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ABSTRACT

Title of Thesis : The Biodegradation of Phenolics Using Mixed Liquor from Passaic Valley Sewerage Commissioners Plant (Newark, New Jersey)

Daewon Pak, Master of Science, 1985

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

The biological degradation of phenol, O-chlorophenol, and 2,6-dichlorophenol was studied at room temperature in aerated 5 liter batch reactors using mixed liquor from Passaic Valley Sewerage Commissioners Plant (Newark, New Jersey). From the concentration versus time data, kinetic rate constants were determined for phenol (at 100 ppm), 2-chlorophenol (at 20 ppm), and 2,6-dichlorophenol (at 10 ppm). Air stripping and adsorption were determined to be insignificant removal mechanisms for the three compounds studied.

It was noted that the biodegradation rates increased after the organisms were acclimated to 100 ppm phenol. In addition, on substrate exposure, the degradation rate increased from the first to second runs. Conversely, the addition of amino acids decreased the rate of biodegradation for 2-chlorophenol and 2,6-dichlorophenol.

The Biodegradation of Phenolics Using Mixed Liquor from Passaic Valley Sewerage Commissioners Plant (Newark, New Jersey)

By

Daewon Pak

Thesis is 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
1985

APPROVAL SHEET

Title of Thesis : The Biodegradation of Phenolics Using Mixed Liquor from Passaic Valley Sewerage Commissioners Plant (Newark, New Jersey)

Name of Candidate : Daewon Pak
Master of Science, 1985

Thesis and Abstract Approved :

Dr. Gordon. A. Lewandowski — Date 5/3/85
Associate Professor
Department of
Chemical Engineering and
Chemistry

Dr. Basil C. Baltzis — Date 5/3/85
Assistant Professor
Department of
Chemical Engineering and
Chemistry

Dr. Joseph W. Bozzelli — Date 5/3/85
Professor
Department of
Chemical Engineering and
Chemistry

VITA

Name : Daewon Pak

Permanent Address :

Degree and Date to be conferred : Master of Science, 1985

Date of Birth :

Collegiate Institutions	Attended Date	Degree	Date of Degree
New Jersey Institute of Tech.	9/82-5/85	M.S.	May, 1985
Hong-Ik University	3/76-2/80	B.S.	Feb, 1980

Major : Chemical Engineering

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I. INTRODUCTION

Industrial discharge to Publicly Owned Treatment Works (POTW's) are presently controlled by categorical standards which essentially dictate the use of best available control technology. This involves an expense to industry that, in the case of organic compounds, may not always be necessary. Considerable data from previous investigators have at least indicated the significant capacity of POTW's to biodetoxify organic wastes. By "biodetoxify" is meant the catalytic oxidation (by microorganism) of the organic compounds to less objectionable end products. If credit is taken for such treatment, industry could realize a considerable cost saving, and thereby improve the conditions for economic development. This approach would be of particular benefit to small quantity generators. Futhermore, since the additional carbon (food) load on the POTW would be small compared to the normal sewage load, little additional sludge would be generated, and the incremental load on the sludge disposal system would be small.

An extensive literature survey of biodegradation has already been undertaken by Colish, Desai, and Gneiding, who recived more than 150 articles. It is apparent that there has not been a very rational approach to data collection in this field. Different types of reactors, different and usually undefined

microbial populations, undetermined products of decomposition, failure to consider compound solubility and vapor pressure, use of a wide variety of units and kinetic models, and terminology which varies from one discipline to another, have made it very difficult to draw conclusions and develope either a fundamental or even emperical picture of the limits of operability.

Two recent pilot-scale studies funded by the U.S. EPA [Petrasek(1983), and Gaudy and Kincannon (1982)] are notable additions to the data base in this field. Yet they also did not determine reaction products, microbial characteristics, or complete partition to air, sludge, and effluent.

The mixed microbial population used in the present study came from the Passaic Valley Sewerage Commissioners (PVSC) plant in Newark, N.J. This plant treats approximately 250 million gallons per day of waste, of which about 30 % comes from industrial sources.

II. LITERATURE REVIEW

A literature search was made to obtain the published results of other researchers who have investigated the ability of activated sludge in municipal wastewater treatment plants to treat toxic organic chemicals.

Petrasek and Kugelman (1983) used two parallel treatment sequences to quantify the behavior and partitioning of organic priority pollutants in a conventioal wastewater system. One treatment sequence was used as the control and the other sequence was spiked with toluene solution containing the 22 compounds of interest (4 pesticides, 3 phenols, 6 phthalates, and 9 polynuclear aromatic hydrocarbons). The sequence of processes used consisted of a sewer simulator, an aerated grit chamber, a primary clarifier and conventional plug flow activated sludge process, which were representive of typical municipal wastewater treatment systems. It was observed in the primary clarifier that partitioning between the wastewater and the sludge occurred, and resulted in a removal of organics by adsorption. The use of octanol/water partition coefficients was suggested as a means of estimating the partitioning of organic compounds to the primary sludge. A comparision of the influent and effluent data indicated that overall removals by the treatment sequence were greater than 97% for most of the compoumds tested.

Kincannon and Stover reported removal mechanisms for biodegradable and non-biodegradable toxic priority pollutants in industrial wastewater. They showed that the most important mechanisms were stripping and biodegradation. Adsorption was not an important mechanism, which contrasts with Petrasek's study. Aromatics were removed by a combination of stripping and biodegradation, and halogenated aliphatics were removed by stripping. Henry's law constants were used to explain the strippability of compounds. It was also found that the removal mechanisms operating in plug-flow systems might be different from those operating in a completely mixed system, with better removal achieved in a completely mixed reactor. It was suggested that microorganisms were in contact with a much higher concentration of pollutant in a plug-flow reactor, and these higher concentrations might cause an inhibition. They also concluded that the removal of specific organic compounds during biological treatment could be significantly impacted by treatment with strong oxidizing agents such as ozone prior to biological treatment.

Petrasek (1981) reported the removal and partitioning of the volatile priority pollutants in conventional wastewater treatment plants consisting of a primary clarifier and a plug-flow activated sludge processes. The removability was good, with TSS and COD removals averaging 94% and 85%, respectively. He also tried to predict the behavior of organic priority pollutants based on knowledge of their structure and physical/chemical

properties such as Henry's law constants and octanol/water partition coefficients. Although the correlation between those parameters was not perfect, higher Henry's law constant generally results in greater stripping rates, and higher octanol/water partition coefficients results in a higher degree of adsorption to biological solids. He suggested that after the development of a more extensive data base, an effort should be made to model the partitioning and removal of organic priority pollutants.

Pellizzari (1982) made an attempt to correlate emissions of organic compounds from biological aeration basins with their aqueous concentrations, and investigated the influence on volatility of the sorption of the compounds on sludge solids. He observed that the concentrations of the compounds in the off-gas were higher at the front end of the aeration basin than at either the middle or back end of the basin, whereas the concentrations in the mixed liquor showed no change across the aeration basin. He concluded that the presence of solids affected the volatility (via a sorption mechanism), and that a simple Henry's law relationship could not be used.

Bishop and Petrasek (1981) studied the pilot scale primary/secondary treatment of raw wastewater spiked with selected priority pollutants. The treatment plant performance on spiked wastewater was compared to its performance using unspiked raw wastewater. The results illustrated that conventional treatment was generally effective in removing selected toxic substances, typically achieving better than 90%

removal of organic and 60-80% removal of the metals. However, certain compounds, most notably lindane, bis-phthalate, phenol, and di-n-butylphthalate, were present in the activated sludge effluent in relatively significant concentration. The three phenols studied were 2,4-dimethylphenol, phenol, and pentachlorophenol. They concluded that 2,4-dimethylphenol and phenol were relatively biodegradable, but pentachlorophenol passed through the treatment plant with little decomposition.

Bishop (1982) also evaluated health and ecosystem effects of effluents from a municipal treatment system. The unspiked raw wastewater exhibited moderately acute toxicity which increased when the priority pollutants were added. Conventional treatment systems essentially eliminated the acute toxicity of the raw wastewater, and reduced (but did not eliminate) the acute toxic effects of the effluent from the spiked wastewater system.

Grady (1983) studied the kinetics of multicomponent substrate removal in suspended growth reactors. Models for the kinetics of biodegradation were divided into two catagories: interactive and non-interactive. Interactive models are based on the premise that one substrate will influence the degradation rate of another. Non-interactive models, on the other hand, assume that the portion of a population carrying out biodegradation of the target substrate is not influenced by the presence of other substrates. He found that the interactive model was more applicable to conventional treatment systems. He also suggested that another important situation which was likely

to be found in the biodegradation of compounds was cometabolism. In other words, a substrate that cannot be used as a sole source of carbon and energy, and would not undergo biodegradation if it were present alone, may be degraded if another utilizable substrate is present.

Gaudy and Kincannon (1982) studied the effects of organic priority pollutants on the performance of activated sludge units at publicly owned treatment works. Batch and continuous flow bench scale activated sludge pilot plants were fed settled municipal sewage. The general approach was to compare the performance of control systems with that of comparable systems dosed with various concentrations of priority pollutants. Twenty-four compounds were studied in batch pilot plants, and eight of these were also studied in continuous flow pilot plants operated at a sludge age of 5 days. Four of the eight were also studied in extended aeration pilot plants. In batch units, operated on a 24 hour fill-and-draw schedule, only pentachlorophenol and 2-chlorophenol caused increases in soluble residual COD at feed concentrations of 5 mg/l. At a feed concentration of 25 mg/l and higher, 4-chloro,3-methyl phenol showed metabolic disturbance. None of the eight compounds tested in continuous flow at a sludge age of 5 days showed increased effluent soluble COD at the 5 mg/l dosage. However, the effluent of the units dosed with phenol and methylene chloride did show increased suspended solids concentrations. Higher dosage levels of several compounds led to higher soluble

COD and suspended solids concentrations in the effluents. Effluents from the extended aeration process were lower in soluble COD and suspended solids than effluents from the other systems. Among the reasons for such results with extended aeration may be a lower mass loading rate and longer solids retention time. The reason for the lower suspended solids concentration is probably due to generally greater abundance of protozoa. In the batch and continuous systems, an increase in dosage of the test compound appeared to cause more serious reduction in protozoan activity than in the extended aeration system. From the analysis made for specific compounds, there was no evidence for excessive pass through of priority pollutants on publicly owned treatment works. Most of the test compounds were removed by either stripping, biodegradation, or attachment to the surface of the biological solids. It was concluded that batch pilot plant operations, although more easily facilitated, cannot be used in place of continuous flow pilot plant studies as a means of gaining information on which to base pretreatment policy regulations. The major analytical techniques employed were COD, supended solids concentration, and specific compound concentration measured by gas chromatography.

Leeuwen (1983) evaluated at various stages of the process the efficiency of municipal plants in the treatment of domestic and industrial waste by analyzing for about 60 organic and inorganic pollutants with possible health implications . It was found that many contaminants could be removed to a large extent

with lime and ferric chloride precipitation before biological treatment. In addition, the precipitation further protected the biological system.

Kincannon (1981) conducted two types of experimental studies to compare the air stripping of compounds in biological and nonbiological systems. Activated sludge obtained from a local municipal treatment plant was preacclimated to a synthetic wastewater containing the single pollutant to be evaluated. The chemical compounds studied were 1,2-dichloropropane, methylene chloride, benzene, ethyl acetate, 1,2-dichloroethane, phenol, and 1,2-dichlorobenzene. In the nonbiological stripping studies, it was observed that all chemicals except phenol were highly stripped. 96-100 % stripping was obtained with ethyl acetate and 1,2-dichloropropane, methylene chloride, benzene, and 1,2-dichloroethane. 80-85 % stripping was observed with ethyl acetate and 1,2-dichlorobenzene. A comparision of stripping in the biological and nonbiological systems show that the fraction stripped to the air of 1,2-dichloropropane, 1,2-dichloroethane, and phenol were approximately the same in both systems. However, it was observed that the other compounds were highly stripped in the nonbiological system and only slightly stripped in the biological systems. In addition, this study concluded that organics that were biodegraded were removed to lower effluent concentrations than compounds removed by stripping alone.

III. OBJECTIVE

The specific objective of this study was to obtain biokinetic rate constants for the biological degradation of phenol, O-chlorophenol, and 2,6-dichlorophenol (2,6-DCP), using a mixed liquor from the Passaic Valley Sewerage Commissioners (PVSC) treatment plant in Newark, New Jersey. This is the largest municipal treatment plant in New Jersey, which routinely receives in its industrial wastes phenolic compounds at concentrations below 100 ppb. It would be desireable to determine the upper level of treatability for these compounds. Rate constants were evaluated from experimental data of substrate concentration vs. time. Ammonia concentration, pH, mixed liquor suspended solids (MLSS) concentration, and chemical oxygen demand (COD) were also monitored during the course of the experiments.

Samples have been preserves for GC/MS analysis. But since a new GC/MS is only now being set up, these analysis will have to be postponed for future work by other.

IV. EXPERIMENTAL APPARATUS AND ANALYTICAL EQUIPMENT

Five-liter cylindrical batch reactors (constructed of lucite) were used in the experiments. Each had a lid with a 3/16" vent hole at the center. Laboratory compressed air was supplied to all the reactors after passing through an activated carbon and glass wool filter, and 1/4" tygon tubing ending in an aquarium diffuser stone. The air flow rate was controlled by rotameters and was usually held constant at 1.0 scfh (500 cc/min). The reactor contents were essentially saturated with oxygen throughout the experiments, and all experiments were run at room temperature.

The analytical equipment consisted of the following :

1. Gas Chromatograph : Tracor 560

   Operating Temperature

Injection	-	300°C
Detector	-	300°C
Oven	-	Substrate Dependent

   Gas Flow Rate

Nitrogen	-	40 cc/min
Hydrogen	-	30 cc/min
Air	-	400 cc/min

2. Automatic Sampler : Tracor, model #770

3. Automatic Injector : Varian, Aerograph

4. G. C. Column : Varian, 6' 1/8" SS 10% SP2100 on 100/200 Supelcoport

5. Electronic Integrator : Hewlett-Packard 3390A

6. pH Meter : Orion Research Model #701/Digital Ionalyzer

7. pH Electrode : Orion Research, Model #91-04

8. Ammonia Electrode : Orion Research, Model #95-10

9. COD Reactor : Hach, Model #16500-10

10. Centrifuge : Damon/Iec, Model #IEC HN-SII

V. EXPERIMENTAL PROCEDURE

A. Air Stripping

Stripping (or adsorption) can be a major removal mechanisms for organic chemicals, and any biodegradation study must be accompanied by examination of these two possible means of substarate disappearance.

Air stripping data, obtained by Colish and Desai, served as the references in the present work. The following procedure was carried out by them.

2.5 liters of deionized water were boiled for 1/2 hours and 2 liters were poured into the reactor. The reactor was covered and water allowed to cool overnight. On the next day, each reactor was spiked to 20 ppm of 2-chlorophenol, or 20, 30, or 40 ppm of 2,6-dichlorophenol, and the air turned on at a flow rate of 500 ml/min. The air passed through a glass wool filter and rotameter before entering the reactor. One or two samples per day were taken over a two week priod. From these experiments, substrate stripping was found to be insignificant during the biodegradation run for all of the compounds examined in the present work.

B. Acclimation of PVSC Sludge to Phenol

Activated sludge was obtained from the Passaic Valley Sewarage Commisioners (PVSC), municipal wastewater treatment

plant in Newark, New Jersey. The PVSC plant, located in a industrial area, treats approximately 250 million gallons per day of a waste that is about 30% industrial and 70% domestic. The plant uses oxygen (rather than air) in its activated sludge system.

The sample of mixed liquor was taken from the monitoring laboratory of the plant. A 10-liter bucket was used for transport of samples. As soon as the mixed liquor was brought to the laboratory, 2 liters were poured into a reactor and immediately provided with air. Before biodegradation runs, a 10 ml sample was taken to measure the fresh sludge concentration, which averaged about 5300-5500 mg dry solids/liter.

A 10,000 ppm phenol stock solution was used to acclimate the sludge to 100 ppm phenol. It contained nitrogen and phosphorus as inorganic nutrients in the form of ammonium carbonate and ammonium phosphate. The solution had a carbon : nitrogen : phosphorus mass ratio of approximately 50 : 14 : 3, and consisted of 1.805 ammonium phosphate, 6.64 g ammonium carbonate, and 10.0 g phenol in 1 liter of distilled water. The reactor was spiked with stock solution to 100 ppm phenol. On the next day, samples were taken from the reactor and the phenol concentration was measured by G.C. If the concentration was greater than 1 ppm, the phenol concentration was monitored until it become less than 1 ppm. The reactor was spiked three times in this fashion with stock solution to acclimate the sludge.

C. Phenol, 2-chlorophenol, and 2,6-DCP Degradation Runs

Two liter of PVSC sludge at room temperature (21-26°C) were placed in the reactor, which was then spiked with one of the compounds. The initial, nominal concentrations were: phenol-100 ppm, 2-chlorophenol-20 ppm, 2,6-DCP-10 ppm. These are in the range of concentration where microorganism can tolerate. A sample was taken every one to two hours depending on the substrate studied until the concentration fell below about 1 ppm. The reactor was then spiked again and samples taken as before. Additional experiments were run in which 10 ppm of total amino acids were added to examine their effect on substrate degradation. These might be used as a cosubstrate or to synthesize the necessary enzymes. The amino acids used were the following at a concentration of 2 ppm each.

1) L-Glutamic Acid
2) L-Histidine Hydrochloride Monohydrate
3) L-Lysine Monohydrochloride
4) L-Arginine Hydrochloride
5) L-Cystein

The following runs were made for phenol

1) Two runs of 100 ppm phenol with fresh sludge
2) Two runs of 100 ppm phenol with fresh sludge and 10 ppm amino acids (2 ppm each)

The following runs were made for 2-chlorophenol. The term "acclimated" or "unacclimated" is used in relation to acclimation

to 100 ppm phenol.

1) Two runs of 20 ppm 2-chlorophenol with unacclimated sludge and no amino acids
2) Two runs of 20 ppm 2-chlorophenol with unacclimated sludge and 10 ppm amino acids (2 ppm each)
3) Two runs of 20 ppm 2-chlorophenol with acclimated sludge and no amino acids
4) Two runs of 20 ppm 2-chlorophenol with acclimated sludge and 10 ppm amino acids (2 ppm each)

The following runs were made for 2,6-DCP. The term "acclimated" or "unacclimated" is used in relation to acclimation to 100 ppm phenol.

1) Two runs of 10 ppm 2,6-DCP with unacclimated sludge and no amino acids
2) Two runs of 10 ppm 2,6-DCP with unacclimated sludge and 10 ppm amino acids (2 ppm each)
3) Two runs of 10 ppm 2,6-DCP with acclimated sludge and no amino acids
4) Two runs of 10 ppm 2,6-DCP with acclimated sludge and 10 ppm amino acids (2 ppm each)

VI. ANALYTICAL PROCEDURE

A. Substrate Analysis

The method of substrate analysis used for the three compounds were similar. After spiking, a 15 ml sample was taken from the reactor every one to two hours until the substrate concentration could no longer be detected. The samples were centrifuged for 4 min, and 10 ml of supernatant were added to a sample vial containing 0.5 ml of internal standard and 0.5 ml of 20,000 ppm copper sulfate. The copper sulfate served as a biocide to stop the reaction. The internal standard used for phenol and 2-chlorophenol was 1000 ppm thymol, and for 2,6-DCP was 100 ppm 1-chloro-2,4-dinitrobenzene. When 2,6-DCP was used as substrate, the amount of internal standard increased to 2 ml since concentration of internal standard is so low. Sample vials were stored in the refrigerator until they could be analyzed by gas chromatography.

The oven temperature of the G.C. depended on the substrate. It was 140^{o}C for phenol, 145^{o}C for O-chlorophenol, and 165^{o}C for 2,6-DCP. The sample injection volume was 3 microliter for all three compounds. The attenuations for phenol, 2-chlorophenol, and 2,6-DCP were 3, 3, and 1 respectively. The retention time was 0.8 min for phenol, 0.85 min for 2-chlorophenol, and 1.2 min for 2,6-DCP. A variation of about +/- 10% was found in the retention times.

B. Hydrogen-Ion Concentration

A pH electrode was used to monitor the hydrogen ion concentration, and pH measurements were recorded whenever a sample was taken. Occasionally, the electrode was removed from the reactor as a check on possible electrode drift and placed in a buffer solution of pH 7. After being adjusted by using the calibration control, the electrode was rinsed with distilled water and placed back in the reactor.

C. Mixed Liquor Suspended Solids

A 10 ml sample was withdrawn from the reactor every one or two hours during each experiment. The fluid was pipetted to numbered and preweighted aluminum dishes. The dishes were then dried at 95 C for 24 hours, and then reweighed. The weight difference was used to calculate the dry solids concentration.

D. Ammonia Concentration

The concentration was measured using an ammonia gas electrode. The manufacture's (Orion) recommended procedure was used. A 0.1 M ammonia chloride standard solution was prepared by adding 0.535 g reagent-grade ammonium chloride to 50 ml distilled water in a 100 ml volumetric flask, stirring to dissolve, and then diluting to volume with distilled water.

Standard solution of 0.01 M, 0.001 M, and 0.0001 M were made by serial dilution of the 0.1 M solution.

The electrode was placed in the 0.001 M standard and 1 ml of 10 M sodium hydroxide was added to raise the pH and liberate free ammonia. The function switch was set to relative millivolts, and the reading was set to 000.0 by adjusting the calibration control. A magnetic stirrer was used throughout the procedure. The electrode was rinsed and placed in the 0.0001 M standard, and 1 ml of 10 M sodium hydroxide was again added. The reading was recorded when it become stable. Other reacings were obtained for the 0.001 M, 0.01 M, and 0.1 M standards by repeating the same procedure.

A calibration curve was prepared by plotting the relative millivolt readings (linear axis) against ppm (log axis) on standard 4-cycle semilogarithmic paper.

It was necessary to dilute the sample initially with distilled water, since only a limited volume of sample was available. 1 ml of sample was pipetted into a sample vial with 9 ml of distilled water. 2 eyedrops of 10 M sodium hydroxide were added. The electrode was placed in the vial with a magnetic bar. The relative millivolts were recorded when the reading stabilized. The experimental value of ammonia concentration was determined from the calibration curve. Every two hours, the electrode was withdrawn and placed in 0.001 M ammonium chloride to check its accuracy.

E. Chemical Oxygen Demand (COD)

The chemical oxygen demand was measured to estimate the amount of organic and oxidizable inorganic matter in a sample. Chemical oxygen demand analysis was performed on the phenol degradation samples to determine if complete mineralization of the compound occurred, or if intermediates were produced.

The theoretical chemical oxygen demand of phenol and thymol can be calculated from a balanced equation for the total oxidation of phenol and thymol to carbon dioxide and water.

$$C_6H_5OH + 7\ O_2 \rightarrow 6\ CO_2 + 3\ H_2O$$
$$C_{10}H_{13}OH + 13\ O_2 \rightarrow 10\ CO_2 + 7\ H_2O$$

From these balanced chemical equations, the theoretical COD (mg oxygen/1000 . mg compound) of phenol and thymol was calculated to be 2380 and 1680 respectively.

The procedure used in this study was a modification of the standard method described in the Federal Register (see references).

A digestion solution was made by adding 7.5 g potassium dichromate, 10.0 g silver sulfate, and 5.0 g mercuric sulfate to a 2.5 l bottle of concentrated sulfric acid. The bottle with magnetic stirrer was placed on a hot plate and heated overnight to dissolve the potassium dichromate and silver sulfate. After both compounds had been 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 16 mm * 100 mm screw-top vials. 2 ml of filtered sample were added and the cap

was screwed on tightly. Slowly the vial was inverted several time to mix. Several blanks containing 2 ml deionized water were also run. Samples were heated for 2 hours at 150°C in a Hach reactor. The vials were removed from the reactor and cooled to room temperature. The contents of each vial was transferred to a 250 ml Erlenmeyer flask that contained approximately 50 ml water (rinsing the inside of the vial several times with water and adding the rinsings to the flask). This solution was then titrated to a bright orange endpoint with 0.0125 N ferrous ammonium sulfate solution (FAS).

The 0.0125 N solution was made by adding 9.8 g ferrous ammonium sulfate (FAS) to approximately 1000 ml deionized water, adding 20 ml concentrated sulfric acid, cooling the solution to room temperature, and finally diluting to 2.0 l with deionized water.

To determine the COD of the sample, the following equation was used.

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

where

A = Volume of FAS used to titrate blank

B = Volume of FAS used to titrate sample

N = Normality of FAS solution

C = Volume of sample ml

VII. RESULTS AND DISCUSSION

The results of the biodegradation runs, listed in Tables 1 to 19 and plotted in Figures 2 to 16.

A. Adsorption

Adsorption is a much more rapid phenomenon than biodegradation, and therfore should appear as a steeper slope in substrate removal curves. Once the surface is saturated, the substrate disappearance curves should reflect biodegradation only. This would imply that the first run would have a steeper slope than the second if adsorption were significant. Since the opposite is true, this would imply that adsorption is not significant for the compounds studies.

B. Hydrogen-ion Concentration

The pH data are plotted in Figures 22, 25-26, and 30-31. The general trend of the pH, in all runs, was to remain relatively constant, never changing by more than +/- 0.15 pH units during a given run. Also, the pH for all runs always remained in the range of 6.8-8.2.

C. Mixed Liquor Suspended Solids

The mixed liquor suspended solids (MLSS) data is included in Tables 1 to 19 and plotted in Figures 21,24, and 29. All the data indicate a roughly constant MLSS during each experiment.

D. Ammonia Concentration

The initial amounts of nitrogen presented in PVSC sludge ranged from 27 ppm ammonia for phenol to 68 ppm ammonia for O-chlorophenol. At these levels, the nutrient requirements of the microorganisms can be adequately satisfied. Although the correlation between the ammonia concentration and time was not clear, the general tendency was a decrease of the ammonia concentration as the substrate was metabolized.

