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ABSTRACT

Title of Thesis : BIODEGRADATION OF PHENOL BY TWO COMPETING MICROBIAL SPECIES IN A SEQUENCING BATCH REACTOR

Yoo-Suk Ko , Master of Science in Chemical Engineering, 1990

Thesis directed by : Dr. Basil C. Baltzis

Dr. Gordon Lewandowski

Biodegradation of a single pollutant by two competing microbial species has been mathematically described for the case where a sequencing batch reactor is used. The model has been solved numerically to predict the behavior of the reactor under different operating conditions and for different kinetic expressions for biodegradation.

Experiments were performed to verify the predictions of the model. Serratia marcescens (ATCC 17991) and Pseudomonas putida (ATCC 31800) were the species used. Experiments were first performed in order to reveal the kinetics of biodegradation for each individual species. The biodegradable substance used was phenol, which was the sole carbon source in the medium. Mixed culture experiments were performed in a 5-liter fill-and-draw reactor. Excellent agreement (both qualitatively and quantatively) was obtained between the experimental data and the model predictions.

BIODEGRADATION OF PHENOL BY TWO COMPETING MICROBIAL SPECIES IN A SEQUENCING BATCH REACTOR

BY

YOO-SUK KO

Thesis submitted to the Faculty of the Graduate School
of the
New Jersey Institute of Technology
in partial Fulfillment of the Requirements for the Degree
of
Master of Science in Chemical Engineering

APPROVAL SHEET

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He would also like to express his gratitude to his parents and fiance for their mental and financial support and love, and for encouraging him to go for further study . Finally, hoping that his fiance will overcome her trouble in her mind and body, he dedicates this thesis to her and his parents.

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I. INTRODUCTION

The performance of many processes and operations can be improved appreciably by controlled periodic operations (unsteady-state processes) [1,2] concerning carrying out for adsorption, ionexchange, particle separation, and various reactions. In many cases including above operations, it has been proved that processes operated periodically have marked increase in performance relative to those of conventional steady continuous flow operations.

In the field of biological wastewater treatment, continuous flow systems (CSTRs) have dominated the technology, and fill-and-draw reactors haven't yet gained wide acceptance. This was originally due to a lack of automated equipment capable of controlling flow, aeration equipment that would resist plugging during start/stop operation, the additional labor costs associated with maintenance and supervision, and the perceived advantages of continuous processes. However, with computer-aided operation of biochemical processes and new techniques in process control, fill-and-draw reactors can be very competitive [3].

Fill-and-draw reactors (also called semibatch or sequencing batch reactors [SBRs]) include five discrete periods : fill, react, settle, draw, and idle. They may be composed of one or more reactors in series.

Quality control in a fill-and-draw reactor is easier to maintain than in a CSTR, especially when the feed is a variable waste. Since the reaction phase is mainly in a batch mode, the reaction products can be

held in the reactor until they are acceptable for discharge. Other operational advantages include : greater flexibility in handling a variable waste, the capability of having both anoxic and aerated periods for control of growth of filamentous organisms, and a much smaller volume for the same throughput [10].

II. LITERATURE REVIEW

The primary investigator responsible for resuscitating fill-and-draw technology has been Robert Irvine at university of Norte Dame. Irvine and Richter [4,5,6,7] conducted simulation studies of fill-and-draw reactor and developed design equations to obtain experimental data in a 4-liter bench scale reactor using a synthetic industrial waste with a soluble TOC of approximately 500 ppm. Their results showed how the design volume for a sequencing batch system changed as a function of the relatively variable mass flow rate, even though the average mass flow rate was the same for all cases investigated.

A study of the effect of fill time vs. react time on sequencing batch reactors was conducted by Dennis and Irvine [8]. The experiments were performed in 4 liter bench-scale plexiglass reactors, the total cycle time was 8 hrs, and the influent feed concentration had a BOD_5 of 400 mg/l. It was found that the average effluent soluble BOD_5 was 3 mg/l in all cases. They concluded that a properly designed fill-and-draw reactor should achieve a higher effluent quality than a CSTR of the same size.

Hsu [9] compared the performance of a 3.5 liter SBR to that of a 4-liter conventional activated sludge unit, using a petrochemical wastewater. Both reactors were operated at the same

hydraulic retention time of 2 days, and the same solids retention time of about 10 days. During the fill phase of the SBR, the feed was instantly added to the drawn-down reactor contents, and so the SBR was simply modelled as a batch reactor, using Monod kinetics. Both the SBR and conventional reactor were shock loaded with phenol at concentrations as high as 200 ppm. The MLSS concentration was higher in the SBR, while soluble BOD_5 and nitrogen in the effluent were lower. However, more pin flocs were observed in the SBR, which contributed to a higher suspended solids concentration in the overflow. This may have been caused by the rapid method of feed addition. Although filamentous growth was clearly present in the conventional unit, its effect was mitigated by an oversized clarifier and a high sludge return rate. There was no filamentous growth in the SBR. It was observed that the performance of the SBR was superior to that of CSTR , even though a more efficient air diffuser was used in the conventional activated sludge unit.

Chang [10] used Monod kinetics for describing phenol biodegradation in a fill-and-draw reactor. It was shown from his study that Pseudomonas putida exhibited substrate inhibition at phenol concentration higher than 60 ppm. At feed concentration of phenol less than 60 ppm, his results showed good agreement between the predictions of mathematical modelling of Monod kinetics and experiments, both during the transient and steady cycles.

Based on the Chang's [10] results, Y.F. Ko [11] used a substrate

inhibition model (Andrews kinetics) to study the dynamic behavior of a fill-and-draw reactor. He solved the model he proposed, numerically and tested its predictions experimentally, using a 5-liter fill-and-draw reactor with phenol as the sole carbon source which was biodegraded by a pure culture of Pseudomanas putida. It was shown that experimental results compared very well to the model predictions and concluded that there were operating conditions under which proper start-up conditions were important for survival of the culture.

Sokol and Howell [12] examined the biodegradation of phenol using pure cultures of Pseudomonas putida growing in continuous culture. Both Monod and Andrews models were applied to the data and the best fit kinetic parameters were determined by regression.

Chiesa and Irvine [13] reported results of a study in which the sludge volume index (SVI) was reduced from about 600 to 50 ppm in a series of batch reactors subjected to different operating strategies. Percent of aerated fill time was decreased successively from 100 % (for a SVI of about 600 ppm) to 0 % (for a SVI of about 50 ppm). They found that the best operating strategy in a SBR was to have a major portion of fill unmixed and unaerated, followed by mixing and aeration during the remaining 15 to 30 minutes of fill time.

Ketchum, Irvine, and Liao [14] studied two different modes of SBR operation. In the first case, all oxygen demands were satisfied, and

in the second, oxygen was limited to that supplied by a constant rate aeration system operating at a rate less than what 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 problems of bulking. They also studied the cost analysis of sequencing batch biological reactors. They concluded that the sequencing batch system required the least cost of the alternatives considered for small facilities in small rural communities.

Aris and Humphrey [15] studied the dynamics of a chemostat in which two organisms compete for a common substrate. They found that the possible steady states depend on the relative disposition of the two specific growth rate curves. It is also shown how the stability of each steady state can be understood and qualitative phase portraits can be drawn for each of the 31 distinct types of situations. Finally, this is one more study indicating that pure and simple competitors can't coexist at a steady state in a single homogeneous vessel.

Yang and Humphrey [16] studied the microbial degradation of phenol by pure and mixed cultures of Pseudomonas putida in batch, phenol-stat, 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 performed an analysis on the stability and dynamic behavior of pure and

mixed cultures. Their results indicated that it should be possible to achieve phenol removal from waste waters down to levels of 1-2 ppm in a single stage system. However, because of the effects of substrate inhibition on the kinetic behavior of microorganisms, long lasting transients can occur. They concluded that the transient behavior of such systems can not be solely determined from the model parameters. They suggested that a consideration of transient size and response characteristics of the organism must be included in the model for proper predictions.

Hill and Robinson [17] studied substrate inhibition kinetics for phenol biodegradation by Pseudomonas putida. 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 function was found to statistically best describe the kinetics. It was particularly shown that wall growth was found to exert a significant effect on the broth biomass concentration and phenol conversion both of which decreased with increasing amounts of wall growth.

Gottschal and Thingstad [18] studied competition between two and three bacterial species under dual substrate limitation in the chemostat via mathematical modelling. They found that Monod kinetics in the model allowed an assessment of the relative importance of the growth parameters for the outcome of the competition. Their study showed how the results of the mathematical description of the

two-membered mixed cultures can be used to predict the outcome of competition of three species competing for two growth-limiting substrates in a three-membered mixed culture. They concluded that facultatively chemolithotrophic bacteria are able to survive under appropriate limiting mixed substrate conditions in the presence of more specialized heterotrophs and obligately chemolithotrophs in spite of their relatively low specific growth rate.

Lauffenburger and Calcagno [19] studied competition between two microbial populations in a non-mixed environment. It was found that bacterial population growth may depend upon the respective cell motility properties in a situation of competition between two bacterial populations for a single chemical nutrient in a non-mixed environment. Anaylsis of this model yielded the following three results : (1) the coexistence state can arise even though one species possesse a smaller intrinsic growth rate constant at all nutrient concentrations, if that same species is sufficiently less motile than the other species. (2) the species with the smaller maximum specific growth rate may grow to a larger population than the other. (3) the possibility of coexistence can be decided essentially from the results of single population growth.

III. OBJECTIVES

The objectives of the present work were to mathematically describe biodegradation of a single substance by two competing organisms, and to experimentally verify the model predictions. This work can be viewed as the first step of an effort to understand and systematically predict results in a unit where biodegradation occurs by a mixed culture. Mixed cultures result in microbial interactions and the one considered here is the most common, i.e. competition. The behavior of a mixed culture is to be predicted from the kinetics of individual species and from the interactions occuring among the various types of organisms present.

Hence, two species namely, Serratia marcescens (ATCC 17991) and Pseudomonas putida (ATCC 31800), have been used. Phenol was the biodegradable substance. The kinetics of phenol biodegradation by Pseudomonas putida were known from a previous study [11]. The kinetics of phenol biodegradation by Serratia marcescens were to be determined in the present study (in batch experiments), followed by experiments using a mixture of S. marcescens and P. putida , in a sequencing batch reactor (SBR).

IV. MATHEMATICAL MODEL

A mathematical model has been derived, assuming that biodegradation of a single pollutant is achieved by two microbial populations competing purely and simply for that substance. The model was converted to a dimensionless form in order to generalize the results and reduce the number of parameters (and hence the numerical work).

The working volume of the reactor was assumed to change linearly with time during the fill and draw phases. This can be seen in Figure 1. Furthermore, the model does not consider a settling phase, and reaction is assumed to occur throughout the cycle.

DERIVATION OF THE MATHEMATICAL MODEL

(1) General

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

$$\frac{dV}{dt} = Q_f - Q \qquad (1)$$

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 the biomass A :

$$\frac{d(Vb_A)}{dt} = Q_f b_{fA} - Qb_A + \mu_A(s)b_A V$$

or

$$V\frac{db_A}{dt} + b_A\frac{dV}{dt} = Q_f b_{fA} - Qb_A + \mu_A(s)b_A V$$

or, by using equation (1)

$$\frac{db_A}{dt} = \frac{Q_f}{V}(b_{fA} - b_A) + \mu_A(s)b_A \qquad (2)$$

Mass balance on the biomass B :

$$\frac{d(Vb_B)}{dt} = Q_f b_{fB} - Qb_B + \mu_B(s) b_B V$$

or

$$V \frac{db_B}{dt} + b_B \frac{dV}{dt} = Q_f b_{fB} - Qb_B + \mu_B(s) b_B V$$

or, by using equation (1)

$$\frac{db_B}{dt} = \frac{Q_f}{V} (b_{fB} - b_B) + \mu_B(s) b_B \qquad (3)$$

The symbols appearing in equations (2) and (3), and not introduced before, are defined as follows:

b_A = concentration of biomass A in the reactor and in the stream exiting the reactor.

b_B = concentration of biomass B in the reactor and in the stream exiting the reactor.

b_{fj} = concentration of biomass j in the feed to the reactor. For all practical purposes, unless there is recycle of solids to the reactor, the value of this quantity is zero, (j = A or B).

$\mu_A(s)$ = specific growth rate of population A. It is a function of the concentration of the hazardous or toxic substance which exerts rate limitation on growth.

$\mu_B(s)$ = specific growth rate of population B.

Mass balance on the rate-limiting substrate (i.e., on the toxic substance which is treated in the unit)

$$\frac{ds}{dt} = \frac{Q_f}{V}(s_f - s) - \frac{\mu_A(s)b_A}{Y_A} - \frac{\mu_B(s)b_B}{Y_B} \qquad (4)$$

The symbols not previously introduced and appearing in equation (4) are defined as follows :

s_f = concentration of the toxic substance in the waste fed into the reactor.

s = concentration of the toxic substance in the reactor and in the stream exiting the reactor.

Y_j = yield coefficient of population j on the toxic substance. It stands for the efficiency with which the biomass converts the substrate into more biomass. The yield is assumed to be constant, (j = A or B).

Clearly, some of the terms appearing in equations (1) through (4) are zero during some of the phases of operation. For instance,there is no input or output from the reactor during the reaction phase. This becomes clear in the following sections when each phase of operation is described separately.

(2) Use of Andrews Kinetics

The Andrews model sometimes referred to as the Haldane model, expresses the specific growth rate as follows:

$$\mu_A = \frac{\hat{\mu}_A\, s}{K_{sA} + s + (s^2/K_{iA})} \quad , \qquad \mu_B = \frac{\hat{\mu}_B\, s}{K_{sB} + s + (s^2/K_{iB})}$$

Where,

$\hat{\mu}_j$ = characteristic constant having units of inverse of time, (j = A or B)

K_{sj} = constant having units of concentration, (j = A or B).

K_{ij} = inhibition constant for population, (j = A or B); it has units of concentration.

$\hat{\mu}_A$, $\hat{\mu}_B$, K_{sA}, K_{sB}, K_{iA}, and K_{iB} are parameters characteristic of the particular substrate and of the given populations.

Equations (1) through (4) can be written in dimensionless form as follows :

$$\frac{dV'}{d\vartheta} = Q_f' - Q' \tag{5}$$

$$\frac{dx_A}{d\vartheta} = -\frac{Q_f'}{V'} x_A + \frac{u}{1 + u + \gamma_A u^2} \beta\, x_A \tag{6}$$

$$\frac{dx_B}{d\vartheta} = -\frac{Q_f'}{V'} x_B + \frac{u}{w + u + \gamma_B u^2} \beta \phi x_B \qquad (7)$$

$$\frac{du}{d\vartheta} = \frac{Q_f'}{V'} (u_f - u) - \frac{u}{1 + u + \gamma_A u^2} \beta x_A - \frac{u}{w + u + \gamma_B u^2} \eta \beta \phi x_B \qquad (8)$$

where,

$u = \frac{s}{K_{sA}}$ = dimensionless concentration of the toxic substance in the reactor and in the stream exiting the vessel.

$u_f = \frac{s_f}{K_{sA}}$ = dimensionless concentration of the toxic substance in the stream fed into the reactor.

$x_A = \frac{b_A}{Y_A K_{SA}}$ = dimensionless concentration of biomass A in the reactor and in the exit of the reactor.

$x_B = \frac{b_B}{Y_A K_{sA}}$ = dimensionless concentration of biomass B in the reactor and in the exit of the reactor.

$Q_f' = \frac{Q_f}{Q_r}$ = dimensionless volumetric flow rate of the stream fed into the reactor (Q_r is a reference volumetric flow rate defined later in this section).

$Q' = \frac{Q}{Q_r}$ = dimensionless volumetric flow rate of the stream leaving the reactor.

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

V_{max} = volume of the reactor contents at the end of the fill phase

$\vartheta = \frac{tQ_r}{V_{max}}$ = dimensionless time.

$\beta = \frac{\hat{\mu}_A V_{max}}{Q_r}$ = a dimensionless measure of the hydraulic residence time.

$\gamma_A = \frac{K_{sA}}{K_{iA}}$ = dimensionless inverse inhibition constant for species A.

$\gamma_B = \frac{K_{sA}}{K_{iB}}$ = dimensionless inverse inhibition constant for species B.

$\eta = \frac{Y_A}{Y_B}$ = ratio of yield coefficients.

$\phi = \frac{\hat{\mu}_A}{\hat{\mu}_B}$ = ratio of parameters.

$w = \frac{K_{sB}}{K_{sA}}$ = ratio of model constants.

(3)Description of the phases of the SBR

Figure (1a) indicates the way the volume of the system changes with time during the various phases. V_{max} is the maximum working volume,(i.e.,the volume of the system at the end of the fill phase),while V_o is the volume of the system at the end of the draw phase.

During the fill phase ($0 \leq t \leq t_1$), the volume increases linearly with time since the system is assumed to be fed at a constant volumetric flow rate. During the react phase ($t_1 \leq t \leq t_2$),as well as during the settling phase ($t_2 \leq t \leq t_2'$),the volume of the system remains constant and equal to V_{max}. During the draw phase ($t_2' \leq t \leq t_3$), the volume of the system decreases linearly with time (because the reactor is assumed to be emptied at a constant flow rate) from the value V_{max} to the original value V_o. During the idle phase ($t_3 \leq t \leq t_3'$), the volume of the system remains constant and equal to V_o. At time $t=t_3'$, the cycle is repeated.

The diagram shown in Figure (1b) indicates the way the volume of the system is assumed to change with time in the present study, in which no settling or idle periods are considered.

