ABSTRACT

Title of Thesis : Kinetics of Biodegradation of Phenol and 2,6-Dichlorophenol

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Biological removal of phenol up to 600 ppm and 2,6-dichlorophenol up to 20 ppm was studied in a well aerated fill-and-draw reactor of 4 l capacity at 26 C using an activated sludge obtained from the municipal treatment plant in Livingston, N.J. Experimental evaluation of kinetic parameters showed that biodegradation of both phenol and 2,6-DCP followed zero-order kinetics. As expected, the lag time for both compounds was considerably reduced when acclimated sludge was used. However, the metabolism of 2,6-DCP was very slow even for preacclimated sludge, requiring about 36 hours to degrade only 15 ppm. This was

markedly improved by the addition of amino-acids to the feed solution, which reduced the degradation time for 15 ppm 2,6-DCP to about 10 hours. The loss of substrate due to air stripping was negligible for both compounds at room temperature.

KINETICS OF BIODEGRADATION OF PHENOL AND 2,6-DICHLOROPHENOL

by
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I. INTRODUCTION

There is increasing public concern over the introduction of harmful pollutants in the drinking water supply. The rapid advances in industrial technology have brought about new man-made organic chemicals which were hitherto unknown to Nature. The ubiquity of this new class of synthetic molecules in our environment is potentially hazardous because most living organisms have yet to adapt themselves to metabolize these molecules.

The most common disposal techniques include landfilling, chemical oxidation, thermal oxidation (incineration) and biological oxidation.

Landfills have been conventionally used to dump all kinds of chemicals. This method has become the center of controversy due to drinking water contamination problems. Landfills do not destroy the waste but merely store it. Therefore, this is not a method of final disposal.

Chemical oxidation makes use of oxidizing agents like ozone, hydrogen peroxide, oxygen, hypochlorous acid and elemental chlorine, which introduce oxygen into the molecular structure of the waste organic compounds, followed by ring cleavage and disintegration leading to the formation of carbon-dioxide and water. Most of these techniques are applicable only to certain types of compounds, and 100 %

degradation may not be realized. Some of them are too expensive, while others require extensive pretreatment. All of the oxidizing agents can form toxic intermediates (some of them chlorinated).

Thermal oxidation uses high temperatures rather than chemical energy to oxidize organic molecules. However, chlorinated organics pose a thermodynamic problem because of the stability of the carbon-chlorine bond in an oxidizing atmosphere. The potential then exists for releasing chlorinated fragments of the original molecule, which may themselves be toxic.

Biological oxidation process can be effectively used to destroy many kinds of toxic and hazardous wastes. Certain fungi and bacteria may be able to utilize the waste as a food source for growth and energy production. In other cases, a chemical subject to microbial action may not contribute to growth or energy production. This is termed cometabolism and requires an additional food source to maintain the microbial population. Biological oxidation requires very little energy input (as opposed to thermal oxidation), since the organisms can utilize enzymes to catalytically oxidize the organic molecule at room temperature. The enzymes are produced by the organisms from a synthesis of appropriate amino-acids. Enzymatic action is extremely specific, with some that cleave aromatic rings by

oxidation, others that dehydrogenate, hydrolyze, etc.

It is of extreme importance to realize that microbes have taken millions of years to develop this capability to metabolize various compounds which are naturally synthesized. As a result, man-made compounds can not be expected to degrade readily unless their structural features were previously encountered in Nature. In many cases, the microbes may fail to produce the necessary enzymes. A molecule that can not penetrate the cell wall, and is not modified by an extracellular enzyme, is recalcitrant.

The present study will concentrate on phenol and its halogenated derivatives. These compounds are relatively non-biodegradable, and their structure is similar to that of many pesticides as well as their precursors and metabolic products. Industrial production of phenolic compounds is very large due to their diverse applications, and phenolic wastewater is generated by production of polymeric resins, pulp and paper mills, oil refining, coal gasification, liquefaction, and coking plants.

It is desired to study the effect of increasing chlorination on biodegradability of aromatic structure. O-chlorophenol has been examined in a prior study in our laboratory. In case of 2,6-dichlorophenol both the ortho positions are blocked by chlorine atoms, investigation of its susceptibility to biodegradation is therefore more

interesting. Virtually no information is available about biological treatment of 2,6-dichlorophenol from literature. Hence, the present work concentrates on biological removal of 2,6-dichlorophenol using activated sludge.

II. LITERATURE CRITIQUE

An extensive amount of literature has already been published on biodegradation of various organic compounds. Kinetic parameters for batch or continuous operation of industrial wastewater treatment plants have also been evaluated. Research has been reported on phenol, o- and p-chlorophenols, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol, but pratically no information is available about 2,6-dichlorophenol except that from Baker, et al (4). Hence, the experimental work and results described in this study marks almost the first attempt to evaluate the biodegradability of 2,6-DCP.

One or more of the following models have been used by most workers in the field to evaluate the kinetic rate constants.

Michaelis Menten model: $-dS/dt = Um.X.S/ [Km + S]$

Haldane model: $-dS/dt = Um.X.S/ [Km + S + S^2/Ki]$

First order model: $-dS/dt = k.S$

Second order model: $-dS/dt = k.S^2$

Grau model: $-dS/dt = k.X.S/So$

where,

So = Initial substrate concentration, ppm.

S = Substrate concentration, ppm.

X = MLSS concentration, ppm.

t = time, hours.

Km = Michaelis Menten (substrate utilization) constant, ppm.

Um = Maximum specific growth rate constant, /hour.

k = Rate constant for 1st order or 2nd order or Grau model.

Ki = Substrate inhibition constant, ppm.

A. PHENOL AND OTHER POLYHYDROXYL BENZENES:

Since phenol is toxic and imparts a bad taste to water even at low concentration, the EPA has set a limit of 1 ppb of phenol in drinking water (84). Experimental and theoretical work on the biodegradation of phenol and other phenolic compounds have been reported since 1950 (7,8,11,14,15,16,18,20,23,24,33,35,36,38,39,41,42,43,45,46, 48,49,52,53,56,57,68,70,84). Though most of this information is about aerobic treatment, Neufeld (52) as well as Evans (23) have studied anaerobic biodegradation and cometabolism of phenols.

Kramer and Doetsch (42) surveyed the degradation of 55 phenolic compounds with a large variety of micro-organisms. Most of the observations they reported have been repeatedly asserted by numerous workers since then. They concluded that the presence of nitro, amino or multiple hydroxyl groups reduced microbial attack. Unfortunately, they were

not able to explain this behavior. Also, the ortho and para positions appeared to be more susceptible to biological attack than the meta position, as demonstrated by the p-cresol/m-cresol or catechol/resorcinol pairs.

In batch and continuous studies on phenol using Pseudomonas putida, Hill, et al (33) observed substrate inhibition, and therefore used the Haldane model to correlate their data (see Table #1). Substrate inhibition with phenol, particularly at concentrations above 360 ppm, has also been reported by a number of workers (8,33,39,43,49,52,53,56,68,84). However, the values of the kinetic parameters obtained and models proposed seem to vary according to the nature of the micro-organism (84), temperature, pH, and other factors. Hill, et al (33) also observed that wall growth on the reactor interior exerted a significant effect on the biomass concentration and phenol conversion, both of which decreased with an increase in wall growth. A similar observation was made by Pawlowsky, et al (57), they concluded that proper kinetic studies must be done before scale-up can proceed, since wall growth greatly influences the substrate concentration. Beltrame, et al (46) found that the data were inconsistent unless the reactor walls were scraped at least twice a day.

Holladay, et al (35,36) studied the biodegradation of phenolic wastes in stirred tank (CSTBR), packed bed (PBBR)

and fluidized bed (FBBR) bio-reactors . The CSTBR was found to be the most vulnerable to shocks, while the utilisation of PBBR for waste treatment was strongly dependent on biomass accumulation. PBBR and FBBR exhibited the largest degradation rates and lowest retention times with a high tolerance for system perturbations. The CSTBR was the most simple to operate but occupied the largest volume with the longest retention time. None of the bioreactors were successful in treating cyanides, nitrates or ammonia. At 99 % conversion, the CSTBR handled up to 1400 mg/l at 2.67 gm phenol/day.l of reactor volume, while the PBBR and FBBR achieved 2 to 3 times the degradation rate but only for 500 and 700 mg/l of phenol, respectively. They also concluded that conversions less than 99 % were mainly due to insufficient dissolved oxygen (DO). The PBBR and FBBR also had operational problems like excessive biomass formation and difficulty in solids separation.

Pawlowsky, et al (56,57) proposed the Haldane model as the best fit for phenol degradation due to substrate inhibition (see Table #1). They found that bacterial composition varied greatly with changes in residence time. When the residence time was 4 hours, filamentous bacteria, algae and fungi predominated. On the other hand, with a residence time of 6 hours spherical bacteria were predominant. The experiments were conducted at 28 C and pH

6.6.

Kim, et al (38,39) made a comprehensive study of the effects of temperature, pH, and salinity on treatability of phenol and methanol. They observed that Y and Km (of Michaelis-Menten model) changed insignificantly with temperature, with values of 1.21 $\pm$ 0.06 and 236 $\pm$ 70 mg/l respectively. The Um for phenol increased from 0.0189 to 0.061 /hr with an increase in temperature from 5 to 21 C according to an Arrehenius relation. The phenol concentration used in the study varied from 650 to 1200 ppm. They proposed an equation of the form $U_{m_2} = U_{m_1} \cdot \theta^{(T_2 - T_1)}$, where θ is the temperature coefficient, which is a function of pH and salinity. (The equation is not very useful since θ is also an unknown function of the temperature range). θ was found to be 1.076 at pH = 7 and salinity =0, for phenol. The temperature effect decreases as pH and salinity become unfavorable to the organisms. They proposed an equation for the cell decay coefficient (Kd) of phenol in terms of Um:

Kd = 0.066xUm.

It was found that Kd increased from 0.002/hr showed that the primary factor affecting decomposition in natural systems was pH. Highly acidic (<4.0) or basic (>9.5) solutions upset the internal pH balance of the microbial cells leading to a rapid decrease in bacterial activity. The optimum pH range was found to be 6.5-8.0. The Um value

increased three fold when solutions were buffered. Effects of pH have also been studied by Yang, et al (84), Omori, et al (54) and Radhakrishnan, et al (60). A significant decrease in pH has been observed by all of them for phenol degradation, which in turn reduced the bacterial growth (54).

The effect of temperature on decomposition rate has also been extensively studied by others (38,39,41,53,60,77). Radhakrishnan, et al (60) observed that for B. Cereus, values of Y and Um both changed significantly with changes in temperature. At initial phenol concentration up to 800 ppm, the values of Y and Um changed from .606 and 0.144 /hr to 0.548 and 0.0937 /hr when the temperature was varied from 40 to 30 C. (This observation conflicts with that made by Kim, et al (39)). Phenol concentration in the range of 400 to 800 ppm was used to study the biodegradation. The temperature coefficient O, in that range was 1.044. In addition, they saw that the effluent concentration was unaffected at 40 C althoush the bacterial concentration increased. Nitrogen deficient systems behave better at lower temperatures since the critical nitrogen level for maximum aerobic activity is lower at lower temperatures. They concluded that in batch cultures, where bacteria grow in the exponential phase with an unlimited food supply, temperature plays a significant role because enzymatic

activity increases and bacteria grow at their maximum growth rate. They also observed that the maximum specific growth rate of the bacteria in the flow reactor was about 4 times greater than that in the batch reactor. The pH did not change much during all the experiments for phenol, signifying that no acidic metabolites accumulated during the experiments. It was more economical to operate at higher dilution rates or lower detention times, provided the DO was maintained. They also concluded that the Michaelis-Menten relation did not hold in continuous flow cultures. For batch experiments, they used a first order model (see Table #1). The experiments were performed under aerobic conditions at pH close to 7.0. The micro-organism used in the study was B. Cereus.

Vela and Rolston (77) studied the effect of temperature on phenol degradation in some detail. Contradictory to other workers they found that the effect of temperature was a very complex phenomenon and can not be described by a simple Arrhenius equation. They concluded that phenol degradation was temperature independent between 10 and 24 C. Below 10 C, the number of phenol degrading bacteria decreased with temperature. The rate of degradation below 10 C, was directly proportional to the rate of growth of bacteria. On the other hand, above 24 C, the rate of phenol degradation was much greater than the

growth rate of bacteria and it increased with temperature. Kostenbader, et al (41) found that in the temperature range of 70 to 100 F, the oxidation efficiency was little affected by temperature provided the phenol loading was kept constant. Otherwise, reducing temperature from 90 to 60 F at 99 % conversion increased the detention time 14 fold at 1/7th the phenol loading.

Neufeld, et al (53) proposed the equation, log(Um) = 5.58 - 1.816/T, for phenol degradation. This is equivalent to an Arrhenius equation.

Yang and Humphery (84) determined that due to inhibition, parameters for phenol degradation were best obtained in batch runs. They also found the Haldane model to be the best fit. The constants at 30 C for P. putida, and I. cutaneum are given in Table #1. These values are quite different from those mentioned previously by other workers. Substrate inhibition was evident at concentrations above 100 ppm. The phenol feed concentration varied from 30 to 500 ppm.

Tabak, et al (73) investigated 104 aromatics. As observed by others (39), the formation of adaptive enzymes to metabolize phenol was indicated by a marked lag in oxygen uptake in respirometric tests.

Wase and Hough (79) found that biodegradation of phenol by yeast Debaryomyces subglobosus was rapid below

1128 ppm (see Table #1). Catechol was formed as an intermediate.

Shivaraman, et al (68) found that yeast, Candida tropicalis, is capable of degrading phenol up to a concentration of 2000 mg/l with an initial cell mass of 440 mg/l. About 90 % of phenol was degraded at a feed concentration of 650 ppm in 24 hours, but with increase in feed concentration, the rate of growth and degradation of phenol decreased. At a neutral pH, the presence of up to even 10,000 ppm ammonium chloride did not affect the degradation.

Luthy, et al (48,49) studied biological oxidation of coking and coal gasification wastewaters. The quenching of gaseous products after pyrolysis results in liquors with very high phenolic (400-3000 ppm), thiocyanate and cyanide contents. The yield coefficients for such liquors were characteristically low due to the presence of inhibitory compounds. He proposed first order kinetics for phenol degradation (see Table #1).

Kostenbader, et al (41) investigated oxidation of coke plant weak ammonia liquors. 99.9 % efficiency was observed at a phenol feed concentration of 2500 ppm. In contrast to Shivaraman's (68) observation, they found that an ammonia concentration of 4000 ppm severely inhibited phenol degradation, but concentrations of about 2000-3000 ppm

actually produced a 60 % increase in phenol removal efficiency. Therefore, controlling ammonia concentration was found to be the key for optimization. They determined the optimum temperature to be 95 F, although the removal efficiency was not much affected by temperature (again contrasting with other investigators).

The studies done by Lee, et al (43) for breakdown of phenol by P. putida in FBBR showed substrate inhibition at phenol concentrations above 200 ppm, with degradation rates of 20-200 ppm/hr.

Because of the inhibitory effects observed, a number of workers like Hill (33), Yang (84) and Pawlowsky (56) used the Haldane model to describe phenol biokinetics. Neufeld, et al (52) in anaerobic media, also observed a maximum in the specific growth rate for phenol. They proposed a Haldane model for anaerobic biodegradation of phenol up to 1500 ppm feed concentration (see Table #1). The specific rate of degradaion was about 160 times slower than those under aerobic conditions. The experiments were conducted in a fill-and-draw reactor with sludge age varying from 30 to 120 days. The temperature was maintained between 32.2-40.5 C, and pH was kept between 7.5 to 8.0. A mixed bacterial culture was used. No release of methane was observed.

In another study by Neufeld (53), aerobic degradation of phenol at feed concentrations below 100 ppm was examined

in an aerated flow reactor, with detention times varying from 4 to 10 hours. The temperature was maintained close to 22 C, and pH was adjusted to 7.0. A Monod model was found to be the best fit (see Table #1).

Beltrame, et al (7), in contrast, found that phenol degradation was not inhibited even at 360 ppm feed concentration in a continuous reactor at 20 C and pH 7.2. They also utilized a Monod model (see Table #1). Though questionable, he suggested that inhibition can occur only in batch studies. Like many other workers, he also concluded that values of the kinetic parameters could change with feed concentration, particularly Um and Km. The change in degradation rate resulting from variations in the feed concentration was so large that he proposed the inclusion of a feed concentration term in the Monod model.

In the study by Lewandowski, et al (45), phenol degradation was found to follow a first order Grau model with a rate constant of 0.04 /hr. Phenol concentration up to 500 ppm was studied in an aerated fill-and-draw reactor at 26 C. They concluded that the addition of a co-substrate (sucrose) did not change the rate constant, but considerably increased the lag time. The pH was maintained close to 7.0 for all the experiments. The seed organism used was obtained from the same source as the one in the present experimental work.

Some amount of work has also been done to study the inhibition to phenol degradation by cyanide and thiocyanates, largely due to their concomitant existence in coal plant wastewaters (8,41,43,49,52,53,64,68). Neufeld, et al (53) found that the presence of CNS- significantly inhibited phenol degradation, obtaining an equation of the form :

$U = 0.275\ S/[\ 2.11 + 1.67\ \sqrt{(SCN)}\ + S]$. Where SCN is thiocyanate concentration. Shivaraman, et al (68) also observed inhibition due to cyanide at CN concentrations greater than 25 ppm. Significant decomposition of thiocyanate was observed, but cyanides were relatively recalcitrant. On the other hand, Bhattacharya, et al (8) did not find any inhibition due to thiocyanate.

Decomposition of di and trihydroxyl phenols like resorcinol, quinol, pyrogallol and phloroglucinol has also been studied by a number of workers (5,11,18,59,73). Chambers, et al (11) found that catechol and resorcinol were readily oxidized, while quinol was slow. Oxygen uptake for them was comparable to phenol while that for phloroglucinol was 1/4th and for pyrogallol it was zero. Trihydroxyl phenols were much more resistant. Tabak, et al (73) observed that the descending order of biodegradability for the above compounds was; phenol, catechol, resorcinol, quinol, phloroglucinol and pyrogallol. Identical

observations were made by Barth (5) and Bunch (10). Pitter (59) obtained degradation rates of 80 mg COD/hr.gm of MLSS for phenol, 57.5 for resorcinol, and 0 for pyrogallol.

B. CHLOROPHENOLS:

Considerable work on chlorophenols indicates the general trend of a sharp decrease in degradability with an increase in chlorine content.

Pitter (59) obtained rates of biodegradation of 25 and 11 mg COD/hr.gm of MLSS for O and P chlorophenol respectively. The experiments were conducted using an activated sludge at pH 7.2 and a temperature of 20 C. The feed concentration was 200 ppm on a COD basis.

Oxygen uptake for most chlorophenols, observed by Chambers (11) was less than 1/3 that for phenol. He conducted respirometric tests using Pseudomonas at 30 C and pH 7. Cell suspensions were omitted from one flask to check that no substrate was lost due to chemical oxidation. The addition of successive chlorine atoms brought lower removal rates. Tabak, et al (73) found that m- and p- chlorophenols were degraded from 100 ppm each to 50 and 66 ppm respectively at a rate at least four times slower than that of phenol.

A detailed review on biodegradation of

2,4-dichlorophenol (2,4-DCP) and 2,4-dichlorophenoxyacetic acid (2,4-D) was made by Tyler, et al (76) using batch and continuous cultures of Pseudomonas NCIb9340. In batch runs with 2,4-D at 25 C and pH from 6.2 to 6.9, the maximum specific growth rate was found to be 0.14 /hr. The same value was obtained for 2,4-DCP at 25 C and pH 7.1-7.8 at concentrations below 25 ppm. Growth for 2,4-DCP was strongly inhibited above a concentrations of 25 ppm, while no inhibition was observed with 2,4-D at concentrations up to even 2000 ppm. At 2,4-DCP concentrations above 25 ppm, the maximum specific growth rate was found to be 0.12 /hr. The data for 2,4-DCP at low feed concentration could fit the Monod model with Um =0.14 /hr and Km = 5.1 mg/l; but at higher concentration, it failed and a linear correlation, U =0.156- 0.00155 S, was proposed. The Haldane model for inhibition kinetics was also tried (see Table #1). A computer fit for the data was obtained for 2,4-DCP but the correlation did not seem to be very good. Batch cultures of Pseudomonas grown on 2,4-DCP showed a sharp increase in lag and total degradation time with an increase in feed concentration. Very similar observations are also made for 2,6-DCP in the present research work.

SJoblad, et al (69) determined that a phenol oxidase was capable of polymerizing 2-chlorophenol, 4-chlorophenol, 2,4-DCP, and 1-bromo-2-chlorophenol. This kind of oxidative

coupling by B. graticola leads to their oxidation at a rapid rate even at room temperature.

Baker and Mayfield (4) did extensive experimental work on chlorophenol degradation. They made sure that o-CP and 2,6-DCP were not photolytically decomposed at 23 C by conducting their experiments in the dark for both aerobic and anaerobic conditions. In aerobic degradation, 2,6-DCP took 18 hours for 100 % decomposition, while 2,4-DCP, and 3,4-DCP took more than 40 and 160 days, respectively for 81 % removal. The o-CP took 36 hours for complete removal, while p-CP and m-CP took more than 20 and 160 days respectively (83 % removal). Increasing the number of Cl atoms consistently reduced the biodegradability, and the presence of Cl in the meta position made the compound much more refractory (which is consistent with theoretical predictions). The experimental work was conducted at 23 C with 100 ppm feed concentration in a clay loam soil for all the compounds. Thus this study was not done in a bioreactor. They noticed that in the absence of micro-organisms, non-biological degradation of chlorophenol did occur, which increased with temperature and was dependent upon the position of Cl atoms (with the ortho position being more susceptible). This mechanism did not appear to involve significant volatilization or photodecomposition. However, like phenol, chlorophenols

could undergo auto-oxidation, though at a much slower rate. They also reported that under anaerobic conditions, micro-organisms were incapable of degrading any chlorophenol.

Identical observations were made by Barth (5), and Bunch (10) about the effects of chlorination on biodegradability. The experiments were done using a Warburg respirometer at 30 C with 100 ppm concentration of o- or m-chlorophenol, 2,4,6-TCP, and 2,6-DCP. 2,4-DCP was also tried with a feed concentration of 60 ppm.

Dence and coworkers (18) studied chlorophenols removal using the fungus Aspergillus fumigatus and came to the same conclusion. 2,4,6-TCP had the greatest effect of all the phenols tested in reducing the growth of the fungus. For the fungi Trichoderma koningii and Penicillium variable, 25 ppm concentration of 2,4,6-TCP was sufficient to prohibit growth; but for Aspergillus niger, Aspergillus fumigatus, Paecilomyces varioti and Cladosporium herbarum, just 10 ppm of 2,4,6-TCP was sufficient for growth inhibition.

All of these fungi could grow on more than 200 ppm of phenol without any serious inhibition. They also confirmed that biodegradability of phenols was structure related, the nature of the substituted group, number of such groups, and their positions, all play important roles. Other workers like Barth (5), Bunch (10), Pitter (59), Tabak (73,74),

Chambers (11) etc, also came to the same conclusions.

Paris, et al (55) used Pseudomonas putida to study the biodegradation of halophenols like o-CP, p-bromophenol, p-cyanophenol, p-cresol, p-nitrophenol, etc., and developed a relationship between rate constants and the electronic, steric or hydrophobic characteristics of the compounds. In contrast to most workers, they found biodegradation to be of 2nd order in terms of substrate concentration and reported rate constants of 7x10(-12) and 1.7x10(-12) l/hr/organism for phenol and p-CP, respectively.

Lewandowski, et al (45) investigated microbial degradation of o-CP up to 40 ppm in a batch reactor with an activated sludge. They found the removal to follow a first order Grau model with a rate constant of 0.002 /hr at 26 C.

Tabak and coworkers (74) studied a large number of chlorophenols and other aromatics for their biodegradability using a static culture-flask procedure. Though they came to almost the same conclusion as others, the usefulness of their data is questionable for a variety of reasons. The static-flask procedure neither simulates what happens in Nature nor does it portray the industrial wastewater treatment plant. They did not provide any information about the cultures used and the concentrations used for the feed compounds were also very low. (less than 15 ppm). Bunch himself has cautioned about the usefulness of such

procedures in developing a commercial design.

Pentachlorophenol (PCP) removal has received special attention because it is being used extensively as a fungicide and bactericide in wood preservaton. According to one estimate (22), 5.5x10(6) gallons of wood preserving wastewater /day loaded with 40-100 ppm PCP is released nationwide which must be treated before discharge due to its toxic properties.

Kirch and Etzel (40) suceeded in removing PCP at 30 ppm feed concentration in the presence of a prescribed nutrient broth (cosubstrate) at a concentration of 1000 ppm. The overall recovery of PCP carbon as carbon-dioxide varied from 2 to 50 %. In the absence of the nutrient broth, the total conversion was reduced by a factor of 2 but the oxidation rate became higher. Hence, they suggested that PCP is probably not a primary substrate in the presence of the nutrient broth, but it is cometabolized by growing cells which use the broth as a primary energy source. The composition of the broth was not specified. However, the addition 20 ppm of phenol as cosubstrate had no effect on the rate of oxidation of PCP. In another study by Etzel and Kirsch (22), an activated sludge under aerobic conditions was used in a continuous flow reactor at 20-25 C and pH of 7.8-8.0. With PCP concentration varying from 20 to 60 ppm, and detention time of 6 to 12 hours, they were able to

obtain 96 to 99+ % removal of PCP from authentic as well as contrived wastewaters. They also described the removal rate for a large number of chlorophenols with the same sludge under identical conditions. Unfortunately, the sludge composition (ie. bacterial culture) is not described.

Wallin, et al (78) also observed that PCP was far more stable compared to phenol for bio-oxidation. In addition, they found that in batch studies with an activated sludge, PCP was not degraded but merely adsorbed by the bacterial flocs. This observation is quite different from those of others.

Wukasch, et al (83) degraded PCP in a continuous flow reactor from a feed concentration of 20 ppm to an effluent concentration of 0.1 ppm, after an acclimation period of 90 days. Unfortunately, they have not mentioned the flow rate at which the PCP was fed to the CSTBR. They also found that above 350 ppb concentration within the reactor, the PCP becomes toxic to the organisms. PCP degradation was found to be first order with a rate constant value of 0.0042 /day. The adsorption of PCP on the flocs was negligible. Similarly , air stripping of PCP was found to be less than 0.05 %. Labelled PCP was used and radioactive carbon-dioxide was measured to study the biodegradation.

Baker and Mayfield (4) also found that PCP took 160 days for 80 % conversion, in aerobic conditions with 100 ppm

initial concentration present in wet soil at 23 C, using a mixed culture. In static culture-flask studies by Tabak et al (74), PCP was found to be degradable even at 10 ppm concentration over a 2-4 week period.

Edgehill and Finn (86) were able to degrade 40-120 ppm PCP in a batch scale activated sludge unit after 7 days acclimation. But, they found a lag time of more than 2 days when bacteria acclimated to 40 ppm PCP were subjected to a shock loading of 120 ppm. The lag time was considerably reduced when a chemostat culture of PCP-degrading bacteria was fed slowly to the mixed liquor.

C. NITROPHENOLS AND OTHER SUBSTITUTED PHENOLS:

Chambers, et al (11) also investigated a variety of nitrophenols. Addition of successive nitro groups reduced the oxygen uptake and removal rate of phenol. The oxygen consumption was 1/3 to 1/4 that of phenol. The degradation of cresols was quite rapid; in fact, faster than even phenol, probably due to the electron releasing effect of the CH_3 groups.

Sjoblad, et al (69) studied the biodegradation of a variety of nitrophenols and amines. They concluded that the nitrophenols and aromatic amines could not be oxidized because of the presence of strong electron withdrawing

nitrogen substituents which prevented the removal of an electron from the phenolic hydroxyl group.

Similar observation was made by Tabak, et al (73). The bio-activity of cresols was found to be more than that of phenol. Other methyl substituted phenols were comparable in biodegradability. Substitution of a CH3 group in the meta position was most favored for degradation, which is exactly opposite to the case of a Cl or NO2 group which withdraw electrons from the ring. In the static flask study, nitrophenol up to 10 ppm was easily degraded in a one week test, but the addition of more NO2 groups sharply reduced the removal rate.

Barth (5) concluded that the addition of a methyl group at the para position resulted in a higher O2 uptake rae but a lower conversion, while o- and m- cresols showed a higher oxygen uptake rate as well as conversion. The presence of chlorine groups deactivated the effect of CH3 in dichloromethylphenol. Nitrophenol decomposed at a much slower rate but with an increase in nitro groups on the ring, the removal efficiency increased, which would be hard to explain on theoretical grounds.

Pitter (59) reported more than 95 % removal of nitrophenol at a rate of 17 mg COD/hr.gm of MLSS. The feed concentration of nitrophenol was 125 ppm. Cresols were removed at the rate of about 55 mg COD/hr.gm of MLSS at 95 %

efficiency, compared to a rate of 80 mg COD/hr.gm of MLSS for phenol.

As previously mentioned, Dence, et al (18) studied the degradation of some substituted phenols using different funsi and bacteria in shaker flasks at 28 C. The pH was maintained at 7.0. The substitution of a CH3 group in trichlorophenol immediately improved the inhibition concentration required for all funsii from 10 ppm to 25-50 ppm. Methyl-catechol was more active than even phenol.

Paris and coworkers (55) found nitrophenol degradation to be of 2nd order with a rate constant of 1x10(-13) l/hr.organism which was 70 fold less than that for phenol. The experiments were done in batch with E. eutida

It is also of interest to view the effect of methoxy groups on phenol or benzene. Most of the above workers observed that the OCH3 group increased the resistance to degradation. The presence of Cl and OCH3 groups together on phenol seem to make it highly recalcitrant (18).

D. BENZENES, CHLOROBENZENES AND NITROBENZENES:

As the theoretical predictions suggest (see the chapter on Structural Chemistry and Biodegradability), the experimental biodegradation rates of benzene, halobenzenes and nitrobenzenes were found to be much slower than for

phenol, by most workers.

Tabak, et al (74) surveyed the biodegradability of benzene, chlorobenzene(CB), 1,2-DCB, 1,4-DCB, trichlorobenzene, and ethylbenzene using static flask tests at 25 C. For benzene, total biodegradation was observed at 5 and 10 ppm feed concentration over a week. Most chlorobenzenes were found to be significantly degradable up to 10 ppm concentration. With increasing chlorination, the total removal as well as removal rate, both decreased. Hexachlorobenzene was most recalcitrant. However, a significant accumulation and loss of biodegradability were observed in second and third generation populations. Nitrobenzene was completely biodegradable up to 10 ppm in all populations over a weeks' time. As expected, the addition of more nitro groups decreased the biodegradability. Of course, the usefulness of such data from the static flask test remains questionable.

In the study of Rittmann and Kobayashi (63), it was found that a very low concentration of chlorobenzenes presented a special problem because the rate of cell growth was less than the minimum necessary to sustain the micro-organisms. The inducible enzymes are not synthesized because the substrate concentration is too low for maintaining the cell metabolism.

In contrast to Tabak, Pitter (59) found that

1,3-dinitrobenzene (DNB) and 1,4-DNB were among the few compounds out of 123 tested that had zero biodegradability. Nitrobenzene was found to be completely degradable at a rate of 14 mg COD/hr.gm of MLSS.

Barth and Bunch (5) tested a variety of nitrobenzenes and chlorobenzenes at 100 ppm according to the method reported previously. As opposed to others, they found that the activity of chlorobenzene to be the lowest and actually below the endogeneous level. Addition of more nitro groups increased the degradability significantly (this is inconsistent on theoretical grounds). Benzene removal was very slow and increasing the chlorine content on the ring decreased the removal rate as expected. All the tests were done using Warburg respirometer. Each test was run over 200 minutes and the oxygen measurements were done every 10 minutes.

In another experiment, Chambers and Tabak (73) used the shaker-flask test for phenol adapted bacteria. 100 ppm each of benzene, chlorobenzene and nitrobenzene as well as their derivatives were degraded at room temperature and at pH of 7.2. Least degradation was observed for nitrobenzene. Though inconsistent with theoretical predictions, increasing the nitro groups on the ring seemed to increase the degradability. The reverse was found to be true for chloro groups. All the rates were much less compared to that of

phenol. In respirometric studies, m-DNB, and p-DNB were removed up to 25 % in 180 minutes while 99 % removal of phenol was observed over the same time period. All the nitro and chlorobenzenes were classified as highly resistant.

In the survey of 104 compounds by Chambers, et al (11) benzene and its halo and nitro derivatives were very slowly oxidized with rates at least 3 to 4 times less than those for the analogous phenolic compounds. The respirometric measurements were conducted at 30 C using Pseudomonas, with a pH of 7.0.

E. BENZOIC ACID AND ITS DERIVATIVES:

In the survey by Chambers, et al (11), the degradation of benzoic acid and its derivatives was found to be quite rapid. However, the addition of nitro and chloro groups readily decreased the removal rate. Similar observations were made by Tabak, et al (73) for 100 ppm concentration of various benzoates.

Barth and Bunch (5) reported high removal rates for benzoic acid at feed concentrations up to 100 ppm, which decreased with increasing substitution of NO2 or Cl groups on the ring.

Pitter (59) observed 99 % removal of benzoic acid at a

rate of 88.5 mg COD/hr.gm of MLSS, compared to about 92 and 50 % removal of nitro and dinitrobenzoic acid at the rates of 20 and 7 mg COD/hr.gm of MLSS respectively. The experiments were conducted under the same conditions as mentioned before.

Shamat and Maier (67) reviewed a number of chlorobenzoic acids (CBAs), and dichlorobenzoic acids in a continuous flow reactor seeded with micro-organisms. o-CBA, p-CBA or 2,4-D were fed at concentration of 50, 111, and 98 ppm respectively. All the experiments were carried in the dark at 20 C. o-CBA and p-CBA had at least 6 to 12 days lag while the lag for DCBAs was even larger. The values of kinetic parameters were evaluated using the Monod model. The values were not very different for batch and continuous runs. The main bacterial species was Pseudomonas. The observed lag was attributed mainly to the low initial biomass concentration and to the unavailability of the enzymes to metabolize the target substrate. In addition, the metabolic capabilities were stable and did not disappear even if the substrate became unavailable. The meta substitution made the benzoic acid even more recalcitrant.

Extensive work on various benzoates up to 16 ppm feed concentration was done by Haller (29) at 30 C. The experiments were done in shaker flasks with an activated sludge. The time lag for degradation was in the order:

benzoic acid < nitrobenzoic acid < chlorobenzoic acid. The presence or absence of an easily metabolized carbon source did not seem to affect the biodegradability.

DiGeronimo, et al (19) observed that all the monochlorobenzenes (MCBs) were rapidly degraded, in the order, o > p > m; while the DCBs were very slow. Addition of a cosubstrate (glucose or sodium benzoate) did not affect the rate of degradation of any of the substrates. The experiments were conducted using an activated sludge die-away system in a well aerated 2 l Erlenmeyer flask at a DO concentration of 6-8 ppm at 25 C and pH of 7.2.

Hartmann, et al (31) explored the degradation of m-CB, p-CB and 3,5-DCB using Pseudomonas, and measuring the rate of oxygen uptake at 28 C. The substrates used were the sole source of carbon and energy. The cells were acclimated for 4 weeks with 3-chlorobenzoate at 3.66 ppm, and then tested with other benzoates. Complete removal was obtained for 4.71 ppm of m-CB and p-CB in 14 and 11 hours while 5.79 ppm of 3,5-DCB were removed in 29 hours. Elimination of organically bound Cl as hydrochloric acid in the system was indicated by accompanying acidification of the medium. Metabolic pathways for their degradation were also suggested (see Figure #6).

E. POLYCHLORINATED BIPHENYLS:

Liu (46) conducted the biodegradation of commercial PCB formulations (Aroclor 1221, 1016, and 1254) in a simulated batch and continuous wastewater treatment process at 20 C and DO content of 2 ppm. The main bacterial population was Pseudomonas sp. Z509. The sludge was acclimated with Aroclor 1221 for 3 days. In the continuous runs, 50 to 100 ppm PCB emulsion was fed at a rate of 13-26 ml/hr in a 10 l tank. This was about 31.2-62.4 mg Aroclor 1221 per day. As the retention times for such runs were high (16-32 days), all the seven mono and dichloro biphenyls of Aroclor 1221 were found to be totally degradable. A number of interesting observations were made. Biphenyl was most easily metabolized, while the addition of successive chlorine atoms sharply reduced the metabolism. The position of Cl substitution on biphenyl molecule is important. Aroclor 1016 and 1254 were much more resistant since they contained a higher percentage of tri and pentachlorobiphenyls. A significant improvement in degradation was observed when the reactor was switched to batch operation.

