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ABSTRACT

Biodegradation of Phenol
and 4-Chlorophenol Using a Single
Species in a Sequencing Batch Reactor

by
Kung-Wei Wang

Biodegradation of phenol and 4-chlorophenol (4CP) using *Pseudomonas putida* (ATCC 17514) was studied in batch and sequencing batch reactors. Batch experiments were first performed in order to reveal the kinetics of biodegradation for each substrate. 4-Chlorophenol was degradable only in the presence of phenol, or immediately after exposure of the organism to phenol. It was postulated that an enzyme induced by the presence of phenol was necessary to initiate degradation of 4-chlorophenol.

A mathematical model was then developed to describe the dynamic behavior of both substrates in a sequencing batch reactor (SBR). The model utilized rate parameters obtained from batch experiments with phenol as sole carbon source, and postulated an additional expression for 4-chlorophenol degradation. The added term for 4CP contained two parameters which were fit by SBR data. The model was tested by operating a 5-liter SBR with 60 ppm phenol and 20 ppm 4CP in the feed. For both transient and steady cycles, there was excellent agreement between the model results and the experimental data.

BIODEGRADATION OF PHENOL AND 4-CHLOROPHENOL USING A SINGLE SPECIES IN A SEQUENCING BATCH REACTOR

by
Kung-Wei Wang

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1. INTRODUCTION

In the field of wastewater treatment, continuous flow systems (CSTRs) have dominated the technology, especially in the biological waste treatment, whereas the performance of many processes and operations can be improved appreciably by controlled unsteady-state operations (periodic processes) [1,2]. Fill-and-draw reactors (also known as sequencing batch reactors) which include five discrete periods : fill, react, settle, draw, and idle are one of the periodical processes. In recent years, semibatch reactors have become more popular in dealing with hazardous waste treatment as a result of automated control, improved decanting mechanisms, and aeration equipment that is resistant to plugging during start/stop operation.

The development of sequencing batch reactors (SBRs) has become more important because quality control is easier to maintain in a SBR than in a CSTR, particularly if the feed is a high strength variable waste. Some advantages of fill-and-draw reactors over CSTRs are as follows [3] : (a) since the SBR operates in a batch mode during the reaction phase, the reaction products can be held in the reactor until they are acceptable for discharge; (b) a much smaller reactor volume is needed for the same throughput; (c) capability of having both anoxic and aerated periods for different redox of characteristics, and control of filamentous organisms.

Chlorophenols are hazardous to the environment, and resistant to biological degradation. They are discharged into the environment in the effluent from pulp and paper mills and from plastic resin manufacture. Chlorophenols are used extensively as antifungal agents, and are often applied as a wood preservative. They are also common degradation products of chlorophenoxy herbicides. The wood shavings from lumber

processes have been used for litter in chicken houses and contain high levels of these chlorophenols [4].

In the present study, *Pseudomonas putida* was obtained from American Type Culture Collection (ATCC #17514). It was used to degrade a mixture of phenol and 4-chlorophenol in a sequencing batch reactor.

2. LITERATURE REVIEW

Irvine and Busch described SBR operation in 1979. Since that time, a U.S. EPA demonstration study has shown that the SBR is an excellent alternative to conventional activated sludge treatment for municipal wastewater. Recently, results from bench-scale studies indicated that the SBR can provide substantial savings in energy and costs by removing organic compounds found in hazardous waste biologically, rather than with activated carbon [5,6,7]. Construction of a 1900 m^3 SBR was completed and operated by Herzbrun *et al.* [8]. TOC degradation averaged 76% and phenol degradation averaged 99.0%.

The Gaudy Center, Iowa wastewater treatment plant was designed to operate as a periodic process rather than as a conventional continuous flow activated sludge system because of the successful operation of the Sequencing Batch Reactor (SBR) at Culver, Ind. Irvine, Murthy, Arora, Copeman, and Heidman [9] studied the full-scale SBR operation, which treated 2500 m^3/day at an average detention time of 26 hours, and organic loading of 0.1 kg BOD_5/kg MLSS·d. Effluent BOD_5 was 10 mg/l, suspended solids was also about 10 mg/l, and NH_4-N at 1 mg/L or less.

Ketchum, Irvine, and Liao [10] studied two different modes of SBR operation. In the first case, all oxygen demands were satisfied, and in the second one, oxygen was limited to that supplied by a constant rate aeration system operating at a rate less than would be needed to meet peak demands. Laboratory studies indicated an operating advantage where peak oxygen demands were not met. This mode of operation appeared to favor growth of nonfilamentous organisms and reduced the problem of bulking.

There were two SBR systems investigated by Irvine, Madan, Barth, Arora, and Ketchum [11]. The system with low organic loading, especially when operated for biological phosphorus removal, developed a bulking but manageable sludge and produced a very high quality effluent. The more highly loaded system was more difficult to operate because the periods of underaeration often resulted in higher effluent phosphorus concentrations, while the periods of extra aeration seemed to produce higher concentrations of effluent suspended solids. These problems can be corrected with microprocessor control of dissolved oxygen levels. The phosphorus concentrations in the effluent for low and high loading were 0.6 ppm and 1.1 ppm, respectively. The corresponding effluent BOD_5 were 3 ppm and 6ppm, and the corresponding effluent suspended solids were 4 ppm and 9 ppm, respectively.

Both Y. F. Ko [12], and Y. S. Ko [13] used a substrate inhibition Andrews model to examine the kinetic behavior of a fill-and-draw reactor. The former studied a pure culture, while the latter worked with mixed cultures. They solved the proposed models numerically and tested its predictions experimentally, using a 5-liter fill-and-draw reactor with phenol as the sole carbon source. It was shown that experimental results matched to the model predictions very well.

Yang and Humphrey [14] studied the microbial degradation of phenol by pure culture of *Pseudomonas putida* ATCC 17514 and mixed cultures in batch, phenol-stat (constant phenol concentration), and continuous culture system. In continuous culture runs, both steady state and transient experiments were performed. They proposed a model for the kinetic behavior of the organisms and used the parameters ($\hat{\mu}$ = 0.6 h^{-1}, K_S = 2 mg/l, K_I = 106 mg/l) of model obtained by nonlinear regression of specific growth rates vs. phenol concentrations to perform an analysis of the stability and dynamic behavior of pure and mixed cultures. These values are quite different from those obtained in the present study with the same organism. The results indicated that it

should be possible to achieve phenol removal from wastewater down to levels of 1-2 ppm in a single stage system.

Hill and Robinson [15] studied substrate inhibition kinetics for phenol degradation by *Pseudomonas putida* ATCC 17484. A pure culture was grown in both batch and continuous culture using phenol as the limiting substrate. Of the two substrate inhibition models examined, the Haldane model ($\mu = \hat{\mu}S/(K_S+S+S^2/K_I)$) was found to statistically best describe the kinetics. In this study, the Haldane equation was linearized such that a plot of $1/\mu$ versus S gave intercept $1/\hat{\mu}$ and slope $1/(K_I\,\hat{\mu})$ at sufficiently high substrate concentrations ($S>>K_S$). Such linearization, of course, yields no information on the values of K_S. It was also shown that wall growth exerted a significant effect on the suspended biomass concentration and phenol conversion, both of which decreased with increasing amounts of wall growth.

G. Molin and I. Nilsson [16] grew *Pseudomonas putida* ATCC 11172 in continuous culture with phenol as the only carbon and energy source. This organism degraded phenol by the metacleavage pathway, which was indicated by the accumulation of 2-hydroxy-muconic semialdehyde (2-HMA). This compound produces a yellow color visually discernible in the fermentation broth and easily recorded by absorbance measurements at 375 nm, even at low concentration (0.05 ppm). 2-HMA is often reported as a metabolite in the degradation of various types of chlorinated aromatic compounds by *Pseudomonas* sp.. Phenol has a potentially inhibitory effect on cell growth, i.e., if the concentration of phenol in the medium is high enough it will cause substrate inhibition following the Haldane model. A chemostat was employed in these experiments using different dilution rates. μ_{max} was found to be 0.4 h^{-1}, which is comparable to 0.5 h^{-1} determined by Hill and Robinson, and 0.6 h^{-1} from the work of Yang and Humphrey for phenol degradation by the same species but different strains.

H. Rubin and S. Schmidt [17] studied the growth of phenol-mineralizing microorganisms in fresh water. Phenol concentrations above 10 ppm were inhibitory to the microorganisms capable of mineralizing phenol. The phenol mineralizers grew in the water samples in the absence of phenol, indicating that there were sufficient indigenous nutrients in the lake water to support growth. There was no difference in the growth rate of the microorganisms in the presence or absence of 1 ng of phenol per ml, whereas the growth was more rapid at 1 μg of phenol per ml than in its absence. There was a correlation between microbial growth and the amount of phenol mineralized at 1 μg but not at 1 ng of phenol per ml. This indicated that 1 ng of phenol per ml was not, but 1 μg of phenol per ml was, a sufficient concentration to increase the rate of growth of the mineralizing microorganisms above that obtained with the indigenous nutrients alone.

T. Ohmori, T. Ikai, Y. Minoda, and K. Yamada [18] reported that *Pseudomonas putida* can metabolize 4-chlorophenol to 4-chlorocatechol, which then employs the metacleavage enzyme 2,3-dioxygenase to produce 2-hydroxy-5-chloromuconic semialdehyde, which accumulates to 10% of the starting substrate. Free chloride amounting to 85% of the substrate is recovered, although a pathway for liberation of the chloride has not been elucidated.

U. Schwien, and E. Schmidt [19] found that *Pseudomonas* spp. B13 can utilize 4-chlorophenol as the sole source of carbon and energy, and with this substrate can cometabolize 2-chlorophenol and 3-chlorophenol completely without accumulation of metabolites. Two species of bacteria were utilized to produce a genetically constructed strain with altered ability to metabolize aromatic compounds. *Alcaligenes* sp. A7, which degrades phenol by the meta pathway and has no activity against chlorophenols, was combined with genetic material from *Pseudomonas* sp. B13 to produce a mutant (designated A7-2) which utilizes phenol by the ortho pathway and also metabolizes 2-,

3-, and 4-chlorophenol as well as 3-chlorobenzoic acid. Three enzymes were isolated, pyrocatechase II and cycloisomerase II, which have high activity for chlorinated substrates, and a third enzyme which functions exclusively in the chloroaromatic pathway to perform a dehalogenating cycloisomerization of chloromuconic acids.

B. R. Folsom, P. J. Chapman, P. H. Pritchard [20] observed that intact cells of *Pseudomonas cepacia* G4 completely degraded trichloroethylene (TCE) following growth with phenol. Degradation kinetics were determined for both phenol, used to induce requisite enzymes, and TCE, the target substrate. Apparent K_S and V_{max} values for degradation of phenol by cells were 8.5μM and 466 nmol/min per mg of protein, respectively. At phenol concentrations greater than 50μM, phenol degradation was inhibited, yielding an apparent second-order inhibitory value, K_{SI}, of 0.45mM as modelled by the Haldane expression. A no-headspace bottle assay was developed, allowing for direct and accurate determinations of aqueous TCE concentration. By this assay procedure, apparent K_S and V_{max} values determined for TCE degradation by intact cells were 3 μM and 8 nmol/min per mg of protein, respectively. Following a transient lag phase, *P. cepacia* G4 degraded TCE at concentrations of at least 300 μM with no apparent retardation in rate. Consistent with K_S values determined for degradation, TCE significantly inhibited phenol degradation.

The kinetics of simultaneous mineralization of p-nitrophenol (PNP) and glucose by *Pseudomonas* sp. which were studied by S. K. Schmidt, K.M. Scow, M. Alexander [21] were evaluated by nonlinear regression analysis. *Pseudomonas* sp. did not mineralize PNP at a concentration of 10 ng/ml but metabolized it at concentrations of 50 ng/ml or higher. The K_S value for PNP mineralization by *Pseudomonas* sp. was 1.1 μg/ml, whereas the K_S values for phenol and glucose mineralization were 0.1 and 0.25 μg/ml, respectively. The addition of glucose to the media did not enable *Pseudomonas* sp. to mineralize 10 ng of PNP per ml but did enhance the degradation of higher

concentrations of PNP. This enhanced degradation resulted from the simultaneous use of glucose and PNP and the increased rate of growth of Pseudomonas sp. on glucose. The dual-substrate model was used to analyze the data because the theoretical assumptions of the Monod equation were not met. Phenol inhibited PNP mineralization and changed the kinetics of PNP mineralization so that the pattern appeared to reflect growth, when in fact growth was not occurring. Thus, the fitting of models to substrate depletion curves may lead to erroneous interpretations of data if the effects of second substrates on population dynamics are not considered.

T. F. Hess, S. K. Schmidt, J. Silverstein, and B. Howe [22] studied *Janthinobacterium* sp. and an actinomycete, both capable of mineralizing 2,4-dinitrophenol (DNP), were used to construct a consortium to mineralize DNP in bench-scale sequencing batch reactors (SBRs). Average K_s values from the Michaelis-Menten model for DNP mineralization by pure cultures of the *Janthinobacterium* sp. and the actinomycete were 0.01 and 0.13 μg/ml, respectively, and the average maximum specific growth rate (μ_{max}) values were 0.06 and 0.23/h, respectively. In the presence of added nitrogen as NH_4Cl, nitrite accumulation in pure culture experiments and in the SBRs was stoichiometric to initial DNP concentration, and the addition of nitrogen enhanced DNP utilization. In the absence of added nitrogen, nitrite accumulation was much less (presumably because of utilization of the nitrogen in DNP), but the utilization rate was also lower. Mineralization of 10 μg of DNP per ml was further enhanced in SBRs by the addition of glucose at concentrations 100 and 500 ppm, but not at 10 ppm. Possible mechanisms for this enhanced DNP mineralization in SBRs were suggested by kinetic analysis and biomass measurements. Average μ_{max} values for DNP mineralization in the presence of 0, 10, 100, 500 μg of glucose per ml were 0.33, 0.13, 0.42, 0.59/h, respectively. In addition, there was greater standing biomass in reactors amended with glucose. At steady-state operation, all SBRs contained

heterogeneous microbial communities but only one organism, an actinomycete, that was capable of mineralizing DNP. This research demonstrates the usefulness of supplemental substrates for enhancing the degradation of toxic chemicals in bioreactors that contain heterogeneous microbial communities.

3. OBJECTIVES

* Extend the results of a previous study, involving a single microbial species and a single pollutant to two pollutants (phenol and 4-chlorophenol).

* Describe the biodegradation of the above in a sequencing batch reactor (SBR) with general mathematical models.

* Study the systems numerically for given kinetics under various operating conditions.

* Test the models experimentally.

* Use the models to design optimization studies in the future.

4. DERIVATION OF THE MATHEMATICAL MODEL

The equations describing the system at any instant of time are the following :

$$\frac{d\,V}{d\,t} = Q_f - Q \qquad (1)$$

The equation above is written under the assumption of constant density, and represents an overall mass-balance.

The symbols appearing in equation (1) are defined as follows :

V = working volume of the reactor.

Q_f = volumetric flow rate of the stream fed into the reactor.

Q = volumetric flow rate of the stream exiting the reactor.

Mass balance on biomass (b) :

$$\frac{d\,(V\,b)}{d\,t} = Q_f\,b_f - Q\,b + \mu_1\,b\,V + \mu_2\,b\,V$$

or

$$V\,\frac{d\,b}{d\,t} + b\,\frac{d\,V}{d\,t} = Q_f\,b_f - Q\,b + \mu_1\,b\,V + \mu_2\,b\,V$$

or, by using equation (1)

$$\frac{d\,b}{d\,t} = \frac{Q_f}{V}\,(b_f - b) + \mu_1\,b + \mu_2\,b \qquad (2)$$

The symbols appearing in equation(2) are defined as following :

b = concentration of biomass in the reactor, and in the stream exiting the reactor.

b_f = concentration of biomass in the feed to the reactor. For all practical purposes, unless there is a recycle of solids to the reactor, the value of this quantity is zero.

μ_1 = specific growth rate of population b on substrate 1.

μ_2 = specific growth rate of population b on substrate 2.

Mass balance on the rate-limiting substrates (i.e., on the toxic substances which are treated in the unit) :

$$\frac{d\,s_1}{d\,t} = \frac{Qf}{V}(s_{1f} - s_1) - \frac{\mu_1\, b}{Y_1} - \frac{\mu_2\, b}{Y_1} \qquad (3)$$

$$\frac{d\,s_2}{d\,t} = \frac{Qf}{V}(s_{2f} - s_2) - \frac{\mu_1\, b}{Y_2} - \frac{\mu_2\, b}{Y_2} \qquad (4)$$

The symbols not previously introduced and showing in equations (3) and (4) are defined as follows :

s_{1f} = concentration of toxic substance 1 in the waste fed to the reactor.

s_{2f} = concentration of toxic substance 2 in the waste fed to the reactor.

s_j = concentration of toxic substance j in the reactor and in the stream exiting the reactor (j = 1 or 2).

Y_j = yield coefficient of population b on the different toxic substances, (j = 1 or 2).

There are some zero terms in above equations during some of the phases of SBR operation. For example, there is no input or output from the reactor during the react phase. This becomes clear in the following sections when each phase of operation is described separately.

Experimentally, it was observed in the present study that the rate of phenol utilization by *Pseudomonas putida* (ATCC 17514) was not affected by the presence of 4-chlorophenol (4CP). Furthermore, the organism grew on phenol only, and not on 4CP. Finally, the rate of 4CP degradation appeared to be a function of the concentration of phenol-induced enzymes.

With these observations in mind, the following equations were proposed in the present study :

Specific growth rate on phenol :

$$\mu_1 = \frac{\hat{\mu}_1 \, s_1}{K_{s1} + s_1 + \frac{s_1^2}{K_1}}$$ (Andrews model for an inhibitory substrate)

Specific growth rate on 4-CP :

$$\mu_2 = 0$$

Pseudo yield coefficient on 4CP :

$$Y_2 = \frac{K_{S2} + s_2}{\alpha \, s_2}$$ (assumed expression)

where,

$\hat{\mu}_1$ = characteristic constant having units of inverse time.

K_{sj} = constant having units of concentration, (j = 1 or2)

K_I = inhibition constant for population b (with units of concentration).

Equations (1) to (4) can be written in dimensionless form as following :

$$\frac{d V'}{d \theta} = Q_f' - Q' \tag{5}$$

$$\frac{d u}{d \theta} = \frac{Q_f'}{V'} (u_f - u) - x \bar{\mu} \tag{6}$$

$$\frac{d v}{d \theta} = \frac{Q_f'}{V'} (v_f - v) - x \alpha \rho \frac{\bar{\mu} v}{(1 + v)} \tag{7}$$

$$\frac{d x}{d \theta} = - \frac{Q_f'}{V'} x + x \bar{\mu} \tag{8}$$

$$\bar{\mu} = \frac{u}{1 + u + \omega u^2}$$

introduce :

$\frac{V}{V_{max}}$ = V' = dimensionless working volume of the reactor.

$\frac{s_1}{K_{s1}}$ = u = dimensionless concentration of phenol in the reactor.

$\frac{s_{1f}}{K_{s1}}$ = u_f = dimensionless concentration of phenol in the feed stream.

$\frac{b}{Y_1 K_{s1}}$ = x = dimensionless concentration of biomass in the reactor.

$\frac{S_2}{K_{s2}} = v =$ dimensionless concentration of 4CP in the reactor.

$\frac{S_{2f}}{K_{s2}} = v_f =$ dimensionless concentration of 4CP in the feed stream.

$\frac{Q_f}{Q_f^* \sigma_1} = Q_f' =$ dimensionless volumetric flow rate of the feed stream.

$\frac{Q}{Q_f^* \sigma_1} = Q' =$ dimensionless volumetric flow rate of the discharge stream.

$\frac{t\, Q_f^* \sigma_1}{Vmax} = \theta =$ dimensionless time.

$\frac{K_{s1}}{K_{I1}} = \omega =$ dimensionless inverse inhibition constant for phenol.

$\frac{\hat{\mu}_1 V_{max}}{Q_f^* \sigma_1} = \beta =$ a dimensionless measure of the hydraulic residence time.

$\frac{K_{s1}}{K_{s2}} Y_1 = \rho =$ ratio of model constants times yield coefficient for phenol.

Yield coefficients for phenol (Y_1) were determined from the slope of biomass concentration vs. phenol concentration plots (Figure 4). Andrews parameters for phenol ($\hat{\mu}_1$, K_{S1}, K_{I1}) were determined from a plot of specific growth (μ_1) vs. the average phenol concentration ($\bar{s}_1$) during the log growth phase (Figures 5.1 and 5.2).

Figure 1.1-(b) indicates the way the volume of the system changes with time during the various phases in which there is no settling or idle period in the present study. The volume and volumetric flow rates appearing in equations (1) through (4) can be expressed as follows for the various phases :

(a) Fill phase ($0 < t \leq t_1$)

$$Q_f = Q_{f,sbr} \; ; \; Q = 0 \; ; \; V = V_o + Q_f \, t$$

(b) React phase ($t_1 \leq t \leq t_2$)

$$Q_f = 0 \; ; \; Q = 0 \; ; \; V = V_{max}$$

(c) Draw phase ($t_2 \leq t \leq t_3$)

$$Q_f = 0 \; ; \; Q = Q \; ; \; V = V_{max} - Q \, (t - t_2)$$

where,

V_{max} is the maximum working volume i.e., the volume of the system at the end of the fill phase.

V_o is the volume of the system at the end of the draw phase.

$Q_{f,sbr}$ is the volumetric flow rate of the feed to SBR.

Using the dimensionless forms in these three periods (see [13] for more detail description) :

(a) Fill phase : $0 < \theta \leq (1 - \delta) \, \sigma_1$

$$Q_f' = \frac{1}{\sigma_3} \; ; \; Q' = 0 \; ; \; V' = \delta + \frac{1}{\sigma_1} \theta$$

(b) React phase : $(1 - \delta) \, \sigma_1 \leq \theta \leq (1 - \delta) \, (1 - \sigma_3)$

$$Q_f' = 0 \; ; \; Q' = 0 \; ; \; V' = 1$$

(c) Draw phase : $(1 - \delta)(1 - \sigma_3) \leq \theta \leq (1 - \delta)$

$$Q_f' = 0 \; ; \; Q' = \frac{1}{\sigma_3} \; ; \; V' = 1 - \frac{1}{\sigma_3}[\theta - (1 - \delta)(1 - \sigma_3)]$$

By defining,

$\sigma_1 = \frac{t_1}{t_3}$ = fraction of total cycle time devoted to the fill phase.

$\sigma_2 = \frac{t_2 - t_1}{t_3}$ = fraction of total cycle time devoted to the react phase.

$\sigma_3 = \frac{t_3 - t_2}{t_3}$ = fraction of total cycle time devoted to the draw phase.

In view of the above, equations (5) through (8) can be written as follows :

(i) Fill phase, $0 < \theta \leq (1 - \delta)\sigma_1$

$$\frac{d\,u}{d\,\theta} = \frac{u_f - u}{\sigma_1\delta + \theta} - x\,\bar{\mu} \tag{9}$$

$$\frac{d\,v}{d\,\theta} = \frac{v_f - v}{\sigma_1\delta + \theta} - x\,\alpha\,\rho\,\frac{\bar{\mu}\,v}{(1 + v)} \tag{10}$$

$$\frac{d\,x}{d\,\theta} = \frac{-x}{\sigma_1\delta + \theta} + x\,\bar{\mu} \tag{11}$$

$$\bar{\mu} = \frac{\beta\,u}{1 + u + \omega\,u^2}$$

(ii) React and Draw phases, $(1 - \delta)\sigma_1 \leq \theta \leq (1 - \delta)$

$$\frac{d\,u}{d\,\theta} = -x\,\bar{\mu} \tag{12}$$

$$\frac{d\,v}{d\,\theta} = -x\,\alpha\,\rho\,\frac{\bar{\mu}\,v}{(1 + v)} \tag{13}$$

$$\frac{d\,x}{d\,\theta} = x\,\bar{\mu} \qquad (14)$$

In the formulation of the problem here, it has been assumed that the biodegradation reaction occurs not only during the react phase, but also during the fill and draw phases as well.

From the original formulation of the problem (equations (1) to (4)), one needs to specify the values of 14 parameters in order to solve the equations and predict the behavior of the system. These parameters are Vo, t_1, t_2, t_3, $\hat{\mu}_1$, K_{s1}, K_{s2} K_I, Y_1, Y_2, s_{1f}, s_{2f}, Q_f, α. In the final formulation of the problem (equations (9) through (14)) in terms of the dimensionless quantities (u, v, and x), one needs to specify the values of 7 parameters, that is, x_f, u_f, v_f, σ_1, σ_3, δ, α. This reduction in the number of parameters from 14 to 7, reduces tremendously the amount of numerical work which needs to be done in order to study the behavior of the system in full detail.

PHENOL BATCH DATA

$$\frac{d\,s}{d\,t} = -\frac{\mu\,b}{Y} = -\frac{\hat{\mu}\,s}{K_S + s + \frac{s^2}{K_I}}\;\frac{1}{Y}\;[Y\,(s_o - s) + b_o]$$

where, $b - b_o = Y\,(s_o - s)$

then,

$$\frac{(K_S + s + \frac{s^2}{K_I})\,Y}{\hat{\mu}\,[Y\,(s_o - s) + b_o]}\,d\,s = -\,d\,t$$

To be integrated between s_o to s, and 0 to t, then the expression is given as following :

$$t = \frac{1}{\hat{\mu}} \ln \left[\frac{b_o + Y(s_o - s)}{b_o}\right] + \frac{1}{Y}\frac{1}{\hat{\mu} K_I}\{(Ys_o + b_o) \ln \left[\frac{b_o + Y(s_o - s)}{b_o}\right] - Y(s_o - s)\} - \frac{Y K_S}{\hat{\mu}} \frac{1}{Ys_o + b_o} \ln \left[\frac{s\, b_o}{s_o (b_o + Y(s_o - s))}\right]$$

The comparisons of the experimental data and predicted results for phenol concentration vs. time are shown in Figure 6.

$$\frac{d\,b}{d\,t} = \mu\, b = \frac{\hat{\mu}\, s}{K_s + s + \frac{s^2}{K_I}} [Y(s_o - s) + b_o]$$

then,

$$\frac{1}{\hat{\mu}} \left[\frac{YK_s}{b(Ys_o + b_o - b)} + \frac{1}{b} + \frac{Ys_o + b_o - b}{bY}\right] d\,b = d\,t$$

To be integrated between b_o to b and 0 to t, then the expression is given as following :

$$t = \frac{1}{\hat{\mu}} \frac{YK_s}{(Ys_o + b_o)} \ln \left[\frac{bYs_o}{b_o (Ys_o + b_o + b)}\right] + \frac{1}{\hat{\mu}} \ln \frac{b}{b_o} \left[1 + \frac{Ys_o + b_o}{Y K_I}\right] - \frac{1}{\hat{\mu}\, Y K_I} (b - b_o)$$

The comparisons of the experimental data and predicted results for biomass concentration vs. time are shown in Figure 7.

