS/l
Use Calc. Rate Const. to deter. Subs Conc v. Time

XEXP	YEXP	YCAL	DY
0.00000	133.69	133.69	0.00000
0.33300	121.01	113.13	-7.8733
0.66700	103.23	92.519	-10.715
1.0000	91.888	71.965	-19.923
1.3330	43.417	51.412	7.9951
1.6670	31.503	30.797	-0.70593
2.0000	15.270	10.244	-5.0262
2.3330	0.00000	-10.310	-10.310

THE SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 96.211

TABLE # 20 - RESULTS OF 2ND 100 PPM PHENOL RUN
DATA REGRESSED USING GRAU EQUATION
REGRESSION PERFORMED WITH 0.00000 HOURS LAG TIME
CHISQR = 0.54178E-01
POLYNOMIAL FITTED IS OF THE DEGREE = 1

XEXP	YEXP	YCAL	DY
0.33300	0.99652E-01	-0.13528	-0.23493
0.66700	0.25850	0.28435	0.25849E-01
1.0000	0.37493	0.70273	0.32780
1.3330	1.1246	1.1211	-0.35439E-02
1.6670	1.4454	1.5407	0.95321E-01
2.0000	2.1696	1.9591	-0.21049

POLYNOMIAL CONSTANTS
A(1)=-0.55366
A(2)= 1.2564
THE CORRELATION COEFFICIENT = 0.96647
SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 0.36120E-01
Kinetic Rate Constants
K = 0.15269 hr-1
Xo = 1100.00000 mg MLSS/l
Use Calc. Rate Const. to deter. Subs Conc v. Time

XEXP	YEXP	YCAL	DY
0.00000	133.69	133.69	0.00000
0.33300	121.01	87.981	-33.026
0.66700	103.23	57.829	-45.405
1.0000	91.888	38.058	-53.830
1.3330	43.417	25.047	-18.370
1.6670	31.503	16.463	-15.040
2.0000	15.270	10.834	-4.4356
2.3330	0.00000	7.1303	7.1303

THE SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 835.52

TABLE # 20 - RESULTS OF 2ND 100 PPM PHENOL RUN
DATA REGRESSED USING HENRI EQUATION
REGRESSION PERFORMED WITH 0.00000 HOURS LAG TIME
CHISQR = 0.28587E-01
POLYNOMIAL FITTED IS OF THE DEGREE = 1

XEXP	YEXP	YCAL	DY
38.078	0.29926	0.29762	-0.16410E-02
45.657	0.38756	0.48169	0.94132E-01
41.799	0.37493	0.38799	0.13062E-01
67.719	0.84370	1.0176	0.17387
61.298	0.86708	0.86161	-0.54720E-02
59.208	1.0848	0.81085	-0.27395

POLYNOMIAL CONSTANTS
A(1)=-0.62726
A(2)= 0.24289E-01
THE CORRELATION COEFFICIENT = 0.88805
SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 0.19058E-01
Kinetic Rate Constants
Km = -41.17104 mg/l
KoXo/Y = 25.82489 1/hr-l-mg biomass
Xo = 1100.00000 mg MLSS/l

TABLE # 20 - RESULTS OF 2ND 100 PPM PHENOL RUN
DATA REGRESSED USING HALDANE EQUATION
REGRESSION PERFORMED WITH 0.00000 HOURS LAG TIME
CHISQR = 99.677
POLYNOMIAL FITTED IS OF THE DEGREE = 2

XEXP	YEXP	YCAL	DY
0.00000	133.69	139.14	5.4500
0.33300	121.01	118.90	-2.1066
0.66700	103.23	98.497	-4.7369
1.0000	91.888	78.049	-13.839
1.3330	43.417	57.496	14.079
1.6670	31.503	36.775	5.2720
2.0000	15.270	16.010	0.74047
2.3330	0.00000	-4.8595	-4.8595