Perhaps the most extensive and systematic study on PCBs was conducted by Furukawa, et al (26). The study was divided into 8 groups depending on the extent and position

of Cl substitution. The effect of cell concentration of species Alcaligenes and Acinetobacter was also studied. They concluded that PCBs containing 2 Cl on either ortho position of a single ring (2,6-) or on both rings (2,2'-) were most recalcitrant. The same total number of Cl atoms degraded faster if present on one ring rather than distributed on both rings. Ring fissure was preferred for the ring with the least number of Cl atom. Yellow colored intermediates (chloro benzoic acids) were formed. The rate and extent of removal sharply decreased with increasing chlorination. Both microbial species were able to degrade mono chlorinated biphenyl at a rate greater than 8.1 ppm/hr after 1 hour of incubation. Dichlorobiphenyls except 2,6-DCB showed a similar rate. 2,6-DCB, 2,3,6-TCB degraded at a rate lower than 0.66 ppm/hr.

Tucker, et al (75) reported that P. putida oxidizes the PCBs through 2,3-dihydro-2,3-dihydroxybiphenyl and benzoic acid route. They used a semi-continuous procedure with a locally obtained activated sludge culture, acclimated over 3 weeks with glucose. The PCBs were dissolved in ethanol due to their low solubility. Measurements were made at intervals of 30 minutes for 48-72 hours. They concluded that the level of chlorination was the most significant factor in biodegradation. The PCBs lost due to stripping were found to be 4.2, 6.1, and 3.6 % for Aroclor 1221, MCS

1043 and Aroclor 1016 respectively. The mono and dichloro compounds were not very difficult to degrade but the rest were much more recalcitrant.

Baxter, et al (6) conducted batch studies (shaker flask) with an activated sludge at 25 C in the dark. The pH was adjusted to 7.00. The feed concentration of PCB was 100 ppm. They also concluded that the degree and pattern of chlorination would determine the extent of biodegradation. Some of the lower chlorinated compounds which appeared to be only slowly degradable when alone, degraded quite readily when present in the mixture (e.g. 4,4'-dichlorobiphenyl present with biphenyl). Cometabolism was suspected for the enhanced biodegradation of certain PCBs when biphenyl was added as a cosubstrate. Organisms which had become adapted to a simple PCB were then capable of metabolizing more complex structures.

Choi, et al (12) used an activated sludge from a municipal sewage treatment plant to study the degradability of PCBs in a continuous system with 5-10 hour retention times. The DO was maintained at 4 ppm. The PCB was dissolved in butanol and emulsified in wastewater using ultrasonics. Contrary to others, they did not observe significant degradation, instead the PCB was found to be adsorbed on the surface of the suspended materials in the sludge. Hence, a high concentration of the PCBs in the

sludge created sludge disposal problems.

Wong and Kaiser (82) analysed various commercial mixtures in a shaker flask study with bacterial species, Achromobacter sp. and Pseudomonas sp.. No inhibition was seen up to 1000 ppm of various Aroclors (1221,1242,1254) in 10,000 ppm glucose medium. 500 ppm Aroclor 1221 and 1242 were easily used as carbon source for growth (this seems to be highly improbable due to their insolubility and stability toward biological attack). Over a period of one month Aroclor 1221 and 1242 were totally degraded into several metabolites of lower molecular weight. Biodegradability was strongly dependent on the extent and position of chlorination. The degradation rates were found to decrease in the order biphenyl > 2-chlorobiphenyl > 4-chlorobiphenyl.

Clark, et al (13) investigated similar PCBs in the shaker flasks with different enriched soil bacteria in the dark at 26 C. The cultures were charged with 7 ppm Aroclor 1242 or other formulations, and the degradation was studied for 5, 10 and 15 days. Identical conclusions were reached about the effects of chlorination. Cometabolism of PCBs in the presence of sodium acetate was also studied. Degradability of the higher chlorinated isomers was greatly enhanced in the presence of a cosubstrate. The bacteria were growing on acetate which was easily metabolized and the enhanced growth improved the PCB oxidation.

Ahmed and Focht (1) were able to isolate two species of Achromobacter using p-chlorobiphenyl and biphenyl as sole carbon source at a concentration of 1000 ppm. The experiments were done at 28 C in a shaker-flask for 36 and 66 hours respectively. The degradation of p-CB was accompanied by the formation of an intermediate p-chlorobenzoic acid. Biphenyl as well as p-CB, both were rapidly degraded. Biphenyl formed benzoic acid as an intermediate. It was proposed that the cleavage of the unsubstituted ring was favored over the one with chlorine attached to it. Chloride was not released. No further degradation was observed since bacteria were incapable of forming enzymes to mineralise the intermediates to carbon-dioxide, water and hydrochloric acid, and the C-Cl bond could not be cleaved.

Herbst and others (32) studied degradation of 2,5,4' trichlorobiphenyl and 2,4,6,2',4'-pentachlorobiphenyl and chloroalkylene-9 at concentrations of 0.178, 0.231 and 0.356 ppm respectively. An activated sludge was obtained from a municipal sewage treatment plant, with experiments performed at 8-14 C in a batch reactor. The PCBs were dissolved in ethanol before being injected. Samples were taken every hour for 6 hours. 90, 15 and 10 % of chloroalkylene-9, TCB and pentachlorobiphenyl were removed respectively. They concluded that all the PCBs were almost undegraded by the

activated sludge, but rather were merely distributed between water and sludge. Thus the isomers with high Cl content were very persistent. These findings are similar to those of other investigators (12).

The study conducted by Reichardt, et al (61) showed that micro-organisms from Alaskan seawater were capable of degrading biphenyl, 2-chlorobiphenyl (2-CB), 3-CB, 4-CB at concentrations of 0.72 ppm, 0.28 ppm, 0.68 ppm and 0.54 ppm respectively at 10 C, without aeration, over 21 days. The rates varied from 1.19x10(-3) ppm/day for biphenyl to 4.225x10(-4) ppm/day for 3-CB. The relative rates for 2-CB, 3-CB and 4-CB were 0.43,0.27 and 0.33 compared to BP. The only significant product was carbon-dioxide. No chlorobenzoic acid or phenylphenol were observed as by-product. The effect of Cl position on the ring was evident from the relative rates. It was concluded that meta substitution had a greater rate retarding effect than para substitution. As the number of non-chlorinated p- and m-positions increase on the biphenyl template, the rate of biodegradability also increases.

Su, et al (71) reviewed a number of Aroclor mixtures for their degradability by an activated sludge in a batch reactor. P. putida, Acetobacter, Acinetobacter , etc. cultures were identified in the sludge. It was found that Aroclor 1242 was more degradable than 1254 due to less

chlorine content in the former. Accumulation of acidic metabolites, mainly chlorinated benzoic acids was observed depending on the nature of the parent PCB. Similar conclusions as mentioned before, concerning the extent and position of Cl on the ring, were made.

G. OTHER MONO AND POLYCYCLIC AROMATICS:

Saeger and Thompson (64) surveyed 32 halogenated biphenylmethanes for their biodegradability using an activated sludge and river water micro-organisms in a magnetically stirred fill-and-draw reactor at room temperature. The results indicated that the DPMs having substituents in only one ring were generally degraded more rapidly. Surprizingly, DPMs with OCH3, CH3 and C2H5 groups in the 2nd ring appeared as refractory as those containing chlorine in both rings. It was suspected that steric factors contributed to the decrease in biodegradability. The presence of up to 2 substituents yielded about 97-99 % removal provided both were on the same ring. More than 2 substituents, or those on different rings, yielded less than 35 % conversion. The DPMs showed the following order of decreasing biodegradability:

2 > 3,4-

3 or 4 or 2,4- > 2,3,4-

2,4,5- > 2,6- > 2,3,6- > 2,4,6-.

Omori, et al (54) used Pseudomonas at 30 C and pH 7.0 in a soil test study with resting cells to measure the degradation of 16 aliphatic and halogenated aromatics in 24 hr tests. All but one were dehalogenated, and the halogen was even cleaved from alkyl side chains of aromatic compounds. The pH fell as halide was removed. This increase in acidity stopped cell growth and further dehalogenation. The removal of Cl from side chains preceeded removal of Cl bound to the ring. They proposed that Cl was replaced by OH before the ring was cleaved (This is theoretically unlikely). Non enzymatic dehalogenation of various compounds was also seen.

SJoblad, et al (69) investigated degradation of anilines, chloroanilines, methoxyanilines, and 1 and 2-naphthol. An enzymatic phenol oxidase from B. graticola was found to be capable of polymerizing naphthols by oxidative coupling, followed by hydrolysis and ring cleavage, but chloro and bromoanilines were not altered by the phenol oxidase. A number of chlorophenoxyacetic acids were found to form chlorophenols as intermediates.

Bailey, et al (85) studied the biodegradation of the fungicide orthophenylphenol (OPP) using an activated sludge in river-die-away tests. 50 % decomposition took 3 hours in the case of the acclimated sludge, 24 hours for virgin

sludge, and a week for the river-die-away procedure. 65 % of the carbon in OPP was converted to carbon-dioxide, while the rest was considered to be metabolized as cell biomass.

In a batch study by Pitter (59) with an activated sludge at 20 C, pH 7.2, and at an initial concentration of 200 mg COD/l, it was found that more than 95 % decomposition of the chloroanilines occured in 120 hours at degradation rates varying from 6 to 17 mg COD/hr.gm of MLSS. 1 and 2-naphthol showed 90 % degradation with a rate of about 39 mg COD/hr.gm of MLSS. The nitroanilines did not show any degradation under these conditions. Significant total degradation of the methylanilines was observed although at a much slower rate. A number of other substituted aromatics were also studied by Pitter.

Under the conditions mentioned previously, Barth, et al (5) studied the degradation of benzaldehyde and its para hydroxyl derivatives. A high level of oxygen uptake was seen for both of them. Replacement of the hydroxyl by a nitro group blocked the activity. Benzamide was also found to be resistant.

Tabak, et al (74) also studied a large number of polycyclic and other substituted aromatics under the conditions aforesaid. Naphthalene was found to be most readily biodegradable. Except for Bis-(2-etylhexyl)phthalate (EHPE) and di-n-octyl phthalate

(DOPE), all others were readily removed at an initial concentration up to 10 ppm. Compounds like anthracene, phenanthrene and other tri and tetracyclic aromatics were highly resistant.

In another study by the same group, the nitroanilines were found to be highly recalcitrant. At 100 ppm feed concentration only 25 % oxidation of nitroaniline was observed in the shaker-flask study using an activated sludge bacteria at room temperature. This was attributed to the NO2 group. The oxidizability of nitroanilines was in the order m < p < o.

Chambers, et al (11) made similar observation in the batch culture studies for benzaldehydes. While aniline was found to be as degradable as toluene, nitroanilines were found to be very resistant. m-NA was most easily degradable among all nitroanilines.

III. STRUCTURAL CHEMISTRY AND BIODEGRADABILITY

This chapter can be divided into 2 parts:

(1) The first part deals with the nature of the carbon-hydroxyl, carbon-chlorine and other related bonds, and their strength, stability, and susceptibility to nucleophilic and electrophilic attack. An attempt is therefore made to predict the general behavior of a variety of substituted aromatics towards biological oxidation on the basis of electron distribution and energy stabilization.

(2) The second part deals with experimental findings of numerous workers about pathways, metabolism, and intermediates for a variety of aromatics subjected to microbial action. Generalized trends have been established based on experimental evidence.

Extensive research work has been done since 1940 concerning the mechanisms of biodegradation, the metabolic pathways involved and enzyme requirements. Chemical reactions involved in biodegradation of an aromatic structure are extremely complex and are not precisely known, though in the last 2 decades considerable progress has been made in prediction of the most probable overall mechanisms consistent with electronic theories of bond formation and structural stability.

In general an electron releasing group like CH_3 on a

benzene ring facilitates bacterial attack and degradation, while an electron withdrawing group like NO2 and Cl retards the activity. The factors affecting the biological degradability can be divided into 3 groups (59):

(1) Physico-chemical factors (temperature, solubility, degree of dispersion of the compound in the medium, pH, and dissolved oxygen content).

(2) Biological factors (history of the microbial culture, its age, manner, and time of its adaptation, toxicity of the compound and effects of other substrates).

(3) Chemical factors (size of the molecule, length of chain, kind, number, and position of the substituents in the molecule and stereochemistry).

The first 2 factors can be controlled and manipulated to some extent. It is the last aspect that really determines a molecule's susceptibility to microbial action and unfortunately nothing can be done about it except to elucidate the structural stability in order to develop and use the most suitable micro-organisms.

Some of these factors can be illustrated by examining the series: phenol, pyrogallol, chlorophenols, nitrobenzenes, anilines, and cresols. But before doing this, it would be helpful to consider the nature of carbon bonds. The bonds between C atoms and N or S or O or H are mostly covalent. The valency electrons are thus shared to

obtain a noble gas structure (nS2nP6).

In the case of benzene, the six C atoms have SP2 hybridization resulting in 3 covalent sigma bonds between adjacent C atoms and H atoms. Resonance occurs between six overlapping 2P orbitals in benzene so that 6 Pi electrons are delocalized over the ring; this arrangement minimizes their energy. This resonance energy stabilization makes benzene least susceptible to biodegradation.

Ideally, the electrons between the C atom and the other bonding atoms should be equally shared, but that is rarely the case due to the higher electronegativity of one of the atoms. The inductive effect arises due to the fact that even in covalent bonds, the electrons are not equally shared. An atom with higher electron affinity (e.g. halogens) pulls the electrons closer to itself. If electrons are pulled away from the ring C by the substituent leading to lower electron density in the ring, the substituent is said to have -I effect. -I and +I effects for different groups are listed below:

-I:: H < C6H5 < OCH3 < I < Br < Cl < F < NO2

+I:: H < CH3 <C2H5 < CH(CH3)2 < C(CH3)3

The electromeric effect is a temporary effect induced by the presence of an attacking substituent in the vicinity of an aromatic ring. For example, adding a 2nd Cl atom to chlorobenzene leads to the release of a lone pair of

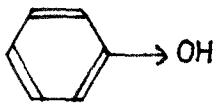
electrons from the Cl atom already present on the ring. It is denoted as +E if the electrons are released to the ring, -E otherwise.

Mesomeric effects arise from the fact that no single structure for a compound is able to describe all the properties of the compound. The actual structure of the chemical lies somewhere between a series of structures. If the electrons are released towards the ring to obtain a most stable structure, the substituent is said to have +M effect (-M otherwise).

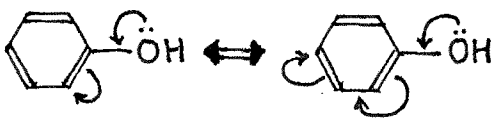
If the net effect of all the above interactions is to withdraw electrons from the benzene ring, then the ring is said to be deactivated towards electrophilic attack. This is the case with halogen or NO2 substituted rings. Table #2 shows the behavior of various groups with some specific examples.

On the other hand, the OH- group has an overall electron releasing effect as follows:

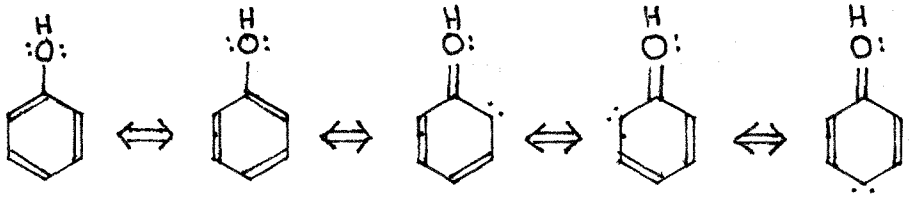
(1) OH- group has a weak -I inductive effect in which Pi electrons are withdrawn from the ring:



(2) Electromeric effect (+E) in which electrons are released toward the ring:



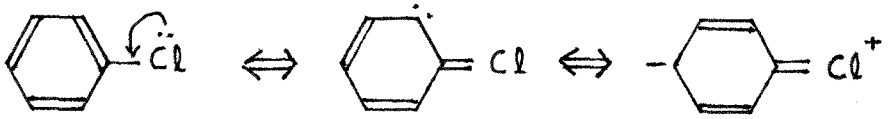
(3) Mesomeric effect (+M) in which a lone pair of electrons of the oxygen atom is released to the benzene ring:

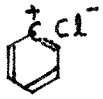


The sum of 'E' and 'M' is called 'T' (Tautomeric effect) which is larger than the inductive effect in the case of OH- substitution. Hence, the ring is activated and the attack of an electrophilic oxygen at the ortho or para position is favored due to a high electron density at these two sites. Therefore, a dihydroxyl alcohol is readily formed. This implies that catechol is the most logical intermediate in the case of phenol oxidation. This is found to be true as supported by a number of experimental evidences. Catechol readily oxidizes to the corresponding carboxylic acids.

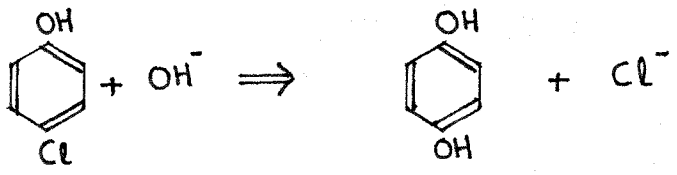
On the other hand, the chlorophenols are far more stable. The C-Cl bond energy is very high. Chlorination of phenol occurs by an electrophilic Cl^+ ion reaction. Phenol exists as a phenoxide ion in even a slightly polar media, again leading to an increased electron density at the ortho and para positions where Cl^+ attack is readily favored. The resulting chlorophenol has a lower resonance energy. One mode of its oxidation requires Cl be replaced by OH or COOH

via nucleophilic substitution. This requires a low electron density at the seat of reaction. This is very difficult due to the effect of Cl which introduces a certain amount of double bond character in the C-Cl bond, thereby increasing the electron density at the chlorine bound carbon atom.



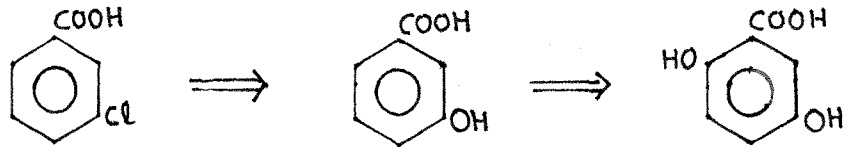
The C-Cl bond length in a ring is 1.70 A while in an aliphatic compound, it is 1.76 A suggesting that Cl is strongly attached to the carbon atom in the ring. This is more unfavorable toward attack by a nucleophilic reagent like OH- which requires a low electron density in the ring carbon atoms and separation of chlorine in sole possession of its bonding electron pair as a negative chloride ion to give  . (This is so because both the electrons forming the C-Cl bond are in reality attached to chlorine alone. Hence, if this bond is to be cleaved the electron pair must be possessed by chlorine because a ring C atom can not hold 2 free electrons).

Therefore reactions like:



must require a very powerful enzymatic action by bacteria.

Chlorine release before ring cleavage has been suggested by a few workers (29) based on based on experimental findings, but that does not agree with present theory. Haller's proposition (29) for m-chlorobenzoic acid degradation via hydrolytic dehalogenation to give gentisic acid as shown below, is very unlikely.



Similarly, oxidation of chlorobenzene via a phenol pathway also does not seem to be probable, because the OH- attack at a chlorine bound carbon atom is not favorable. As discussed later, it is therefore more appropriate to consider the attack of an electrophilic oxygen on the ring, and subsequent ring cleavage, before chlorine release. Since the further addition of Cl atoms continues to withdraw electrons from the ring, it reduces the probability of oxygen attack. This explains the recalcitrance of polychlorinated aromatics toward microbial attack.

The nitro group also has an electron withdrawing characteristic when attached to a ring. This makes nitrobenzene less susceptible to degradation than benzene. The example of p-chloronitrobenzene is interesting because the electron withdrawing nitro group, with a higher electronegativity than the Cl group, opens the way for

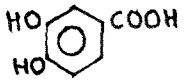
attack of nucleophilic OH^- at the p position, leading to the formation of nitrophenol. This is shown below.

The presence of a methyl group on the aromatic ring produces only a +I effect. The pi electrons are released in the ring. Electrophilic oxygen attack is readily favored, and hence, xylene is more rapidly oxidized than toluene, which in turn is more rapidly biodegraded than benzene. The same is found to be true about the cresol and phenol pair.

Similarly, nitrophenol can be expected to be relatively easily biodegradable compared to nitrobenzene due to the electron releasing effect of the OH group. Aniline is less biodegradable due to the electron withdrawing character of the NH2 group. Nitroaniline is even more recalcitrant due to the presence of 2 electron withdrawing groups of comparable strength.

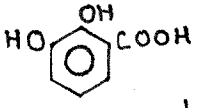
Most bacteriologist and biochemists have unanimously accepted that dihydroxylation is a first step toward enzymatic fission of the benzene ring (14,15,16,24,25,26, 28,51,70,79,84). Although the compounds that undergo a ring fission may vary in their nuclear substituents, they all have 2 hydroxyl groups. The hydroxyl groups may be ortho to each other as in catechol (29,33,84) and protocatechuic acid

(14) or para to each other as in gentisic and homogentisic acid. These structures are shown below.



Protocatechuic acid

Gentisic acid



Homogentisic acid

Catechol has been found to be an essential intermediate before ring fission in the microbial degradation of phenol, benzene, naphthlene, o-cresol, phenanthrene, anthracene, benzoic acid, etc. Protocatechuic acid has been found to be an intermediate prior to ring fission for m-cresol, p-cresol, phthalic acid, p-hydroxybenzoic acid and p-aminobenzoic acid (14,15,16,23,24,28). Thus, catechol and protocatechuate play a critical role, and most studies have concentrated on these two compounds. Evans, et al (24) and Gibson (28) have proposed reaction schemes for degradation of a variety of aromatics via catechol and protocatechuate.

The enzymes catalyzing the hydroxylation of the aromatic ring have been termed as mixed-function oxidases. In such reactions one atom of oxygen is incorporated into the substrate molecule. In the presence of a suitable electron donor (like NADH2, the reduced form of Nicotinamide Adenine Dinucleotide) the other atom of the oxygen molecule is reduced to water. If XH2 is the donor,

R-H + O2 + XH2 ----> ROH + H2O + X

OR

R-H + NAD(P)H + H+ + O2 -----> ROH + NAD(P)++ + H2O

Hypothetical pathways for the formation of catechol from benzene are shown in Figure #1 (28). A similar pathway is also proposed for protocatechuate from benzoate. Further degradation of both can occur in numerous ways.

However, no general consensus exists among bacteriologists about position, nature, and products of ring fission. This is because, the action of enzymes is extremely specific and depends on physical factors like pH, and temperature. Different strains of even the same species, like Pseudomonas, have shown different types of ring cleavage (16,25). This is so because different bacterial species activate molecular oxygen in different ways. Oxygen has two unpaired electron spins and the molecule is paramagnetic (pseudo-paramagnetic). Since carbon in the organic compound is in a singlet state, concerted reaction with oxygen are spin forbidden. In order to react, oxygen must be activated in a controlled manner by means of an enzyme-oxygenase. A scheme for the degradation of protocatechuate by E. cutida is shown in Figure #8 (28).

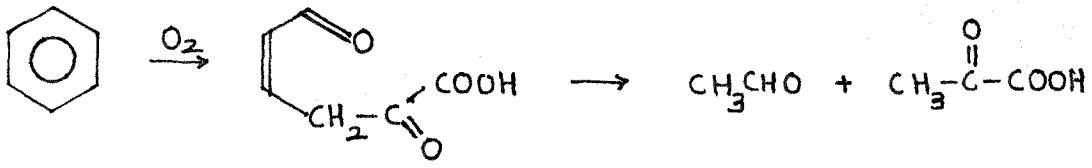
It is now confirmed that further metabolism of catechol can occur at least via 2 paths as detected by Feist, et al (25). A large number of pathways are proposed for further oxidation, it is not possible to describe all of them, although the most general schemes are presented:

(1) Catechol is cleaved in the ortho position by 1,2-oxygenase between two carbon atoms bearing the OH group and metabolized via the B-ketoadipate pathway as shown in Figure #2.

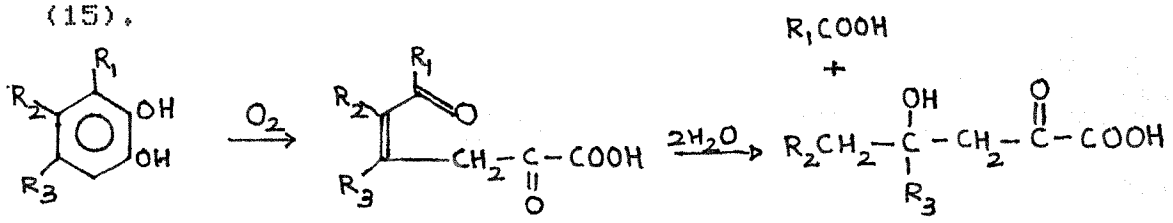
(2) The meta cleavage of catechol by 2,3-oxygenase between the carbon atom bearing the OH group and the adjacent carbon atom carrying a carbon side-chain. This is shown in Figure #3. This is the most common pathways in micro-organisms (16,28). Catechol is converted to pyruvate without evolution of carbon-dioxide, and the only consumption of oxygen is that needed for ring fission. The detailed action of 2,3-dioxygenase is discussed by Gibson, et al (28). The oxygen combines with ferrous in the enzyme to form perferyl ion. The oxygen is then activated, and catechol is attacked to form a peroxide intermediate which undergoes molecular rearrangement to form 2-hydroxymuconic semialdehyde.

Metabolism of toluene and isopropylbenzene by P. putida leads to the formation of orthodihyroxy compounds in which the side chains are left intact. It is therefore extremely likely that many aromatics undergo an enzymatic hydroxylation of the aromatic nucleus in preference to degradation of the side chain (28). This was also observed

by Dagley, et al (15). The cleavage of cresol after addition of another OH group occured with the methyl group intact as shown below (15).



On the other hand, another strain of P. putida was able to cleave methyl catechol only after converting the methyl group to carboxyl. Dagley also proposed a general scheme for the oxidation of substituted catechol by meta cleavage (15).



Dagley, Evans and Ribbons (16) also proposed most likely pathways for degradation of benzoates. They found that micro-organisms which degrade polycyclic aromatic compounds like naphthlene, anthracene, and phenanthrenes employ a stepwise end-ring attack giving alternate routes (16). That is, the attack on the second or third ring would depend upon the nature and position of the first ring cleaved because the electron distribution would be significantly different in different cases.

Evans, et al (24) found that P. fluorescens produced

catechol as an intermediate in the degradation of benzoic acid. They concluded that B-ketoadipate was the central metabolite in the biodegradation of benzene, phenol, catechol, and even protocatechuate. A similar conclusion was also drawn by Stanier, et al (70). Harris, et al (30) showed identical results while degrading phenols with yeast.

McKinney, et al (51) studied the degradation of phenol, o-, m-, and p-cresols, benzoic acids, etc. in an aerated batch reactor using an activated sludge. Warburg respirometric measurements showed different metabolic pathways. Phenol metabolism was found to be via B-oxidation, where catechol was not a required intermediate. This is shown in Figure #7.

An excellent discussion about the metabolism and ultimate fate of other man-made chemicals has been given by Dagley (14). Two factors play an important role. First, the ability of microbial enzymes to accept the chemical as substrate. Second, the ability of this substrate to induce or depress the synthesis of necessary degradative enzymes. Any naturally occuring substitution readily activates the ring for bacterial attack. For example, the p-methoxy group in the pesticide methoxychlor is far more susceptible to biodegradation than the p-chloro group in DDT because DDT is not synthesized in Nature.

Alexander (2) and Evans (23) found that degradation of

chlorocatechol and chlorophenols is via ortho fission. The cleavage occured before chlorine release, and the lactonizing enzyme that released chloride ion from the substrate has different properties from the corresponding enzymes for chlorine-free substrate. Unfortunately, they have not shown any degradation pathway.

Haller (29) found that m- and p- chlorophenol both degraded via 4-chlorocatechol with a wide variety of bacteria. But, m-chlorobenzoic acid could degrade via 3-chlorocatechol, 5-chloro-2,3-dihydroxy benzoic acid, or even by immediate hydrolytic dehalogenation through gentisic acid.

The study of Saeger and Thompson (64) showed that phenylacetic acid was a likely intermediate in the oxidation of diphenylmethanes. A number of workers (10,11,59,73,74) have reported very high resistance of trihydroxyl alcohols compared to phenol and catechol. This might be attributed to the fact that ring cleavage could not occur due to the lack of suitable oxygenase to form carboxylate since all the attack sites are stable due to uniform electron distribution.

Hartmann and coworkers (31) have proposed degadation routes for chlorobenzoic acids (CBA). As suspected, the pathways are quite different from benzoic acid. Chlorophenols would be expected to behave in a similar way.

Chlorocatechols are found to be central metabolites for all of them. Identification of CBA pathways is important because they are found to be intermediates in cometabolism of PCBs (1,13,61). These routes for 3-CBA, 4-CBA and 3,5-DCBA are shown in Fisure #6. Chloromuconolactones were considered as intermediates prior to the chlorine liberation step in the desradation of all the CBAs, but they could not experimentally verify their existence in all cases. 4-chloromuconolactone was detected in desradation of 3-chlorobenoate with Pseudomonas sp.B13. The desradation of DCBA and DCP was also considered as occurins via the dichlorocatechol route.

In the case of PCBs, the pathways are diverse since the enzymatic attack would stronsly depend on the number and location of Cl atoms on each rins. However, a seneralized route was proposed by Ahmed, et al (1).

Anaerobic metabolism of phenol, and other aromatics, is quuite different from that of aerobic attack (23,52). The bacteria use inorsanic electron acceptors instead of oxysen, and the enzymes produced are (predictably) quite different. The cleavase of the aromatic nucleus requires that extensive pi electron delocalisation must be altered by hydration or hydrosenation because no oxysen is available. For benzoic acid, Evans (23) proposed anaerobic photometabolism usins Rhodopseudomonas palustris; nitrate

respiration using Moraxella sp.; and a methanogenic fermentation route using mixed cultures. These are shown in Figure #4.

They concluded that anaerobic degradation can occur only after complete reduction of the benzene ring followed by hydrolytic cleavage. Hydrogen, carbon-dioxide and methane would be released in the process.

Neufeld, et al (52) reported the findings of a number of other workers on the anaerobic decomposition of phenol, p-cresol, resorcinol, and benzoate. Total conversion of all carbon to carbon-dioxide and methane was reported via a number of intermediates. Two distinct steps were, hydrogenation followed by alicyclic ring-fission and methanogenesis of alicyclic byproducts. On the other hand, anaerobic non-methanogenic phenol degradation showed distinct substrate inhibition above 686 ppm feed concentration. The metabolism is shown in Figure #5.

IV. EXPERIMENTAL APPARATUS

A. REACTOR SET-UP:

The main set-up consisted of two working reactors and one acclimation tank.

The accumulated activated sludge was immediately aerated in a 5 gallon glass tank. Another five gallon tank was used to supply nutrients and acclimating substrate (500 ppm phenol) using a peristaltic pump (Sage instruments, model 375A, Div. Orion Research, fitted with one mm id tubes) at a rated of 1.225 l/day. The feed lines were of 1/2" Tygon tubing.

The two reactors were of lucite, each one of approximately four liter capacity. Reactor-1 consisted of 6" o.d. and 5" i.d. tube of height 13.5" mounted on 9.6"x10" base. A lid, 6.75"x6.5", with two 3/16" holes at center, was provided. The reactor-2 consisted of 6" o.d. and 5" i.d. tube of height 14.6" mounted on 7"x7.4" base. Three holes of 1/2" diameter on a lid of dimension 7"x7.4" were provided.

Laboratory compressed air was supplied to all the reactors after passing it through a glass wool filter. The air to reactor-1 passed through a rotameter which had a flow range of 0-8 l/min. While the air to reactor-2 entered via

a rotameter with a flow range of 0-1.25 l/min.

The schematic diagram of a typical reactor assembly is shown in Figure #9.

B. ANALYTICAL EQUIPMENT:

During each experimental run, the following parameters were measured:

(1) pH

(2) Sludge concentration (as MLSS)

(3) Substrate concentration

(4) Chemical Oxygen Demand (COD)

In the sample preservation and biocide experiments, the pH was approximated using litmus paper. Later on, in phenol and 2,6-DCP runs, accurate pH measurements were accomplished with a pH meter (Orion Research, model 701A, Cambridge, Mass.; accuracy up to 3 decimal digits).

The sludge concentration was measured by a dry weight technique using an oven at 95 C.

The substrate concentration was measured using:

(1) UV Spectrophotometer (Perkin-Elmer model #571)

(2) Gas Chromatographs (Tracor model #565, with model #770 Auto-injector, or, model #560 with manual injection). Both the GCs were connected to Hewlett Packard 3390A integrators. The chromatographs had 5'x1/8" S.S.

column packed with 5% SP 2100 on 100/120 Supelcoport.

The chemical oxygen demand was measured using a COD reactor heater (Hach model #16500) at 150 C and Kimax glass digestion reagent vial with teflon-lined cap (both supplied by Hach Chemical Company, P.O. Box 907, Ames, Iowa).

An ammonia test kit was periodically used to measure ammonia concentration in the sludge.

C. MATERIALS:

All the chemicals, phenol, 2,6-DCP and other nutrients used, were reagent grade. Phenol was obtained from J. T. Baker & Co. and had 99.8 % purity. 2,6-DCP was obtained from Aldrich Chemicals Co. and had 98 % purity.

The activated sludge was obtained from the Livingston Wastewater Treatment Plant (N.J.). The MLSS of the fresh sludge was about 2500 to 2600 ppm.

The nutrients were supplied in a C/N/P ratio of 50/14/3. A number of other proportions like 7.6/1 (C/N), 100/10/2 (C/N/P), 50/10/1 (C/N/P) and 100/23/4.6 (C/N/P) have also been recommended (7,39,52,60). It is necessary to have a slight excess of N and P when performing carbon substrate degradation studies, so that the organisms are not N or P limited.

For each gram of phenol, 0.17 gms of ammonium

dihydrogen phosphate and 0.56 grams of ammonium nitrate were added. Similar proportions were also used for 2,6-DCP.

L-Glutamic Acid (LGA), L-Histidine Hydrochloride Monohydrate (LHHM), L-Lysine Monohydrochloride (LLMC), L-Arginine Hydrochloride (LAH), and L-Cysteine (LCN) were supplied by Aldrich Chemicals Co. and had an average purity above 98 %.

V. EXPERIMENTAL PROCEDURES

All the experiments were run at room temperature (26 C).

The GC conditions depended on the substrate. In the case of phenol, the oven, injector, and detector temperatures were 120, 250, and 300 C, respectively. In the case of 2,6-DCP these temperature were 155, 300, and 300 C, respectively. The sample injection size, for both the compounds was 3 ml. The attenuation for phenol was varied from 0 to 3 while that for 2,6-DCP was kept at 0. The peak width for both was 0.04 cm. Thymol was used as internal standard for phenol analysis. In the case of 2,6-DCP, the runs made before 1/1/83 had phenol as internal standard. This internal standard did not give good accuracy and peak separation. Hence, phenol was replaced by 1-chloro-2,4-dinitrobenzene. The retention time for 2,6-DCP varied from 0.81 to 1.19 minutes, while that for phenol varied from 0.59 to 0.80. The peak shift allowance for phenol and 2,6-DCP were kept at 10 and 15 %, respectively.

The flow rates of carrier gas (nitrogen), hydrogen and air were set at 40, 30, and 400 cc/min (at STP), respectively.

The process air was supplied to the acclimation tank at a rate sufficient to keep the solids in suspension and

maintain a saturated DO level. After 3/1/83, the sludge was also fed with 20 ppm amino-acids, once a week. The filtered air was supplied to both the reactors during all the runs at a constant rate of 1 l/min through a diffuser stone. The sludge was completely mixed in all the reactors.

A. AIR STRIPPING EXPERIMENTS:

Any biodegradation study must be accompanied by examination of all other possible means of substrate disappearance.

Two possibilities exist, besides biodegradation.

(1) Air stripping of substrate.

(2) Adsorption of the substrate on bacterial flocs, and reactor walls.

As mentioned before, most workers have found that for phenol, chlorophenols, and nitrophenols, the evaporation loss was less than 3 % at air flow rates ranging from 0.5-1.0 l/min. (6,22,83,90-92).

Air stripping data was obtained in the present study for 2,6-DCP at air flow rates similar to that used in biodegradation itself (1 l/min, see Tables #3,4,5).