The volume and volumetric flow rates appearing in equations (1) through (4) can be expressed as follows for the various phases:

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

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

where, $Q_{f,SBR}$ is the volumetric flowrate of the feed to the

SBR,

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

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

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

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

It is also true (under the assumption of constant density) that the volume fed into the SBR during the fill phase must be equal to the volume exiting the SBR during the draw phase, i.e.,

$$[Q_{f,SBR}]t_1 = Q\,[t_3 - t_2] \qquad (9)$$

By defining,

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

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

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

and using relation (9), it is clear that

$$Q_f' = Q'_{f,SBR} = \frac{1}{\sigma_1} \text{ , and } Q' = \frac{1}{\sigma_3} \qquad (10)$$

At the end of the fill phase , the volume of the reactor reaches its maximum value, V_{max}. Hence,

$$1 = \delta + Q_f' \, \vartheta_1$$

or, by using the first relation (10)

$$1 = \delta + \frac{1}{\sigma_1} \vartheta_1 \text{ ,or } \quad \vartheta_1 = (1 - \delta) \, \sigma_1 \tag{11}$$

where, $$\delta = \frac{V_o}{V_{max}}$$

i.e., the fraction of the vessel which is occupied by mixed liquor at the end of the draw phase.

From the definition of σ_3, one can see that

$$\vartheta_3 \sigma_3 = \vartheta_3 - \vartheta_2 \tag{12}$$

At the end of the draw phase,the volume of the system is equal to its minimum value, V_o ,and thus one can write

$$\delta = 1 - Q' \, (\vartheta_3 - \vartheta_2)$$

or by using the second relation (12)

$$\delta = 1 - \frac{1}{\sigma_3} (\vartheta_3 - \vartheta_2) \tag{13}$$

Combining relations (12) and (13), one gets

$$\vartheta_3 = 1 - \delta \tag{14}$$

Relations (13) and (14) also result in

$$\vartheta_2 = (1 - \delta)(1 - \sigma_3) \tag{15}$$

Using relations (10), (11), (14) and (15) one can now describe the volumetric flow rate and the volume of the system during the three phases as follows:

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

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

React phase, $(1 - \delta)\sigma_1 \leq \vartheta \leq (1 - \delta)(1 - \sigma_3)$

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

Draw phase, $(1 - \delta)(1 - \sigma_3) \leq \vartheta \leq 1 - \delta$

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

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

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

$$\frac{dx_A}{d\vartheta} = -\frac{1}{\delta\,\sigma_1 + \vartheta}\,x_A + \frac{u}{1 + u + \gamma_A u^2}\,\beta\,x_A \qquad (16)$$

$$\frac{dx_B}{d\vartheta} = -\frac{1}{\delta\,\sigma_1 + \vartheta}\,x_B + \frac{u}{1 + u + \gamma_B u^2}\,\phi\,\beta\,x_B \qquad (17)$$

$$\frac{du}{d\vartheta} = -\frac{1}{\delta\,\sigma_1 + \vartheta}(u_f - u) - \frac{u\,\beta\,x_A}{1 + u + \gamma_A u^2} - \frac{\eta\,u\,\phi\,\beta\,x_B}{w + u + \gamma_B u^2} \qquad (18)$$

(ii) React and Draw phase $[\ (1-\delta)\,\sigma_1 \le \vartheta \le (1-\delta)\ ]$

$$\frac{dx_A}{d\vartheta} = \frac{u}{1 + u + \gamma_A u^2}\,\beta\,x_A \qquad (19)$$

$$\frac{dx_B}{d\vartheta} = \frac{u}{w + u + \gamma_B u^2}\,\phi\,\beta\,x_B \qquad (20)$$

$$\frac{du}{d\vartheta} = -\frac{u}{1 + u + \gamma_A u^2}\,\beta\,x_A - \frac{u}{w + u + \gamma_B u^2}\,\phi\,\beta\,\eta\,x_B \qquad (21)$$

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

In the original formulation of the problem (equations (1) through (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 V_0, t_1, t_2, t_3, $\hat{\mu}_A$, $\hat{\mu}_B$, K_{sA}, K_{sB}, K_{iA}, K_{iB}, Y_A, Y_B, s_f, Q_f.

The values of V_{max} and Q are not independent parameters, since $V_{max} = Q_f t_1 + V_0$ and $Q\ (t_3 - t_2) = Q_f t_1$. In the final formulation of the problem (equations (16) through (21)) in terms of the dimensionless quantities (u, x_A, and x_B), one needs to specify the values of 7 parameters (i.e., x_f, u_f, σ_1, σ_3, γ_1, γ_2, δ). This reduction in the number of the 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.

V. EXPERIMENTAL APPARATUS

A. Batch System

All experiments were conducted in a constant temperature shaker bath at 28 °C. The inoculated solutions were placed in 250 ml flasks on the rotary shaker (Model G-24 New Brunswick Scientific Company, New Brunswick, NJ). The only aeration was that transferred through the cotton plug by shaking.

B. SBR System

All experiments were conducted at room temperature (approximately 21 °C). The reactor was a 15 cm diameter, 5-liter capacity, cylindrical vessel constructed of Lucite, capped with a removable lid. An effluent port was installed two liters above the bottom, with a solenoid valve to control the discharge of treated wastewater.

Aeration alone provided the agitation and there was no mechanical stirring.

Laboratory compressed air was passed through a series of filters and activated carbon before entering the reactor. The volume of air was regulated by two needle-valve rotameters,with a solenoid valve on each air line. To increase the efficiency of air/liquid contact, a porous diffuser stone was placed on the end of each air line at the bottom of the reactor.

A microprocessor (Omron, Sysmac-PO Sequence controller) controlled the system (feed peristaltic pump, air solenoid valves, and decant solenoid valve). Any combination of fill, react, and draw period times could easily be programmed into the computer. The output setting and programming of the sequence controller are described in Appendix A.

A schematic diagram of the fill-and-draw reactor assembly is depicted in Figure 2.

C. Analytical Equipment

(1) Varian Model 3370 Gas Chromatograph with flame ionization detector. operating temperatures :

oven = 140 °C

injector port = 210 °C

detector = 250 °C

(2) GC column - mr 81439 , glass , 3 % sp-2100 on 100/120 Supelcoport , Supelco.

(3) Hewlett - Packard 3390 Electronic Integrator

(4) Waters HPLC & Waters 600E system controller

(5) HPLC column - No : 3064B Length : 15 cm

OD : 1/4 in ID : 4.6 mm

particle size : 5 μ Packing : LC-8

Solvent - 60 : 40 MeOH : H_2O

Cat No : 5 - 8220 Supelco

Mobile phase A : 1 % CH_3COOH in H_2O

Mobile phase B : 1 % CH_3COOH in MeOH

(6) Waters 715 ULTRA WISP sample processor

(7) Water 994 programmable Photodiode Array detector

(8) Waters 5200 printer plotter

(9) Mc micro-software linked to Star Nx-1000 Printer

(10) DO & PH meter Orion Research , Model 701A

(11) Spectrophotometer Bausch & Lomb ,spectronic 20,70

VI. EXPERIMENTAL PROCEDURES

In order to have a clear test of the mathematical model under consideration, it was necessary to obtain well defined rate parameters for the microbial populations employed in the reactor. As a result, pure cultures of Serratia marcescens (ATCC 17991) and Pseudomonas putida (ATCC 31800) were first used in this study. The growth parameters obtained from batch experiments were used to test the model in the SBR, when a mixed culture of these species was used.

A stock culture was prepared by transfering a loop of dried biomass into Difco-Bacto nutrient broth and placed outside for about 24 hrs, and then stored at 4 ^{o}C in a refrigerator. The primary culture was prepared by transfering a loop of stock culture to 2.5 ml of sterilized defined medium solution diluted with 47.5 ml of distilled water (i.e., a final concentration of 50 ppm phenol). The inoculated culture was then placed in a 250 ml flask and incubated for approximately one day at 28 ^{o}C in a rotary shaker bath (rotating at 250 rpm). The medium was aerated by virtue of the shaking process.

A secondary culture was prepared by transfering 2.5 ml of sterilized defined medium solution diluted with 47.5 ml of distilled water. The inoculated culture was then placed in a 250 ml flask stoppered with cotton plug and incubated for 12 to 14 hours at 28 ^{o}C in a rotary shaker bath (rotating at 250 rpm). The medium was repeated for tertiary cultures in order to ensure that the culture had fully adapted to growth on the phenol medium and that phenol was the sole carbon

source. Both cultures followed above procedures to be adapted in both batch and SBR experiments.

A. Formulation of Defined Medium

Many papers have proposed formulations of phenol defined growth medium solutions for which there is often little or no fundamental growth justification. Modified Gaudy's growth medium solution was used in this work. The composition of the solution, shown in Table 1, contained carbon, nitrogen, and phosphate as nutrients. Phenol was the sole carbon source, and ammonium sulfate/potassium phosphate provided nitrogen, phosphorus and buffer.

B. Determination of Andrews Parameters

The growth parameters of Pseudomonas putida were determined from previous work [11], and those of Serratia marcescens were obtained from batch experiments conducted in the present study on tertiary cultures in shaker flasks, which involved measuring the optical density of the cultures on exposure to different initial phenol concentrations (Table 2). When the phenol concentrations are high (e.g., 100 ppm, 140 ppm, 180 ppm, 220 ppm), one needs to take a lot of samples (it takes a long time for microbes to degrade the phenol), and when the phenol concentrations are low (e.g., 5 ppm, 10 ppm), samples have to be taken more rapidly. Hence, a pre-run is required to get a rough idea of the growth behavior of the Serratia marcescens, minimize the amount of sample volume taken from the reactor. Pre-runs were conducted at initial different phenol concentrations of 10, 60, 140, 220 ppm. The

minimum sampling volume required to measure the optical density with the Spectronic 20 is 2.5 ml. From the pre-run data, for initial phenol concentrations of 140 and 220 ppm samples were taken every one and half to 2 hours, whereas for initial phenl concentrations of 10 and 60 ppm samples were taken every 30 minutes.

The optical density was converted to biomass concentration (Table 3) by using a calibration curve for each species (Figure 3). Semi-log plots of biomass concentration (ppm) vs. time (hr) were used to obtain the initial slope for each value of the initial phenol concentration (Figures 4-1 to 4-9). The initial slope is the specific growth rate, which was plotted versus the initial phenol concentration (Figure 6). The yield coefficient Y was also determined by plotting the biomass concentration (Table 3) versus phenol concentration (Table 4), as shown in Figures 5-1 to 5-9.

C SBR Experiments

Growth was allowed to proceed initially in a batch mode (periodically adding defined medium solution when the phenol was depleted), until the biomass concentration was between 56 and 77 % of its predicted maximum steady cycle value. In those experiments where the S. marcescens was expected to wash out of the reactor, the initial biomass concentration was set at 20 to 25 mg/l. Once these initial values were achieved, the SBR sequence was initiated. In the SBR experiments, 2 liters of diluted defined medium solution at the appropriate phenol concentration, were inoculated in the 5-liter Lucite

reactor with the tertiary shaker flask culture. SBR 1 & 2 experiments were conducted with only tertiary Serratia marcescens at feed phenol concentration of 100 ppm, and SBR 3 & 4 experiments were conducted with tertiary Pseudomonas putida & Serratia marcescens at the same feed concentration as in SBR 1 & 2 experiments. The SBR experiments with a pure culture of Serratia marcescens were performed in order to confirm that this species follows the same dynamic behavior as exhibited by Pseudomonas purida [11].

During the fill phase, the feed pumping rate was adjusted so that the reactor volume increased from 2 liters to 4 liters during the fill period. The dissolved oxygen was in the range of 6.9 - 7.4 ppm in the SBR 1 & 2 experiments and 6.6 - 7.2 ppm in the SBR 3 & 4 experiments. The pH was maintained at the range 6.8-7.4 using a potassium phosphate buffer.

In the react phase, the feed pump was shut off, aeration was continued, and samples were taken periodically to measure optical densities and phenol concentrations.

In the cases (SBR 1 & 2) where only S. marcescens was used, the optical density was measured by using a Spectronic 20, and subsequently, the sample was filtered with millipore filter paper to remove microbes and other suspended substances in order to measure the phenol concentration via auto-injection using GC or HPLC. In the cases (SBR 3 & 4) where both S. marcescens and P. putida were used, the optical density of the samples was measured as before, and a loop of sample was streaked on an agar plate to obtain a relative colony count. The color of

S.marcescens is red ; it has red pigment (prodigiosins), while that of P.putida is creamy. Phenol concentration was measured as before.

At the end of the react phase, aeration was continued, and the decant solenoid valve was opened to completely discharge 2 liters of mixed liquor. By continuing aeration during discharge, there was a predicted loss of biomass, which was recovered by growth during the fill and react phases (i.e., in cases where the biomass was not eventually washed-out). This was done to simplify the reactor operation and make it easier to compare the results with the mathematical model. In a real operation, there would be a quiescent settling period prior to discharge.

D . Analytical Prodedures

(1) Biomass Growth

Growth of the organisms was determined by measuring the optical density of the mixed liquor using a spectrophotometer at a wave length of 540 nm, and distilled water as the reference sample. The optical density was then converted to biomass concentration using a calibration curve (Figure 3) obtained earlier [11], and confirmed in this study through the following procedure:

S. marcescens and P. putida were grown independently in a standard nutrient broth, harvested towards the end of the logarithmic growth phase (after about two days), and serially diluted. The turbidity of each dilution (1/10,2/10,3/10/4/10,5/10) was determined spectrophotometrically.

For the dry weight determination of cell mass, three 10 ml samples were taken from the original culture solution (undiluted) and pipetted into three numbered aluminum dishes. The water was then evaporated in an oven at 95 ^{o}C for 24 hours, and the samples reweighed to determine the concentration of suspended solids in the original sample. The suspended solids concentration was assumed to be the same as the biomass concentration. The concentrations of the serially diluted samples were determined by dividing the dry weight value by the dilution volume. The data points of the confirmation test are also shown in Figure 3, which shows good agreement with previous study [11].

In order to obtain the number of relative colonies at every time interval, samples were taken to measure the optical density and streaked

on agar plates, which were then incubated at 30 °C for 20-24 hours. A number of visible colonies having different colors appeared on the agar plate. Knowing the relative concentration from the colony count, and the total concentration from spectrophotometric measurements, the biomass concentration of each species could be determined.

For all experiments in this study , in order to avoid a significant reduction in volume caused by taking large samples from the reactor, 3 ml samples were taken each time (the minimum sample volume for measuring the optical density is 2.5 ml). Samples were taken periodically from the reactor and added to 10 ml cuvettes for immediate measurement of the optical density. After taking each biomass sample, the sampling pipets were carefully cleaned to avoid reactor contamination. The same samples were streaked, filtered, and kept in the refrigerator to measure phenol concentration. In handling the cuvettes, the following procedure was used:

a. The cuvettes were rinsed two or three times with distilled water to clean them.

b. The lower parts of cuvettes were kept clean by maintaining them free of liquids, dirt, smudges, and finger prints, and were wiped clean with lint-free tissue.

(2) Substrate Analysis

When manual injection (Batch experiments) was used (right after the optical density was measured), the sample was analyzed immediately by gas chromatography (at least two times for each sample). 0.5 ml of a

500 ppm thymol solution was added to the sample as an internal standard. The accuracy of the analysis is about +/-1 ppm.

When auto-injection was used to measure phenol concentration, samples were filtered through a 0.45 μm millipore filter to remove the organisms and then collected in the refrigerator at 4 °C until they were loaded onto the autoinjector.

Distilled water blanks were injected into the gas chromatography between samples.

When HPLC was used to measure phenol concentrations, mobile phase A was 1 % acetic acid in HPLC water, and mobile phase B was 1 % acetic acid in methanol. The ratio of the two mobile phases was 50 : 50. A solution composed of 40 % HPLC water and 60 % methanol was used to clean the column.

VII. EXPERIMENTAL RESULTS AND DISCUSSION

From the batch runs in shaker flasks, results were obtained for the specific growth rates, yield coefficeints, and Andrews parameters for Serratia marcescens (ATCC 17991) used for biodegradation of phenol as the sole carbon source. The specific growth rates, and Andrews parameters for Pseudomonas putida (ATCC 31800) were determined in a previous study [11]. These values are given in Tables 5 and 6 for phenol concentrations of 5 to 220 ppm. The Andrews parameters for Serratia marcescens were : $\hat{\mu}$= 0.493 (1/hr), K_s= 29.128 (ppm), K_i= 35.605 (ppm), and Y = 0.260 mg biomass/mg substrate.

From Figure 6, both cultures exhibited substrate inhibition at phenol concentrations above 20 to 60 ppm. However, the specific growth rate for P.putida is always higher than that of S.marcescens. Therefore, in any reactor containing both species, S.marcescens will always wash out, according to the model prediction.

A pure culture of S. marcescens was utilized in the SBR for two runs, and mixed cultures of S. marcescens and P. putida were used in two other runs. These results are given in Tables 7, 8, 9,and 10. The prediction from theory is as follows :

RUN-SBR 1 & 2 : survival for S. marcescens in a steady cycle.

RUN-SBR 3 & 4 : washout for S. marcescens and survival for P. putida in a steady cycle.

Figures 7-1, 7-2, 8-1, and 8-2 show results during transient cycles, and Figures 7-3, 7-4, 8-3, and 8-4 indicate results of phenol

degradation and biomass growth during steady cycle with S. marcescens only. In Figures 9-1 to 10-8, results are shown from transient and steady cycles using a mixture of the cultures.