5. EXPERIMENTAL APPARATUS

A. Batch Systems

There were two batch reactors in all experiments. Batch system A consisted of 250 ml flasks placed on a controlled environment incubator shaker (Model # G-25) at 28°C. The only aeration was transferred through the cotton plug by shaking. Batch system B was a 15-cm diameter, 5-liter capacity, cylindrical vessel constructed of Lucite, capped with a removable lid. Aeration was provided by laboratory compressed air passed through a series of filters, and activated carbon, and then through a bottom diffuser. Batch system B experiments were conducted at room temperature (approximately 21°C).

B. Sequencing Batch Reactor (SBR) System

All experiments were run at room temperature (approximately 21°C). The reactor was a 15-cm ID, 5-liter Lucite cylindrical vessel, capped with a removal lid. An effluent port was installed two liter above the bottom, with a solenoid valve to control the discharge of treated wastewater. Laboratory compressed air was passed through a series of filters, and activated carbon, before entering the reactor through a bottom diffuser. The volume of air was regulated by two needle-valve rotameters, with a solenoid valve on each air line. To increase the contact efficiency between air and liquid, a porous diffuser stone was placed on the end of each air line at the bottom of the reactor. Aeration also provided the only agitation. There was no mechanical stirring.

A microprocessor (Omron, Sysmac-PO sequence controller) controlled the system which included feed peristaltic pump, air solenoid valves, and decant solenoid valve. Any combination of time periods associated with fill, react, and draw phases could be programmed into the microprocessor. The output setting and programming of the sequence controller are described in detail in the Appendix A of [17].

C. Analytical Equipment

Waters High-Performance Liquid Chromatograph System, with a Waters 600E System Controller, Waters 715 Ultra Wisp Sample Processor, Waters Tunable Absorbance Detector.

Column : Alltech Econosphere C8 5μ, 4.6mm i.d. x 150 mm (Cat.#70090)

Alltech Direct Connect Refillable Guard Column : using for protecting column

Orion Model SA720 pH\ISE meter for measuring DO value, by using O_2 electrode (Model # 97-08-99)

Orion Model EA920 Expandable ionAnalyzer for detecting pH value with Model 91-56 combination pH electrode.

Varian DMS 200 UV-VISIBLE Spectrophotometer.

6. EXPERIMENTAL PROCEDURES

6.1 Acclimatizing the Culture in Phenol Only

In order to have an unambiguous test of the mathematical model, it was necessary to obtain well-defined, constant rate parameters for the microbial population employed in the reactor. As a result, a pure culture of *Pseudomonas putida* (ATCC 17514) was used in this study. The growth parameters were obtained from experiments in batch system B.

A stock culture was prepared by transferring a loop of dried biomass into BBL nutrient broth and placed in incubator for about 24 hours, then stored at 4°C in the refrigerator. The primary culture was prepared by transferring 2 ml stock culture to 88 ml of sterilized defined medium solution, and 10 ml of phenol stock solution of 1000 ppm (i.e. final concentration of 100 ppm phenol). The inoculated culture was then placed in 250 ml flasks in the incubator shaker (at 250 rpm) for 12 hours at 28°C. The medium was aerated by virtue of the shaking process only.

A secondary culture was prepared by transferring 10 ml of primary solution to 80 ml of sterilized defined medium, and 10 ml of phenol stock solution of 1000 ppm (i.e. final concentration of 100 ppm phenol). The inoculated culture was then placed in 250 ml flasks stoppered with cotton plugs, and incubated for 12 hours at 28°C in a shaker apparatus at 250 rpm. The procedure was repeated for a tertiary culture, in order to ensure that the culture had fully adapted to growth on the phenol medium, and that phenol was the sole carbon source. Any desired phenol concentration in the primary, secondary, and tertiary cultures could be prepared by adjusting the volume of phenol stock solution.

For all experiments, in order to prevent contamination, all glassware (such as flasks, pipets, etc.) were autoclaved at 121 °C under pressure, while the Lucite reactors were washed thoroughly with 75% methanol solution.

6.2 Acclimatizing the Culture in 4-Chlorophenol (4CP) Only

The process of preparing primary, secondary, and tertiary cultures with 4CP was the same as above, except that 4CP at a concentration of 20 ppm was the sole carbon source.

6.3 Acclimatizing the Culture in Both Phenol and 4-Chlorophenol

Two procedures were tested :

(1) The same procedure of section 6.1 using the phenol-acclimated tertiary culture, followed by development of secondary and tertiary cultures on 4CP as sole carbon source.

(2) The same procedure of section 6.1 using both phenol (at 100 ppm) and 4CP (at 20 ppm) to prepare primary, secondary, and tertiary cultures.

6.4 Formulation of Defined Medium

Many formulations of medium solutions had been proposed for which there was often little or no fundamental justification. The composition of the phenol defined medium solution used in the present study (Table 1) had been suggested by Gaudy (25),

and was also used for 4CP experiments. Ammonium sulfate /potassium phosphate provided nitrogen, phosphorus, and buffer.

6.5 Determination of Andrews Parameters

The growth parameters of a pure culture of *Pseudomonas putida* (ATCC 17514) were obtained from experiments at room temperature (approximately 21 °C) using the 5-liter Lucite reactor of batch system B on tertiary cultures. It involved measuring the optical density of the culture on exposure to different initial concentrations of phenol (Table 2). The optical density could be converted to biomass concentration by carrying out a calibration curve in Figure 2. Semi-log plots of biomass concentration (ppm) vs. time (hour) were used to determine the slope of the exponential growth phase at different initial phenol concentrations (Figure 3). The slope is the specific growth rate, which was then plotted vs. the average phenol concentration during the exponential growth phase (Figures 5-1 and 5-2). In addition, the yield coefficients (Table 3) were also determined by plotting the biomass concentration versus phenol concentration, as shown in Figure 4. Andrews parameters (Table 4) could then be obtained by regressing these data.

6.6 SBR Experiments

2 liters of defined medium solution were inoculated with a phenol-acclimated tertiary culture in the 5-liter Lucite reactor at room temperature (around 21 °C). Biomass was grown in a batch mode with phenol added periodically over approximately 6 hours. Once the biomass concentration reached a predetermined value, experiments were run with phenol only (Table 9.1), and with both phenol and 4CP (Table 10.2). A

stripping test was conducted before starting the SBR experiments (Table 10.1). There was no significant effect due to air stripping.

During the fill phase, the feed rate was fixed while increasing the reactor volume from 2 to 4 liters. The aeration rate of one air line was 350 cc/min, and there were two air lines for each SBR experiment. The dissolved oxygen (DO) concentrations in the reactor were in the range 6 to 8 mg/l in SBR 1, and 7 to 9 mg/l in SBR 2. The pH was kept constant at 7.1 by using potassium phosphate buffer. DO and pH values were monitored continuously at all times during the cycles.

In the react phase, the feed pump was shut off, aeration continued, and samples taken periodically for optical density and substrate concentration. At the end of the react phase, aeration was still continued, and the decant solenoid valve was opened to completely discharge 2 liters of solution without input. There was a predicted loss of biomass during withdrawal of the mixed liquor. The biomass could be recovered due to growth during the fill and react phases. After draw-down was complete, the cycle started over again with fill. In a real operation, there would be a quiescent settling period with the air off before draw down. However, since only a kinetic model is presented here, aeration was continued.

It was necessary to streak a loop of solution on a nutrient agar plate (incubated at 30°C for one day) to determine whether any significant contamination had occurred during each cycle.

6.7 Analytical Procedures

6.7.1 OD/Biomass Calibration Curve

The growth of microbes was determined by measuring the optical density (OD) of the mixed liquor using a spectrophotometer at a wavelength of 540 nm, and deionized water as the reference sample. The optical density could be converted into biomass concentration using a calibration curve which was obtain earlier [23], and confirmed in this study through the following procedures. *Pseudomonas putida* (ATCC 17514) was grown in a BBL nutrient broth, harvested towards the end of the logarithmic growth phase (after about 36 hours), and diluted in different ratios. The turbidity of each dilution (1/10, 2/10, 3/10, 4/10, 5,10) was determined spectrophotometrically.

Three samples (10ml, 15ml, 20ml) were taken from the original, undiluted culture solution to determine the dry weight of cell mass. These samples were pipetted into three numbered, preweighed aluminum dishes, then dried in an oven at 95 °C for 24 hours. Before reweighing, the dishes were put in a desiccator and allowed to cool for about 30 minutes. The difference in weight between dried dish and tare determined the biomass concentration. The biomass concentration of each serially diluted sample was then determined by dividing the dry weight by each dilution ratio. The data points of the confirmation test are also shown in Figure 2, which coincided with the previous calibration curve.

6.7.2 Substrate Analysis

The instrument using for substrate analysis was a Waters HPLC. Mobile phase A (1% acetic acid in methanol) : Mobile phase B (1% acetic acid in Mill-Q water). Ratio of A : B was 43 : 57, run isocratically. The flow rate was 1 ml/min. The UV detector was set at 280 nm, 0.5 AUFS. The data were processed by PE Nelson chromatography software rev 5.10, interfaced with 760 series Model 2600.

Calibration curves for phenol and 4CP were needed before running any experiment. There were six standard points employed for each substrate calibration curve. At the beginning of each HPLC run, standards of 50ppm of phenol and 4CP were injected to check the calibration curves. Right after the optical density was measured, one drop of 6N HCl was added to each culture sample to kill the microorganisms and shift the phenolics to the unionized form. The sample was then filtered through a 0.45 μm millipore filter paper to remove the microbes and other suspended substances, in order to prevent plugging of the chromatographic column. The prepared samples were then run immediately on the HPLC.

7. RESULTS AND DISCUSSIONS

7.1 Andrews Model Parameters of *Pseudomonas putida* (ATCC 17514) in Batch Experiments Using Phenol only

From batch runs in the 5-liter reactor, results were obtained for the specific growth rates, yield coefficients, and Andrews parameters for *Pseudomonas putida* (ATCC 17514) utilizing phenol as the sole carbon source. The Lucite reactor was used in order to be certain that there was no oxygen limitation, such as might exist in the shaker flasks. These data are given in Tables 2 and 3 for phenol concentrations of 15 ppm to 130 ppm, and the results are shown in Figures 3 and 4. Differences in the number of points measured during the exponential growth phase, and the corresponding variations in the average phenol concentration, resulted in some variation in the regressed Andrews parameters (Table 4, Figures 5.1 and 5.2). Case B uses the entire data set shown in Figure 3, while case A uses only the initial slope during the exponential growth phase.

In the present study, a new method was developed to detect the reliability of Andrews kinetics parameters obtained from regression of specific growth rates versus phenol concentrations. The new approach is to compare the theoretical with experimental data for phenol consumption or biomass growth vs. time. The results are shown in Figure 6 (phenol consumption rate) and Figure 7 (biomass growth rate). Biomass growth data were always fit by the regressed Andrews parameters better than phenol consumption data. A possible reason is that the OD values (biomass concentrations) were detected right after the samples were taken from the reactors, while phenol concentrations must wait about 15 - 30 mins before running the HPLC. In

the mean while, there may be some active enzyme still available for phenol degradation. As a result, the experimental phenol concentrations would be smaller than the predicted values (except for Run 6-3b), and this error would be more pronounced at low concentrations.

7.2 Batch Experiments Using 4-Chlorophenol only

Biomass was acclimated to 4CP only, without phenol pretreatment, which was followed by the procedure in section 6.2. The results are shown in Table 5. For the runs shown in Table 6, the biomass was first acclimated to phenol as per the procedure in section 6.3-(1). Both acclimation methods were unable to sustain degradation of 4CP. However, the results shown in Table 6 suggested that continued 4CP degradation would require the presence of phenol.

7.3 Evidence for Phenol Inducing Enzyme Utilized for Degradation of 4-Chlorophenol

The following experiments were studied to further confirm the need for a phenol-induced enzyme to degrade 4CP. All experiments were conducted at a total solution volume of 100 ml, including growth medium, biomass, and phenol/4CP stock solution.

Data shown in Tables 7, 7.1, and 7.2 prove that 4CP cannot induce the enzymes needed to initiate biodegradation with the organism. In Table 7, degradation did not take place until after phenol addition. Further more, 4CP could be degraded

immediately after prior phenol exposure, but continued spiking with 4CP alone resulted in no further degradation.

7.4 Degradation Trend of 4CP with Phenol, and Effect of Phenol/4CP Concentration on Optical Density (Biomass Concentration)

Phenol and 4CP simultaneously were put into solution in different concentration ratios. Results are shown in Table 8. In run 8-1, the degradation of phenol was faster than 4CP, even though the phenol concentration was two times greater. Also the data trend in Figure 8 showed that there was almost no degradation of 4CP in the beginning of the run, indicating a lag period and diauxic phenomena. In runs 8-4 to 8-6, the phenol concentrations were reduced to less than that of 4CP. This proved to be phenol-deficient, since as soon as the phenol was depleted the degradation rate of 4CP slowed down.

Normally, a degradation of 10ppm phenol would increase optical density about 0.020 units. Runs 8-4 to 8-6 show only a minor increase of about 0.001 to 0.003 OD units after phenol was exhausted, even though more than 10 ppm 4CP were subsequently degraded. This also indicated that 4CP could not support growth of *Peudomonas putida* by itself.

7.5 Phenol Run in SBR

The phenol-acclimated (section 6.1) pure culture was utilized in the SBR with phenol as the sole carbon source. The experimental conditions and results are shown in Tables 9 and 9.1. Figures 9.1 to 9.4 compare theoretical curves based on the batch

Andrews parameters with experimental points in the SBR. The Andrews parameters which best represented the SBR data were based on the initial slope in the exponential growth phase. The transient cycle and steady cycle of phenol and biomass concentration versus time are shown in Figures 9.1, 9.2, and 9.3, 9.4 respectively. In SBR Run 1, it required about 7 cycles to reach steady cycle. There was good agreement between theory and experiment.

7.6 Phenol and 4CP Run Simultaneously in SBR

The phenol and 4CP-acclimated (section 6.3-(2)) pure culture was utilized in SBR Run 2 with both phenol and 4CP as the carbon sources. The experimental conditions and results are shown in Tables 10 and 10.2. Figures 10.1 to 10.4 compare theoretical curves based on the batch Andrews parameters, with experiments using 60 ppm phenol and 20 ppm 4CP in the feed. The results of the transient cycle and steady cycle are shown in Figures 10.1, 10.2, and 10.3, 10.4 respectively. It required about 6 cycles or less to reach the steady cycle, due to the initial biomass concentration being close to the steady-state value.

The SBR experimental data for phenol alone (both biomass and substrate) agreed very well with the predicted curves based on batch parameters. However, the experimental data for 4CP did not match well with the fitted curve, especially on the first cycle (Figure 10.1). It was therefore postulated that there was a 30 minutes time lag between a given phenol concentration and its corresponding enzyme concentration. This improved the fit (Figures 10.5 and 10.6), although there is still some discrepancy.

8. CONCLUSIONS AND RECOMMENDATIONS

* *Pseudomonas putida* (ATCC 17514) can degrade phenol as sole carbon source, but can not degrade 4-Chlorophenol (4CP) as sole carbon source.

* 4CP can be degraded only in the presence of phenol, apparently due to enzyme induction by phenol.

* 4CP does not contribute to biomass growth.

* A mathematical model was derived, which describes this process.

* The model was verified experimentally in a SBR.

* It is recommended that a previously derived mixed population model be extended to included mixed substrates in order to build a model that is more representative of real applications.

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TABLE 1. PHENOL AND 4CP DEFINED MEDIUM SOLUTION

AMMONIUM SULFATE	500 mg
MAGNESIUM SULFATE	100 mg
FERRIC CHLORIDE	0.5 mg
MANGANESE SULFATE	10 mg
1.0M POTASSIUM PHOSPHATE BUFFER SOLUTION (pH 7.2)	50 ml
TAP WATER	100 ml
DISTILLED WATER	add to volume of 1.0 liter

Table 2.

OPTICAL DENSITY AND SUBSTRATE CONCENTRATION V.S. TIME
(*Pseudomonas putida*, ATCC17514)

RUN 2-1

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)	DISSOLVED OXYGEN (PPM)
0.000	17.2	0.060	16.403	7.77
0.167	16.2	0.063	17.223	7.80
0.333	14.8	0.066	18.043	7.68
0.500	12.4	0.070	19.137	7.50
0.667	10.2	0.074	20.230	7.64
0.833	8.5	0.079	21.597	7.45
1.000	6.2	0.084	22.964	7.34
1.167	3.3	0.090	24.604	7.23
1.333	0.0	0.096	26.245	***
1.500	0.0	0.101	27.612	8.10

*** no data taken at this time

RUN 2-2

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)	DISSOLVED OXYGEN (PPM)
0.000	41.5	0.071	19.410	7.79
0.250	36.3	0.076	20.777	6.95
0.500	31.3	0.082	22.417	8.00
0.750	26.4	0.090	24.604	7.58
1.000	19.5	0.100	27.338	7.15
1.250	13.9	0.114	31.166	7.08
1.500	6.3	0.134	36.633	6.90
1.750	0.0	0.156	42.648	7.11
1.917	0.0	0.167	45.655	7.90

*** no data taken at this time

RUN 2-3

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)	DISSOLVED OXYGEN (PPM)
0.00	74.0	0.061	16.676	7.9
0.50	72.0	0.065	17.770	8.0
1.00	69.2	0.070	19.137	7.8
1.50	65.0	0.078	21.324	7.7
1.75	62.7	0.084	22.964	7.5
2.00	60.0	0.091	24.878	7.6
2.25	55.4	0.097	26.518	7.4
2.50	52.5	0.104	28.432	7.3
2.75	48.7	0.112	30.619	7.1
3.00	43.2	0.122	33.353	7.0
3.25	37.6	0.131	35.813	6.9
3.50	30.4	0.140	38.274	6.7
3.80	21.0	0.153	41.828	6.4
4.00	13.5	0.161	44.015	6.6
4.30	***	0.174	47.569	6.2

*** no data taken at this time

RUN 2-4

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)	DISSOLVED OXYGEN (PPM)
0.000	116.5	0.066	18.043	8.70
0.500	113.7	0.072	19.684	8.25
1.000	110.5	0.078	21.324	8.25
1.667	105.9	0.088	24.058	8.07
2.000	102.7	0.094	25.698	8.00
2.333	98.6	0.099	27.065	7.97
2.667	92.2	0.106	28.979	7.80
3.000	87.0	0.115	31.439	7.65
3.667	77.6	0.136	37.180	7.50
4.000	73.7	0.148	40.461	7.50
4.333	66.5	0.164	44.835	7.40
4.667	57.6	0.181	49.482	7.20
5.000	49.8	0.196	53.583	7.10
5.500	36.9	0.223	60.964	6.70
6.000	21.9	0.255	69.713	6.60
6.417	***	0.300	82.015	6.02

*** no data taken at this time

RUN 2-5

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)	DISSOLVED OXYGEN (PPM)
0.000	140.1	0.062	16.950	9.78
0.500	141.0	0.063	17.223	9.80
1.000	137.2	0.063	17.223	9.78
1.500	136.5	0.064	17.496	9.75
2.000	133.9	0.065	17.770	9.67
2.333	131.7	0.067	18.317	9.52
2.667	130.6	0.069	18.863	9.40
3.000	129.0	0.071	19.410	9.01
3.667	127.0	0.078	21.324	8.64
4.000	126.1	0.081	22.144	8.31
4.333	124.8	0.084	22.964	8.40
4.667	122.0	0.088	24.058	8.35
5.000	118.6	0.093	25.425	8.32
5.333	115.6	0.097	26.518	8.19
5.667	112.4	0.104	28.432	8.11
6.000	109.5	0.111	30.345	8.06
6.250	105.1	0.117	31.986	8.05
6.500	100.6	0.123	33.626	8.00
6.750	95.6	0.130	35.540	7.90
7.000	91.0	0.136	37.180	7.80
7.500	84.5	0.150	41.007	7.80
7.750	***	0.158	43.194	7.55
8.133	***	0.172	47.022	7.40

*** no data taken at this time

RUN 2-6

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY	BIOMASS CONCENTRATION (PPM)	DISSOLVED OXYGEN (PPM)
0.000	62.3	0.042	11.482	***
0.500	59.8	0.043	11.755	***
1.000	60.1	0.046	12.576	***
1.333	***	0.047	12.849	***
1.500	58.0	0.049	13.396	***
1.750	57.3	0.052	14.216	***
2.250	54.9	0.058	15.856	***
3.000	48.9	0.069	18.863	7.45
3.500	44.9	0.082	22.417	7.80
4.000	37.7	0.096	26.245	7.60
4.333	32.5	0.107	29.252	7.50
4.667	27.0	0.123	33.626	7.06
5.000	20.0	0.137	37.453	6.70
5.250	13.9	0.149	40.734	6.69
5.500	***	0.170	46.475	7.40

*** no data taken at this time

TABLE 3.1 SPECIFIC GROWTH RATE AND YIELD COEFFICIENT VS. PHENOL CONCENTRATION (INITIAL SLOPE IN EXPONENTIAL GROWTH PHASE)

NO.	PHENOL CONCENTRATION (PPM)	SPECIFIC GROWTH RATE IN EXPONENTIAL GROWING PERIOD, (1/HOUR)	YIELD COEFFICIENT	YIELD COEFFICIENT (AVERAGE)
1	13.20	0.324	0.568	
2	31.35	0.338	0.460	
3	62.30	0.264	0.547	**0.525**
4	101.35	0.179	0.548	
5	122.30	0.133	0.500	

TABLE 3.2 SPECIFIC GROWTH RATE AND YIELD COEFFICIENT VS. PHENOL CONCENTRATION (ALL DATA IN EXPONENTIAL GROWTH PHASE)

NO.	PHENOL CONCENTRATION (PPM)	SPECIFIC GROWTH RATE IN EXPONENTIAL GROWING PERIOD, (1/HOUR)	YIELD COEFFICIENT	YIELD COEFFICIENT (AVERAGE)
1	11.20	0.348	0.568	
2	25.10	0.404	0.460	
3	45.10	0.285	0.547	**0.537**
4	66.20	0.238	0.548	
5	106.75	0.169	0.500	
6	35.60	0.305	0.600	

TABLE 4. PARAMETERS OF ANDREWS MODEL

	$\hat{\mu}$ (1/HOUR)	K_s (PPM)	K_i (PPM)	CONCENTRATION 0F μ_{max}, (PPM)
INITIAL SLOPE	1.68	41.7	12.3	22.65
ALL DATA	1.55	27.9	12.4	18.60

TABLE 5 BATCH BIODEGRADATION OF 4CP WITHOUT PHENOL ACCLIMATION

Run 5-1

PROCESS DESCRIPTION	TIME (HOURS)	4CP CONCENTRATION (PPM)
primary culture on 4CP	0	19.3
	23	19.6
	111	13.2
	135	12.9
	159	9.8
secondary culture on 4CP	0	18.0
	24	22.2
	89	18.66
	141	21.3
	191	21.0

Run 5-2

PROCESS DESCRIPTION	TIME (HOURS)	4CP CONCENTRATION (PPM)
primary culture on 4CP	0	5.4
	22	0.0
add 4CP 0.2ml ** to primary culture	0	5.0
	17	3.1
	25	2.3
spike again with 4CP	0	6.8
	15	7.0

** 1000ppm of stock solution of4cp.

TABLE 6 BATCH BIODEGRADATION OF 4CP USING PHENOL ACCLIMATED TERTIARY CULTURES

Run 6-1

PROCESS DESCRIPTION	TIME (HOURS)	4CP CONCENTRATION (PPM)
48ml growth medium + 2ml 4cp** + 40ml tertiary culture on phenol	0	18.3
	5	7.2
add 1ml 4cp** to tertiary culture	0	16.0
	2	14.0
	13	0.0
spike again with 4cp**	0	25.0
	3	23.0
	4	22.0
	8	24.0

** 1000ppm of stock solution of 4cp.

Run 6-2

PROCESS DESCRIPTION	TIME (HOURS)	4CP CONCENTRATION (PPM)
88ml growth medium + 2ml 4cp** + 10ml tertiary culture on phenol	0.0	20.0
	8.5	15.0
	23.0	10.6
	32.0	10.3

** 1000ppm of stock solution of 4cp.

Run 6-3

PROCESS DESCRIPTION	TIME (HOURS)	4CP CONCENTRATION (PPM)
6ml growth medium + 4ml 4cp** + 90ml tertiary culture on phenol	0	33.0
	11	0.0
88ml growth medium + 2ml 4cp** + 10ml tertiary culture on phenol	0	21.4
	10	19.8
	23	19.9
	34	20.2

** 1000ppm of stock solution of 4cp.

Run 6-4

PROCESS DESCRIPTION	TIME (HOURS)	4CP CONCENTRATION (PPM)
86ml growth medium + 4ml 4cp** + 10ml tertiary culture on phenol	0	40.5
	11	40.1
	22	38.0
	35	33.0
	46	38.2

** 1000ppm of stock solution of 4cp.

TABLE 7 BATCH DATA SUGGESTING PHENOL-INDUCED ENZYMES

PROCESS DESCRIPTION	TIME (HOURS)	CONCENTRATION(PPM)	
		4CP	PHENOL
primary culture on 4CP	0	17.0	
	5	13.0	
	18	12.0	
secondary culture on 4CP	0	20.0	
	51	19.0	
add 15ml phenol* to secondary culture	0	18.5	138.5
	21	0.0	0.0

* 1000ppm of stock solution of phenol

THE LAST SOLUTION ABOVE WAS THEN UTILIZED BELOW.

TABLE 7.1

PROCESS DESCRIPTION	TIME (HOURS)	CONCENTRATION (PPM)	
		4CP	PHENOL
43ml growth medium+5ml phenol*+2ml 4cp**+50ml of last solution (table 7)	0	16.0	53.0
	24	1.0	0.0
add 2ml 4cp**	0	14.0	
	28	3.0	
48ml growth medium + 2ml 4cp** + 50ml of last solution (table 7)	0	16.0	
	9	15.5	
	40	19.0	
	67	19.0	
add phenol* 2ml	0	18.5	26.0
	16	0.0	0.0
38ml growth medium+10ml phenol*+2 ml 4cp**+50ml of last solution (table 7)	0	22.0	95.0
	24	1.0	0.0
add 2ml 4cp**	0	14.0	
	28	0.7	

* 1000ppm of stock solution of phenol
** 1000ppm of stock solution of 4cp.