The reactor tubing and diffuser stone were sterilized by repeated cleaning with hydrogen peroxide, soap solution

and water. 3.5 l of deionized water was boiled for 1/2 hour. The solubility of 2,6-DCP in water is less than 150 ppm, hence a 100 ppm solution was prepared in distilled water. This preparation was then used to charge the reactor after diluting it to the required concentration. 2 l of diluted solution was used at the start of each experiment. The experiments were conducted at 20, 30 and 40 ppm of 2,6-DCP. Each experiment was carried out over 6-7 days. Samples were withdrawn once or twice each day. The samples for 20 and 40 ppm concentration were analyzed immediately by GC (model #560), while those for 30 ppm were refrigerated till the experiment was over and then analyzed by GC (model #565).

B. SAMPLE PRESERVATION EXPERIMENTS:

The objective of this study was to determine the most effective way of storing the samples during an experimental run for later analysis.

The reactor was washed and sterilized, and charged with two liters of activated sludge and phenol. Samples were taken at the beginning of each experiment as well as at fixed time intervals. Five samples were taken for each observation and treated as follows:

(1) Sample-1 was centrifuged for 10 minutes at 1200 rpm,

immediately analyzed by GC, and then stored in a refriserator.

(2) Sample-2 was treated identically as sample-1, except that isopropanol was added to inactivate the bacteria. (10 % isopropanol concentration in the sludge).

(3) Sample-3 was treated identically to sample-1 except that after centrifusing, the decanted solution was vacuum filtered throush a 0.45 Um Millipore paper and then refriserated.

(4) Sample-4 was treated identically to sample-3 except that filteration was followed by addition of isopropanol to a sample concentration of 10 %.

(5) Sample-5 was treated like Sample-3, but was not refriserated.

Periodic analysis was done to detect the sample deterioration.

Sample-1 (without a biocide) showed progressive desradation. However, the rate of phenol removal is still very slow (0.14 ppm/hr) compared to the rate beins measured

in the bioreactor (170-213 ppm/hr, see Table # 29-34). Even though this sample was the least preserved, its rate of degradation is less than the error in GC analysis during an actual run. Millipore filtration was more effective than isopropanol in sterilizing the samples.

However, the use of isopropanol to kill the bacteria was discontinued primarily because it interfered with the phenol peak on the GC. In most runs with phenol, and all the runs of 2,6-DCP, 3 drops of 1000 ppm copper sulfate was used instead as a biocide. Two experiments were performed to determine the effectiveness of copper sulfate. 1000 ppm copper sulfate was added to centrifused reactor samples involving 2,6-DCP, and then refrigerated. The samples were analyzed periodically by GC, and the results are shown in Table #8. No significant loss of 2,6-DCP was observed. Hence, in all the biodegradation runs, copper sulfate was used as bactericide.

C. 2,6-DCP IONIZATION EXPERIMENTS:

The aim of these experiments was to determine the effect of variation of pH on observed readings on the gas chromatograph.

For this purpose, standard solutions of 15 and 20 ppm 2,6-DCP were made up with distilled water.

1-chloro-2,4-dinitrobenzene was used as an internal standard. The pH of the solution was varied by adding a drop of dilute NaOH or HCl solution, and 2,6-DCP concentration measured by GC.

It was found that the observed readings on the GC were not significantly affected (see Table #9).

D. UV DEGRADATION EXPERIMENTS

The objective of these experiments was to determine the degradation of 2,6-DCP due to its exposure to UV radiations for a prolonged time period. For this purpose, two standard solutions of 15 and 20 ppm 2,6-DCP were prepared to which 20 ppm internal standard (1-chloro-2,4-dinitrobenzene) was added. Both the solutions were immediately analyzed on a GC. Each mixture was then stored in a closed vial at room temperature near a window, facing direct sun-light for more than a week. Each solution was analyzed again (see Table #10). No significant loss of 2,6-DCP was observed due to UV radiations from the sun over a week's time.

E. COD DETERMINATION EXPERIMENTS

Chemical Oxygen Demand (COD) represents the amount of

oxygen consumed in the oxidation of organic and oxidizable inorganic matter in waste-waters. The theoretical COD can be calculated from a balanced equation for total oxidation of the organic matter in to carbon-dioxide and water. Knowing the theoretical and experimental COD, it is possible to estimate the extent of mineralization of the organic waste.

The theoretical COD for 2,6-DCP can be calculated from the following equation.

$$C_6H_3Cl_2OH + 6.5O_2 \longrightarrow 6CO_2 + 2Cl + 2H_2O$$

Hence 1.276 mg oxygen/mg of 2,6-DCP are required for total carbon oxidation.

The experimental COD was determined according to the procedure of Jirka, et al (96). A digestion solution was made-up by adding 7.5 gms potassium dichromate, 10.0 gms silver sulfate and 5.0 gms mercuric sulfate to a 2.5 l bottle of concentrated sulfuric acid. The mixture was magnetically stirred and heated overnight to dissolve the salts. 5 ml of cooled digestion mixture was pipetted in a screw top vial to which 3 ml of sample (or blank) was added. The cap was screwed tightly on the vial. Three such vials were prepared for each sample. Water was used as blank in same amount as any sample. All the vials were then placed in a Hach dry-bath reactor heater for 2 hours and heated to 150 C. A 0.025 N solution of ferrous ammonium sulfate (FAS)

was prepared by dissolving 19.6 gms of FAS in 1980 ml water to which 20 ml concentrated sulfuric acid was added.

After heating, the samples and blanks were cooled to room temperature. The contents of a vial were emptied in a 250 ml flask containing 50 ml of deionized water. The vial was repeatedly rinsed with deionized water which was also added to the flask. 5-6 drops of Ferrion were added as an indicator. 0.03 gms of mercuric sulfate were also added to reduce the chloride ion interference. The mixture acquired a greenish color. It was then titrated vs FAS solution in a burette to a bright orange end-point.

The experimental COD was determined from the following equation:

COD (mg/l) = (V1-V2)×N×8000/sample size

where N is the normality of FAS solution (0.025 N), sample size is the amount of sample used for each titration (3 ml), and V1 and V2 are the volume of FAS solution consumed in ml by blank, and sample respectively.

E. PHENOL AND 2,6-DCP BIODEGRADATION:

The sludge was preacclimated to 500 ppm phenol for all the runs. During acclimation, 0.17 grams of ammonium dihydrogen phosphate, 0.56 grams of ammonium nitrate, and 0.50 grams of phenol were added per liter of sludge, every

day or alternate day for more than 15 days. In addition, the acclimation tank was fed continuously with 500 ppm phenol (and a calculated amount of nutrients) at a rate of 1.225 l/day.

Before conductins experiments with 2,6-DCP, the phenol acclimated sludse was spiked with 15 ppm 2,6-DCP in a batch reactor periodically for more than 15 days. No dropwise addition of 2,6-DCP was made in the reactors. Thus, for phenol and 2,6-DCP the sludse ase was at least 15 days and 45 days, respectively. The pH was measured once or twice per week in the acclimation tank, and whenever a chanse was observed, sodium bicarbonate or ammonium bicarbonate were added as buffer. Ammonia concentration was not measured. All the runs were started with 2 l of total liquor in the reactor, aerated at a rate of 1 l/min. All the experiments were conducted at 26-27 C. Before spikins in the bach ractor, it was checked that phenol concentration in the sludse was zero.

No amino-acids were added in phenol runs. In certain runs with 2,6-DCP (15 ppm concentration) amino-acids were added at a total concentration of 20 ppm. Without considerins the effects of amino-acids, the C/N/P ratio was kept at 50/14/3 at the start of each experiment.

At the completion of a run, if a significant pH chanse was observed, sodium bicarbonate was added to the reactor to

readJust the pH between 7.0 to 7.5 before proceedins to the next run. The pH of the reactor medium was measured approximately every half hour in the case of phenol. In the case of 2,6-DCP, the measurements were less frequent because the desradation rates were much less compared to phenol.

The mixed liquor suspended solid (MLSS) concentration was measured periodically by withdrawins 10 ml of liquor and emptyins it into a preweished aluminium dish. The dish was then dried at 95 C in an oven for about 12-24 hours. The final weisht was then used to calculate the dry solids concentration.

Samples (15 ml) for substrate analysis were taken from the reactors at fixed time intervals. The samples were centrifused for 10 minutes at 1200 rpm. In the runs with phenol, 0.5 ml of 1000 ppm Thymol was added to 9.5 ml of sample as an internal standard for the GC analysis. In the runs with 2,6-DCP, 2 ml of 100 ppm 1-chloro-2,4-dinitrobenzene was added in 8 ml of sample as an internal standard. For all samples after 1/1/83, copper sulfate was also added as a biocide.

The followins runs were made for phenol:

(1) Two runs each with 250 ppm initial phenol concentration. (see Table #29 and 30).

(2) Two runs each with 400 ppm initial phenol concentration (see Tables #31,32).

(3) Two runs each with 600 ppm initial phenol concentration (see Tables #33,34).

In the runs with 2,6-DCP, the followins amino-acids were used to examine the possibility of cometabolism or substrate inhibition:

(1) L-Glutamic Acid (LGA).

(2) L-Histidine Hydrochloride Monohydrate (LHHM).

(3) L-Lysine Monohydrochloride (LLMC).

(4) L-Arsinine Hydrochloride (LAH).

(5) L-Cysteine (LCN).

The followins runs were made for 2,6-DCP (The sludse was acclimated to phenol for all the 2,6-DCP runs. Hereforth, the term 'acclimated' or 'unacclimated' is used in relation to acclimation to 2,6-DCP only). Appropriate amounts of ammonium dihydrosen phosphate and ammonium nitrate were also added at the besinnins of each run:

(1) One run of 10 ppm 2,6-DCP with unacclimated sludse, no amino-acids added (see Table #23).

(2) Two runs of 15 ppm 2,6-DCP with acclimated sludse, no amino-acids added (see Tables #11,12).

(3) Two runs each of 10, 15, and 20 ppm 2,6-DCP with acclimated sludse, all amino-acids added at a total concentration of 20 ppm (Tables #13,14,24-27).

(4) Two runs of 15 ppm 2,6-DCP with acclimated sludse, L-Histidine Hydrochloride Monohydrate (LHHM),

L-Lysine Monohydrochloride (LLMC), and L-Arginine Hydrochloride (LAH) added (total concentration 20 ppm, see Tables #15,16).

(5) Two runs of 15 ppm 2,6-DCP with acclimated sludge, LHHM, and LAH added (total concentration 20 ppm, see Tables #17,18).

(6) Two runs of 15 ppm 2,6-DCP with acclimated sludge, LHHM, and LLMC added (total concentration 20 ppm, see Tables #19,20).

(7) Two runs of 15 ppm 2,6-DCP with acclimated sludge, L-Glutamic Acid (LGA), and L-Cysteine (LCN) added (total concentration 20 ppm, see Tables #21,22).

(8) One run of 28 ppm 2,6-DCP with acclimated sludge, all amino-acids added (total concentration 20 ppm, see Tables #28).

In the case of 2,6-DCP, samples were withdrawn after 45 minutes to one hour depending upon the initial substrate concentration. In some runs, pH and MLSS were only measured infrequently.

A UV spectrophotometer was used in a number of 2,6-DCP runs prior to 1/1/83. These runs are not listed here because they were not made to evaluate kinetic parameters, but were intended to determine and identify the nature of intermediates, if present. Water was used as a blank. The spectrum was varied from 230 nm to 320 nm. The scanning

speed and chart speed were 12 nm/min and 10 nm/cm respectively. The ranse was varied from 1.0 to 1.50. The absorbence of various standard solutions of 2,6-DCP were measured and plotted, and the samples were compared with these standards.

VI. ANALYSIS OF DATA AND RESULTS

A. AIR STRIPPING:

Substrate stripping was found to be insignificant during the biodegradation runs (see Tables #3,4,5).

Leighton and Colo measured the distribution coefficient (K=Yi/Xi) for 23 chlorinated hydrocarbons in dilute air-water systems in the temperature range 0 to 30 C. The distribution coefficients for benzene, chlorobenzene, toluene, and o-chlorotoluene have been mentioned (Unfortunately, no phenolics were reported). They also proposed an expression of the form;

$\ln K = A - B/T$;

where A and B are constants, and T is absolute temperature. The values of A and B are listed in the Table below. Using these values, the infinite dilution activity coefficients for benzene, toluene, chlorobenzene, and o-chlorotoluene are calculated at 27 C and 1 atmosphere (assuming the fugacity coefficient in the vapor phase to be unity. This may introduce an error of 5 % in the value of the activity coefficients). An increase in chlorination leads to a sharp increase in the value of the activity coefficient. The measured values agreed with predictions based on the UNIFAC method.

COMPOUND	A	B	VAPOR PRESSURE	ACTIVITY COEFFICIENT
---	---	K	mm Hg	---
Benzene	19.02	3964	103.00	2453.37
Chlorobenzene	16.83	3466	13.94	10670.03
Toluene	18.46	3751	32.03	9165.02
o-Chlorotoluene	17.18	3545	6.12	26508.90

Due to the presence of two chlorine groups, the infinite dilution activity coefficient for 2,6-DCP is also expected to be very high. On the other hand, the value for phenol would not be high due to its polar nature.

Perhaps the most extensive and systematic work to determine the substrate removal mechanism has been done by Kincannon, et al (90), and Stover, et al (91). Kincannon, investigated the disappearance of 8 aliphatic and 7 aromatic hydrocarbons and their chloro and nitro derivatives. They reported that stripping was the major removal mechanism for most aliphatic hydrocarbons and their derivatives, all of which were essentially non-polar. All the polar compounds (phenol, 2,4-DCP, 2,4-dinitrophenol) studied by them, were removed only by biodegradation. 99 % removal efficiency was obtained for all of them, and loss due to stripping was virtually zero. The removal mechanisms were the same in batch and flow reactors. However, the total removal

efficiency was considerably poorer in batch reactors (particularly for 2,4-DNP). They sussested that if the compound is strippable, it is more likely to be stripped from a batch reactor than from a CSTBR. Sisnificant strippins was found in the case of benzene, and its chloro derivatives like 1,2-DCB, and 1,3-DCB. In the case of benzene and its chloro derivatives, the value of Henry's Law Constant (90,91) is of the order of 10(-3) atm.m^3 /mole. Therefore, sisnificant loss occurred because of strippins. On the other hand, nitrobenzene, phenol, and its derivatives had Henry's Law Constants of the order of 10(-5) atm.m^3 /mole, and practically no strippins was observed for these compounds.

The followins Table summarizes the findinss of Kincannon (90) and Stover (91).

COMPOUND	% OVERALL REMOVAL	% STRIPPING	% SORPTION	% BIODEGRADATION
Benzene	99.9	16	0	84
1,2-DCB	+99.9	22	0	78
1,3-DCB	99.5	**	**	**
Nitrobenzene	97.8	0	0	100
Phenol	+99.9	0	0	100
2,4-DCP	95.2	0	0	100
2,4-DNP	+99.3	0	1	99

Based on the values of Henry's Law Constant, corresponding values of the infinite dilution activity coefficient of each compound can be calculated. The values for phenol and 2,4-DCP are not very accurate because of the difficulty of extrapolating low pressure data given in the Handbook of Chemistry and Physics, and Lange's Handbook of Chemistry (94,95). These values are calculated at atmospheric pressure and 27 C.

COMPOUND	VAPOR PRESSURE	HENRY'S LAW CONSTANT	ACTIVITY COEFFICIENT
---	mm Hg	Atm.m^3/mole	---
Benzene	103.00	6.0x10(-3)	2459.54
1,2-DCB	1.6856	1.9x10(-3)	47593.81
1,3-DCB	2.2628	2.6x10(-3)	48514.41
Nitro-benzene	0.3033	2.4x10(-5)	3341.13
Phenol	0.8790 0.4191	1.3x10(-6)	62 to 131
2,4-DCP	0.30 to 0.20	4.2x10(-5)	5911 to 8866

Using the values of infinite dilution activity coefficients calculated above, the substrate loss due to

air-stripping can be computed using the equation:

Y = Ps.X.r/P, where r is the infinite dilution activity coefficient and Ps and P are vapor pressure and total pressure, respectively.

Therefore, stripping rate (moles/hr) = Yxair flow rate (moles/hr). The maximum theoretical loss of phenol and 2,6-DCP due to air-stripping in the experimental concentration range were found to be 0.393 ppm/hr and 0.844 ppm/hr, respectively; while the experimental loss of these compounds were 0.14 and 0.044 ppm/hr, respectively. Therefore, it can be concluded that the air leaving the bio-reator was less than 30 % saturated with respect to the substrate. Hence, air-stripping is neglisible.

In the case of pentachlorophenol (PCP), Wukasch, et al (83) found that loss of PCP due to air stripping was below 0.05 %. Similar observations were made by Etzel, et al (22) about PCP, and Baxter, et al (6) about PCBs.

However, caution should be exercized when considering the possibility of stripping in the presence of activated sludge. Stover, et al (91) found that several compounds that showed little or no stripping while present in a biological reactor, were completely stripped under identical conditions without the biological population (This seems rather implausible). On the contrary, Gaudy et al (88,89) found that a variety of aromatics, which were not stripped

in the absence of a microbial population, were lost as unidentified compounds in the presence of an activated sludge. Kincannon, et al (90) reported that removal mechanisms for 1,3-dichlorobenzene could not be identified because it was converted to two unidentified chemicals which were then stripped out of the liquor at room temperature.

In the present work, the 2,6-DCP runs did not last more than 12 hours, and phenol runs did not last longer than 7 hours. Phenol and 2,6-DCP losses due to air stripping were below the sensitivity of the GC analysis (about 1.5 ppm) for a single run (see Tables #3,4,5).

B. ADSORPTION:

At 600 ppm feed concentration, it was found that no phenol disappeared for a prolonged period of time (45 minutes, see Figure #13). This time lag implies that no surface adsorption was occuring for preacclimated sludge. A similar conclusion can be made about 2,6-DCP, for which disappearance was negligible over the first 1.5 hours at a feed concentration of 15 ppm (see Figure #19). These observations are in line with the findings of Kincannon, et al (90), and Stover, et al (91). In their extensive research on identification of removal mechanisms, they concluded that loss of substrate due to adsorption of any

kind in the case of benzene, phenol, and its derivatives was essentially negligible.

C. pH:

The pH measurements for phenol showed that the reaction proceeded with a significant decrease in pH, probably due to the formation of carbon-dioxide and carbonic-acid or due to nitrification of ammonium (see Figures #14,15,16,17). No buffer was added while the experiment was in progress, although it was corrected with sodium bicarbonate before repeating the run. This behavior has also been reported by numerous other workers (38,39,54,84). In the case of 2,6-DCP degradation without addition of amino-acids, the reaction proceeded with a decrease in pH. But when amino-acids were added, in most cases the reaction proceeded with a significant increase in pH. This could be due to decarboxylation of the amino-acids or due to air-stripping of HCl formed during the bioreactions (see Figures #28-34).

The 2,6-DCP ionization experiments showed that the experimental variation of pH had little effect on the GC readings, implying that negligible amount of 2,6-DCP was ionized.

D. MIXED LIQUOR SUSPENDED SOLIDS:

The MLSS concentration of fresh sludge from the Livingston Wastewater Treatment Plant was about 2600 ppm and the sludge appeared greenish brown in color. After its acclimation to phenol over 15 days, the greenish cast disappeared. However, after further acclimation to 2,6-DCP there was no observed color change.

The bacterial population was found to be very heterogeneous (93). Though the bacteria have not been completely identified as yet, a rich variety of eucaryotic organisms were noticed, including ciliated protozoa (Colacium, Vorticella, Epistylis), flagellated protozoa (Lionotus, Peranema) and amoeba (Valcampfia, Polychaos, Mayorela). In addition, rotifers that feed on protozoa and bacteria, and even watermites and flatworms that feed on other micro-organisms, have been detected under the microscope. This was found in fresh sludge as well as in the sludge acclimated to 500 ppm phenol.

It was observed that the MLSS concentration varied somewhat during a run (see Tables 11-28, and 29-34). No well-defined pattern could be established for the variation in sludge concentration, and the most probable reason is the measurement technique. It was difficult to measure the precise dry weight in a 10 ml sample of reactor liquor.

Probably because of the relatively short duration of the runs, no significant decrease in MLSS was observed even when 2,6-DCP was fed at a much lower carbon level (15 ppm vs 250-600 ppm phenol).

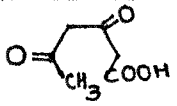
E. LAG TIME:

Practically no time lag was observed for phenol up to 400 ppm, since the sludge was already acclimated to 500 ppm (see Figures #11,12). But, the lag time was evident at 600 ppm feed concentration, and the rate of disappearance was also considerably reduced (see Figure #13).

As expected, for 2,6-DCP, the lag time for unacclimated sludge (as well as acclimated sludge without addition of amino-acids) was quite large (4 hrs, see Figure #19). The rate of degradation seemed to be linear with 2,6-DCP concentration. Addtion of amino-acids reduced the time lag to less than 1.5 hours, and increased the removal rate (see Figures #20-27).

E. METABOLIC INTERMEDIATES:

2,6-DCP gives two absorption peaks at frequencies of 278 and 285 nm. Auto-oxidation of 2,6-DCP was evident because the UV spectrophotometric analysis showed the

presence of an intermediate at 300 nm which increased rapidly even in air stripping runs (see Figure #39). These peaks partially overlapped in both spectrophotometric and gas chromatographic analysis for air stripping as well as biodegradation experiments (see Figure #10). An attempt was made to identify the peak at 300 nm. 3,5-dioxohexanoic acid () was the only six carbon structure to give a peak in the vicinity of 300 nm. However this compound is not very likely because of two reasons:

(1) Since the peaks were overlapping in both the detection methods, the structure of the intermediate should be more similar to that of 2,6-DCP.

(2) Fundamentally, the carbon-chlorine bond cannot be easily cleaved just by a supply of air at room temperature because this bond is very stable.

A more likely intermediate would be dichlorocatechol. Formation of dichlorocatechol has been already reported in the degradation of dichlorobenzoate (31). On same grounds, 2,6-DCP would be expected to form 2,6-dichlorocatechol. Unfortunately, the maximum UV absorbance peak for this compound could not be found in the literature.

Further experiments showed that a freshly prepared solution of 20-100 ppm 2,6-DCP, left overnisht at room

temperature in a closed bottle, would oxidize by itself resulting in the same UV peak shift.

During the biodegradation runs, the peak separation of 2,6-DCP and the intermediate was better defined by GC after most of the 2,6-DCP had degraded. Because of the intermediate peak (see Figures #10,39), the GC was occasionally unable to identify the proper reference peak. The accuracy of the GC was about 1.5-2 ppm. Furthermore, as the run proceeded the size of the unknown peak appeared to go through a maximum.

G. COD DETERMINATION EXPERIMENTS

The accuracy of the data was tested by determining the theoretical and experimental COD for different standard solutions of pure 2,6-DCP. The experimental data was not very consistent and an average of 25 % deviation from the theoretical value was observed for standard solutions. At such a low level of carbon content, the above procedure does not seem to be very accurate (see Table #35). The following table shows the % deviation measured in theoretical and experimental values of COD for standard solutions of 2,6-DCP.

STD. 2,6-DCP CONCN (PPM)	COD (THEOR) MG/L	COD (EXPTL) MG/L	% DEVIATION ***
25	31.90	36.67	+15.05
20	25.52	33.33	+30.47
15	19.14	18.00	-5.96
10	12.76	20.00	+56.74
7.5	9.57	11.11	+16.09

B. KINETICS OF BIODEGRADATION:

First and second order kinetic models did not fit the data for either compound, which display convex curves in the initial region and a straight line later on (see Figures #11-13, and 18-33).

An attempt was made to fit the data to a Monod model. The Lineweaver-Burk (53), Hanes (20), and Henri (72) linearizations of the Monod model were tried.

Lineweaver-Burk differential form:

$$-dt/dS = (Km/Um.X)[1/S] + 1/[Um.X],$$

so that at constant X, a plot of $-1/[dS/dt]$ vs $1/S$ would be a straight line with slope $(Km/Um.X)$ and intercept $1/[Um.X]$.

Hanes differential form:

$$-S[dt/dS] = (Km/Um.X) + S/[Um.X],$$

so that at constant X, a plot of $-S/[dS/dt]$ vs S would be a straisht line with slope $(1/Um.X)$ and intercept $Km/[Um.X]$.

Henri integral form:

$$[\ln S/S_o]/t = (S_o-S)/[Km.t] + Um.X/Y.Km,$$

so that at constant X, a plot of $1/t[\ln S/S_o]$ vs $(S_o-S)/t$ would be a straisht line with slope 1/Km and intercept Um.X/Y.Km.

In all the above methods, an assumption is made that MLSS concentration remains constant. In the present work, an averase variation of 7-8 % was observed for 2,6-DCP experiments. For phenol, the MLSS variation was about 11.5 %. The standard deviation for phenol and 2,6-DCP varied from about 12 to 100 ppm (of MLSS) with an averase value of 50 ppm. The differential methods required evaluation of dS/dt. This was done sraphically by plottins S vs t. A smooth curve was drawn and slopes were measured at different substrate concentrations. All the above models save nesative values of Km. The method of Gates and Marlar (72) was not tried due to the failure of Henri's linearization method.

Inhibition models (senerally Haldane) have been successfully emploved by a number of workers for phenol (33,52,56,84), particularly at concentrations above 360 ppm.

Haldane model: $-dS/dt = Um.X.S/[Km + S + S^2/Ki]$

The observations in the present work also show that at feed concentrations higher than that for acclimation, a significant lag period was observed (see see Figure #13).

For S << Ki, the Haldane model can be linearized to obtain a Lineweaver-Burk form of straight line. This also did not seem to fit the data.

For $S + S^2/Ki >> Km$, the Haldane equation reduces to:

$-dt/dS = 1/[Um.X] + S/[Um.X.Ki]$,

This equation was also tried, and it also failed to give a good fit.

Therefore, in spite of the fact that the curves often seem convex, the experimental data is best fit (in terms of correlation coefficient) by a straight line over most of its range. Hence, the rate of substrate disappearance was nearly constant for most runs, and a zero-order kinetic expression seemed to fit the data best.

For the sludge acclimated to 500 ppm phenol, the average degradation rate for feed concentrations below 500 ppm (4 observations) was 206.45 ppm/hr, with a range of 198.98 to 214.92 ppm/hr. It has been confirmed by a number of researchers that degradation rates are often a function of feed and biomass concentration. Hence, the average degradation rate per unit dry mass of organisms is 0.0738 ppm/hr.ppm of MLSS (for an MLSS of 2780 ppm). These rates are based on a linear regression of data points which did

not show a time lag. The correlation coefficients for all regressions were above 0.97.

The behavior of the 600 ppm runs was quite different. An appreciable lag time was observed although a zero order kinetic model appeared to be a best fit during the period of falling substrate concentration. The average rates of phenol degradation during and after the lag period were found to be 82.87 ppm/hr (0.03280 ppm/hr.ppm of MLSS), and 188.1 ppm/hr (0.0768 ppm/hr.ppm of MLSS), respectively. The average MLSS concentrations in these two periods were 2520 and 2450 ppm respectively. The correlation coefficients were above 0.99.

In the case of 2,6-DCP, for unacclimated sludge, without addition of amino-acids, the zeroth order kinetics fit gave rates that varied from 0.16 to 0.45 ppm/hr. For sludge acclimated for at least 15 days, the rate increased to 0.54 ppm/hr (1.893x10(-4) ppm/hr.ppm of MLSS, MLSS = 2850 ppm).

The addition of one or more of amino-acids (L-Glutamic acid, L-Histidine Hydrochloride Monohydrate, L-Lysine Monohydrochloride, L-Arginine Hydrochloride, L-Cysteine) significantly affected the biodegradability of 2,6-DCP. Though the exact effect of amino-acids on 2,6-DCP metabolism was not determined, it seems that addition of these acids helped the bacteria to synthesize the required extracellular

enzymes. Once these enzymes were manufactured, bacterial attack on the substrate increased significantly. The lag time decreased with repeated exposure using different amino-acids, to the point that the S vs t relationship became a straight line with no apparent lag time (see Figures #25,26). Unfortunately, no specific conclusions could be reached about the effects of individual amino-acids because the time for complete degradation did not vary significantly with different acids. The variation that was observed, could be due to other physical factors like pH, repeated exposures, etc. (see Figures #23-27). The average degradation rate for 2,6-DCP was 1.62 ppm/hr (5.43x10(-4) ppm/hr.ppm of MLSS) at an average MLSS concentration of 2980 ppm. The rates and MLSS concentration varied from 1.55 to 1.74 ppm/hr, and 2890 to 3000 ppm, respectively.

Although 2,6-DCP feed concentrations of 10, 15, and 20 ppm behaved similarly, a feed concentration of 25 ppm caused a sharp drop in degradation rate to (1.01 ppm/hr), even though all the amino-acids were added (see Figure #27).

When the feed concentration was varied from 10 to 25 ppm for 2,6-DCP, and from 250 to 600 ppm for phenol, the total time for complete degradation increased exponentially. At 25 ppm concentration, the time required for complete removal of 2,6-DCP was twice as much as that for 20 ppm concentration. Similar behavior was seen for phenol (see

Fisures #35,36).

The table on the next pase summarizes the experimental findinss of the present research work. Compared to the rates reported in literature, the rate of biodesradation of phenol in the present experimental work is found to be sisnifiantly hisher. Little information is available about the rate of biodesradation of 2,6-DCP from literature. Baker, et al (4) found that 2,6-DCP was rapidly biodesradable at 100 ppm feed concentration at a rate of 5.56 ppm/hr in aerobic media. In the case of 2,4-DCP, only 81 % desradation was observed over 40 days at a feed concentration of 100 ppm. In the present work, the rate of biodesradation of 2,6-DCP varied from 0.445 to 2.32 ppm/hr dependins on the experimental conditions. The experimental procedure in the present work and that of Baker, et al, are however, significantly different. (They studied the biodesradation of 2,6-DCP in a clay soil where 100 ppm of the substrate was added. The soil was kept in a sealed flask to prevent any volatilization. The flask was incubated at 23 C. No nutrients were provided).

COMPOUND	FEED CONCN	AVG. K	MLSS	ACCLIM SLUDGE?	AMINO ACID ADDED?
	PPM	PPM/HR	mg/l		
Phenol	250	206.95	2.74	Yes	No
Phenol	400	211.44	2.77	Yes	No
Phenol	600	188.15	2.46	Yes	No
2,6-DCP	10	0.445	2.71	No	No
2,6-DCP	10	1.677	2.87	Yes	Yes,all
2,6-DCP	15	0.538	2.85	Yes	No
2,6-DCP	15	1.552	2.98	Yes	Yes,all
2,6-DCP	15	2.325	3.02	Yes	LHHM, LLMC LAH
2,6-DCP	15	1.220	2.95	Yes	LHHM, LAH
2,6-DCP	15	1.388	2.87	Yes	LHHM, LLMC
2,6-DCP	15	1.636	2.97	Yes	LGA,LCN
2,6-DCP	20	1.623	3.10	Yes	Yes,all
2,6-DCP	25	1.005	2.77	Yes	Yes,all

VII. CONCLUSIONS AND COMMENTS:

Experiments were conducted on the biodegradability of phenol and 2,6-DCP in batch reactors with activated sludge obtained from the Livingston (N.J.) Wastewater Treatment Plant. The following results were obtained:

(1) Loss of substrate due to air stripping is negligible, but as noted by Gaudy, et al (88,89), a distinct possibility exists that the stripping of a compound in the presence of micro-organisms could be quite different. Athough the compound is relatively non-volatile, the intermediates formed by microbes may be rapidly stripped from the solution (90).

(2) For both the substrates, unacclimated sludge showed a much larger lag-time and a very slow removal rate as compared to acclimated sludge. A sharp drop in rate was also observed when the acclimated sludge was exposed to a higher substrate concentration.

(3) For acclimated sludge, it was found that phenol and 2,6-DCP follow zero-order kinetics. At concentrations up to 500 ppm phenol, and 20 ppm 2,6-DCP, the average degradation rates were 0.0738,

and 1.893x10(-4) ppm/hr.ppm of MLSS, respectively.

(4) Addition of amino-acids to 2,6-DCP significantly improved the biodegradability. When 20 ppm (total) of L-Glutamic acid, L-Histidine Hydrochloride Monohydrate, L-Lysine Monohydrochloride, L-Arginine Hydrochloride, and L-Cysteine were added to the aqueous solution, 2,6-DCP removal (for feed concentration up to 20 ppm) averaged 5.4305x10(-4) ppm/hr.ppm of MLSS (a three-fold increase).

(5) 2,6-DCP showed an additional unidentified peak in both UV spectrophotometry or GC analysis, upon exposure to air. It is important to identify this potential intermediate because it may be the first step in biodegradation. GC analysis indicated that the size of this peak generally went through a maximum as the reaction proceeded.

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TABLES

Table #1 : Literature survey summary

One or more of the followins models have been tried by most of the workers to obtain the kinetic rate constants.

Haldane model: $-dS/dt = Um.X.S/ [Km + S + S^2/Ki]$
Monod model: $U = Um.S/ [Km + S]$
Grau model: $-dS/dt = k.X.S/So$
First order model: $-dS/dt = k.S$
Second order model: $-dS/dt = k.S^2$
where,
So = Initial substrate concentration, ppm.
S = Substrate concentration, ppm.
X = MLSS concentration, ppm.
t = time, hours.
Km = Michaelis-Menten (substrate saturation) constant, ppm.
Um = Maximum specific growth rate constant, ppm.
k = Rate constant for 1st order or 2nd order or Grau model.
Ki = Substrate inhibition constant, ppm.