The curves in the SBR experiments are not fitted curves, but rather are predicted from the numerical integration of the mathematical model equations. Andrews parameters which were determined in this study and previous work [11] were combined with the SBR equations to predict phenol biodegradation and biomass growth. The curves shown in Figures 7-1 to 10-8 are therefore not interpolated curves but theoretically predicted curves.

For the first case (RUN - SBR 1 & 2), it takes about seven cycles to reach the limit cycle for biomass growth and phenol degradation. However, in the second case (Run - SBR 3 & 4) it takes thirty-seven cycles and S. marcescens is always washed out.

It was shown from RUN - SBR 3 & 4 that even though the initial biomass concentrations of both cultures were different, the steady-cycle results of phenol biodegradation and organism growth were the same for both runs. In the case of P.putida, the results for both runs were quantitatively the same regardless of the initial biomass concentration (as predicted by theory).

The trend of biomass growth curves in RUN - SBR 1 & 2 was not smooth, and indicated some fluctuations. This might be caused by the attachment of biomass on the bottom of the reactor. Most bacteria have external structures containing fimbriae or pili for attachment to surfaces.

In the cases where S. marcescesn was used only, there was maximum difference of 26 % between theory and experiment in both transient and steady cycles (Figure 7 & 8). However, there was excellent agreement in Run-SBR 3 & 4 with maximum 11 % difference between theory and experiment (Figure 9 & 10).

VIII. CONCLUSIONS AND RECOMMENDATIONS

A. CONCLUSION

* A mathematical model of pure and simple competition between two microorganisms has been verified in a SBR with an inhibitory substrate.

* It was observed that survival or washout of biomass largely depend on the initial conditions in the experiments with S. marcescens only, while S. marcescens is always washed-out in the experiments with S. marcescens and P. putida.

* It was shown that the results of phenol degradation and biomass growth in the SBR runs under different initial conditions, are identical provided that survival of at least one species is achieved.

B. RECOMMENDATION

The mixed culture which was used will never result in coexistence of both species since the specific growth rate curves do not cross. Therefore, another species capable of degrading phenol, with a different colony color, whose specific growth rate curve crosses that of either P. putida or S. marcescens, has to be employed in order to experimentally

test the model for coexistence.

Additionally, an extension of the mixed population model to mixed substrates is required in order to study more complex cases that are more likely to occur in real applications. With multiple substrates, one would have to also consider possible preferential uptake phenomena (for the substrates).

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TABLE 1

PHENOL DEFINED GROWTH MEDIUM SOLUTION

Phenol	1000	mg
Ammonium Sulfate	500	mg
Magnesium Sulfate	100	mg
Ferric Chloride	0.5	mg
Manganese Sulfate	10	mg
1.0 M Potassium Phosphate (dibasic) plus 1.0 M Potassium Phosphat (monobasic) Buffer Solution (PH 7.2)	30	ml
Tap Water	100	ml
Distilled Water	to volume of 1 liter	

TABLE 2

OPTICAL DENSITY vs TIME

(Serratia marcescens , ATCC 17991)

Time (hr)	OPTICAL DENSITY , UOD (at different initial phenol concentrations, ppm) 5	10	20	60	80	100	140	180	220
0	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007
1.0		0.008		0.008	0.008	0.008			
2.0		0.009	0.010		0.009	0.009	0.009	0.008	0.008
2.5									
3.0			0.012	0.011		0.010		0.009	0.009
3.5									
4.0			0.014	0.013			0.011	0.010	0.010
4.5									
5.0	0.008	0.011	0.017	0.015	0.014		0.012		
5.5									
6.0		0.013	0.020	0.018	0.016	0.014			0.011
7.0	0.009			0.021	0.018	0.016	0.014	0.013	
8.0		0.015		0.025	0.020	0.018	0.015	0.014	0.011
9.0	0.010			0.028	0.023	0.020	0.017	0.015	0.012
10	0.011	0.018		0.034	0.026	0.023	0.019	0.016	0.013

TABLE 3

BIOMASS CONCENTRATION vs TIME

(Serratia marcescens , ATCC 17991)

Time (hr)	BIOMASS CONCENTRATION , ppm (at different initial phenol concentrations, ppm) 5	10	20	60	80	100	140	180	220
0	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82
1.0		2.08		2.08	2.08	2.08			
2.0		2.34	2.60		2.34	2.34	2.34	2.08	2.08
2.5									
3.0			3.12	2.86		2.60		2.34	2.34
3.5									
4.0			3.64	3.38			2.86	2.60	2.60
4.5									
5.0	2.08	2.86	4.42	3.86	3.58		3.12		
5.5									
6.0		3.38	5.20	4.68	4.16	3.64			2.86
7.0	2.34			5.58	4.68	4.16	3.64	3.38	
8.0		3.89		6.50	5.20	4.68	3.89	3.64	2.80
9.0	2.60			7.28	5.98	5.20	4.42	3.89	3.12
10	2.86	4.68		8.84	6.76	5.98	4.94	4.16	3.38

TABLE 4

SUBSTRATE CONCENTRATION vs TIME

(Serratia marcescens , ATCC 17991)

Time (hr)	SUBSTRATE CONCENTRATION , ppm (at different initial phenol concentrations, ppm) 5	10	20	60	80	100	140	180	220
0	4.83	10.17	19.78	59.88	78.50	97.25	140.08	177.32	218.29
1.0		9.28		57.82	77.49	96.26			
2.0		8.37	17.20		76.17	95.27	138.57	175.98	217.27
2.5									
3.0			15.58	54.78		94.01		175.23	216.72
3.5									
4.0			13.70	52.71			136.39	174.55	216.16
4.5									
5.0	2.79	5.52	11.79	50.20	71.10		135.49		
5.5									
6.0		4.65	9.75	47.21	68.89	89.62			214.98
7.0	2.17			43.97	67.06	87.36	133.02	171.42	
8.0		3.14		40.56	64.00	85.14	131.62	170.89	213.26
9.0	1.58			35.68	60.00	82.57	129.97	169.66	212.40
10	1.30	1.94		30.00	56.22	79.20	127.65	168.37	211.37

TABLE 5

SPECIFIC GROWTH RATE AND YEILD COEFFICIENT vs PHENOL CONCENTRATION FOR Serratia marcescens (ATCC 17991)

Phenol Conc (ppm)	SPECIFIC GROWTH RATE (1/hr)	YIELD COEFFICIENT
5	0.0436	0.269
10	0.0898	0.314
20	0.1696	0.319
60	0.1579	0.236
80	0.1326	0.227
100	0.1156	0.224
140	0.0969	0.244
180	0.0842	0.265
220	0.0595	0.215

YIELD AVERAGE : 0.260 mg biomass/mg substrate; it was used for prediction and obtained from the plot of biomass vs. phenol concentration.

TABLE 6

A. Andrews Parameters for S. Marcescens(ATCC 17991)

K_i	:	35.605	(ppm)
K_s	:	29.128	(ppm)
$\hat{\mu}$	:	0.493	(1/hr)

B. Andrews Parameters for P. Putida (ATCC 31800) [11]

K_i	:	51.000	(ppm)
K_s	:	47.101	(ppm)
$\hat{\mu}$	:	1.395	(1/hr)

TABLE 7

RESULTS OF RUN-SBR1
(Serratia Marcescens, ATCC 17991)

Experimental Strategies :

fill time	= 1 hr ; (σ_1=0.2)
react time	= 3 hrs, 53 min
draw time	= 7 min ; (σ_3=0.0233)
total cycle time	= 5 hrs
phenol concentration in feed	= 100 ppm ; (u_f=3.4331)
initial phenol concentration	= 0 ppm ; (u_o=0)
initial biomass concentration	= 15.7 ppm ; (x_o=2.0731)
initial reactor volume	= 2 liters
volume after fill phase	= 4 liters
volume after draw-down phase	= 2 liters ; (δ=0.5)
feed flow rate	= 2 lit/hr ; (β=13.95)

Cycle	Time (hrs)	Biomass Conc (S.Marcescens)	Substrate Conc (ppm)	pH	DO (ppm)
1	0	15.60	0	6.80	7.15
	0.33	13.24	18.17		
	0.67	11.14	27.79		
	1.0	9.46	34.78	7.00	7.20
	1.33	11.70	29.32		
	1.67	12.98	21.34	7.04	7.05
	2.0	14.30	14.65		
	2.5	16.38	8.02		
	3.0	18.20	5.32	7.01	6.91
	4.0	18.72	4.07		
	4.5	19.26	3.12		
1..2	5.0	19.52	2.56	7.10	7.37

Cycle	Time (hrs)	Biomass Conc (S.Marcescens)	Substrate Conc(ppm)	pH	DO (ppm)
2	0.33	16.88	16.34	7.00	7.28
	0.67	14.82	23.66		
	1.0	13.52	30.97	7.08	6.97
	1.33	14.94	16.29		
	1.67	15.60	11.58		
	2.0	16.88	8.15	7.00	7.10
	2.5	17.16	5.23		
	3.0	17.55	3.21	7.01	7.25
	3.5	17.81	2.76		
	4.0	17.94	2.61	7.05	7.21
2..3	5.0	18.20	2.52	7.03	7.18
3	0.33	15.72	12.17	6.82	7.30
	0.67	15.08	20.31		
	1.0	14.56	26.27	7.03	7.15
	1.33	15.20	21.83		
	1.67		14.02		
	2.0	16.12	7.36	7.05	7.25
	2.5	17.04	6.27		
	3.0	17.69	4.83	7.09	7.20
	3.5	17.81	3.92		
	4.0	17.94	3.06	7.08	7.26
3.4	5.0	18.08	2.97		

Cycle	Time (hrs)	Biomass Conc (S.Marcescens)	Substrate Conc(ppm)	pH	DO (ppm)
6	0.33	16.88	11.39	6.82	7.30
	0.67	15.60	15.32		
	1.0	15.34	23.37	7.30	7.39
	1.33	15.60	15.81		
	1.67	15.92	10.36		
	2.0	17.42	4.27	7.34	7.23
	2.5	17.54	3.08		
	3.0	17.80	2.97	7.36	7.21
	3.5	17.94	2.74		
	4.0	18.08	2.61		
6..7	5.0	18.08	2.26	7.39	7.37
7	0.33	16.88	13.23	7.10	7.27
	0.67	16.76	17.33		
	1.0	15.60	24.80	7.11	7.08
	1.33	15.92	15.31		
	1.67	16.12	9.24		
	2.0	18.38	4.07	7.12	7.13
	2.5	18.90	3.21		
	3.0	19.29	3.03	7.11	7.24
	3.5	19.55	2.88		
	4.0	19.81	2.41	7.10	7.23
	5.0	20.28		7.16	7.18

TABLE 8

RESULTS OF RUN-SBR2
(Serratia Marcescens, ATCC 17991)

Experimental Strategies :

fill time	= 1 hr ; (σ_1=0.2)
react time	= 3 hrs, 53 min
draw time	= 7 min ; (σ_3=0.0233)
total cycle time	= 5 hrs
phenol concentration in feed	= 100 ppm ; (u_f=3.4331)
initial phenol concentration	= 0 ppm ; (u_o=0)
initial biomass concentration	= 14.3 ppm ; (x_o=1.8882)
initial reactor volume	= 2 liters
volume after fill phase	= 4 liters
volume after draw-down phase	= 2 liters ; (δ=0.5)
feed flow rate	= 2 lit/hr ; (β=13.95)

Cycle	Time (hrs)	Biomass Conc (S.Marcescens)	Substrate Conc (ppm)	pH	DO (ppm)
1	0	14.30	0	6.80	7.42
	0.33	11.18	19.46		
	0.67	10.28	30.27		
	1.0	9.88	35.60	6.93	7.31
	1.33	12.22	28.59		
	1.67	14.22	19.46		
	2.0	16.64	10.48	7.12	7.25
	2.5	18.46	4.25		
	3.0	18.98	3.39	7.12	7.21
	4.0	19.24	2.90		
	4.5	———	———		
1..2	5.0	———	———	7.13	7.27

Cycle	Time (hrs)	Biomass Conc (S.Marcescens)	Substrate Conc(ppm)	pH	DO (ppm)
2	0.33	15.86	18.79	7.19	7.16
	0.67	14.82	26.50		
	1.0	13.78	29.89	7.23	7.14
	1.33	17.16	19.97		
	1.67	19.94	9.07		
	2.0	21.32	3.77	7.21	7.08
	2.5	21.84	2.37		
	3.0	22.10	2.20	7.18	7.02
	3.5	22.36	1.89		
	4.0	22.64	1.43	7.23	7.01
2..3	5.0	———	——	7.29	7.08
3	0.33	18.20	18.00	7.26	7.32
	0.67	17.16	24.96		
	1.0	16.90	27.25	7.30	7.27
	1.33	19.50	16.38		
	1.67	22.10	7.37		
	2.0	23.14	3.47	7.25	7.23
	2.5	23.66	2.72		
	3.0	23.92	1.62	7.16	7.20
	3.5	24.16	1.32		
	4.0	24.42	0	7.13	7.20
3.4	5.0	———	——		

Cycle	Time (hrs)	Biomass Conc (S.Marcescens)	Substrate Conc(ppm)	pH	DO (ppm)
6	0.5	20.28	17.32	7.21	7.33
	1.0	18.46	25.06		
	1.5	23.14	7.86		
	2.0	24.44	2.47	7.24	7.34
	2.5	24.66	2.03		
	3.0	24.92	1.52	7.24	7.35
	3.5	25.18	0.82		
	4.0	25.18	0.82		
	5.0	25.18	0	7.21	7.37

TABLE 9

RESULTS OF RUN-SBR3
(P. Putida ,ATCC 31800 & S. Marcescens, ATCC 17991)

Experimental Strategies :

fill time	= 1 hr ; (σ_1=0.2)
react time	= 3 hrs, 53 min
draw time	= 7 min ; (σ_3=0.0233)
total cycle time	= 5 hrs
phenol concentration in feed	= 100 ppm ; (u_f=2.1231)
initial phenol concentration	= 0 ppm ; (u_o=0)
initial S.marcescens concentration	= 24.3 ppm ; (x_{Bo}=1.8039)
initial P.putida concentration	= 16.2 ppm ; (x_{Ao}=1.2026)
initial reactor volume	= 2 liters
volume after fill phase	= 4 liters
volume after draw-down phase	= 2 liters ; (δ=0.5)
feed flow rate	= 2 lit/hr ; (β=13.95)

Cycle	Time (hrs)	P.Putida Conc(ppm)	S.Marcescens Conc (ppm)	Substrate Conc (ppm)	pH	DO (ppm)
1	0	16.17	24.28	0	6.90	6.85
	0.25	13.75	19.26	16.23		
	0.50	12.85	16.88	23.87		
	0.75	11.90	15.06	28.22		
	1.0	11.64	13.94	30.39	7.10	6.90
	1.25	13.20	14.22	23.22		
	1.50	14.50	15.06	17.03		
	1.75	15.78	15.90	10.93		
	2.0	16.32	16.18	7.08	7.14	6.75
	2.5	17.22	16.88	2.71		
	3.0	17.88	17.15	1.23	7.11	6.70
	3.5	18.14	17.56	0.75		
	4.0	18.40	17.84	0	7.12	6.68

Cycle	Time (hrs)	P.Putida Conc (ppm)	S.Marcescens Conc (ppm)	Substrate Conc (ppm)	pH	DO (ppm)
2	0.25	15.02	13.67	16.02	6.91	6.80
	0.5	13.72	11.70	24.46		
	0.75	13.07	10.16	29.24		
	1.0	12.81	9.32	31.75	6.97	6.85
	1.25	14.24	9.74	25.15		
	1.5	15.54	10.02	18.64		
	1.75	16.84	10.58	12.76		
	2.0	17.62	10.86	8.08	6.97	6.90
	2.5	18.92	11.28	2.85		
	3.0	19.18	11.42	1.13	6.95	6.81
	3.5	19.70	11.98	0.56		
	4.0	19.83	12.12	0	6.95	6.80
3	0.25	16.45	9.32	16.53	6.95	7.21
	0.5	15.02	8.06	24.53		
	0.75	14.50	6.94	29.31		
	1.0	14.11	6.66	31.88	7.00	7.11
	1.25	15.54	6.80	25.26		
	1.5	17.10	7.10	18.61		
	1.75	18.66	7.78	12.71		
	2.0	19.70	8.06	8.10	7.03	7.18
	2.5	20.74	8.34	2.98		
	3.0	21.13	8.62	1.15	7.03	7.12
	3.5	21.26	8.76	0.57		
	4.0	21.39	8.90	0	7.03	7.08

Cycle	Time (hrs)	P.Putida Conc (ppm)	S.Marcescens Conc (ppm)	Substrate Conc (ppm)	pH	DO (ppm)
6	0.25	21.00	3.86	16.35	6.92	6.90
	0.5	18.92	3.16	23.99		
	0.75	17.62	3.02	28.32		
	1.0	17.75	2.32	30.46	6.97	6.87
	1.25	19.57	2.56	23.24		
	1.5	21.65	2.84	16.38		
	1.75	22.30	3.02	10.58		
	2.0	24.12	3.16	6.14	6.96	6.71
	2.5	25.28	3.44	2.11		
	3.0	25.68	3.58	0.92	6.95	6.75
	3.5	25.95	3.72	0		
	4.0	25.98	3.72	0	6.96	6.76
7	0.25	21.78	2.32	15.94	6.97	7.03
	0.5	20.22	1.90	23.71		
	0.75	19.18	1.62	28.87		
	1.0	18.92	1.48	31.48	7.06	6.91
	1.25	20.74	1.62	22.53		
	1.5	22.82	1.74	15.85		
	1.75	24.25	1.90	10.02		
	2.0	25.42	2.04	5.82	7.05	6.90
	2.5	26.33	2.18	1.98		
	3.0	26.72	2.32	0.48	7.04	6.88
	3.5	26.98	2.46	0		
	4.0	27.24	2.56	0	7.02	6.86