THE LAST SOLUTION ABOVE WAS THEN UTILIZED BELOW

TABLE 7.2

PROCESS DESCRIPTION	TIME (HOURS)	CONCENTRATION (PPM)	
		4CP	PHENOL
60ml growth medium + 2ml phenol**+ 1ml 4cp** +37 ml of last solution (table 7.1)	0	12.0	16.0
	9	0.2	0.0
	22	0.0	0.0
add 2ml 4cp**	0	17.0	
	16	16.0	
	35	13.0	
add 2ml phenol*	0	13.0	25.0
	16	0.0	0.0
60ml growth medium + 1ml 4cp** + 39 ml of last solution (table 7.1)	0	13.5	
	9	16.0	
	22	11.0	
	40	14.0	
	59	12.5	
	78	13.0	
add phenol* 2ml	0	13.0	25.0
	16	0.0	0.0

* 1000ppm of stock solution of phenol

** 1000ppm of stock solution of 4cp.

TABLE 8
DEGRADATION OF PHENOL AND 4CP TOGETHER

RUN 8-1 35.5ML GROWTH MEDIUM + 1.5ML 4CP + 3ML PHENOL + 10ML ACCLIMATED CULTURE (EXPOSED THREE TIMES TO PHENOL @ 60 PPM, AND 4CP @ 30 PPM)

TIME (HOURS)	CONCENTRATION (PPM)		OPTICAL DENSITY (UOD)
	PHENOL	4CP	
0	60.0	29.0	0.105
1	58.8	28.9	0.110
2	54.8	26.4	0.116
4	46.0	26.3	0.129
5	38.7	24.5	0.156
6	20.0	23.6	0.196
7	4.6	15.3	0.211
8	0.0	3.1	0.230
8.5	0.0	1.5	0.241

RUN 8-2 37ML GROWTH MEDIUM + 1.5ML 4CP + 1.5ML PHENOL + 10ML ACCLIMATED CULTURE (EXPOSED THREE TIMES TO PHENOL @ 60 PPM, AND 4CP @ 30 PPM)

TIME (HOURS)	CONCENTRATION (PPM)		OPTICAL DENSITY (UOD)
	PHENOL	4CP	
0	30.0	27.9	0.105
1	29.7	28.6	0.109
2	27.5	25.7	0.114
4	17.6	23.4	0.129
5	11.8	20.7	0.145
6	2.4	11.0	0.163
7	0.0	1.7	0.169

RUN 8-3 37.75ML GROWTH MEDIUM + 1.5ML 4CP + 0.75ML PHENOL + 10ML ACCLIMATED CULTURE (EXPOSED THREE TIMES TO PHENOL @ 60 PPM, AND 4CP @ 30 PPM)

TIME (HOURS)	CONCENTRATION (PPM)		OPTICAL DENSITY (UOD)
	PHENOL	4CP	
0	15.2	27.3	0.107
1	15.0	27.7	0.112
2	13.9	25.8	0.115
4	9.5	24.4	0.128
5	6.5	19.7	0.132
6	2.0	13.8	0.139
7	0.0	5.7	0.142
8	0.0	3.3	0.143
9.5	0.0	1.0	0.143

RUN 8-4 38ML GROWTH MEDIUM + 1.5ML 4CP + 0.5ML PHENOL + 10ML ACCLIMATED CULTURE (EXPOSED THREE TIMES TO PHENOL @ 60 PPM, AND 4CP @ 30 PPM)

TIME (HOURS)	CONCENTRATION (PPM)		OPTICAL DENSITY (UOD)
	PHENOL	4CP	
0.0	11.8	26.2	0.066
2.0	10.5	27.3	0.068
3.0	9.5	26.2	0.068
4.0	9.1	26.7	0.070
5.5	6.6	23.5	0.073
6.5	5.4	23.2	0.075
7.5	3.3	17.7	0.076
8.5	1.5	16.2	0.075
9.5	0.5	14.0	0.076
10.5	0.0	9.4	0.078
11.5	0.0	5.4	0.078
12.5	0.0	3.8	0.079
15.5	0.0	0.7	0.080

RUN 8-5 37.25ML GROWTH MEDIUM + 2ML 4CP + 0.75ML PHENOL + 10ML ACCLIMATED CULTURE (EXPOSED THREE TIMES TO PHENOL @ 60 PPM, AND 4CP @ 30 PPM)

TIME (HOURS)	CONCENTRATION (PPM)		OPTICAL DENSITY (UOD)
	PHENOL	4CP	
0.0	16.0	36.0	0.066
2.0	15.0	37.0	0.068
4.0	14.5	36.5	0.072
5.5	12.9	37.0	0.072
7.5	8.5	34.3	0.073
8.5	5.9	30.7	0.081
9.5	3.4	26.0	0.081
10.5	1.2	19.5	0.084
11.5	0.1	14.6	0.083
12.5	0.0	10.8	0.084
15.5	0.0	1.9	0.085

RUN 8-6 38.75ML GROWTH MEDIUM + 1ML 4CP + 0.25ML PHENOL + 10ML ACCLIMATED CULTURE (EXPOSED THREE TIMES TO PHENOL @ 60 PPM, AND 4CP @ 30 PPM)

TIME (HOURS)	CONCENTRATION (PPM)		OPTICAL DENSITY (UOD)
	PHENOL	4CP	
0.0	5.7	17.1	0.066
2.0	4.9	17.5	0.067
3.0	4.3	17.2	0.068
4.0	3.7	16.6	0.068
5.5	2.4	14.4	0.069
6.5	1.4	11.2	0.071
7.5	0.4	8.4	0.071
8.5	0.0	5.9	0.072
9.5	0.0	4.7	0.071
10.5	0.0	2.0	0.072
11.5	0.0	1.2	0.073
12.5	0.0	1.2	0.072
15.5	0.0	0.4	0.070

TABLE 9. OPERATING CONDITIONS OF SBR RUN 1 IN PHENOL ONLY

EXPERIMENTAL CONDITIONS		EXPERIMENT CONDITIONS IN DIMENSIONLESS FORM
fill time	1 hours	0.400
react time	1 hours 20 mins	0.533
draw time	10 minutes	0.067
total cycle time	2.5 hours	1.000
phenol concentration in feed	94 ppm	2.254
initial phenol concentration	0 ppm	0.000
initial biomass concentration	60.144 ppm	2.747
initial reactor volume	2 liter	
volume after fill phase	4.0 liter	
volume after draw-down phase	2 liter	0.500
feed flow rate	2.0liter/hour	8.400

TABLE 9.1 EXPERIMENTAL RESULTS OF SBR RUN 1 IN PHENOL ONLY

CYCLE NO.	TIME (HOURS)	BIOMASS CONCENTRATION (PPM)	PHENOL CONCENTRATION (PPM)	DISSOLVED OXYGEN (PPM)	pH
1	0.00	60.144	0.0	***	***
	0.25	49.482	15.0	***	***
	0.50	45.288	21.0	7.9	7.12
	0.75	41.461	25.8	7.5	7.13
	1.00	40.547	28.2	6.0	7.10
	1.25	43.828	19.9	5.3	7.08
	1.50	47.108	12.6	5.0	7.11
	1.75	49.115	6.9	5.7	7.07
	1.92	52.000	2.1	5.4	7.10
	2.17	54.200	0.0	6.3	7.08
	2.33	55.240	0.0	***	***
1..2	2.5	53.350	0.0	***	***
6	0.00	48.4	0.0	***	***
	0.25	42.6	12.5	***	***
	0.50	38.7	22.2	7.8	7.14
	0.75	35.6	28.5	7.7	7.14
	1.00	34.0	32.5	7.5	7.13
	1.25	37.1	26.8	7.5	7.11
	1.50	39.0	20.2	7.1	7.13
	1.75	42.1	14.5	7.2	7.10
	2.00	46.7	8.7	7.2	7.12
	2.17	48.6	4.2	7.0	7.09
	2.33	50.5	0.0	6.7	7.08
6..7	2.50	49.9	0.0	7.3	7.11

*** no data taken at this time

continued..

continued TABLE 9.1

CYCLE NO.	TIME (HOURS)	BIOMASS CONCENTRATION (PPM)	PHENOL CONCENTRATION (PPM)	DISSOLVED OXYGEN (PPM)	pH
7	0.00	49.7	0.0	7.3	7.11
	0.25	42.6	13.0	***	***
	0.50	39.9	21.5	7.7	7.09
	0.75	26.9	27.0	8.3	7.07
	1.00	34.2	32.3	7.4	7.10
	1.25	36.5	25.5	7.3	7.10
	1.50	38.5	19.5	7.2	7.07
	1.75	40.9	13.1	7.1	7.05
	2.00	44.5	7.8	6.8	7.06
	2.17	47.4	4.0	6.6	7.05
	2.33	49.2	0.0	***	***
7..8	2.50	50.1	0.0	***	***

*** no data taken at this time

TABLE 10. OPERATING CONDITION OF SBR RUN 2 IN BOTH PHENOL AND 4CP

EXPERIMENTAL CONDITIONS		EXPERIMENT CONDITIONS IN DIMENSIONLESS FORM
fill time	1 hours	0.333
react time	1 hours 50 mins	0.611
draw time	10 minutes	0.056
total cycle time	3 hours	1.000
phenol concentration in feed	61.3 ppm	1.470
4CP concentration in feed	19.4 ppm	
initial phenol concentration	0 ppm	0.000
initial 4CP concentration	0 ppm	0.000
initial biomass concentration	33.35 ppm	1.523
initial reactor volume	2 liter	
volume after fill phase	4 liter	
volume after draw-down phase	2 liter	0.500
feed flow rate	2 liter/hour	10.080

TABLE 10.1 STRIPPING TEST OF PHENOL AND 4CP

TIME (HOURS)	CONCENTRATION (PPM)	
	PHENOL	4CP
0.0	67.6	25.6
6.5	67.3	26.4

TABLE 10.2 EXPERIMENTAL RESULTS OF SBR RUN 2 IN BOTH PHENOL AND 4CP

CYCLE NO.	TIME (HOURS)	BIOMASS CONCENTRATION (PPM)	SUBSTRATE CONCENTRATION		DISSOLVED OXYGEN (PPM)	pH
			PHENOL	4CP		
1.	0.00	33.353	0.0	0.0	6.9	7.02
	0.25	27.065	10.9	5.7	8.2	7.01
	0.50	25.471	14.4	7.6	8.5	7.04
	0.75	24.878	17.5	9.1	8.6	7.05
	1.00	23.784	18.9	9.9	***	***
	1.25	25.698	16.5	9.6	8.3	7.03
	1.50	27.065	13.2	9.3	8.2	7.02
	1.75	28.432	10.3	8.8	***	***
	2.00	29.525	6.6	7.8	***	***
	2.25	30.892	4.2	7.1	8.0	7.00
	2.50	31.712	2.2	5.3	7.4	6.98
	2.83	33.899	0.0	3.1	6.9	6.90
1..2	3.00	33.626	0.0	2.4	8.2	7.05
6.	0.00	33.353	0.0	2.4	8.2	7.05
	0.25	27.065	10.5	5.7	8.5	7.07
	0.50	25.698	14.6	7.8	8.7	7.08
	0.75	24.331	17.3	8.9	***	***
	1.00	23.511	18.7	9.8	8.3	7.02
	1.25	24.604	16.2	9.5	7.7	7.03
	1.50	26.792	12.5	8.9	7.5	6.95
	1.75	28.158	9.8	8.2	7.1	6.91
	2.00	28.979	7.2	7.6	***	***
	2.25	30.072	3.5	6.4	***	***
	2.50	31.712	1.6	5.0	***	***
	2.83	33.353	0.0	2.8	8.4	7.10
6..7	3.00	33.353	0.0	1.8	***	***

*** no data taken at this time

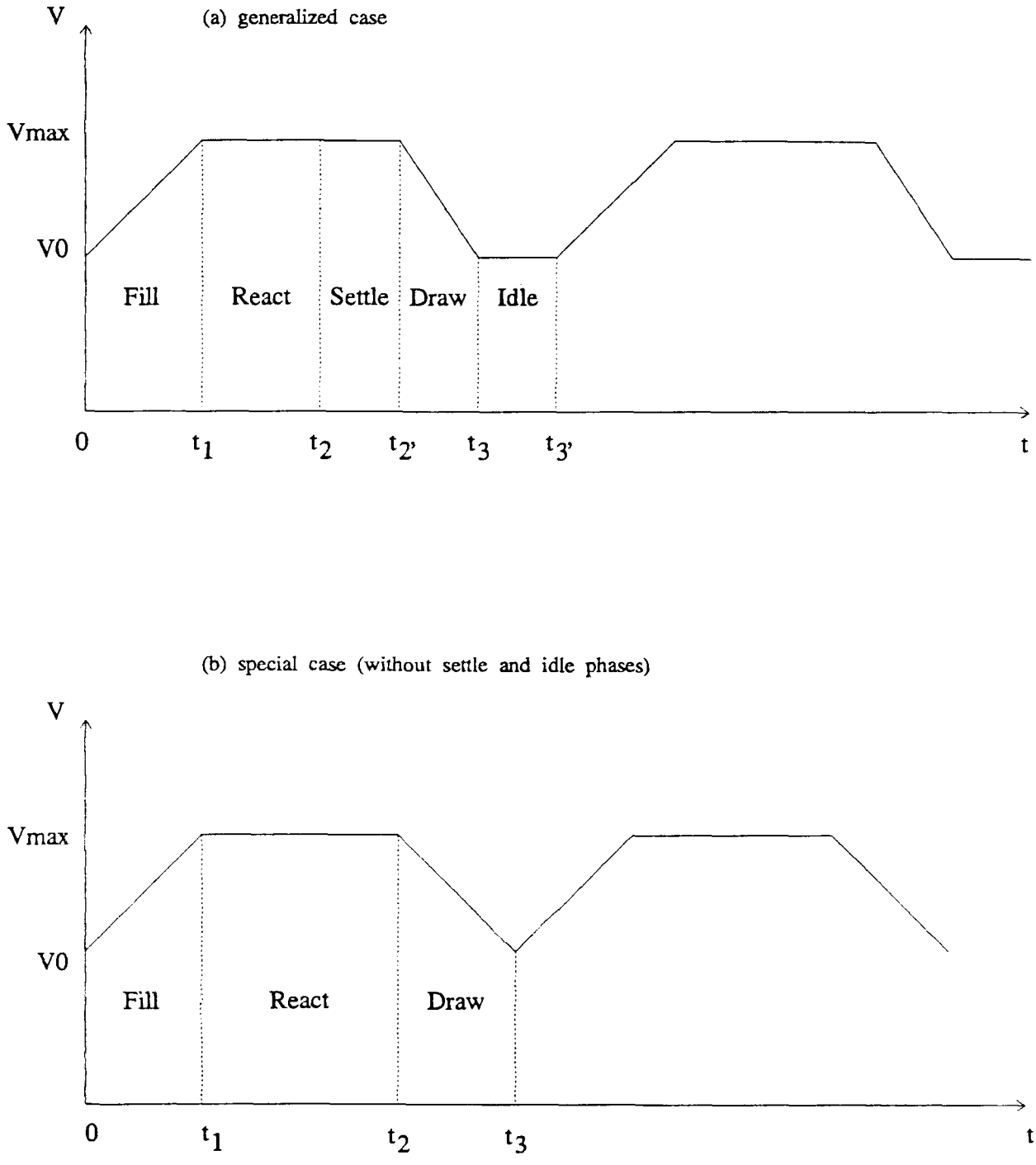
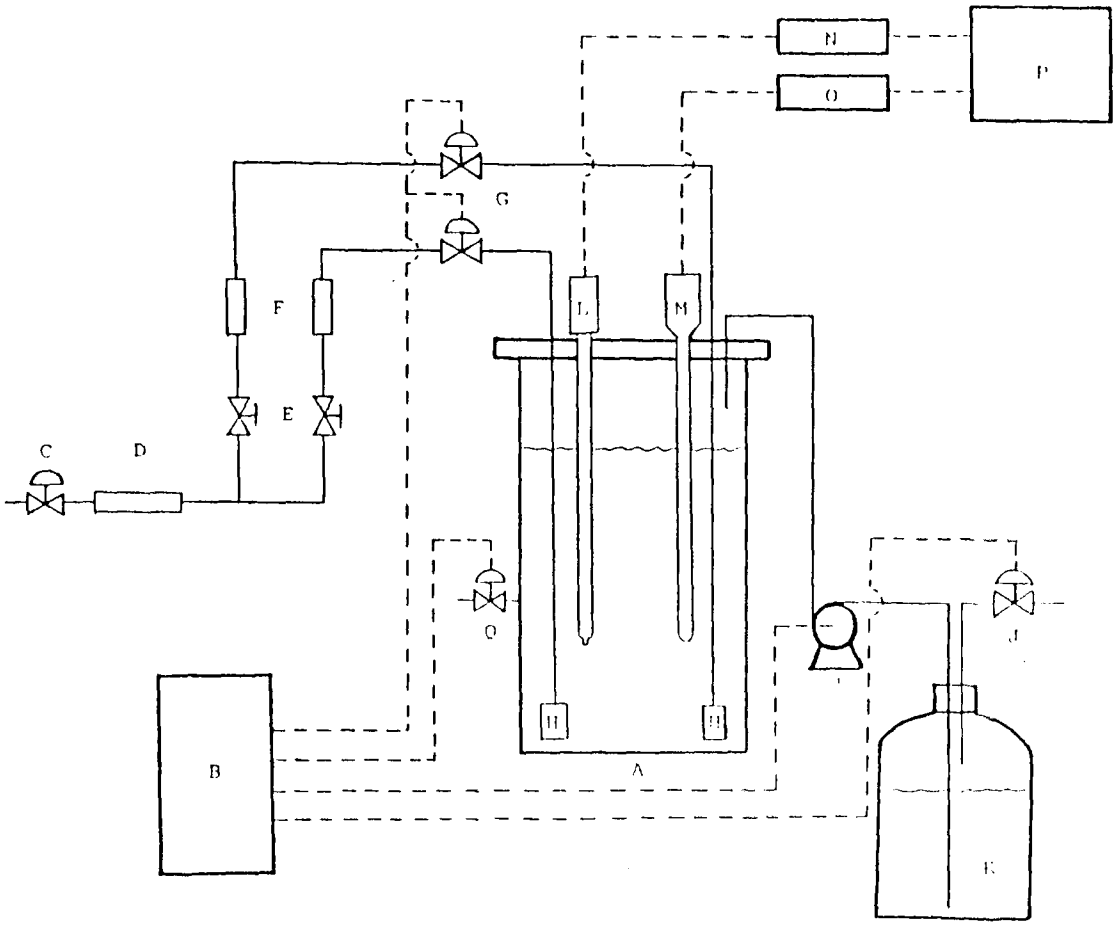


FIGURE 1.1 QUALTATIVE REPRESENTATION OF THE VOLUME CHANGE IN THE SEQUENCING BATCH REACTOR DURING CYCLES

FIGURE 1.2 SCHEMATIC DIAGRAM OF SEQUENCING BATCH REACTOR



A. REACTOR
B. MICROPROCESSOR
C. MAIN VALVE
D. AIR FILTER
E. ROTAMETER CONTROL
F. ROTAMETER
G. AIR SOLENOID VALVES
H. DIFFUSER STONE
I. INFLUENT PUMP
J. FEED SOLENOID VALVE
K. FEED BOTTLE
L. pH ELECTRODE
M. D.O. ELECTRODE
N. pH INDICATOR
O. D.O. INDICTOR
P. RECORDER
Q. DECANT SOLENOID VALVE

FIG. 2 CALIBRATION CURVE FOR DETERMINATION OF BIOMASS CONCENTRATION AS A FUNCTION OF OPTICAL DENSITY

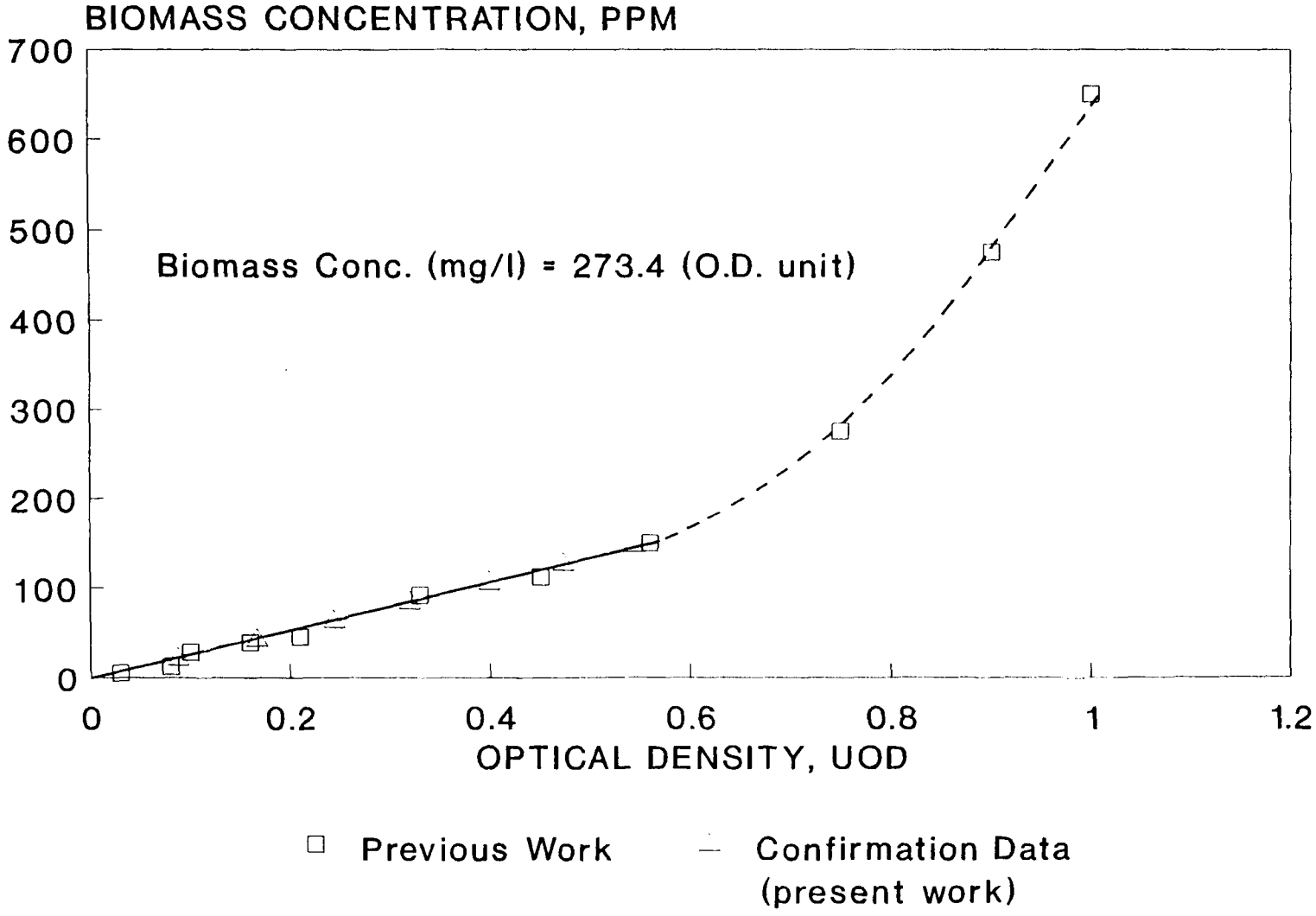
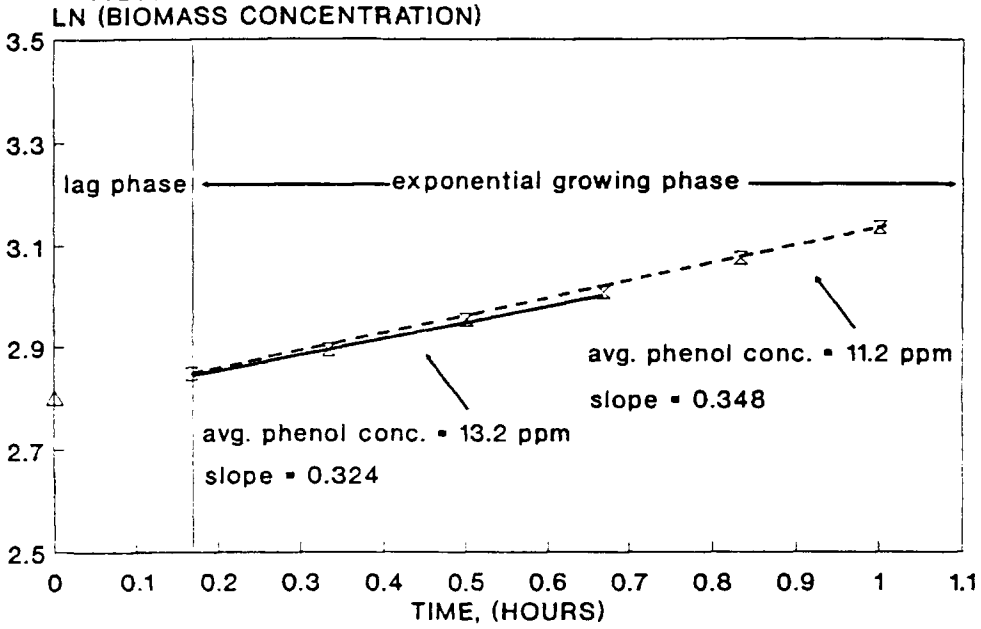
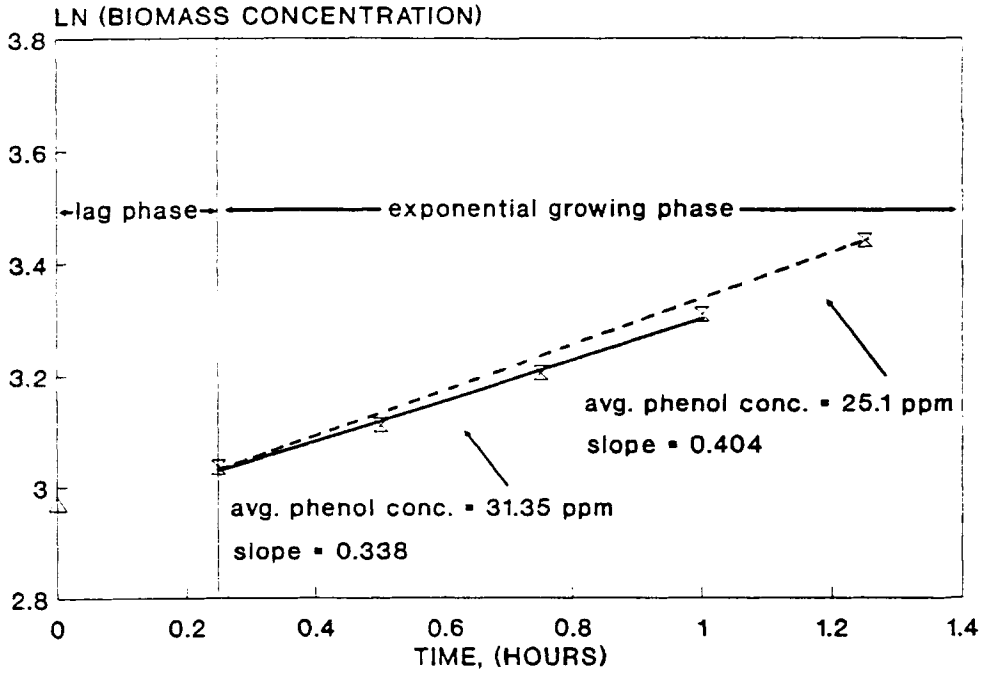