REF #	COMPD.	CONC PPM	MODEL	COMMENTS (Temp., pH, organisms etc.)
33	Phenol	200	Haldane, Um=0.534, Km<1.0, Ki=470, Y=0.52	30 C, pH=6.5, Pseudomonas putida
33	Phenol	700	Haldane, Um=0.481, Km<1.0, Ki=840, Y=0.52	30 C, pH=6.5, ATCC17484
52	Phenol	1500	Haldane, Um=0.08, Km=700, Ki=966, Y=.82-1.22, Kd=3.45x10(-4)	32-40 C, pH=7.5-8.0, mixed culture, anaerobic
56	Phenol	800	Haldane, Um=0.66, Km=86.7, Ki=34.2, Y=0.616	28 C, pH=6.6, filamentous bacteria, 4 hr residence time

REF #	COMPD.	CONC PPM	MODEL	COMMENTS (Temp., pH, organisms etc.)
56	Phenol	800	Haldane, Um=1.01, Km=160, Ki=14.7, Y=0.545	28 C, pH=6.6, spherical bacteria, 6 hr residence time
84	Phenol	500	Haldane, Um=0.464, Km=1.66, Ki=380, Y=0.85	30 C, pH=4.5, T. cutaneum
84	Phenol	500	Haldane, Um=0.567, Km=2.38, Ki=106, Y=0.85	30 C, pH=6.0, Pseudomonas putida
87	Phenol	-	Haldane, Um=0.29, Km<1.0, Ki=110, Y=0.59	27 C, pH=7.0, Bacterium NCIB8250
7	Phenol	360	Monod, Um=0.17, Km=245, Y=0.45, Kd=0.008	20 C, pH=7.2, mixed culture
20	Phenol	60	Monod, Um=0.63, Km=30, Y=0.47, Kd=0.05	24 C, pH=7.0, coal gasification wastewaters
38	Phenol	650	Monod, Um=0.019, Km=236, Kd=0.002, Y=1.21	5 C, pH=7.1, mixed culture
38	Phenol	720	Monod, Um=0.070, Km=236, Kd=0.006, Y=1.21	23 C, pH=7.05, mixed culture
38	Phenol	790	Monod, Um=0.072, Km=236, Kd=0.007, Y=1.21	28 C, pH=6.9, mixed culture
53	Phenol	<100	Monod, Um=0.287, Km=2.11, Kd=0.01, Y=1.20	22 C, pH=7.0, mixed culture

REF #	COMPD.	CONC PPM	MODEL	COMMENTS (Temp., pH, organisms etc.)
79	Phenol	100	Monod, Um=.328, Km=24.96, Y=0.011	30 C, Yiest Debaryomyces subslobosus
45	Phenol	500	Grau, k=0.04 /hr	26 C, pH=7.0, mixed culture
60	Phenol	800	Exponential, Um=0.144, Y=0.606	40 C, pH=7.0, Bacillus Cereus
60	Phenol	800	Exponential, Um=0.0937, Y=0.548	30 C, pH=7.0, Bacillus Cereus
49	Phenol	400	1st order, k=0.004 1/mg.day, Y=0.13, Kd=8.33x10(-4)	**, mixed culture
55	Phenol	***	2nd order, k=7x10(-12) 1/hr per organism	**, mixed culture
55	p-CP	***	2nd order, k=1.7x10(-12) 1/hr per organism	**, mixed culture
76	2,4-DCP	25	Monod, Um=0.14, Km=5.1, **OR** Haldane, Um=0.228, Km=11.7, Ki=35.7	25 C, pH=7.1-7.8, Psuedomonas NCIB9340
83	PCP	20	1st order, k=1.75x10(-4) /hr	**, mixed culture

REF #	COMPD.	CONC PPM	MODEL	COMMENTS (Temp., pH, organisms etc.)
67	2,4-D	111	Monod, Um=0.0958, Km=5.4, Y=0.14	20 C, pH=7.0, Pseudomonas putida
55	nitro phenol	***	2nd order, k=1x10(-13) 1/hr per organism	**, mixed culture
67	p-CBA	50	Monod, Um=0.05, Km=1.1, Y=0.25	20 C, pH=7.0, Pseudomonas putida
67	o-CBA	98	Monod, Um=0.042, Km=2.4, Y=0.22	20 C, pH=7.0, Pseudomonas putida
67	m-CBA	*	Monod, Um=0.025, Km=2.0, Y=0.14	20 C, pH=7.0, Pseudomonas putida
67	2,5-DCBA	*	Monod, Um=0.025, Km=1.5, Y=0.16	20 C, pH=7.0, Pseudomonas putida
67	3,5-DCBA	*	Monod, Um=0.002, Km=25.3	20 C, pH=7.0, Pseudomonas putida

Table #2 : Interaction of various substituents with the ring carbon atom

TYPE	SUBSTITUENT	EFFECT	POSITION OF OXYGEN ATTACK
1	Cl	Deactivation	ortho, para
2	NO2	Deactivation	meta
3	OH	Activation	ortho, para
4	COOH	Deactivation	meta
5	CH3	Activation	ortho, para
6	NH2	Deactivtion	meta

Table #3. Air strippins of 2,6-DCP at 40 ppm
7/28/82

DATE	TIME	TIME FROM START	CONCN
****	HOURS	HOURS	PPM
7/28/82	9:45 am	0:00	40.538
7/28/82	8:00 pm	10:15	39.900
7/29/82	9:45 am	24:00	38.339
7/29/82	8:00 pm	34:15	38.235
7/30/82	9:45 am	48:00	37.576
7/30/82	8:00 pm	58:15	37.429
7/31/82	9:45 am	72:00	36.594
8/1/82	10:00 am	96:15	35.947
8/2/82	9:45 am	120:00	35.195
8/2/82	8:00 pm	130:15	34.487
8/3/82	12 noon	146:15	33.869
8/3/82	8:00 pm	154:15	32.480
8/4/82	8:00 pm	178:15	30.647

Table #4. Air stripping of 2,6-DCP at 30 ppm
8/11/82

DATE	TIME	TIME FROM START	CONCN
****	HOURS	HOURS	PPM
8/11/82	10:30 am	0:00	30.232
8/11/82	7:30 pm	9:00	28.770
8/12/82	10:30 pm	24:00	28.516
8/12/82	8:00 pm	33:30	27.519
8/13/82	10:30 am	48:00	26.971
8/13/82	8:00 pm	57:30	25.387
8/14/82	2:30 pm	76:00	24.082
8/15/82	11:00 am	96:30	25.281
8/15/82	7:30 pm	105:00	23.788
8/16/82	11:45 am	121:15	22.623
8/16/82	8:00 pm	129:30	19.816
8/17/82	7:30 pm	153:00	17.641

Table #5. Air strippins of 2,6-DCP at 15 ppm
3/5/83

DATE	TIME	TIME FROM START	CONCN
****	HOURS	HOURS	PPM
3/5/83	4:15 pm	0:00	18.696
3/6/83	2:15 pm	22:00	15.731
3/6/83	7:45 pm	27:30	17.313
3/7/83	10:45 am	42:30	17.813
3/7/83	9:00 pm	52:45	17.638
3/8/83	12:30 pm	68:15	18.353
3/8/83	5:30 pm	73:15	17.915
3/8/83	9:30 pm	77:15	18.461
3/9/83	1:00 pm	92:45	17.681
3/10/83	11:00 am	114:45	16.882
3/10/83	7:15 pm	123:00	17.483
3/11/83	9:30 pm	149:15	16.427

Table #6. Sample preservation experiment-1

DATE	TIME	TIME FROM START	SAMPLE TYPE	CONCN PHENOL
****	HOURS	HOURS	****	PPM
8/11/82	8:49 pm	0:00	Sample-1	366.060
	10:02 pm	0:00	Sample-2	384.230
	9:31 pm	0:00	Sample-3	341.900
	9:00 pm	0:00	Sample-4	380.870
8/16/82	11:23 am	110:34	Sample-1	344.440
	1:05 pm	111:03	Sample-2	373.850
	11:41 am	110:00	Sample-3	342.840
	1:32 pm	112:32	Sample-4	374.220
8/26/82	1:04 pm	352:15	Sample-1	302.510
	1:31 pm	351:29	Sample-2	355.780
	2:36 pm	353:05	Sample-3	319.490
	2:00 pm	353:00	Sample-4	353.850
9/02/82	2:35 pm	521:46	Sample-1	289.750
	2:01 pm	519:59	Sample-2	333.180
	2:15 pm	520:44	Sample-3	307.290
	1:54 pm	520:54	Sample-4	356.150

Table #7. Sample preservation experiment-2

DATE	TIME	TIME FROM START	SAMPLE TYPE	CONCN PHENOL
****	HOURS	HOURS	****	PPM
9/4/82	1:20 pm	0:00	Sample-1	285.170
	12:30 pm	0:00	Sample-2	205.630
	2:09 pm	0:00	Sample-3	207.600
	10:44 am	0:00	Sample-4	200.790
	11:54 am	0:00	Sample-5	248.400
9/8/82	11:45 am	94:25	Sample-1	277.330
	12:30 pm	96:00	Sample-2	197.200
	10:50 am	92:41	Sample-3	201.231
	12:45 pm	98:01	Sample-4	203.250
	1:15 pm	97:19	Sample-5	241.100
9/20/82	10:00 am	380:40	Sample-1	235.870
	12:43 pm	384:13	Sample-2	199.570
	11:10 am	381:01	Sample-3	208.960
	12:34 pm	385:50	Sample-4	190.080
	1:50 pm	385:56	Sample-5	239.310

Table #8. Biocide experiments

BIOCIDE-1, 10 % ISOPROPANOL IN SLUDGE

DATE	TIME	TIME FROM START	CONCN PHENOL
****	HOURS	HOURS	PPM
9/14/82	5:49 pm	0:00	285.610
9/15/82	4:20 pm	22:31	000.000

BIOCIDE-2, 10 % ISOPROPANOL IN SLUDGE

DATE	TIME	TIME FROM START	CONCN PHENOL
****	HOURS	HOURS	PPM
9/17/82	7:59 pm	0:00	975.480
9/18/82	8:30 pm	24:31	969.730

BIOCIDE-3, 25 % ISOPROPANOL IN SLUDGE

DATE	TIME	TIME FROM START	CONCN PHENOL
****	HOURS	HOURS	PPM
9/19/82	9:45 am	0:00	152.870
9/20/82	6:30 pm	20:45	138.230

BIOCIDE-4, 1000 PPM COPPER SULFATE

DATE	TIME	TIME FROM START	CONCN 2,6-DCP
****	HOURS	HOURS	PPM
2/21/83	7:00 pm	0:00	15.429
2/27/83	3:01 pm	140:01	16.229

BIOCIDE-5, 1000 PPM COPPER SULFATE

DATE	TIME	TIME FROM START	CONCN 2,6-DCP
****	HOURS	HOURS	PPM
2/20/83	5:45 pm	0:00	13.556
2/27/83	5:56 pm	168:08	11.574

Table #9. 2,6-DCP ionization experiments
(15 PPM 2,6-DCP)
8/21/83

pH	CONCN (PPM)
6.6	15.636
10.7	15.193
5.4	14.828

(20 PPM 2,6-DCP)
8/21/83

pH	CONCN (PPM)
6.7	24.429
10.2	23.879
4.6	24.906

Table #10. UV degradation experiments
(20 PPM 2,6-DCP)

DATE	TIME	TIME FROM START	2,6-DCP CONCN
	HOURS	HOURS	PPM
8/21/83	5:50 pm	0:00	22.180
8/30/83	9:45 pm	219:55	20.910

(15 PPM 2,6-DCP)

DATE	TIME	TIME FROM START	2,6-DCP CONCN
	HOURS	HOURS	PPM
8/21/83	5:50 pm	0:00	13.983
8/30/83	8:40 pm	218:50	15.270

Table #11. Biodegradation of 15 ppm 2,6-DCP, (no amino acids added, acclimated sludge) 2/14/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
9:15 am	0:00	2.77	7.03	16.924
10:00	0:45	****	7.01	17.099
10:45	1:30	2.78	6.96	17.394
11:45	2:30	****	6.92	16.812
1:00 pm	3:45	2.81	6.80	16.479
1:45	4:30	****	6.75	16.076
2:30	5:15	2.82	6.67	15.601
3:15	6:00	****	6.63	15.421
4:00	6:45	****	6.61	14.872
4:45	7:30	2.85	6.57	14.401
6:15	9:00	2.89	6.51	13.599
7:30	10:15	2.89	6.50	12.919
8:15	11:00	****	6.49	12.554
9:00	11:45	2.91	6.46	12.03
9:45	12:30	****	6.44	11.788
10:45	13:30	2.90	6.43	11.112
8:15 am	23:00	2.90	6.15	6.212
9:00	23:45	****	6.14	5.791
9:30	24:15	****	6.12	5.404

10:30	25:15	2.89	6.12	5.462
11:30	26:15	****	6.12	4.509
12:30 pm	27:15	2.88	6.12	3.864
1:30	28:15	****	6.12	3.430
2:30	29:15	****	6.13	3.398
4:30	31:15	2.87	6.15	1.552
5:30	32:15	****	****	0.000

Table #12. Biodegradation of 15 ppm 2,6-DCP,
(no amino-acids added, acclimated sludge)
2/14/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
9:15 am	0:00	2.79	7.20	16.145
10:00	0:45	****	7.18	16.386
10:45	1:30	2.79	7.12	16.583
11:45	2:30	****	7.07	16.357
1:00 pm	3:45	2.81	6.98	15.709
1:45	4:30	****	6.93	15.951
2:30	5:15	2.82	6.88	15.188
3:15	6:00	****	6.92	14.702
4:00	6:45	****	6.86	14.851
4:45	7:30	2.83	6.90	13.562
6:15	9:00	2.85	6.76	12.754
7:30	10:15	2.86	6.69	12.170
8:15	11:00	****	6.67	11.659
9:00	11:45	2.86	6.65	11.448
9:45	12:30	****	6.63	10.954
10:30	13:15	2.87	6.61	10.414
8:15 am	23:00	2.89	6.40	5.867
9:00	23:45	****	6.37	5.379
9:30	24:15	****	6.33	4.910

10:30	25:15	2.89	6.34	4.372
11:30	26:15	****	6.29	4.034
12:30 pm	27:15	2.88	6.28	3.434
1:30	28:15	****	6.32	2.451
2:30	29:15	****	6.32	2.481
4:30	31:15	2.88	6.29	0.973
5:30	32:15	****	****	0.000

Table #13. Biodegradation of 15 ppm 2,6-DCP,
(acclimated sludge, all amino-acids added -20 ppm total-)
2/16/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
10::00 am	0:00	2.84	6.32	15.567
11:00	1:00	****	6.53	14.358
12 noon	2:00	2.93	6.66	14.374
1:00	3:00	****	6.79	13.157
2:00	4:00	2.95	6.81	11.935
3:00	5:00	****	6.92	11.191
3:45	5:45	3.01	7.01	10.313
4:30	6:30	****	7.10	9.273
5:15	7:15	3.01	7.12	8.299
6:35	8:35	3.01	7.17	6.312
7:15	9:15	****	7.20	5.125
7:45	9:45	3.00	7.20	4.757
8:30	10:30	2.99	7.23	2.691
9:15	11:15	****	7.22	0.000

Table #14. Biodegradation of 15 ppm 2,6-DCP,
(acclimated sludge, all amino-acids added -20 ppm total-)
2/16/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
10 am	0:00	2.85	6.61	14.369
11:00	1:00	****	6.70	13.896
12 noon	2:00	2.97	6.74	12.828
1:00 pm	3:00	****	6.83	12.603
2:00	4:00	3.02	6.93	11.577
3:00	5:00	****	7.07	10.387
3:45	5:45	3.01	7.10	9.649
4:30	6:30	****	7.18	8.900
5:15	7:15	3.04	7.18	7.624
6:35	8:35	3.03	7.20	5.284
7:15	9:15	****	7.24	4.146
7:45	9:45	3.02	7.26	3.407
8:30	10:30	3.02	7.28	2.733
9:15	11:15	****	7.31	0.000

Table #15. Biodegradation of 15 ppm 2,6-DCP, (acclimated sludge, amino-acids LHHM, LLMC, and LAH added -20 ppm total-)
2/17/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
9:00 am	0:00	2.86	6.63	15.734
10:00	1:00	****	6.97	15.309
11:00	2:00	2.94	7.21	14.577
12 noon	3:00	****	7.41	12.554
1:00	4:00	2.98	7.53	10.426
2:00	5:00	****	7.55	7.703
2:50	5:50	2.99	7.65	6.160
3:30	6:30	****	7.66	4.386
4:15	7:15	3.00	7.72	2.628
5:15	8:15	2.99	7.74	0.00

Table #16. Biodegradation of 15 ppm 2,6-DCP, (acclimated sludge, amino-acids LHHM, LLMC, and LAH added -20 ppm total-)
2/17/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
9:00 am	0:00	2.88	7.07	14.852
10:00	1:00	****	7.21	14.399
11:00	2:00	3.04	7.34	13.780
12 noon	3:00	****	7.39	12.066
1:00 pm	4:00	3.12	7.51	9.407
2:00	5:00	****	7.53	7.579
2:50	5:50	3.15	7.58	5.428
3:30	6:30	****	7.59	3.681
4:15	7:15	3:15	7.59	1.047
5:15	8:15	3:15	7.56	0.00

Table #17. Biodegradation of 15 ppm 2,6-DCP, (acclimated sludge, amino-acids LHHM, and LAH added -20 ppm total-)
2/19/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
12 noon	0:00	2.80	6.70	15.243
12:45 pm	0:45	****	6.76	14.980
1:30	1:30	2.86	6.90	14.250
2:15	2:15	****	7.09	13.878
3:00	3:00	2.91	7.24	12.472
3:45	3:45	****	7.30	12.147
4:30	4:30	3.00	7.37	11.250
6:00	6:00	****	7.30	9.461
6:30	6:30	3.16	7.44	8.677
7:00	7:00	****	7.49	8.064
7:45	7:45	3.19	7.50	7.330
8:30	8:30	****	7.51	6.274
9:15	9:15	2.99	7.51	5.117
10:00	10:00	****	7.54	3.641
10:45	10:45	3.17	****	2.568
11:15	11:15	****	7.48	1.505

Table #18. Biodegradation of 15 ppm 2,6-DCP, (acclimated sludge, amino-acids LHHM, and LAH added -20 ppm total-)
2/19/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
12 noon	0:00	2.74	6.80	14.975
12:45 pm	0:45	****	6.94	14.695
1:30	1:30	2.80	7.06	13.931
2:15	2:15	****	7.09	13.332
3:00	3:00	2.90	7.24	12.350
3:45	3:45	****	7.30	11.458
4:30	4:30	2.92	7.37	10.826
6:00	6:00	****	7.44	8.979
6:30	6:30	2.96	7.47	8.591
7:00	7:00	****	7.50	7.766
7:45	7:45	2.96	7.52	6.993
8:30	8:30	****	7.54	5.866
9:15	9:15	2.97	7.53	4.652
10:00	10:00	****	7.55	3.427
10:45	10:45	2.96	****	2.289
11:15	11:15	****	7.56	1.419

Table #19. Biodegradation of 15 ppm 2,6-DCP, (acclimated sludge, amino-acids LHHM, and LLMC added -20 ppm total-) 2/20/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
10:30 am	0:00	2.66	6.92	14.988
11:15	0:45	****	7.16	14.067
12 noon	1:30	2.75	7.30	12.698
12:45 pm	2:15	****	7.43	11.874
1:30	3:00	2.78	7.55	10.747
2:15	3:45	****	7.60	9.617
3:00	4:30	2.88	7.63	8.870
3:45	5:15	****	7.65	7.457
5:00	6:30	2.93	7.70	5.369
5:45	7:15	****	7.71	4.231
6:30	8:00	2.98	7.73	2.883
7:15	8:45	****	7.73	1.799
8:00	9:30	3.00	7.72	0.795
8:45	10:15	****	7.72	0.397
9:30	11:00	3.01	7.72	0.105
10:15	11:45	****	****	0.000

Table #20. Biodegradation of 15 ppm 2,6-DCP, (acclimated sludge, amino-acids LHHM, and LLMC added -20 ppm total-) 2/20/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
10:30 am	0:00	2.70	7.14	15.048
11:15	0:45	****	7.30	14.153
12 noon	1:30	2.76	7.48	12.582
12:45	2:15	****	7.57	11.560
1:30 pm	3:00	2.80	7.65	10.350
2:15	3:45	****	7.69	9.529
3:00	4:30	2.87	7.72	8.696
3:45	5:15	****	7.78	7.318
5:00	6:30	2.94	7.84	5.506
5:45	7:15	****	7.83	4.228
6:30	8:00	2.96	7.80	2.899
7:15	8:45	****	7.80	1.786
8:00	9:30	2.97	7.79	0.760
8:45	10:15	****	7.80	0.356
9:30	11:00	2.97	7.81	0.095
10:15	11:45	****	****	0.000

Table #21. Biodegradation of 15 ppm 2,6-DCP, (acclimated sludge, amino-acids LGA, and LCN added -20 ppm total-) 2/21/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
11:00 am	0:00	2.75	6.72	15.284
11:30	0:30	****	6.42	12.378
12 noon	1:00	2.83	6.49	12.182
12:45 pm	1:45	****	6.53	11.084
1:30	2:30	2.92	6.42	9.718
2:15	3:15	****	6.32	8.157
3:00	4:00	3.00	6.27	7.196
3:45	4:45	****	6.17	5.660
4:30	5:30	3.11	5.95	****
5:55	6:55	****	5.79	2.442
6:30	7:30	3.09	5.72	1.722
7:15	8:15	****	5.62	0.181
8:00	9:00	3.08	5.50	0.000
8:45	9:45	****	5.45	****
9:30	10:30	****	5.42	****

Table #22. Biodegradation of 15 ppm 2,6-DCP, (acclimated sludge, amino-acids LGA, and LCN added -20 ppm total-)
2/21/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
11:00 am	0:00	2.80	6.82	15.008
11:30	0:30	****	6.69	12.517
12 noon	1:00	2.85	6.71	11.941
12:45 pm	1:45	****	6.59	11.239
1:30	2:30	2.97	6.56	9.346
2:15	3:15	****	6.47	7.940
3:00	4:00	3.03	6.37	6.727
3:45	4:45	****	6.32	5.402
4:30	5:30	3.05	6.18	****
5:55	6:55	****	6.12	2.233
6:30	7:30	3.05	6.08	1.366
7:15	8:15	****	6.04	0.109
8:00	9:00	3.02	6.00	0.000
8:45	9:45	****	5.98	****
9:30	10:30	****	5.96	****

Table #23. Biodegradation of 10 ppm 2,6-DCP,
(unacclimated sludge, no amino-acids added)
2/23/83 2/24/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
7:30 am	00:00	2.59	7.15	9.994
8:15	00:45	****	7.16	9.788
9:00	01:30	****	7.12	9.187
9:45	02:15	****	7.08	8.853
10:30	03:00	2.61	7.09	8.595
11:15	03:45	****	7.06	8.289
12 noon	04:30	****	6.96	8.120
1:00 pm	05:30	****	6.91	7.993
2:00	06:30	2.66	6.88	7.828
3:00	07:30	****	6.84	7.655
3:45	08:15	****	6.78	7.599
4:30	09:00	****	6.79	7.426
5:30	10:00	2.73	6.77	7.321
6:15	10:45	****	6.74	7.177
7:50	12:20	****	6.71	7.088
8:45	13:15	****	6.69	6.995
9:45	14:15	2.75	6.72	6.902
10:30 pm	15:00	****	6.69	6.859
8:45 am	25:15	2.81	6.47	4.989

10:30	27:00	****	6.44	4.257
12 noon	28:30	2.77	6.43	3.873
2:00 pm	30:30	****	6.45	2.362
3:30	32:00	****	6.37	1.535
4:15	32:45	2.73	6.35	1.186
5:45	34:15	****	6.33	0.925
7:30	36:00	****	6.32	0.323
8:30	37:00	2.72	****	0.000 2nd peak

Table #24. Biodegradation of 10 ppm 2,6-DCP,
(acclimated sludge, all amino-acids added -20 ppm total-)
2/24/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
7:30 am	00:00	2.76	6.81	9.902
8:00	00:30	****	6.88	9.903
8:30	01:00	2.79	7.01	9.469
9:00	01:30	****	7.04	9.251
9:30	2:00	****	7.13	8.925
10:30	03:00	2.92	7.12	7.594
11:00	3:30	****	7.16	7.198
12 noon	04:30	****	7.18	5.228
01:00 pm	05:30	2.92	7.19	2.274
02:00	06:30	****	7.19	0.836
3:00	7:30	****	7.16	0.000
4:30	9:00	****	****	1.326 (2nd peak)

Table #25. Biodesradation of 10 ppm 2,6-DCP,
(acclimated sludge, all amino-acids added -20 ppm total-)
2/23/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
7:30 am	0:00	2.81	6.91	10.093
8:00	0:30	****	7.05	09.955
8:30	1:00	2.88	7.18	9.600
9:00	1:30	****	7.11	10.278
9:30	2:00	****	7.20	8.929
10:30	3:00	2.93	7.27	7.799
11:00	3:30	****	7.28	6.902
12 noon	4:30	****	7.30	5.327
01:00 pm	5:30	2.95	7.31	2.841
02:00	6:30	****	7.32	0.884
3:10	7:40	****	7.35	0.000

Table #26, Biodegradation of 20 ppm 2,6-DCP,
(acclimated sludge all amino-acids added -20 ppm total-)
2/25/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
11:30 am	0:00	2.89	6.77	20.489
01:00 pm	1:30	****	7.07	19.606
02:00 pm	2:30	3.01	7.27	17.972
2:45	3:15	****	7.39	17.747
3:30	4:00	3.08	7.46	16.899
4:15	4:45	****	7.42	16.413
5:45	6:15	3.17	7.59	13.934
6:15	6:45	****	7.64	13.390
7:30	8:00	3.23	7.65	10.755
8:30	9:00	****	7.69	9.546
9:15	9:45	3.23	7.70	8.438
10:00	10:30	****	7.70	6.158
10:45	11:15	3.22	7.65	3.215
11:15	11:45	****	7.69	2.111
12 midnite	12:30	3.21	7.68	0.181
2nd peak				

Table #27. Biodegradation of 20 ppm 2,6-DCP,
(acclimated sludge, all amino-acids added -20 ppm total-)
2/25/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
11:30 am	0:00	2.90	7.01	19.969
01:00 pm	1:30	****	7.20	19.468
2:00	2:30	2.95	7.40	17.962
2:45	3:15	****	7.44	17.334
3:30	4:00	3.02	7.50	16.759
4:15	4:45	****	7.53	14.664
5:45	6:15	3.10	7.57	12.641
6:15	6:45	****	7.62	11.960
7:30	8:00	3.13	7.61	10.483
8:30	9:00	****	7.63	8.563
9:15	9:45	3.13	7.64	7.520
10:00	10:30	****	7.65	5.683
10:45	11:15	3.15	7.66	2.925
11:15	11:45	****	7.67	1.803
12 midnite	12:30	3.15	7.68	00.169
2nd peak				

Table #28. Biodegradation of 25 ppm 2,6-DCP,
(acclimated sludge, all amino-acids added -20 ppm total-)
2/27/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
11:30 AM	0:00	2.74	6.35	26.244
12:15 pm	0:45	****	6.79	25.792
1:00	1:30	2.75	7.06	23.696
1:45	2:15	****	7.20	23.890
2:30	3:00	2.74	7.22	23.065
3:15	3:45	****	7.23	25.984
4:00	4:30	2.77	7.27	21.570
5:15	5:45	****	7.30	20.507
6:00	6:30	2.78	7.33	20.014
6:45	7:15	****	7.35	19.671
7:30	8:00	2.78	7.36	18.686
8:15	8:45	****	7.39	17.912
9:00	9:30	2.81	7.39	17.087
10:15	10:45	****	7.38	15.604
11:00	11:30	2.83	7.43	14.653
11:45	12:15	****	7.43	14.100

Table #29. Biodegradation of 250 ppm phenol,
(acclimated sludge, no amino-acids added)
1/20/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
3:30 pm	0:00	2.63	6.49	250.080
3:45	0:15	****	6.45	230.640
4:00	0:30	2.69	6.24	185.700
4:15	0:45	****	6.04	142.500
4:30	1:00	3.02	5.76	102.660
4:45	1:15	****	5.72	023.429
5:00	1:30	2.81	5.69	000.000

Table #30. Biodegradation of 250 ppm phenol,
(acclimated sludge, no amino-acids added)
1/26/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
6:30 pm	0:00	2.53	7.42	248.650
6:45	0:15	****	7.36	228.490
7:00	0:30	2.61	7.18	185.140
7:15	0:45	****	6.95	138.570
7:30	01:00	2.89	6.84	091.897
7:45	01:15	****	6.80	006.451
8:00	01:30	2.71	6.72	000.000

Table #31. Biodegradation of 400 ppm Phenol,
(acclimated sludge, no amino-acids added)
1/20/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
9:30 pm	0:00	2.52	7.09	397.300
9:45	0:15	****	****	384.000
10:00	0:30	****	7.07	346.790
10:15	0:45	2.64	****	311.990
10:30	1:00	****	7.01	288.490
10:45	1:15	****	****	255.470
11:00	01:30	2.96	6.95	154.980
11:15	01:45	****	****	103.500
11:30	2:00	****	6.79	031.643
11:45	2:15	2.91	****	000.000
12 midnite	2:30	****	6.64	****

Table #32. Biodegradation of 400 ppm phenol,
(acclimated sludge, no amino-acids added)
1/21/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
6:45 pm	0:00	2.50	6.94	391.97
7:00	0:15	****	****	383.280
7.15	0:30	****	6.87	346.590
7:30	0:45	2.63	****	314.670
7:45	1:00	****	6.71	260.800
8:00	1:15	****	****	205.570
8:15	1:30	3.02	6.54	144.330
8:30	1:45	****	****	059.023
8:45	2:00	****	6.36	000.000
9:00	2:15	2.96	6.30	****

Table #33. Biodegradation of 600 ppm phenol,
(acclimated sludge, no amino-acids added)
1/23/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
4:30 pm	0:00	2.38	7.51	606.420
4:45	0:15	****	****	603.410
5:00	0:30	****	7.52	591.79
5:15	0:45	****	****	582.290
5:30	01:00	2.48	7.52	574.240
5:45	01:15	****	****	566.970
6:00	01:30	****	7.45	548.260
6:15	01:45	****	****	525.500
6:30	02:00	2.51	7.38	507.750
6:45	02:15	****	****	483.430
7:00	02:30	****	7.29	462.590
7:15	02:45	****	****	461.730
7:30	03:00	2.54	7.23	432.91
7:45	03:15	****	****	401.060
8:00	03:30	****	7.11	393.930
8:15	03:45	****	****	366.860
8:30	04:00	2.54	7.08	353.330
8:45	04:15	****	****	321.210
9:00	04:30	****	7.04	296.760

9:15	04:45	****	****	269.190
9:30	05:00	2.50	7.02	235.000
10:00	05:30	****	****	160.410
10:15	05:45	****	6.98	****
10:30	06:00	2.40	****	75.963
11:00	06:30	****	6.99	****

Table #34. Biodegradation of 600 ppm phenol, (acclimated sludge, no amino-acids added) 1/24/83

TIME	TIME FROM START	MLSS	pH	CONCN
HOURS	HOURS	GM/LITER	****	PPM
3:45 pm	0:00	2.37	7.69	619.030
4:00	0:15	****	****	610.530
4:15	0:30	****	7.67	605.670
4:30	0:45	****	****	592.530
4:45	01:00	2.43	7.63	582.170
5:00	01:15	****	****	553.420
5:15	01:30	****	7.57	544.880
5:30	01:45	****	****	524.640
5:45	02:00	2.47	7.50	506.670
6:00	02:15	****	****	481.680
6:15	02:30	****	7.48	474.920
6:30	02:45	****	****	451.350
6:45	03:00	2.50	7.29	434.250
7:00	03:15	****	****	406.360
7:15	03:30	****	7.17	392.330
7:30	03:45	****	****	372.600
7:45	04:00	2.49	7.07	358.810
8:00	04:15	****	****	323.210
8:15	04:30	****	7.10	288.360

8:30	04:45	****	****	261.000
8:45	05:00	2.46	7.01	222.870
9:00	05:15	****	****	180.750
9:15	05:30	****	7.00	151.650
9:30	05:45	****	****	087.167
9:45	06:00	2.39	6.99	005.340
10:00	06:15	****	****	000.000

Table #35. COD determination experiments for 15 ppm 2,6-DCP (acclimated sludge, no amino-acids added)
9/10/83

Sample size = 3 ml
Normality of FAS = .025 N
Blank reading = 11.00 ml

TIME	READING	COD (EXPTL)
HOUR	ML	MG/L
9:15 am	10.70	20.00
10:45	10.70	20.00
1:00 pm	10.75	16.67
2:30 pm	10.67	22.00
4:00	10.80	13.33
7:30	10.90	****
9:00	10.75	16.67
10:45	10.90	****
8:15 am	10.90	****
10:30 am	11.00	****
12:30 pm	10.95	****

"****" indicates that COD values were not experimentally determinable.