E. Chemical Oxygen Demand

The results of the COD analysis for phenol are listed in Tables 1 - 4 and Figures 34 through 37. These show that COD decreased with substrate concentration and indicate nearly complete mineralization of phenol. The error for the COD method is about +/- 20 ppm. When the substrate concentration falls below this level, the results of COD measurement are unreliable. This probably explains the leveling off of COD results in Figures 36 and 37. Since the concentrations of 2-chlorophenol and 2,6-DCP were always below 20 ppm, COD determination for these substrates was not attempted.

F. Substrate Concentration and Kinetics

Raw kinetic data, in the form of substrate concentration versus time, for phenol, 2-chlorophenol, and 2,6-DCP runs are presented in Tables 1 to 19 and Figures 2 through 20.

Four mathmatical models were used to correlate the experimental data ; zero-order, first-order, Monod , and Haldane (the last two assuming constant biomass). A linear regression program (see Appendix) was used to determine the kinetic rate constants. The degree of fit was evaluated using the average absolute residual, calculated from

$$\text{Absolute average residual} = \frac{(Ccal - Cexp)^2}{Np - 1}$$

where

Ccal = Concentration calculated from kinetic model

Cexp = Concentration obtained from experiment

Np = Number of data

The zero-order kinetic model assumes that the rate of substrate disappearance, dS/dt, is constant and independent of substrate concentration. In differential form, it is given by :

$$-dS/dt = K$$

or, in integrated form :

$$So - S = Kt$$

where

S = Substrate concentration at time t (mg/l)

So = Initial substrate concentration (mg/l)

K = Zero-order kinetic rate constant (mg/l hr)

t = Time (hr)

The first-order kinetic model assumes that the rate of substrate disappearance, -dS/dt, is proportional to substrate

concentration. In differential form, it is given by :

$$-dS/dt = KS$$

or, in integrated form :

$$\ln So - \ln S = Kt$$

where

S = Substrate concentration at time t (mg/l)

So = Initial substrate concentration (mg/l)

K = First-order kinetic rate constant (1/hr)

Using the monod model, assuming a constant biomass concentration, the rate of substrate utilization is :

$$-dS/dt = K1*S/(K2 + S)$$

or, in integrated form :

$$(So - S) + K2*(\ln So - \ln S) = K1*t$$

where

S = Substrate concentration at time t (mg/l)

So = Initial substrate concentration (mg/l)

K1 = Rate constant (mg/l hr)

K2 = Substrate utilization constant (mg/l)

t = Time (hr)

The Haldane model for substrate inhibition kinetics, when a constant biomass concentration is assumed, is given by :

$$-dS/dt = K1*S/(K2 + S + S/K3)$$

or, in integrated form :

$$(So - S) + K2*(\ln So - \ln S) + 1/2K3*(So^2 - S^2) = K1*t$$

where

S = Substrate concentration at time t (mg/l)

So = Initial substrate concentration (mg/l)

K1 = Kinetic rate constant (mg/l hr)

K2 = Substrate saturation constant (mg/l)

K3 = Inhibition constant (mg/l)

t = time (hr)

Generally, the expression for zero-order kinetics best represents the rates of substrate utilization for phenol, 2-chlorophenol, and 2,6-dichlorophenol. First-order kinetics also show a capability of fitting the experimental data. The absolute average residual of the zero-order equation for phenol run is 3.4-246.31, for 2-chlorophenol is 0.36-5.25, and for 2,6-dichlorophenol is 0.029-0.53. The absolute average residual of the first-order equation for phenol run is 31.9-470.15, for 2-chlorophenol is 0.11-4.14, and for 2,6-dichlorophenol is 0.04-55.94. Generally, the absolute average residual of the zero-order kinetic equation is less than that of the first-order kinetic equation (Table 20-22).

The Haldane and Monod equations very often represent the degradation data with relatively small absolute average residuals, but one or more of their kinetic constants are negative. This makes the constants physically meaningless.

With amino acids added, the rate of substrate degradation for 2-chlorophenol and 2,6-dichlorophenol is decreased by about 20 %, based on zero order rate constants (Fig 4, 9-10, 17-18).

In the case of 2-chlorophenol runs with acclimated sludge, the rate of substrate degradation (zero order rate constant) is increased by 20 %, but the first order rate constants is decreased.

Using acclimated sludge, the rate of substrate degradation (zero order constant) for O-chlorophenol is increased by a factor of 30 - 40 and for 2,6-DCP increased by about 30 % in comparision to using unacclimated sludge (Fig 11-12, 19-20).

From a comparision of the first and second runs of each substrate, the rate of substrate degradation (zero-order rate constant) of the second run increased by a factor of 3 to 5 over that of the first run.

VIII. CONCLUSION

1. PVSC sludge by itself can significantly degrade phenol, 2-chlorophenol, and 2,6-DCP at concentrations up to 100 ppm, 20 ppm, and 10 ppm, respectively.
2. For 2-chlorophenol and 2,6-dichlorophenol at initial concentrations of 20 ppm and 10 ppm respectively, the addition of amino acids decreased the degradation rate by about 20 % since amino acids was used before substrate.
3. The degradation rate for 2-chlorophenol increased by a factor of 30-40, and for 2,6-dichlorophenol is by 30 % when the sludge was previously acclimated to 100 ppm phenol.
4. The substrate degradation rates increased by a factor of 3 to 5 between the first and second runs
5. The zero-order kinetic model can successfully represent phenol, 2-chlorophenol, and 2,6-DCP degradation data.
6. The first-order kinetic model also was capable of fitting the experimental data of phenol, 2-chlorophenol, and 2,6-dichlorophenol.
7. Both the Monod and Haldane models were unable to describe the phenol, 2-chlorophenol, and 2,6-DCP degradation data, since regression of data yielded negative values for the rate constants.

IX. REFERENCE

1. Bishop D. F., "The Role of Municipal Wastewater Treatment in Control of Toxics", presented at the NATO/CCMS Meeting (September 28-30, 1982)
2. Bishop D. F., Petrasek A. C., "Control of Specific Organic and Metal Contaminants by Municipal Wastewater Treatment Processes" (1982)
3. Colish J. "Biodegradation of Phenol and O-chlorophenol Using Activated Sludge Bacteria", M.S. thesis, N.J.I.T., Newark, NJ (1984)
4. Desai S., "Kinetics of Biodegradation of Phenol and 2,6-dichlorophenol", M.S. thesis, N.J.I.T. (1983)
5. Federal Registar, (4/21/80), P. 26811
6. Gaudy A. F. Jr., Gaudy E. T., Microbiology, Mcgraw Hill, 1980
7. Gaudy A. F. Jr., Kincannon D. F., "Treatment Compartibility of Municipal Waste and Biologically Harzardous Industrial Compounds", EPA-600/S2-82-075 (November, 1982)
8. Kincannon D. F., Stover E. L., "Removal Mechanisms for Biodegradable and Nonbiodegradable Toxic Priority Pollutants in Industrial Wastewaters" (1981)
9. Kincannon D. F., Stover E. L., "Biological Treatment of Organic Compounds Found in Industrial Aqueous Effluents", presented at ACS national meeting, Atlanta, GA (March 29 - April 3, 1981)

10. Leslie Grady Jr. C. P., "Multicomponent Substrate Removal in Suspended Growth Reactors", 1983 AICHE Diamond Jubilee Conference (October 31 - November 4, 1983)

11. Lewandowski G. A., Personal Communication, N.J.I.T. Newark, NJ (1983)

12. Monod J., "The Growth of Bacterial Cultures", Annual Review of Microbiology, Vol 3, P 371 (1949)

13. Orion Research Incorporated, "Instruction Manual, Ammonia Electrode, Model 94-17B"

14. Petrasek A. C., Kugelman I. J., "Fate of Toxic Organic Compounds in Wastewater Treatment Plants", JWPCF Vol 55, No 10, October, 1983

15. Petrasek A. C., "Removal and Partitioning of the Volatile Priority Pollutants in Conventional Wastewater Treatment Plant", MERL Cincinnati, Ohio (October, 1981)

16. Pellizzari E. D., "Volatile Organics in Aeration Gases at Municipal Treatment Plants", EPA-600/S2-82-056 (August 1982)

17. Short T. E., "Proceedings of the Conference on Combined Municipal/Industrial Wastewater Treatment", EPA-600/89-81-021 (Feb. 1983)

18. Stover E. L., "Biological Treatability of Specific Organic Compounds Found in Chemical Industry Wastewaters", presented at the 36th Purdue Industrial Waste Conference, (May 12 - 14, 1981)

19. Sundstorm D. W., Klei H. E., Wastewater Treatment, Prentice-Hall Inc., Englewood Cliffs, NJ (1979)

Table #1 - First 100 ppm Phenol Biodegradation Run
(No Amino Acid, Temp - 22°C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	PH	Ammonia Conc (ppm)	COD (mg/l)	Substrate Conc (ppm)
AM 11:00	0:00	4026	7.48	27.2	102.5	99.09
PM 12:00	1:00	3962	7.53			96.76
1:00	2:00	3754	7.54			94.48
2:00	3:00	3505	7.55			91.48
3:00	4:00	3270	7.55	27.3	87.8	86.22
4:00	5:00	3545	7.57			82.67
5:00	6:00	3268	7.58			76.66
6:25	7:25	3595	7.58	28.3	71.0	67.93
AM 9:45	22:45	3393	7.67			10.66
11:00	24:00	3506	7.79	17.5	11.1	7.54

* Experiment date : May 31, 1984

Table #2 - Second 100 ppm Phenol Biodegradation Run
(No Amino Acid, Temp - 21°C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	COD (mg/l)	Substrate Conc (ppm)
AM 10:00	0:00	7.82	16.1	105.5	104.59
11:00	1:00	7.82		86.8	85.22
PM 12:00	2:00	7.77		66.8	60.49
1:00	3:00	7.73	10.2	22.75	22.12
2:00	4:00	7.67		8.61	6.62
3:00	5:00	7.58	5.3		4.78
4:00	6:00	7.49			4.05
5:00	7:00	7.68	3.06	6.51	0.79

* Experiment date : June 1, 1984

Table #3 - First 100 ppm Phenol Biodegradation Run
(10 ppm Amino Acid, Temp - 24°C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	COD (mg/l)	Substrate Conc (ppm)
PM 3:00	0:00	5220	7.5	26.8	111.9	108.27
4:00	1:00	4800	7.58		95.9	99.43
5:00	2:00	4890	7.55		82.56	73.73
6:00	3:00	4950	7.47		56.73	53.73
7:00	4:00	4540	7.48		43.23	24.76
8:00	5:00	4300	7.72	21.2	38.4	12.62
AM 9:15	18:15	4840	8.02	42.7	32.1	7.2
10:00	19:00	4620	8.01			6.76
11:00	20:00	4710	8.04			5.83
PM12:00	21:00	4550	8.06			5.3
1:00	22:00	4630	8.08			4.36
2:00	23:00	4520	8.11			1.09
3:00	24:00	4600	8.13	39.0	30.0	0.72

* Experiment date : Oct 1, 1984

Table #4 - Second 100 ppm Phenol Biodegradation Run
(10 ppm Amino Acid, Temp - 24°C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	COD (mg/l)	Substrate Conc (ppm)
AM 9:55	0:00	8.43	41.9	98.3	96.37
10:55	1:00	8.35			87.69
11:55	2:00	8.24		85.84	80.69
PM 12:55	3:00	8.17			67.89
1:55	4:00	8.14	40.4	50.00	46.26
2:55	5:00	8.13		31.61	20.34
3:55	6:00	8.24			9.83
4:55	7:00	8.29			7.36
5:55	8:00	8.32	34.9	24.7	6.03

* Experiment date : Oct 3, 1984

Table #5 - First 20 ppm 2-chlorophenol Biodegradation Run
(Unacclimated Sludge, No Amino Acid, Temp - 22°C,)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
PM 12:25	0:00	4064	7.44	25.4	18.49
2.25	2:00	3184	7.65		16.50
4:25	4:00	3427	7.65		15.58
6:25	6:00	4008	7.76	31.2	14.44
AM 8:25	20:00	4184	7.92	45.9	8.77
10:25	22:00	4053	7.86		7.77
PM 12:25	24:00	3751	7.86		6.75
2.25	26:00	4077	7.87		6.41
4:25	28:00	4085	7.89		5.76
6.25	30:00	3812	7.92	51.3	5.68
AM 10:25	46:00	4106	7.92	61.5	3.66
PM 12:25	48:00	4225	7.93		3.00
2:25	50:00	4168	7.92		2.93
4:25	52:00	3892	7.95		2.60
6:40	54:15	4333	8.00	49.3	2.08

* Experiment date : May 29, 1984

Table #6 - Second 20 ppm 2-chlorophenol Biodegradation Run
(Unacclimated Sludge, No Amino Acid, Temp - 23°C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
PM 2:25	0:00	8.10	51.9	21.01
4:25	2:00	8.14		15.32
6.25	4:00	8.13	48.56	10.44
AM 10:25	20:00	7.99		3.17
PM 12:25	22:00	7.99		2.12
2.25	24:00	7.98	49.90	0.45

* Experiment date : June 2, 1984

Table #7 - First 20 ppm 2-chlorophenol Biodegradation Run
(Unacclimated Sludge, 10 ppm Amino Acid, Temp - 25°C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
AM 10:45	0:00	3140	7.63	32.4	19.08
PM 12:45	2:00	3080	7.64		18.11
2:45	4:00	2960	7.69		17.65
4:55	6:10	3120	7.69		16.39
6:45	8:00	3010	7.69	38.3	15.32
AM 9:55	23:10	3030	7.72	43.8	12.81
11:45	25:00	2970	7.69		11.47
PM 2:00	27:15	2950	7.68		10.89
3:45	29:00	3010	7.68		10.33
5:45	31:00	2930	7.67		9.56
7:45	33:00	2680	7.62	43.8	9.24
AM 9:45	47:00	2850	7.64		7.43
PM 12:45	50:00	2830	7.65		5.53
2:45	52:00	2970	7.65		4.56
4:45	54:00	2530	7.65	41.6	3.81

* Experiment date : July 31, 1984

Table #8 - Second 20 ppm 2-chlorophenol Biodegradation Run
(Unacclimated Sludge, 10 ppm Amino Acid, Temp - 25°C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
PM 2:25	0:00	8.10	27.8	19.99
4:25	2:00	8.14		18.37
6:35	4:10	8.14		17.44
8:25	6:00	8.13	28.3	15.93
AM 10:25	20:00	7.99	28.3	8.25
PM 12:25	22:00	7.99		4.77
2.25	24:00	7.98		3.50
4:25	26:00	7.98	28.6	1.90

* Experiment date : Aug 3, 1984

Table #9 - First 20 ppm 2-chlorophenol Biodegradation Run
(Acclimated sludge, No Amino Acid, Temp - 25°C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
AM 9:31	0:00	3920	7.82	68.1	19.91
10.06	0:35	3540	7.83		14:00
10:33	1:02	3520	7.88	71.2	5.72
11:01	1:30	3500	7.91		2.86
11:31	2:00	3580	7.95	71.4	2.02

* Experiment date : July 6, 1984

Table #10 - Second 20 ppm 2-chlorophenol Biodegradation Run
(Acclimated Sludge, No Amino Acid, Temp - 23°C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
AM 10:31	0:00	7.90	60.5	18.56
11:03	0:32	7.86		13.37
11:34	1:03	7.90	63.5	7.84
PM 12:01	1:30	7.97		3.19
12:34	2:03	7.99	63.5	1.72

* Experiment date : July 7, 1984

Table #11 - 20 ppm 2-chlorophenol Biodegradation Run
(Acclimated Sludge, 10 ppm Amino Acid, Temp - 24°C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
PM 5:25	0:00	4210	7.27	41.9	19.81
5:55	0:30	4160	7.20		14.70
6:25	1:00	4160	7.19	42.7	7.50
6:55	1:30	4110	7.26	44.3	3.28

* Experiment date : Sept 14, 1984

Table #12 - First 10 ppm 2,6-dichlorophenol Biodegradation Run
(Unacclimated Sludge, No Amino Acid, Temp - 25°C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
AM 10:35	0:00	3830	8.06	52.0	10.83
PM 12:35	2:00	4010	8.09		10.59
2:35	4:00	3930	8.11		10.13
4:35	6:00	4110	8.12		9.66
6:35	8:00	4320	8.12	60.0	9.04
AM 10:35	24:00	4060	8.10	68.7	8.22
PM 12:35	26:00	4070	8.08		8.11
2:35	28:00	4100	8.09		7.58
4:35	30:00	3920	8.06		7.46
6:35	32:00	4050	8.10	68.7	6.73
AM 10:35	48:00	4040	7.96	61.3	5.24
PM 12:35	50:00	4210	7.93		5.27
2:35	52:00	3920	7.92		5.25
4:35	54:00	3750	7.93		4.91
6:35	56:00	3870	7.98		4.77
AM 10:35	72:00	3860	7.99	64.2	4.20
PM 12:35	74:00	3780	7.98		3.98
2:35	76:00	3940	8.01		3.54
4:35	78:00	3670	8.01		3.35
6:35	80:00	3720	8.01	74.6	2.46
Following day		3700	7.91	74.9	0.00

* Experiment date : Aug 21, 1984

Table #13 - Second 10 ppm 2,6-dichlorophenol Biodegradation Run
(Unacclimation Sludge, No Amino Acid, Temp - 23°C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
PM 1:23	0:00	6.75	20.2	9.62
3:23	2:00	6.75		8.73
5:23	4:00	6.82		8.43
7:23	6:00	6.91		7.36
9:23	8:00	6.90	20.3	6.56
AM 10:23	21:00	6.86	23.0	0.00

* Experiment date : Aug 27, 1984

Table #14 - First 10 ppm 2,6-dichlorophenol Biodegradation Run
(Unacclimated Sludge, 10 ppm Amino Acid, Temp - 25°C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
PM 12:15	0:00	3840	7.96	29	9.93
2:15	2:00	3760	8.12		9.59
4:15	4:00	3860	8.13		9.21
6:15	6:00	3810	8.14	36	8.66
AM 10:15	22:00	3890	8.14	62	7.62
PM 12:15	24:00	3930	8.13		7.23
2:15	26:00	4070	8.22		7.24
4:15	28:00	3970	8.23		7.17
6:15	30:00	3880	8.19	68.2	7.35
AM 10:15	46:00	4060	8.15	60	6.74
PM 12:15	48:00	4030	8.12		6.45
2:15	50:00	4250	8.14		6.43
4:15	52:00	3930	8.13		5.60
6:15	54:00	4020	8.25	68.4	5.18
AM 10:15	70:00	3950	7.96		1.23

* Experiment date : Aug 20, 1984

Table #15 - Second 10 ppm 2,6-dichlorophenol Biodegradation Run
(Unacclimated Sludge, 10 ppm Amino Acid, Temp - 23°C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
PM 1:05	0:00	7.65	39.8	9.84
3:05	2:00	7.69		9.28
5:05	4:00	7.66		8.87
7:05	6:00	7.66		8.12
9:05	8:00	7.64	38.4	7.43
AM 10:05	21:00	7.36	39.9	0.27

* Experiment date : Aug 27, 1984

Table #16 - First 10 ppm 2,6-dichlorophenol Biodegradation Run
(Acclimated Sludge, No Amino Acid, Temp - 23°C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
AM 11:10	0:00	4250	7.54	43.6	9.59
PM 1:10	2:00	4030	7.53		8.99
3:10	4:00	4150	7.48		8.76
5:10	6:00	4090	7.48		8.44
7:10	8:00	4300	7.47	46.3	8.37
AM 10:10	23:00	3720	7.47	47.6	6.96
PM 12:10	25:00	3990	7.49		6.90
2:10	27:00	4140	7.53		6.87
4:10	29:00	4000	7.51		6.56
6:10	31:00	3620	7.56	50.3	5.88
AM 10:10	47:00	3870	7.50	50.3	0.08

* Experiment date : Sept 12, 1984

Table #17 - Second 10 ppm 2,6-dichlorophenol Biodegradation Run
(Acclimated Sludge, No Amino Acid, Temp - 24°C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	Substarte Conc (ppm)
AM 11:05	0:00	7.31	49.7	9.41
PM 1:05	2:00	7.34		8.99
3:05	4:00	7.34		8.91
5:05	6:00	7.40		8.71
9:15	10:10	7.37	48.3	7.52
AM 10:05	23:00	7.26		1.35
PM 12:05	25:00	7.27	46.7	0.00

* Experiment date : Sept 17, 1984

Table #18 - First 10 ppm 2,6-dichlorophenol Biodegradation Run
(Acclimated Sludge, 10 ppm Amino Acid, Temp - 23 °C)

Time Sample Taken (hr)	Time from Start (hr)	MLSS (mg/l)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
AM 10:50	0:00	4630	7.47	55.0	10.27
PM 12:50	2:00	4420	7.48		9.60
2:50	4:00	4380	7.43		9.40
4:50	6:00	4330	7.43		8.53
6:50	8:00	4480	7.42	54.4	8.10
AM 9:50	23:00	4220	7.40	56.2	7.63
11:50	25:00	4010	7.46		7.47
PM 1:50	27:00	3870	7.48		7.35
4:05	29:15	3920	7.53		7.21
5:50	31:00	3740	7.52		6.89
7:50	33:00	4140	7.52	56.8	6.36
AM 8:50	46:00	3950	7.61	61.78	0.00

* Experiment date : Aug 12, 1984

Table #19 - Second 10 ppm 2,6-dichlorophenol Biodegradation Run
(Acclimated Sludge, 10 ppm Amino Acid, Temp - 24 °C)

Time Sample Taken (hr)	Time from Start (hr)	pH	Ammonia Conc (ppm)	Substrate Conc (ppm)
AM 10:45	0:00	7.18	50.3	9.66
PM 1:00	2:15	7.26		8.13
2:45	4:00	7.28		7.54
4:45	6:00	7.26		7.13
9:15	10:30	7.29	48.7	5.96
AM 9:45	23:00	7.08	45.0	0.00

* Experiment date : Aug 17, 1984

Table #20 - Results of Kinetic Studies of Phenol

# of Run	Amino Acids	Kinetic Model	Absolute Average Residual	Rate Constant
I	None	Zero	3.401	K = 3.93947
		First	157.521	K = 0.11010
		Monod	4.657	K1= 3.45176
				K2= -3.90755
		Haldane	3.487	K1= -0.45286
				K2=-21.58365
				K3=-100.2408
II	None	Zero	246.315	K = 15.65494
		First	434.407	K = 0.69266
		Monod	283.730	K1= 25.74300
				K2= 11.94846
		Haldane	103.448	K1= -7.69221
				K2=-18.76881
				K3=-71.98814
I	10 ppm	Zero	138.457	K = 4.05857
		First	31.928	K = 0.16905
		Monod	251.264	K1= 5.79805
				K2= 5.03485
		Haldane	191.038	K1= 2.10038
				K2= -1.21070
				K3=-106.5762
II	10 ppm	Zero	70.357	K = 13.19361
		First	470.151	K = 0.39891
		Monod	57.865	K1= 9.26530
				K2=-10.30448
		Haldane	19.488	K1= -4.07115
				K2=-20.43471
				K3=-70.36179

Table #21 - Results of Kinetic Studies of 2-chlorophenol

# of Run	Type of Sludge	Amino Acids	Kinetic Model	Absolute Average Residual	Rate Constant
I	Unaccl	None	Zero	2.7894	K = 0.2765
			First	0.1167	K = 0.0374
			Monod	4.1363	K1=-0.3242
					K2=-17.536
			Haldane	1.2397	K1= 0.0660
					K2=-2.3610
					K3=-20.325
II	Unaccl	None	Zero	3.9485	K = 0.7152
			First	1.0199	K = 0.1253
			Monod	10.767	K1= 0.9429
					K2= 0.3002
			Haldane	0.1673	K1= 0.3228
					K2=-0.5778
					K3=-20.839
I	Unaccl	10 ppm	Zero	0.3761	K = 0.2615
			First	1.0550	K = 0.0259
			Monod	0.5898	K1= 0.2687
					K2=-0.5319
			Haldane	0.3060	K1= 0.0450
					K2=-3.9558
					K3=-26.347
II	Unaccl	10 ppm	Zero	0.5260	K = 0.6474
			First	3.9282	K = 0.0775
			Monod	0.2091	K1= 0.5394
					K2=-1.8858
			Haldane	0.7078	K1= 0.2240
					K2=-3.0588
					K3=-35.478
I	Accl	None	Zero	5.2560	K = 9.3859
			First	2.1740	K = 1.2330
			Monod	5.5930	K1= 3.7188
					K2=-5.6815
			Haldane	0.0380	K1=-3.1116
					K2=-5.8630
					K3=-18.454

Table #21 - continued

II	Accl	None	Zero	1.5500	K = 8.7719
			First	4.1450	K = 1.2380
			Monod	1.1900	K1=14.1186
					K2= 4.2540
			Haldane	0.4640	K1=-6.7117
					K2=-7.2432
					K3=-13.094
I	Accl	10 ppm	Zero	0.3698	K =11.2520
			First	3.8420	K = 1.2062
			Monod	0.2453	K1=13.8483
					K2= 2.2272
			Haldane	0.5093	K1=-12.862
					K2=-10.352
					K3=-11.333

Table #22 - Results of Kinetic Studies of 2,6-dichlorophenol

# of Run	Type of Sludge	Amino Acids	Kinetic Model	Absolute Average Residual	Rate Constant
I	Unaccl	None	Zero	0.1612	K = 0.0932
			First	0.1653	K = 0.1519
			Monod	0.2399	K1= 0.0813
					K2=-1.4243
			Haldane	0.1122	K1= 0.0084
					K2=-2.8525
					K3=-15.02
II	Unaccl	None	Zero	0.029	K = 0.3747
			First	0.044	K = 0.0468
			Monod	0.003	K1= 0.1040
					K2=-5.9520
			Haldane	0.012	K1=-0.0009
					K2=-4.0854
					K3=-16.633
I	Unaccl	10 ppm	Zero	0.1512	K = 0.0699
			First	0.1263	K = 0.0093
			Monod	0.0357	K1= 0.0028
					K2=-7.6801
			Haldane	0.0128	K1= 0.0015
					K2=-3.6944
					K3=-15.567
II	Unaccl	10 ppm	Zero	0.2747	K = 0.4682
			First	14.057	K = 0.1799
			Monod	0.0058	K1= 0.2482
					K2=-1.2120
			Haldane	0.1765	K1=-0.0253
					K2=-1.4346
					K3=-9.7691
I	Accl	None	Zero	0.0447	K = 0.0978
			First	0.0431	K = 0.0127
			Monod	0.0114	K1=-0.0010
					K2=-0.0808
			Haldane	0.0015	K1= 0.0019
					K2=-3.0059
					K3=-15.673