FIGURE 3 SPECIFIC GROWTH RATES OF *PSEUDOMONAS PUTIDA* (ATCC 17514)

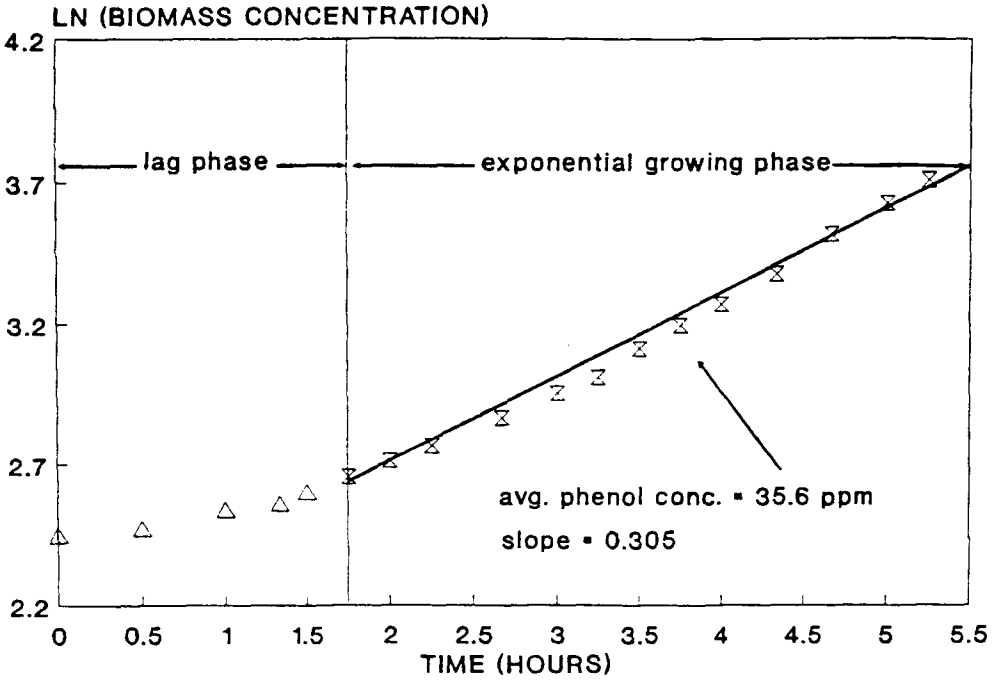
RUN 3-1 AT INITIAL PHENOL CONC. OF 17.2 PPM



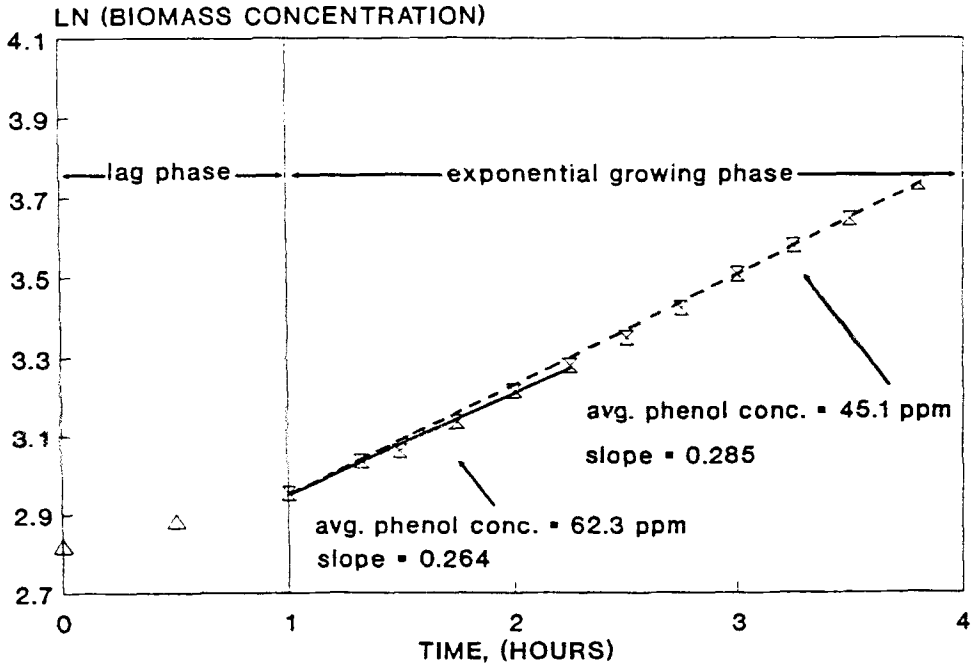
RUN 3-2 AT INITIAL PHENOL CONC. OF 41.5 PPM



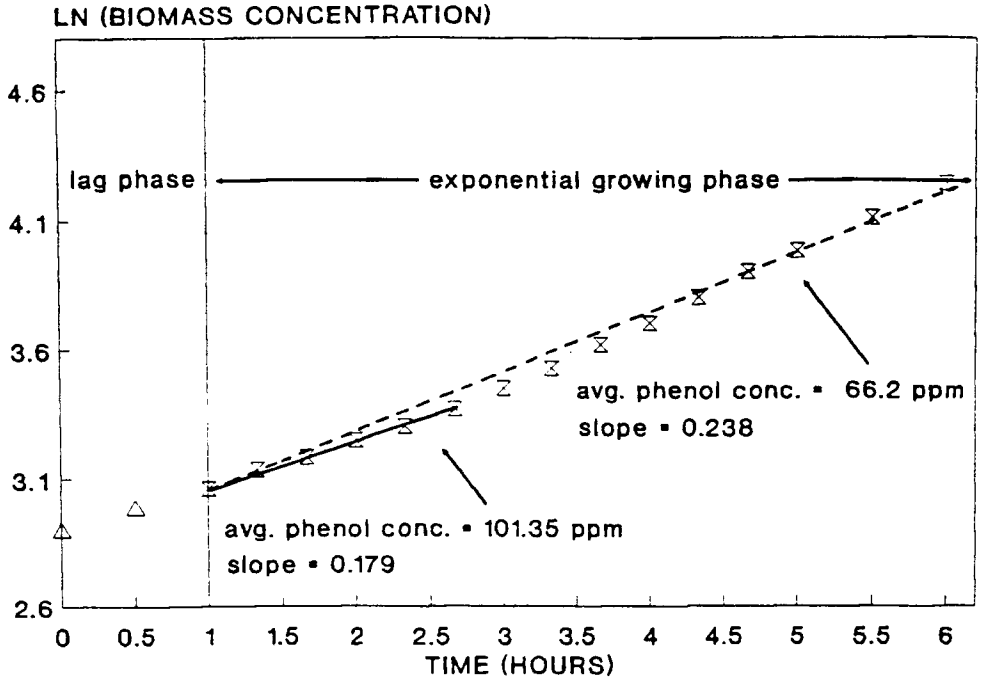
RUN 3-3 AT INITIAL PHENOL CONC. OF 62.3 PPM



RUN 3-4 AT INITIAL PHENOL CONC. OF 74 PPM



RUN 3-5 AT INITIAL PHENOL CONC. OF 116.5 PPM



RUN 3-6 AT INITIAL PHENOL CONC. OF 140.4 PPM

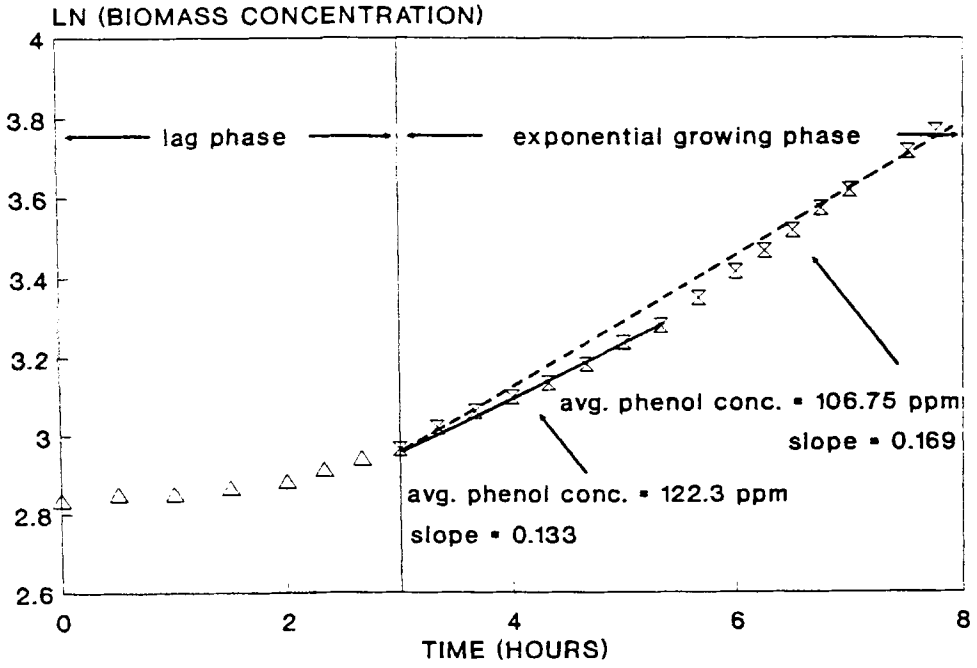
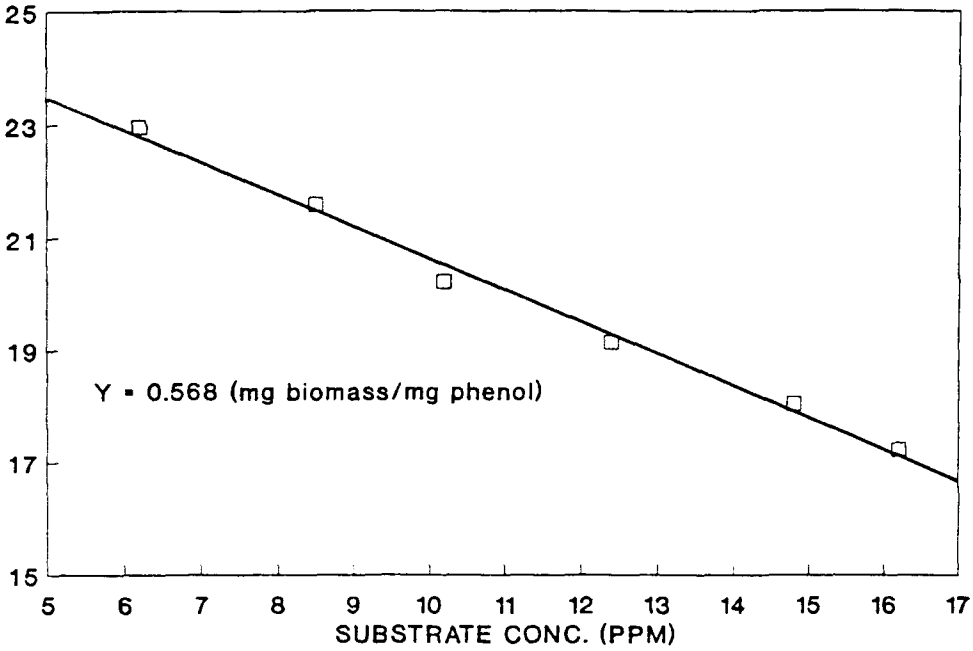
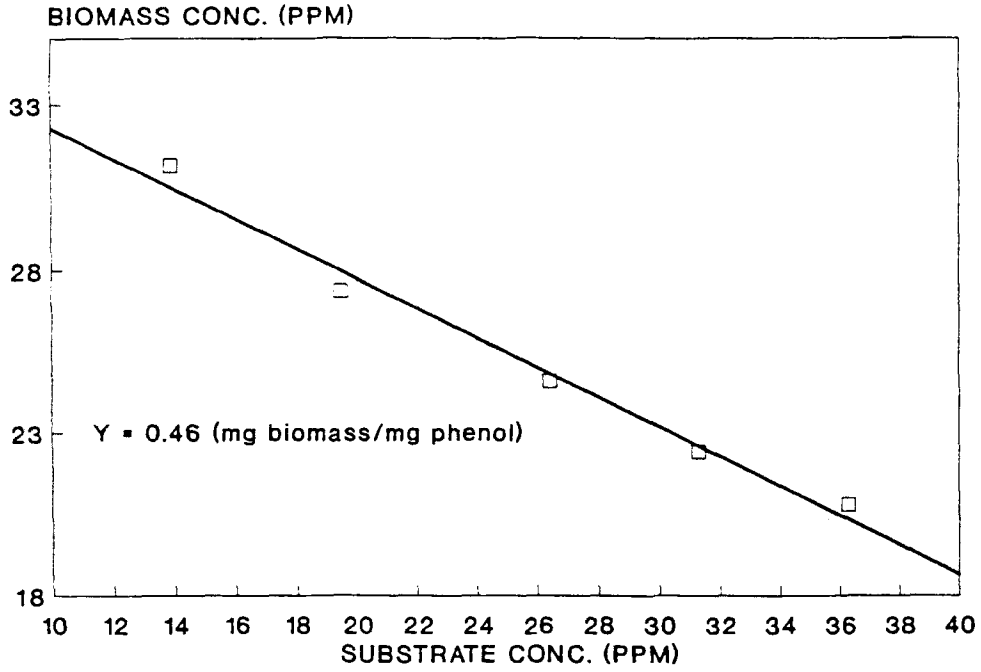


FIGURE 4 YIELD COEFFICIENTS

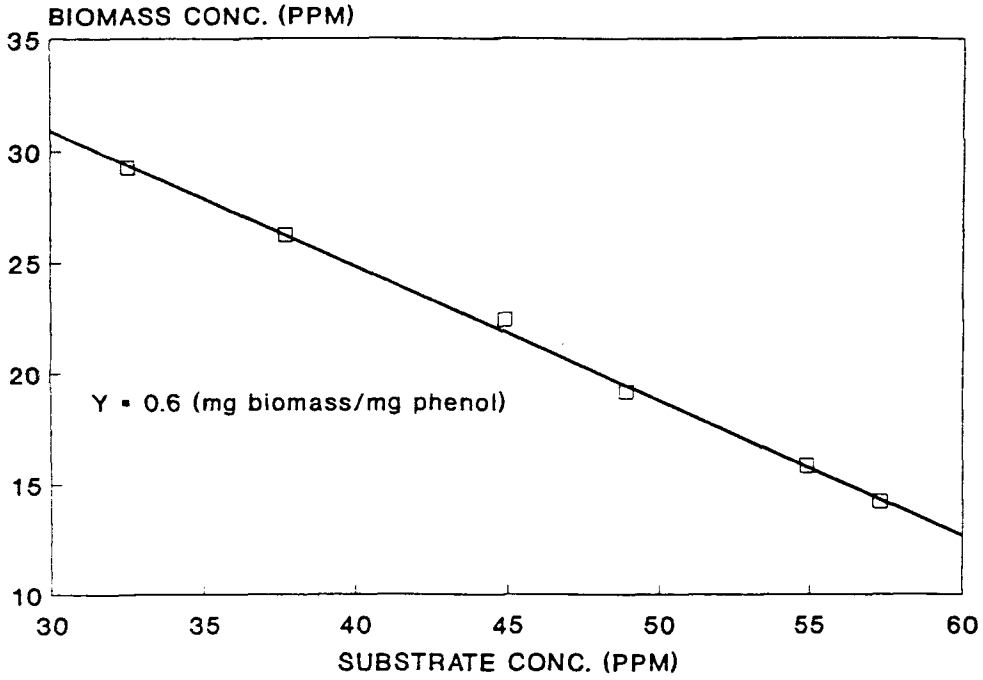
RUN 4-1 AT INITIAL PHENOL CONC. OF 17.2 PPM



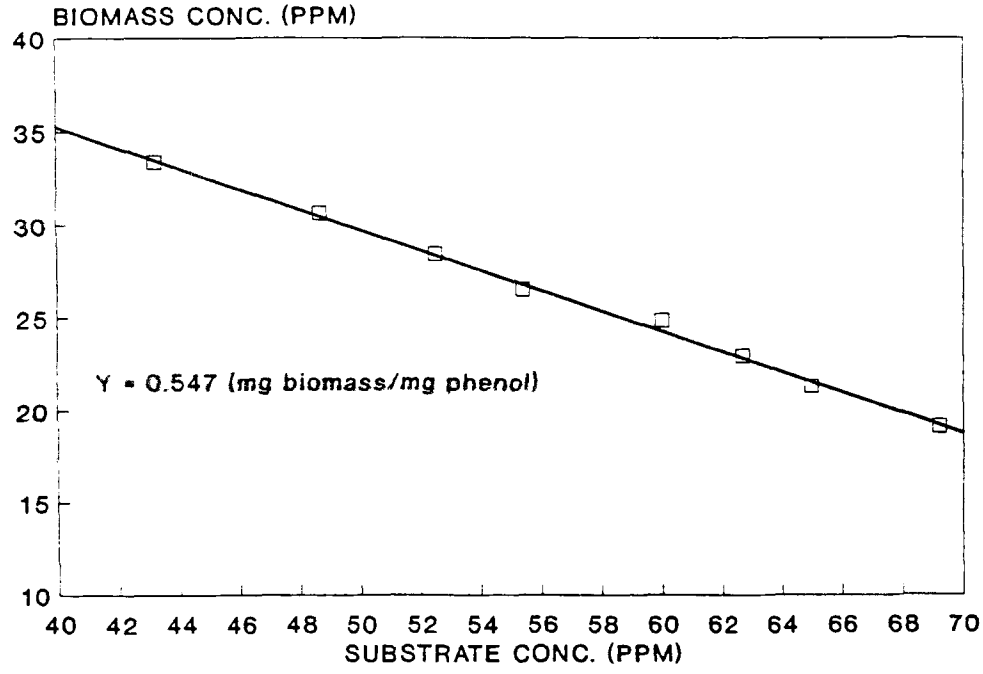
RUN 4-2 AT INITIAL PHENOL CONC. OF 41.5 PPM



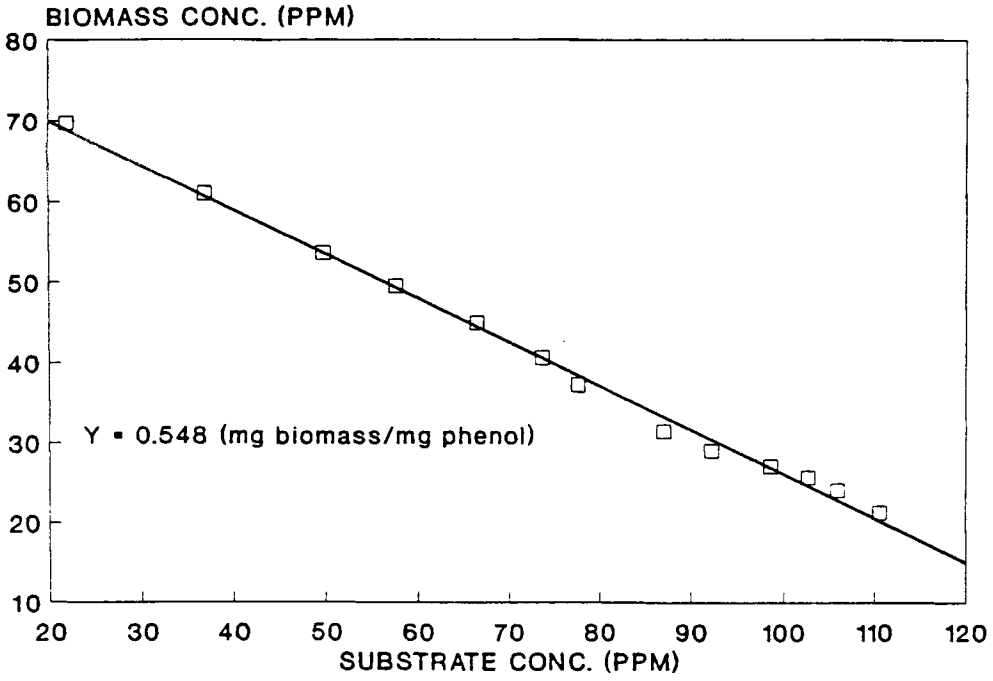
RUN 4-3 AT INITIAL PHENOL CONC. OF 62.3 PPM



RUN 4-4 AT INITIAL PHENOL CONC. OF 74 PPM



RUN 4-5 AT INITIAL PHENOL CONC. OF 116.5 PPM



RUN 4-6 AT INITIAL PHENOL CONC. OF 140.4 PPM

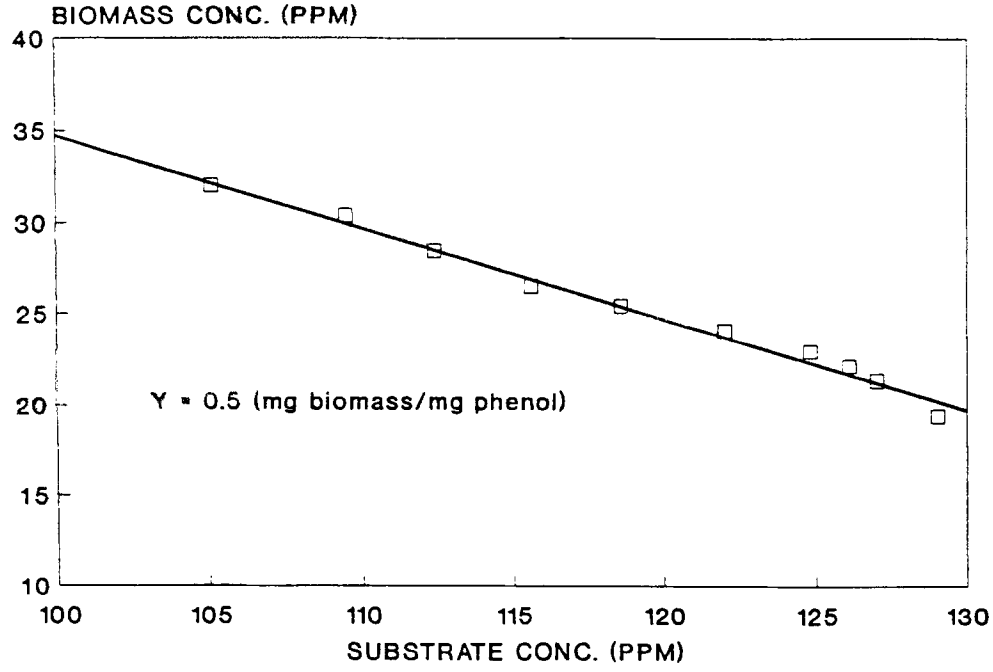
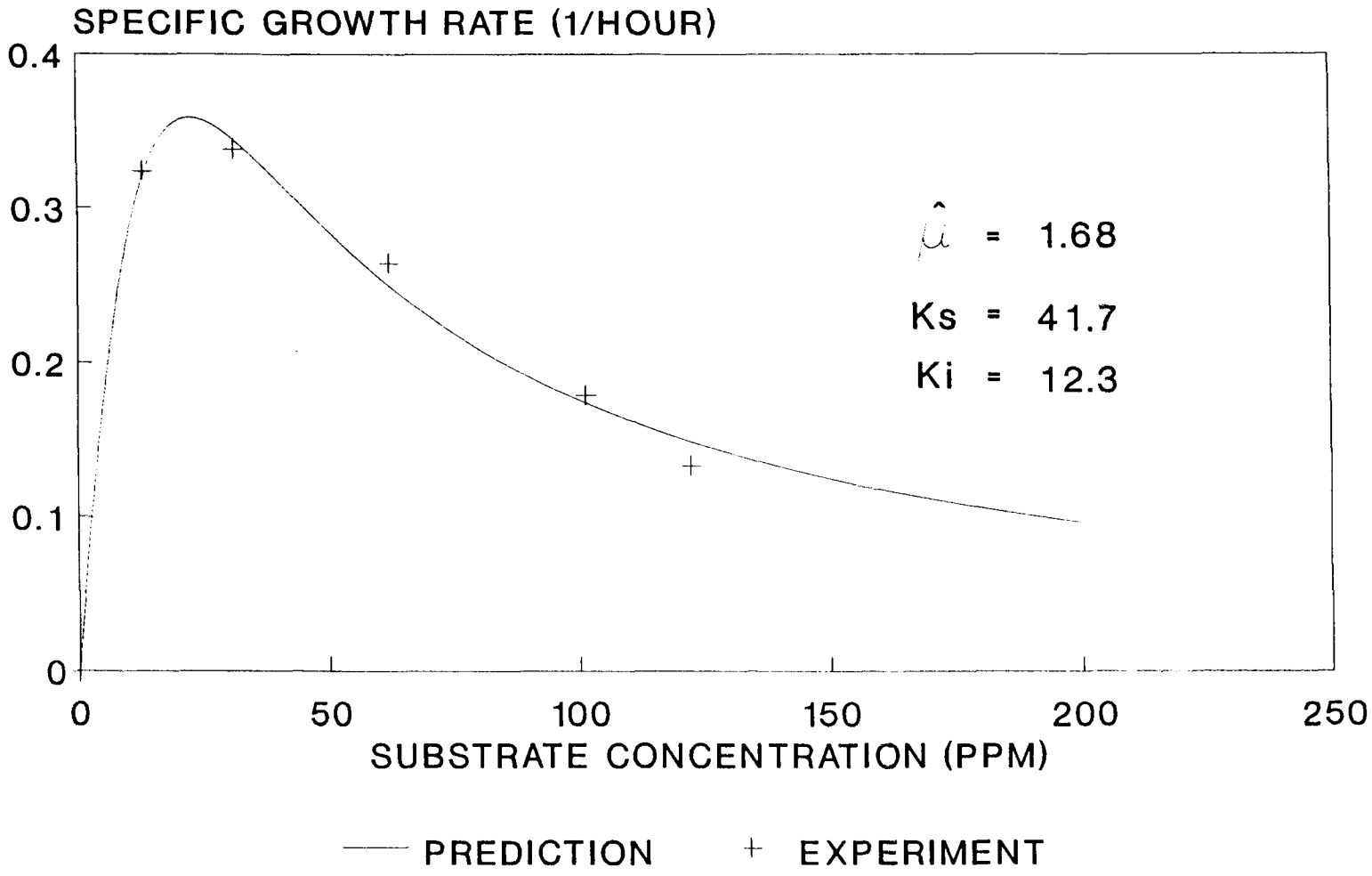
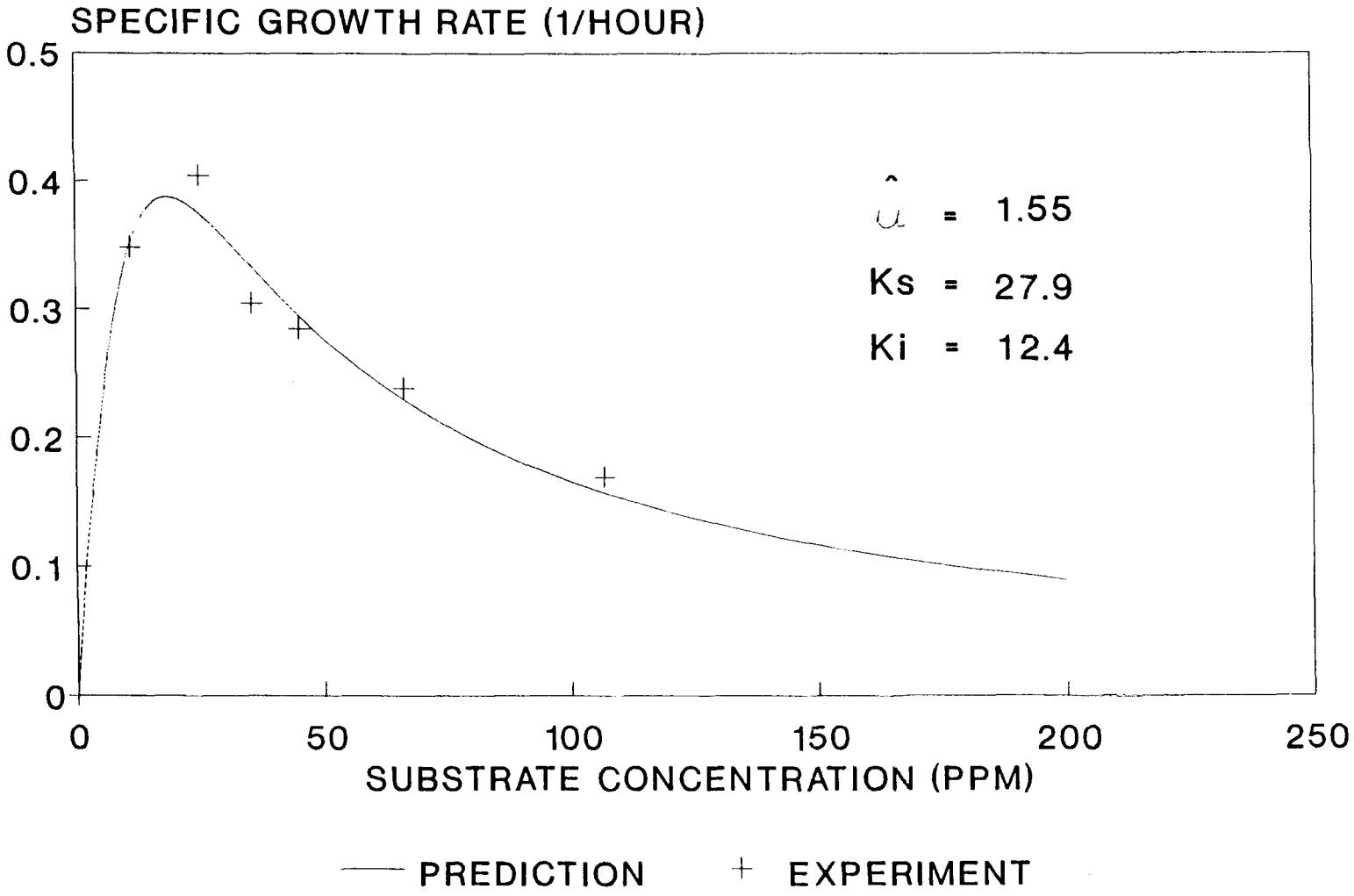