FIGURES

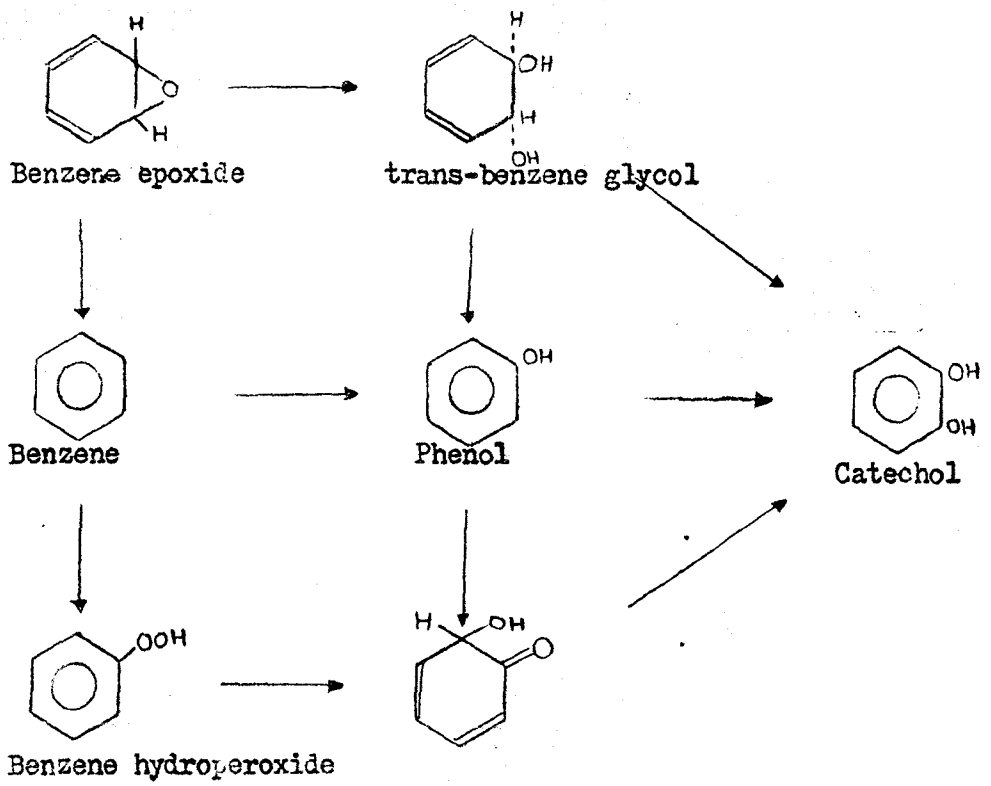


Figure #1 Pathways for degradation of benzene to catechol

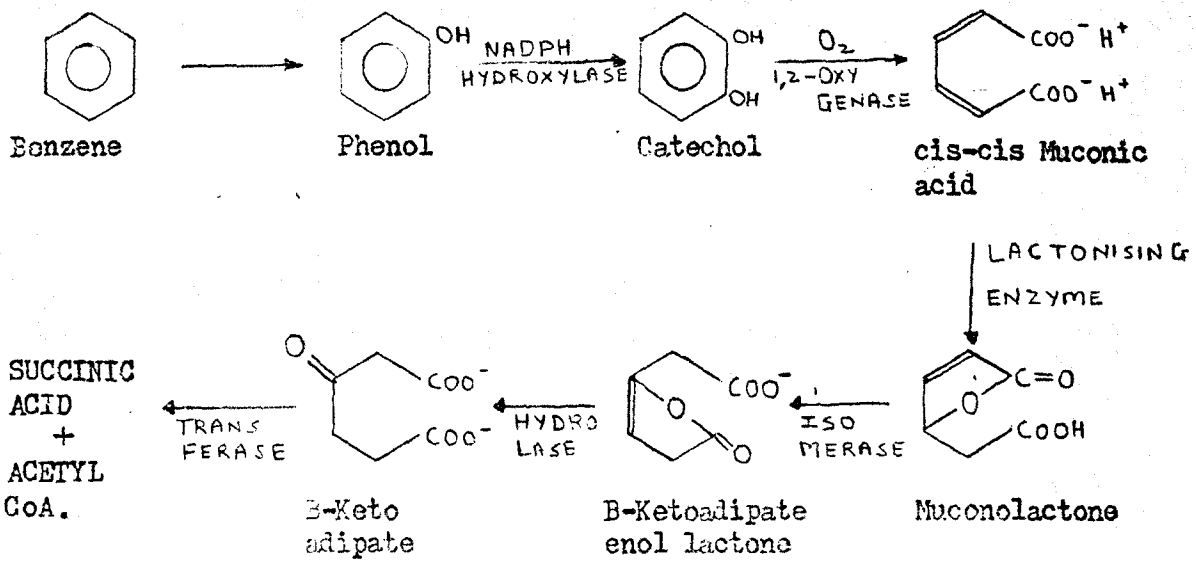


Figure #2 Pathway for ortho fission of catechol

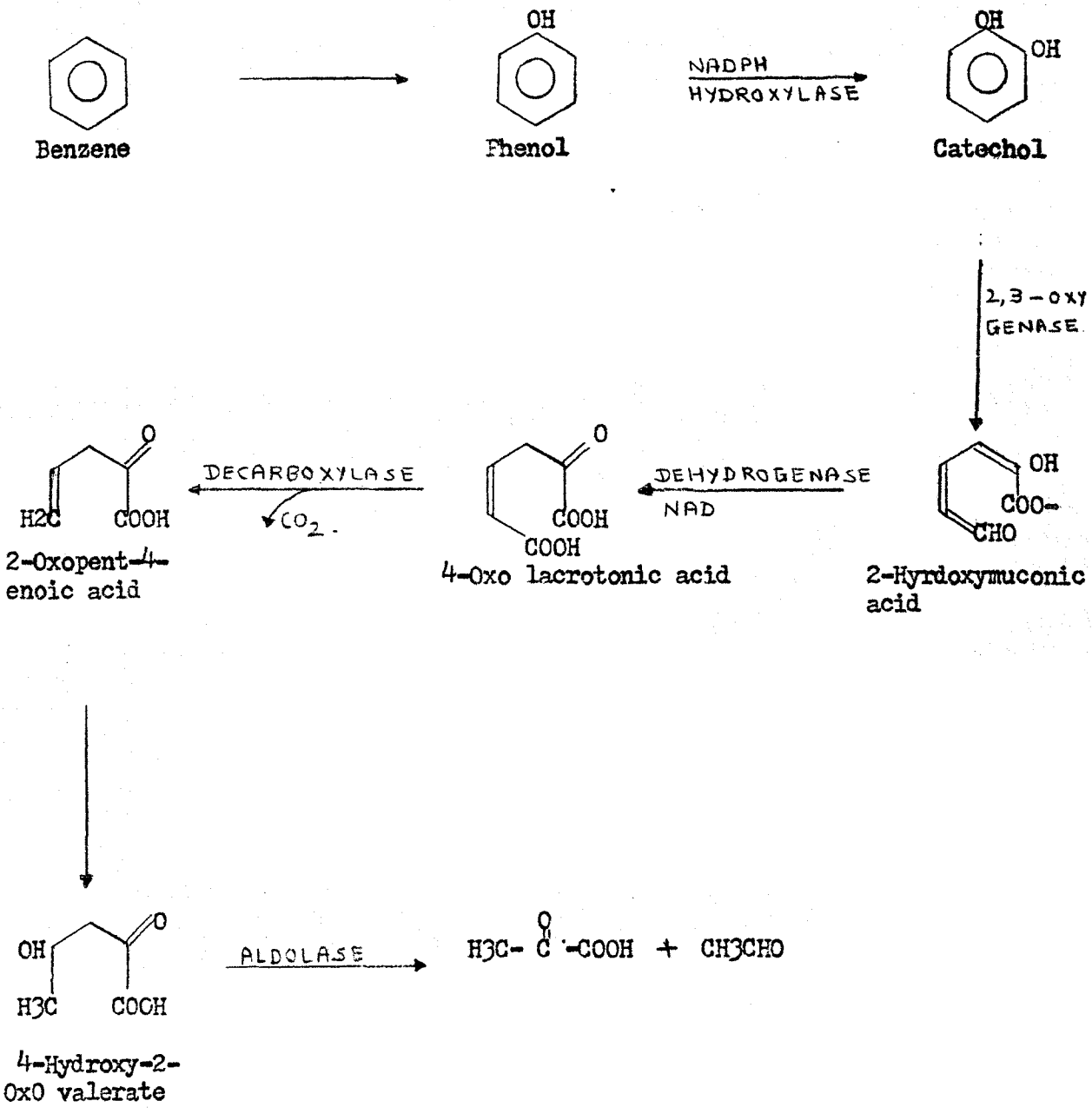
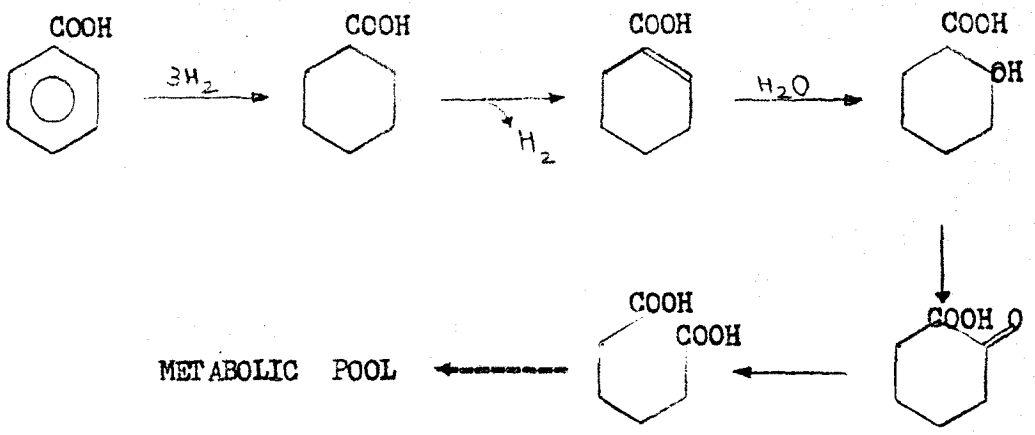
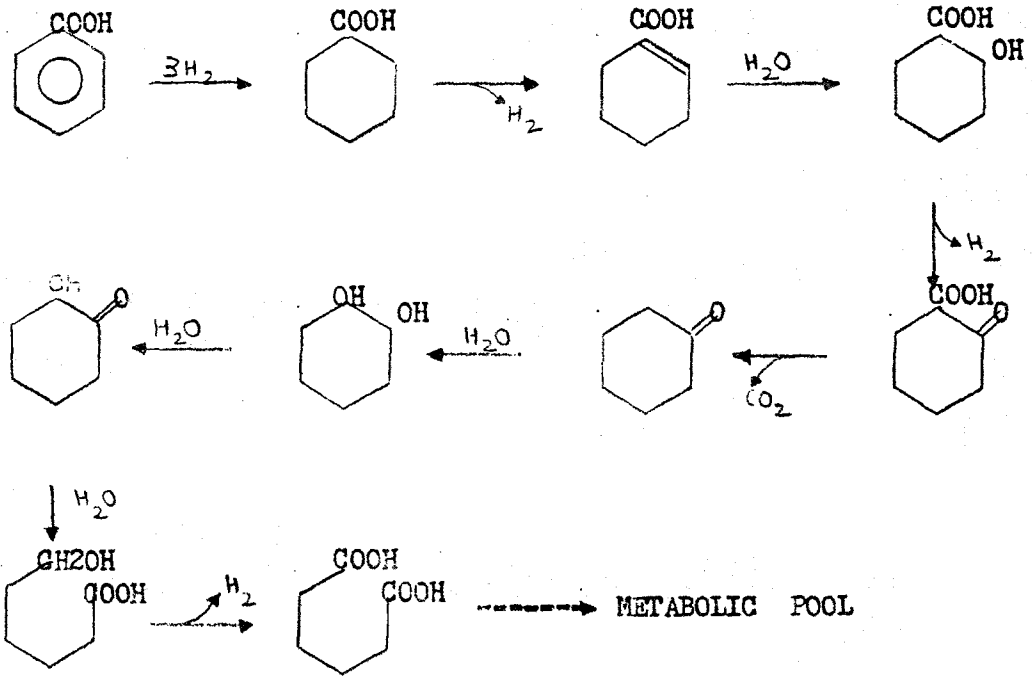


Figure #3 Pathway for meta fission of catechol

PHOTOMETABOLISM:



NITRATE RESPIRATION (BENZOIC ACID):



NITRATE RESPIRATION (PHENOL):

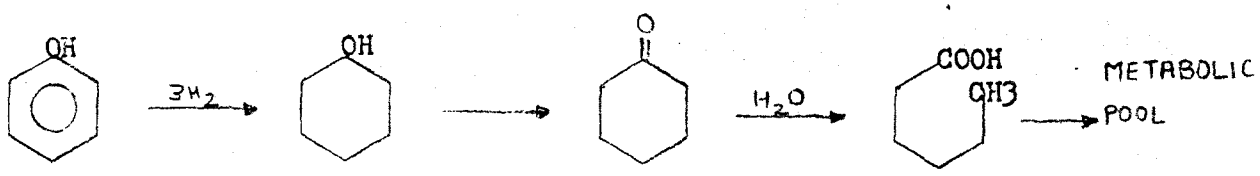


Figure #4 Pathways for anaerobic metabolism of aromatics --CONTINUED--

METHANOGENIC FERMENTATION:

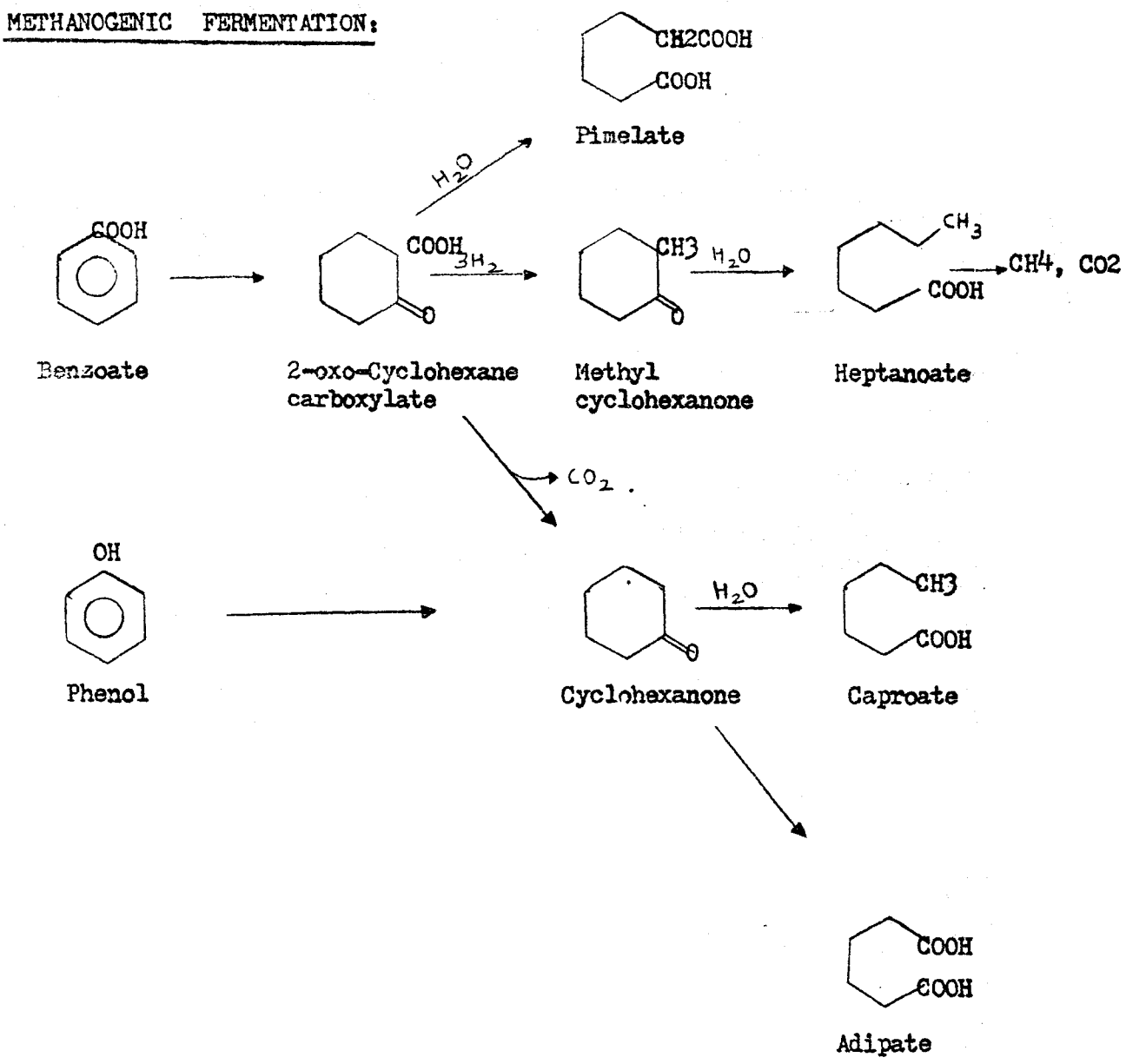


Figure #4 Pathways for anaerobic metabolism of aromatics

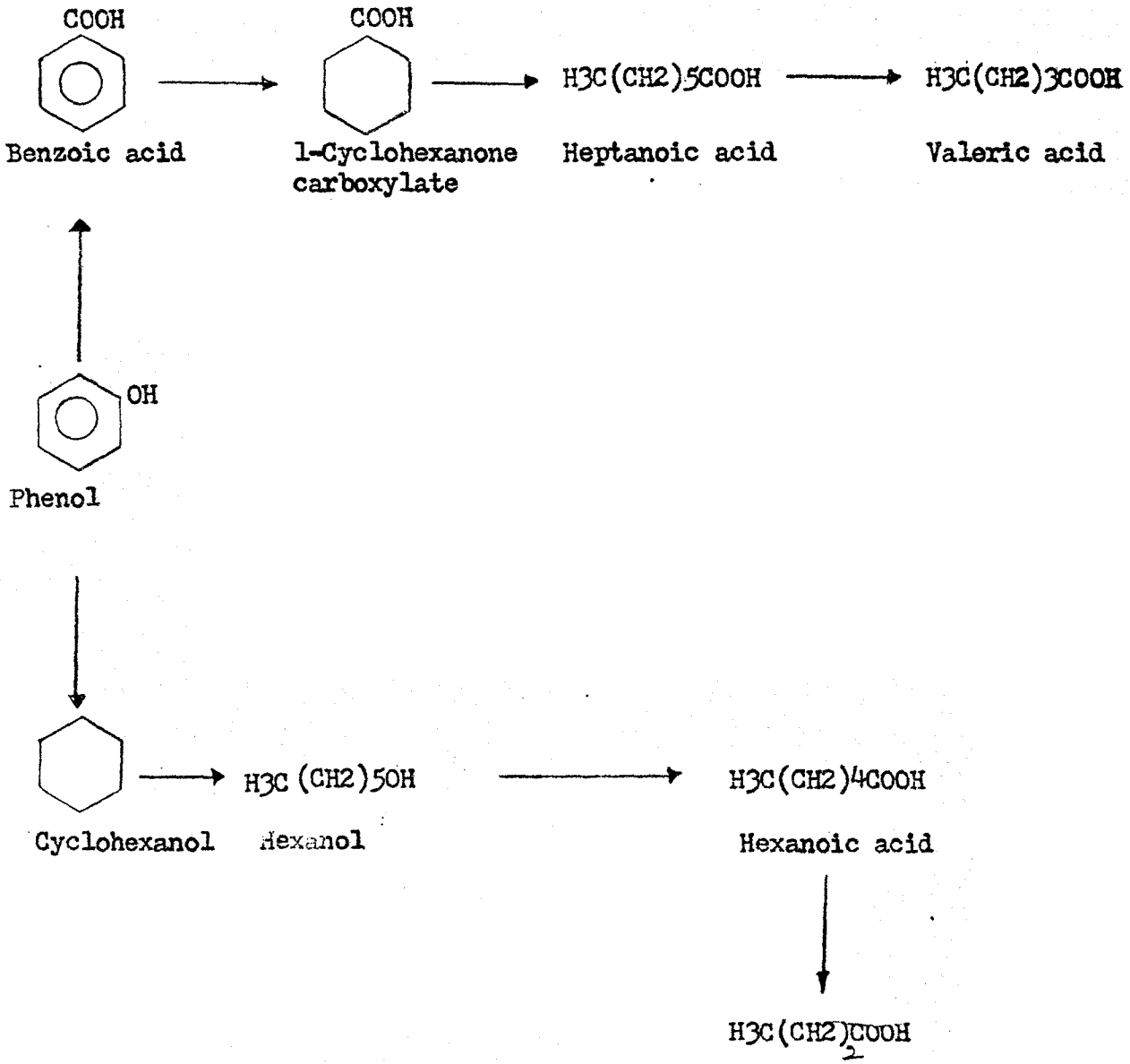


Figure #5 Pathway for anaerobic metabolism of phenol

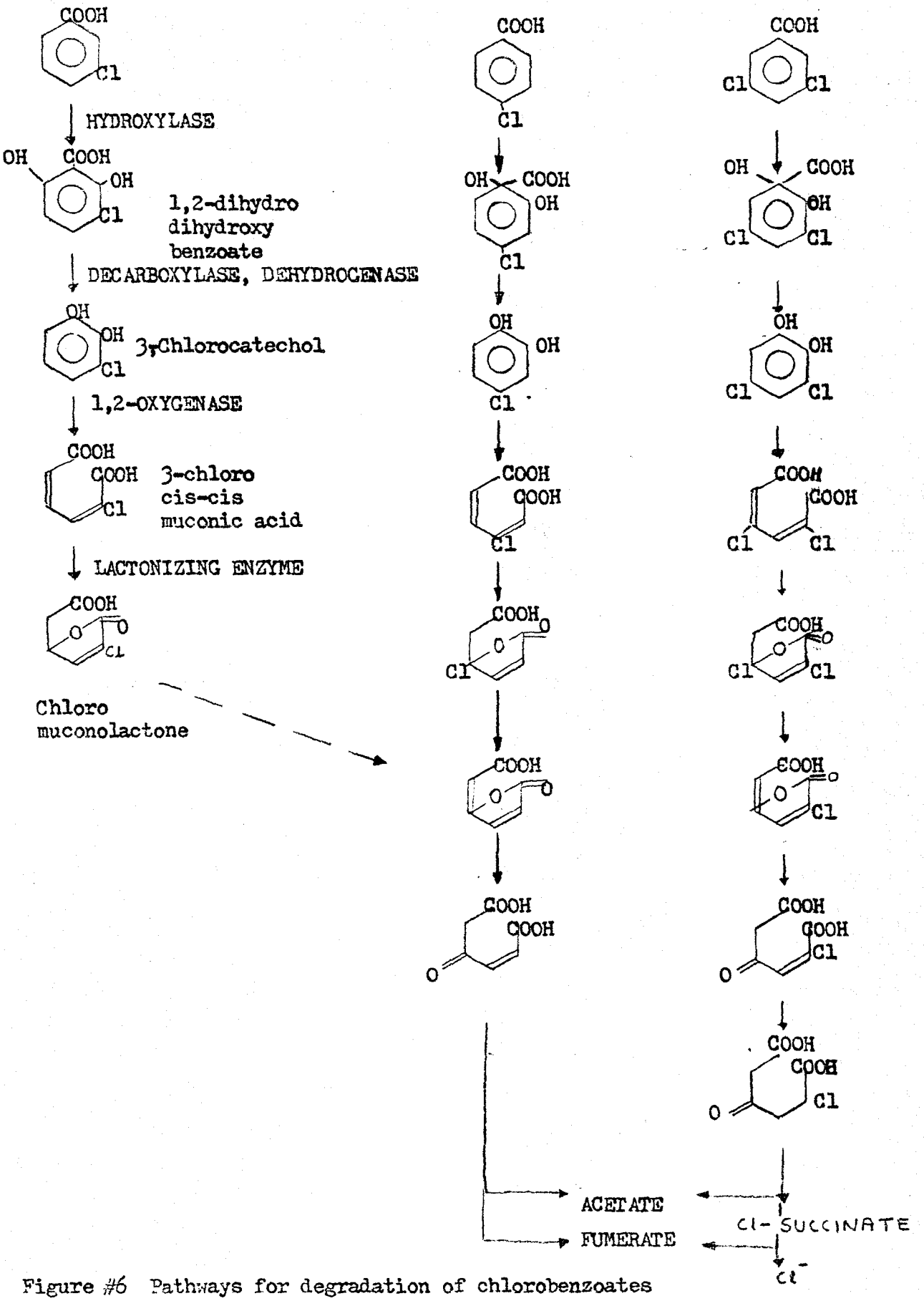


Figure #6 Pathways for degradation of chlorobenzoates

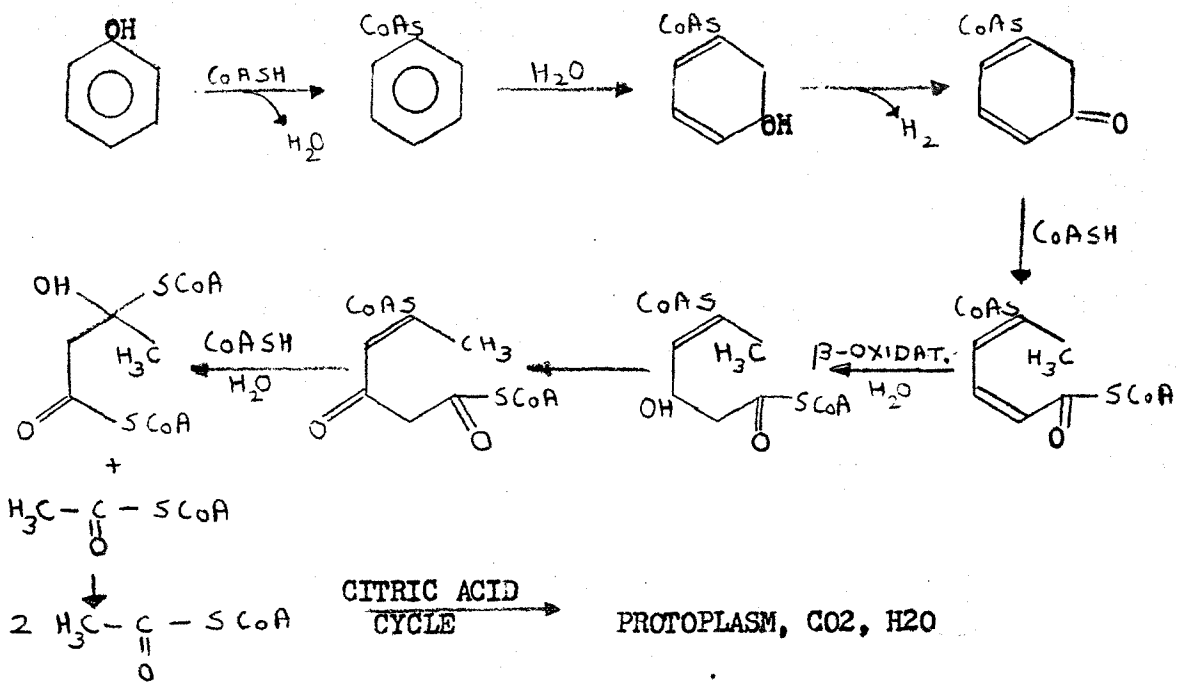


Figure #7 Pathway for anaerobic metabolism of phenol via B-oxidation

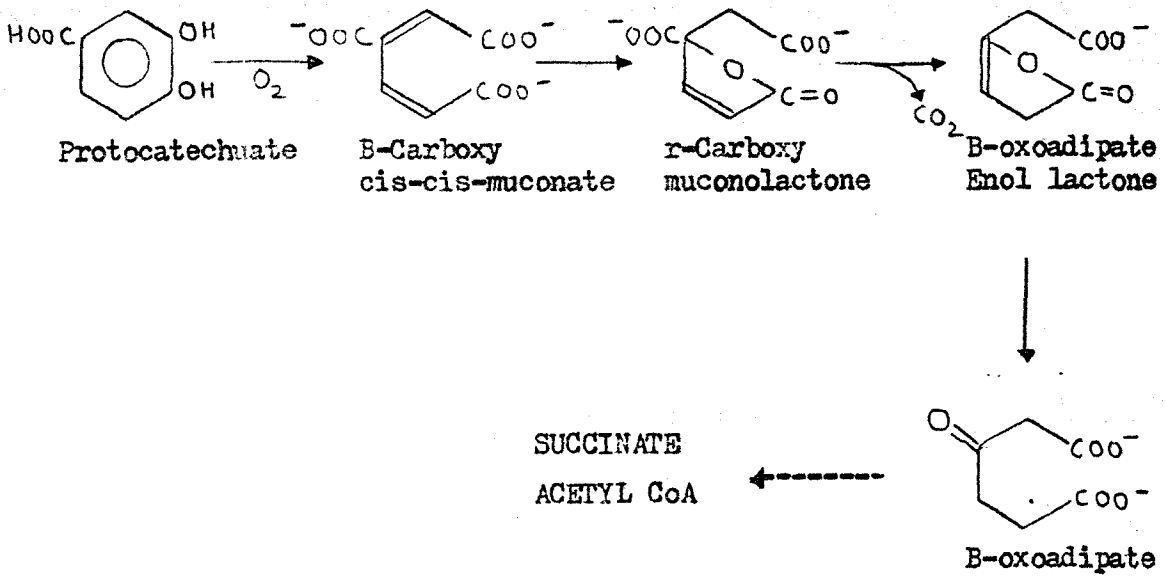


Figure #8 Pathway for degradation of protocatechuate

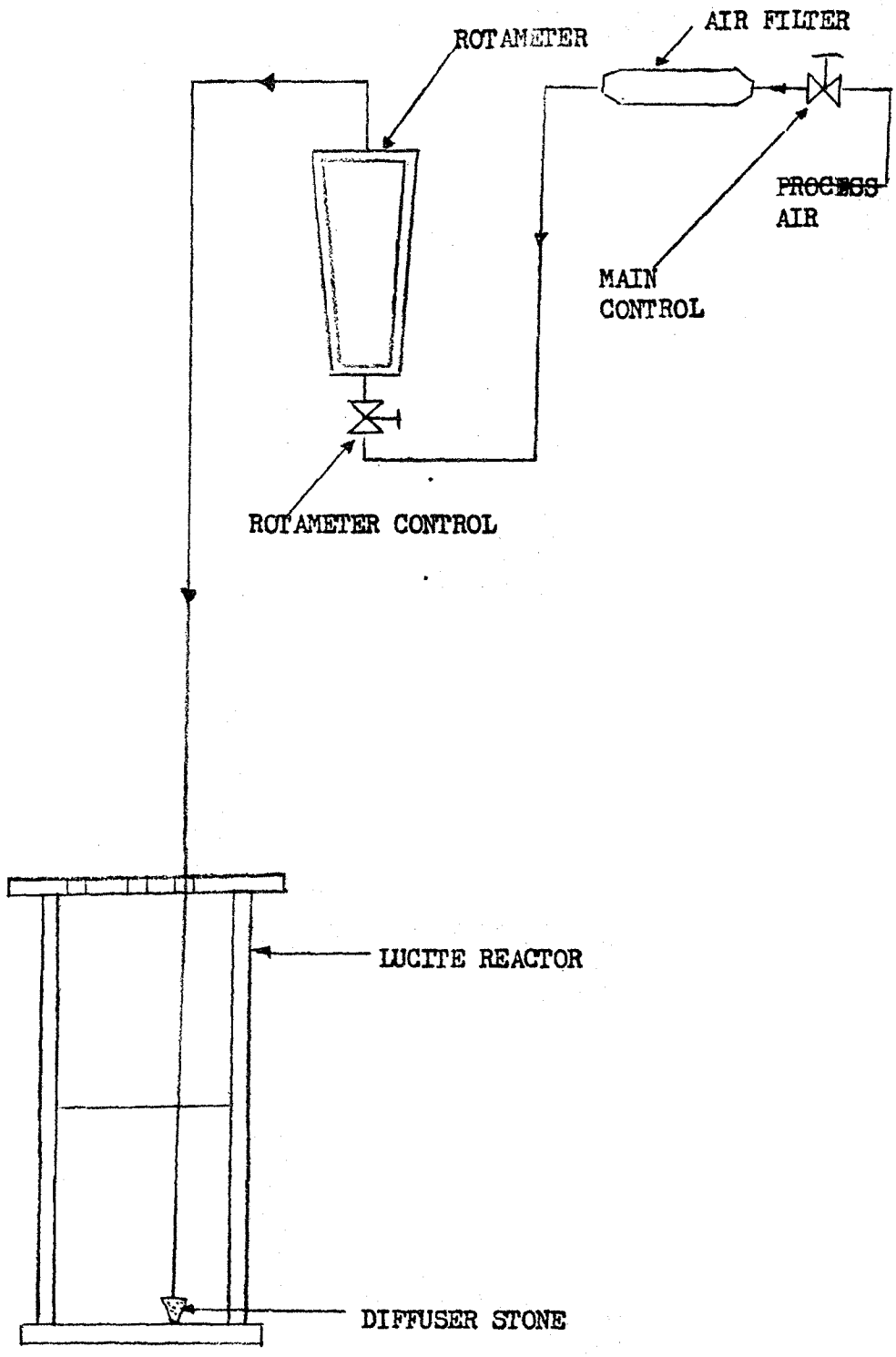


Figure #9 Typical reactor set-up

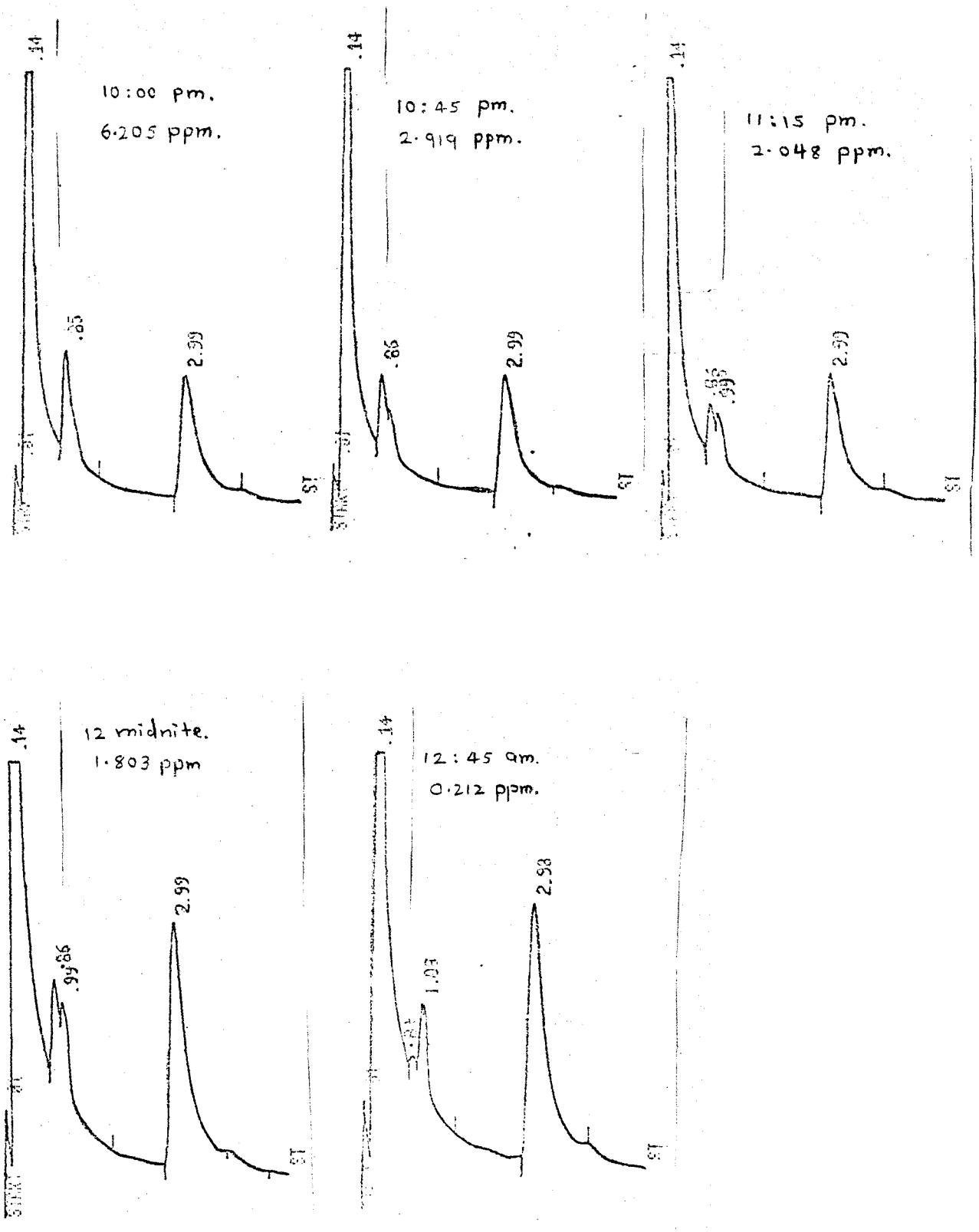


Figure #10 GC output showing overlapping of peaks for 2,6-DCP and its metabolic intermediate (Table #24, 2/25/83).

Figure #11. Plots of concentration vs time for 250 ppm phenol

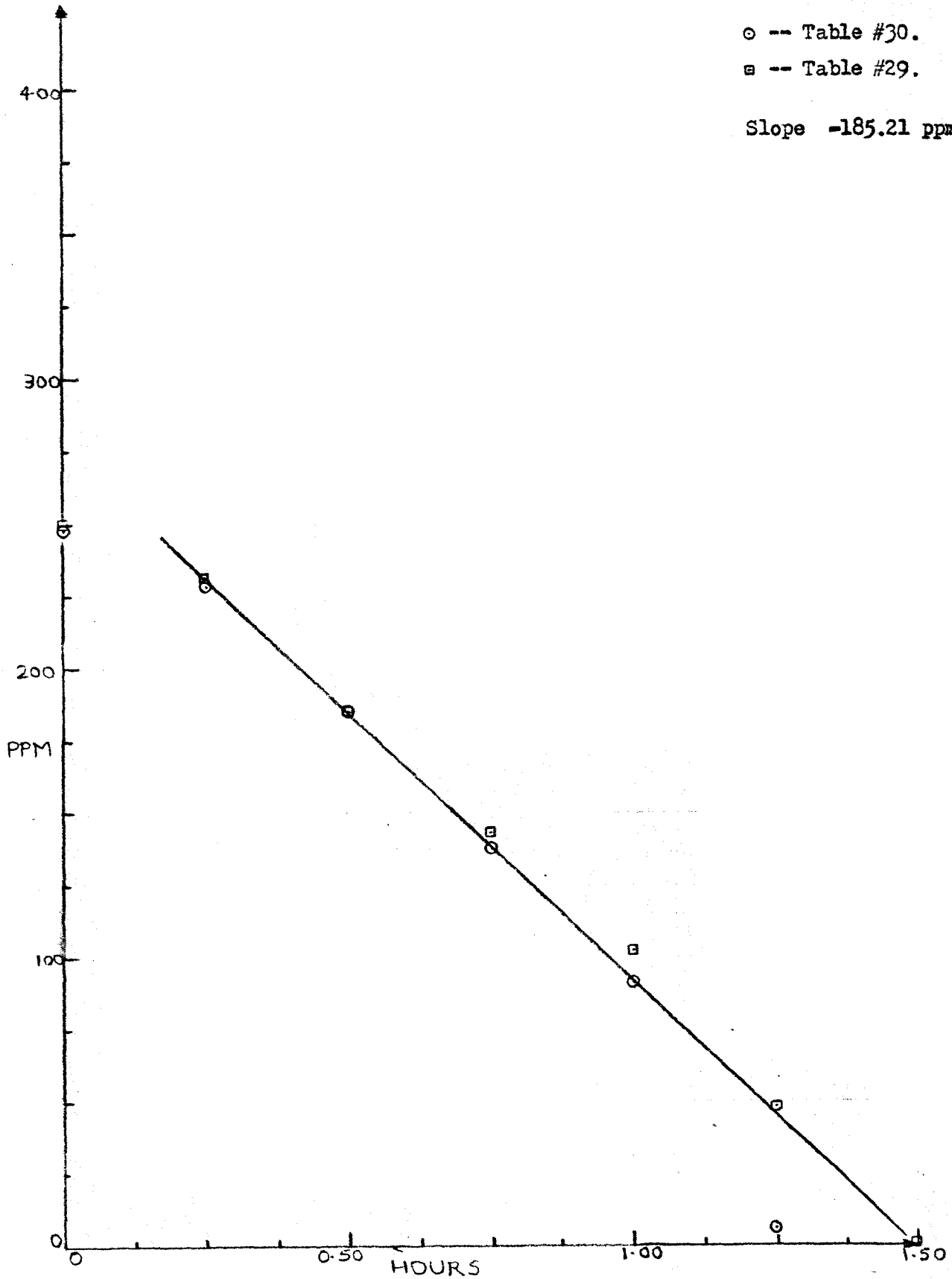


Figure #12. Plots of concentration vs time for 400 ppm phenol

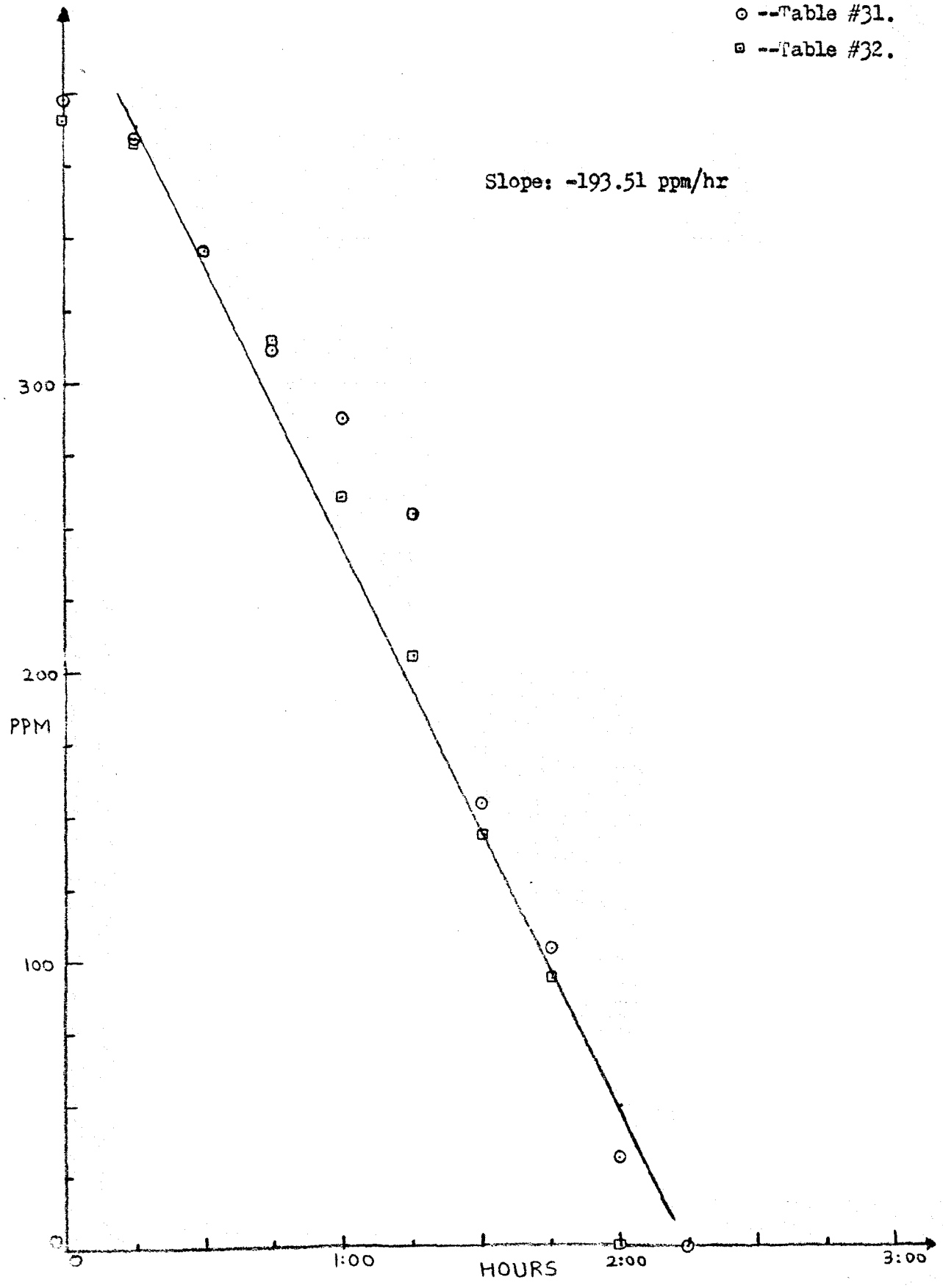


Figure #13. Plots of concentration vs time for 600 ppm phenol

⊙ -- Table #33.