TABLE 10

RESULTS OF RUN-SBR4
(P. Putida ,ATCC 31800 & S. Marcescens, ATCC 17991)

Experimental Strategies :

fill time	= 1 hr ; (σ_1=0.2)
react time	= 3 hrs, 53 min
draw time	= 7 min ; (σ_3=0.0233)
total cycle time	= 5 hrs
phenol concentration in feed	= 100 ppm ; (u_f=2.1231)
initial phenol concentration	= 0 ppm ; (u_o=0)
initial S.marcescens concentration	= 21.64 ppm ; (x_{Bo}=1.6064)
initial P.putida concentration	= 15.40 ppm ; (x_{Ao}=1.1432)
volume after fill phase	= 4 liters
volume after draw-down phase	= 2 liters ; (δ=0.5)
feed flow rate	= 2 lit/hr ; (β=13.95)

Cycle	Time (hrs)	P.Putida Conc(ppm)	S.Marcescens Conc (ppm)	Substrate Conc (ppm)	pH	DO (ppm)
1	0	15.39	21.64	0	6.96	7.21
	0.25	12.16	16.88	17.43		
	0.50	11.38	14.22	24.95		
	0.75	———	———	———		
	1.0	9.82	11.42	33.63	7.08	7.09
	1.25	11.64	12.96	26.23		
	1.50	12.94	13.51	18.85		
	1.75	13.98	14.22	13.99		
	2.0	15.57	14.77	9.06		
	2.5	16.84	15.34	3.22		
	3.0	17.36	15.61	1.27	7.09	7.00
	3.5	17.88	15.90	0.87		
	4.0	17.88	15.90	0	7.06	7.01

Cycle	Time (hrs)	P.Putida Conc (ppm)	S.Marcescens Conc (ppm)	Substrate Conc (ppm)	pH	DO (ppm)
2	0.25	13.46	11.98	17.27	7.06	6.99
	0.5	12.16	10.02	26.61		
	0.75	11.38	8.90	29.98		
	1.0	9.82	8.06	32.04	7.13	6.93
	1.25	13.20	9.04	24.63		
	1.5	14.89	9.60	16.77		
	1.75	———	———	———		
	2.0	17.36	10.30	9.47	7.14	6.95
	2.5	18.66	10.86	3.48		
	3.0	19.18	11.14	1.15	7.14	6.91
	3.5	19.44	11.28	0.58		
	4.0	19.44	11.28	0	7.11	6.90
6	0.25	19.70	3.02	14.55	7.01	7.03
	0.5	18.01	2.32	24.69		
	0.75	16.97	2.04	28.41		
	1.0	16.19	1.76	32.04	7.09	6.91
	1.25	18.53	2.32	24.63		
	1.5	20.71	2.56	16.77		
	1.75	22.82	2.84	10.83		
	2.0	24.12	3.02	6.30	7.10	6.93
	2.5	25.42	3.30	2.32		
	3.0	25.94	3.44	0.88	7.08	6.94
	3.5	25.94	3.44	0		
	4.0	25.94	3.44	0	7.13	6.92

Cycle	Time (hrs)	P. Putida Conc (ppm)	S. Marcescens Conc (ppm)	Substrate Conc (ppm)	pH	DO (ppm)
7	0.25	21.52	2.04	16.18	7.07	6.98
	0.5	18.93	1.90	24.46		
	0.75	17.89	1.76	28.63		
	1.0	17.09	1.48	31.46	7.10	6.93
	1.25	19.96	1.76	23.13		
	1.5	———	———	———		
	1.75	23.60	2.04	10.42		
	2.0	25.16	2.32	6.22	7.11	6.93
	2.5	26.46	2.56	2.73		
	3.0	26.98	2.84	0.66	7.11	6.90
	3.5	26.98	2.84	0		
	4.0	26.98	2.84	0	7.13	6.89

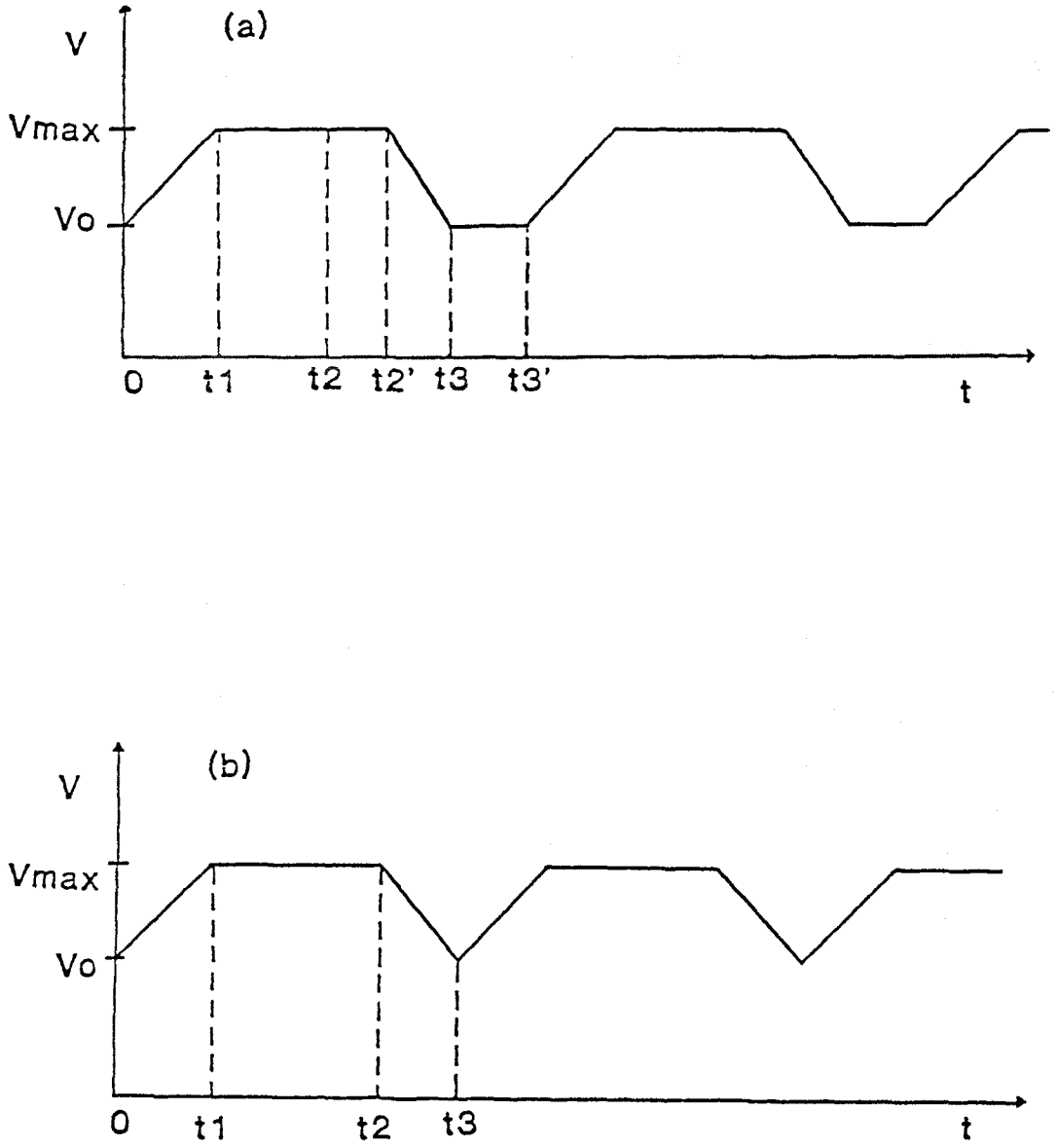
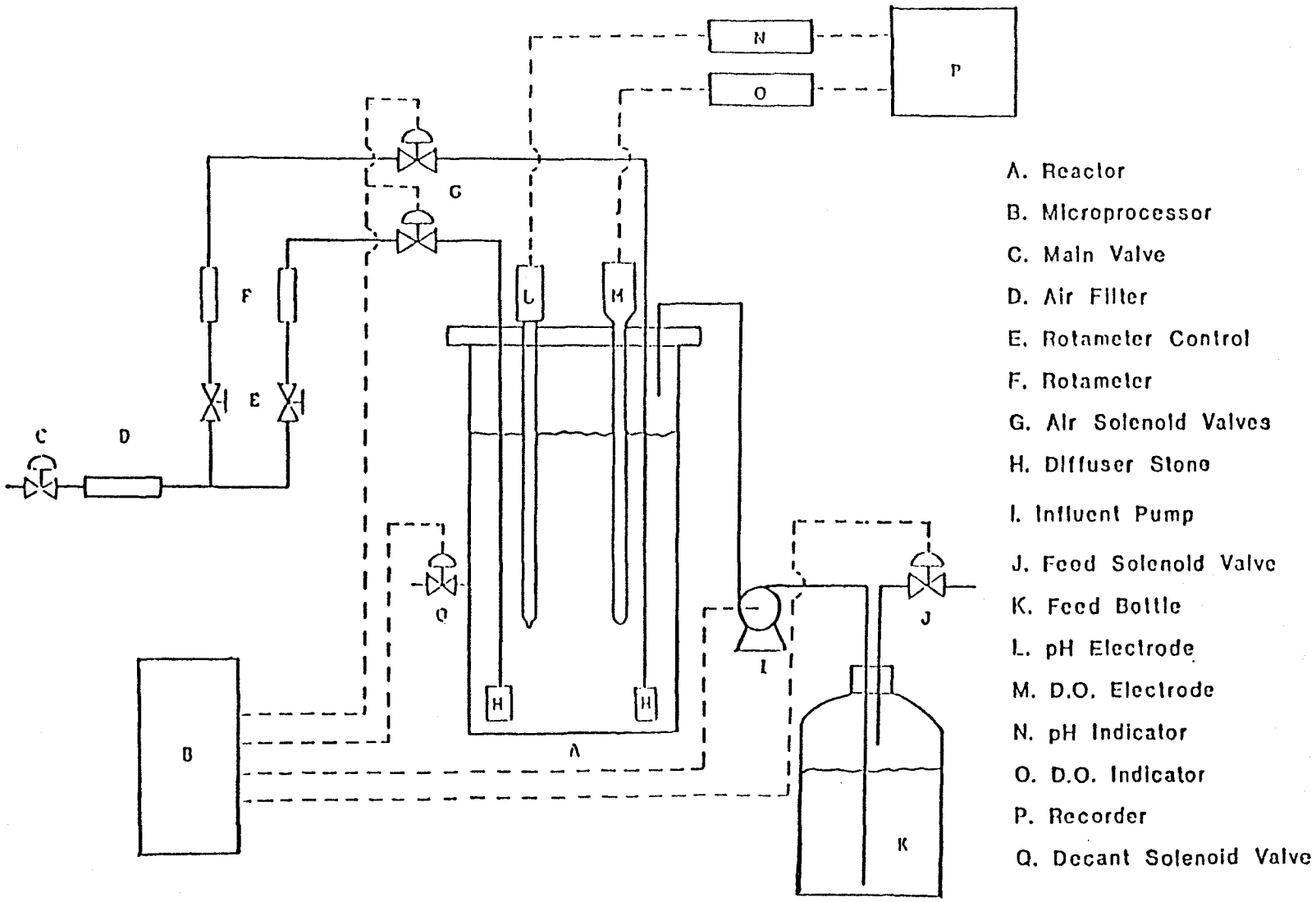
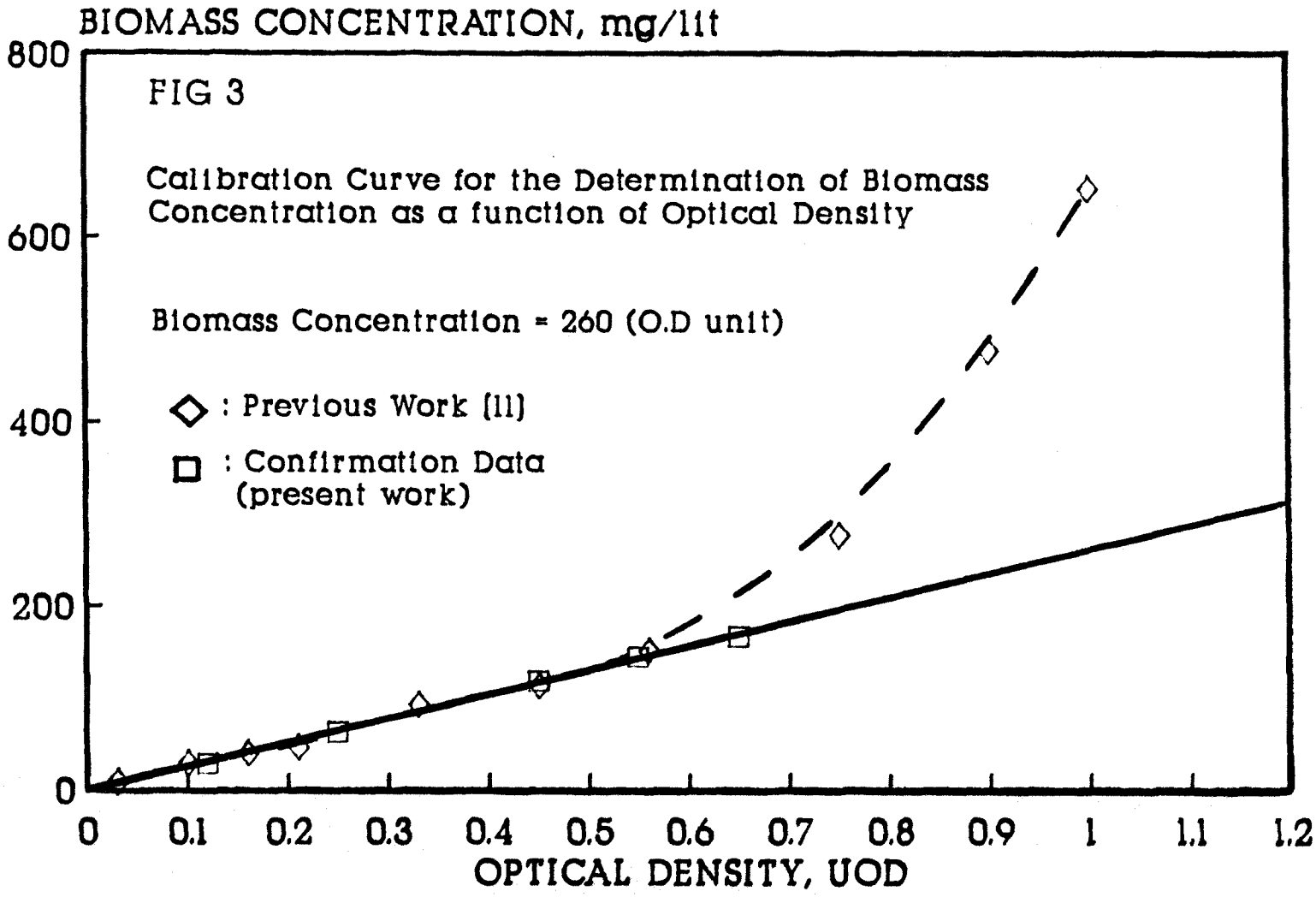


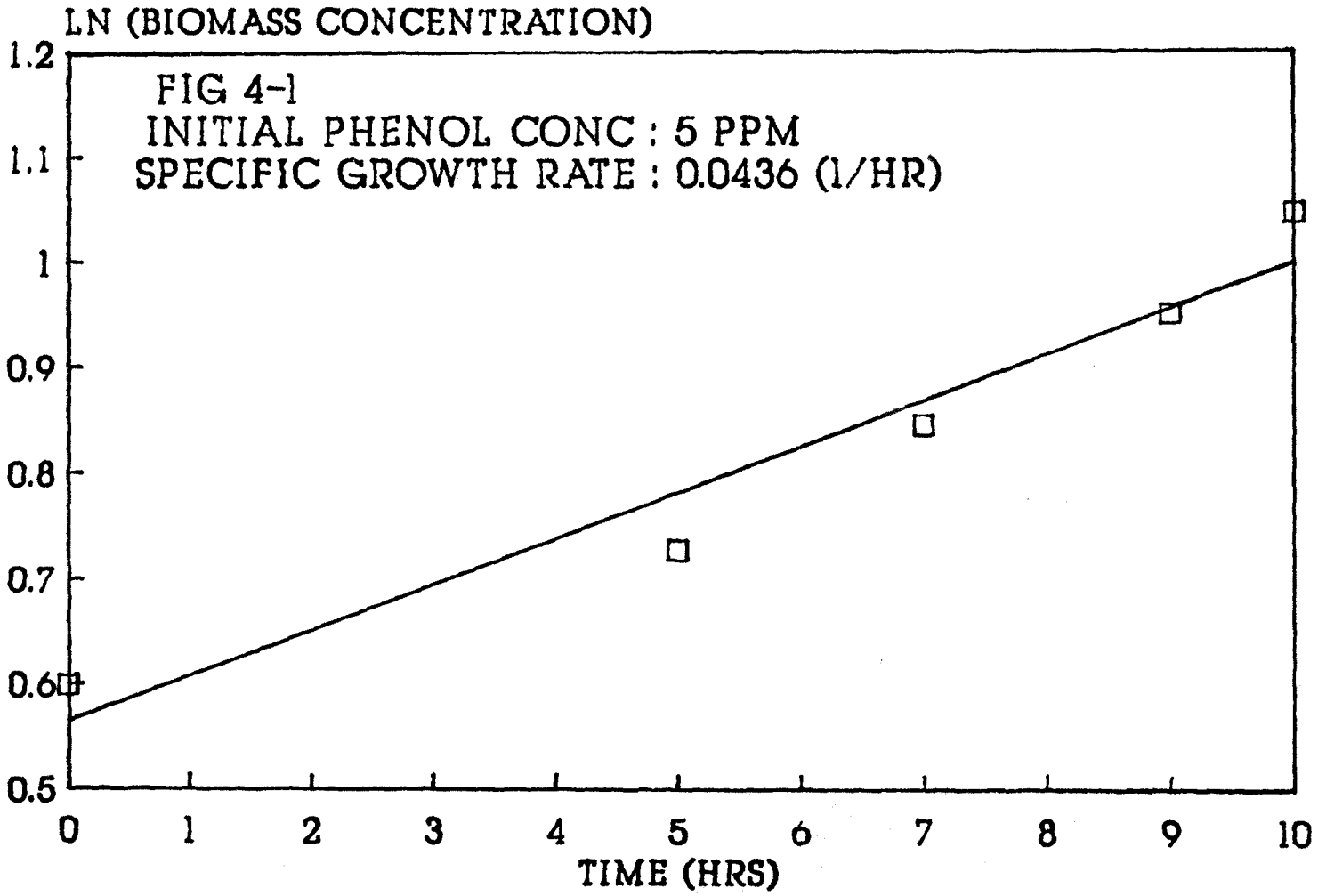
Figure 1 - Qualitative Representation of the Volume Change During Cycles: (a) generalized case; (b) special case (with no settle phase).