FIG. 5.1 SPECIFIC GROWTH RATE VS. AVERAGE PHENOL CONC.* FOR ANDREWS MODEL PARAMETERS



* during initial slope of log growth phase

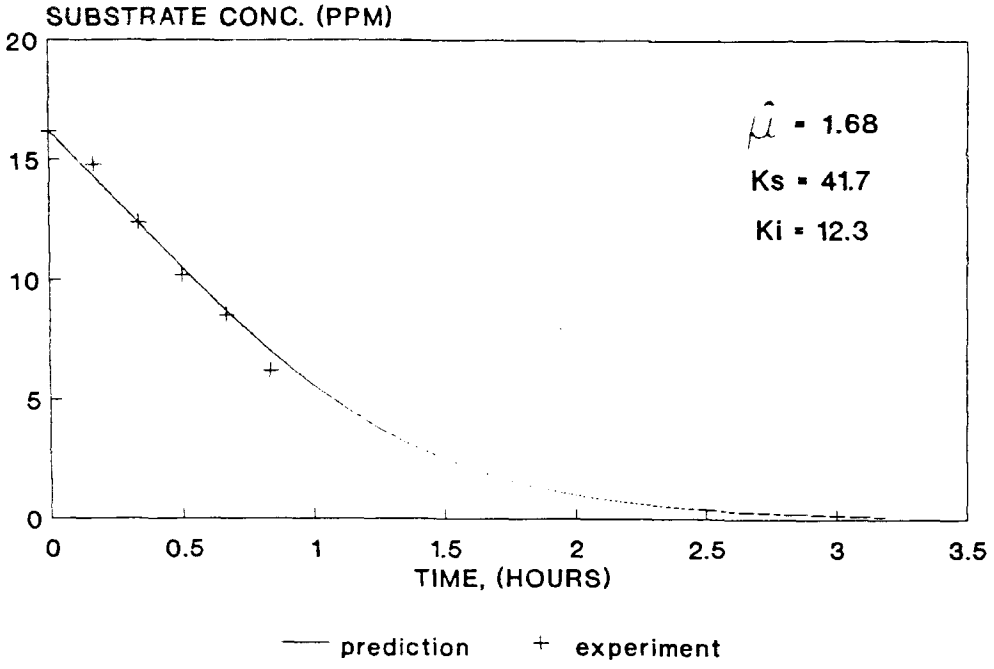
FIG. 5.2 SPECIFIC GROWTH RATE VS. AVERAGE PHENOL CONC.* FOR ANDREWS MODEL PARAMETERS



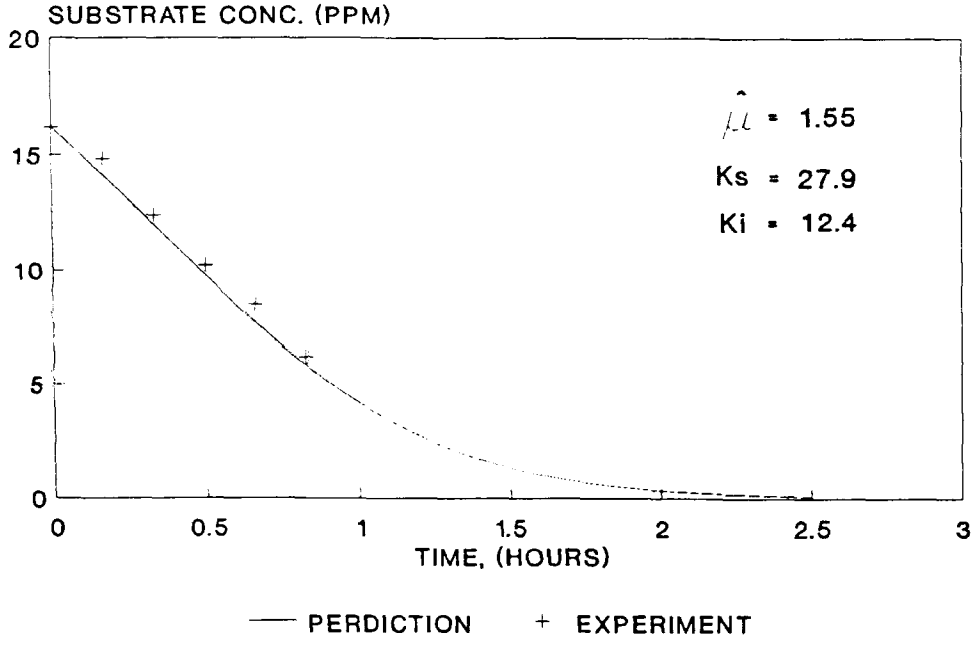
* using full data set in log growth phase

FIGURE 6 PHENOL CONCENTRATION VS. TIME

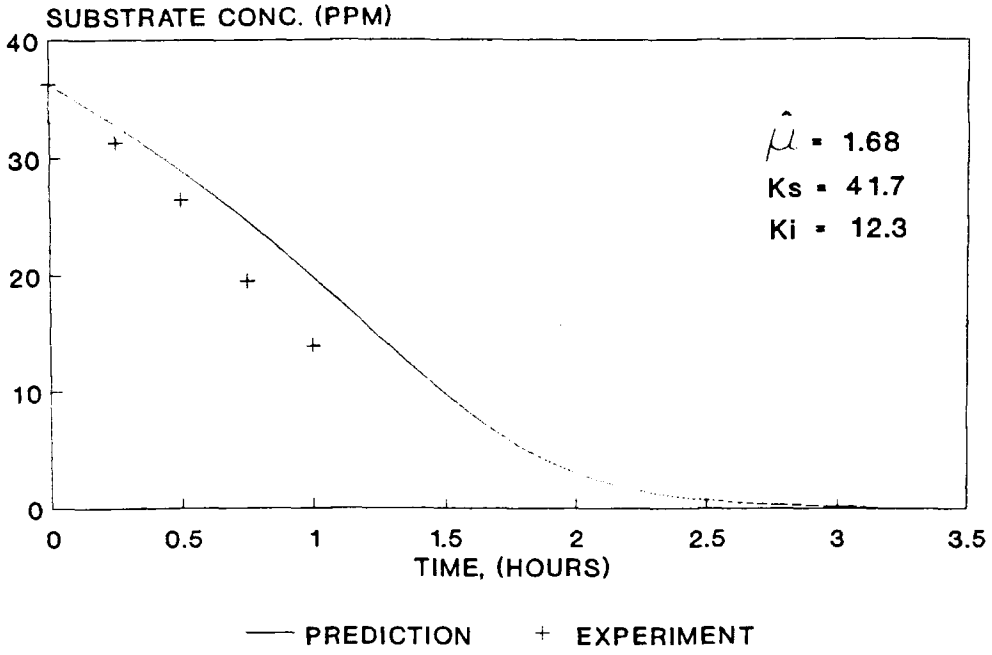
RUN 6-1a AT INITIAL PHENOL CONC. OF 17.2 PPM



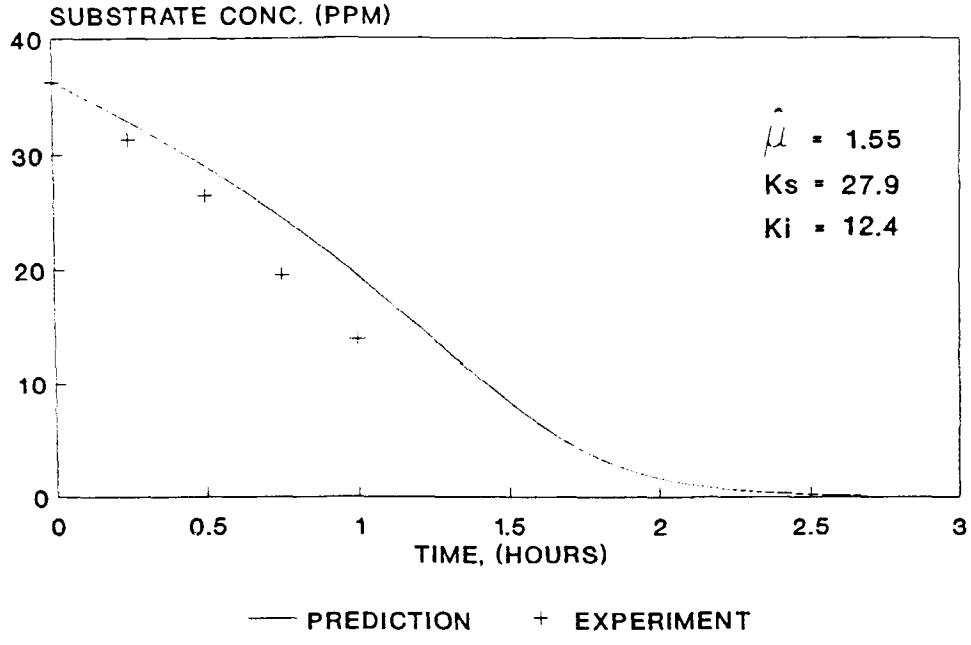
RUN 6-1b



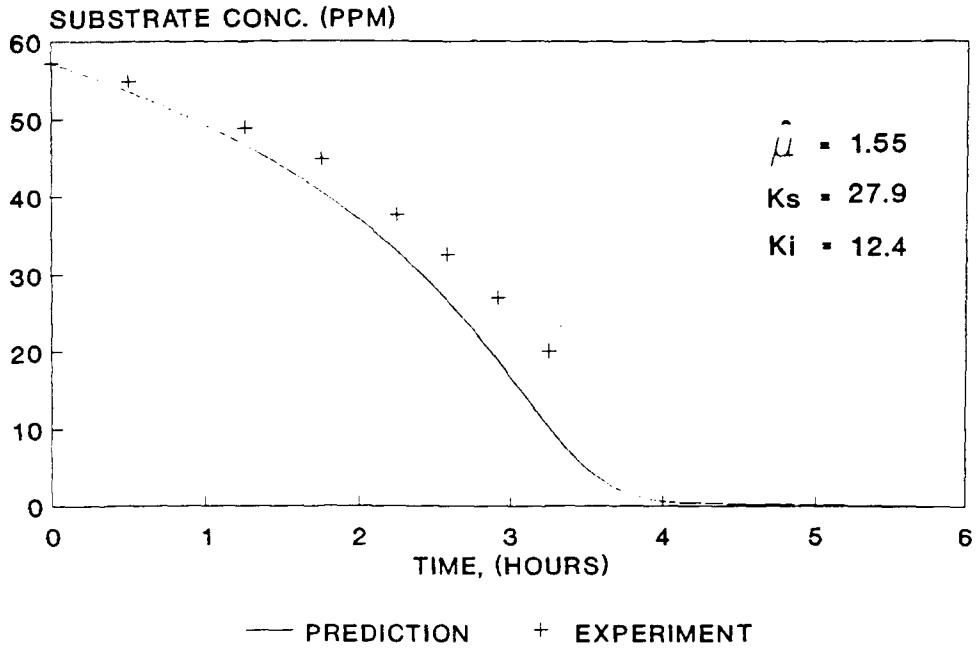
RUN 6-2a AT INITIAL PHENOL CONC. OF 41.5 PPM



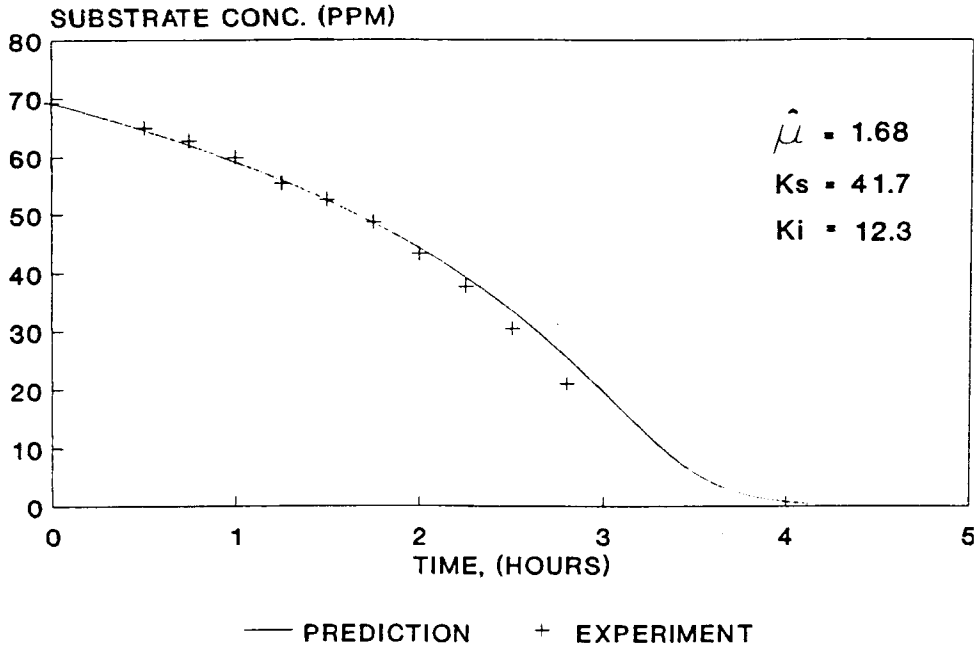
RUN 6-2b



RUN 6-3b AT INITIAL PHENOL CONC. OF 62.3 PPM

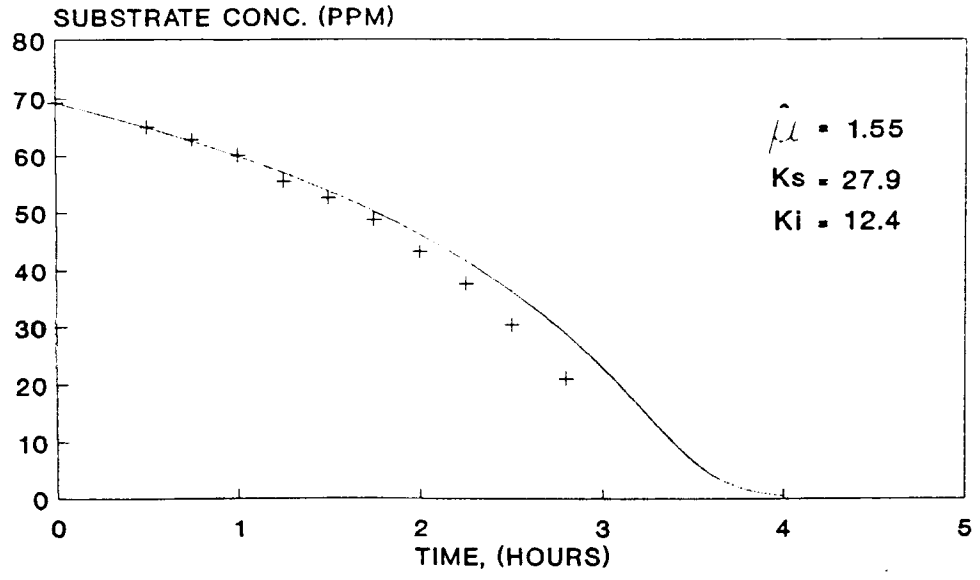


RUN 6-4a AT INITIAL PHENOL CONC. OF 74 PPM



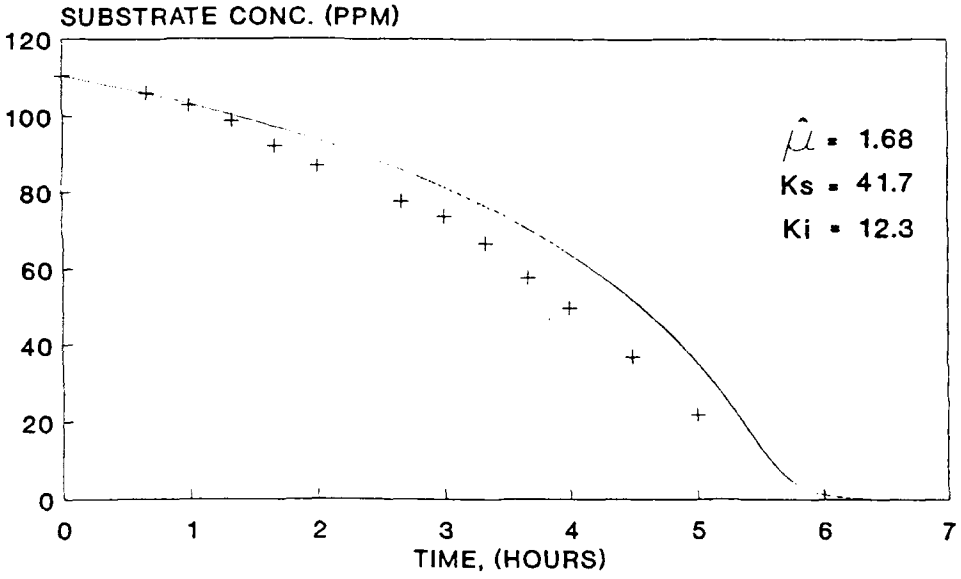
PREDICTION + EXPERIMENT

RUN 6-4b

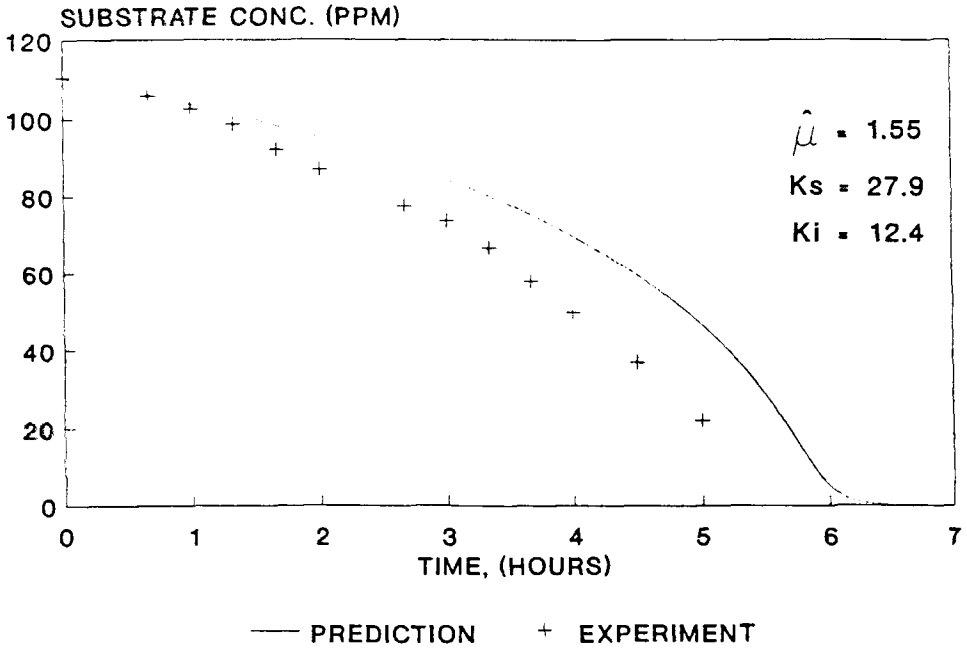


PREDICTION + EXPERIMENT

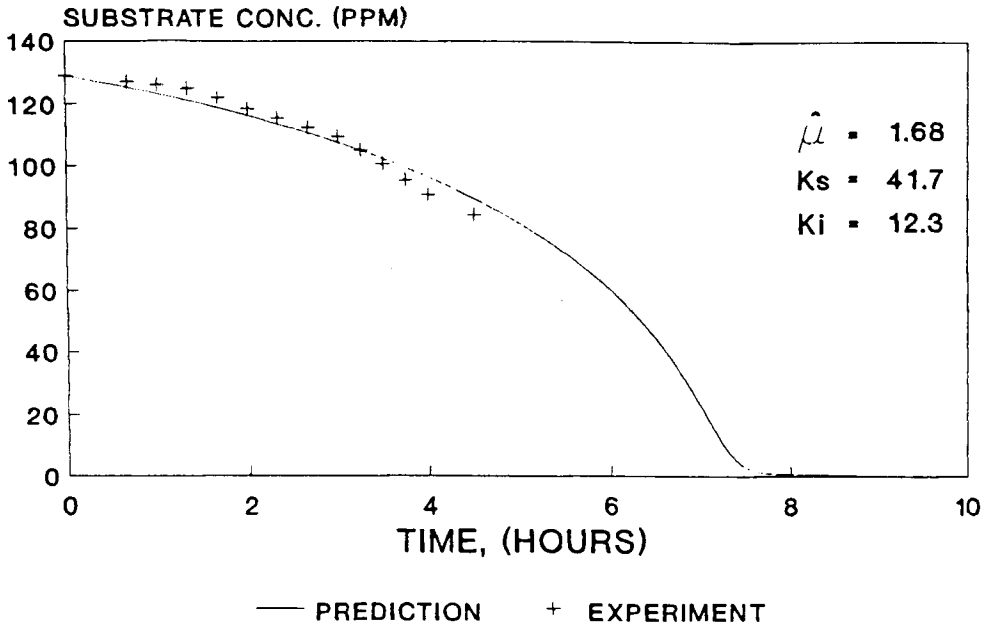
RUN 6-5a AT INITIAL PHENOL CONC. OF 116.5 PPM