⊡ -- Table #34.

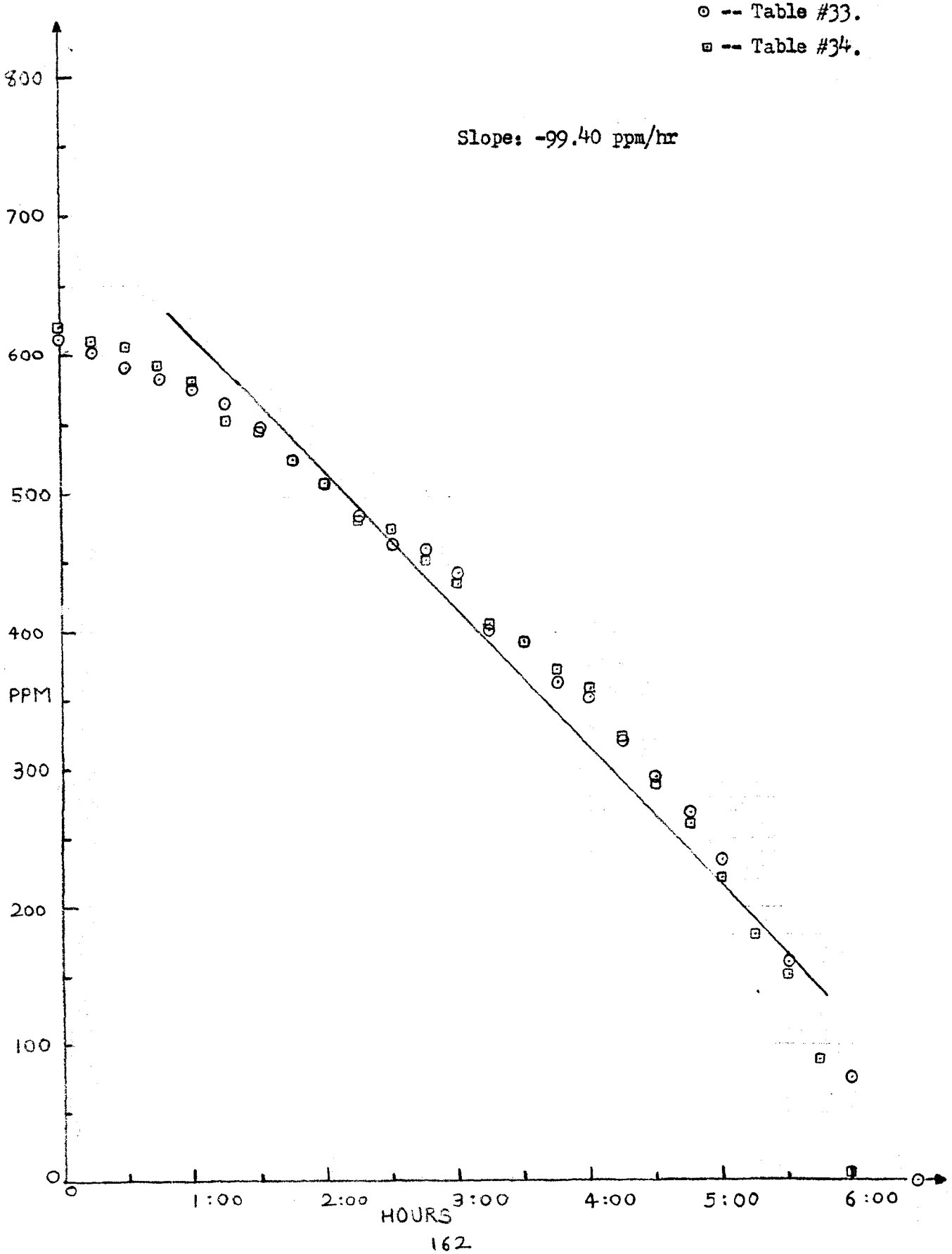


Figure #14. Plot of pH vs time for 250 ppm phenol

⊙ -- Table #29.

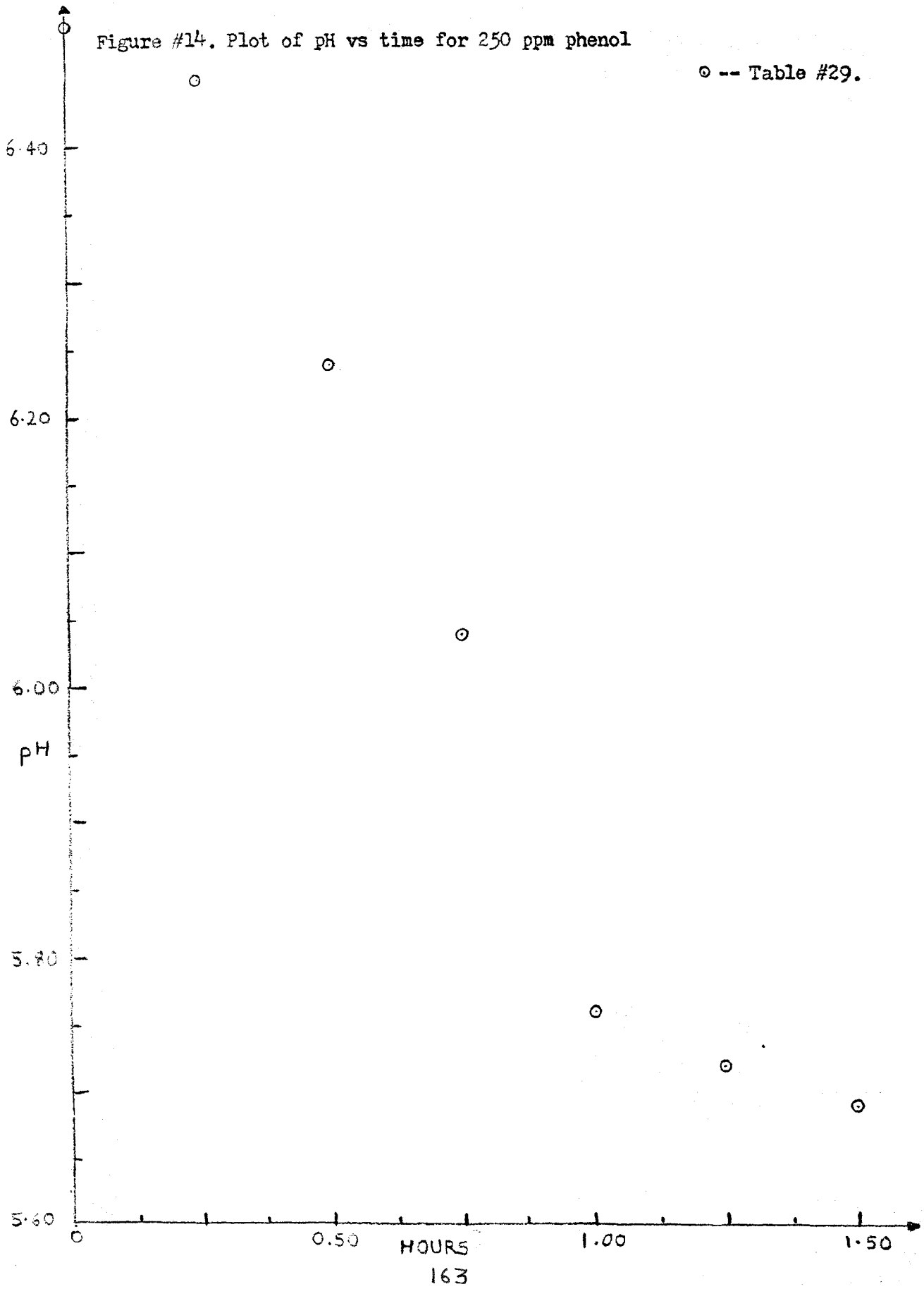


Figure #15. Plot of pH vs time for 250 ppm phenol

⊙ -- Table #30.

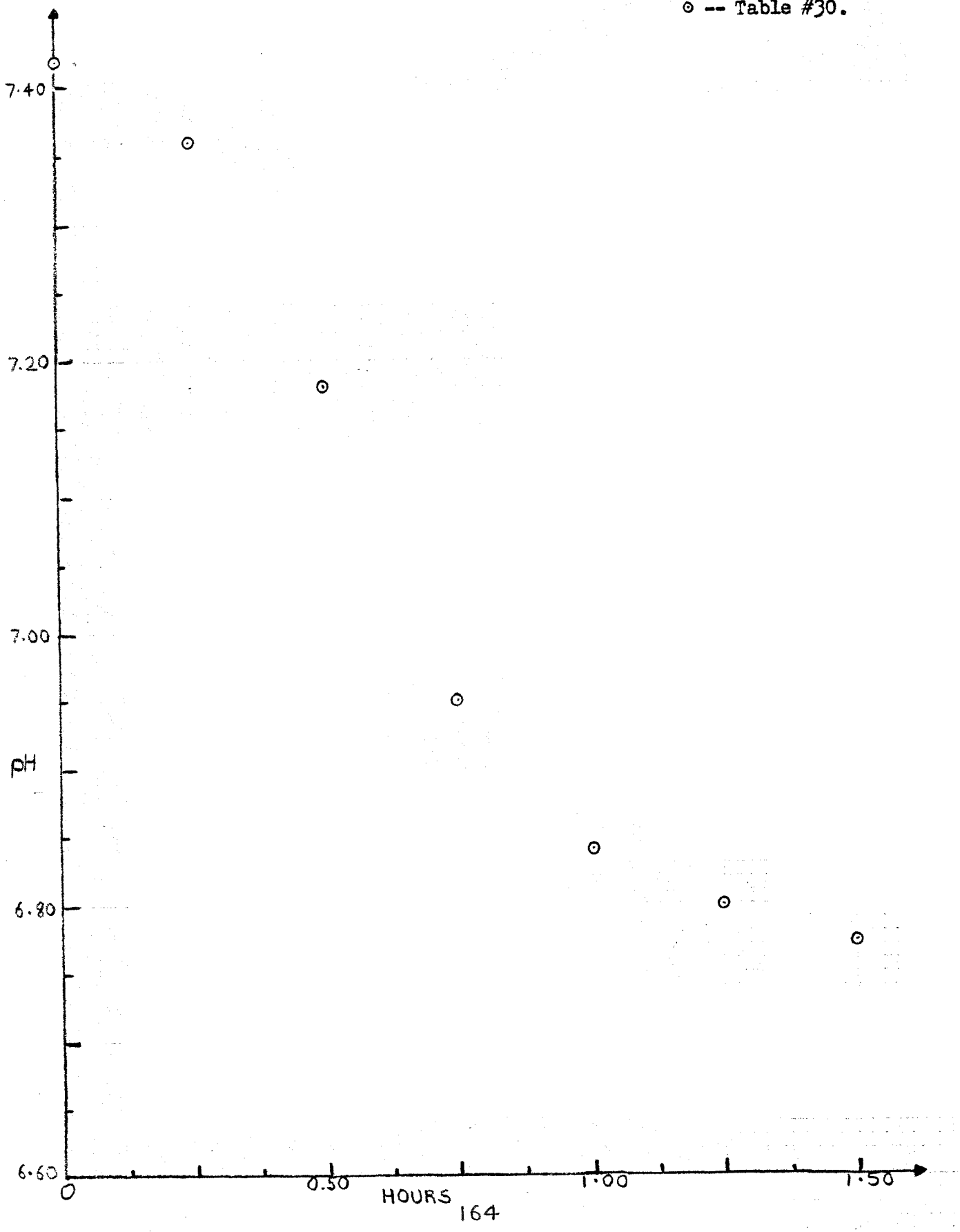


Figure #16. Plots of pH vs time for 400 ppm phenol

⊙ -- Table #31.

⊡ -- Table #32.

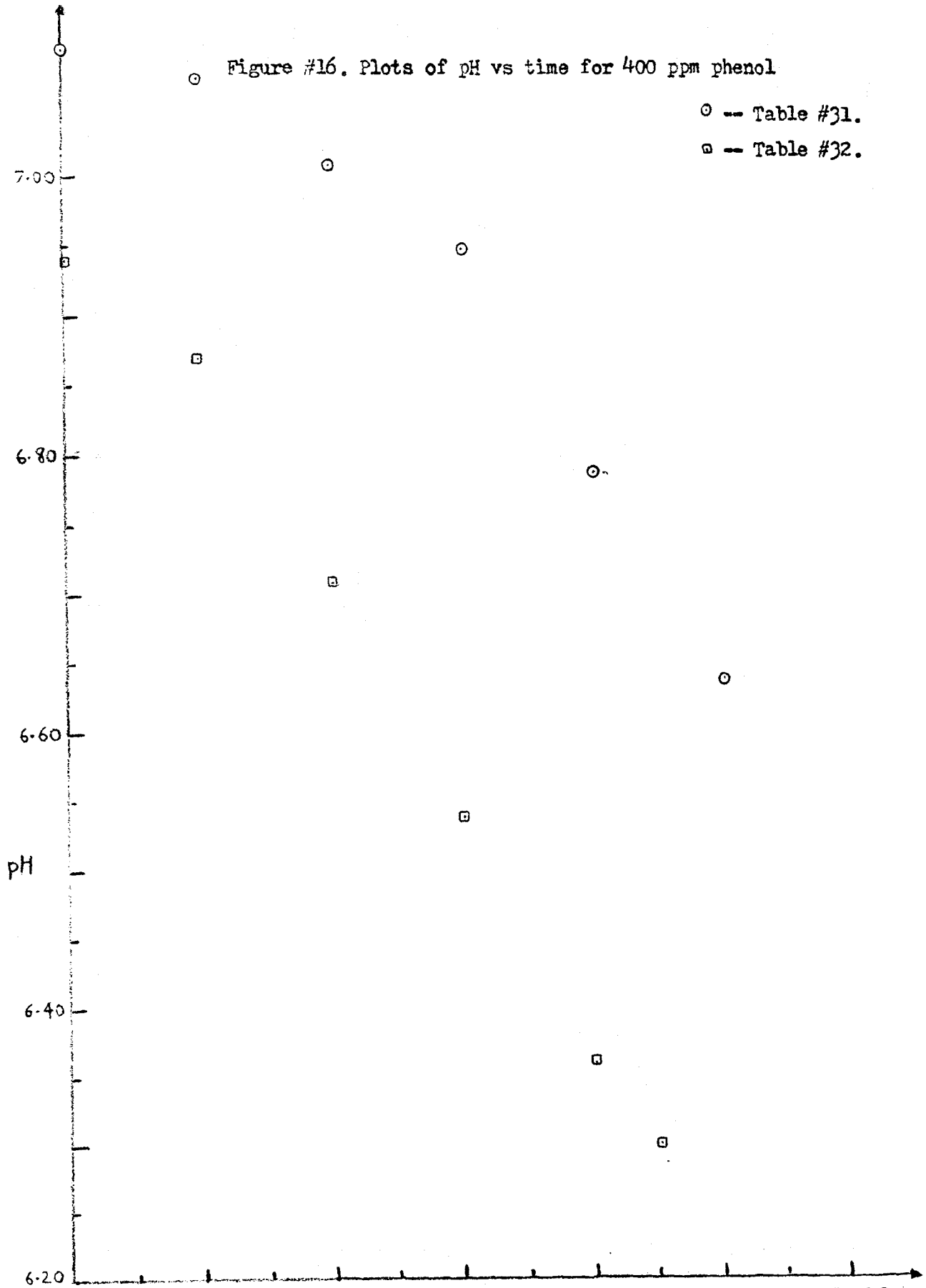


Figure #17. Plots of pH vs time for 600 ppm phenol

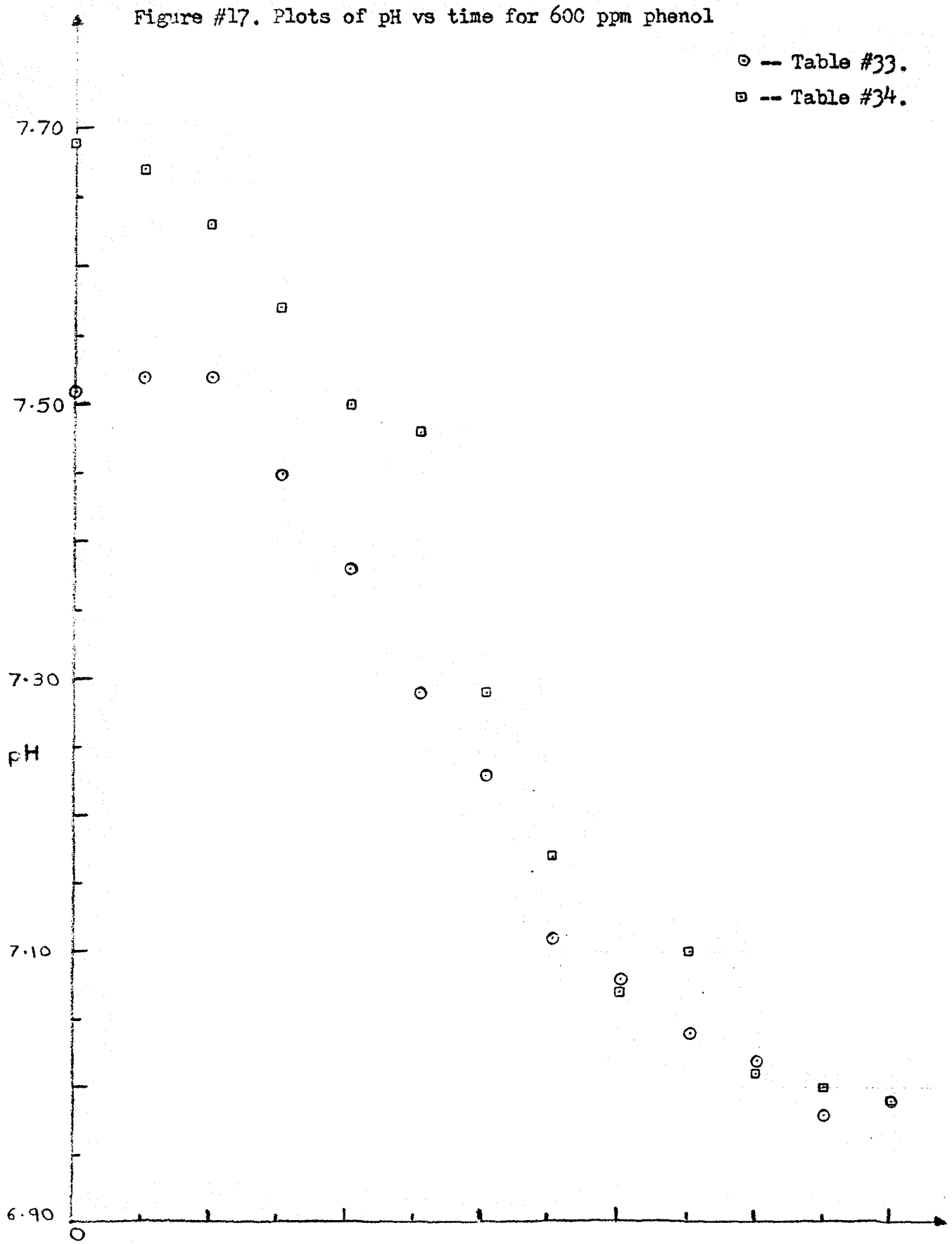


Figure #18. Plot of concentration vs time 10 ppm 2,6-DCP,
(no amino-acid added, unacclimated sludge)

⊙ -- Table #23.

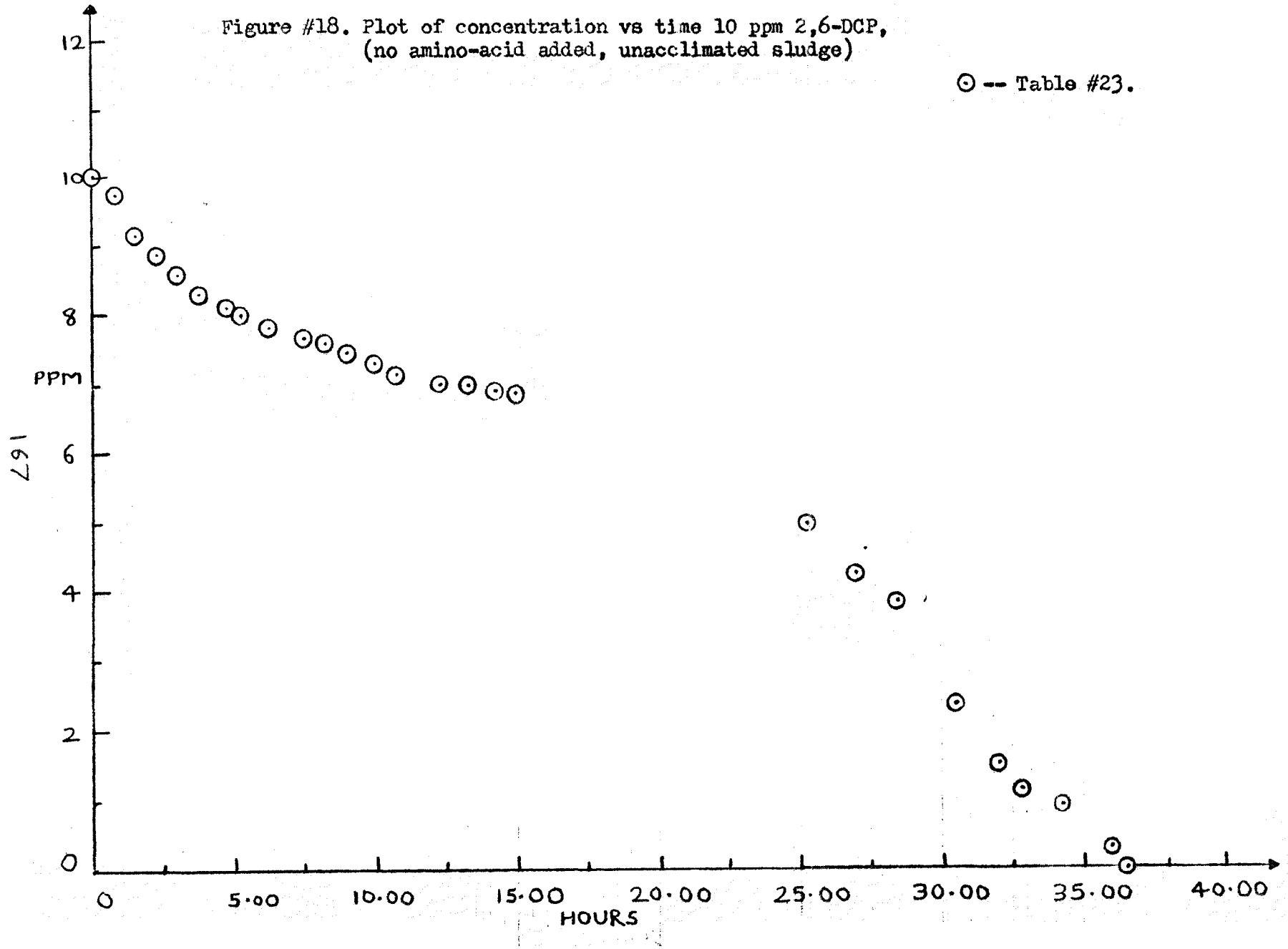


Figure #19. Plots of concentration vs time for 15 ppm 2,6-DCP, (no amino-acids added, acclimated sludge)

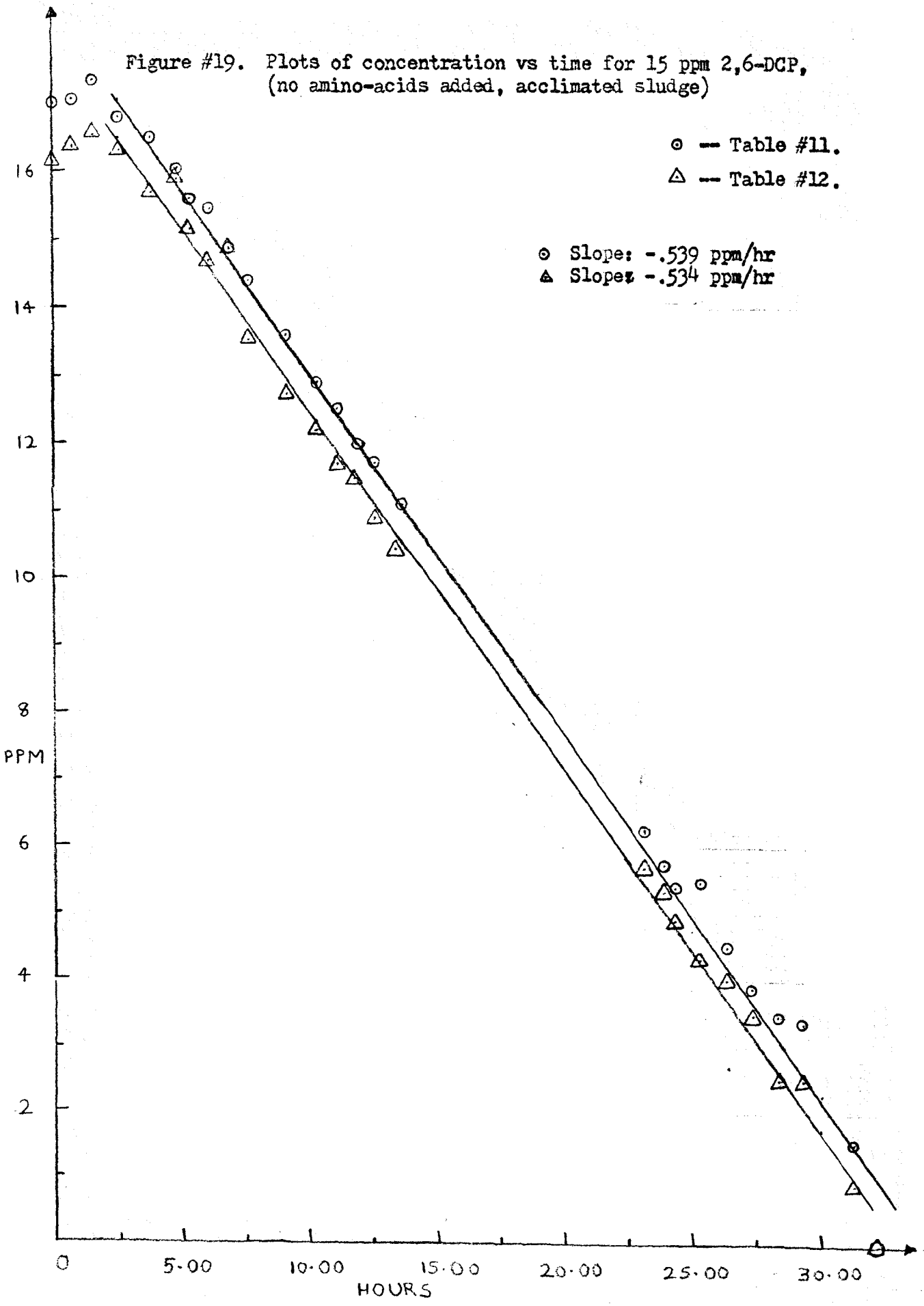


Figure #20. Plots of concentration vs time for 10 ppm 2,6-DCP, (all amino-acids added, acclimated sludge)

⊙ -- Table #24.

△ -- Table #25.

Slope: -1.714 ppm/hr

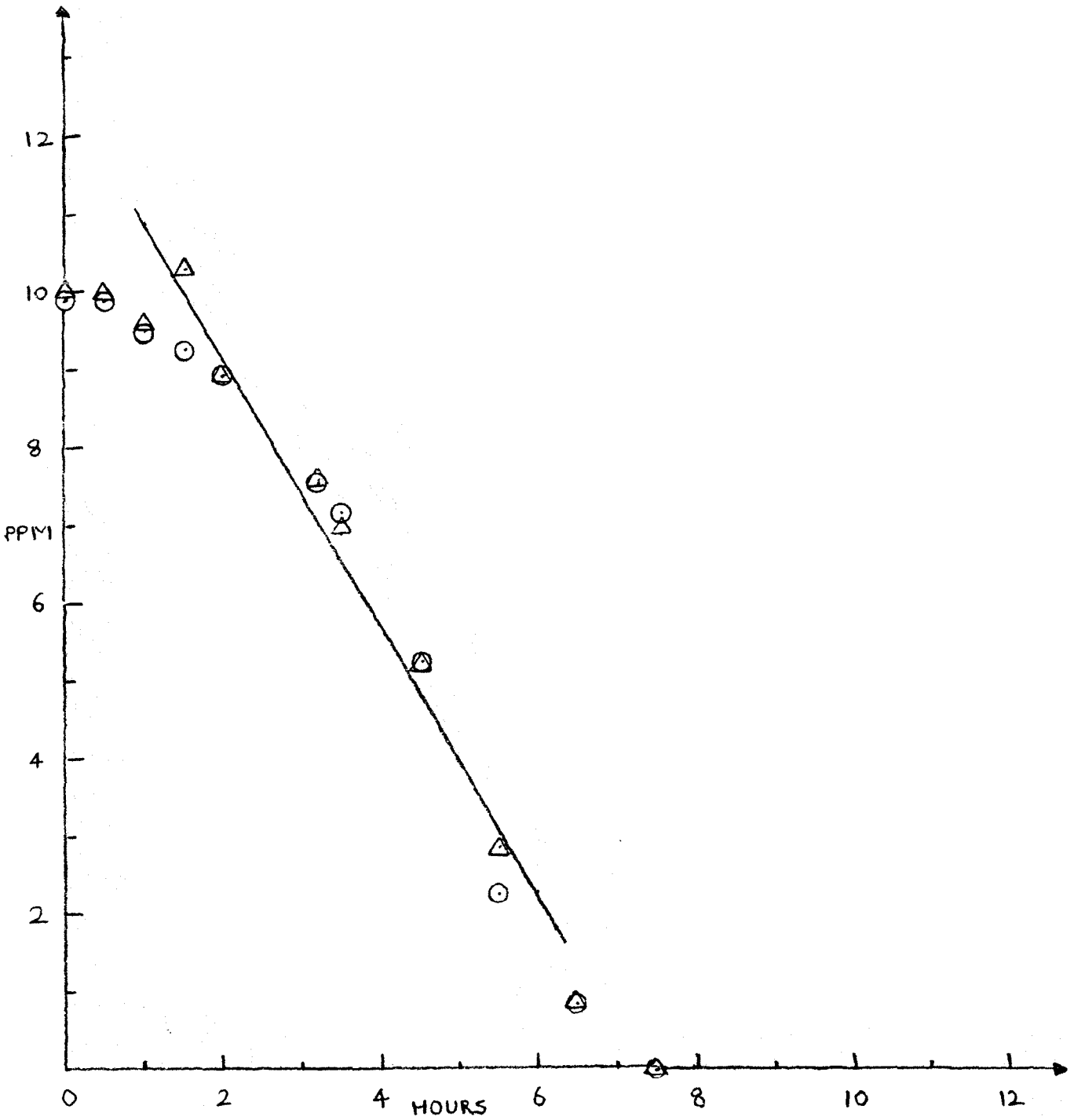


Figure #21. Plots of concentration vs time for 15 ppm 2,6-DCP, (all amino-acids added, acclimated sludge)

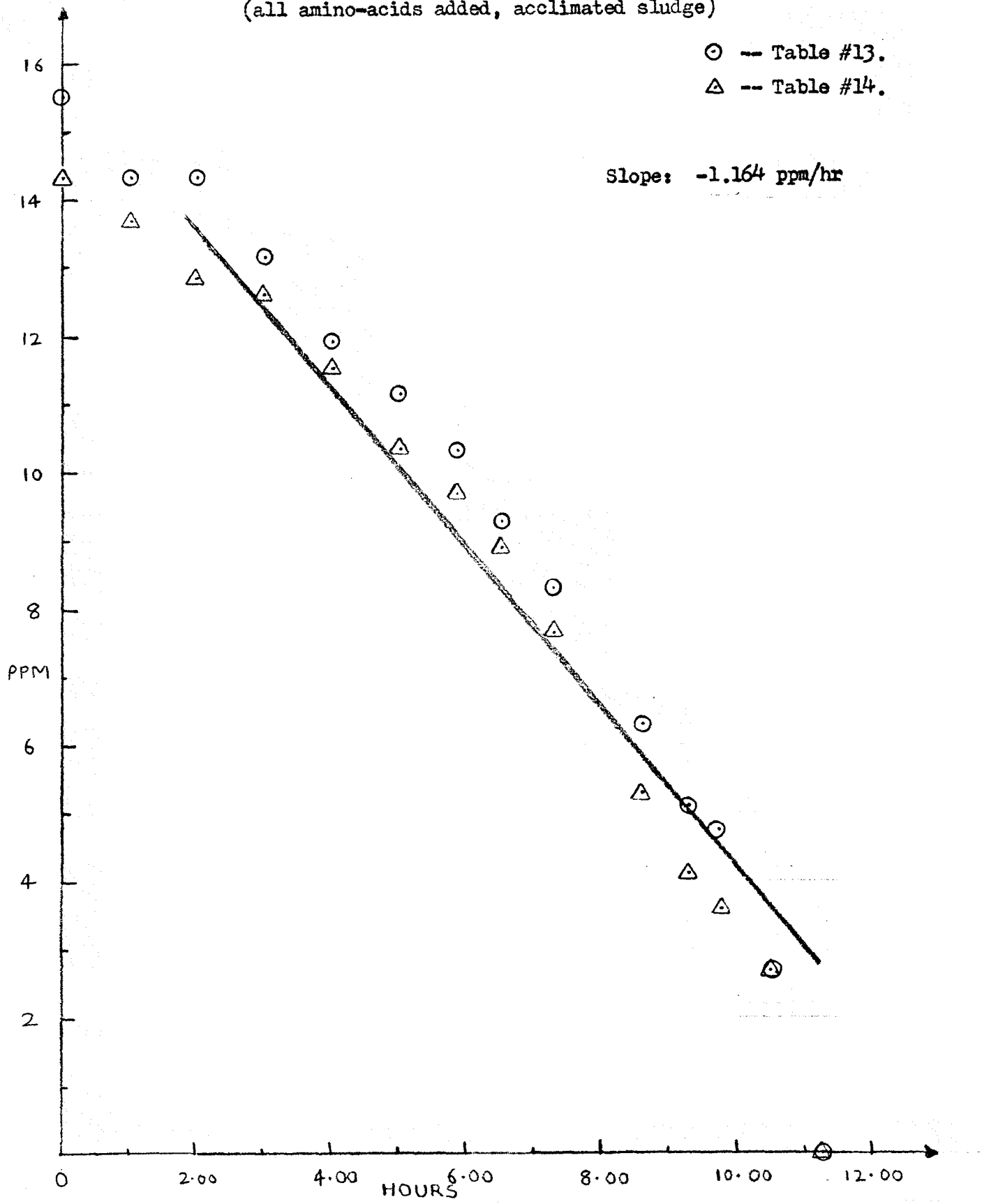


Figure #22. Plots of concentration vs time for 20 ppm 2,6-DCP, (all amino-acids added, acclimated sludge)

⊙ -- Table #26.