Table #22 - continued

II	Accl	None	Zero	0.5344	K = 0.3879
			First	28.693	K = 0.2081
			Monod	0.5380	K1= 0.2781
					K2=-0.4221
			Haldane	2.8035	K1=-0.5341
					K2=-0.6510
					K3=-6.6744
I	Accl	10 ppm	Zero	0.1579	K = 0.0875
			First	0.1472	K = 0.0107
			Monod	0.0092	K1=-0.0151
					K2=-9.4866
			Haldane	0.0018	K1= 0.0006
					K2=-4.1390
					K3=-16.937
II	Accl	10 ppm	Zero	0.1953	K = 0.3981
			First	55.946	K = 0.2999
			Monod	0.2087	K1= 0.3864
					K2=-0.1133
			Haldane	0.0169	K1= 0.0602
					K2=-0.5520
					K3=-10.436

Figure #1 - Diagram of Reactor Setup

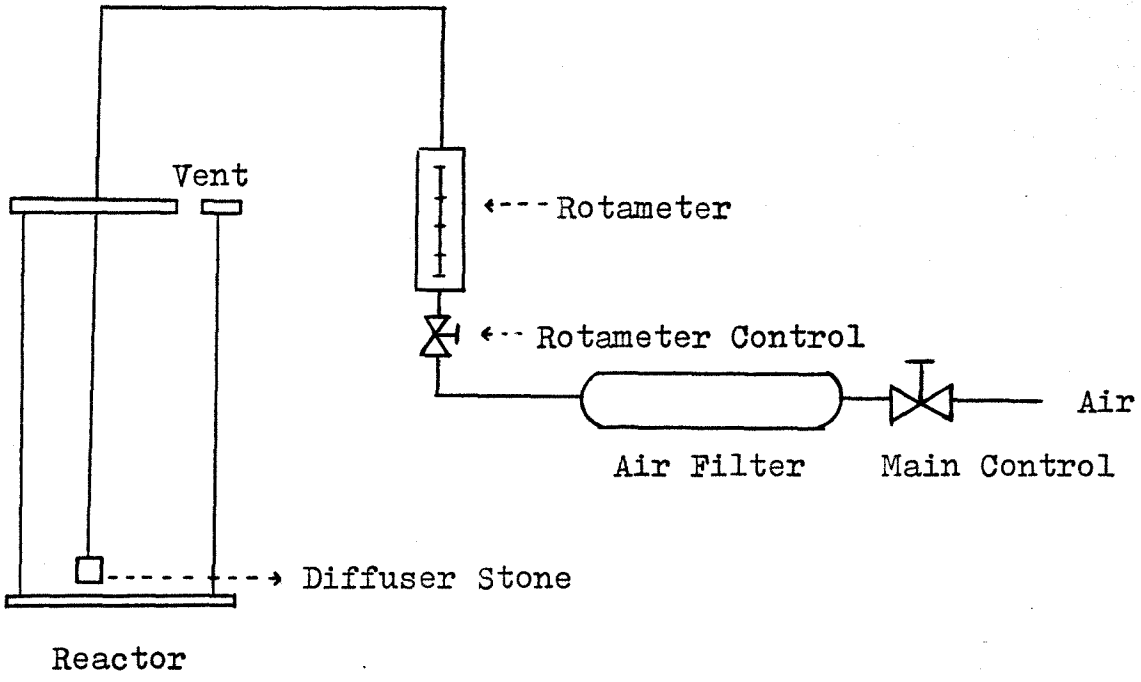


Figure #2 - Result of 100 ppm Phenol Biodegradation Run
(Without Amino Acids)

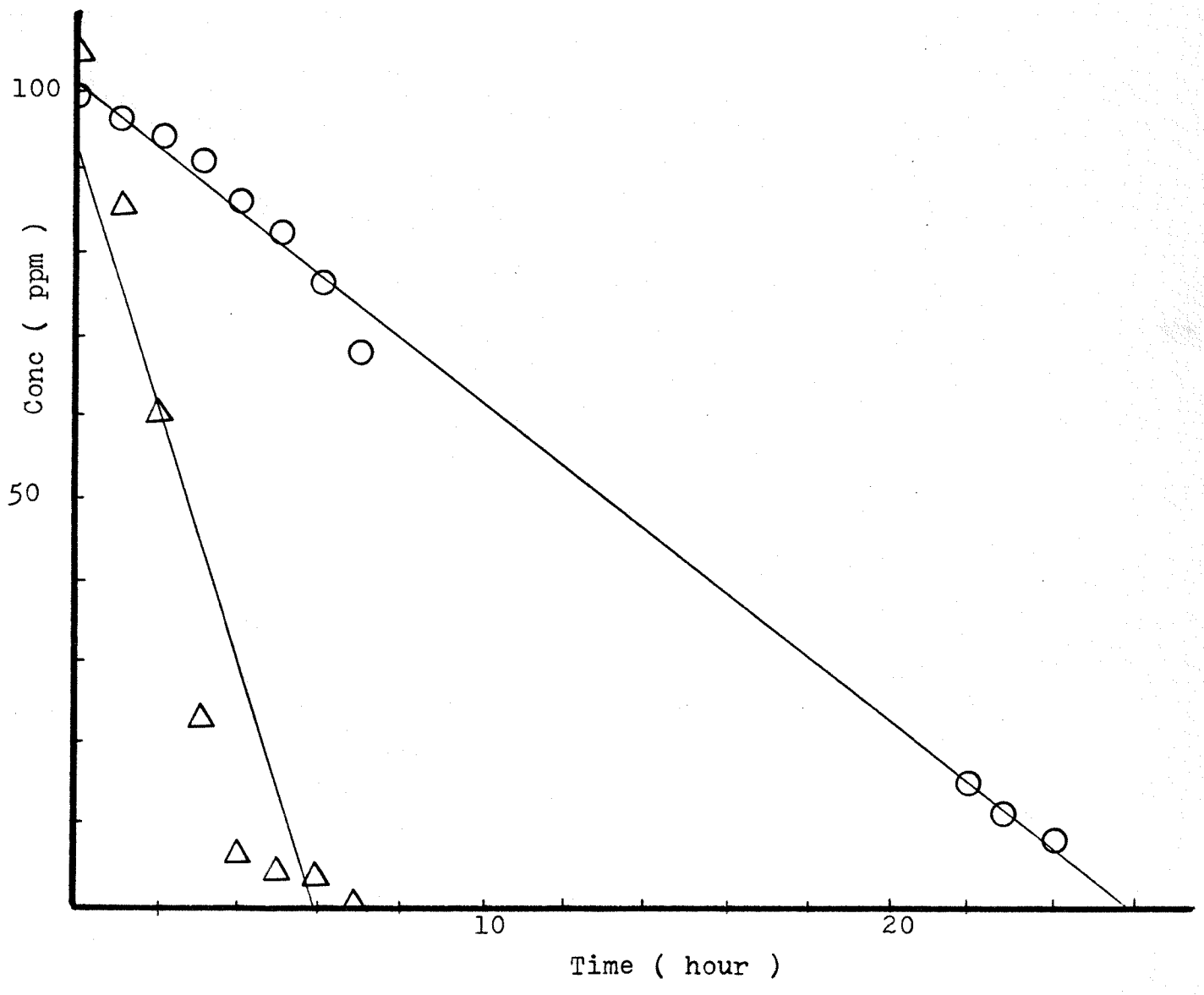


Figure #3 - Result of 100 ppm Phenol Biodegradation Run
(With Amino Acids)

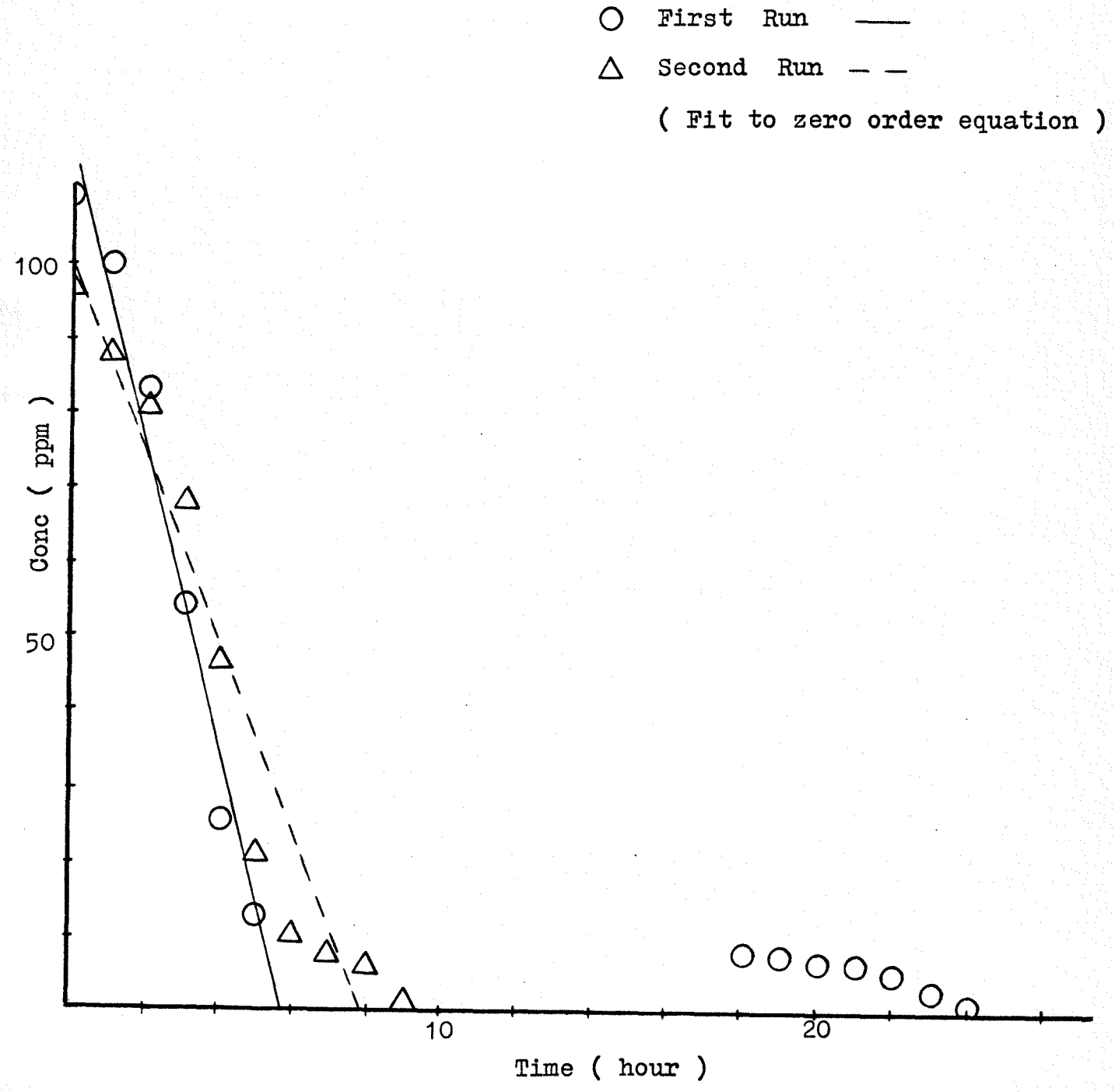


Figure #4 - Result of 100 ppm Phenol Biodegradation Run

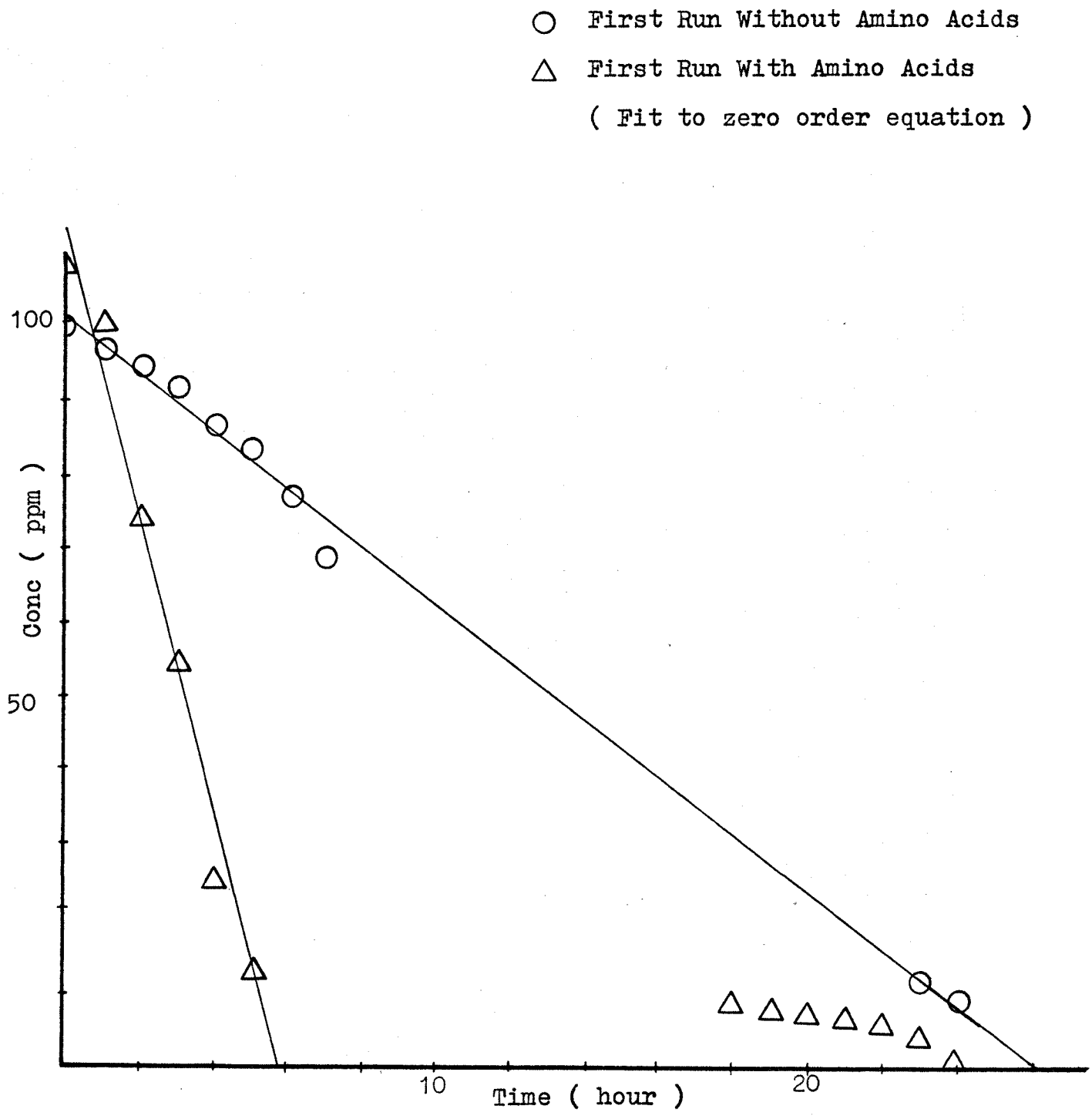


Figure #5 - Result of 20 ppm 2-chlorophenol Run
(Unacclimated Sludge, No Amino Acids)

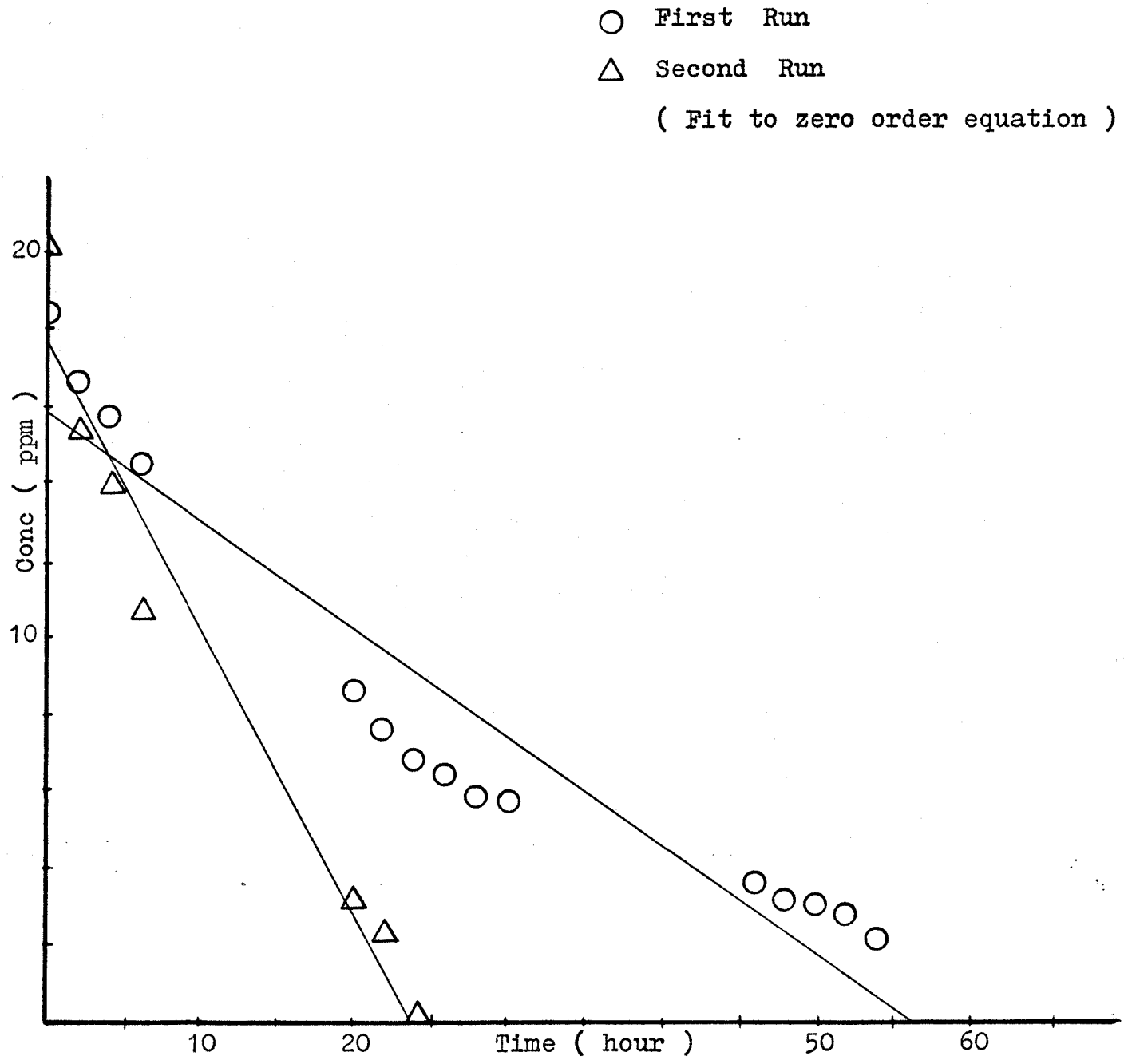


Figure #6 - Result of 20 ppm 2-chlorophenol Run
(Unacclimated Sludge, 10 ppm Amino Acids)

○ First Run
△ Second Run
(Fit to zero order equation)

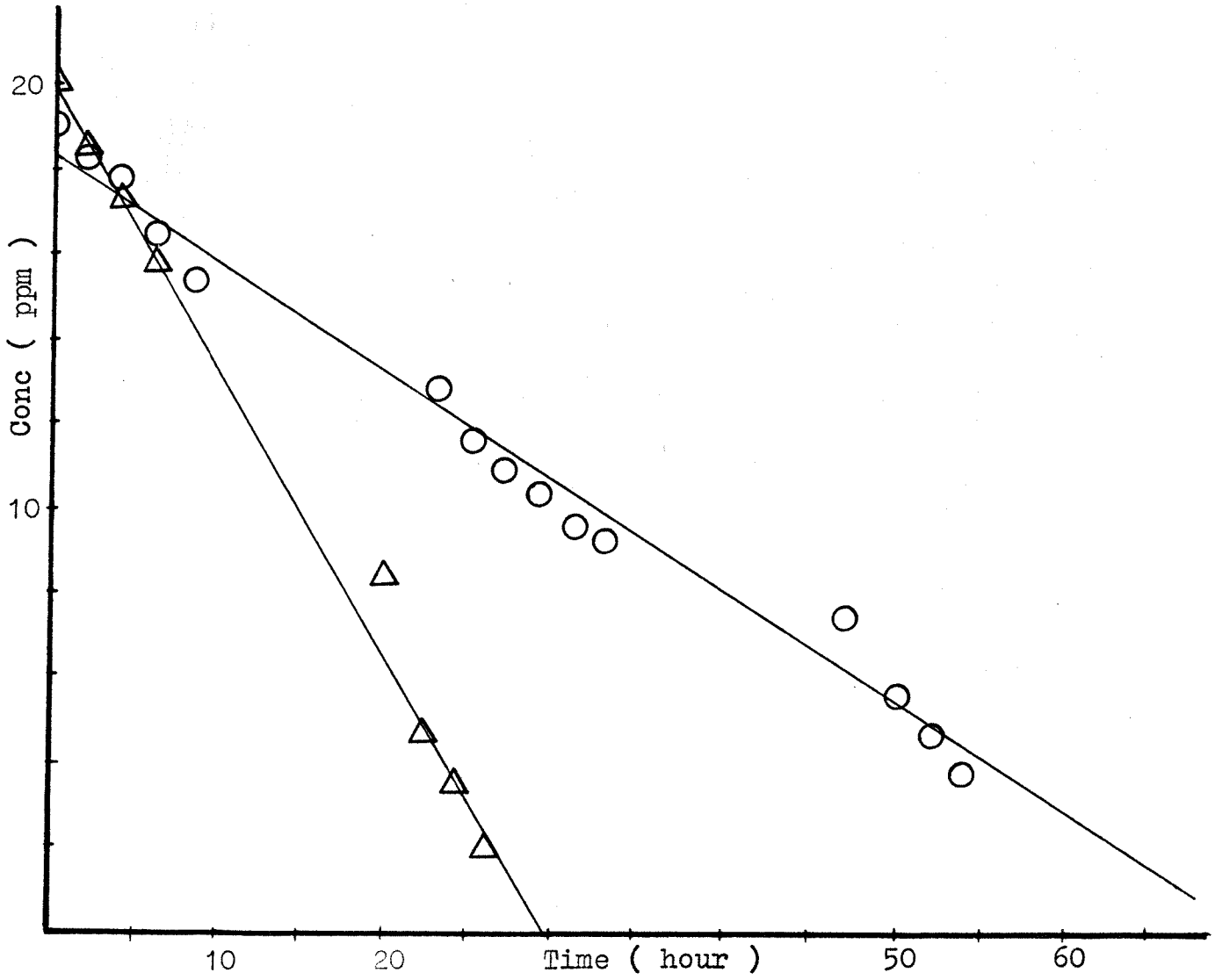


Figure #7 - Result of 20 ppm 2-chlorophenol Run
(Acclimated Sludge, No Amino Acids)

First Run
Second Run
(Fit to zero order equation)

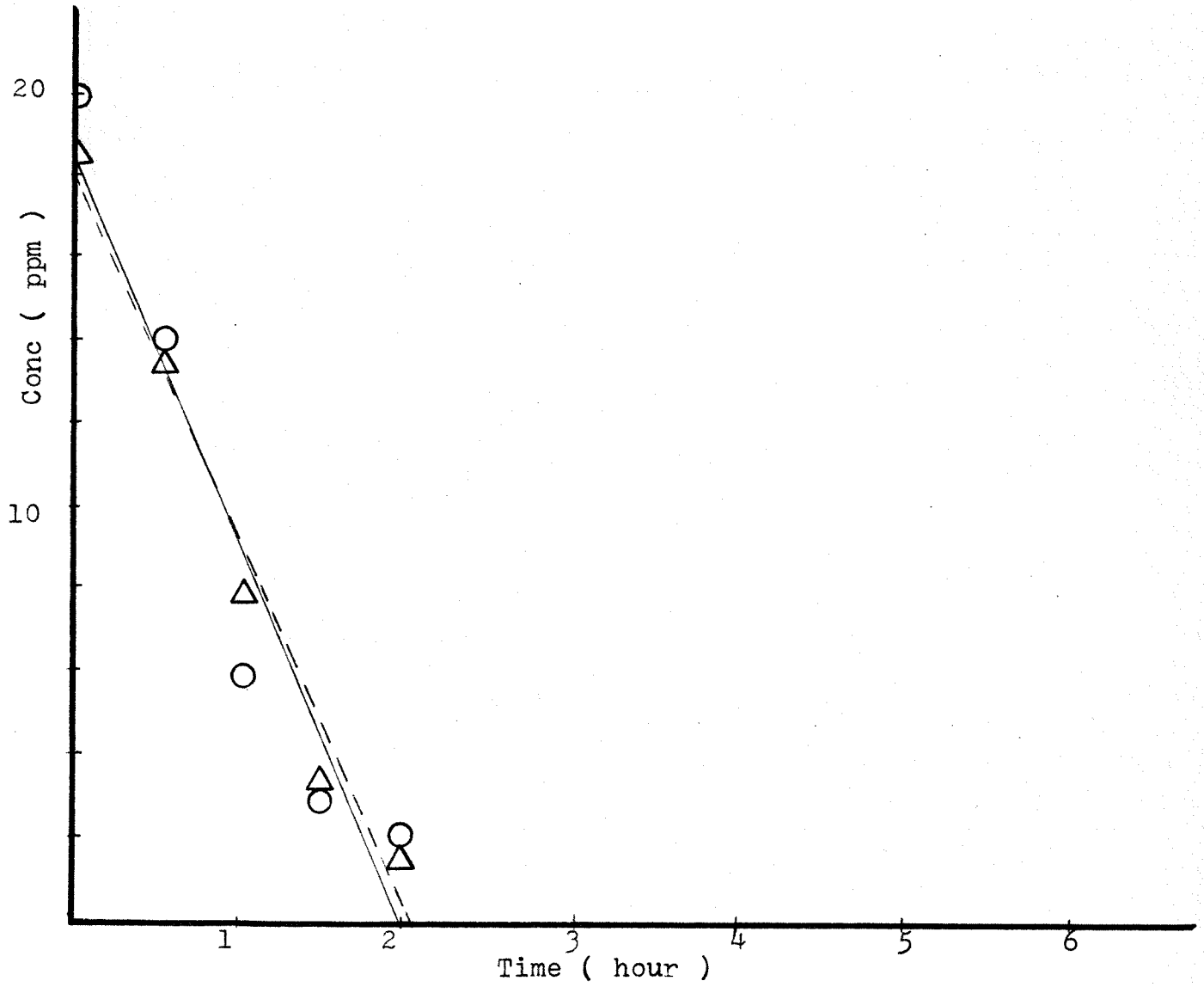


Figure #8 - Result of 20 ppm 2-chlorophenol Run
(Acclimated Sludge, 10 ppm Amino Acids)