RUN 6-5b



RUN 6-6a AT INITIAL PHENOL CONC. OF 140.4 PPM



RUN 6-6b

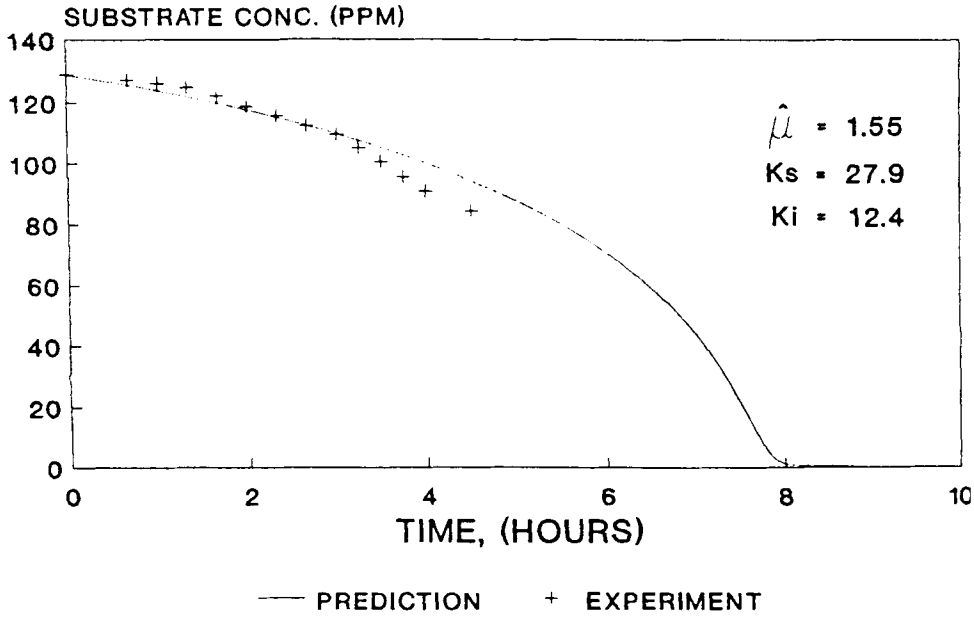
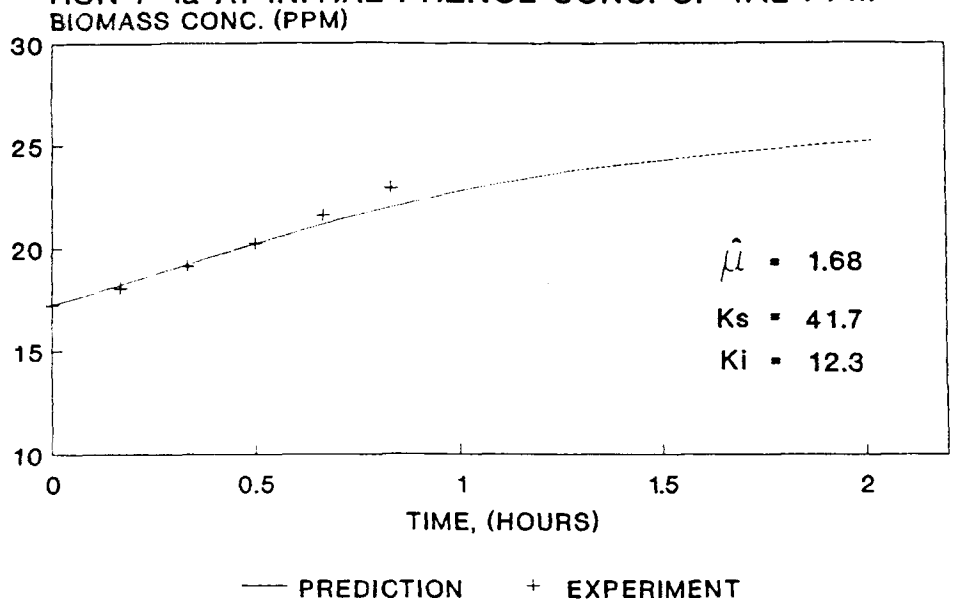
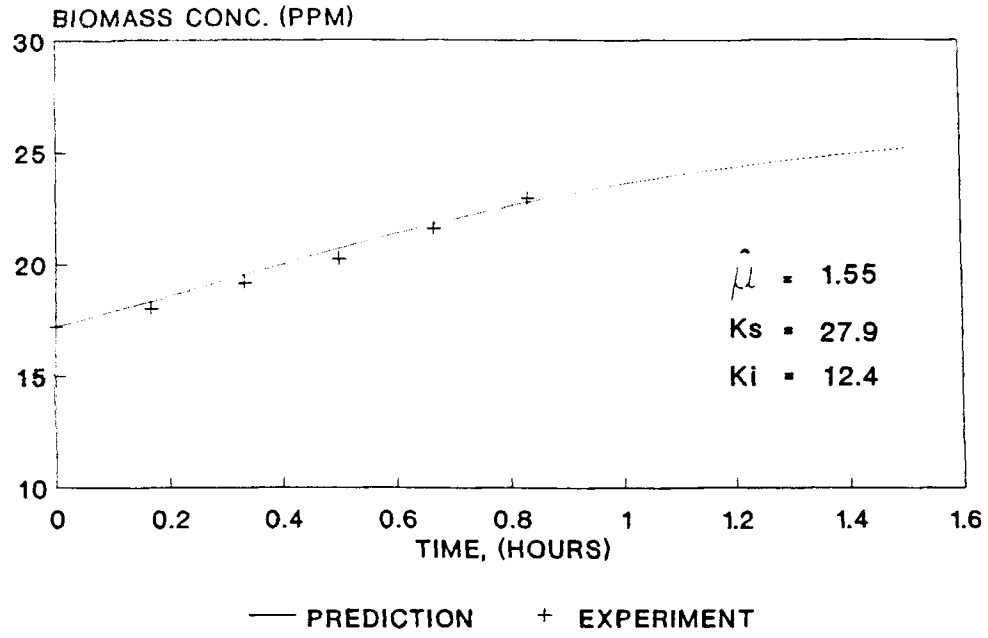


FIG. 7 BIOMASS CONCENTRATION VS. TIME (CORRESPONDING TO FIGURE 6)

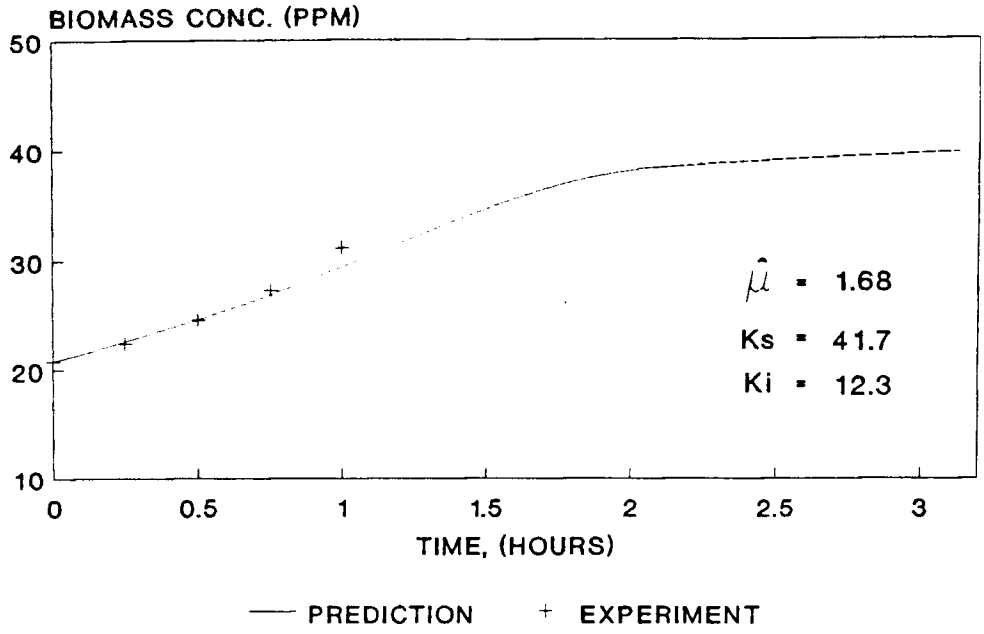
RUN 7-1a AT INITIAL PHENOL CONC. OF 17.2 PPM



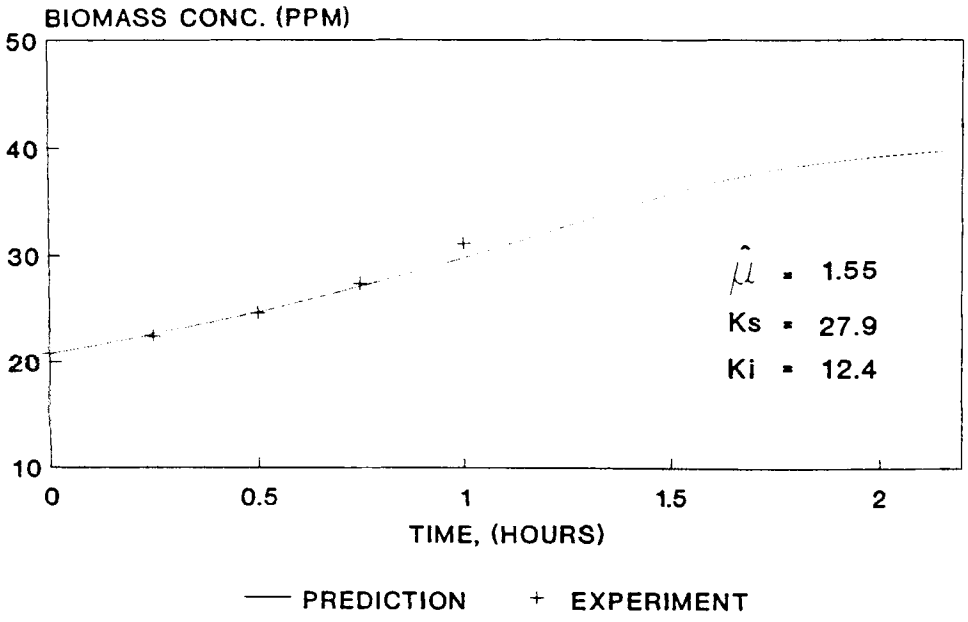
RUN 7-1b



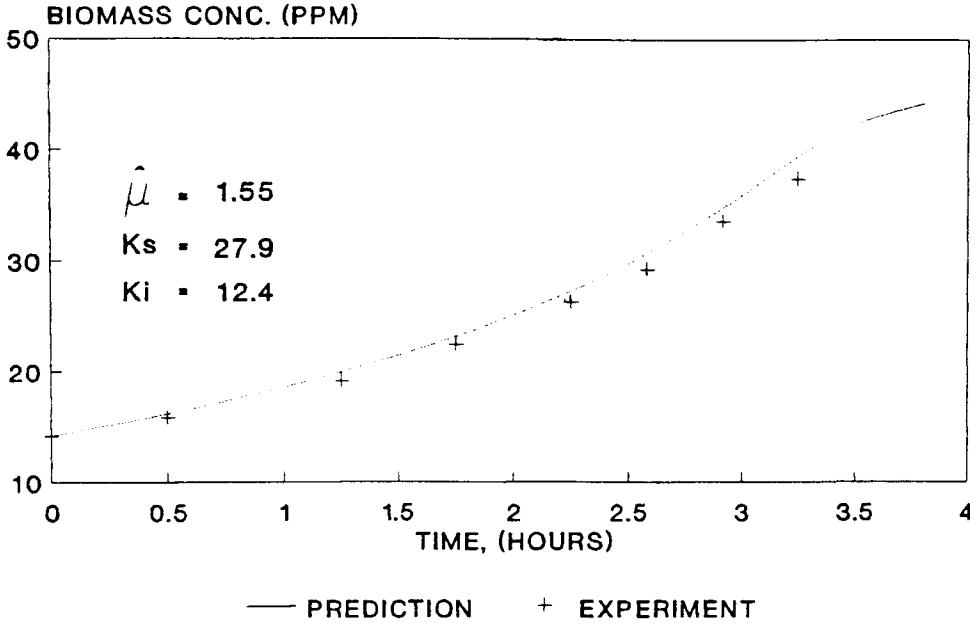
RUN 7-2a AT INITIAL PHENOL CONC. OF 41.5 PPM



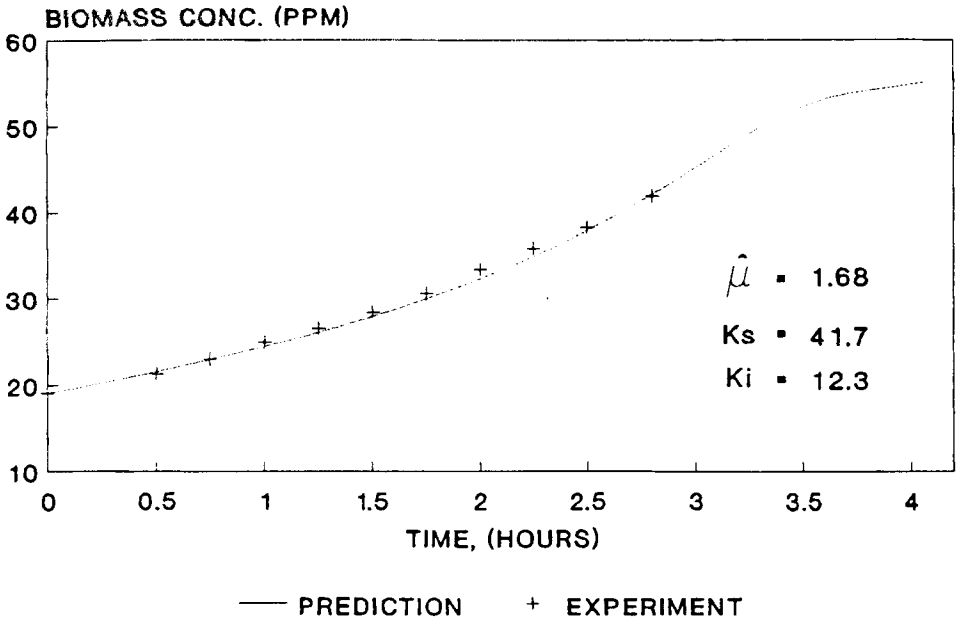
RUN 7-2b



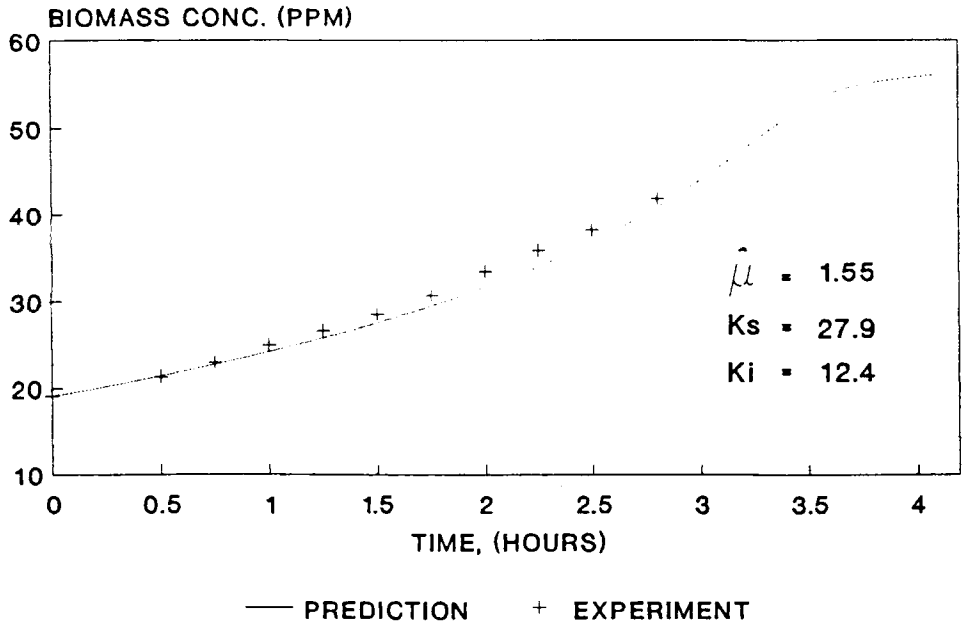
RUN 7-3b AT INITIAL PHENOL CONC. OF 62.3 PPM



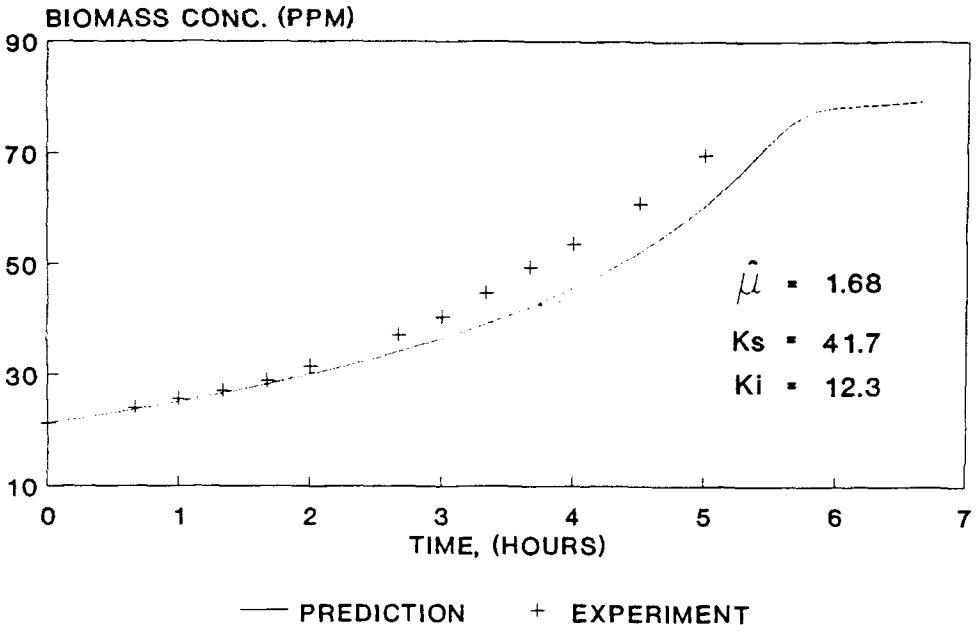
RUN 7-4a AT INITIAL PHENOL CONC. OF 74 PPM



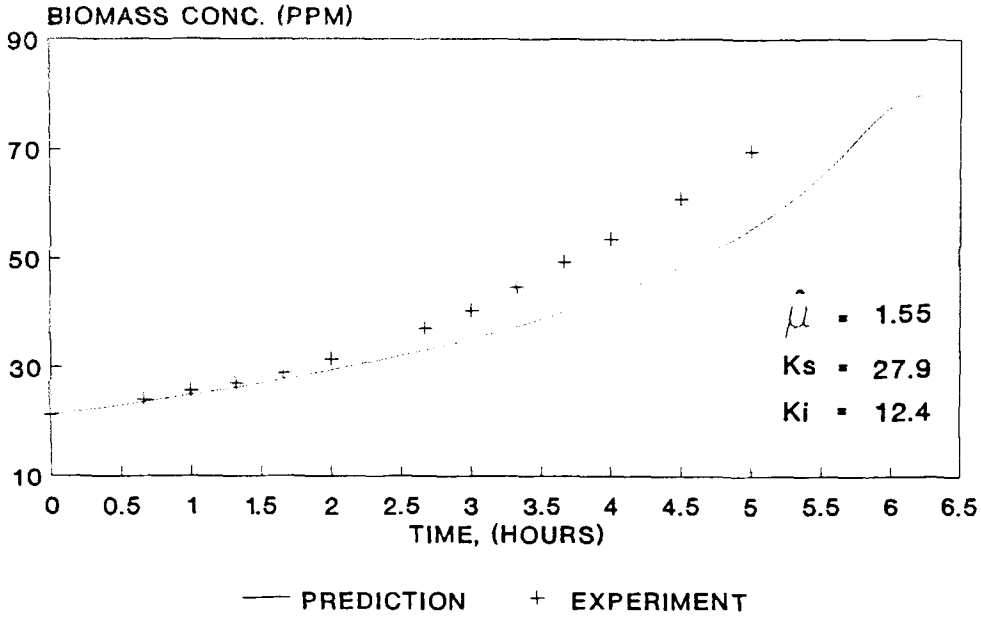
RUN 7-4b



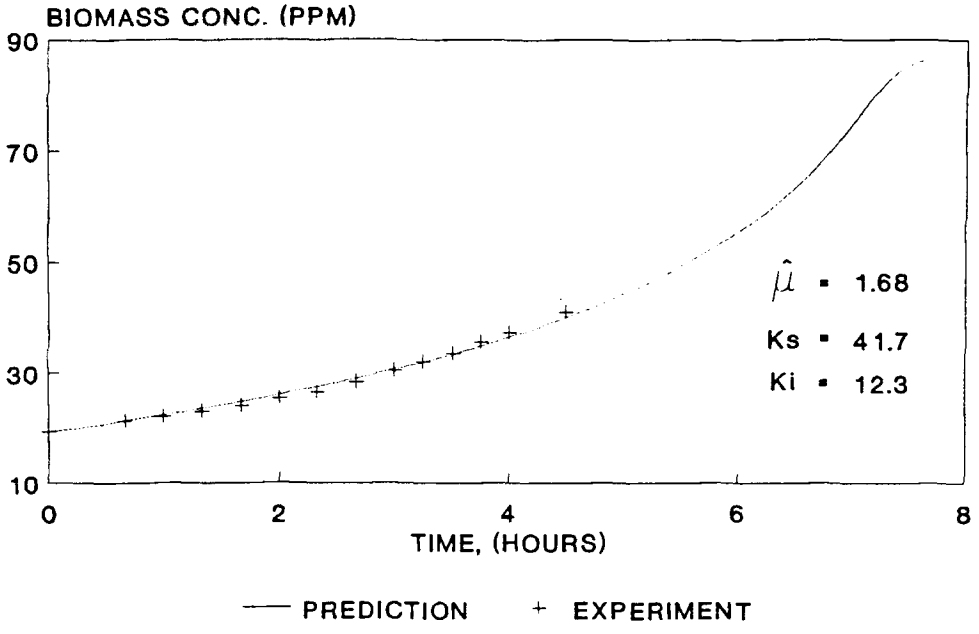
RUN 7-5a AT INITIAL PHENOL CONC. OF 116.5 PPM