△ -- Table #27.

Slope: -1.385 ppm/hr

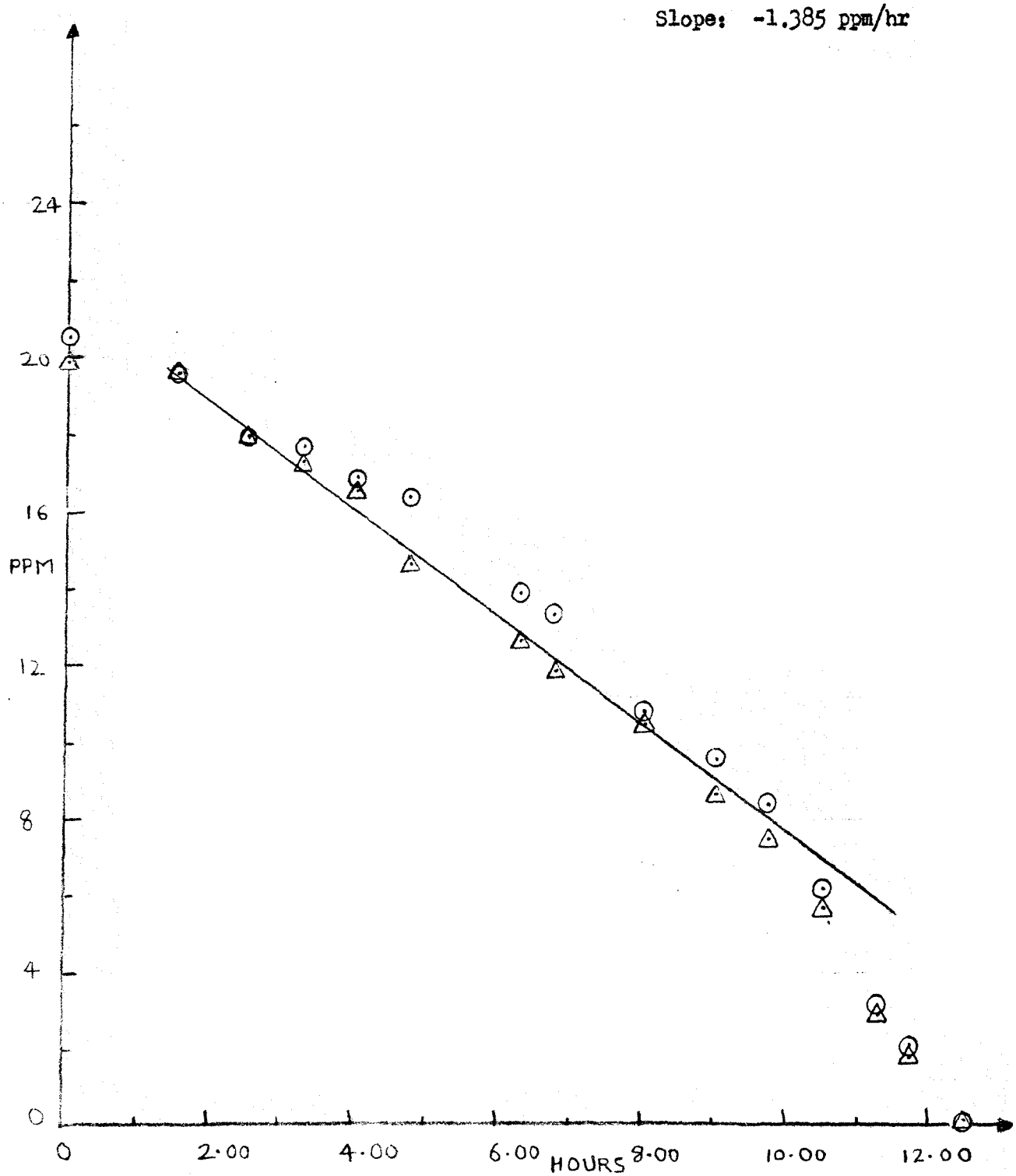


Figure #23. Plots of concentration vs time for 15 ppm 2,6-DCP, (amino-acids LHHM, LLMC, and LAH added, acclimated sludge)

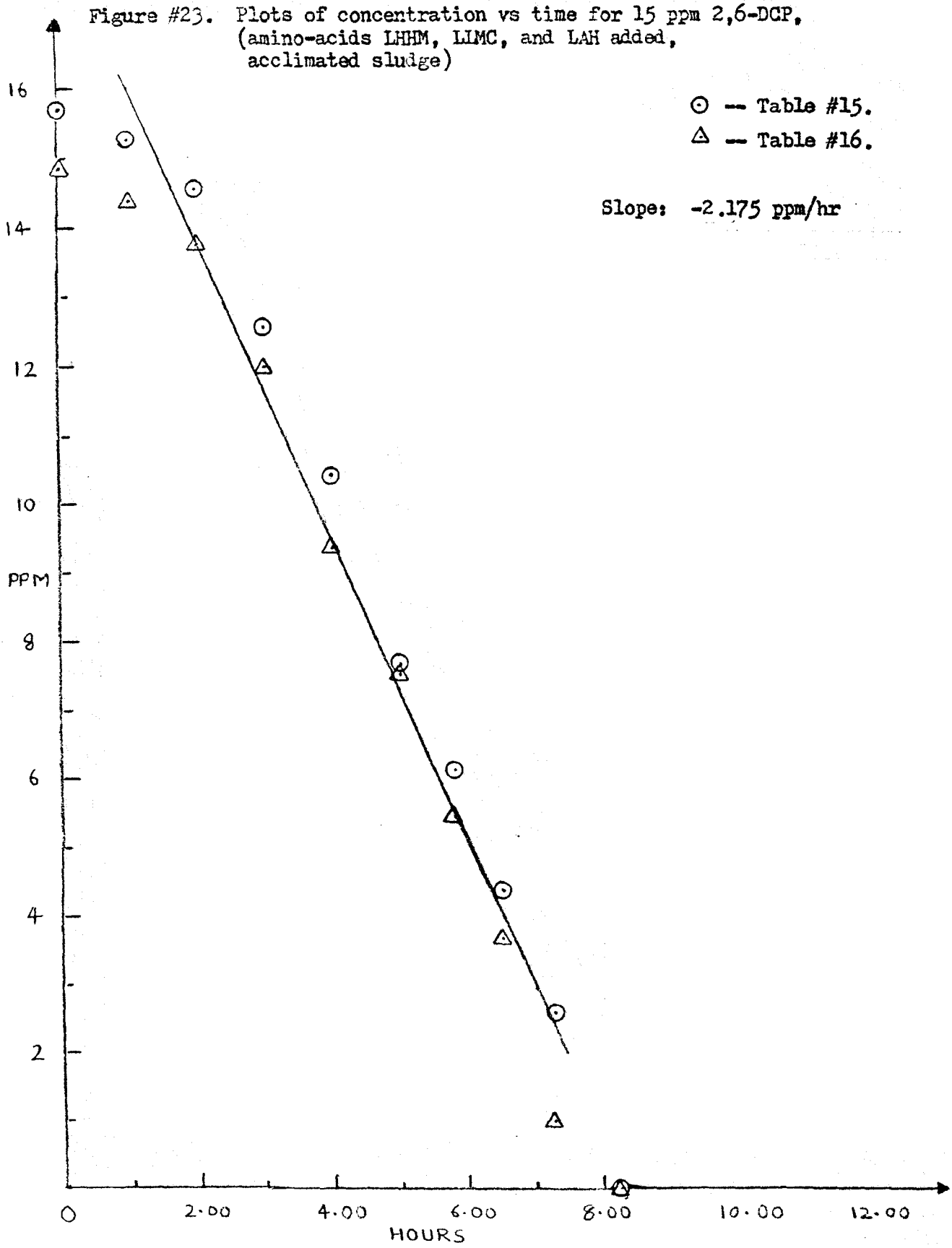


Figure #24. Plots of concentration vs time for 15 ppm 2,6-DCP, (amino-acids LHHM, and LAH added, acclimated sludge)

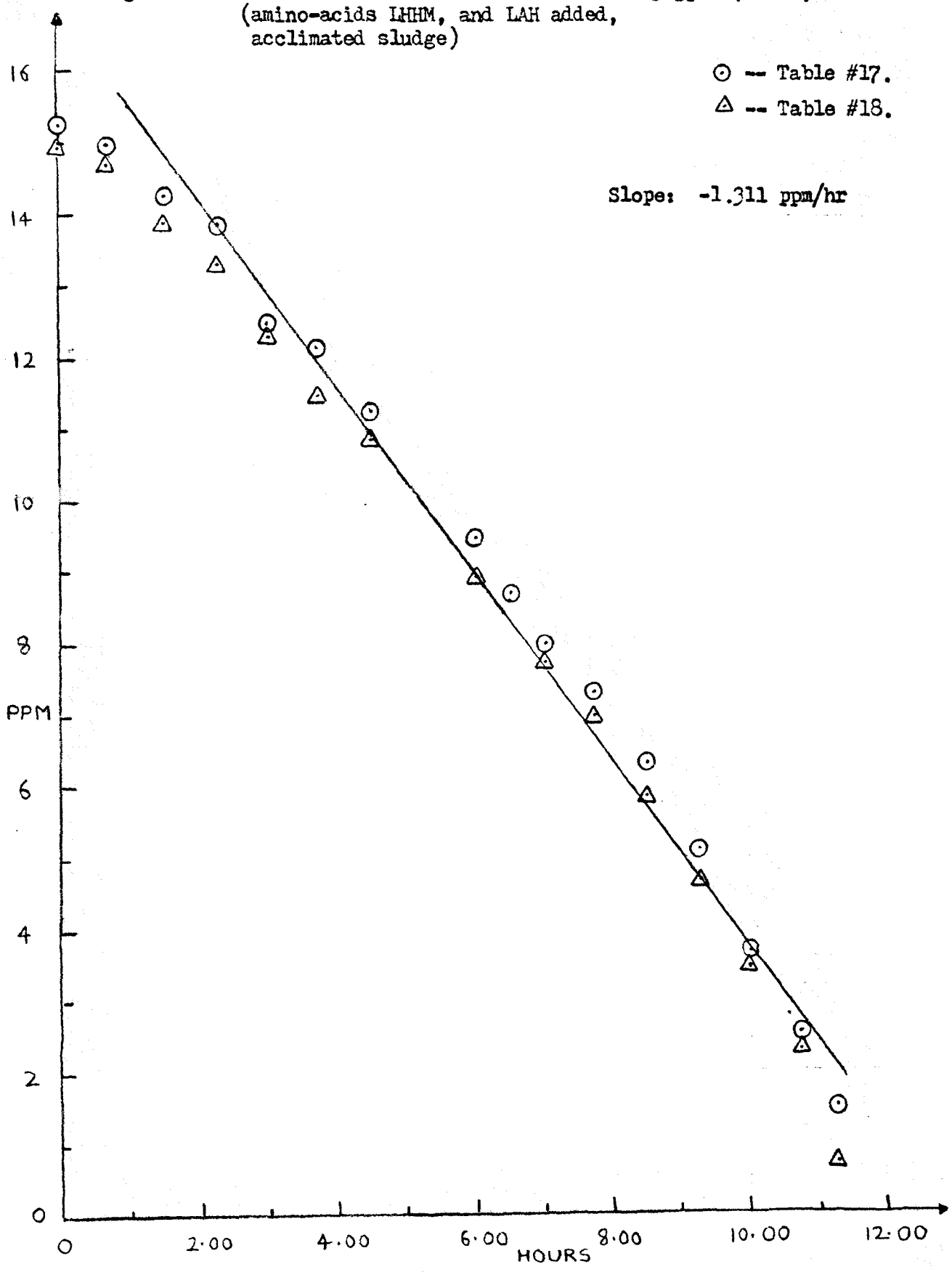


Figure #25. Plots of concentration vs time for 15 ppm 2,6-DCP, (amino-acids LHHM, and LIMC added, acclimated sludge)

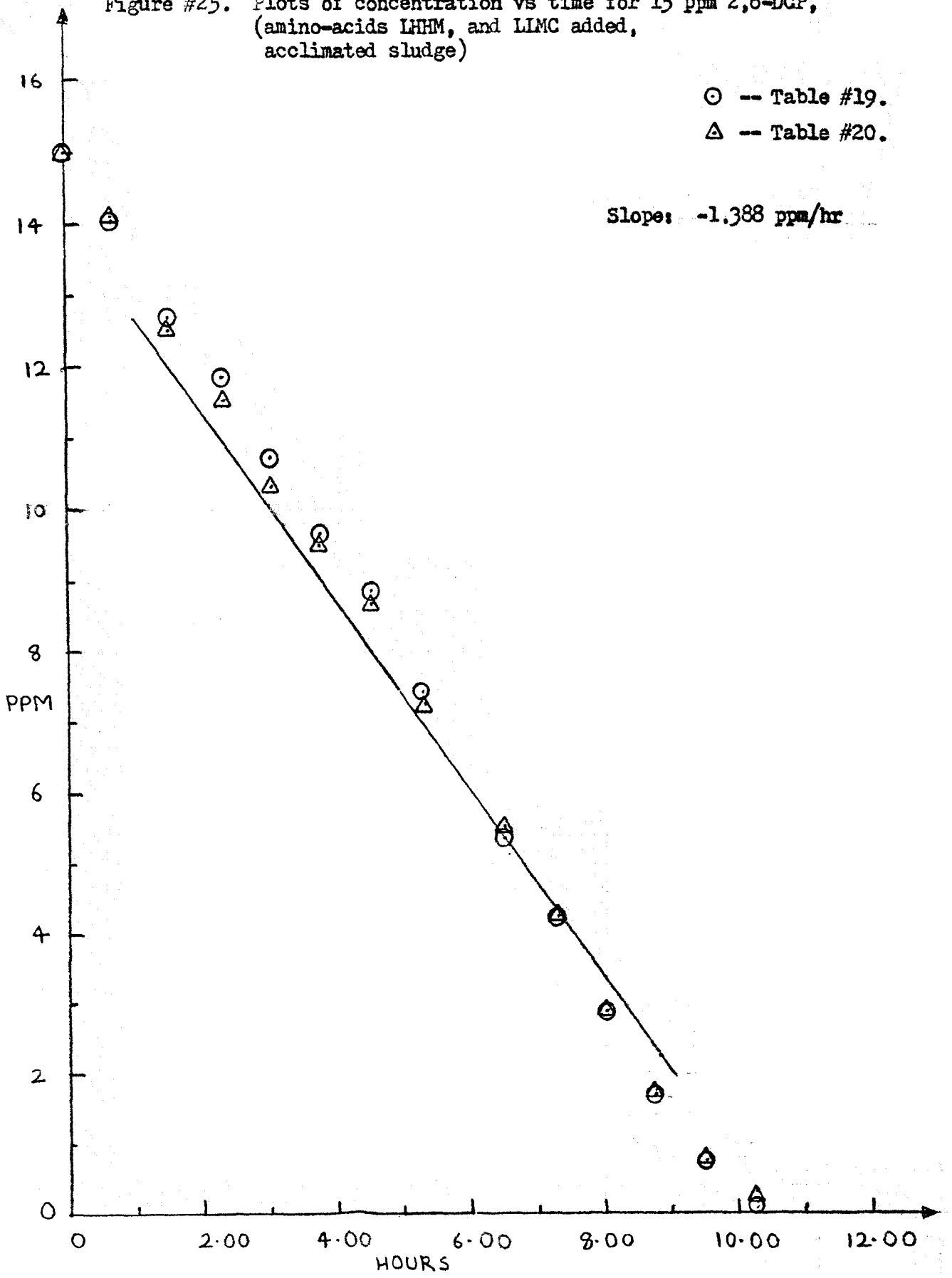


Figure #26. Plots of concentration vs time for 15 ppm 2,6-DCP, (amino-acids LGA, and LCN added, acclimated sludge)

⊙ -- Table #21.

△ -- Table #22.

Slope: -1.636 ppm/hr

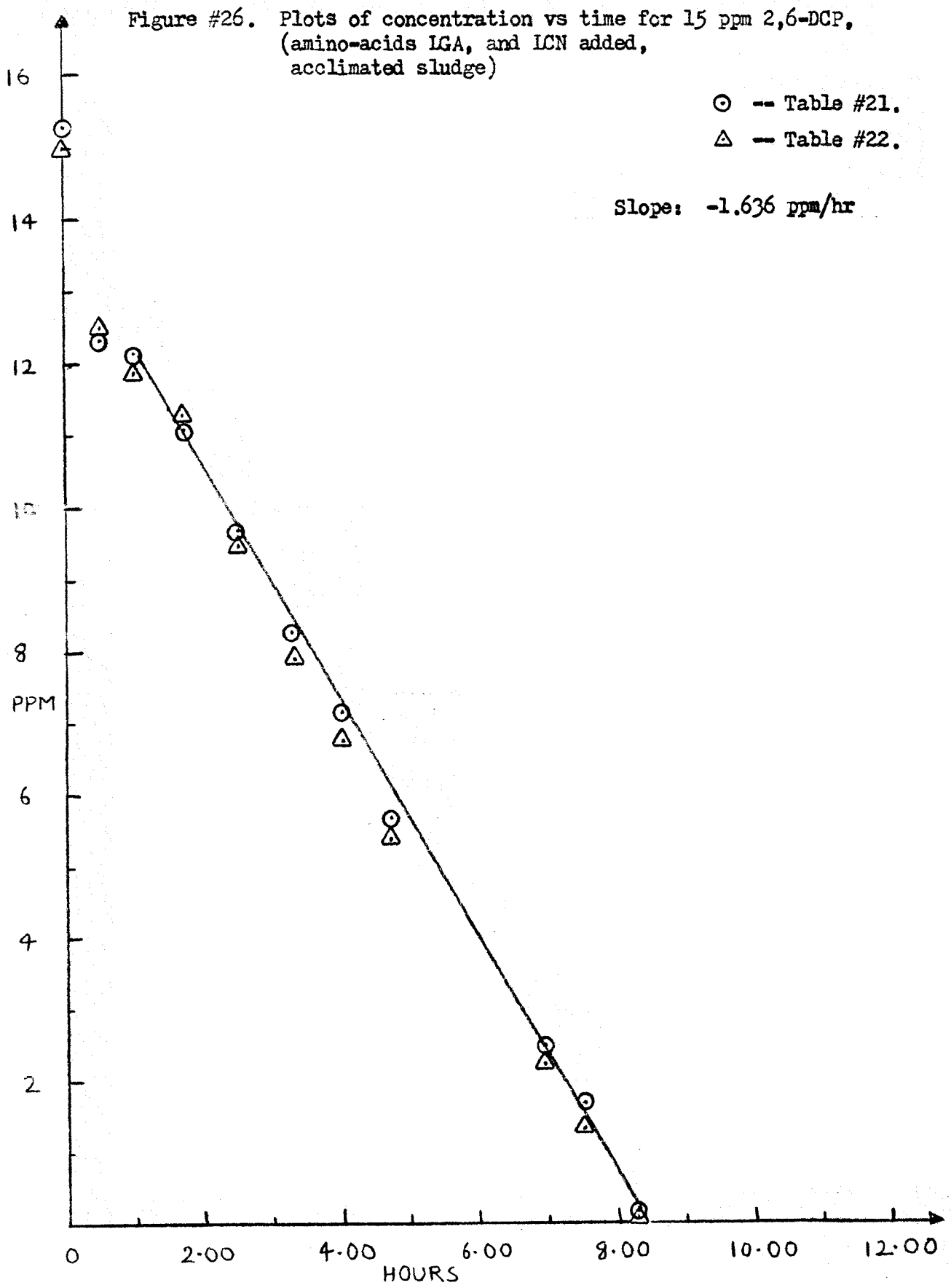


Figure #27. Plot of concentration vs time for 25 ppm 2,6-DCP, (all amino-acids added, acclimated sludge)

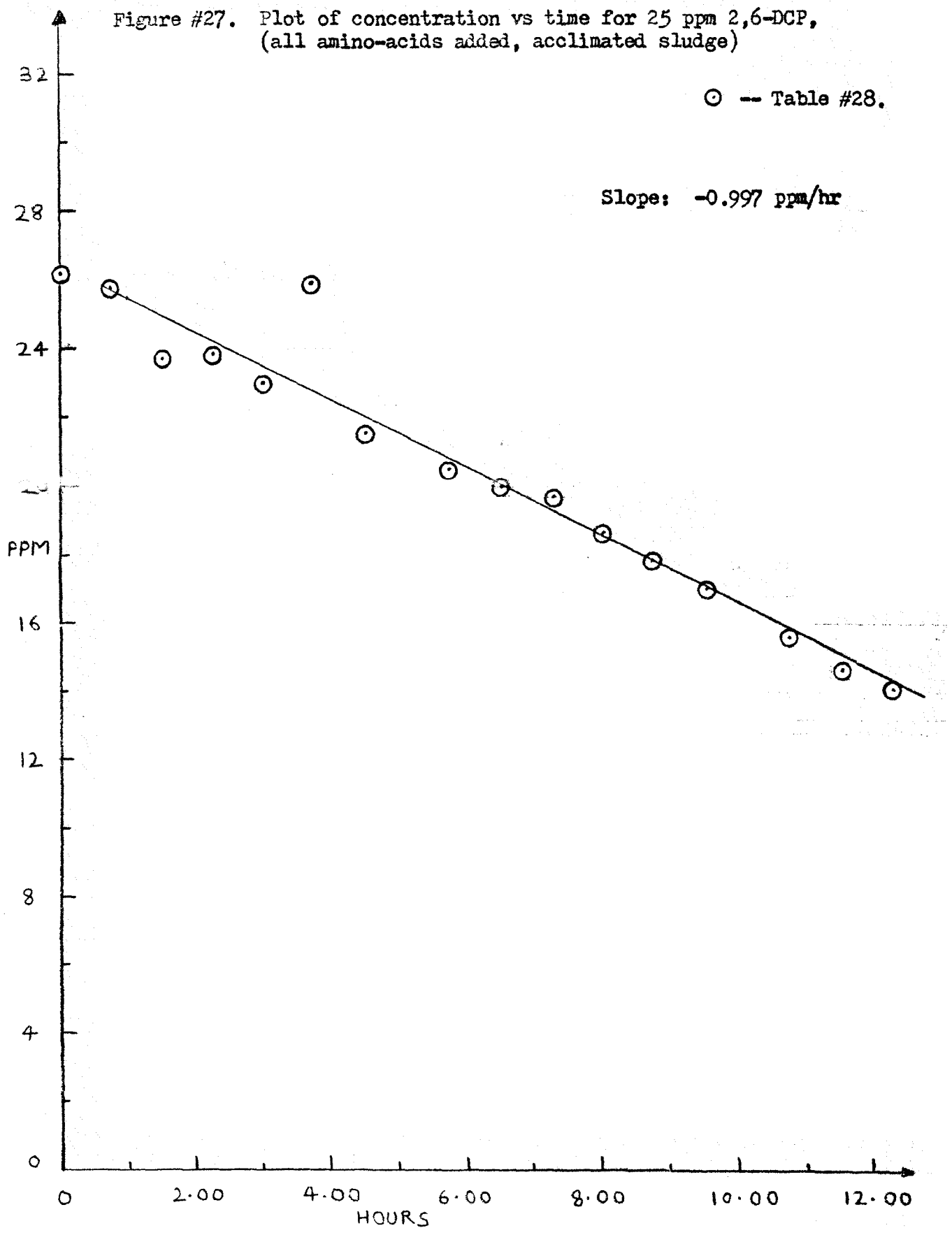


Figure #28. Plots of pH vs time for 10 ppm 2,6-DCP,
(no amino-acids added, unacclimated sludge)

⊙ -- Table #23.

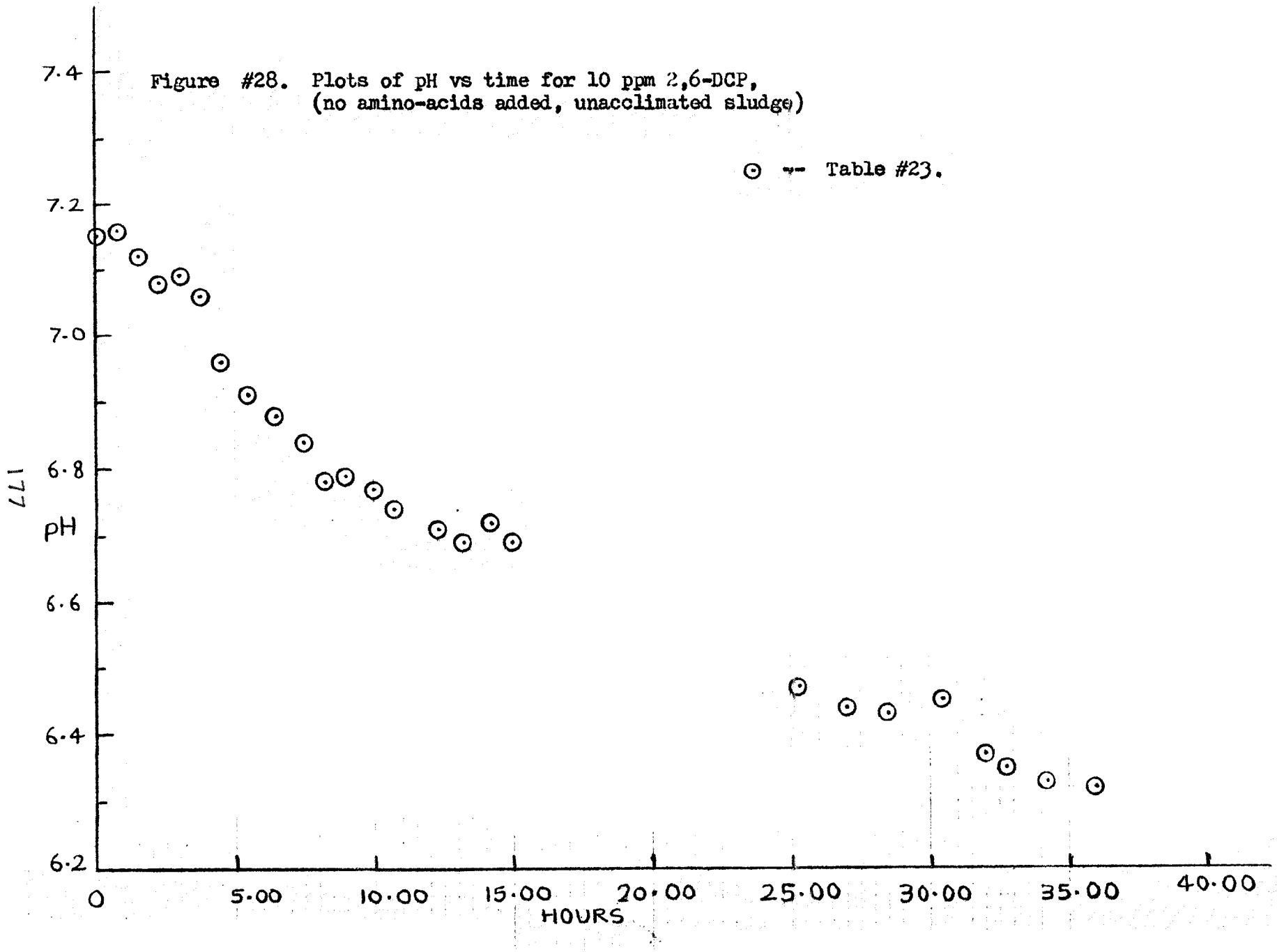


Figure #29. Plots of pH vs time for 15 ppm 2,6-DCP, (no amino-acids added, acclimated sludge)

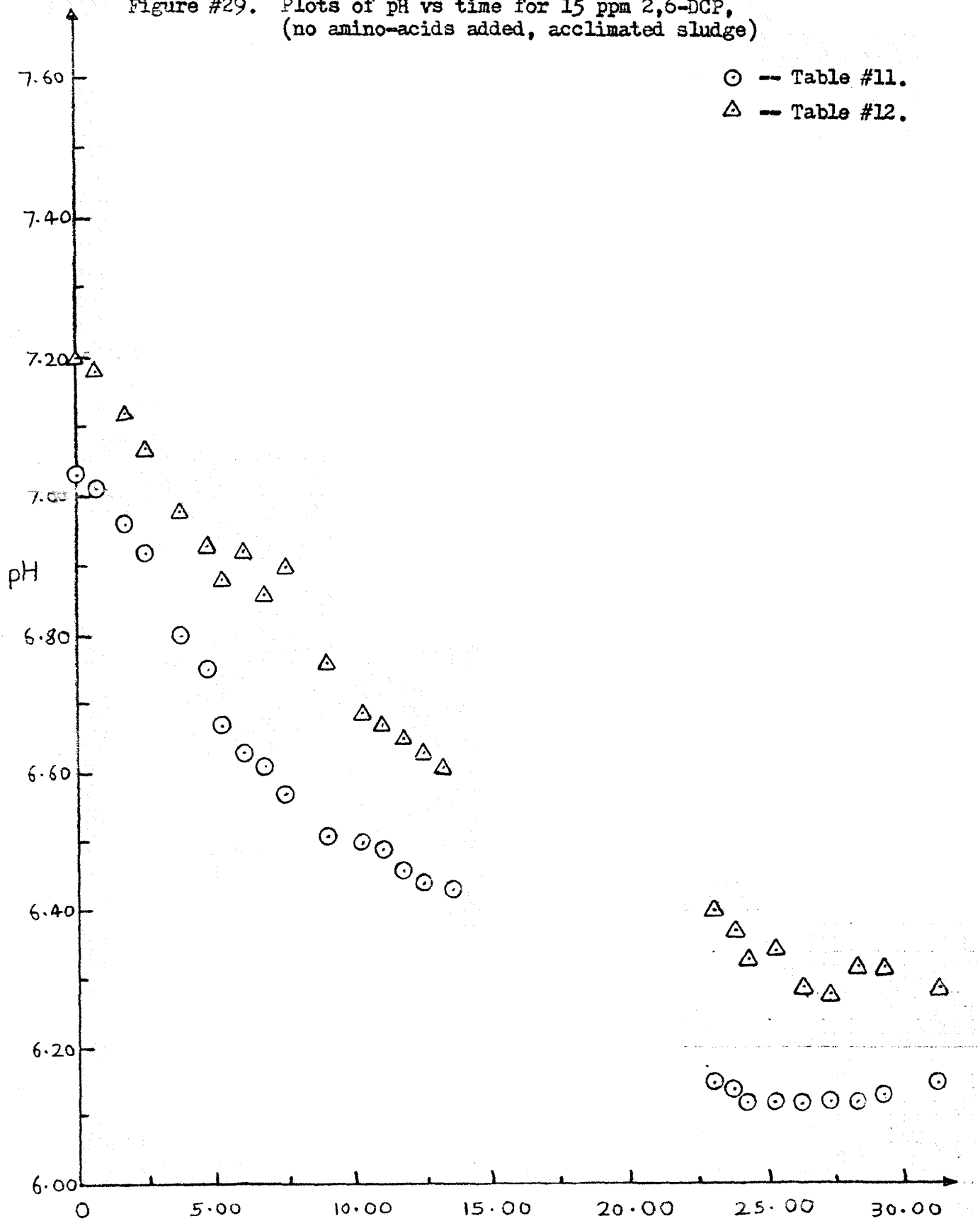


Figure #30. Plots of pH vs time for 15 ppm 2,6-DCP,
(all amino-acids added, acclimated sludge)

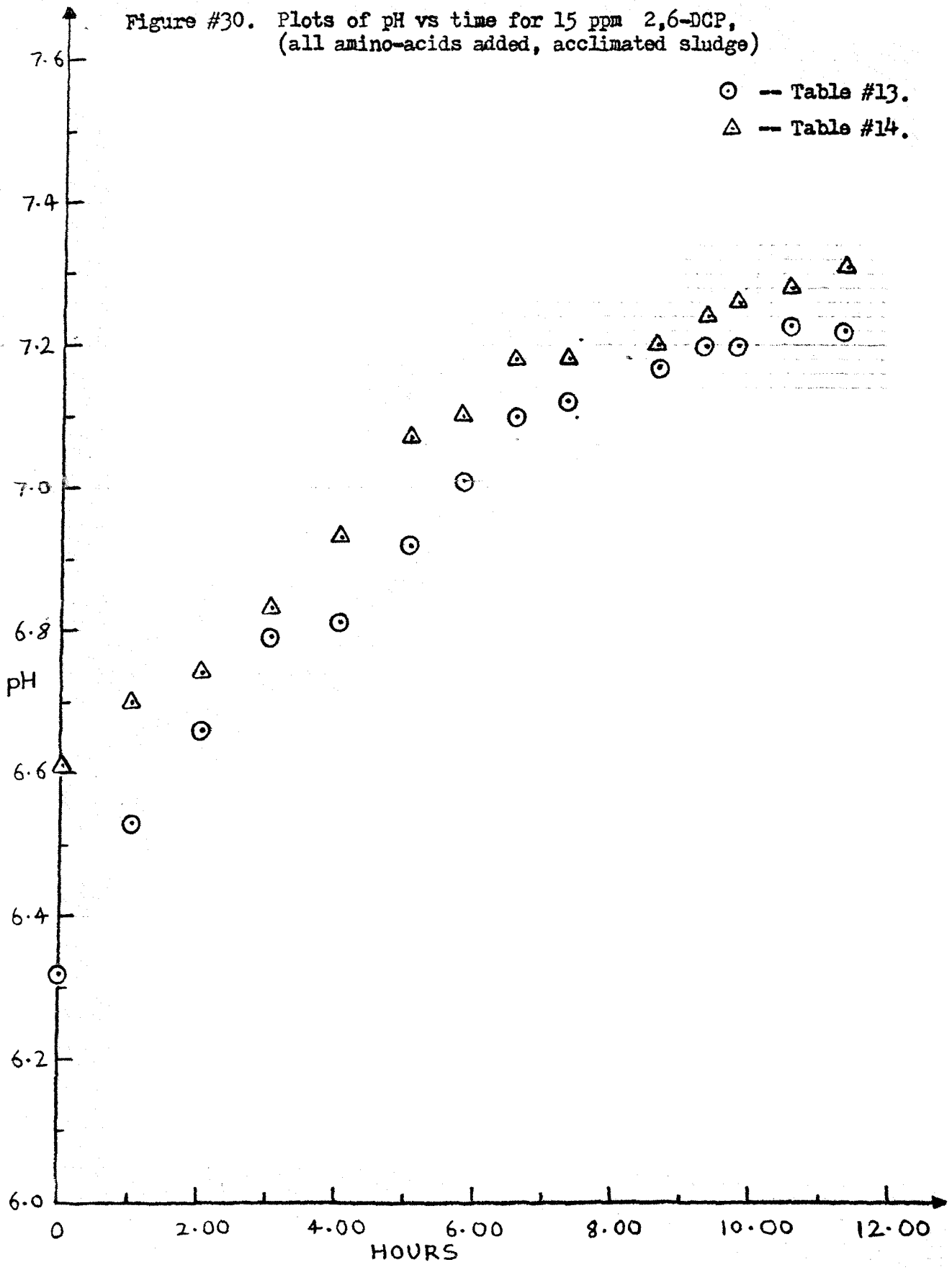


Figure #31. Plots of pH vs time for 15 ppm 2,6-DCP, (amino-acids LHHM, LLMC, and LAH added, acclimated sludge)