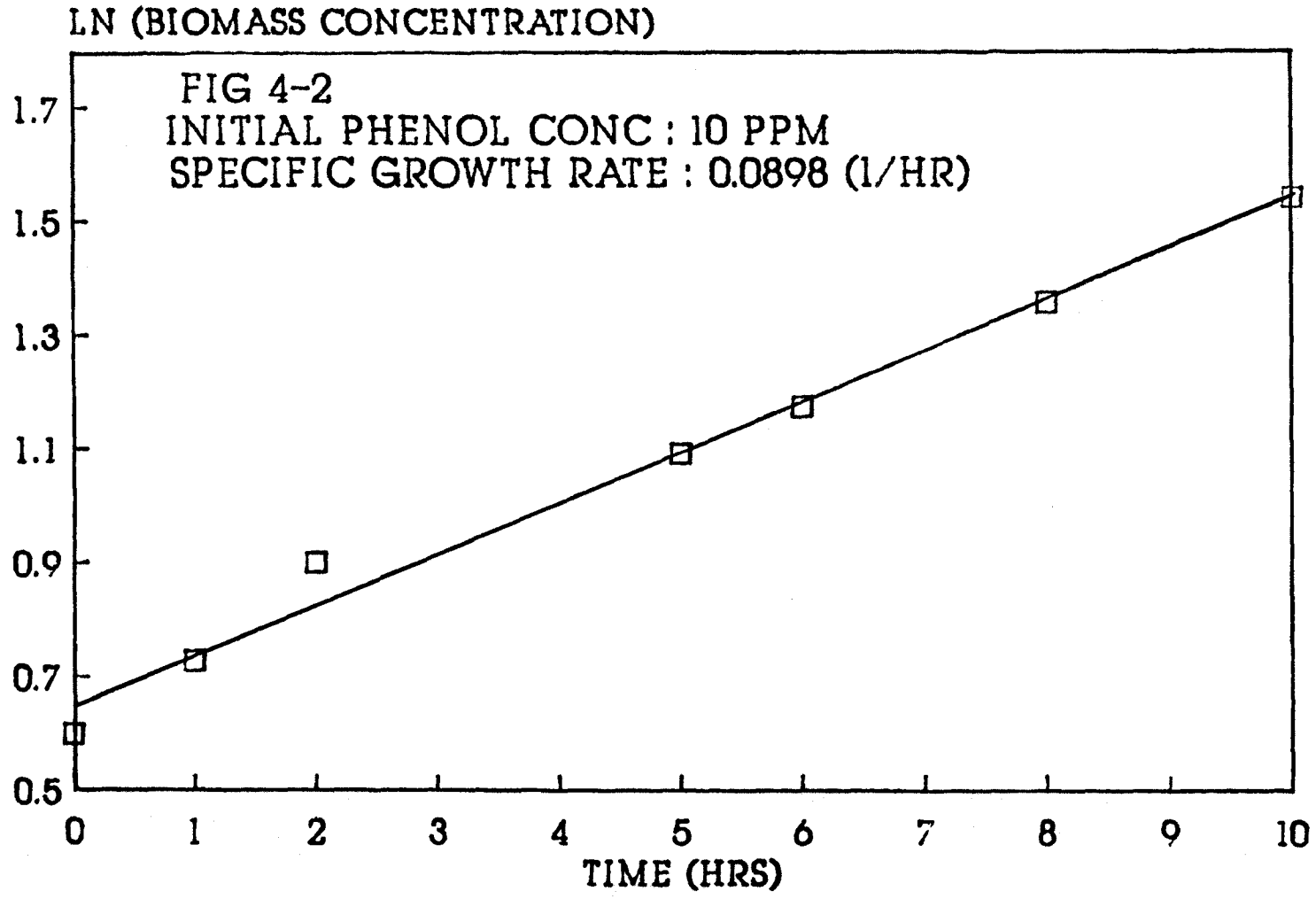


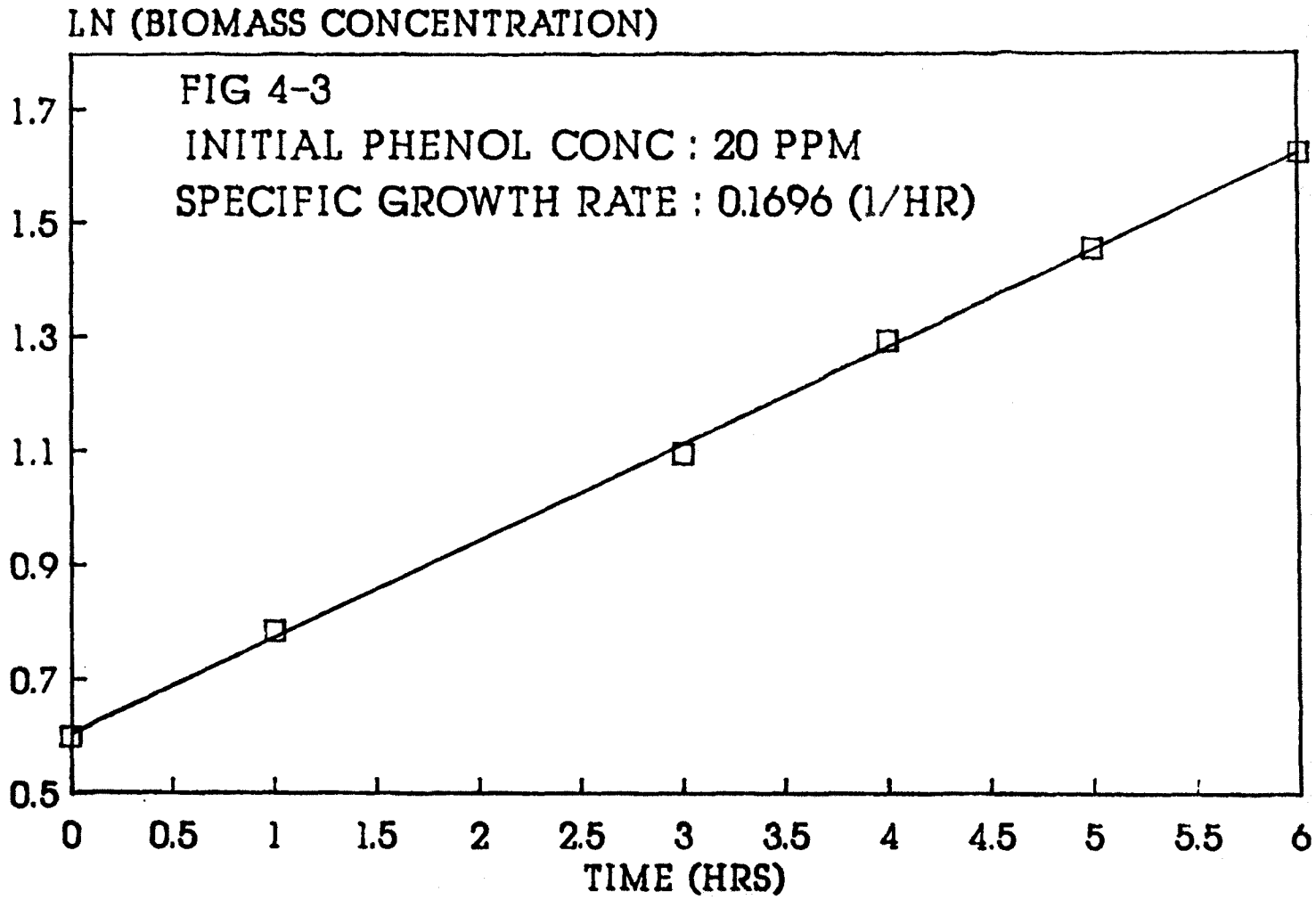
A. Reactor
B. Microprocessor
C. Main Valve
D. Air Filter
E. Rotameter Control
F. Rotameter
G. Air Solenoid Valves
H. Diffuser Stone
I. Influent Pump
J. Feed Solenoid Valve
K. Feed Bottle
L. pH Electrode
M. D.O. Electrode
N. pH Indicator
O. D.O. Indicator
P. Recorder
Q. Decant Solenoid Valve

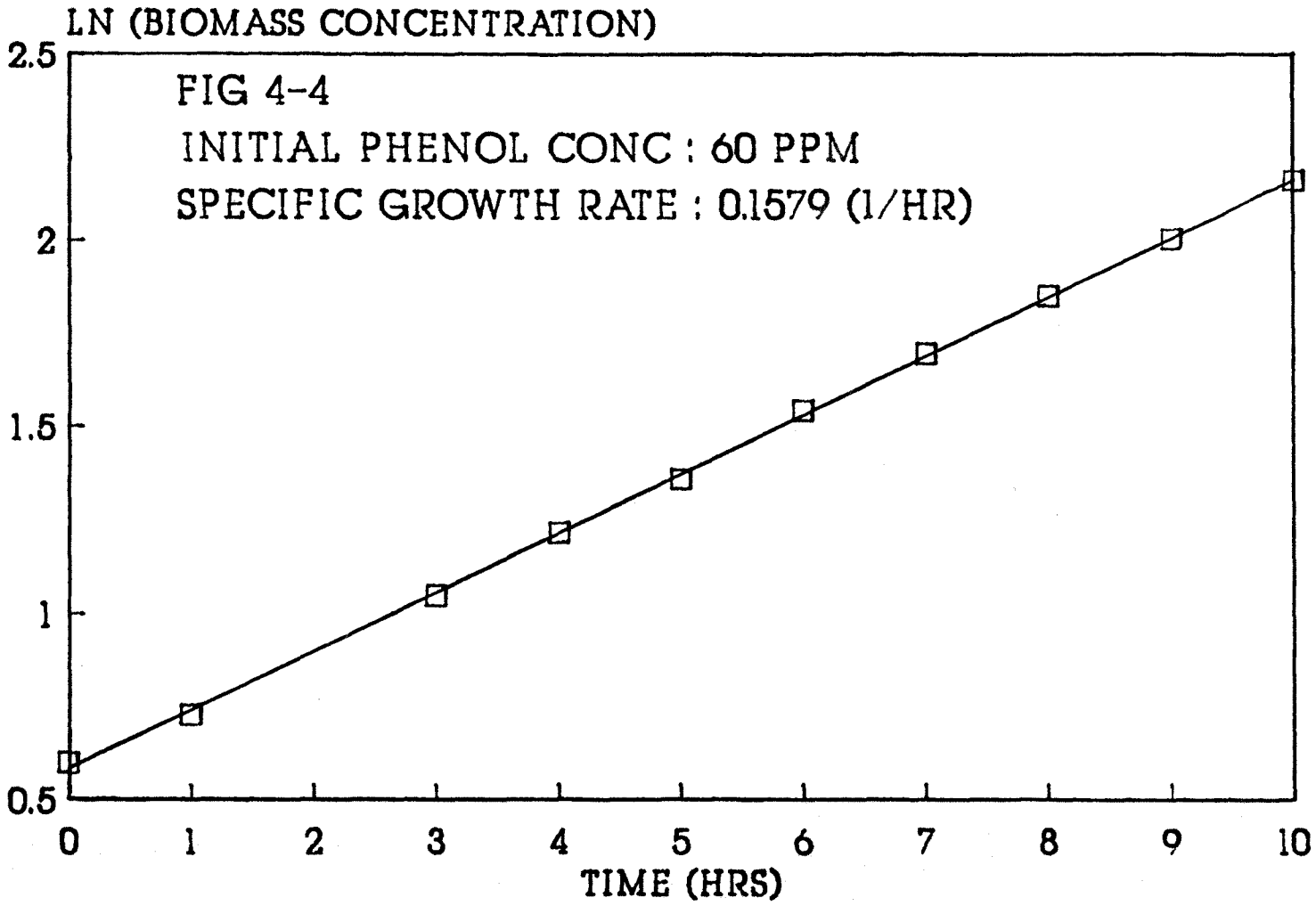
Figure 2 : Schematic Diagram of Sequencing Batch Reactor

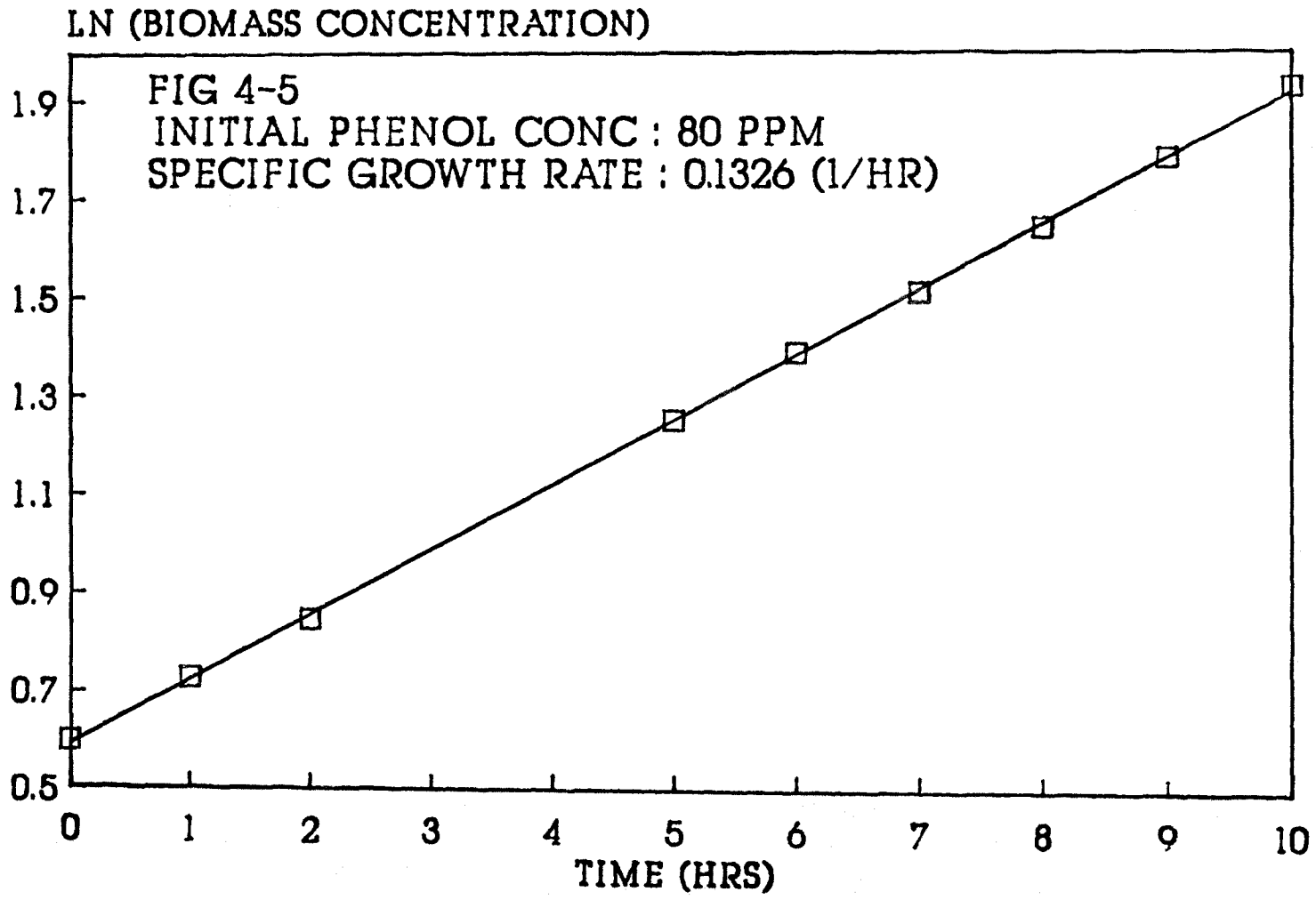

BIOMASS CONCENTRATION, mg/lit
800
600
400
200
0
FIG 3
Calibration Curve for the Determination of Biomass Concentration as a function of Optical Density
Biomass Concentration = 260 (O.D unit)
: Previous Work [11]
: Confirmation Data (present work)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
OPTICAL DENSITY, UOD