RUN 7-5b

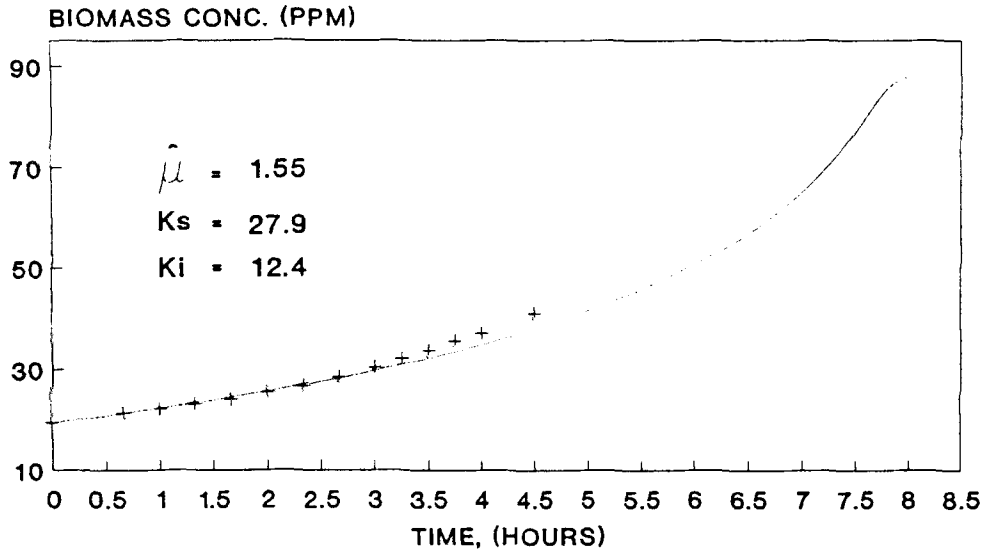


RUN 7-6a AT INITIAL PHENOL CONC. OF 140.4 PPM



— PREDICTION + EXPERIMENT

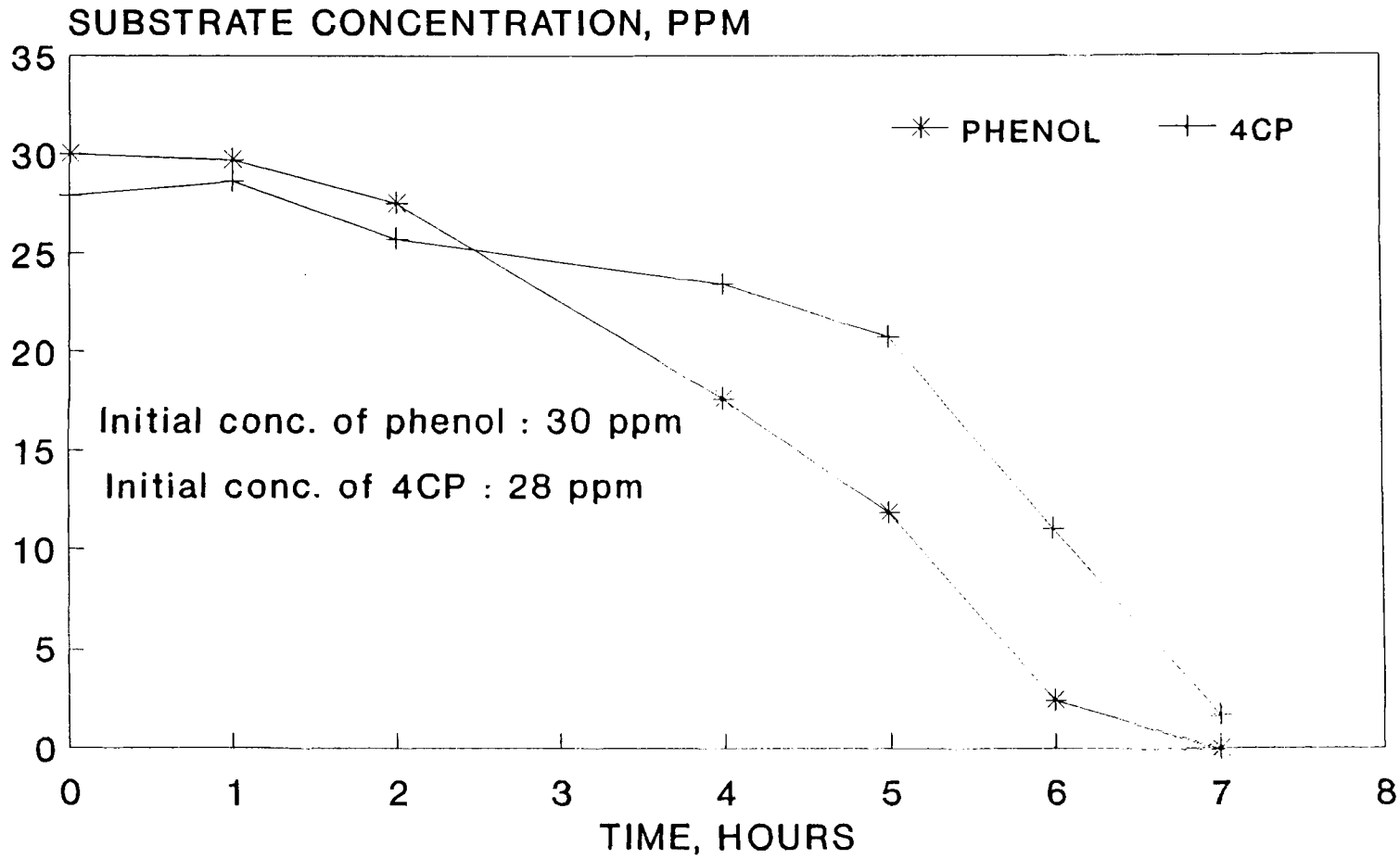
RUN 7-6b



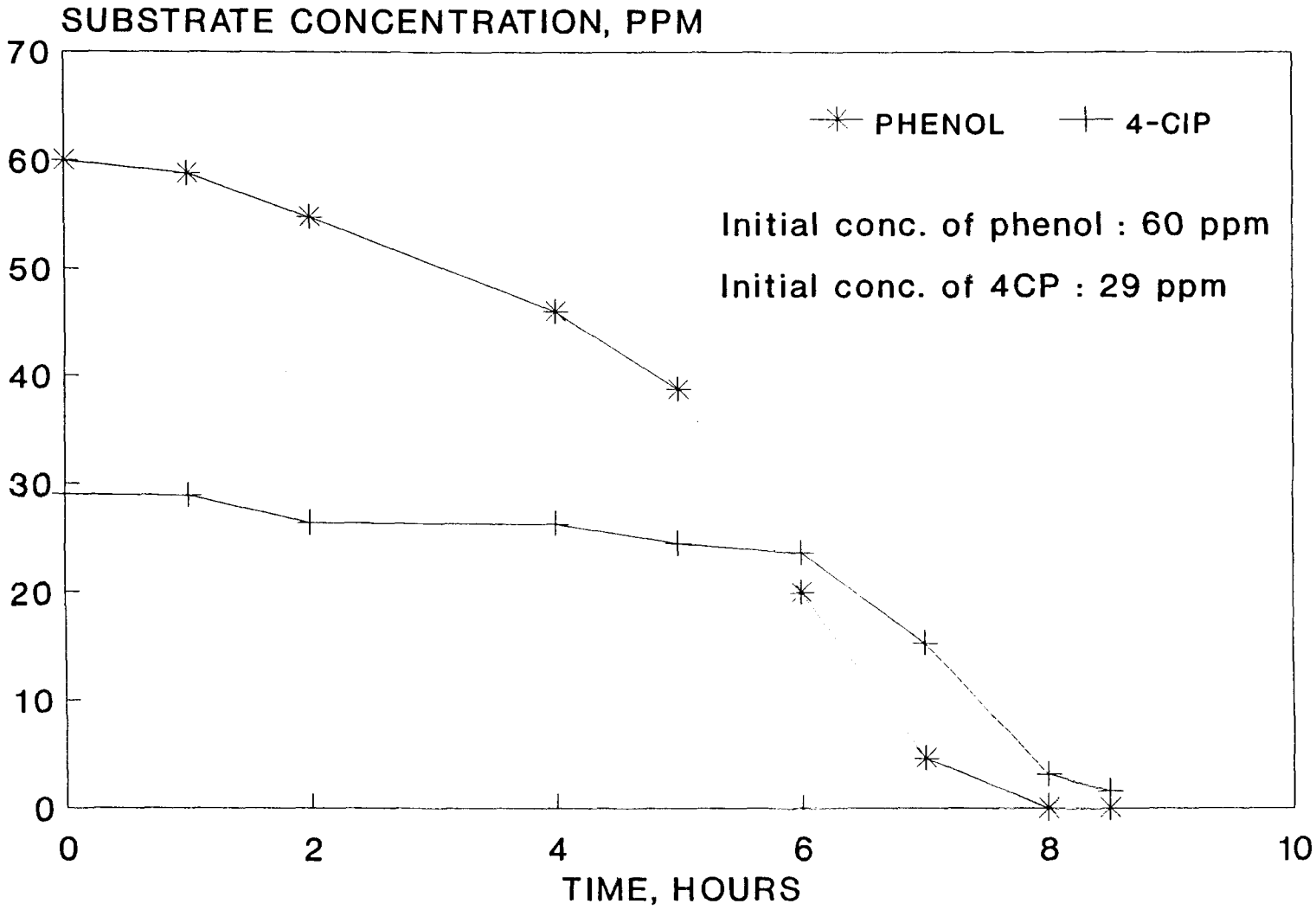
— PREDICTION + EXPERIMENT

FIG. 8 BIODEGRADATION TENDENCY OF 4CP IN THE PRESENCE OF PHENOL

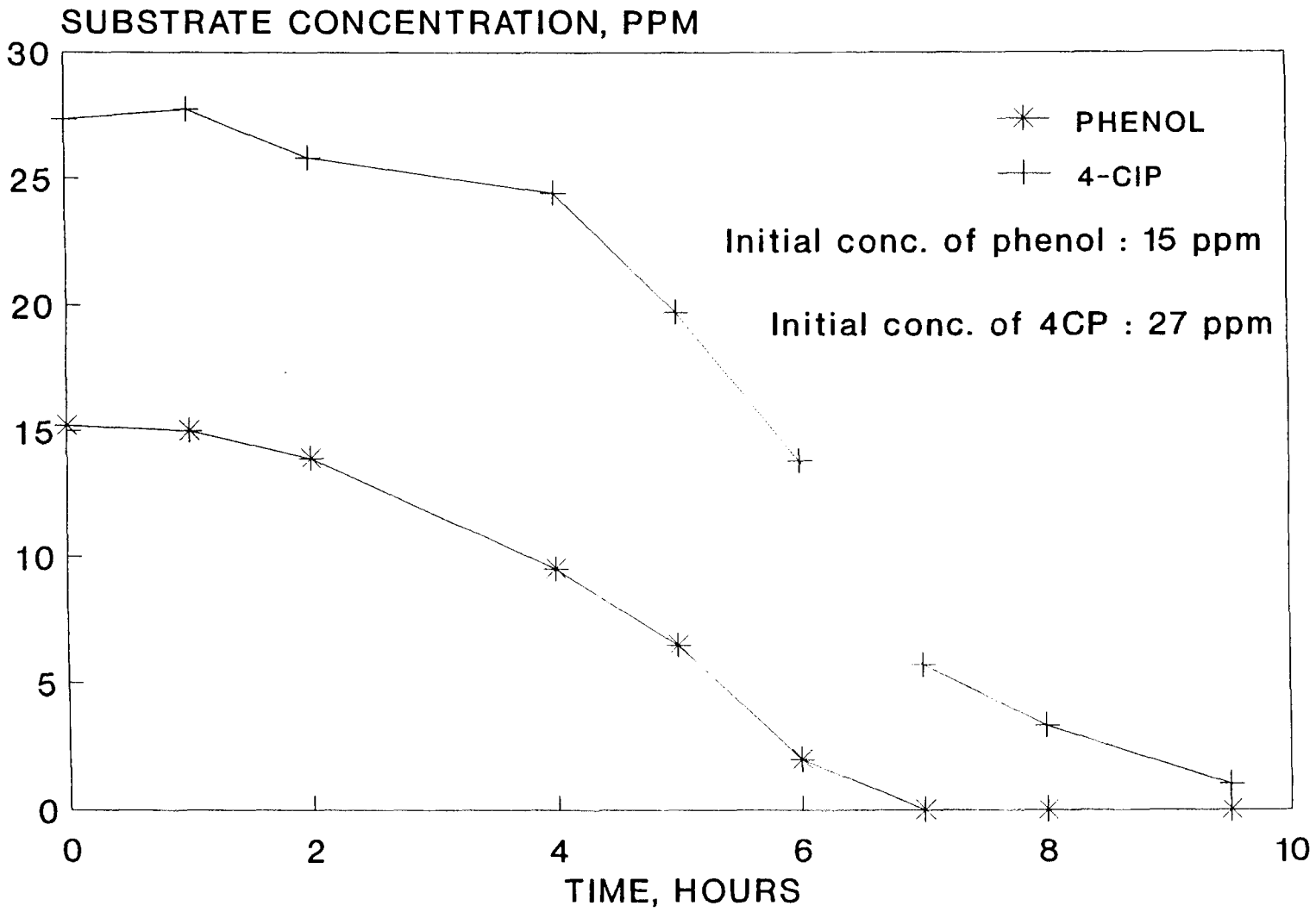
RUN 8-1



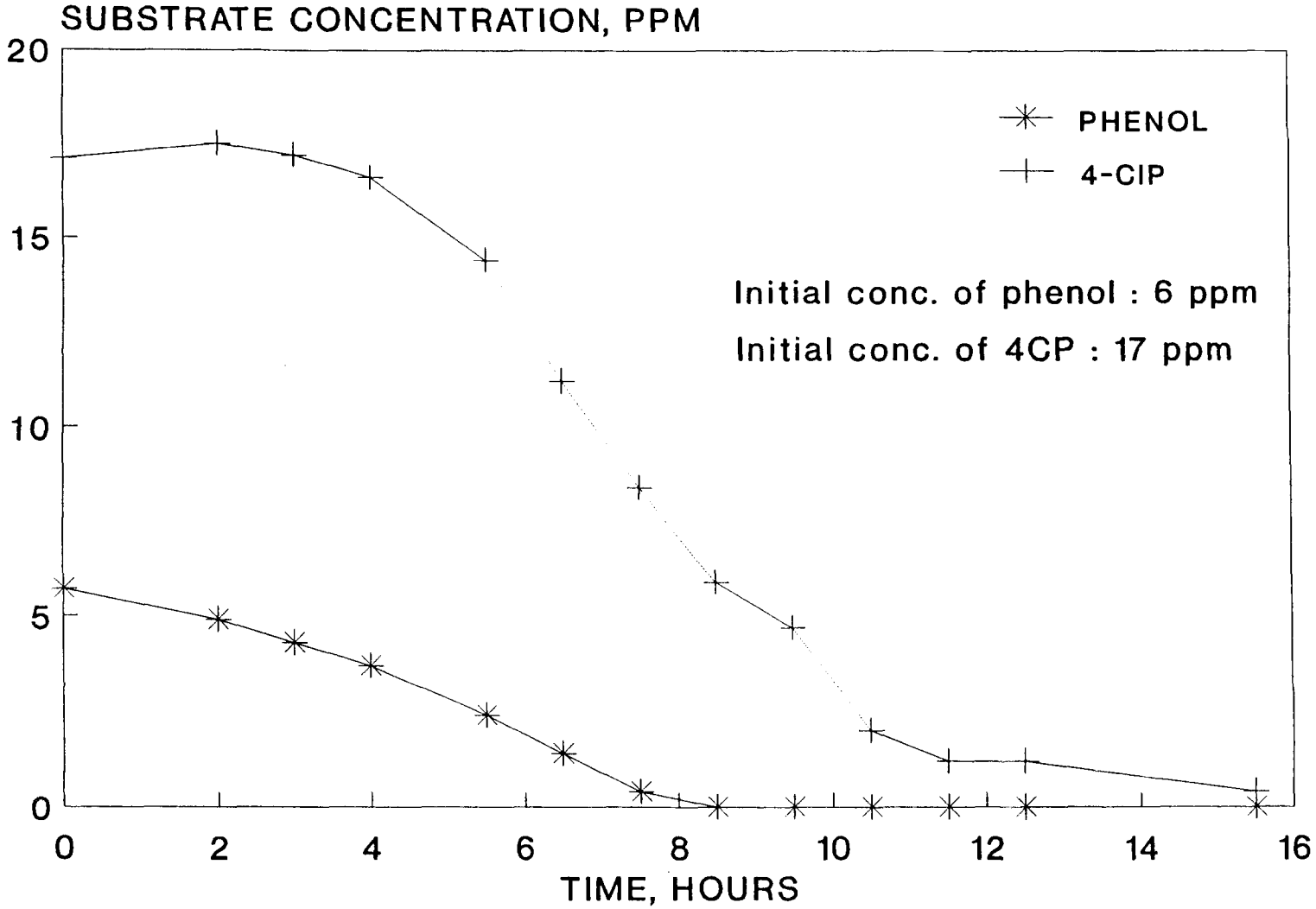
RUN 8-2

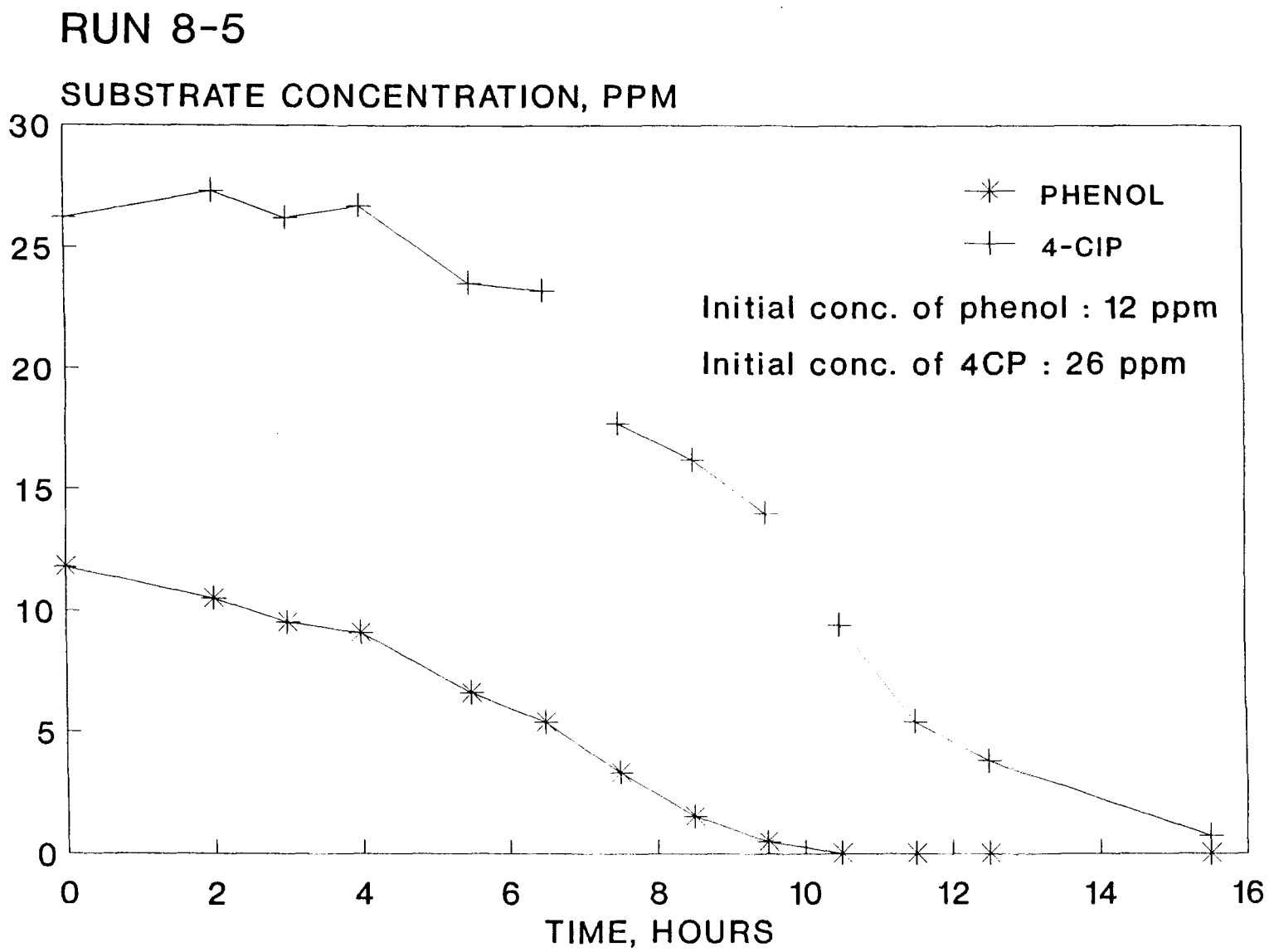

SUBSTRATE CONCENTRATION, PPM
70
60
50
40
30
20
10
0
PHENOL
4-CIP
Initial conc. of phenol : 60 ppm
Initial conc. of 4CP : 29 ppm
0
2
4
6
8
10
TIME, HOURS

RUN 8-3


SUBSTRATE CONCENTRATION, PPM
30
25
20
15
10
5
0
0
2
4
6
8
10
TIME, HOURS
PHENOL
4-CIP
Initial conc. of phenol : 15 ppm
Initial conc. of 4CP : 27 ppm

RUN 8-4


SUBSTRATE CONCENTRATION, PPM
20
15
10
5
0
0
2
4
6
8
10
12
14
16
TIME, HOURS
PHENOL
4-CIP
Initial conc. of phenol : 6 ppm
Initial conc. of 4CP : 17 ppm


RUN 8-5
SUBSTRATE CONCENTRATION, PPM
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
TIME, HOURS
PHENOL
4-CIP
Initial conc. of phenol : 12 ppm
Initial conc. of 4CP : 26 ppm

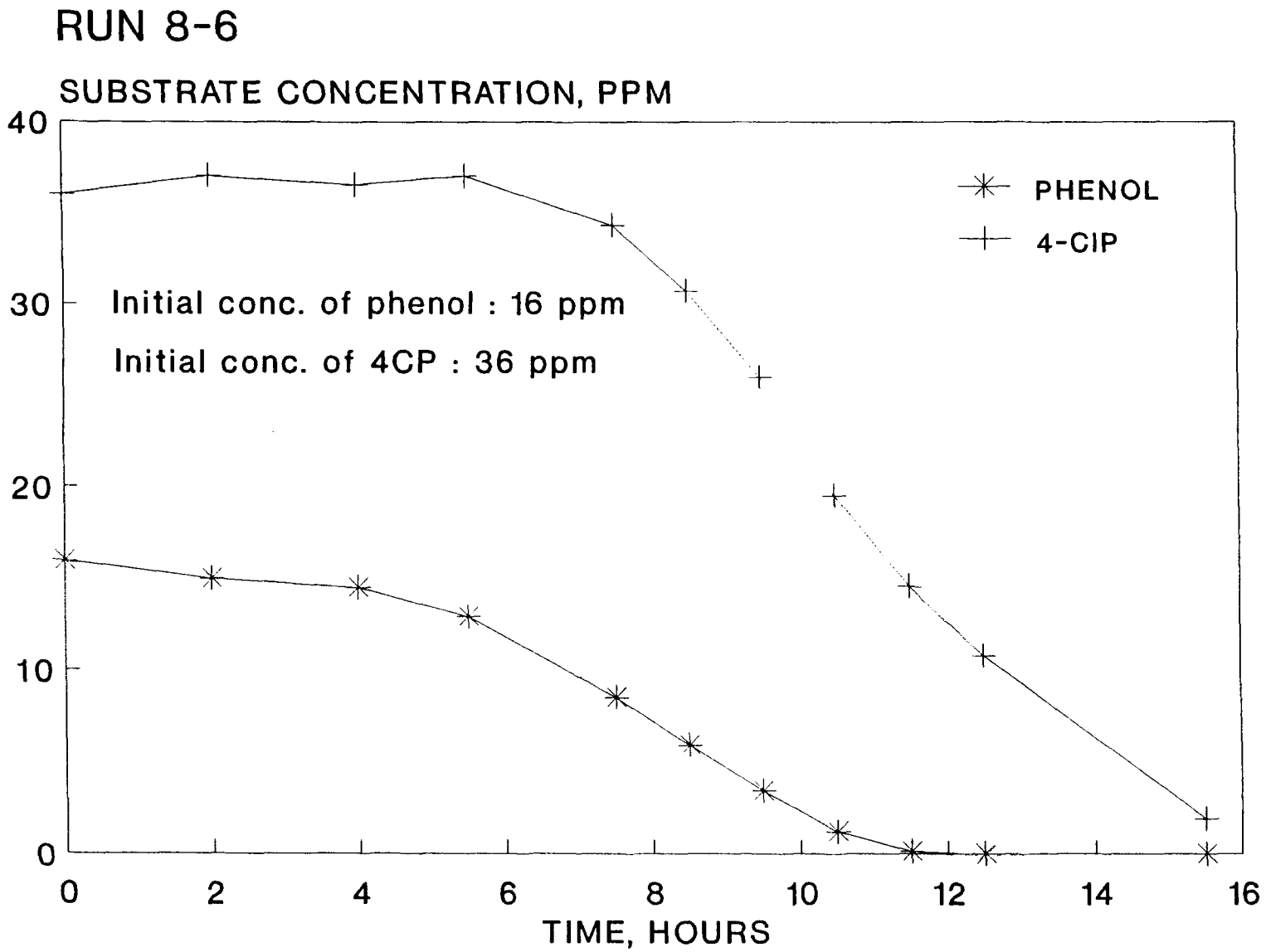

RUN 8-6
SUBSTRATE CONCENTRATION, PPM
40
30
20
10
0
PHENOL
4-CIP
Initial conc. of phenol : 16 ppm
Initial conc. of 4CP : 36 ppm
0
2
4
6
8
10
12
14
16
TIME, HOURS