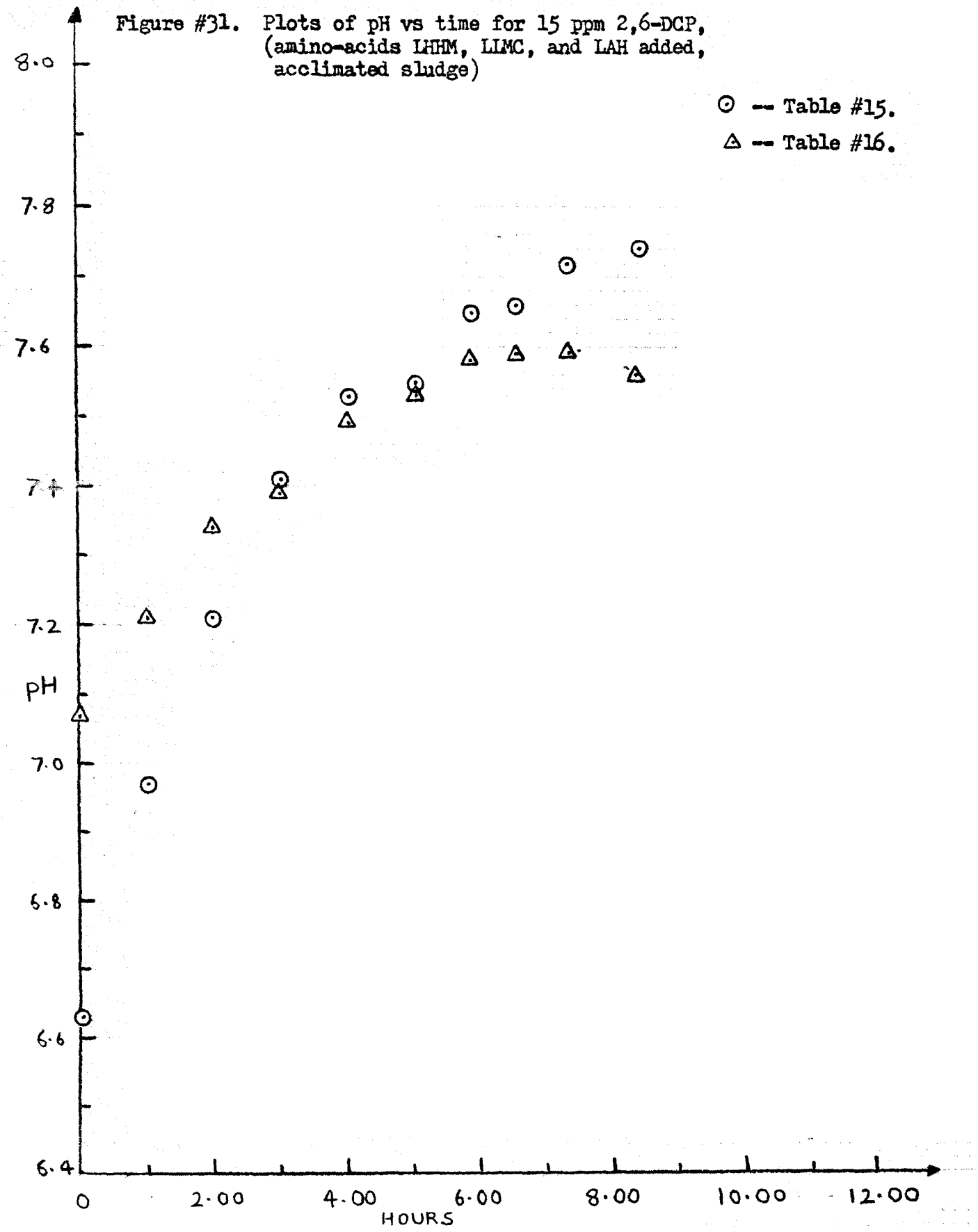


Figure #32. Plots of pH vs time for 15 ppm 2,6-DCP, (amino-acids LHHM, and LAH added, acclimated sludge)

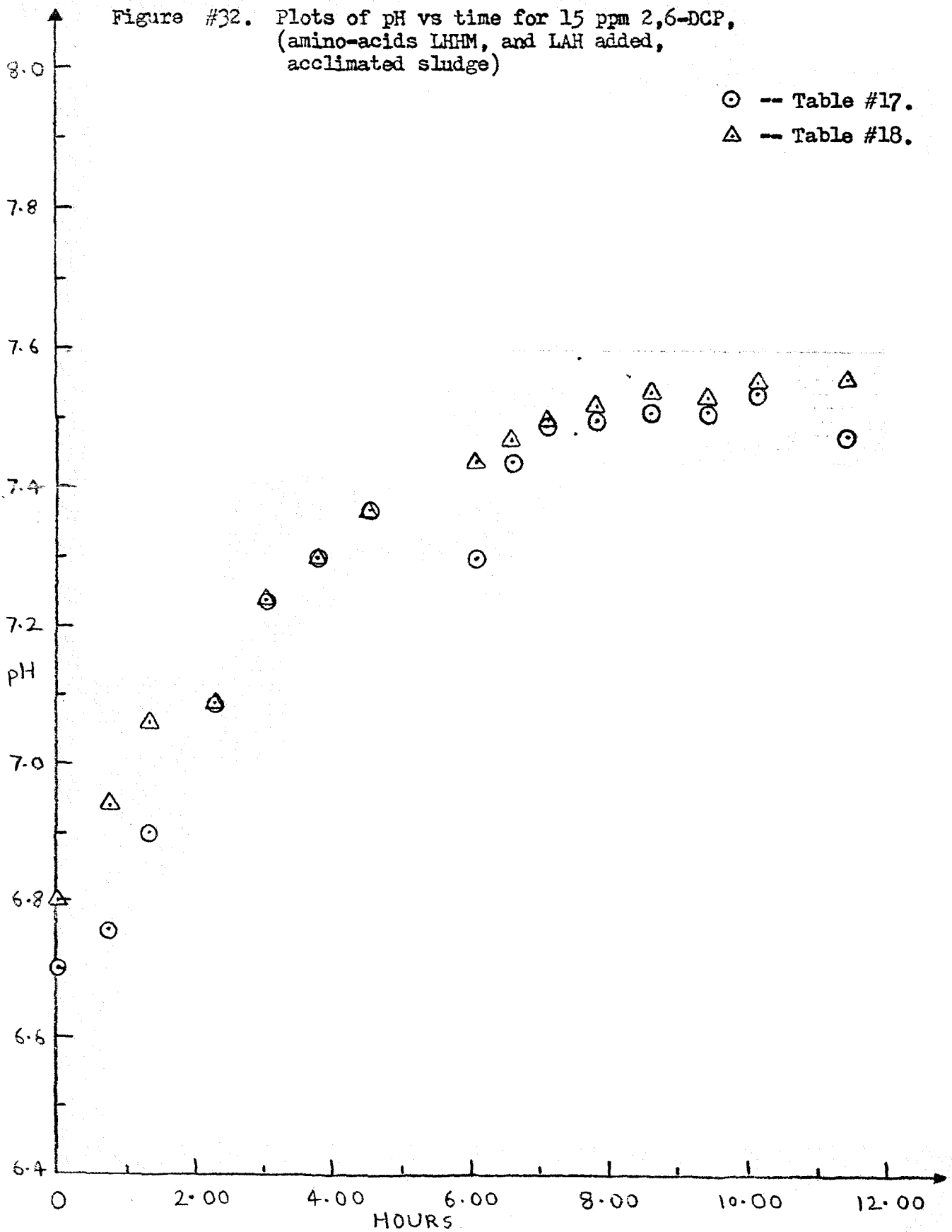


Figure #33. Plots of pH vs time for 15 ppm 2,6-DCP, (amino-acids LGA and LGN added, acclimated sludge)

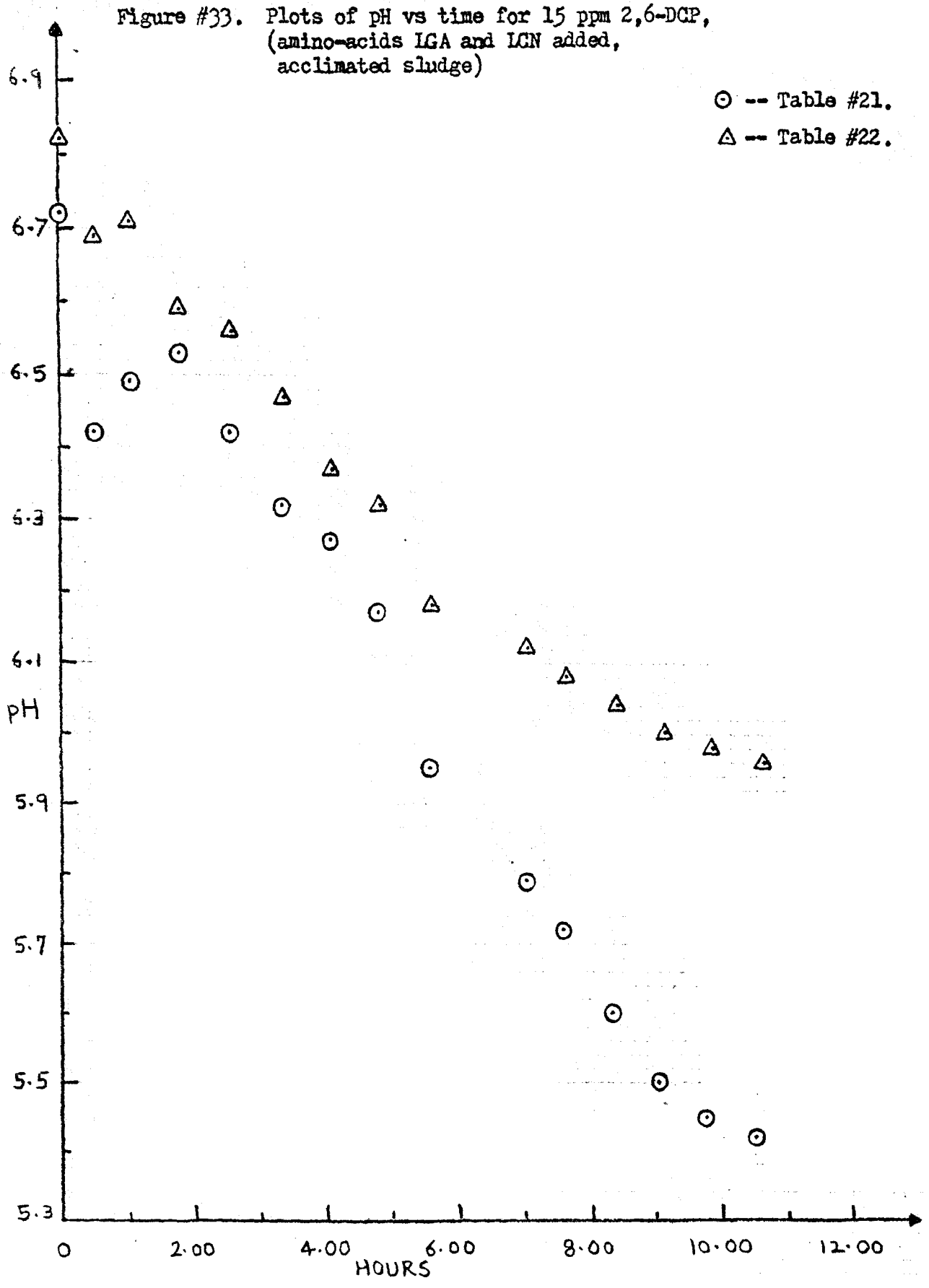


Figure #34. Plot of pH vs time for 25 ppm 2,6-DCP, (all amino-acids added, acclimated sludge)

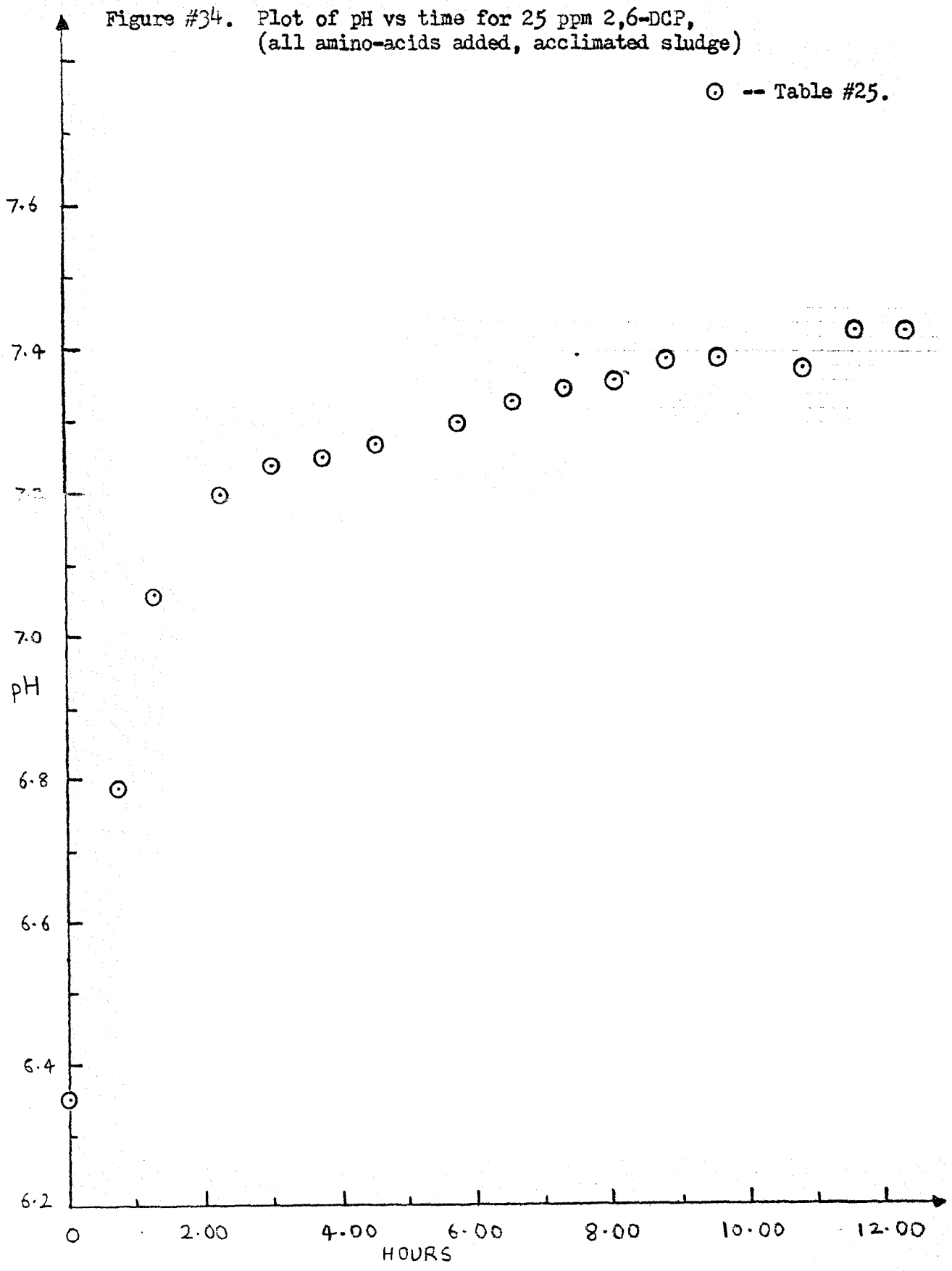


Figure #35. Plot of initial concentration vs complete degradation time for phenol

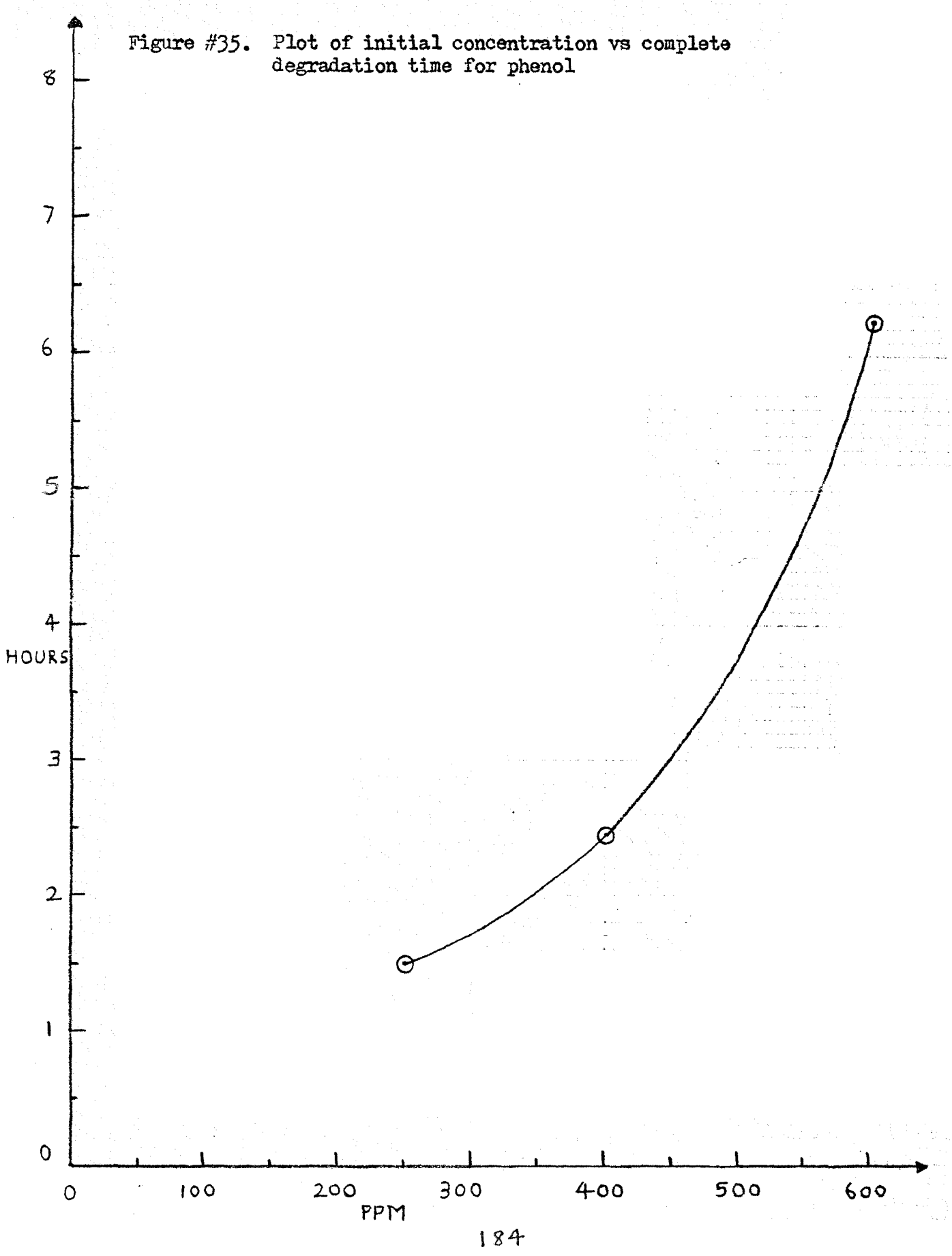
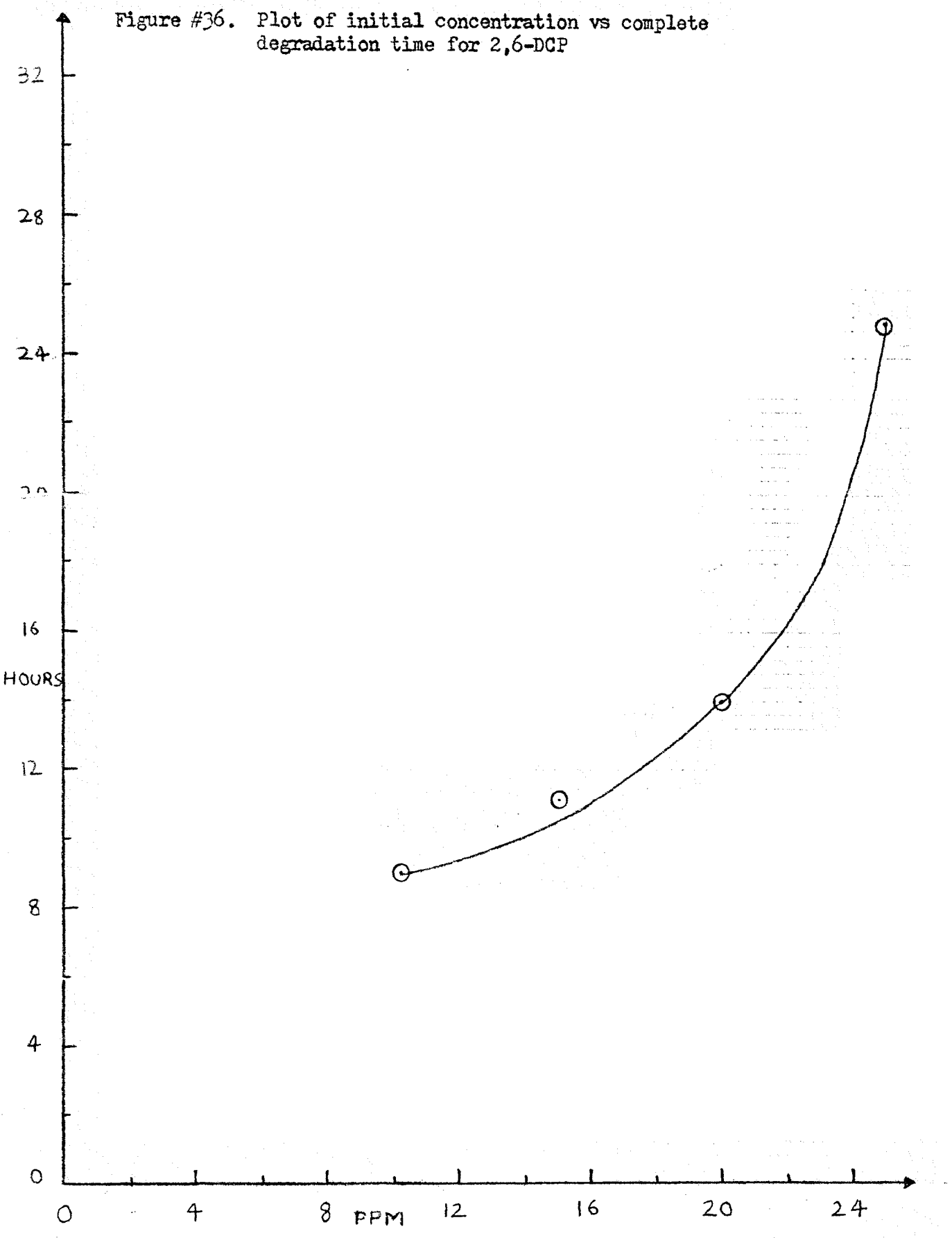


Figure #36. Plot of initial concentration vs complete degradation time for 2,6-DCP



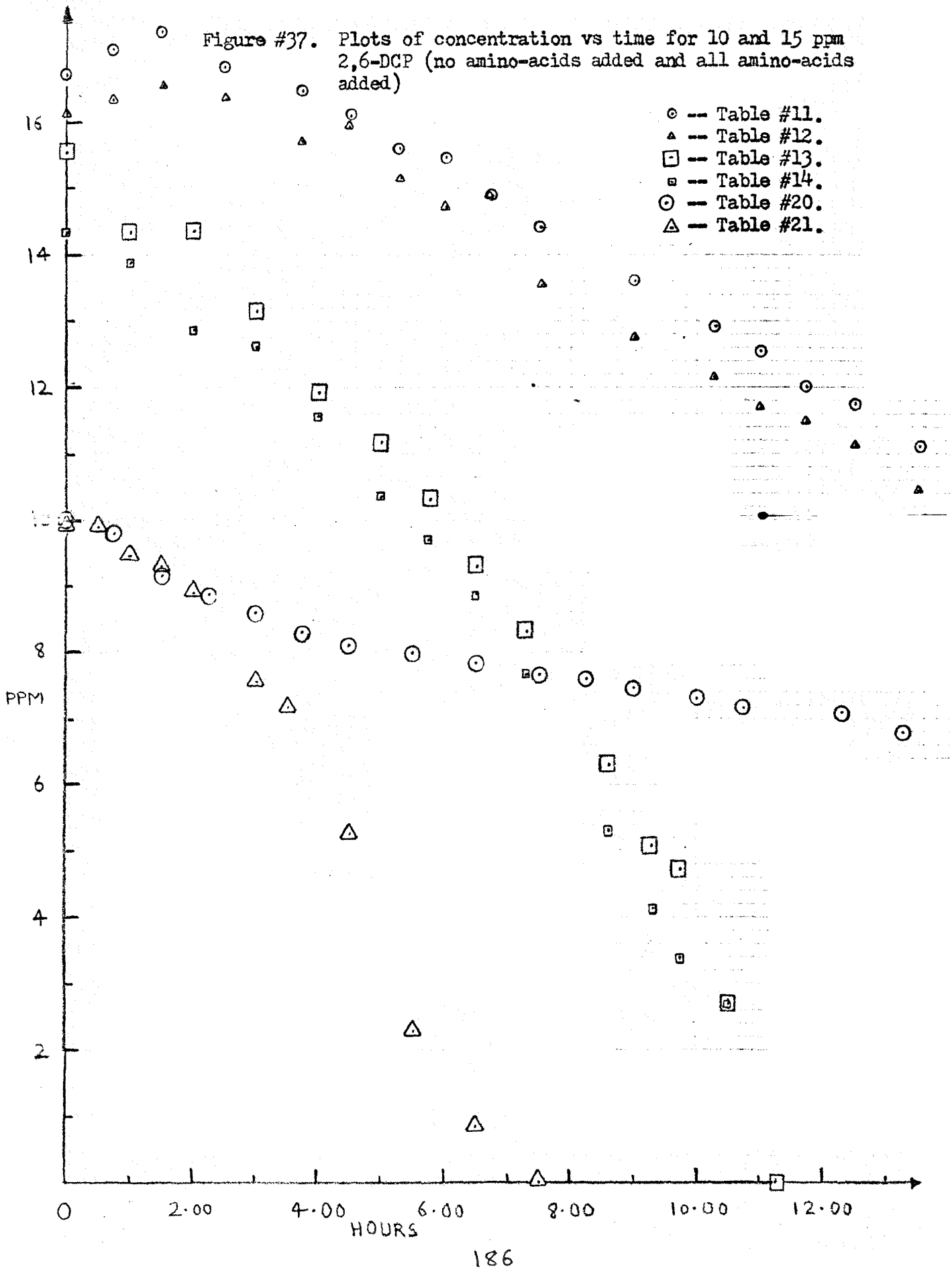


Figure #37. Plots of concentration vs time for 10 and 15 ppm 2,6-DCP (no amino-acids added and all amino-acids added)

Figure #38. Plots of concentration vs time for 10, 15, 20, and 25 ppm 2,6-DCP (all amino-acids added, acclimated sludge)

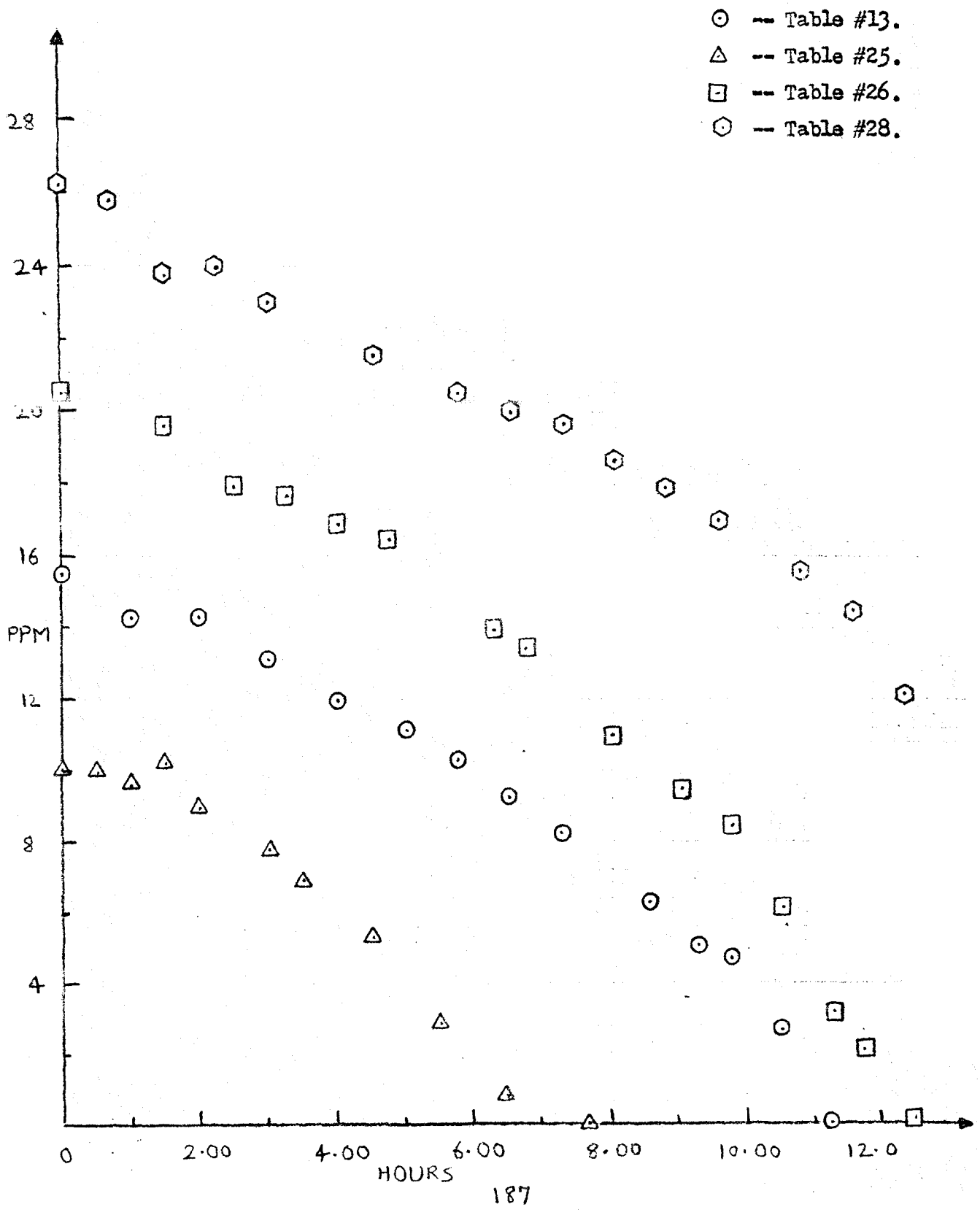
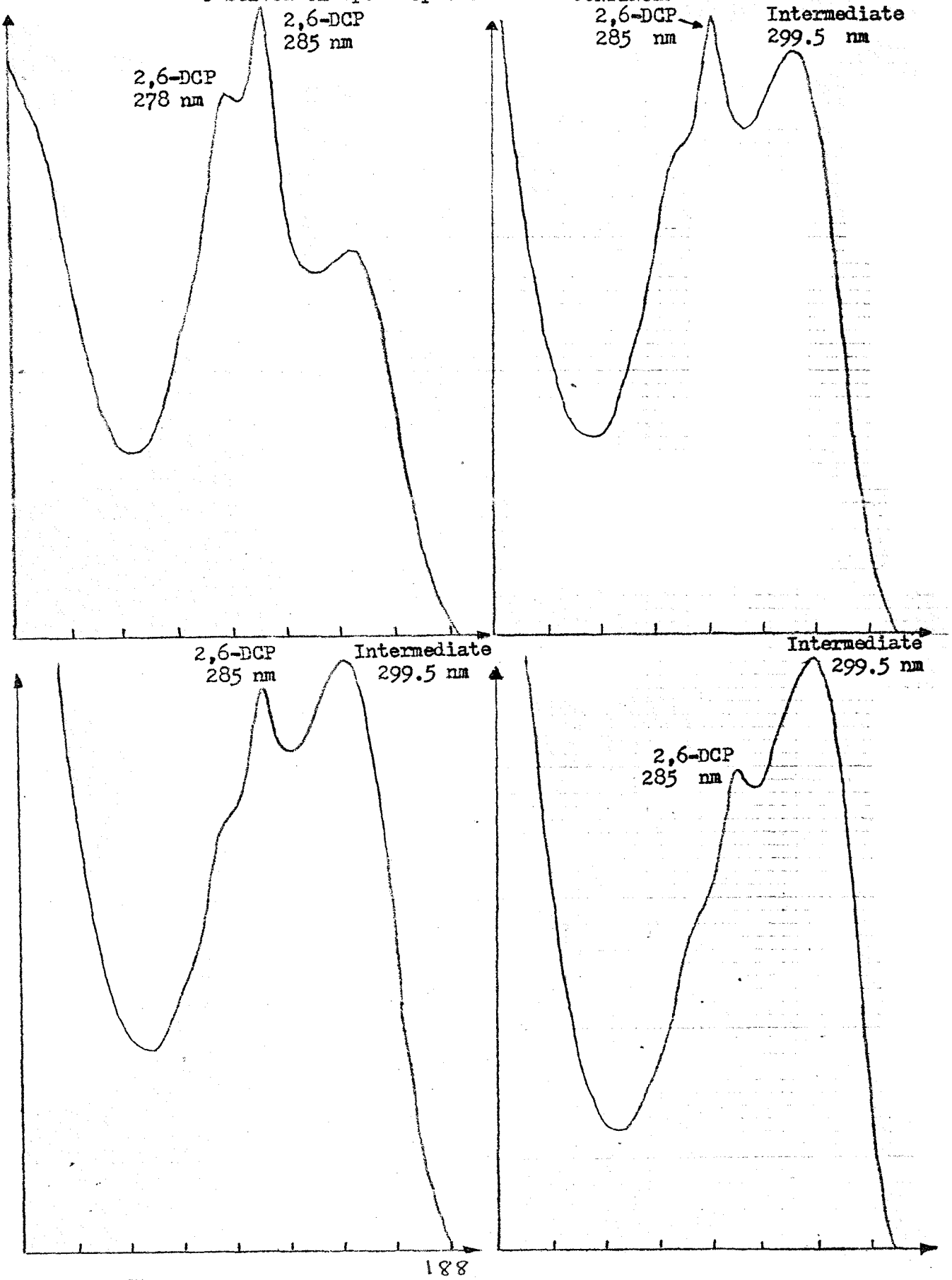


Figure #39. Progressive oxidation of 2,6-DCP as observed on spectrophotometer - CONTINUED --



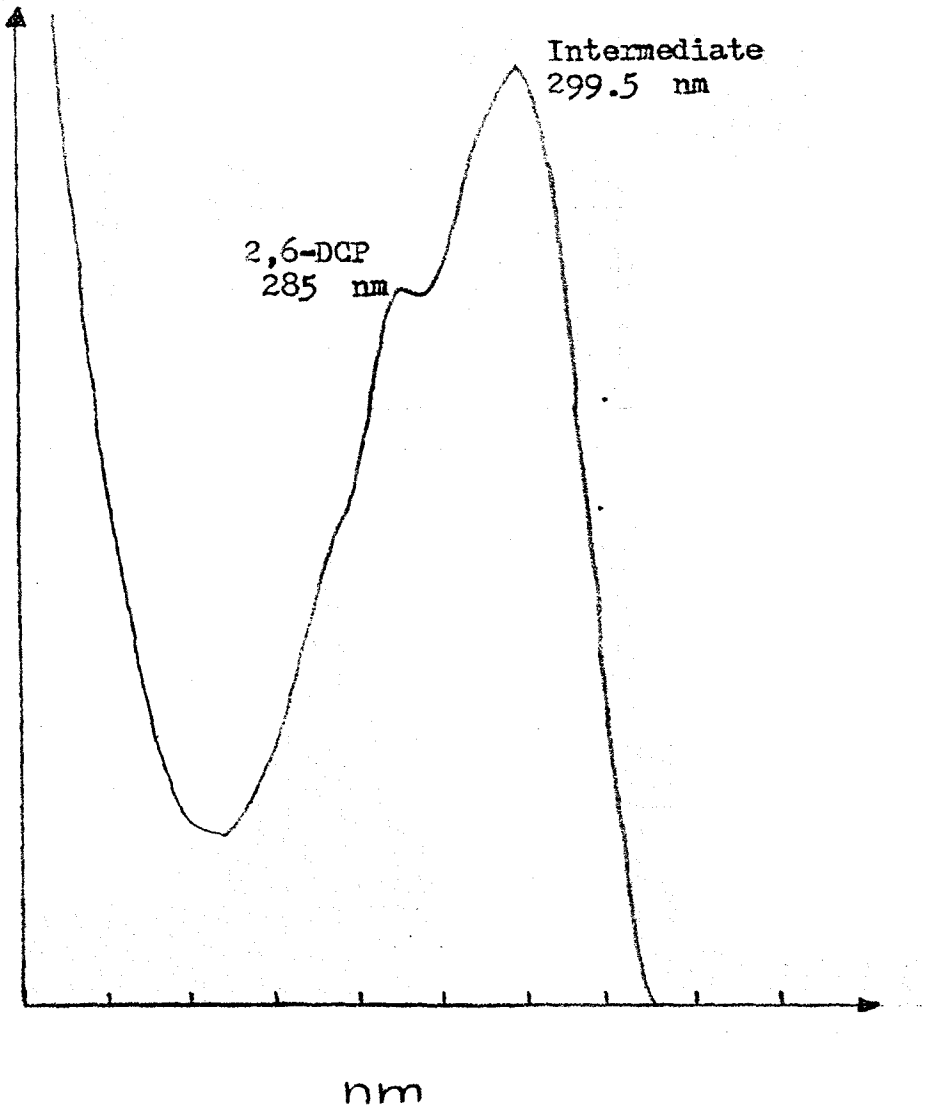


Figure #39. Progressive oxidation of 2,6-DCP as observed on spectrophotometer.