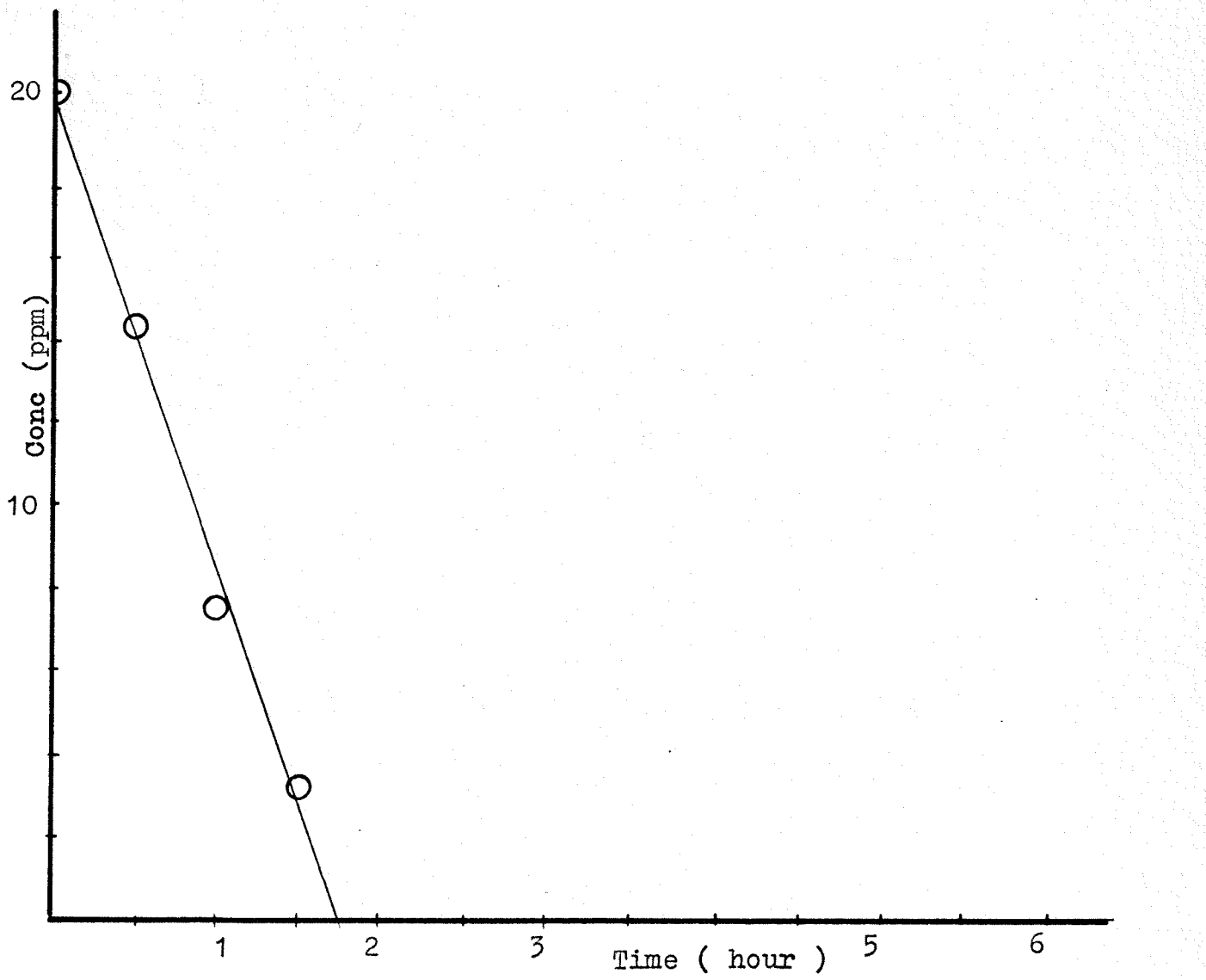


Figure #9 - Result of 20 ppm 2-chlorophenol Run

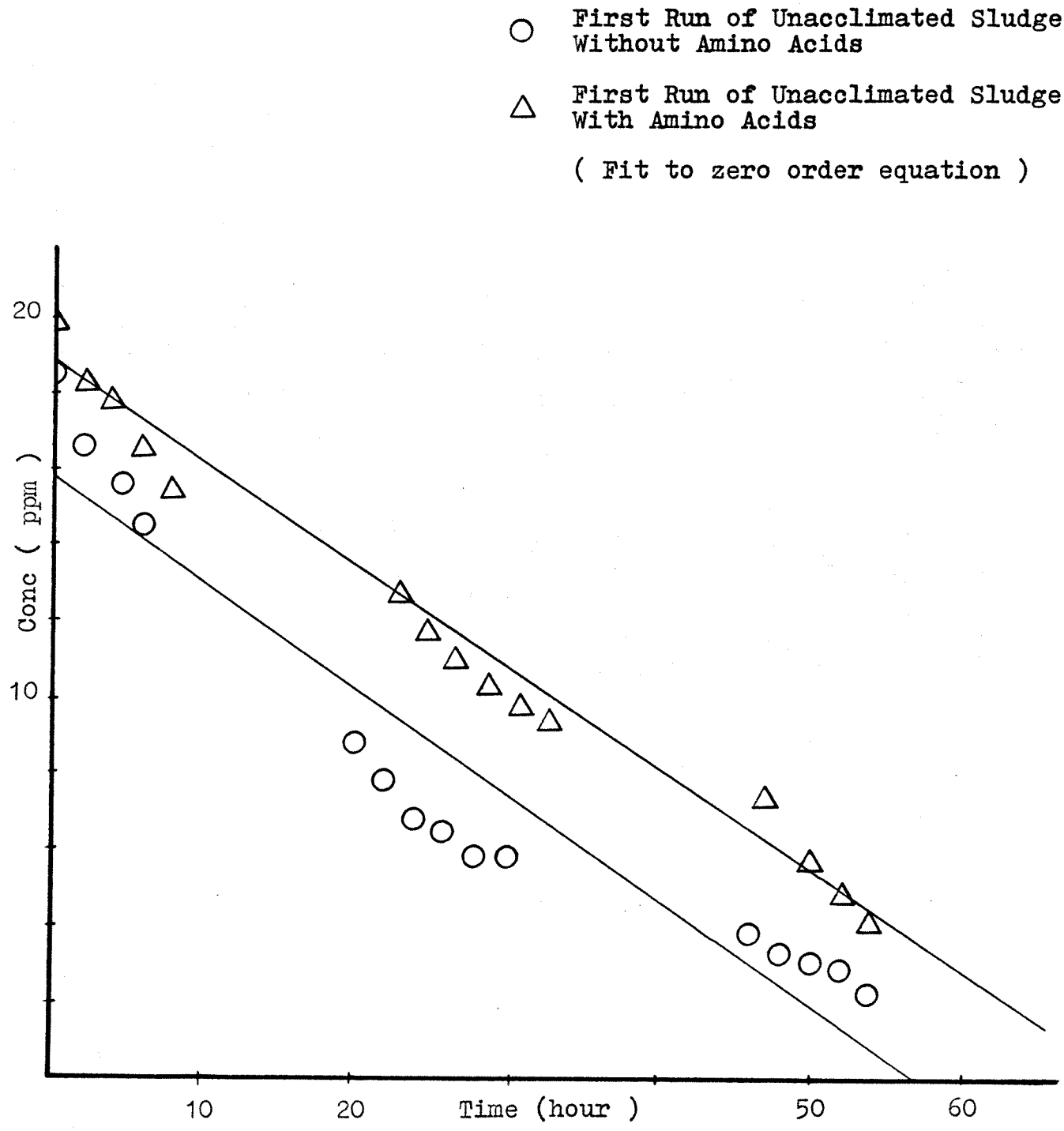


Figure #10 - Result of 20 ppm 2-chlorophenol Run

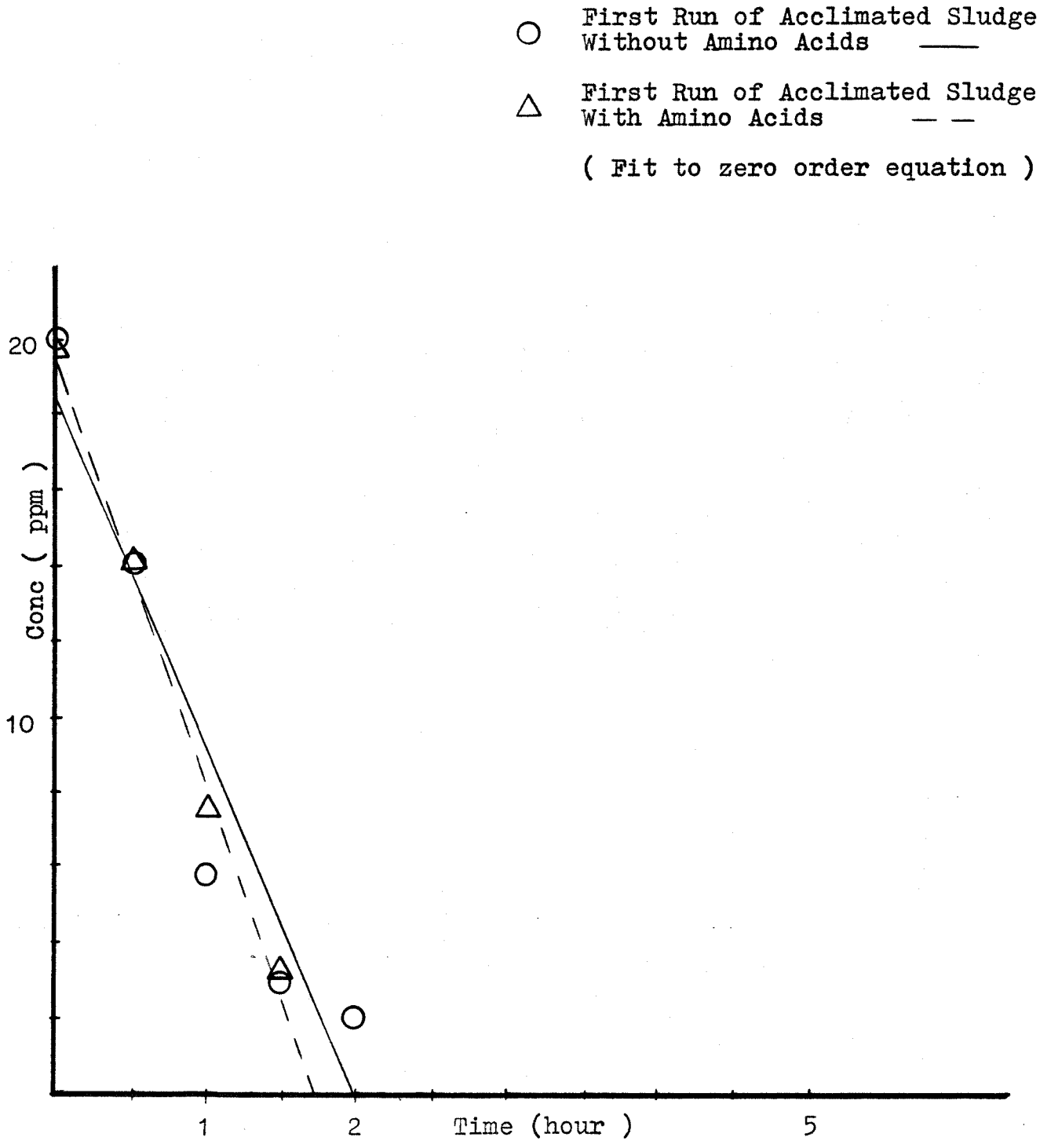


Figure #11 - Result of 20 ppm 2-chlorophenol Run

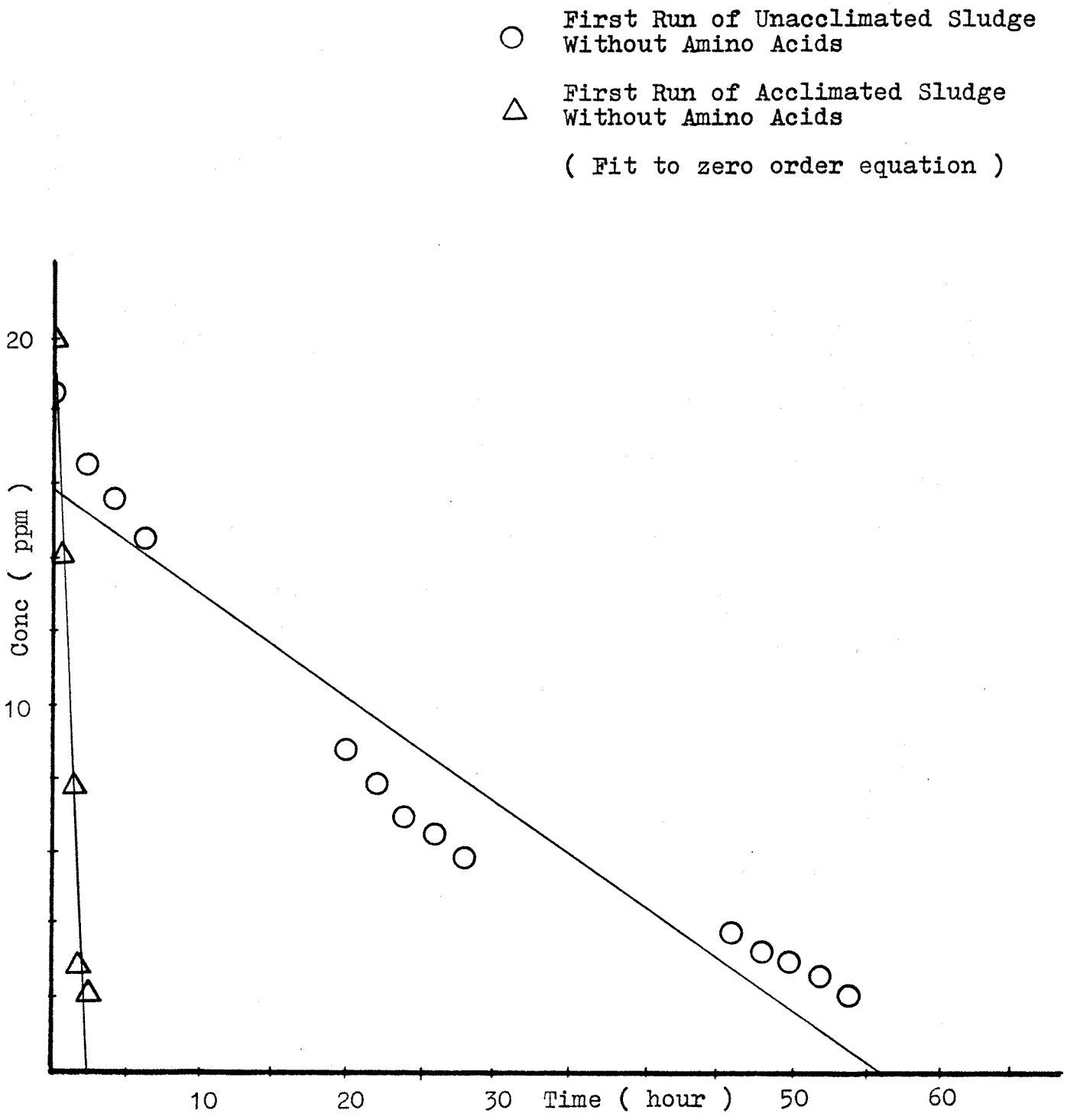


Figure #12 - Result of 20 ppm 2-chlorophenol Run

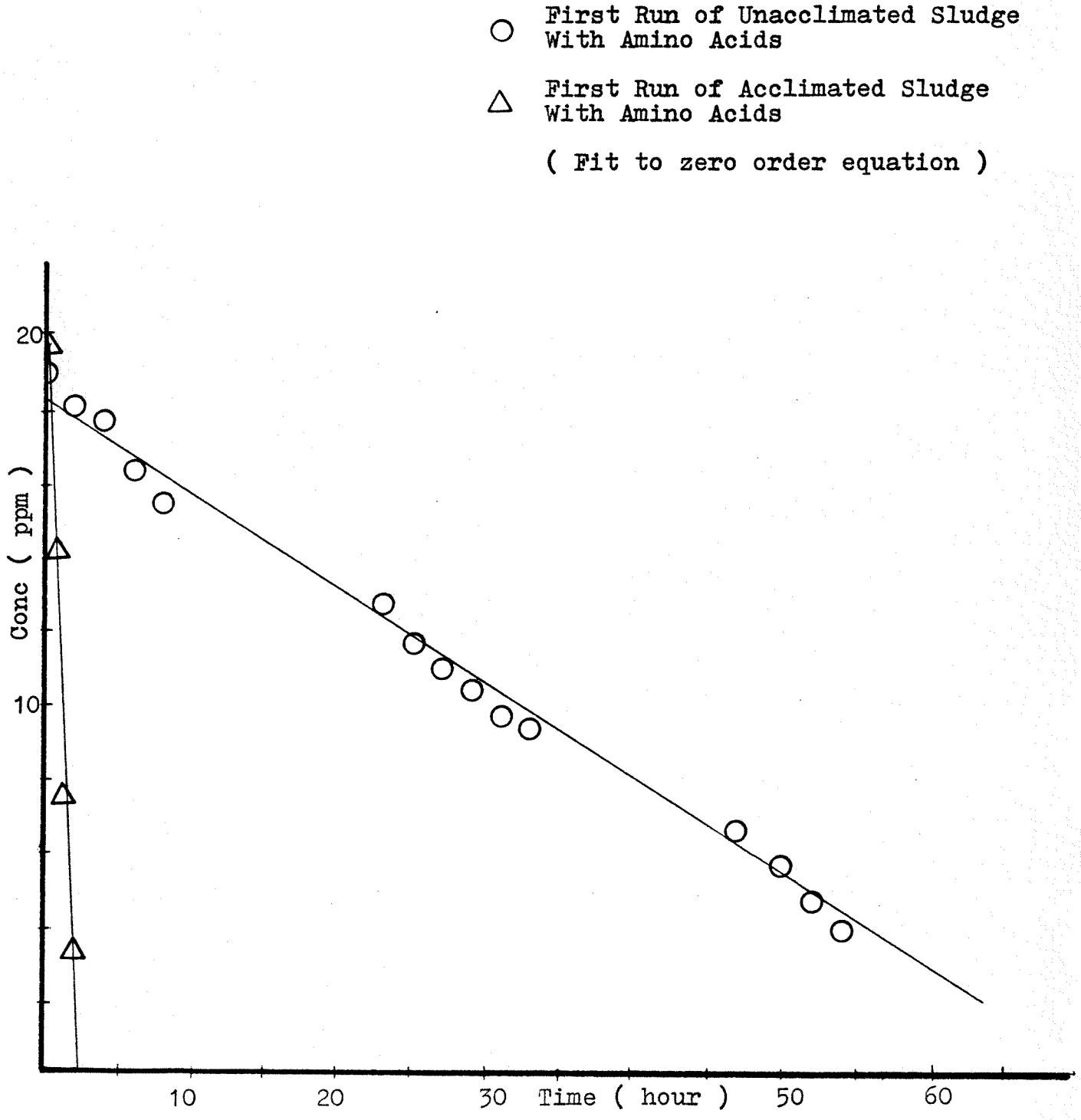


Figure #13 - Result of 10 ppm 2,6-dichlorophenol

(Unacclimated Sludge, No Amino Acids)

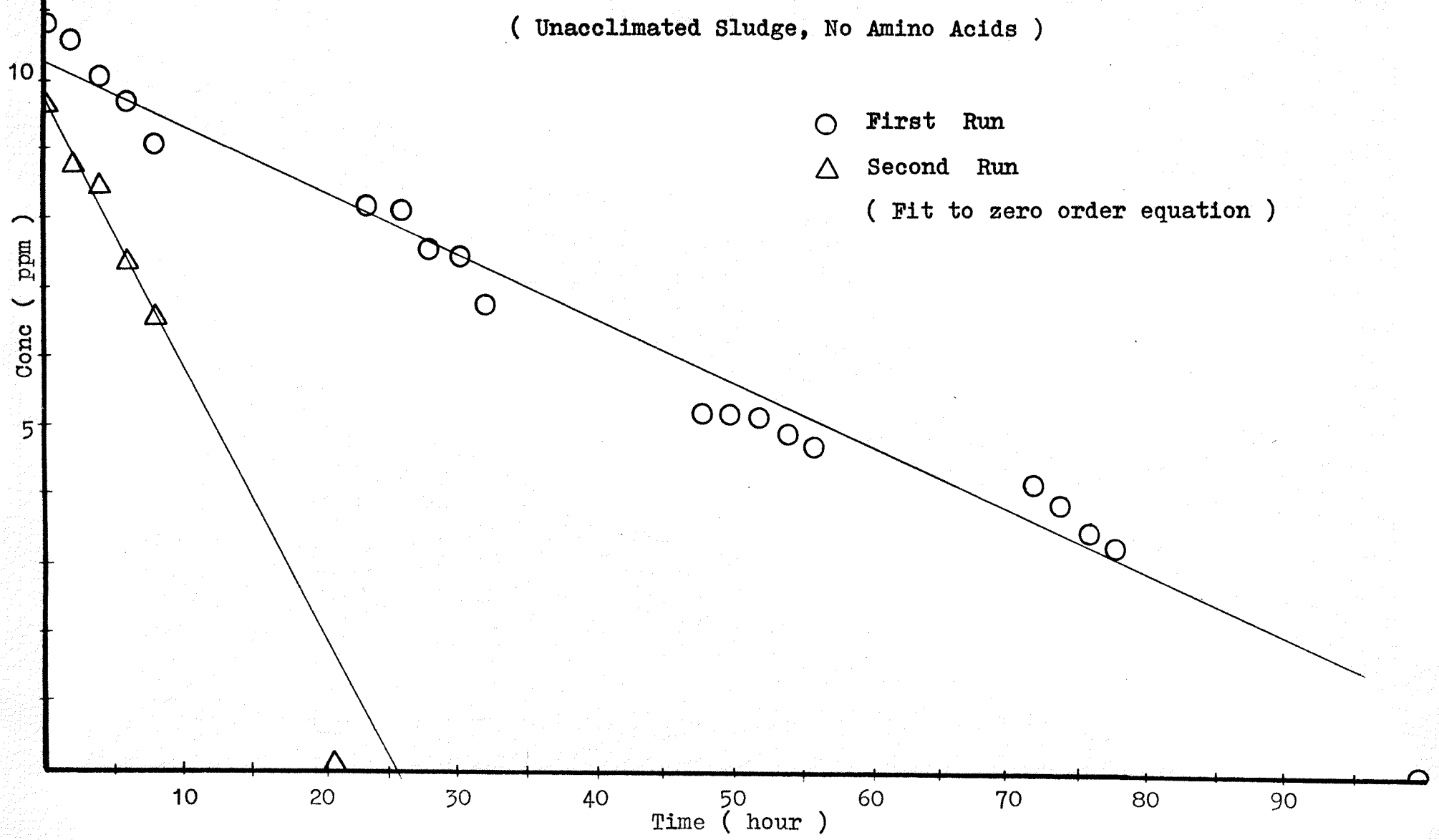


Figure #14 - Result of 10 ppm 2,6-dichlorophenol Run
(Unacclimated Sludge, 10 ppm Amino Acids)