FIG. 9.1 FIRST CYCLE OF SBR RUN 1 IN PHENOL ONLY

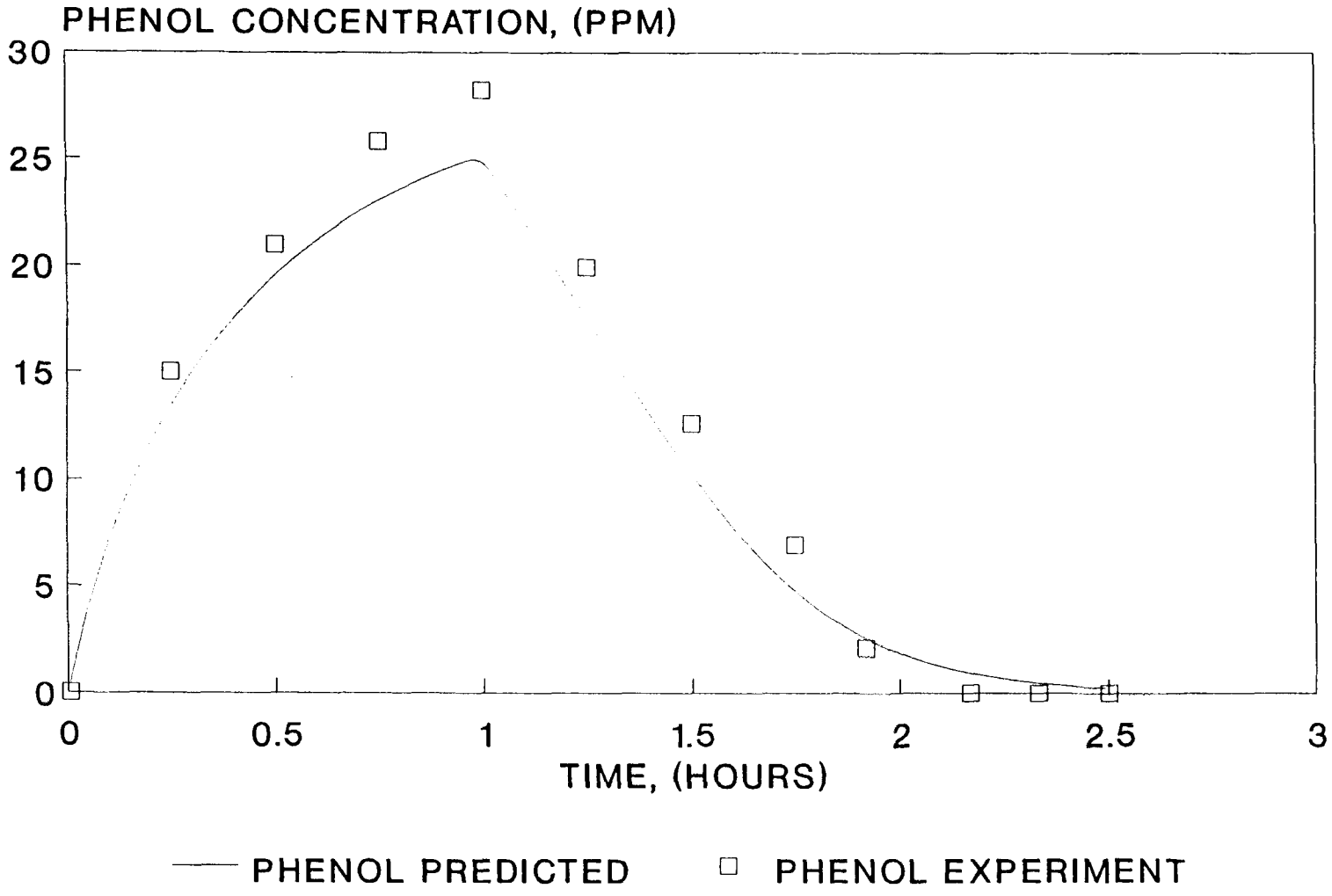


FIG. 9.2 FIRST CYCLE OF SBR RUN 1 IN PHENOL ONLY

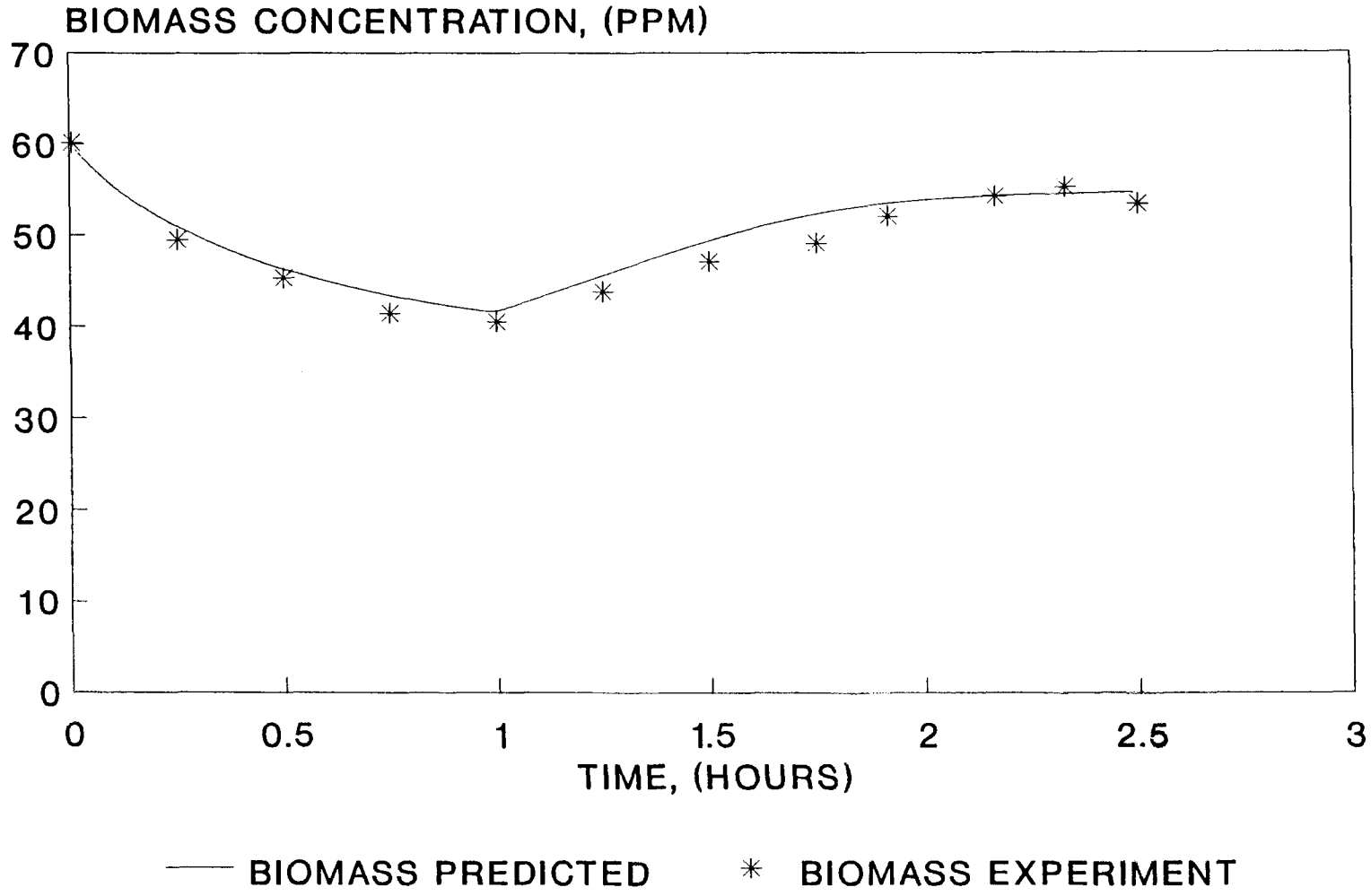


FIG. 9.3 STEADY CYCLE OF SBR RUN 1 IN PHENOL ONLY

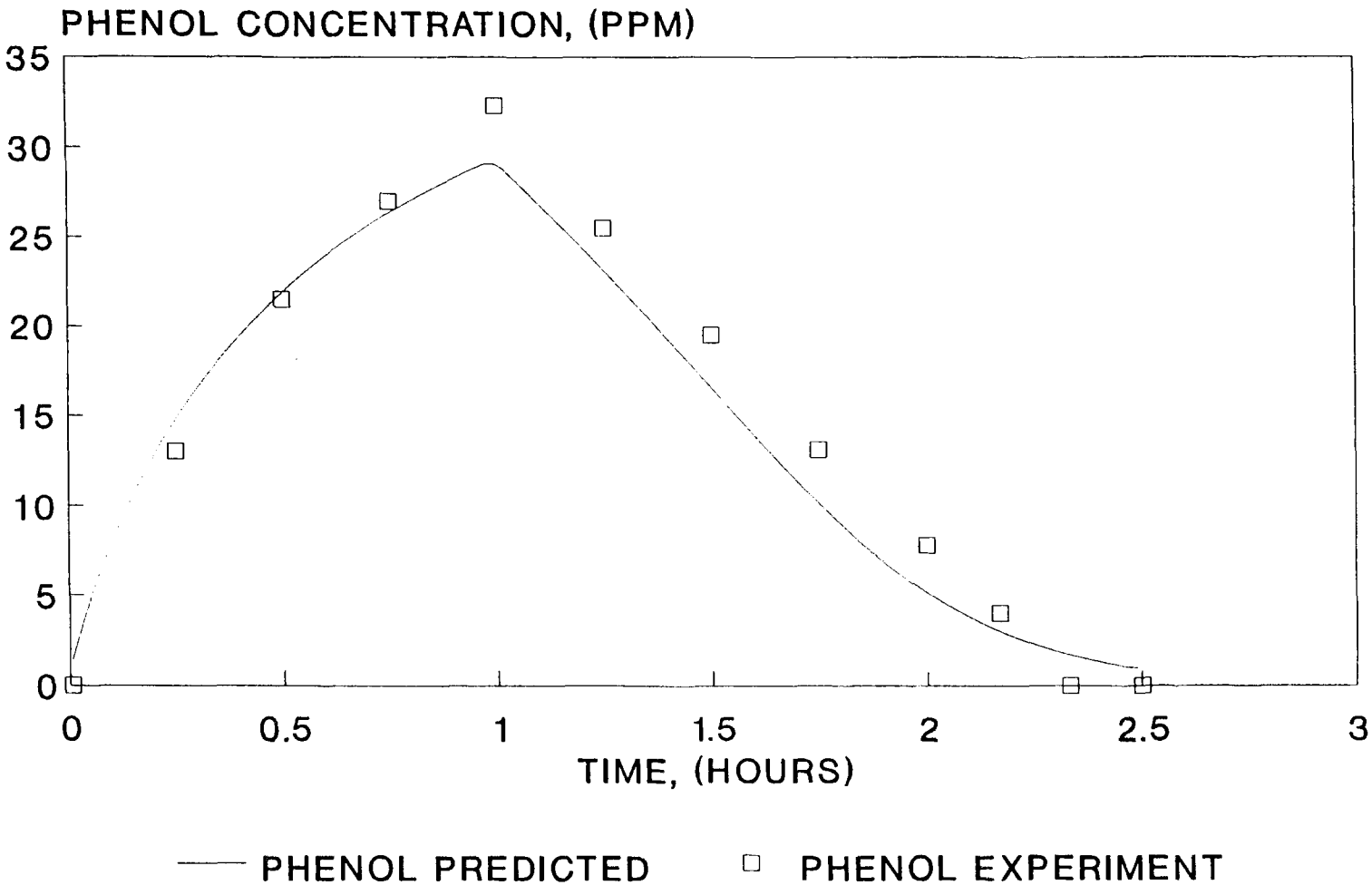


FIG. 9.4 STEADY CYCLE OF SBR RUN 1 IN PHENOL ONLY

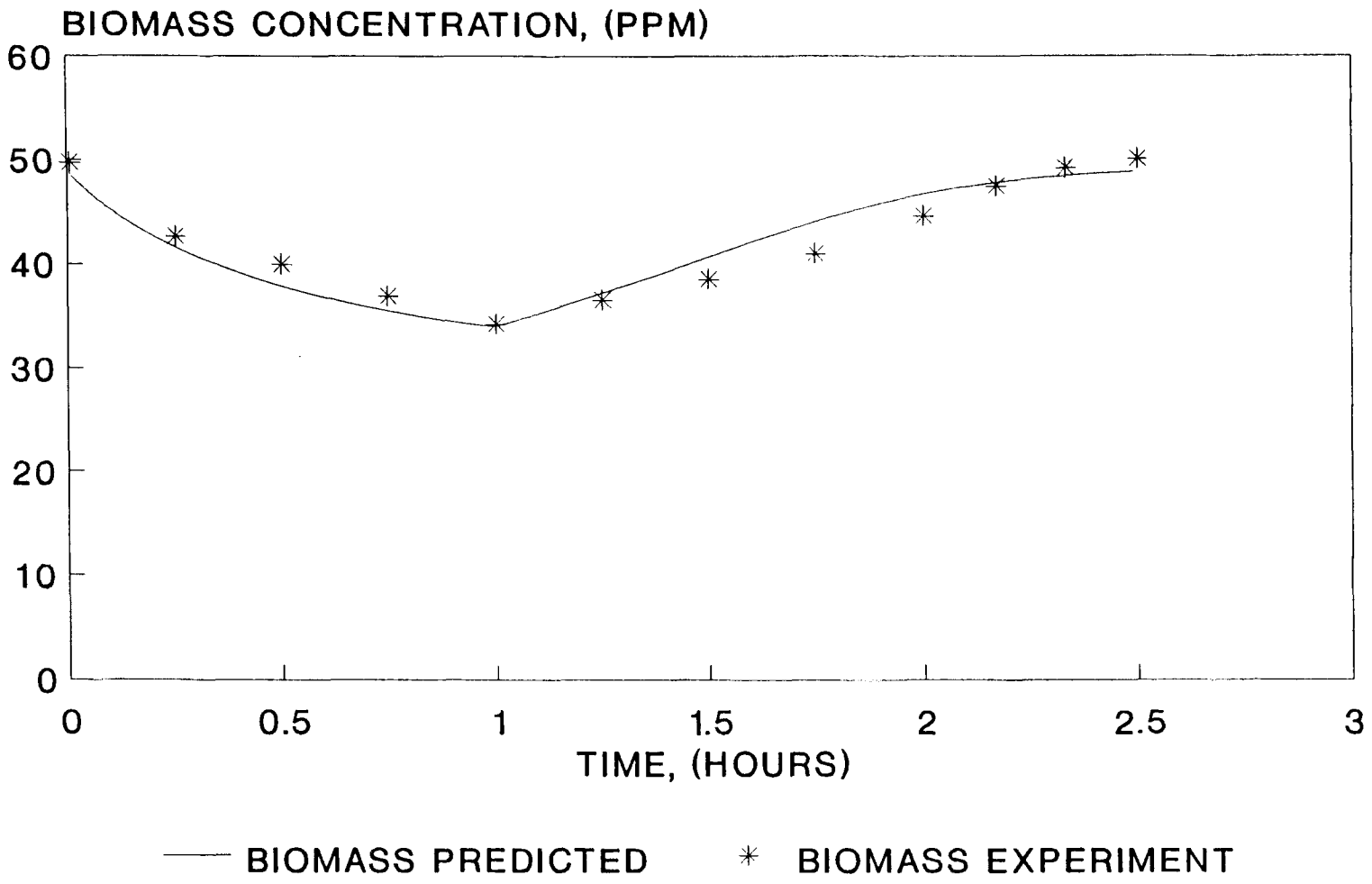


FIG. 10.1 FIRST CYCLE OF SBR RUN 2 IN PHENOL AND 4CP

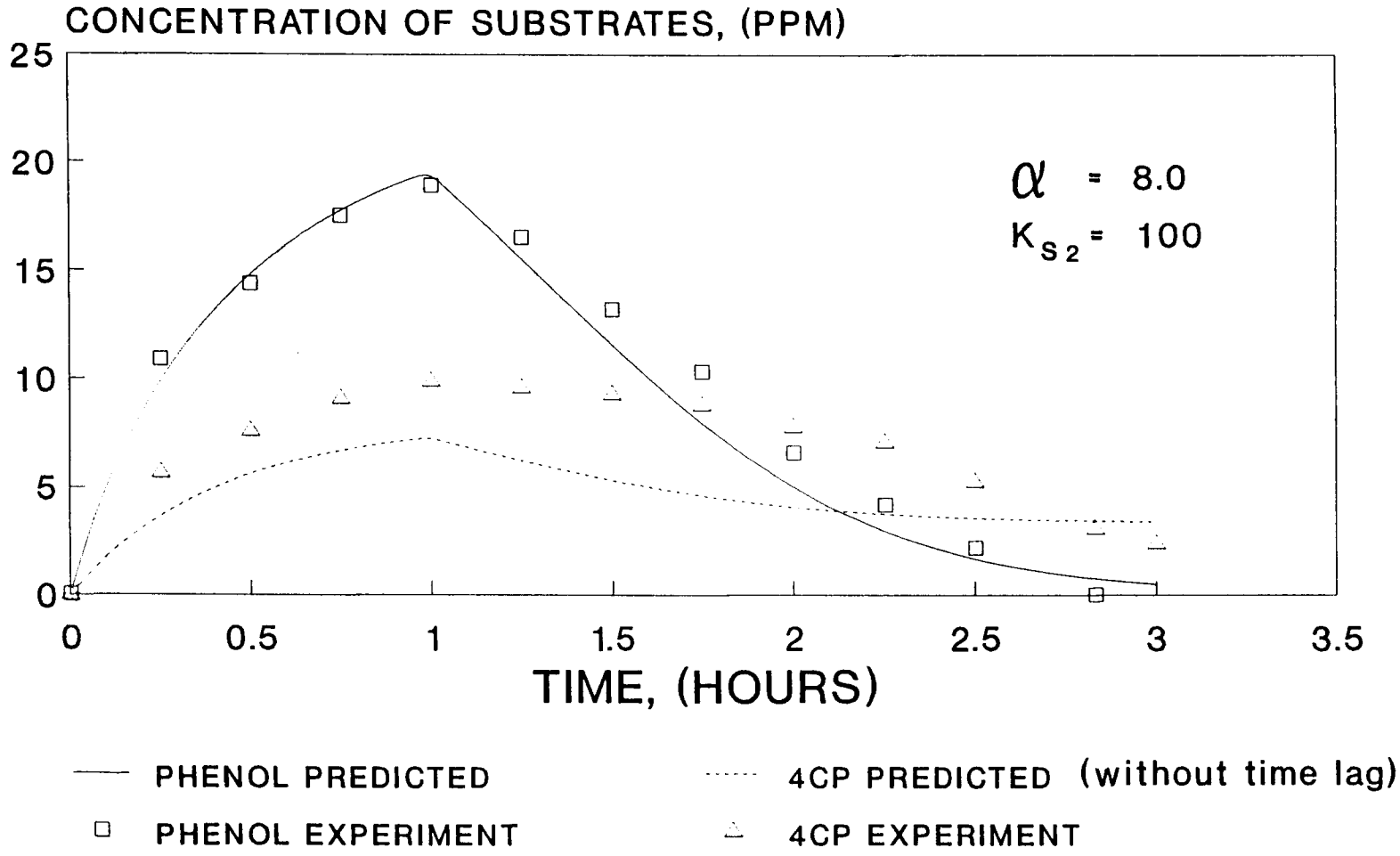


FIG. 10.2 FIRST CYCLE OF SBR RUN 2 IN PHENOL AND 4CP

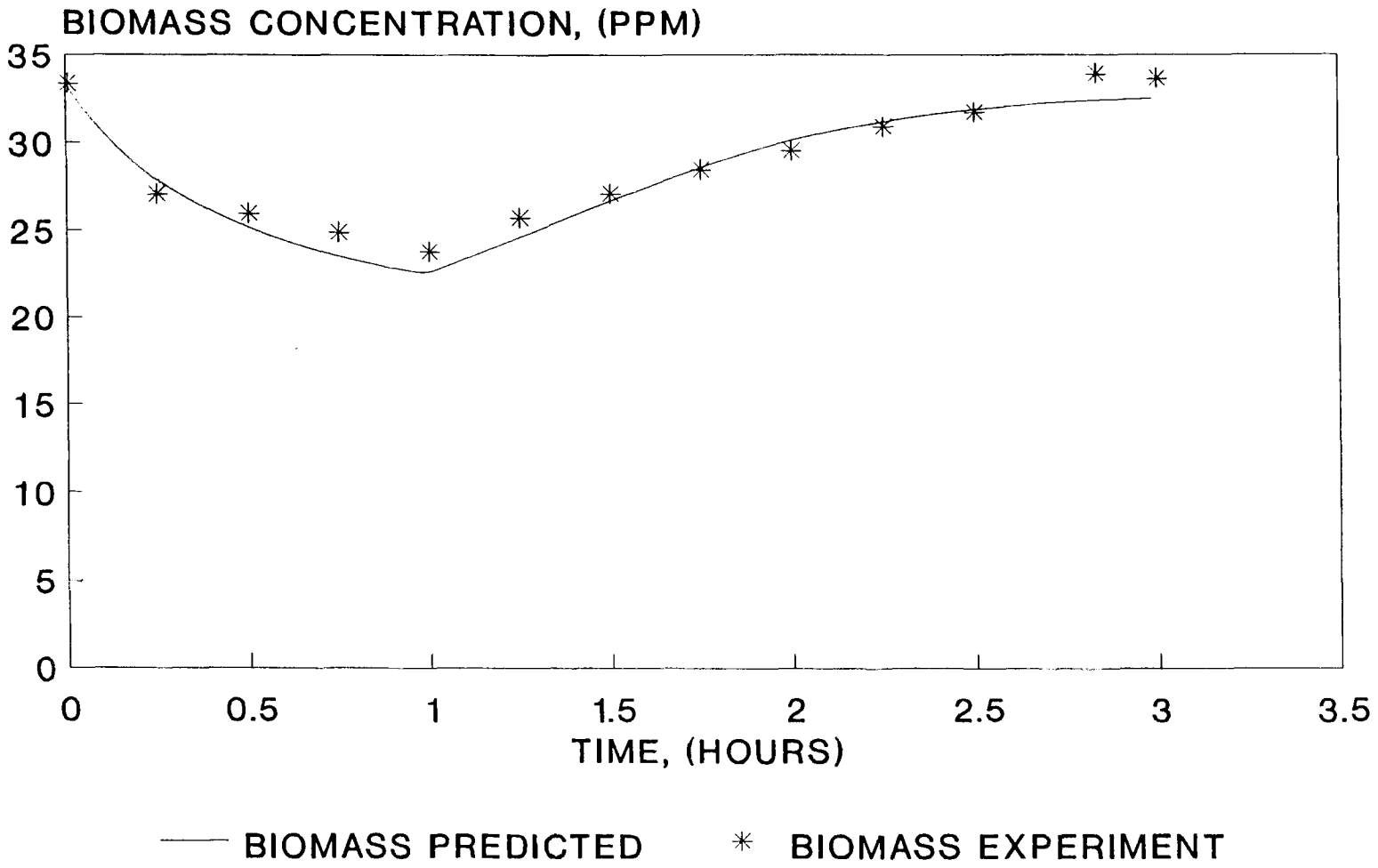


FIG. 10.3 STEADY CYCLE OF SBR RUN 2 IN PHENOL AND 4CP

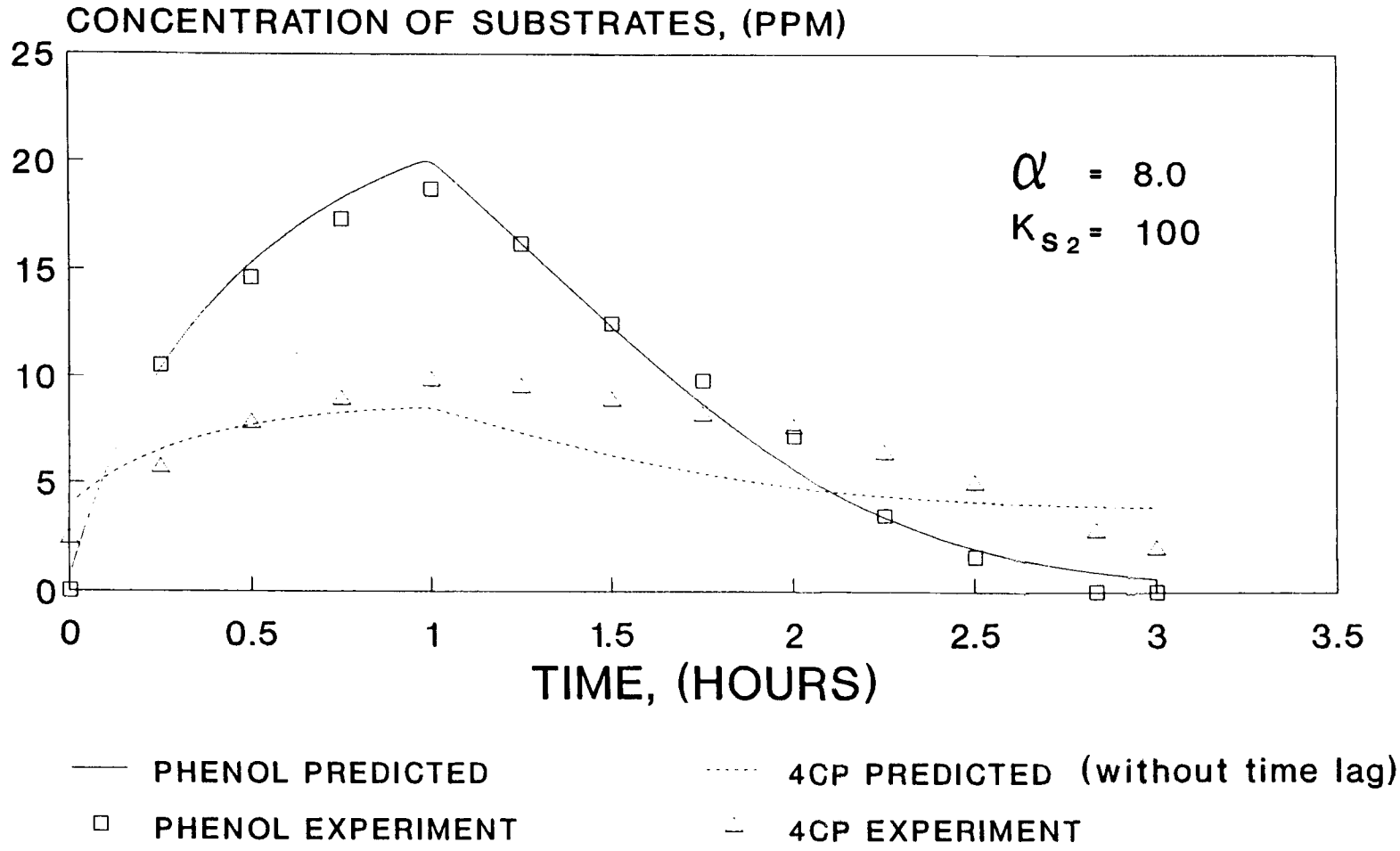


FIG. 10.4 STEADY CYCLE OF SBR RUN 2 IN PHENOL AND 4CP

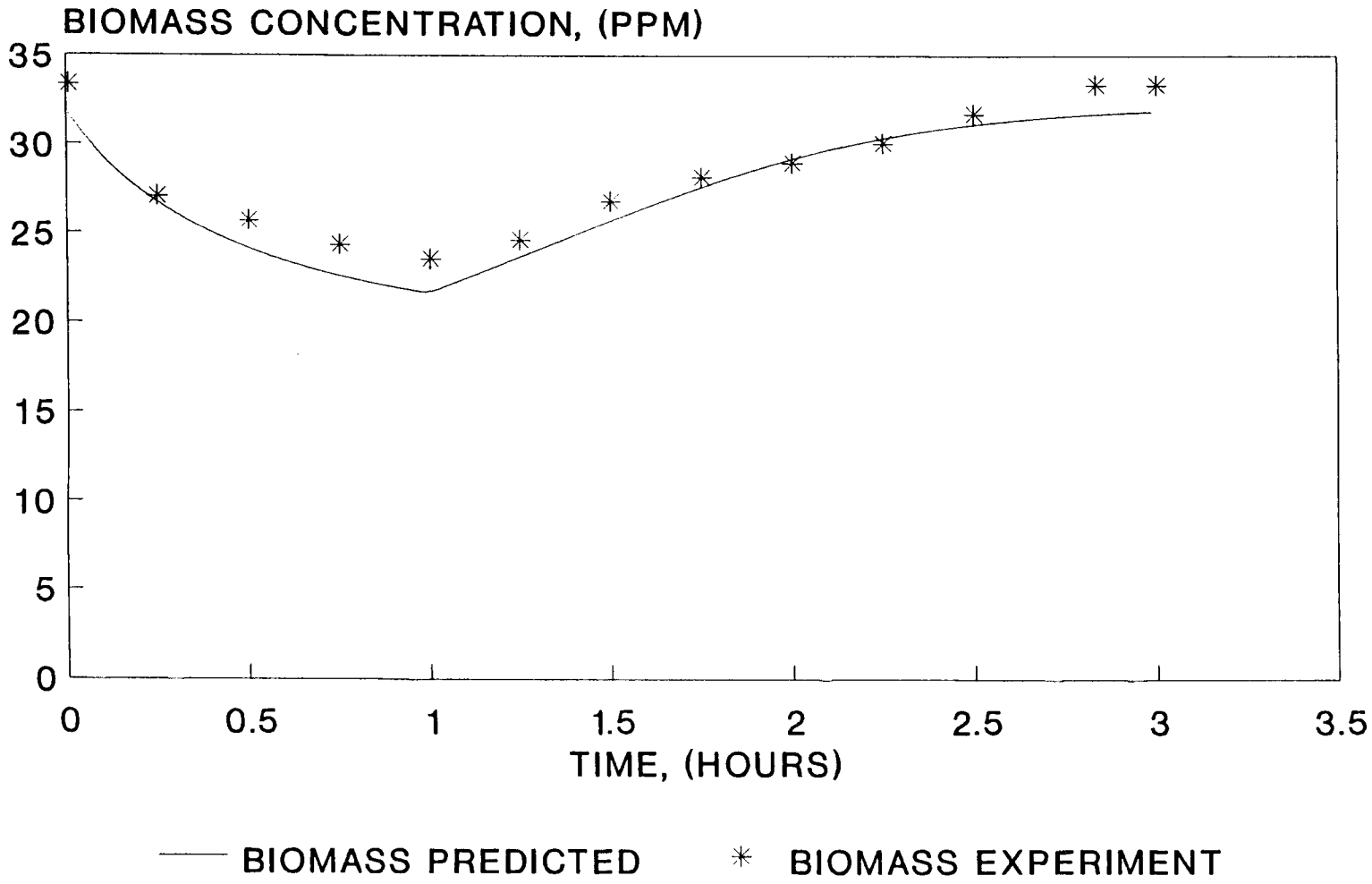


FIG. 10.5 FIRST CYCLE OF SBR RUN 2 IN PHENOL AND 4CP

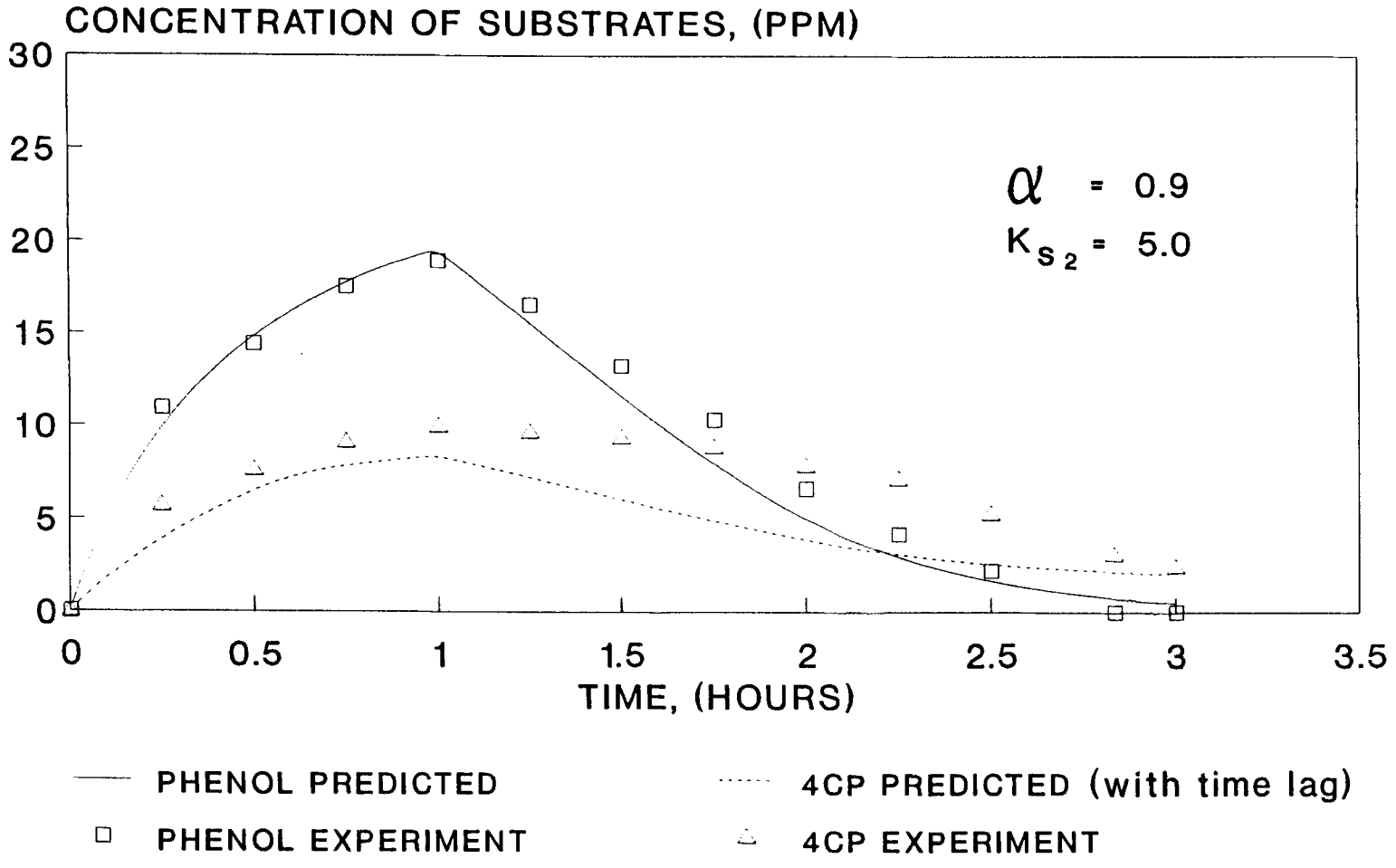


FIG. 10.6 STEADY CYCLE OF SBR RUN 2 IN PHENOL AND 4CP