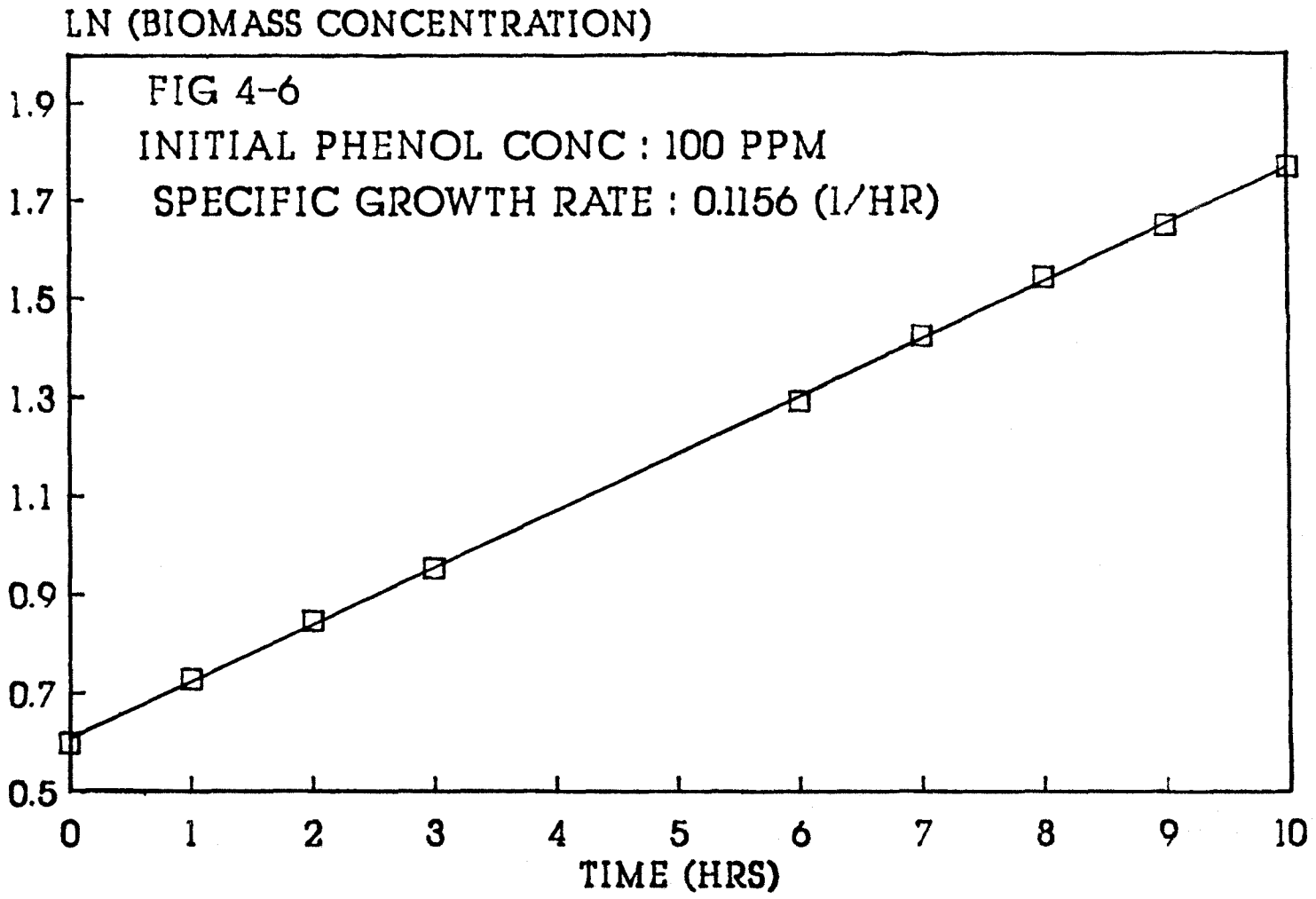

LN (BIOMASS CONCENTRATION)
FIG 4-1
INITIAL PHENOL CONC : 5 PPM
SPECIFIC GROWTH RATE : 0.0436 (1/HR)
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0
1
2
3
4
5
6
7
8
9
10
TIME (HRS)

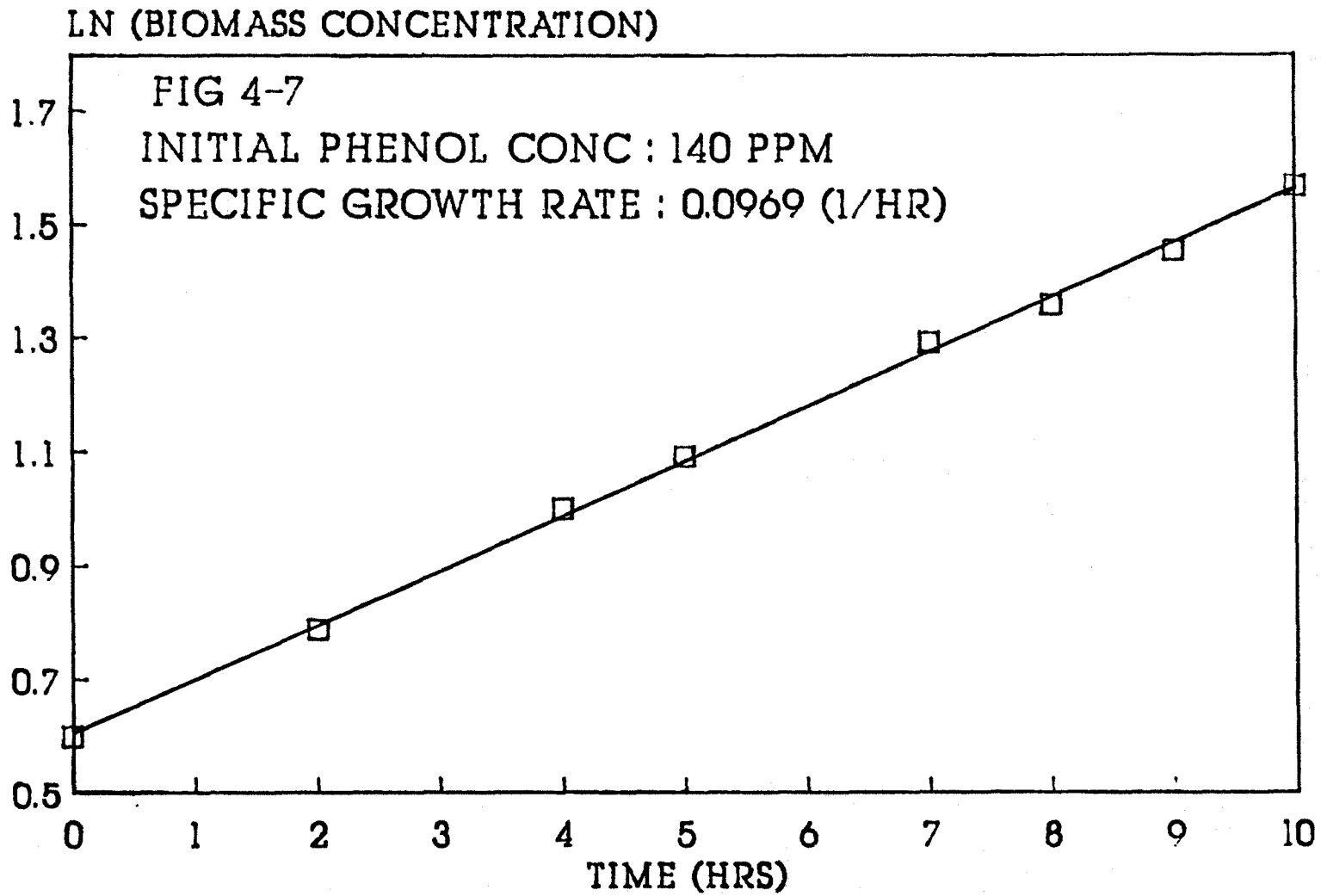

LN (BIOMASS CONCENTRATION)
FIG 4-2
INITIAL PHENOL CONC : 10 PPM
SPECIFIC GROWTH RATE : 0.0898 (1/HR)
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0
1
2
3
4
5
6
7
8
9
10
TIME (HRS)

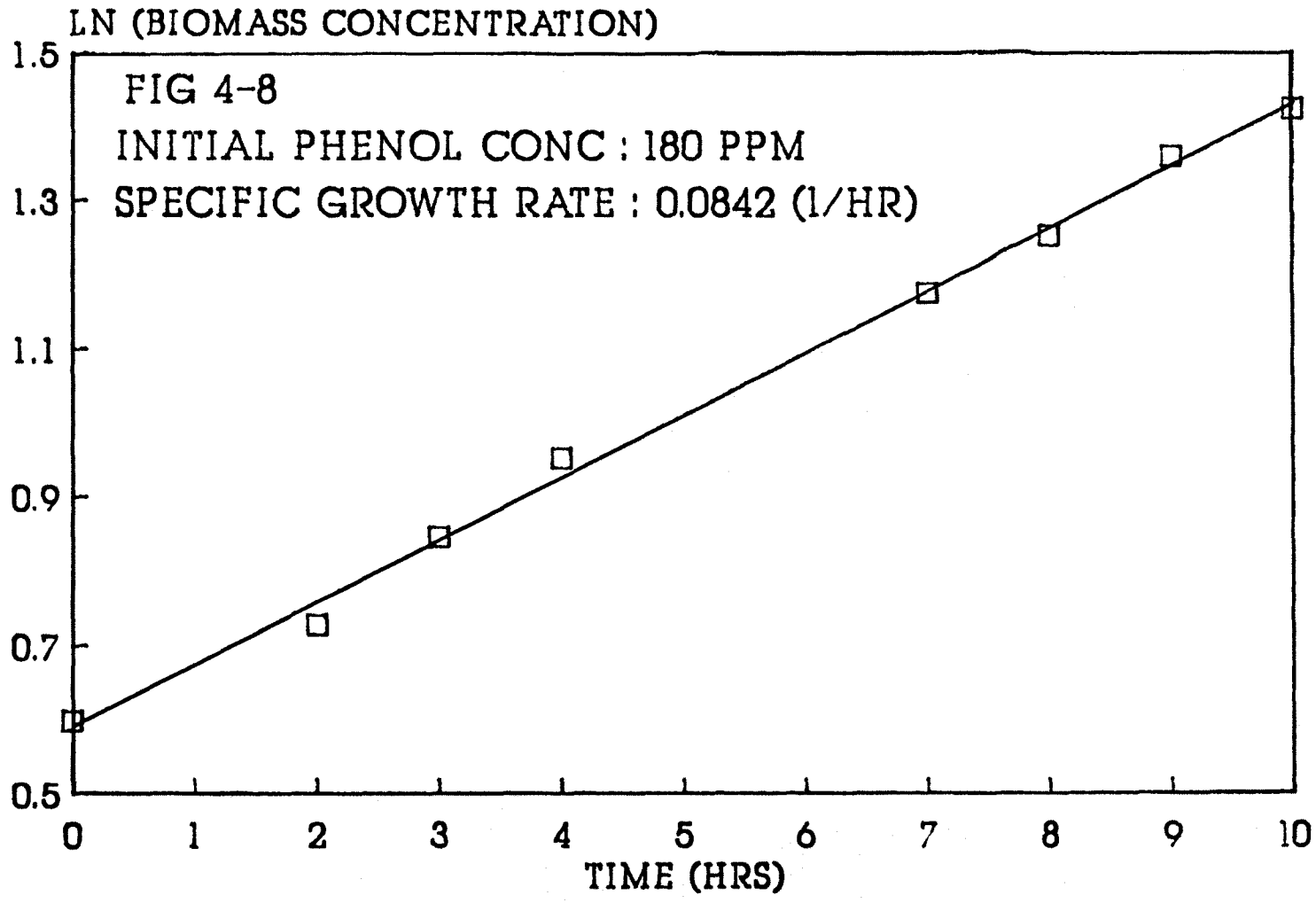

LN (BIOMASS CONCENTRATION)
FIG 4-3
INITIAL PHENOL CONC : 20 PPM
SPECIFIC GROWTH RATE : 0.1696 (1/HR)
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
TIME (HRS)

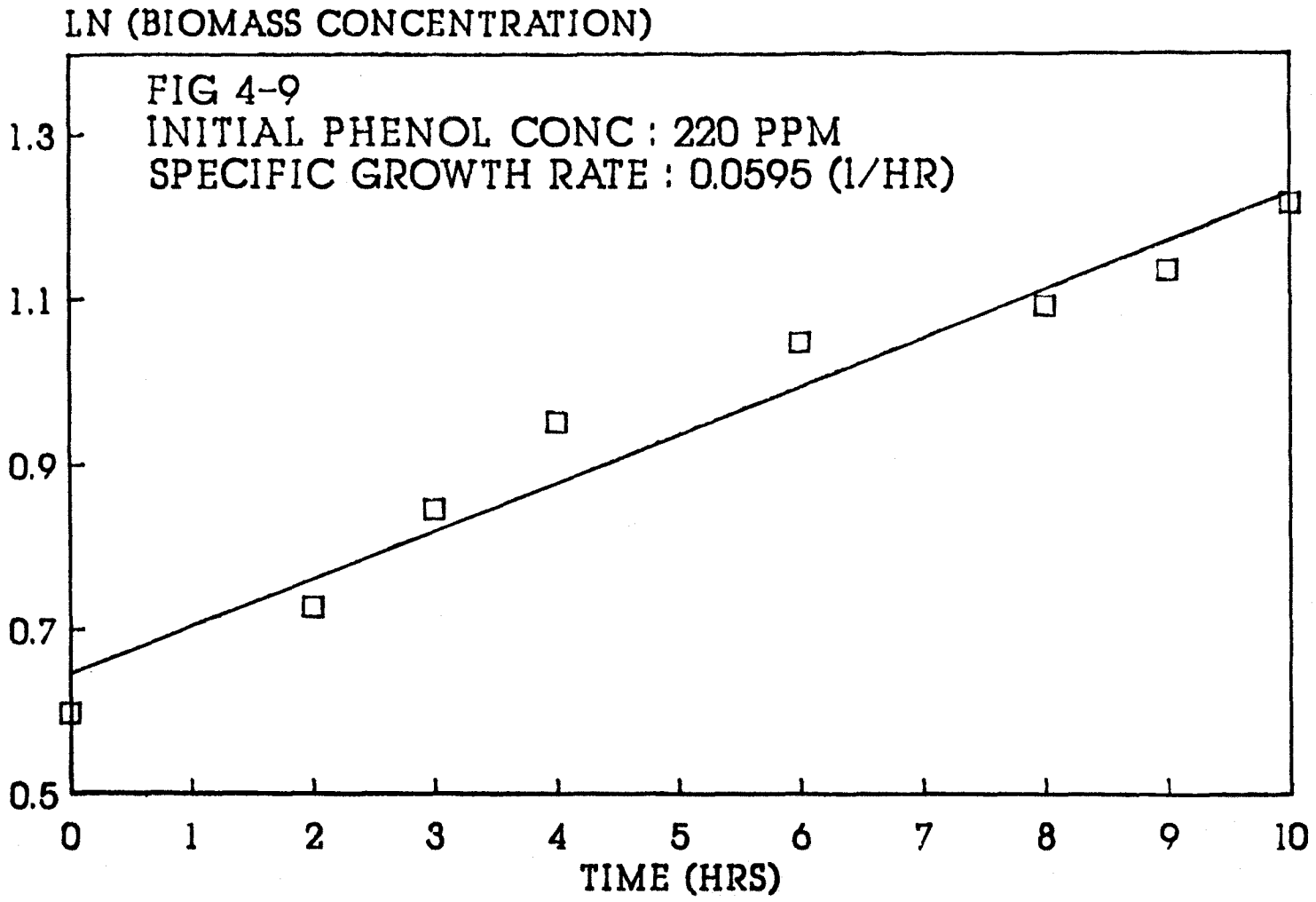

LN (BIOMASS CONCENTRATION)
FIG 4-4
INITIAL PHENOL CONC : 60 PPM
SPECIFIC GROWTH RATE : 0.1579 (1/HR)
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
8
9
10
TIME (HRS)