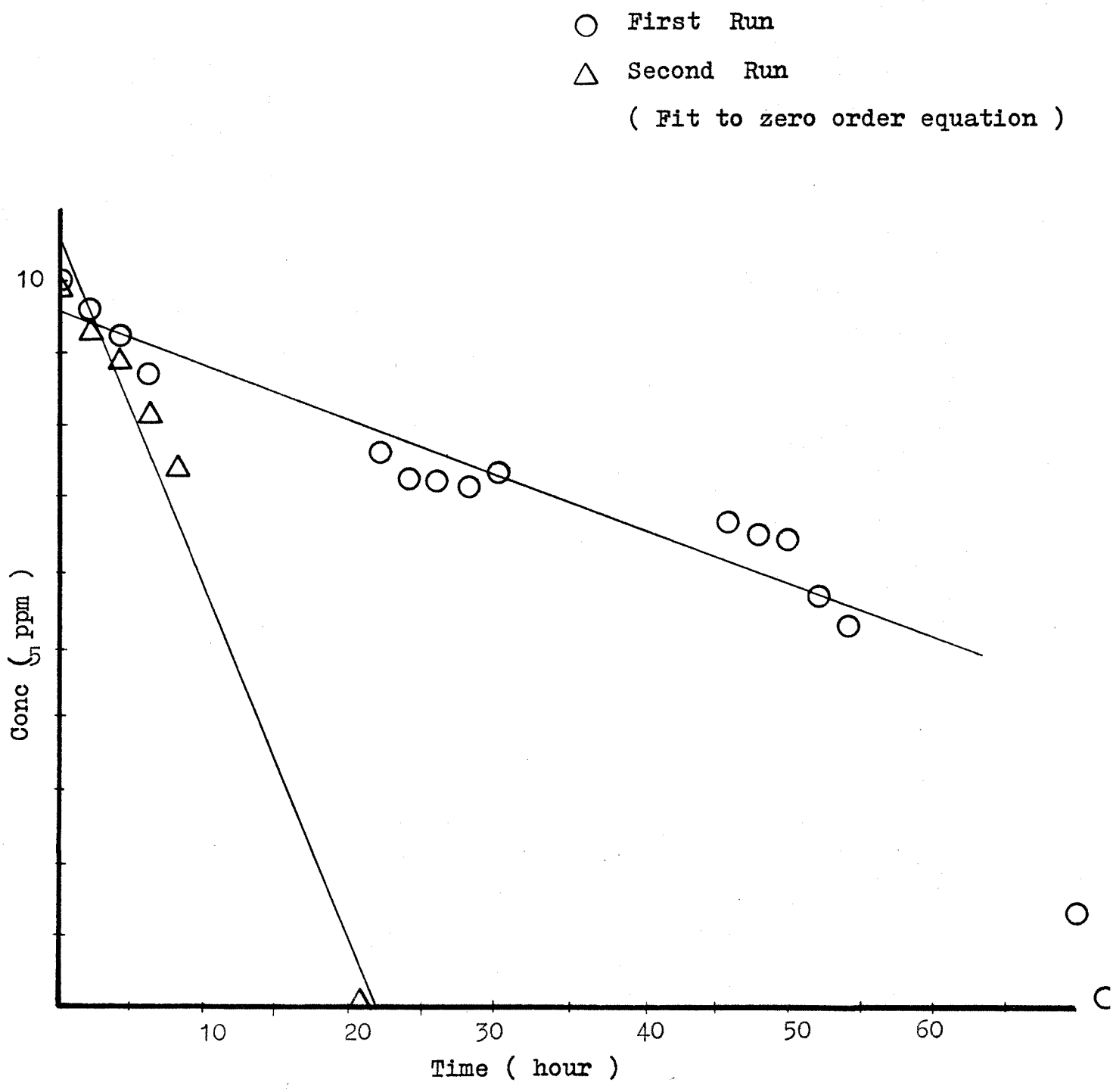


Figure #15 - Result of 10 ppm 2,6-dichlorophenol Run
(Acclimated Sludge, No Amino Acids)

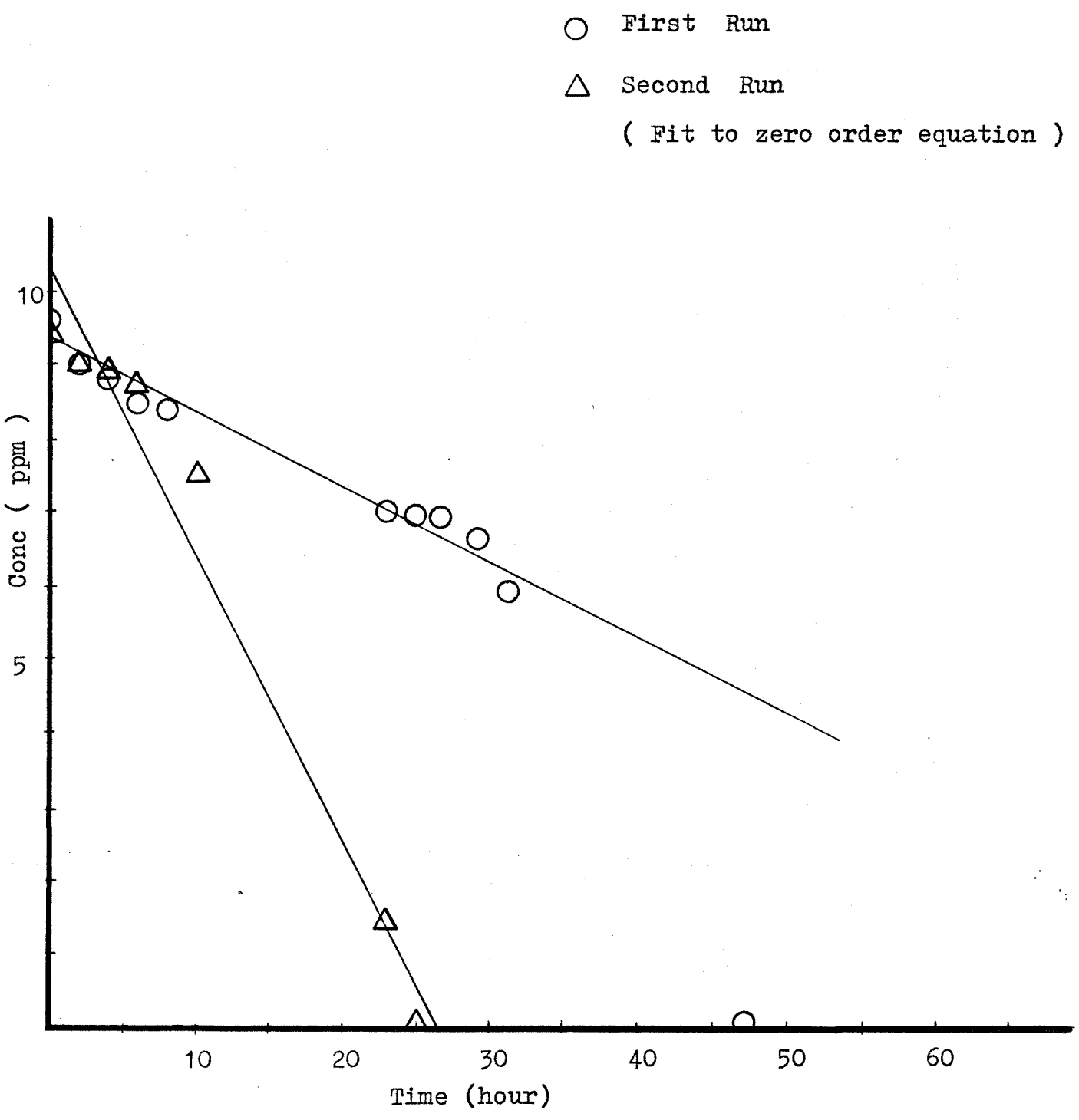


Figure #16 - Result of 10 ppm 2,6-dichlorophenol Run
(**Acclimated Sludge,** 10 ppm Amino Acids)