POLYNOMIAL CONSTANTS
A(1)= 139.14
A(2)= -60.612
A(3)=-0.47553
SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 62.283
Plot -dt/ds versus substrate conc.
CHISQR = 0.91038E-09
POLYNOMIAL FITTED IS OF THE DEGREE = 1

XEXP	YEXP	YCAL	DY
133.69	0.16498E-01	0.16464E-01	-0.33889E-04
121.01	0.16413E-01	0.16415E-01	0.19670E-05
103.23	0.16327E-01	0.16345E-01	0.17155E-04
91.888	0.16243E-01	0.16300E-01	0.56501E-04
43.417	0.16160E-01	0.16109E-01	-0.51107E-04
31.503	0.16078E-01	0.16062E-01	-0.15456E-04
15.270	0.15996E-01	0.15998E-01	0.21197E-05
0.00000	0.15916E-01	0.15938E-01	0.22665E-04

POLYNOMIAL CONSTANTS
A(1)= 0.15938E-01
A(2)= 0.39351E-05
THE CORRELATION COEFFICIENT = 0.98101
SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 0.10010E-08
Kinetic Rate Constants
K = 254120.50000 mg/l
Ki = 4050.26680 mg/l
Tmax = 2.16592 hrs
Xo = 1100.00000 mg MLSS/l
Use Calc. Rate Const. to deter. Subs Conc v. Time

XEXP	YEXP	YCAL	DY	INTR
0.00000	133.69	133.69	-0.15259E-04	1
0.33300	121.01	113.41	-7.5945	10
0.66700	103.23	92.977	-10.257	10
1.0000	91.888	72.503	-19.385	10
1.3330	43.417	51.926	8.5088	10
1.6670	31.503	31.183	-0.32008	10
2.0000	15.270	10.397	-4.8734	10
2.3330	0.00000	-10.497	-10.497	10

THE SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 93.137

TABLE # 20 - RESULTS OF 2ND 100 PPM PHENOL RUN
REGRESSION PERFORMED WITH 0.00000 HOURS LAG TIME
DATA REGRESSED USING GATES & MARLAR EQ.
AA = 0.001000
CHISQR = 0.33212E-01
POLYNOMIAL FITTED IS OF THE DEGREE = 1

XEXP	YEXP	YCAL	DY
0.37836E-01	0.29926	0.30711	0.78577E-02
0.44975E-01	0.38756	0.49661	0.10905
0.40949E-01	0.37493	0.38973	0.14804E-01
0.64835E-01	0.84370	1.0238	0.18005
0.58364E-01	0.86708	0.85200	-0.15073E-01
0.55957E-01	1.0848	0.78811	-0.29670

POLYNOMIAL CONSTANTS
A(1)=-0.69719
A(2)= 26.544
THE CORRELATION COEFFICIENT = 0.86857
SUM OF DY SQUARED DIVIDED BY THE # OF PTS = 0.22141E-01
Kinetic Rate Constants
Km = -41.15984 mg/l
Ko = 0.02531 hr -1
Xo = 1100.00000 mg MLSS/l
Y = 1.10000 mg biomass/mg substrate

Regression Scorecard

TABLE # 20 - RESULTS OF 2ND 100 PPM PHENOL RUN

METH # 1 = Zero-order equation
METH # 2 = Grau kinetic equation
METH # 3 = Equation # 4-23
METH # 4 = Henri equation
METH # 5 = Haldane equation
METH # 6 - 15 = Gates & Marlar Method with A= 0.1 - 1.0

	# OF HR OF LAG	
Meth #	0.000	0.333
1	11	11
2	11	11
3	11	11
4	0	0
5	11	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0

Explanation of Regression Scorecard Codes

A 1 in units column = ABS(R) > 0.95
A 1 in tens column = rate constants have correct sign
A 1 in hundreds column = sum DY**2/NP < 5.0
A score of 0 = data failed to meet any "good fit" criterion