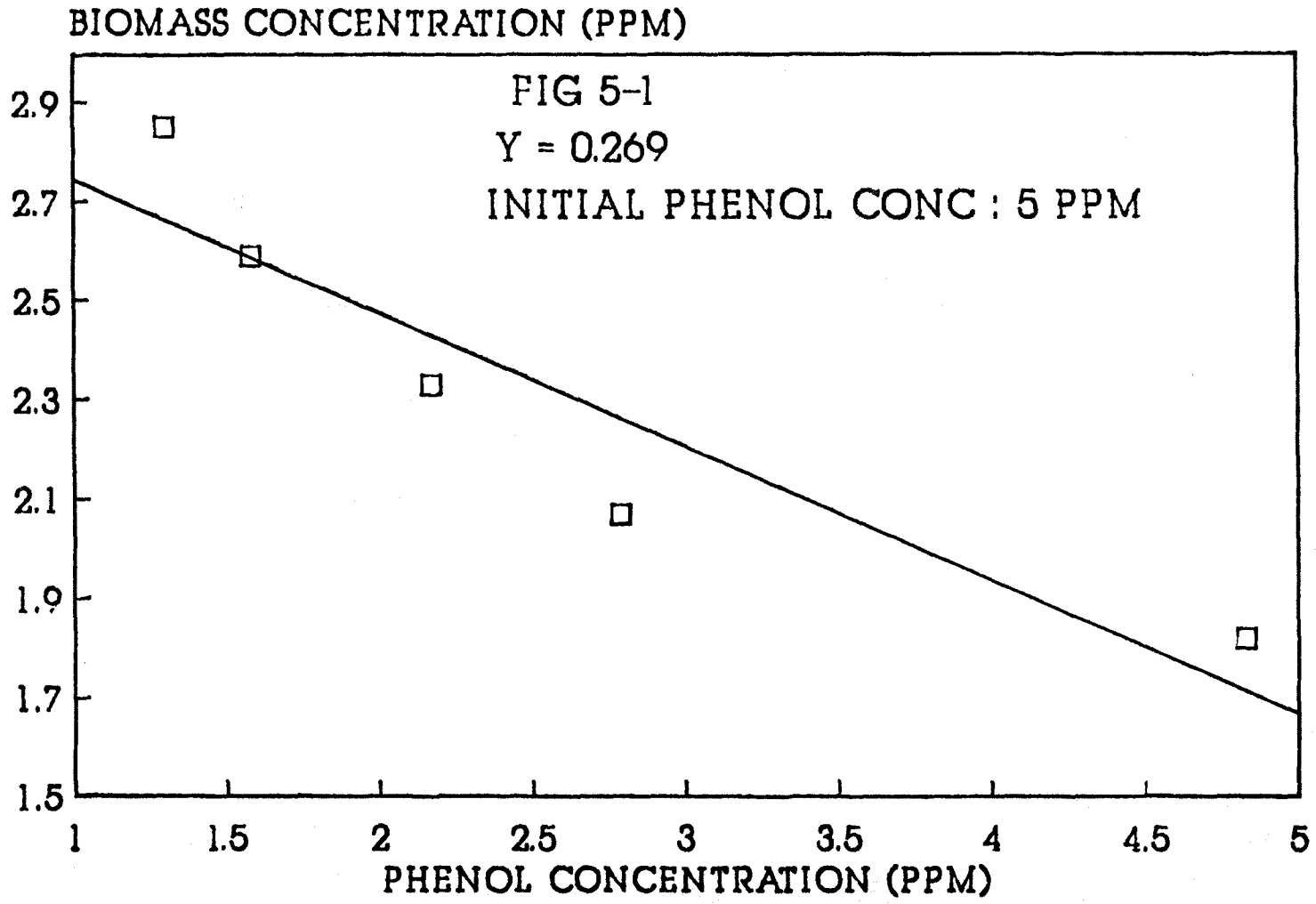

LN (BIOMASS CONCENTRATION)
FIG 4-5
INITIAL PHENOL CONC : 80 PPM
SPECIFIC GROWTH RATE : 0.1326 (1/HR)
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0
1
2
3
4
5
6
7
8
9
10
TIME (HRS)

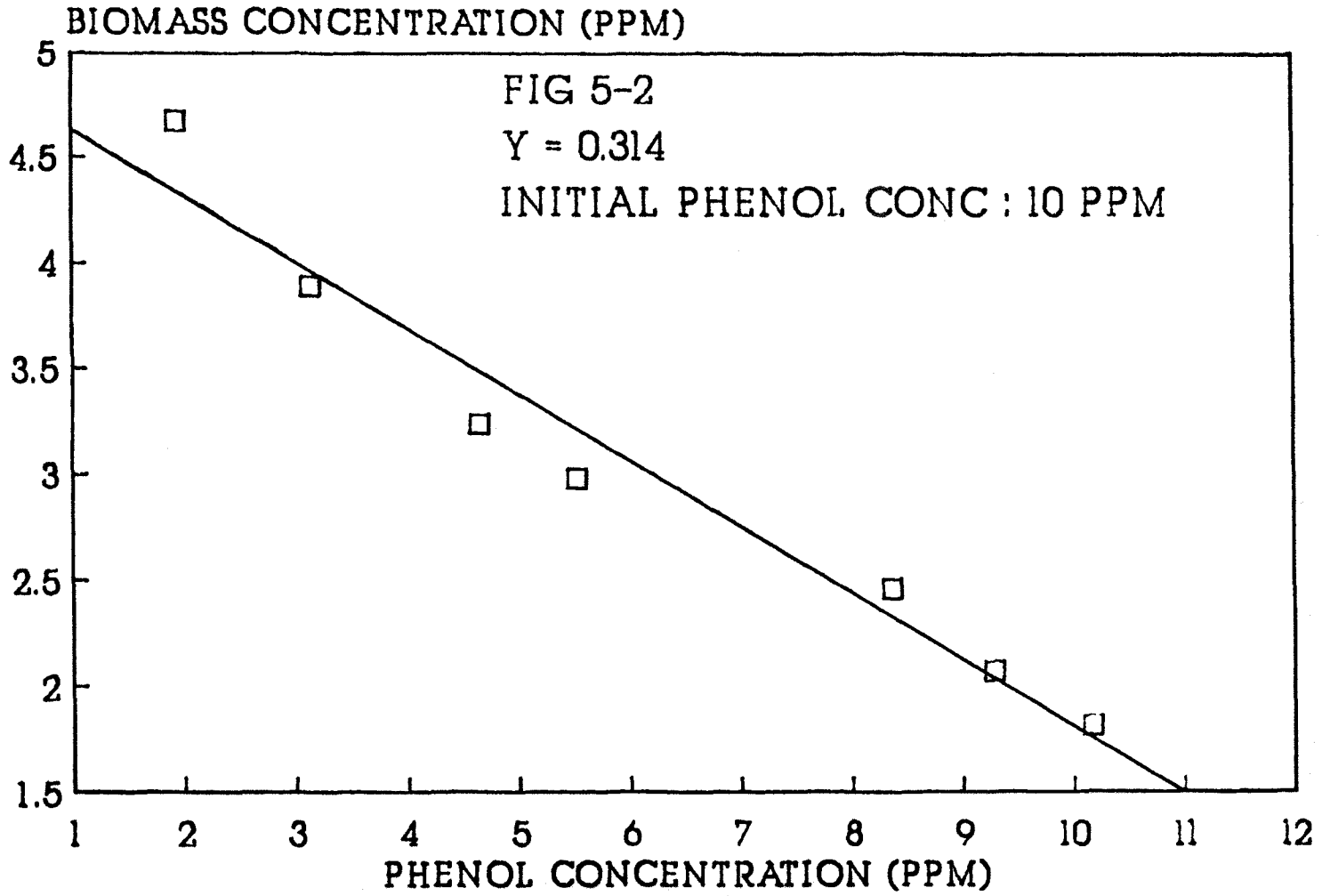

LN (BIOMASS CONCENTRATION)
FIG 4-6
INITIAL PHENOL CONC : 100 PPM
SPECIFIC GROWTH RATE : 0.1156 (1/HR)
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0
1
2
3
4
5
6
7
8
9
10
TIME (HRS)

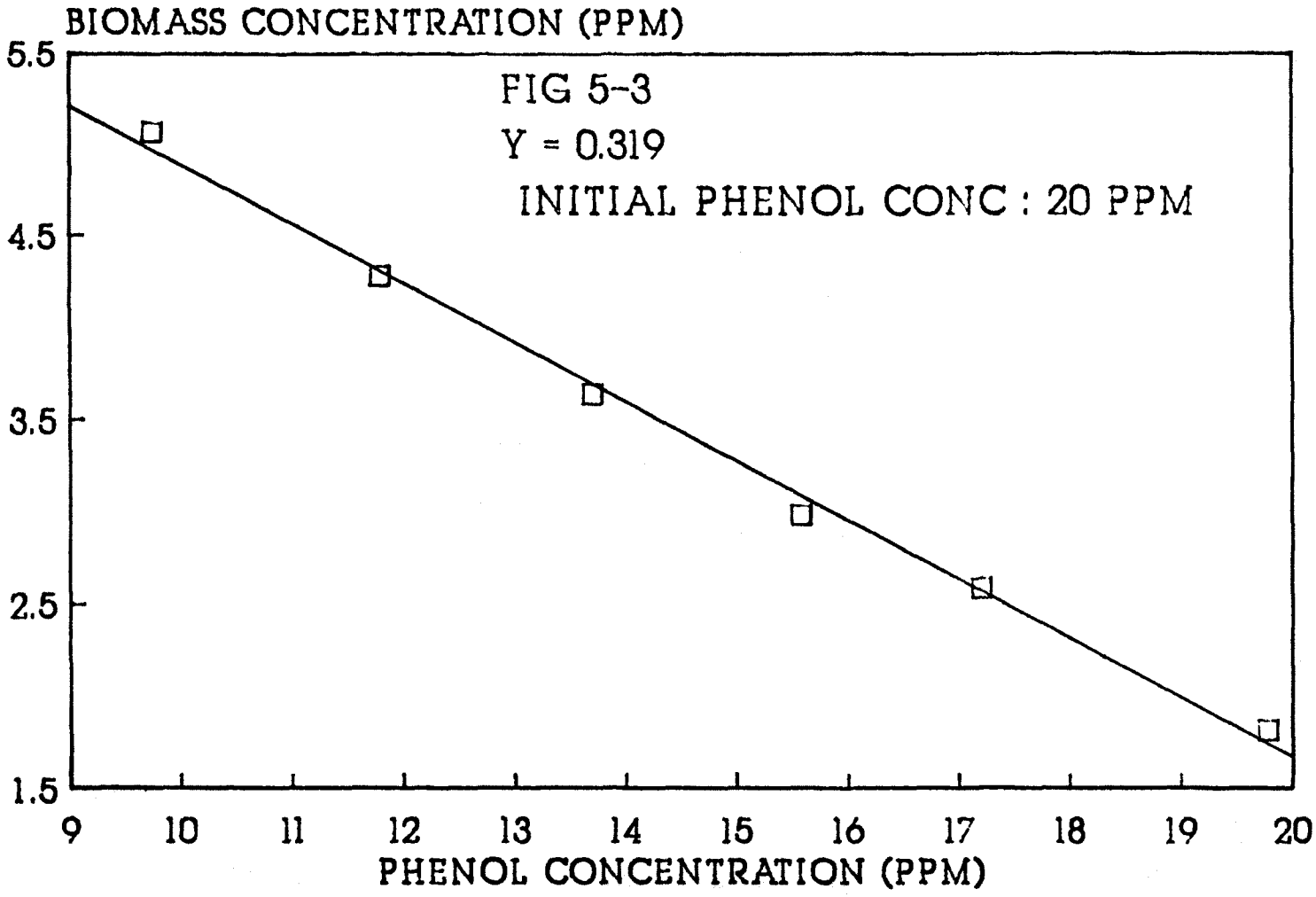

LN (BIOMASS CONCENTRATION)
FIG 4-7
INITIAL PHENOL CONC : 140 PPM
SPECIFIC GROWTH RATE : 0.0969 (1/HR)
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0
1
2
3
4
5
6
7
8
9
10
TIME (HRS)

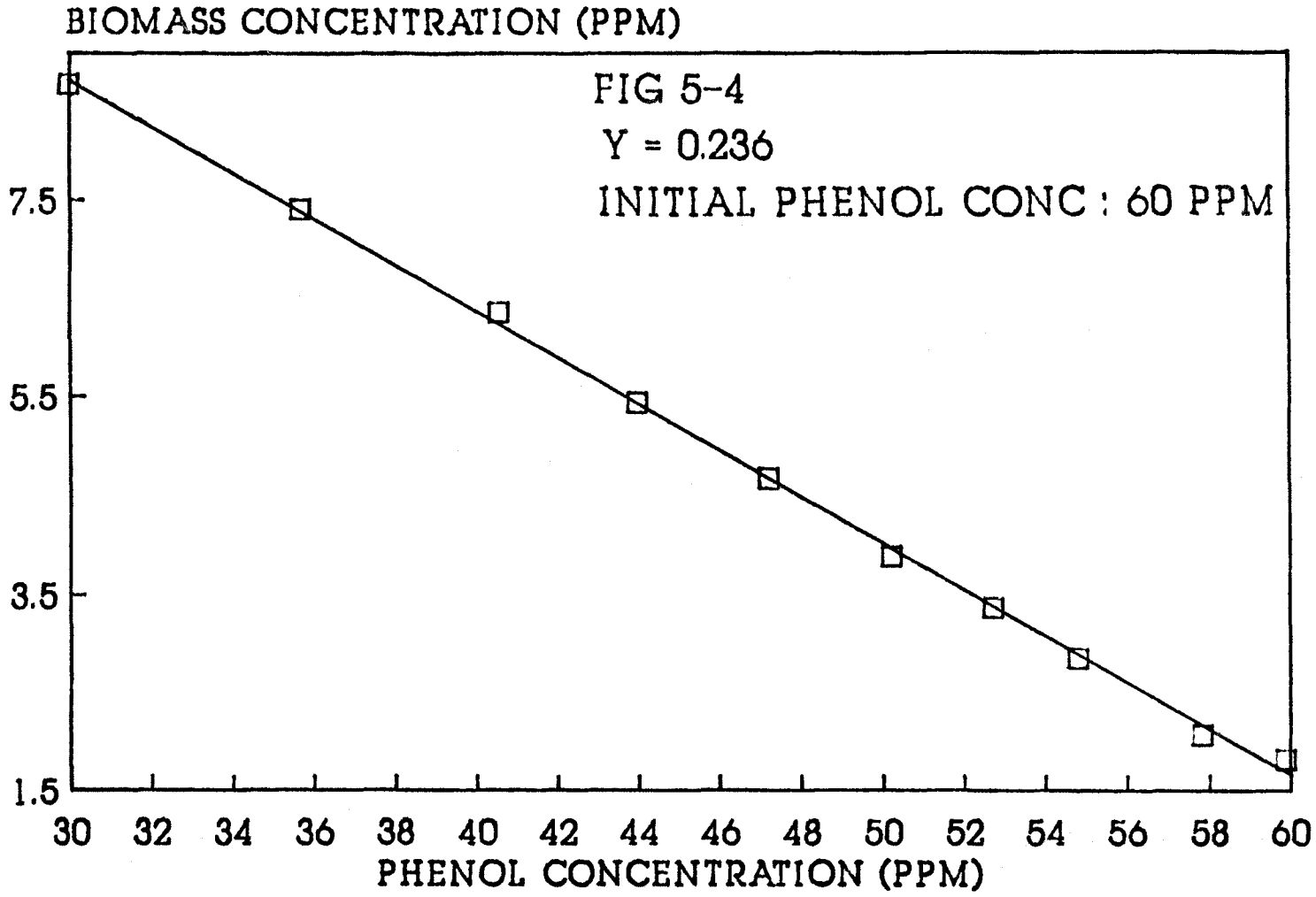

LN (BIOMASS CONCENTRATION)
FIG 4-8
INITIAL PHENOL CONC : 180 PPM
SPECIFIC GROWTH RATE : 0.0842 (1/HR)
1.5
1.3
1.1
0.9
0.7
0.5
0
1
2
3
4
5
6
7
8
9
10
TIME (HRS)

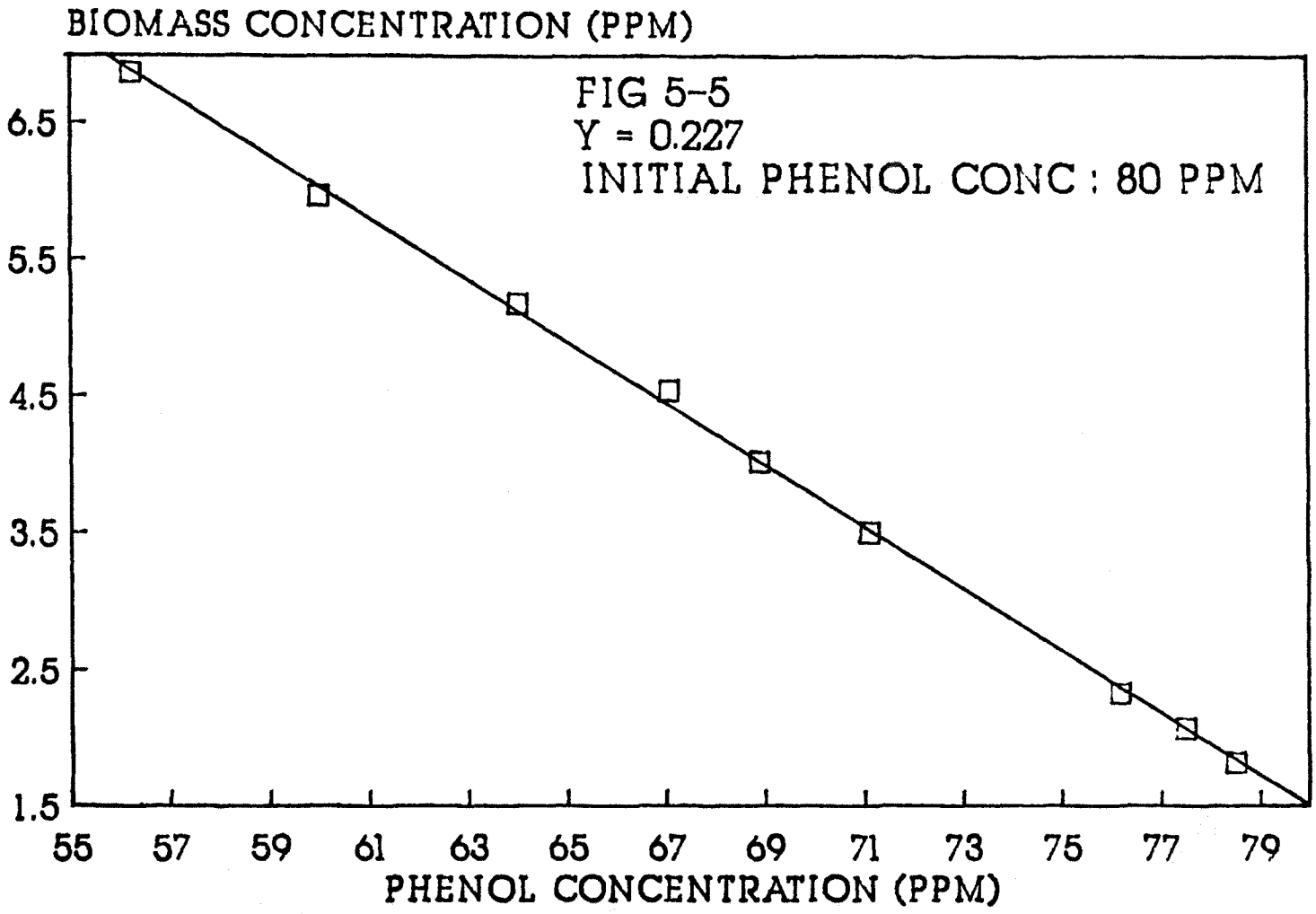

LN (BIOMASS CONCENTRATION)
FIG 4-9
INITIAL PHENOL CONC : 220 PPM
SPECIFIC GROWTH RATE : 0.0595 (1/HR)
1.3
1.1
0.9
0.7
0.5
0
1
2
3
4
5
6
7
8
9
10
TIME (HRS)