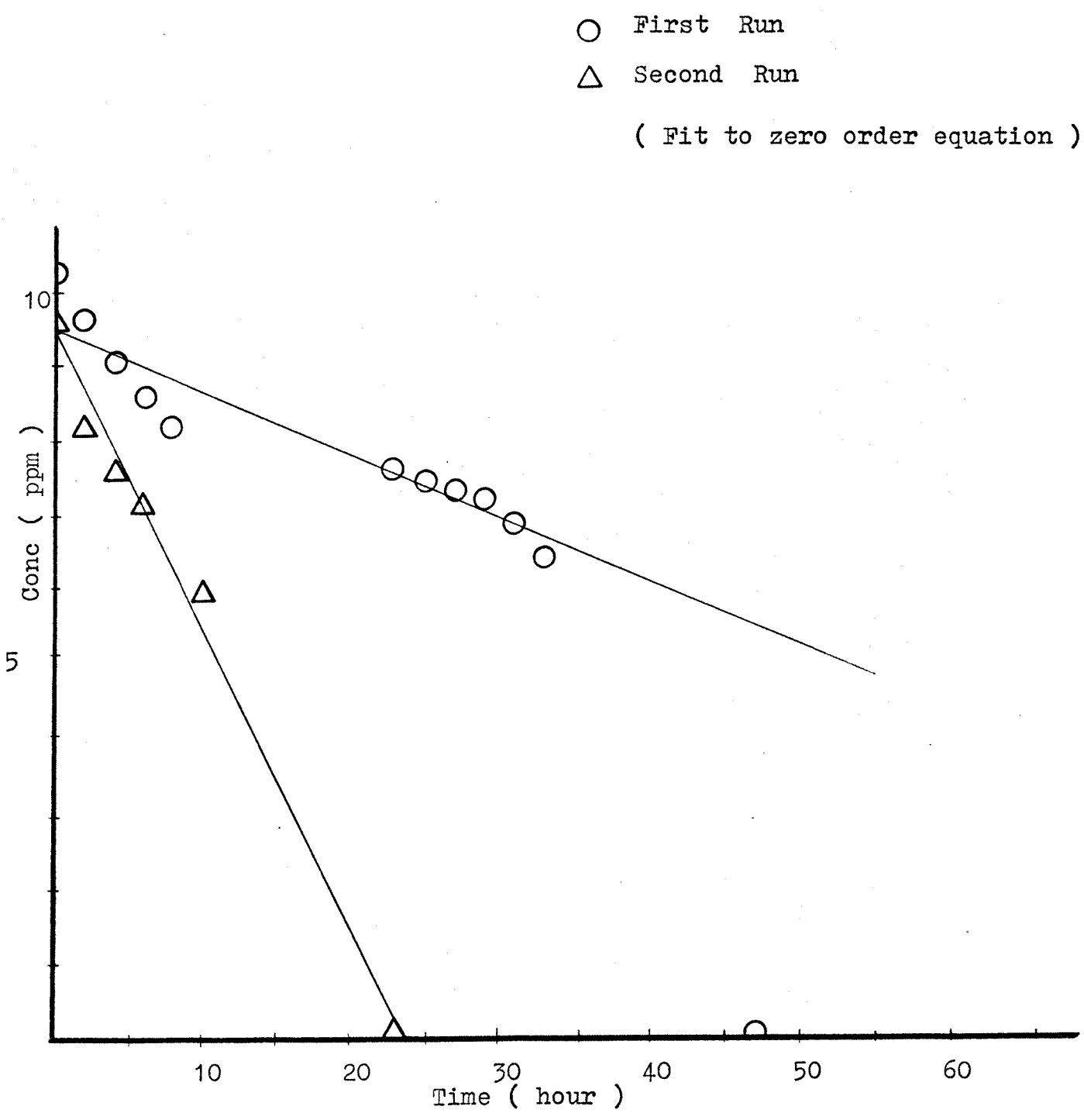


Figure #17 - Result of 10 ppm 2,6-dichlorophenol

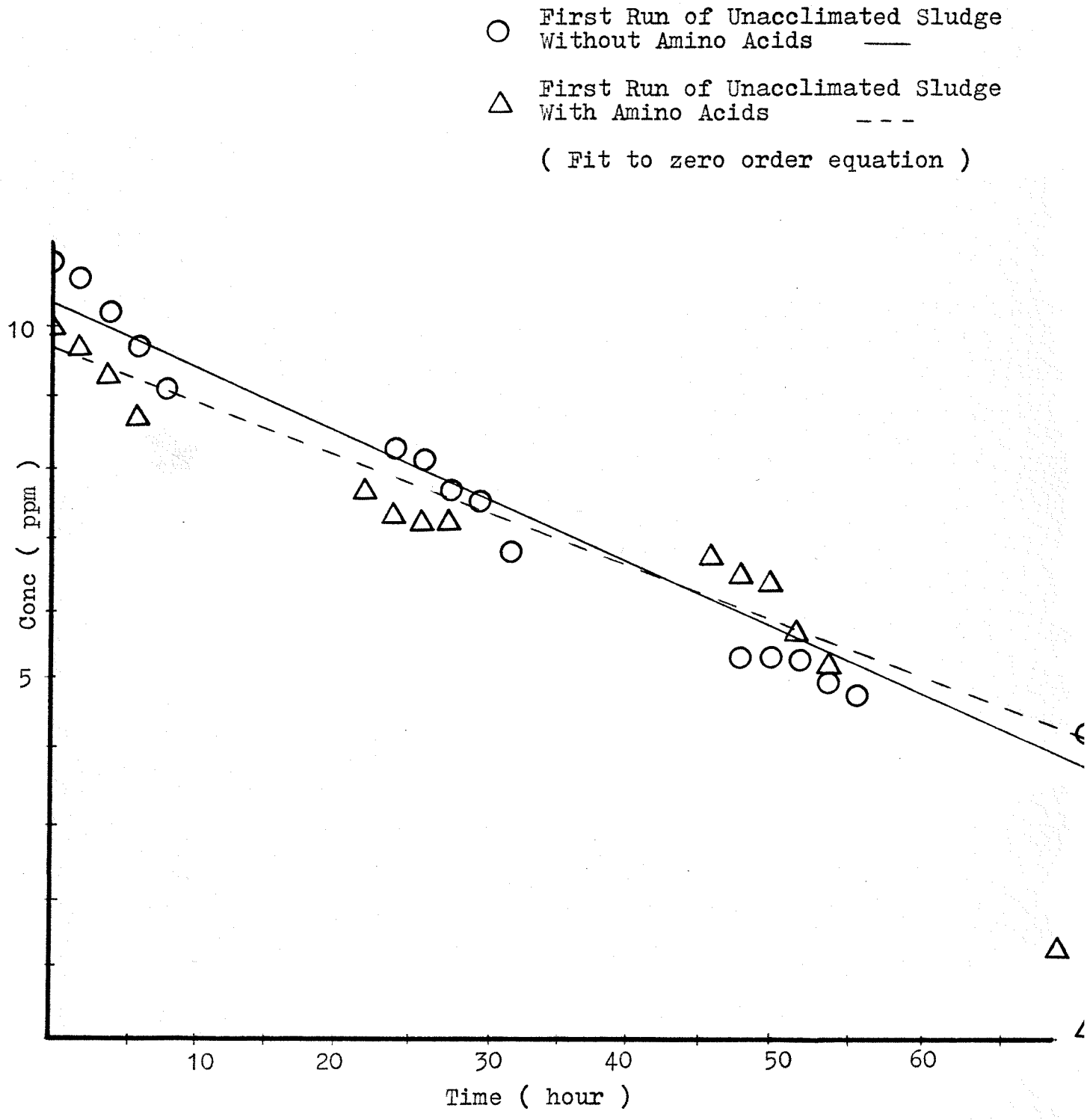


Figure #18 - Result of 10 ppm 2,6-dichlorophenol Run

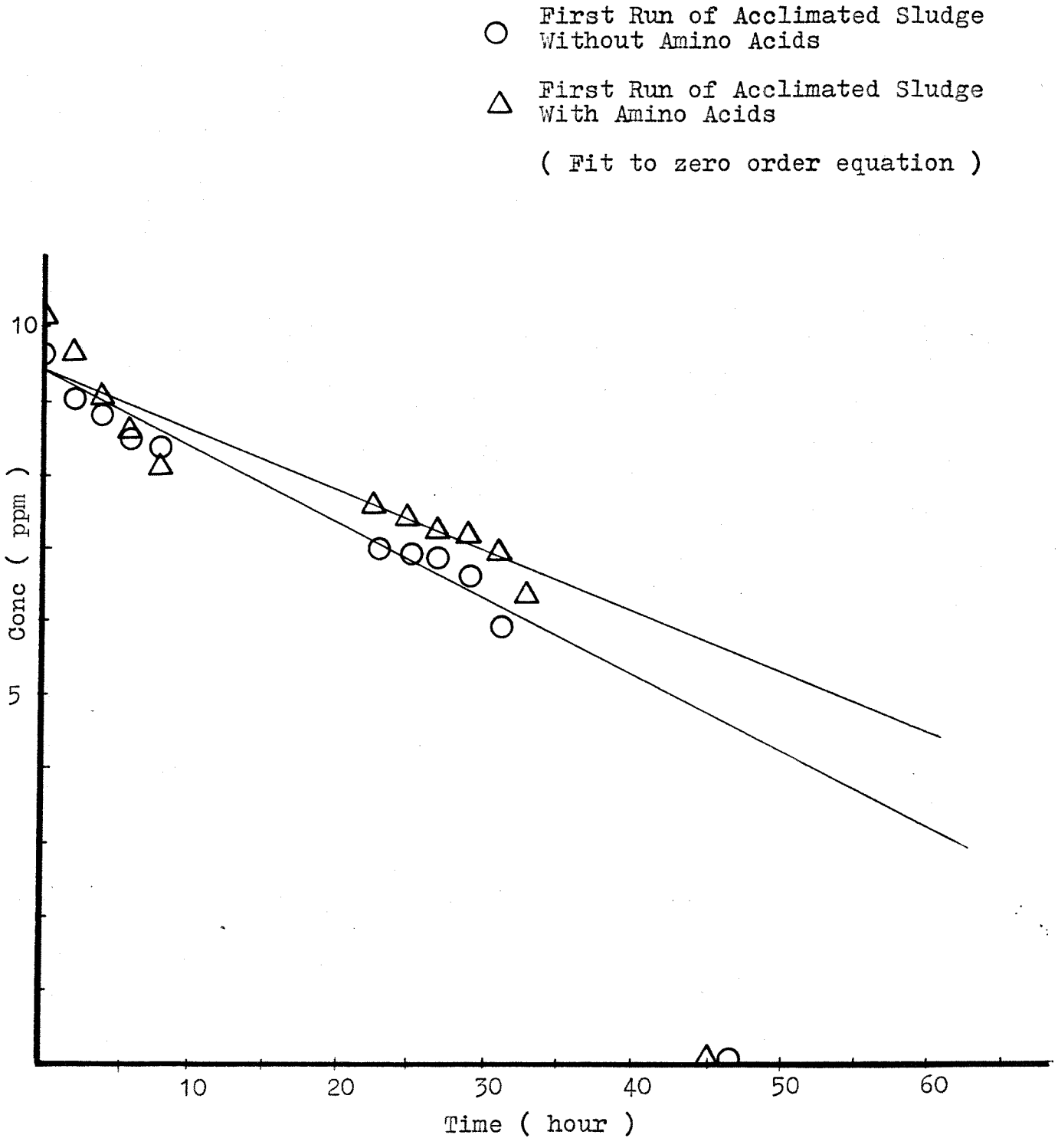


Figure #19 - Result of 10 ppm 2,6-dichlorophenol Run

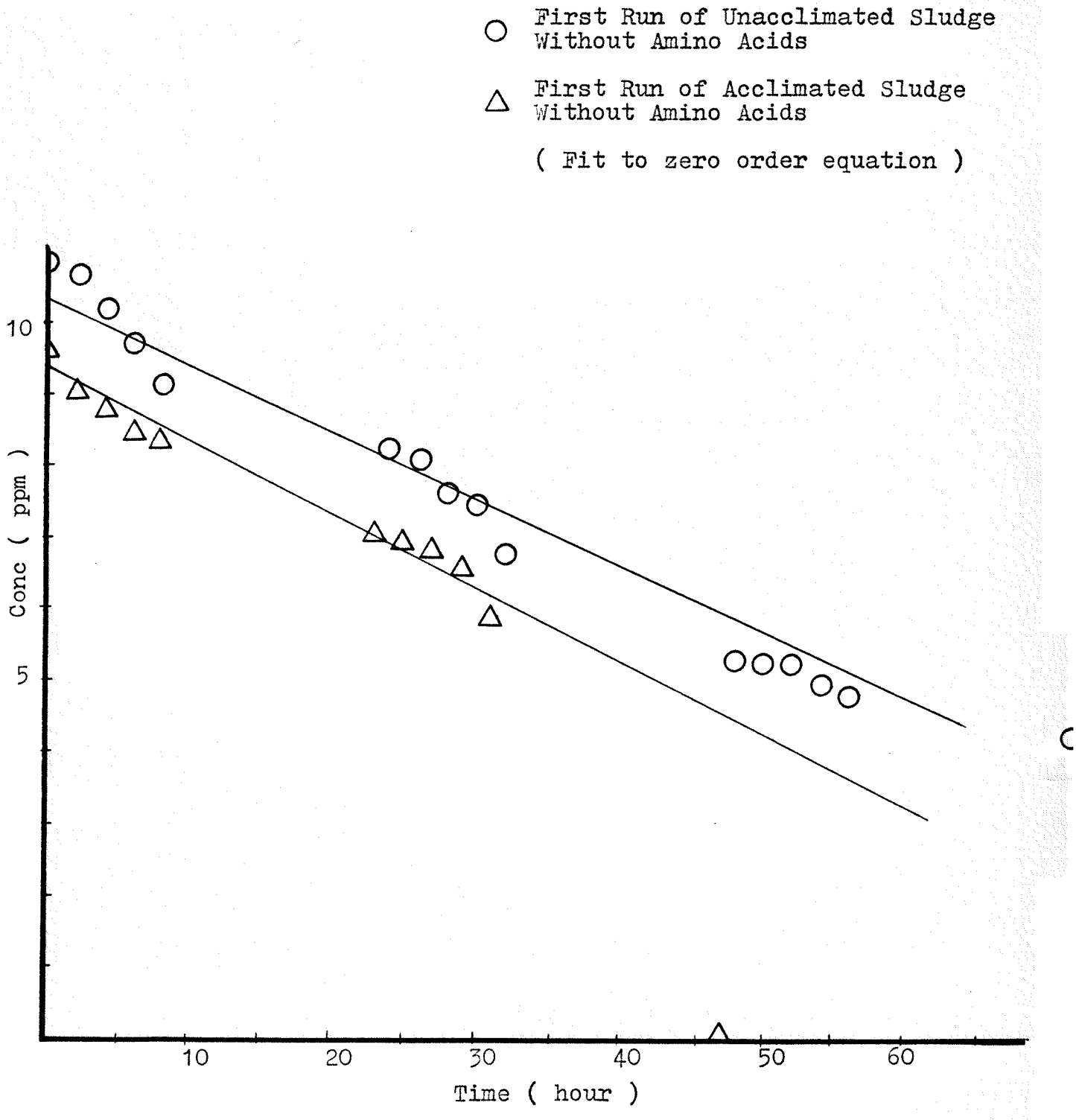


Figure #20 - Result of 10 ppm 2,6-dichlorophenol

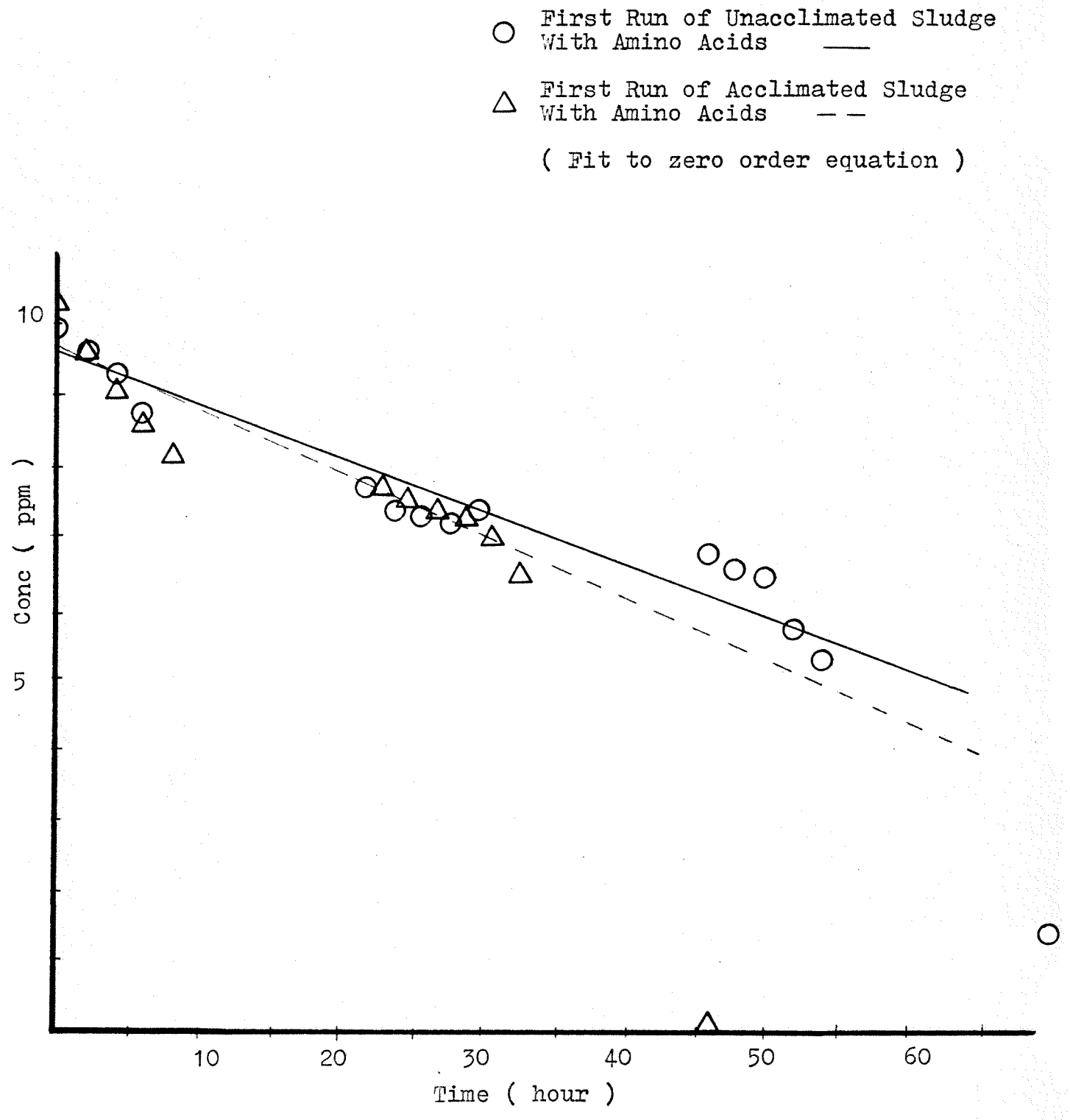


Figure #21 - MLSS Concentration of 100 ppm Phenol Run

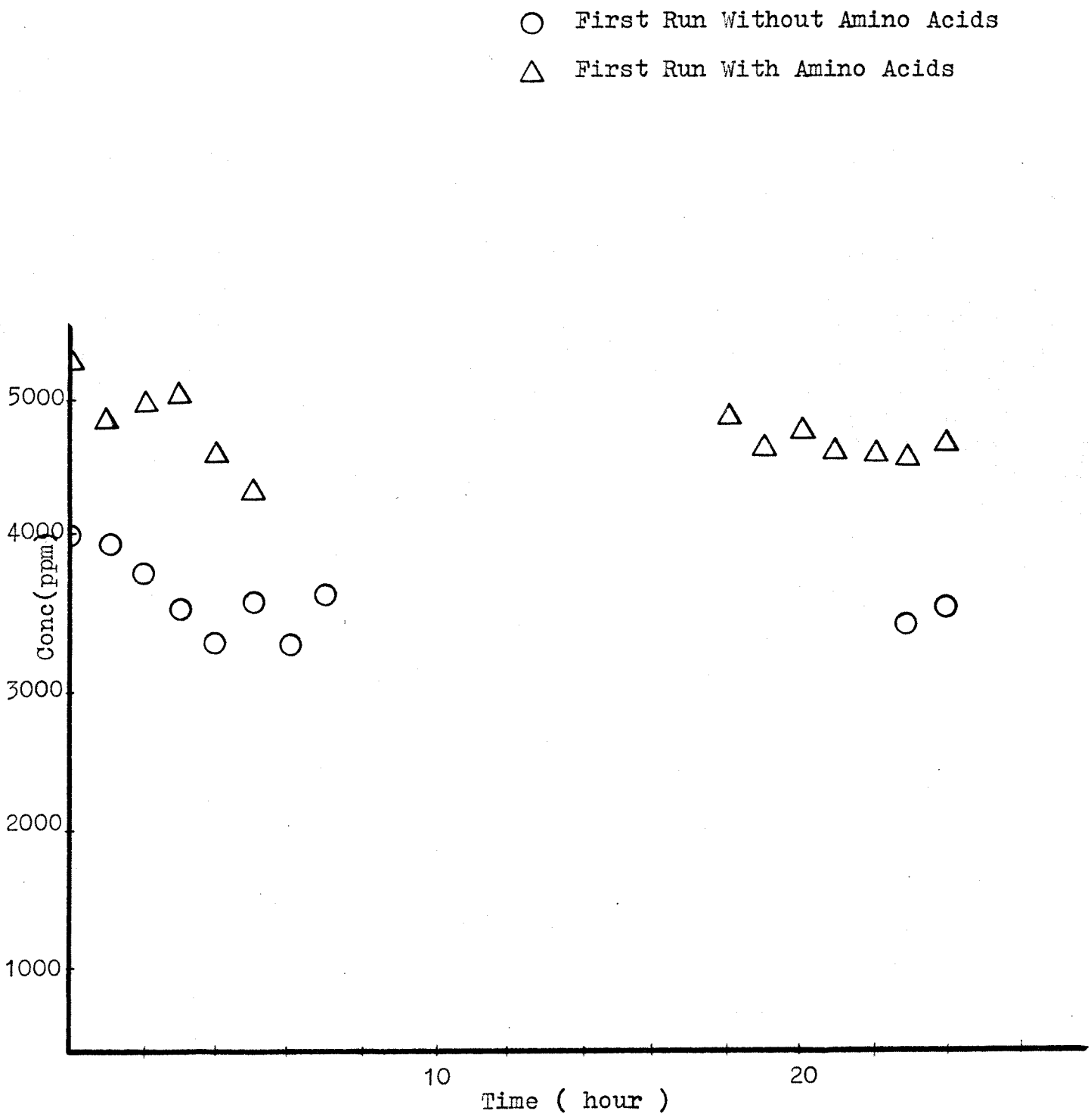


Figure #22 - pH vs. Time of 100 ppm Phenol Run

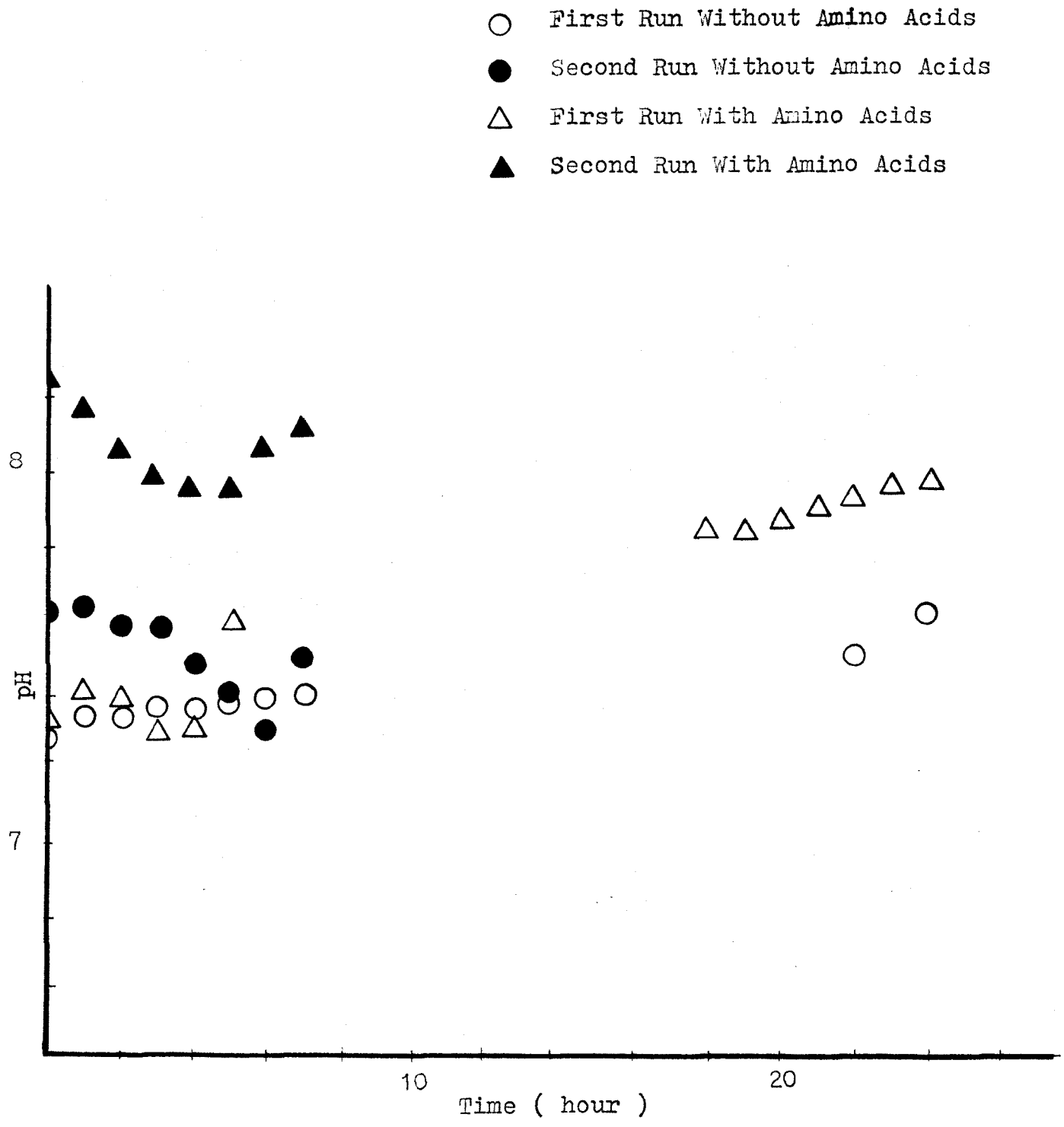


Figure #23 - Ammonia Concentration of 100 ppm Phenol Run

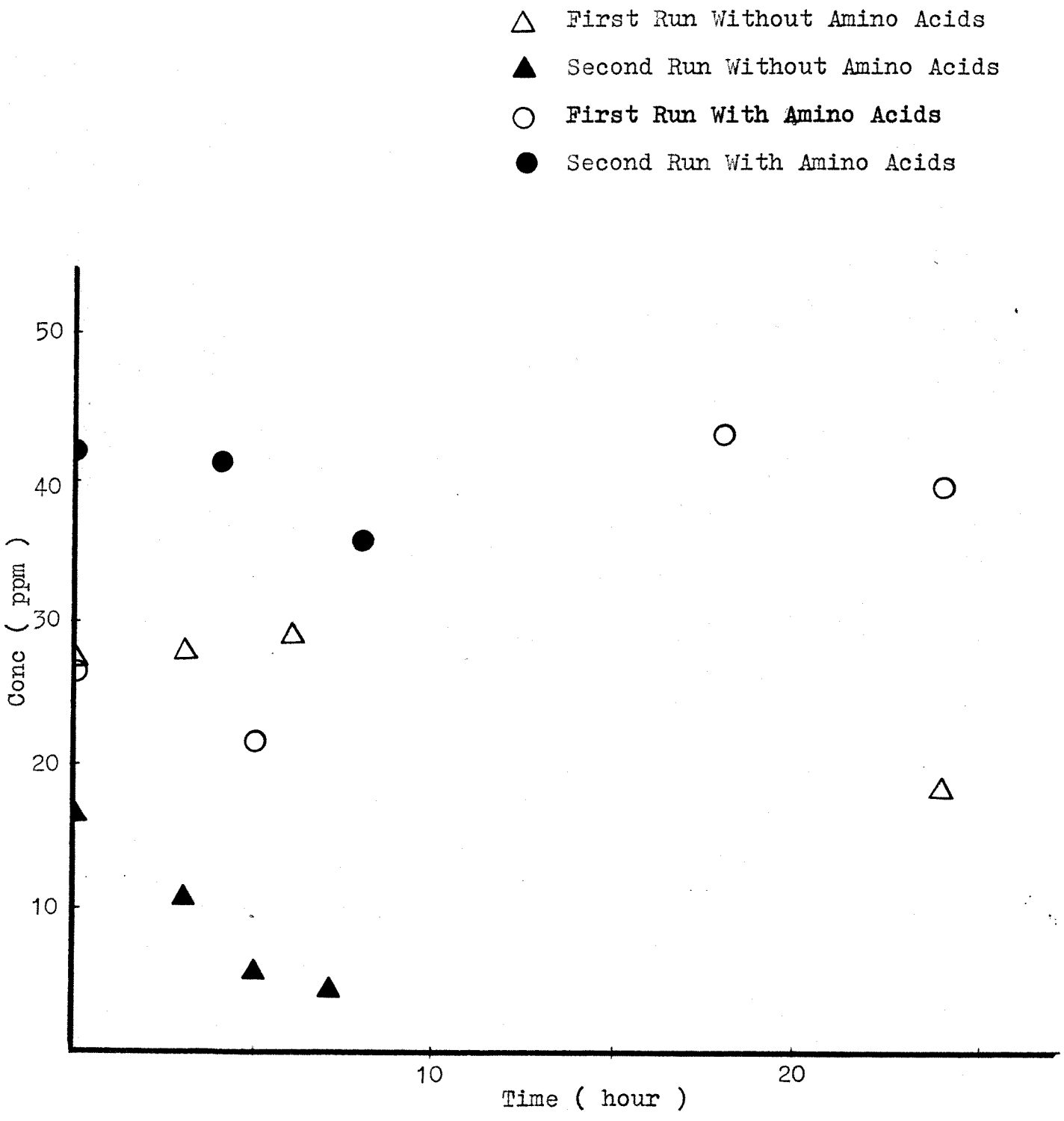


Figure #24 - MLSS Concentration of 2-chlorophenol Run

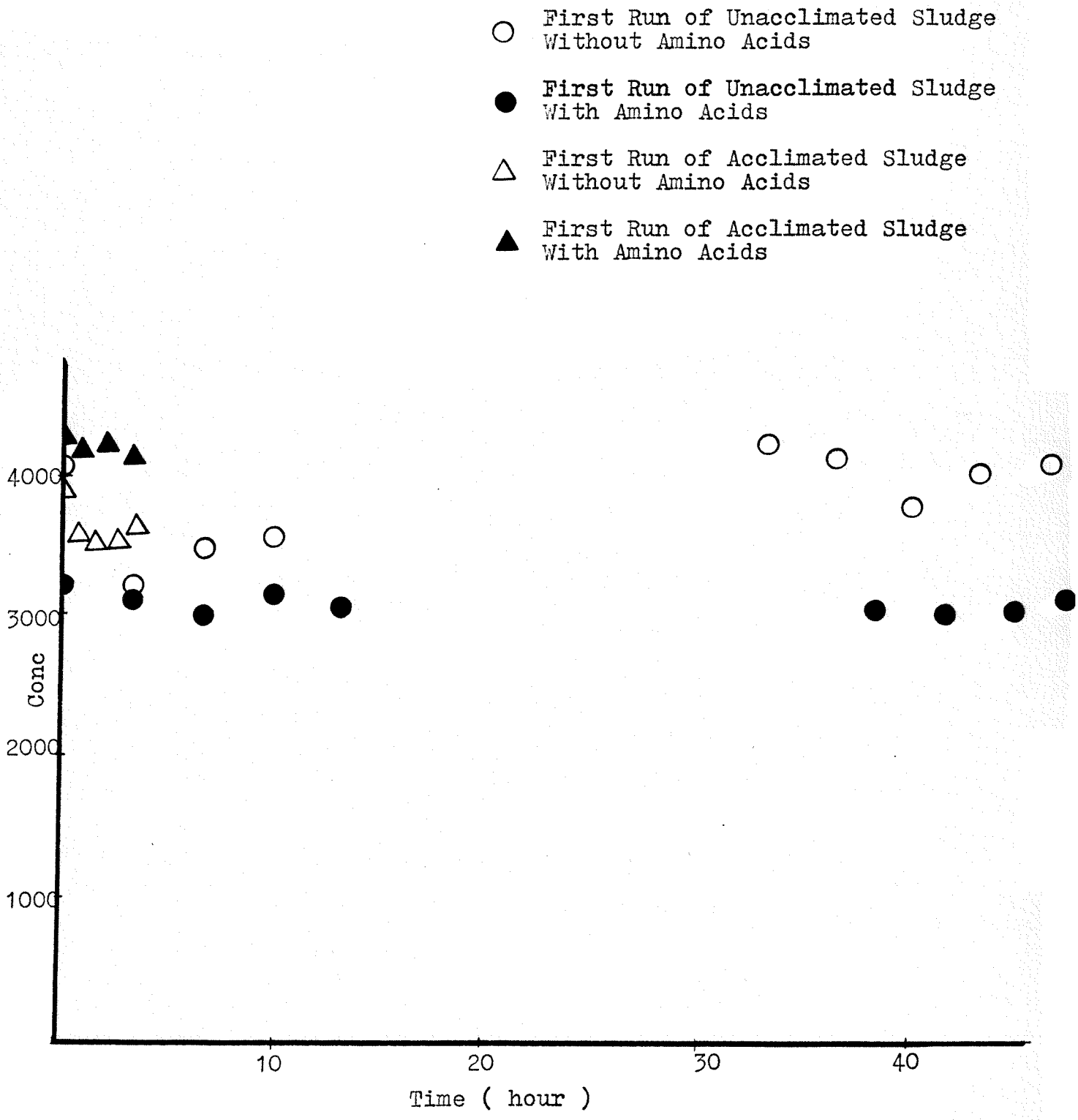


Figure #25 - pH vs. Time of 20 ppm 2-chlorophenol Run

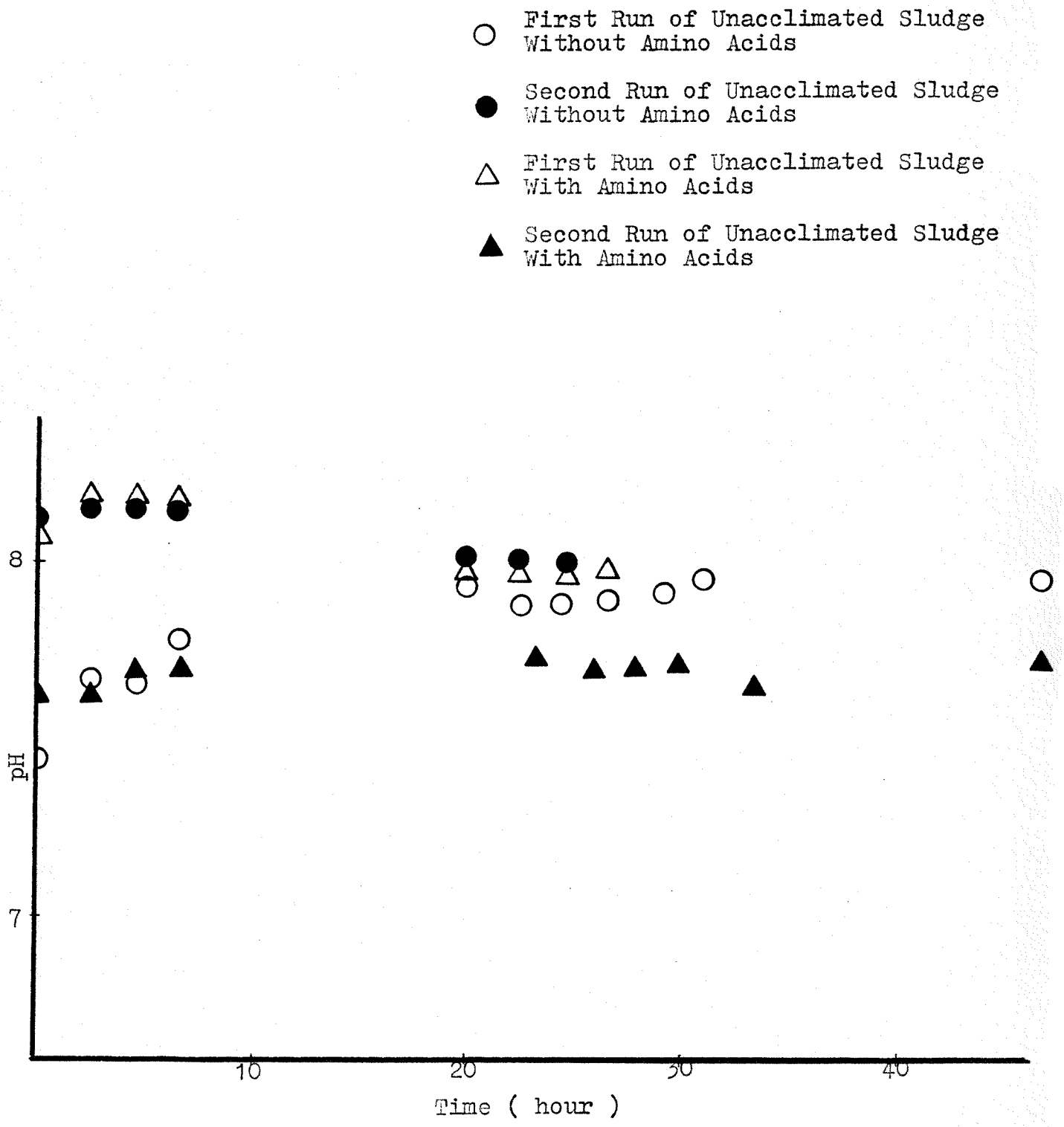


Figure #26 - pH vs. Time of 20 ppm 2-chlorophenol Run

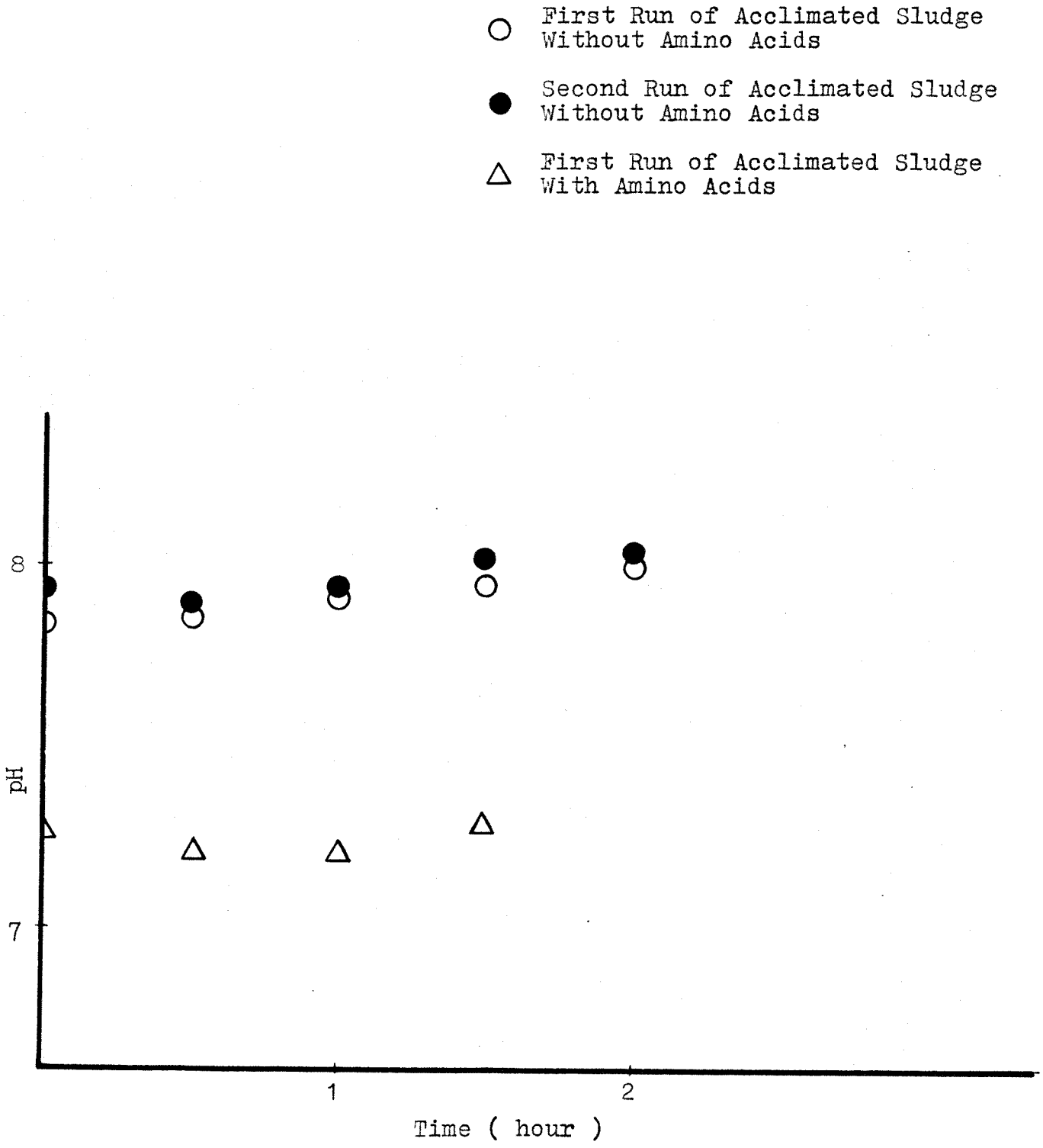


Figure #27 - Ammonia Concentration of 2-chlorophenol Run

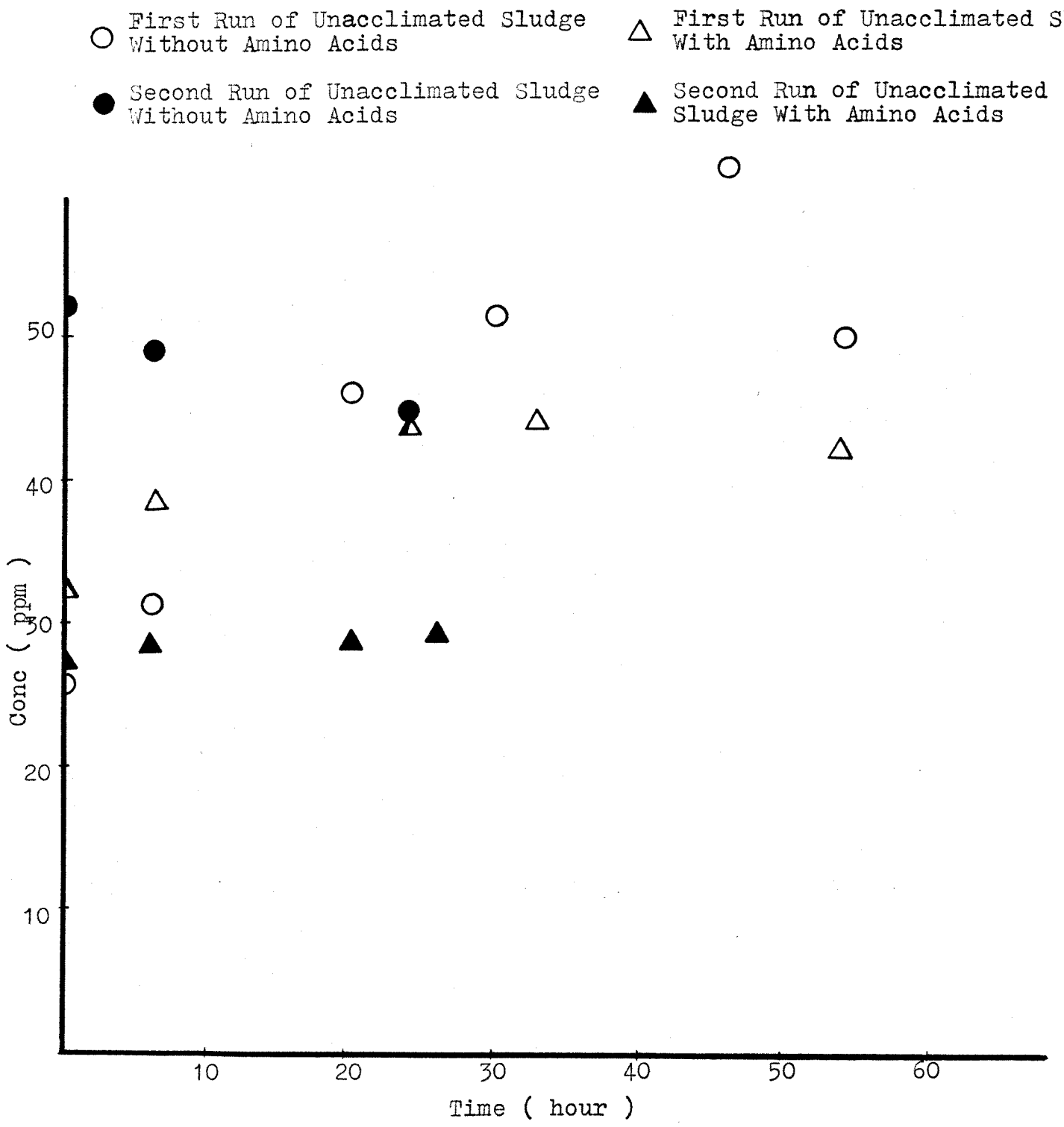


Figure #28 - Ammonia Concentration of 2-chlorophenol Run

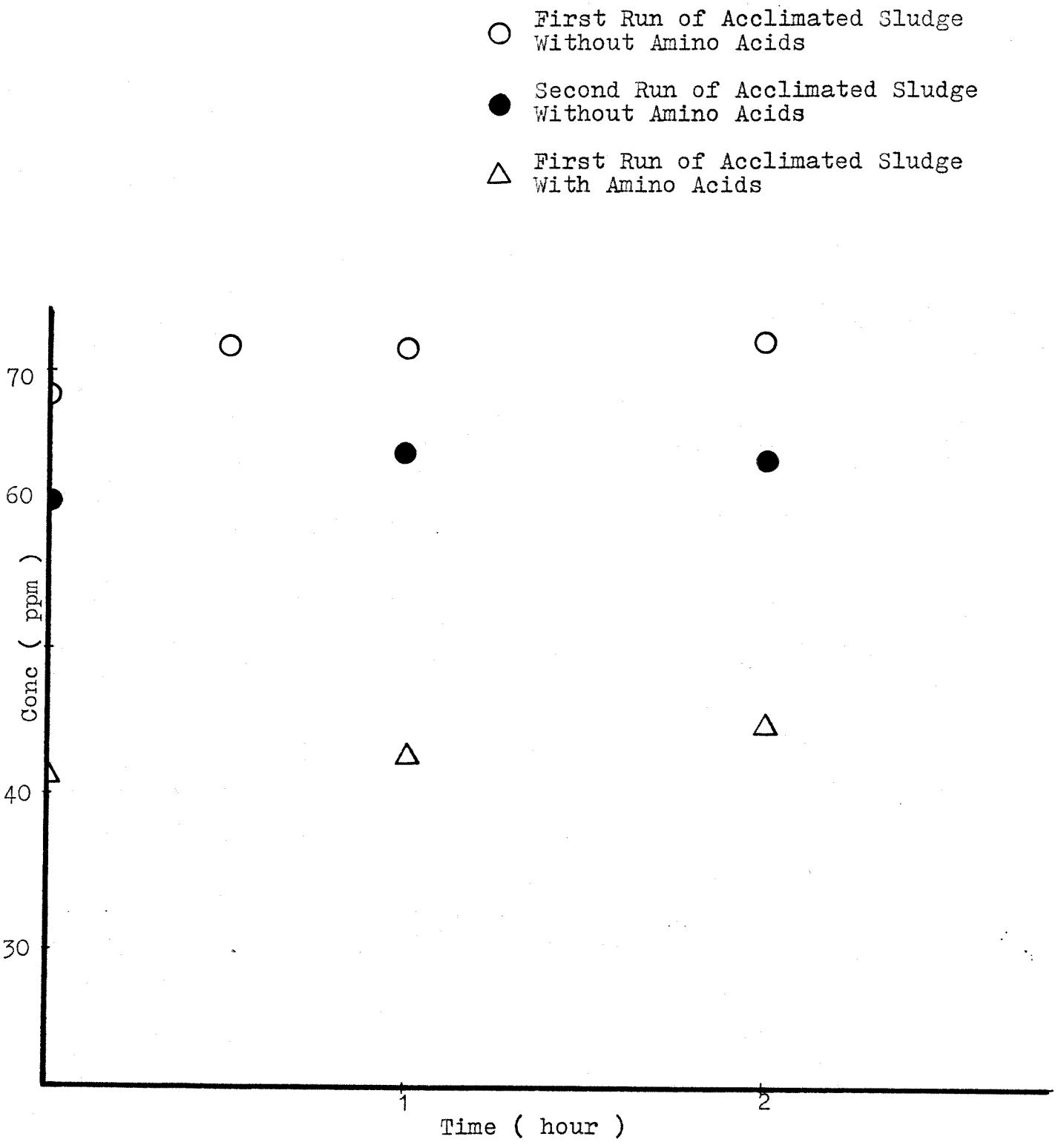


Figure #29 - MLSS Concentration of 10 ppm 2,6-DCP Run

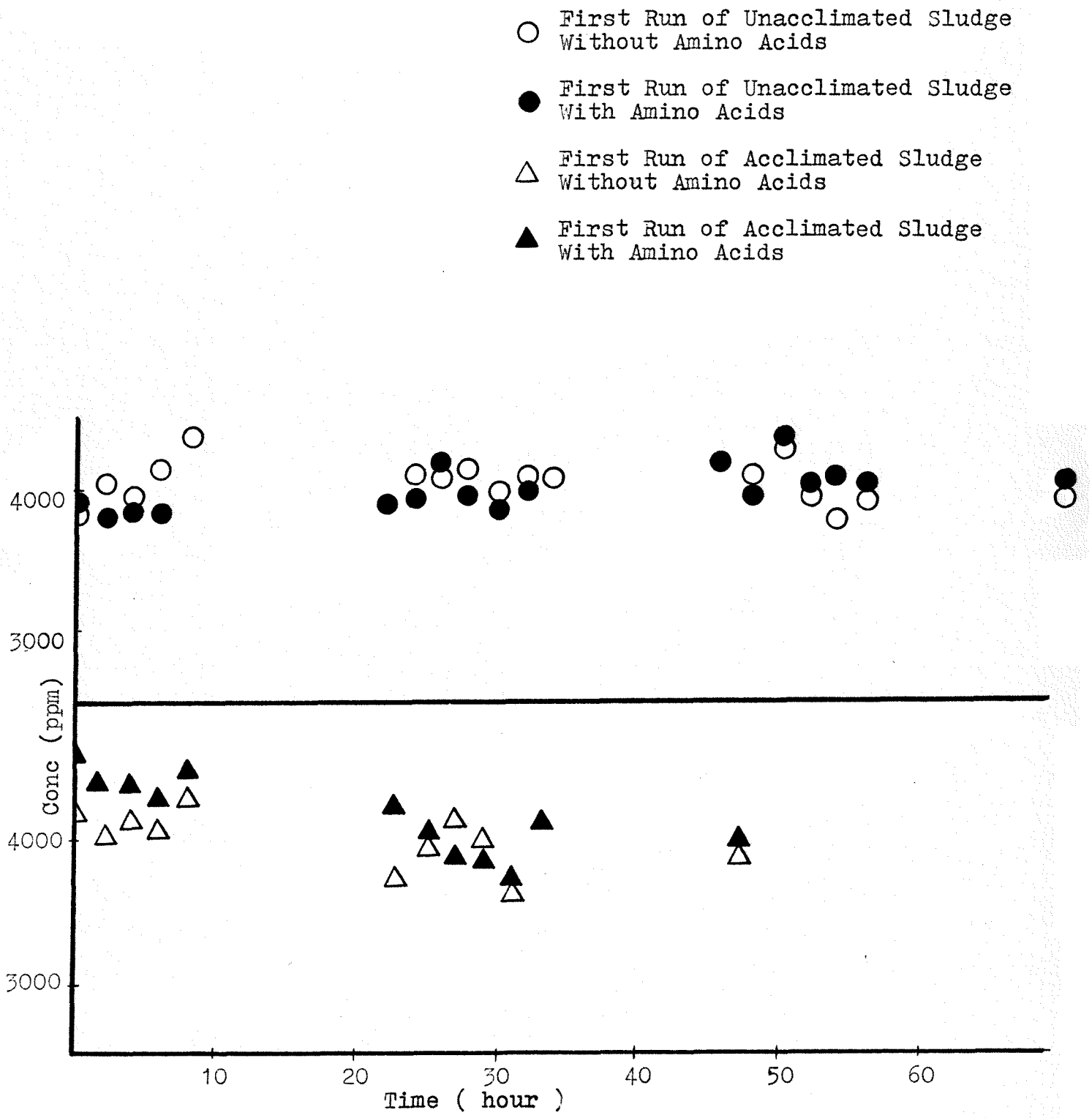


Figure #30 - pH vs. Time of 10 ppm 2,6-DCP Run

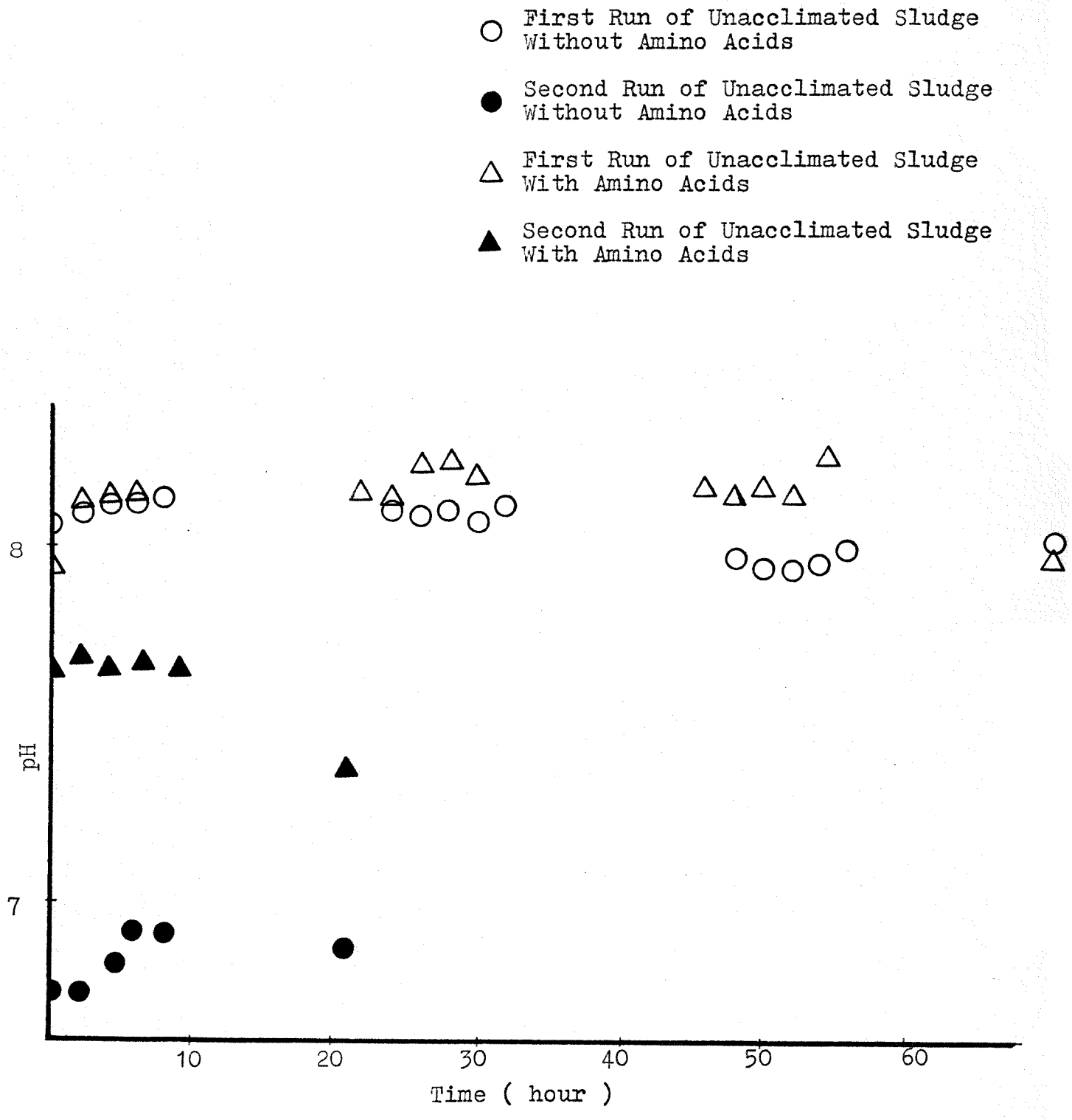


Figure #31 - pH vs. Time of 10 ppm 2,6-DCP Run

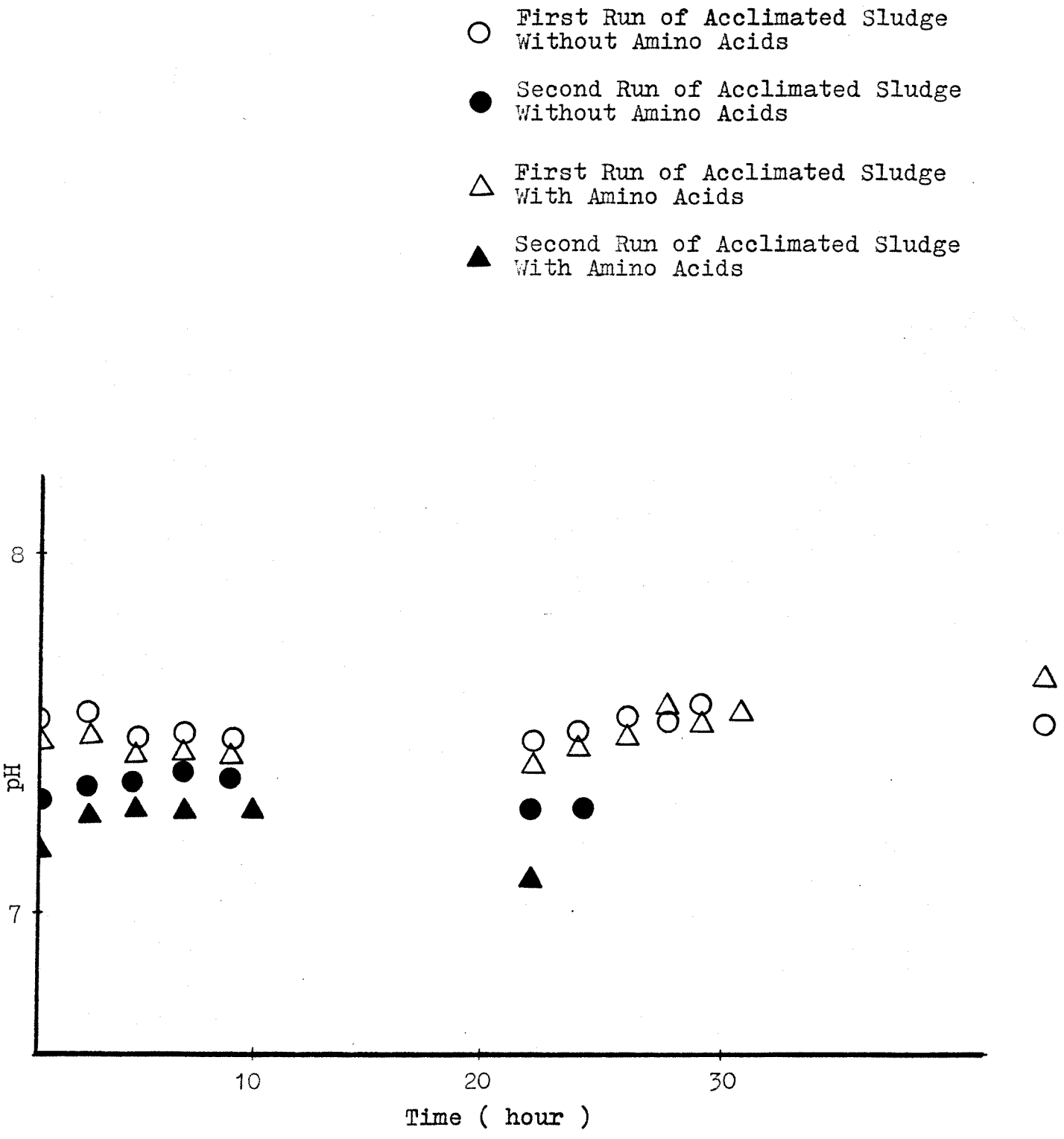


Figure #32 - Ammonia Concentration of 10 ppm 2,6-DCP Run

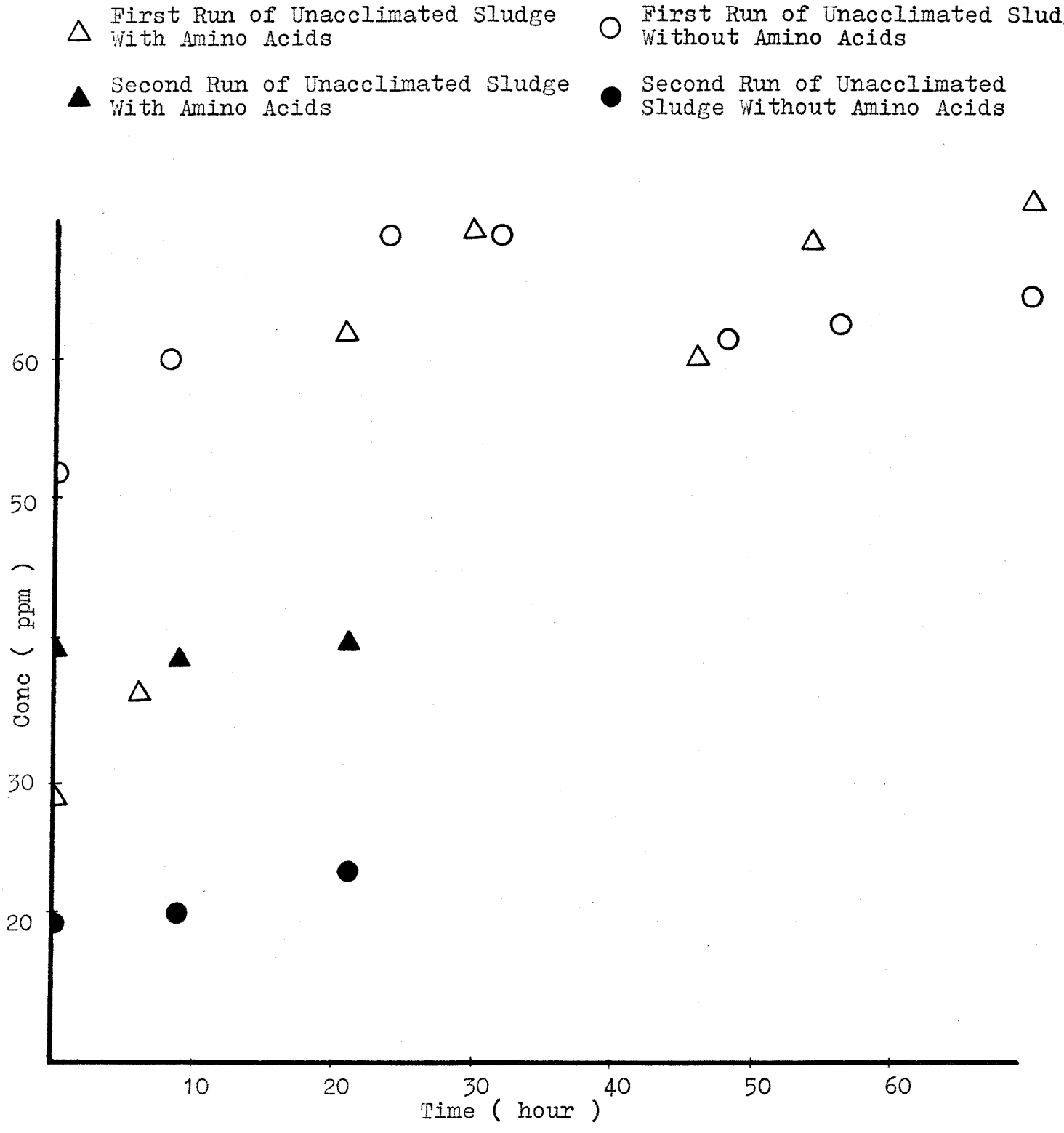


Figure #33 - Ammonia Concentration of 10 ppm 2,6-DCP Run

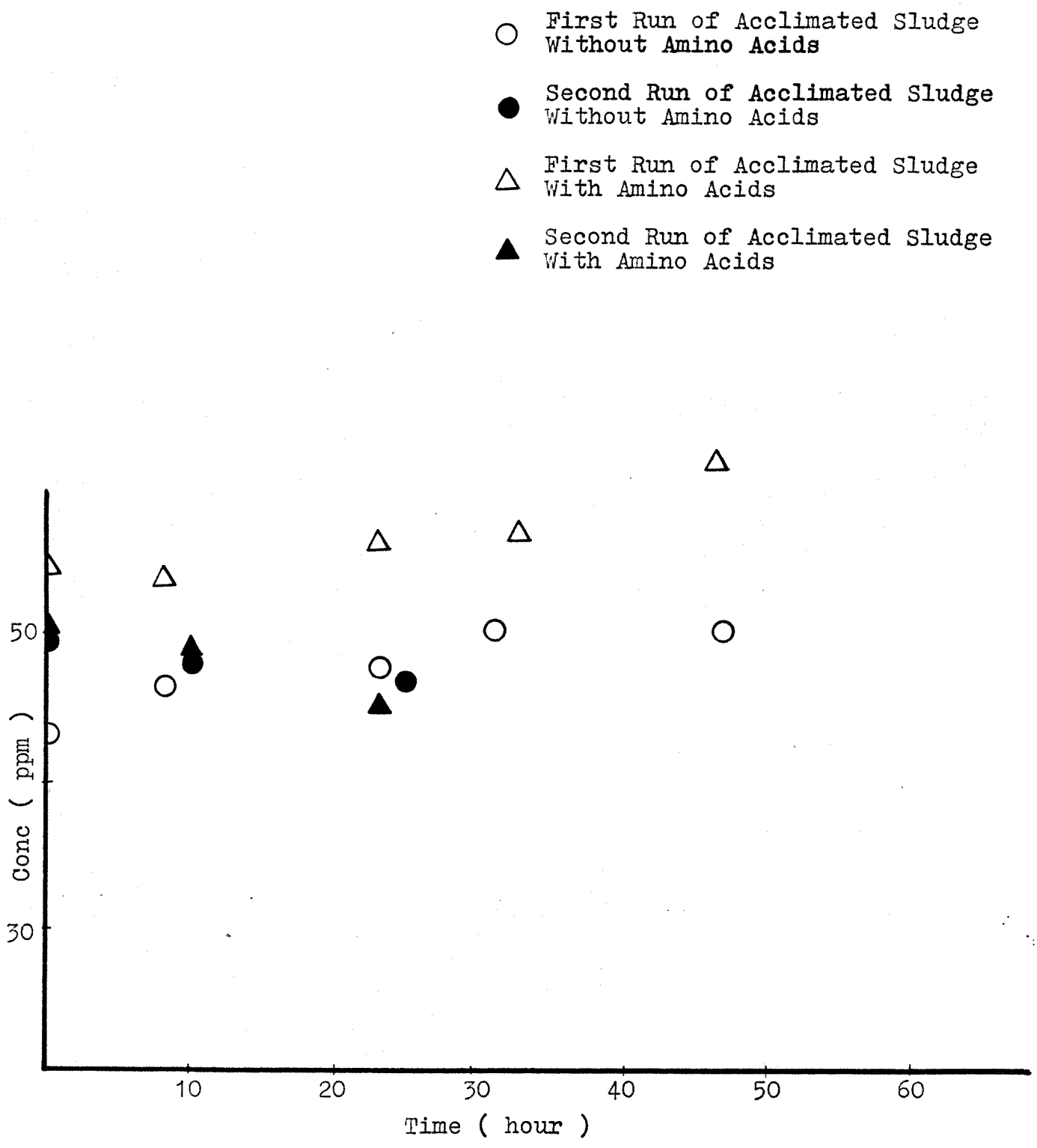


Figure #34 - Result of COD of First 100 ppm Phenol Run

(Without Amino Acids)

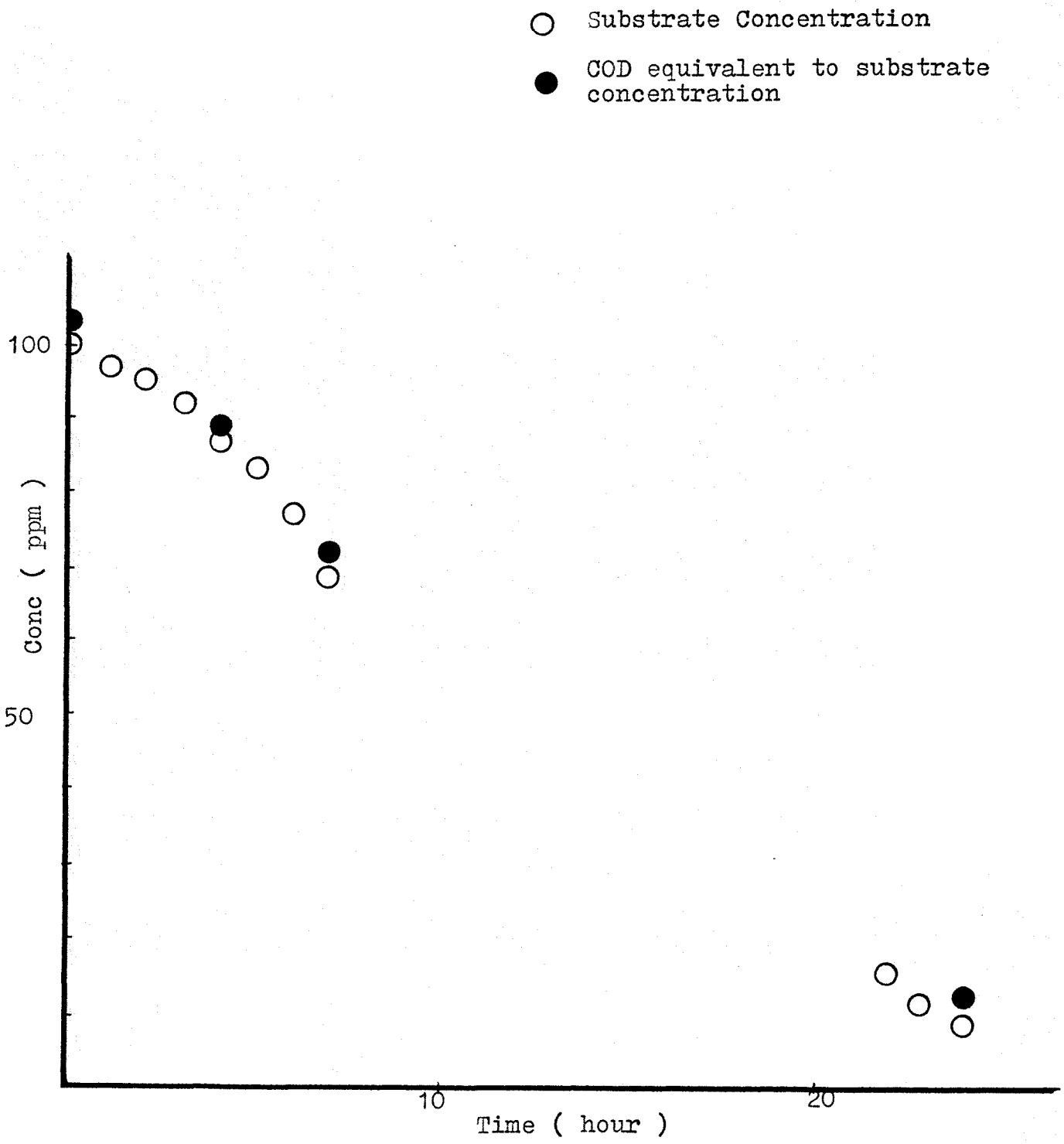


Figure #35 - Result of COD of Second 100 ppm Phenol Run
(Without Amino Acids)

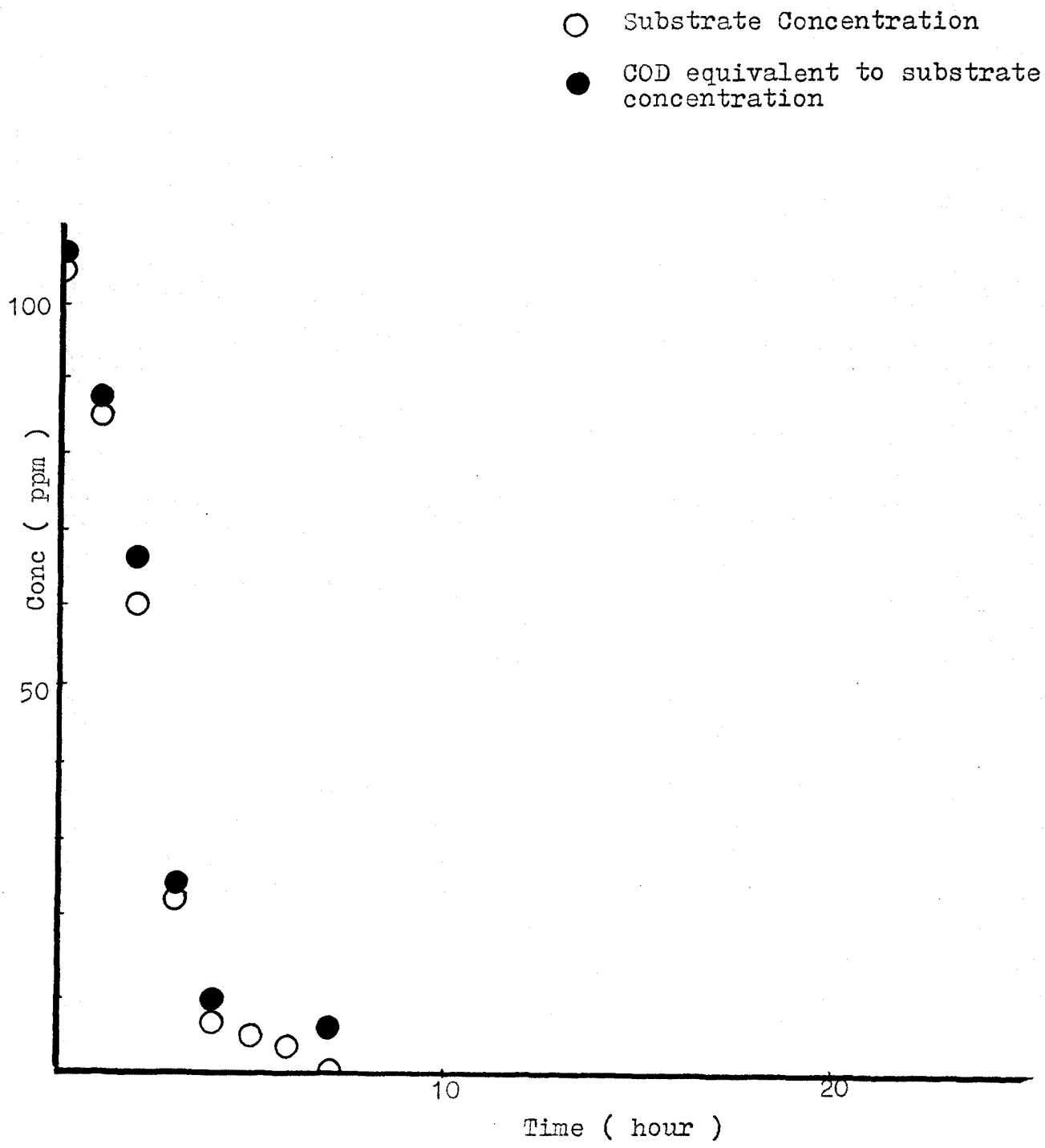


Figure #36 - Result of COD of First 100 ppm Phenol Run
(With Amino Acids)

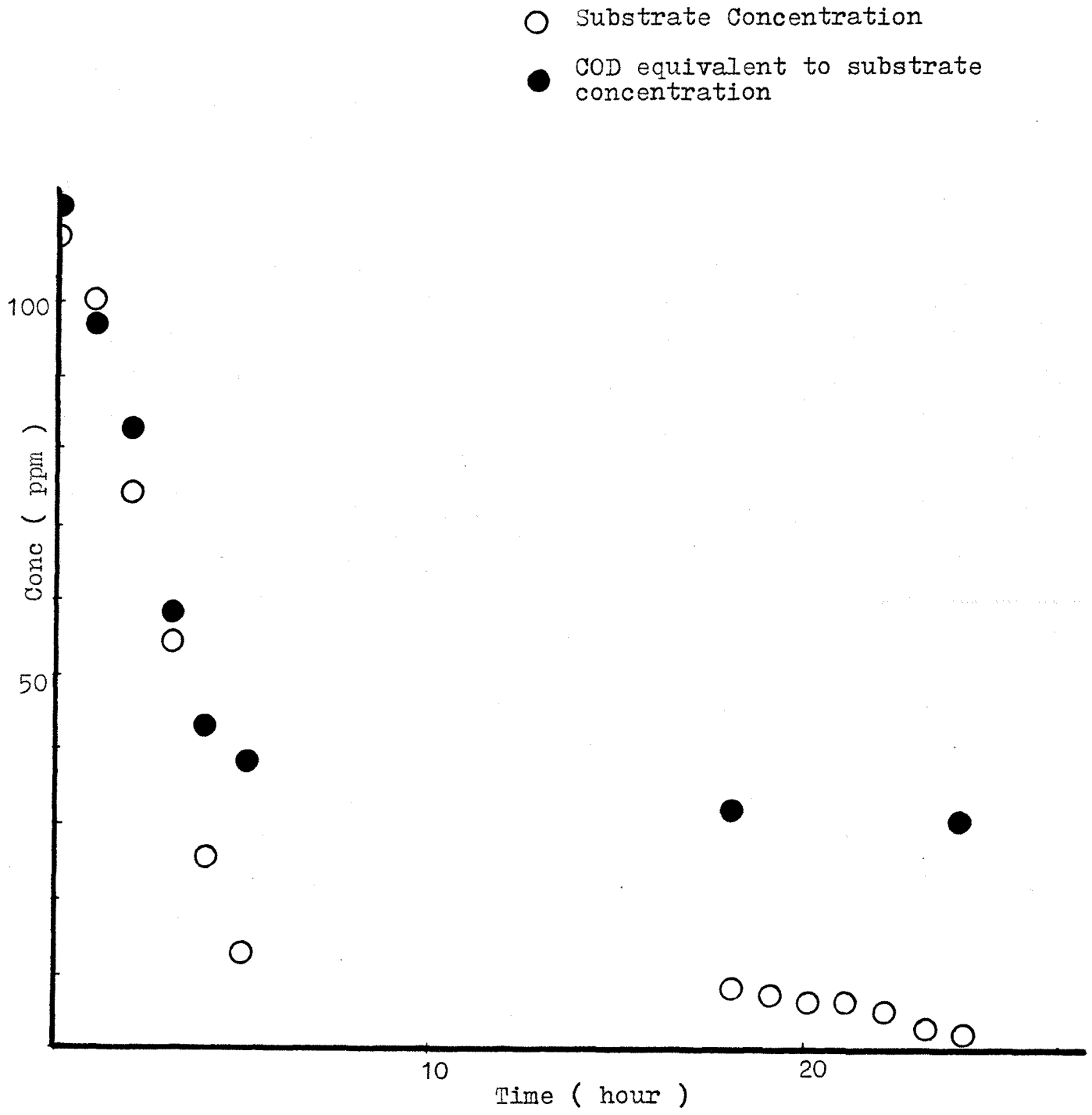


Figure #37 - Result of COD of Second 100 ppm Phenol Run
(With Amino Acids)

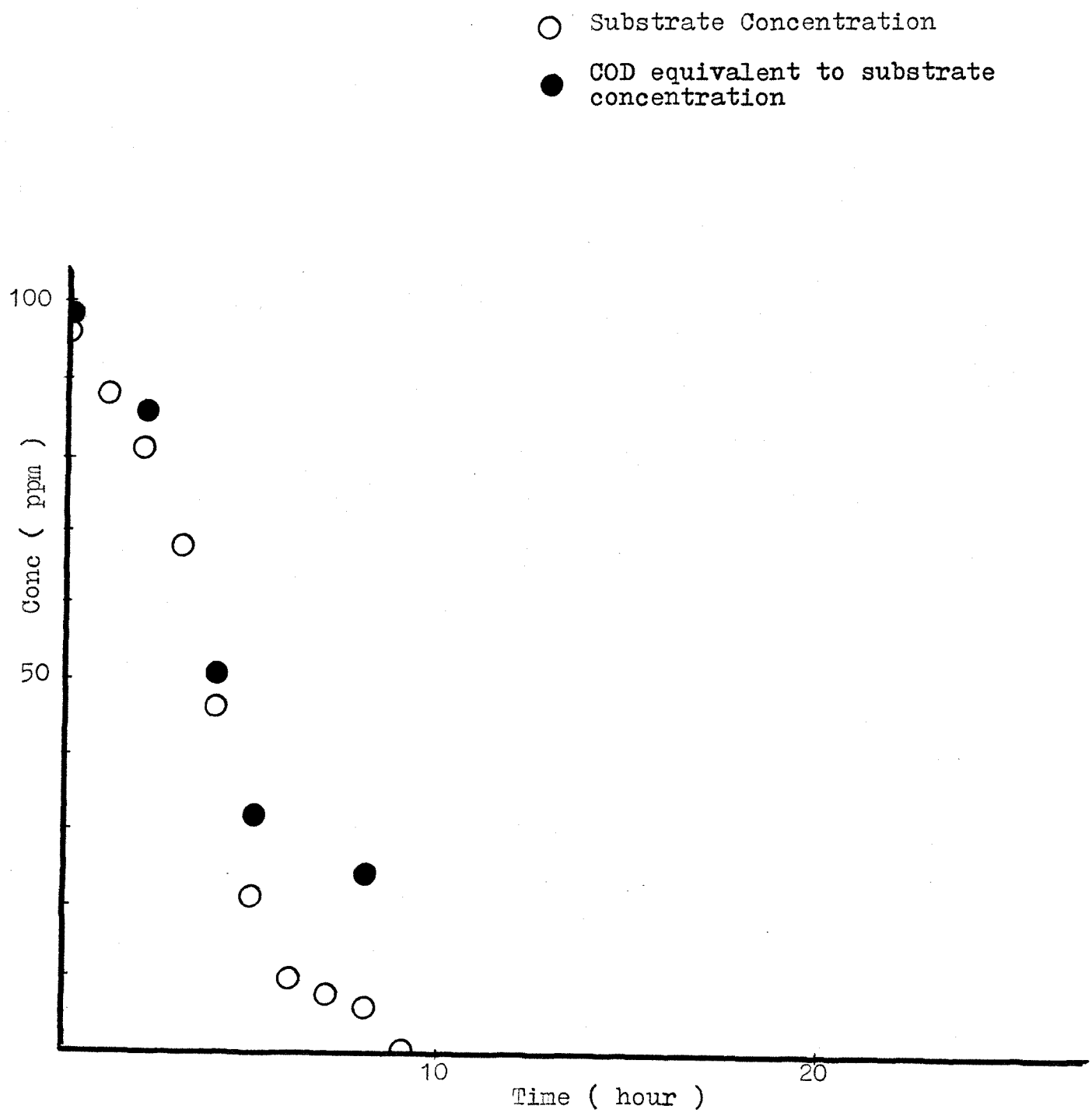
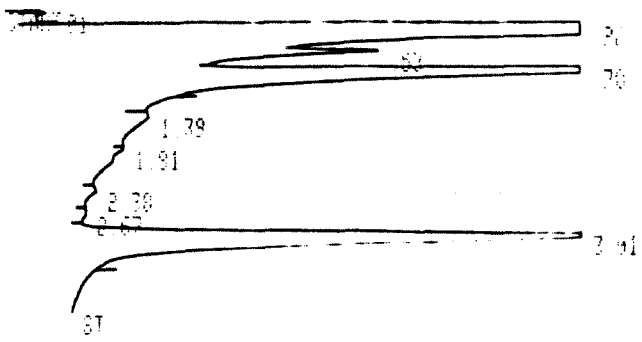


Figure #38 - Example of GC Analysis

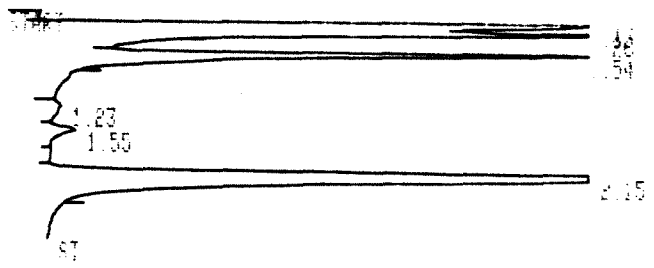


RUN # 2681 SEP/28/84 02:35:11

ISTD

RT	AREA	TYPE	CAL#	AMOUNT
0.78	787500	VB	1R	59.602
3.01	746670	PB	2S	45.450

* 59.602 ppm Phenol

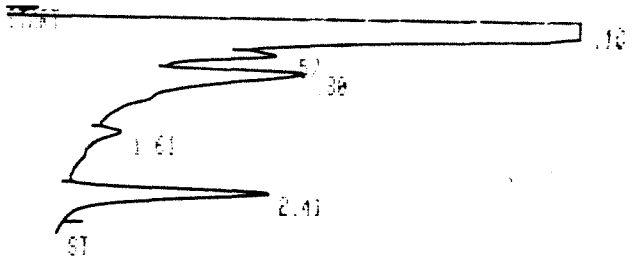


RUN # 660 AUG/08/84 14:13:59

ISTD

RT	AREA	TYPE	CAL#	AMOUNT
0.54	280800	BB	1R	20.185
2.15	822010	PB	2S	45.454

* 20.185 ppm 2-chlorophenol



RUN # 1200 AUG/15/84 15:41:20

ISTD

RT	AREA	TYPE	CAL#	AMOUNT
0.80	55581	PP	1R	10.388
2.41	58806	PB	2S	16.000

* 10.388 ppm 2,6-dichlorophenol

APPENDIX 1 COMPUTER PROGRAMING

```
C
C     ************************************************************
C     *                                                          *
C     *       PROGRAM      REGRESS                               *
C     *                                                          *
C     ************************************************************
C
C     Written by    :   Daewon   Pak
C
C     Purpose       :   To fit the substrate concentration versus
C                       time  data  to  the  following  kinetic
C                       equations and determine the rate constant
C
C                       1)  Zero-order rate equation
C                       2)  First-order rate equation
C                       3)  Monod equation
C                       4)  Haldane equation
C
C     Data input    :   NP      =  No of point
C                       TM(I)   =  Time
C                       CN(I)   =  Substrate concentration
C                       Note    :  time is in hour
C                                  substrate concentration is  in
C                                  ppm
C
      DIMENSION  CN(30), TM(30), CY(30), CNL(30), DY(30)
      DIMENSION  X(30), Y(30), Z(30)
      READ(5,*)  NP
      DO  101  I = 1 , NP
        READ(5,*) T , CO
        TM(I) = T
        CN(I) = CO
101   CONTINUE
C
C     TO CALCULATE ZERO ORDER RATE CONSTANT
C
      CALL  REGRESS  (CN, TM, NP, DK, CY, DB)
      DK = -DK
      DO  102  J = 1 , NP
        DY(J) = CN(J) - CY(J)
102   CONTINUE
      CALL  AAR  (DY, NP, R)
      WRITE(6,201)  DK
      WRITE(6,220)  R
      WRITE(6,202)
      DO  103  K = 1 , NP
        WRITE(6,203)   TM(K), CN(K), CY(K), DY(K)
103   CONTINUE
C
C     TO CALCULATE FIRST-ORDER RATE CONSTANT
C
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      DO  104  L = 1 , NP
        A = CN(L)
        CY(L) = ALOG(A)
104   CONTINUE
      CALL  REGRESS  (CNL, TM, NP, DK, CY, DB)
      DO  108  I = 1 , NP
        A = CY(I)
        CY(I) = EXP(A)
108   CONTINUE
      DO  105  M = 1 , NP
        DY(M) = CN(M) - CY(M)
105   CONTINUE
      CALL  AAR  (DY, NP, R)
      DK = -DK
      WRITE(6,204)  DK
      WRITE(6,220)  R
      WRITE(6,202)
      DO  106  N = 1 , NP
        WRITE(6,203)  TM(N), CN(N), CY(N), DY(N)
106   CONTINUE
C
C     TO FIT SUBSTRATE CONCENTRATION VS. TIME DATA TO MONOD
C     MODEL
C
      NPN = NP - 1
      DO  107  I = 1 , NPN
        A = CN(1)/CN(I+1)
        X(I) = TM(I+1)
        Y(I) = ALOG(A)
        Z(I) = CN(I)-CN(I+1)
107   CONTINUE
      CALL  REGRES2  (X, Y, Z, NPN, CY, B, C)
      DO  118  K = 1 , NPN
        X(K+1) = CN(1) - CY(K)
118   CONTINUE
      X(1) = CN(1)
      DO  109  N = 1 , NP
        DY(N) = CN(N) - X(N)
109   CONTINUE
      CALL  AAR  (DY, NP, R)
      C = -C
      WRITE(6,205)   B
      WRITE(6,206)   C
      WRITE(6,220)   R
      WRITE(6,202)
      DO  110  K = 1 , NP
        WRITE(6,203)  TM(K), CN(K), X(K), DY(K)
110   CONTINUE
C
C     TO FIT SUBSTRATE CONCENTRATION VS. TIME DATA TO HALDANE
C     MODEL
C
```

```
      DO  111  I = 1 , NPN
        A1 = (CN(1)-CN(I+1))*(CN(1)+CN(I+1)
        A2 = CN(1)/CN(I+1)
        X(I) = TM(I+1)/A1
        Y(I) = ALOG(A2)/A1
        Z(I) = 1/(CN(1)+CN(I+1))
111   CONTINUE
      CALL  REGRES3  (X, Y, Z, NPN, B, C, D, CY)
      DO  112  N = 1 , NPN
        Z(N+1) = 1/CY(N)-CN(1)
112   CONTINUE
      Z(1) = CN(1)
      DO  113  M = 1 , NP
        DY(M) = CN(M) - Z(M)
113   CONTINUE
      CALL  AAR  (DY, NP, R)
      C = -C
      D = 1/(-2*D)
      WRITE(6,207)  B
      WRITE(6,208)  C
      WRITE(6,209)  D
      WRITE(6,220)  R
      WRITE(6,202)
      DO  114  L = 1 , NP
        WRITE(6,203)  TM(L), CN(L), Z(L), DY(L)
114   CONTINUE
201   FORMAT(' ',//,'ZERO ORDER REACTION , K =',2X,F10.5)
202   FORMAT(' ','TIME',7X,'CON(EXP)',5X,'CON(CAL)',7X,'DY',/)
203   FORMAT(' ',F5.2,6X,F7.3,6X,F7.3,5X,F7.3)
204   FORMAT(' ',//,'FIRST ORDER REACTION , K =',2X,F10.5)
205   FORMAT(' ',//,' MONOD MODEL , K1 =',2X,F10.5)
206   FORMAT(' ','                K2 =',2X,F10.5)
207   FORMAT(' ',//,' HALDANE MODEL , K1 =',2X,F10.5)
208   FORMAT(' ','                  K2 =',2X,F10.5)
209   FORMAT(' ','                  K3 =',2X,F10.5)
220   FORMAT(' ','ABSOLUTE AVERAGE RESIDUAL =',2X,F10.5,/)
      STOP
      END
```

```
      SUBROUTINE  REGRES  (CN, TM, NP, DK, CY, DB)
C
C     PURPOSE  :    TO REGRESS SUBSTRATE CONCENTRATION  VS.  TIME
C                   DATA ACCORDING TO ZERO-ORDER AND  FIRST-ORDER
C                   EQUATION
C
C     VARIABLE LISTING
C
C                   CN  :  SUBSTRATE CONCENTRATION
C                   TM  :  TIME
C                   NP  :  NO. OF POINT
C                   DK  :  RATE CONSTANT
C                   CY  :  DIFFERENCE  BETWEEN  CALCULATED  AND
C                          EXPERIMENT VALUE OF CONCENTRATION
C
      DIMENSION  CN(30), TM(30), CY(30)
      SUMX=0
      SUMY=0
      SUMXY=0
      SUMX2=0
      DO  800  I = 1 , NP
        SUMX=SUMX+TM(I)
        SUMY=SUMY+CN(I)
        SUMXY=SUMXY+XY
        SUMX2=SUMX2+TM(I)**2
800   CONTINUE
      DNU=NP*SUMXY-SUMX*SUMY
      DEN=NP*SUMX2-SUMX**2
      DK=DNU/DEN
      DNU=SUMX2*SUMY-SUMXY*SUMX
      DB=DNU/DEN
      DO  900  J = 1 , NP
        CY(J)=DK*TM(J)+DB
900   CONTINUE
      RETURN
      END
```

```
      SUBROUTINE  REGRES2  (X , Y, Z, NPN, CY, B, C)
C
C     PURPOSE  :  TO REGRESS SUBSTRATE CONCENTRATION VS. TIME
C                 DATA ACCORDING TO MONOD MODEL
C
      DIMENSION  X(30), Y(30), Z(30), CY(30)
      SUMX2=0
      SUMY2=0
      SUMXY=0
      SUMXZ=0
      SUMYZ=0
      DO  501  I = 1 , NPN
        XY=X(I)*Y(I)
        XZ=X(I)*Z(I)
        YZ=Y(I)*Z(I)
        SUMX2=SUMX2+X(I)**2
        SUMY2=SUMY2+Y(I)**2
        SUMXY=SUMXY+XY
        SUMXZ=SUMXZ+XZ
        SUMYZ=SUMYZ+YZ
501   CONTINUE
      DNU=SUMXZ*SUMY2-SUMXY*SUMYZ
      DEN=SUMX2*SUMY2-SUMXY**2
      B=DNU/DEN
      DNU=SUMX2*SUMYZ-SUMXZ*SUMXY
      C=DNU/DEN
      DO  502  J = 1 , NPN
        CY(J)=B*X(J)+C*Y(J)
502   CONTINUE
      RETURN
      END
```

```
      SUBROUTINE  REGRES3  (X, Y, Z, NPN, B, C, D, CY)
C
C     PURPOSE  :   TO REGRESS SUBSTRATE CONCENTRATION  VS.  TIME
C                  DATA ACCORDING TO HALDANE MODEL
C
      DIMENSION   X(30), Y(30), Z(30), CY(30)
      SUMX=0
      SUMY=0
      SUMZ=0
      SUMXY=0
      SUMXZ=0
      SUMYZ=0
      SUMX2=0
      SUMY2=0
      DO  601  I = 1 , NPN
        XY=X(I)*Y(I)
        XZ=X(I)*Z(I)
        YZ=Y(I)*Z(I)
        SUMX=SUMX+X(I)
        SUMY=SUMY+Y(I)
        SUMZ=SUMZ+Z(I)
        SUMXY=SUMXY+XY
        SUMXZ=SUMXZ+XZ
        SUMYZ=SUMYZ+YZ
        SUMX2=SUMX2+X(I)**2
        SUMY2=SUMY2+Y(I)**2
601   CONTINUE
      T1=NPN*SUMX2*SUMY2+SUMX*SUMY*SUMXY*2
      T2=SUMY2*SUMX**2+SUMX2*SUMY**2+NPN*SUMXY**2
      DEN=T1-T2
      T1=NPN*SUMY2*SUMXZ+SUMY*SUMZ*SUMXY+SUMX*SUMY*SUMYZ
      T2=SUMX*SUMY2*SUMZ+SUMXZ*SUMY**2+NPN*SUMXY*SUMYZ
      DNU=T1-T2
      B=DNU/DEN
      T1=NPN*SUMX2*SUMYZ+SUMX*SUMY*SUMXZ+SUMX*SUMXY*SUMZ
      T2=SUMYZ*SUMX**2+SUMX2*SUMY*SUMZ+NPN*SUMXZ*SUMXY
      DNU=T1-T2
      C=DNU/DEN
      T1=SUMX2*SUMZ*SUMY2+SUMX*SUMYZ*SUMXY+SUMXZ*SUMXY*SUMY
      T2=SUMX*SUMY2*SUMXZ+SUMY*SUMX2*SUMYZ+SUMZ*SUMXY**2
      DNU=T1-T2
      D=DNU/DEN
      DO  602  K = 1 , NPN
        CY(K)=B*X(K)+C*Y(K)+D
602   CONTINUE
      RETURN
      END
```

```
      SUBROUTINE  AAR  (DY, NP, R)
C
C     PURPOSE  :  TO CALCULATE THE ABSOLUTE AVERAGE RESIDUAL
C
      DIMENSION   DY(30)
      SUM=0
      DO  901  K = 1 , NP
        DUM=DY(K)
        DUM=DUM**2
        SUM=SUM+DUM
901   CONTINUE
      R=SUM/(NP-1)
      RETURN
      END
```