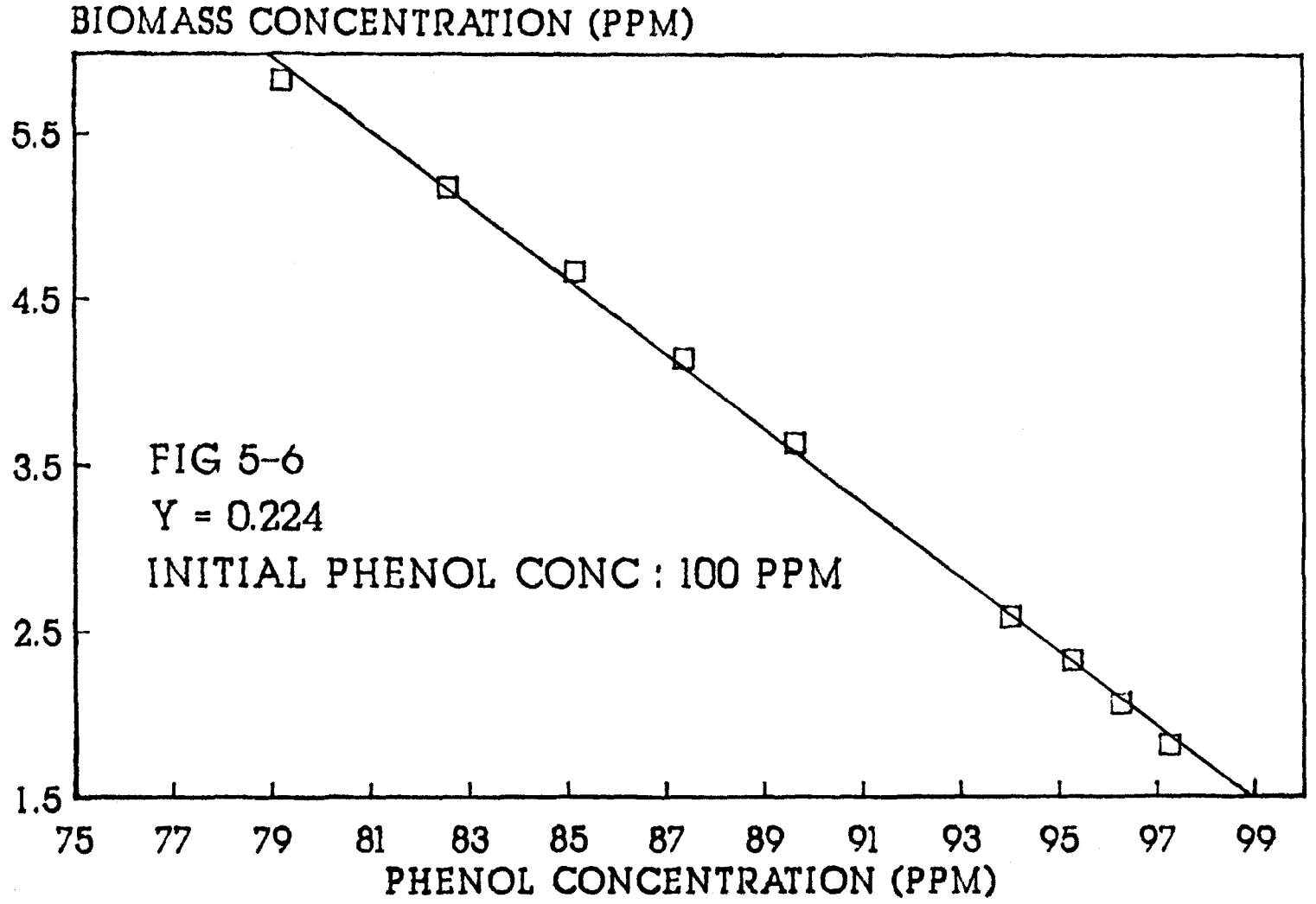

BIOMASS CONCENTRATION (PPM)
FIG 5-1
Y = 0.269
INITIAL PHENOL CONC : 5 PPM
2.9
2.7
2.5
2.3
2.1
1.9
1.7
1.5
1
1.5
2
2.5
3
3.5
4
4.5
5
PHENOL CONCENTRATION (PPM)

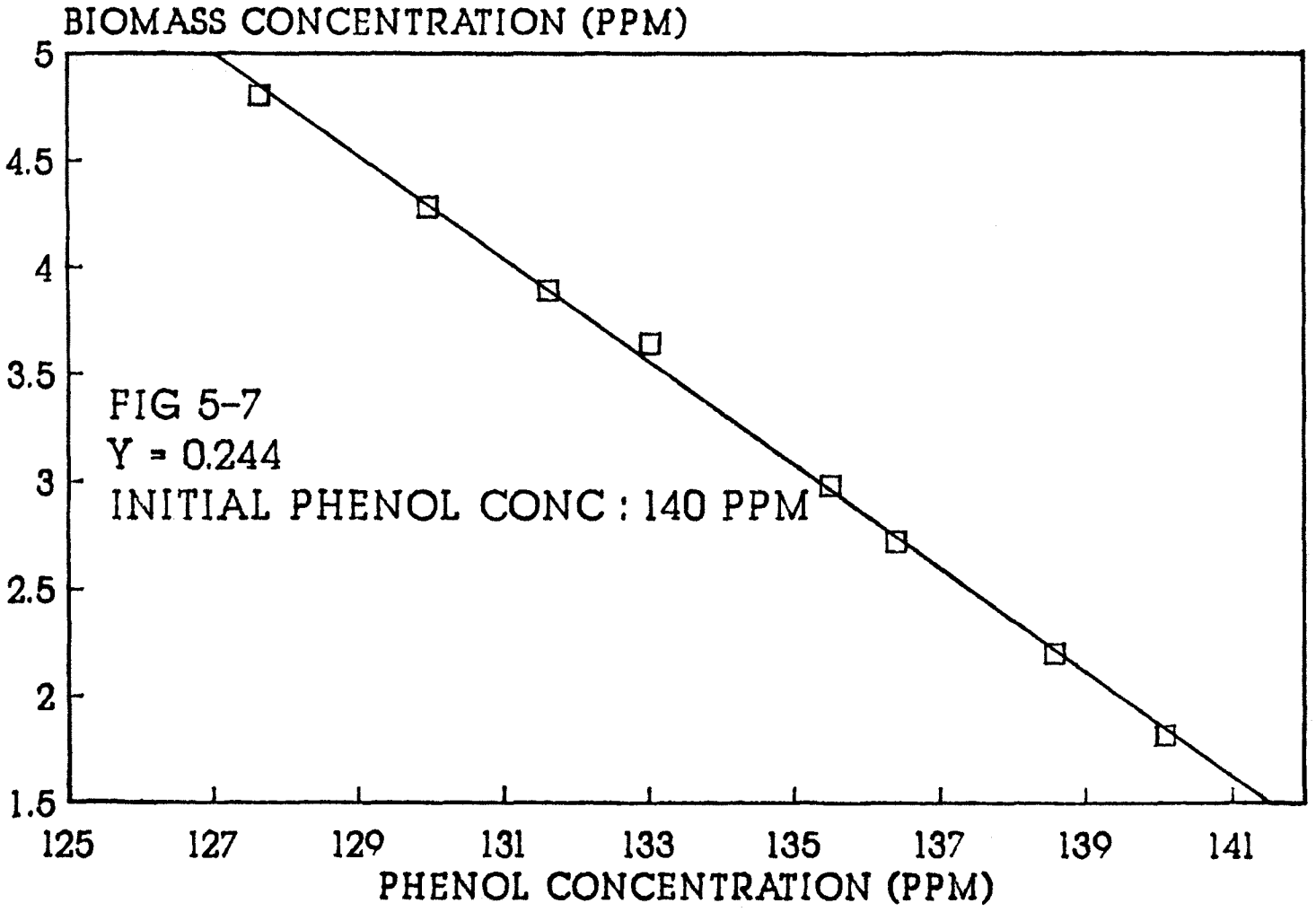

BIOMASS CONCENTRATION (PPM)
FIG 5-2
Y = 0.314
INITIAL PHENOL CONC : 10 PPM
5
4.5
4
3.5
3
2.5
2
1.5
1
2
3
4
5
6
7
8
9
10
11
12
PHENOL CONCENTRATION (PPM)

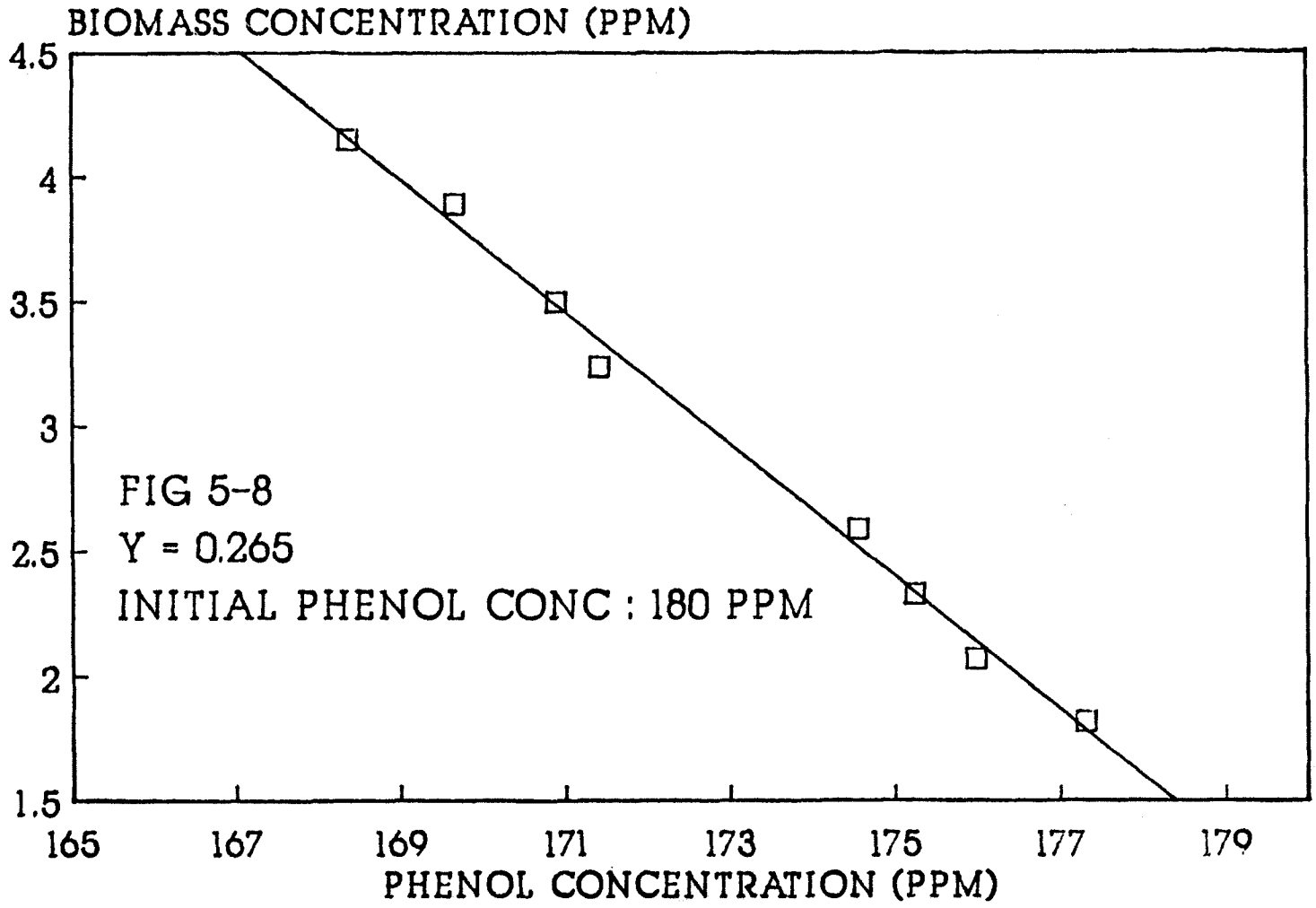

BIOMASS CONCENTRATION (PPM)
FIG 5-3
Y = 0.319
INITIAL PHENOL CONC : 20 PPM
5.5
4.5
3.5
2.5
1.5
9
10
11
12
13
14
15
16
17
18
19
20
PHENOL CONCENTRATION (PPM)

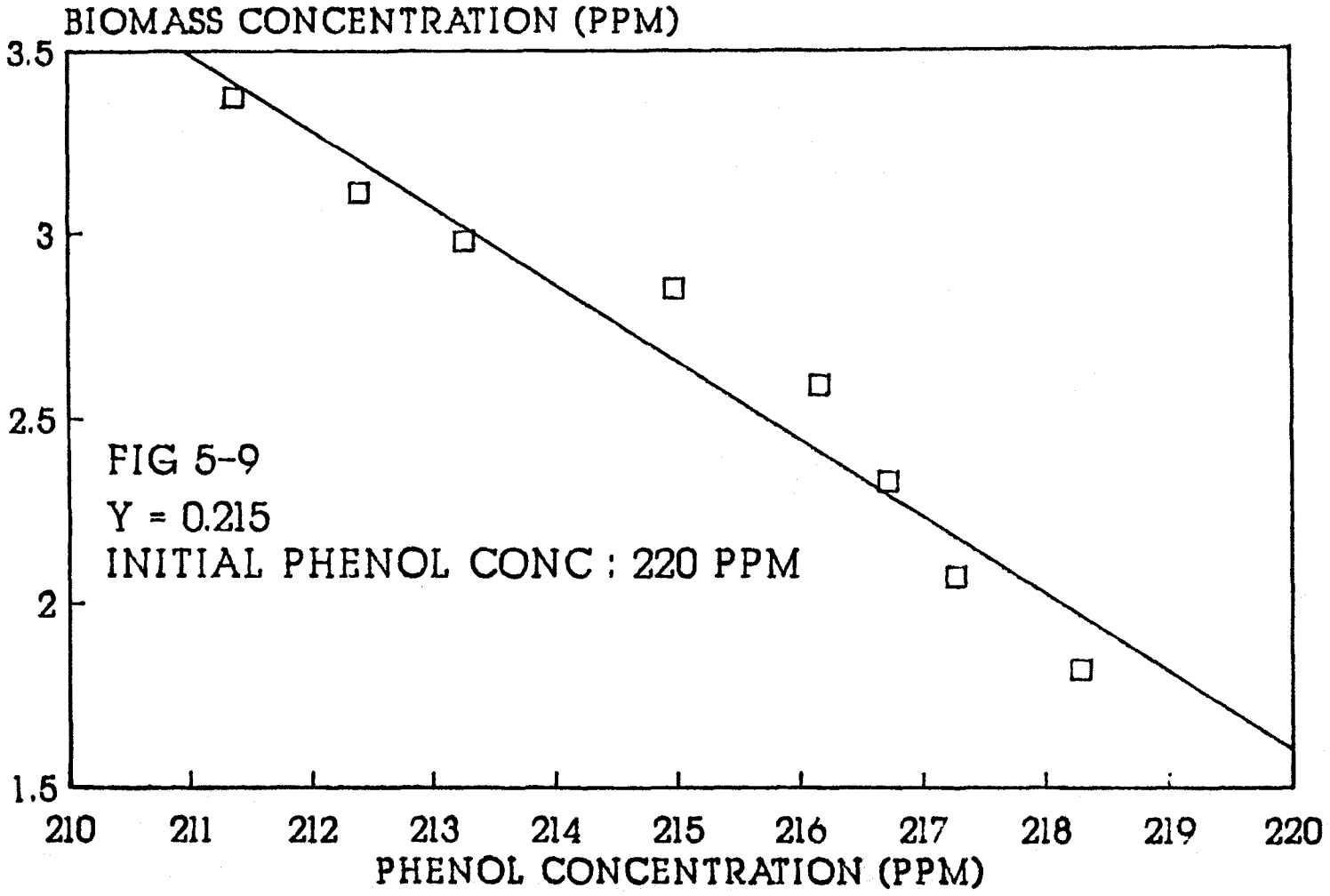

BIOMASS CONCENTRATION (PPM)
FIG 5-4
Y = 0.236
INITIAL PHENOL CONC : 60 PPM
7.5
5.5
3.5
1.5
30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
PHENOL CONCENTRATION (PPM)

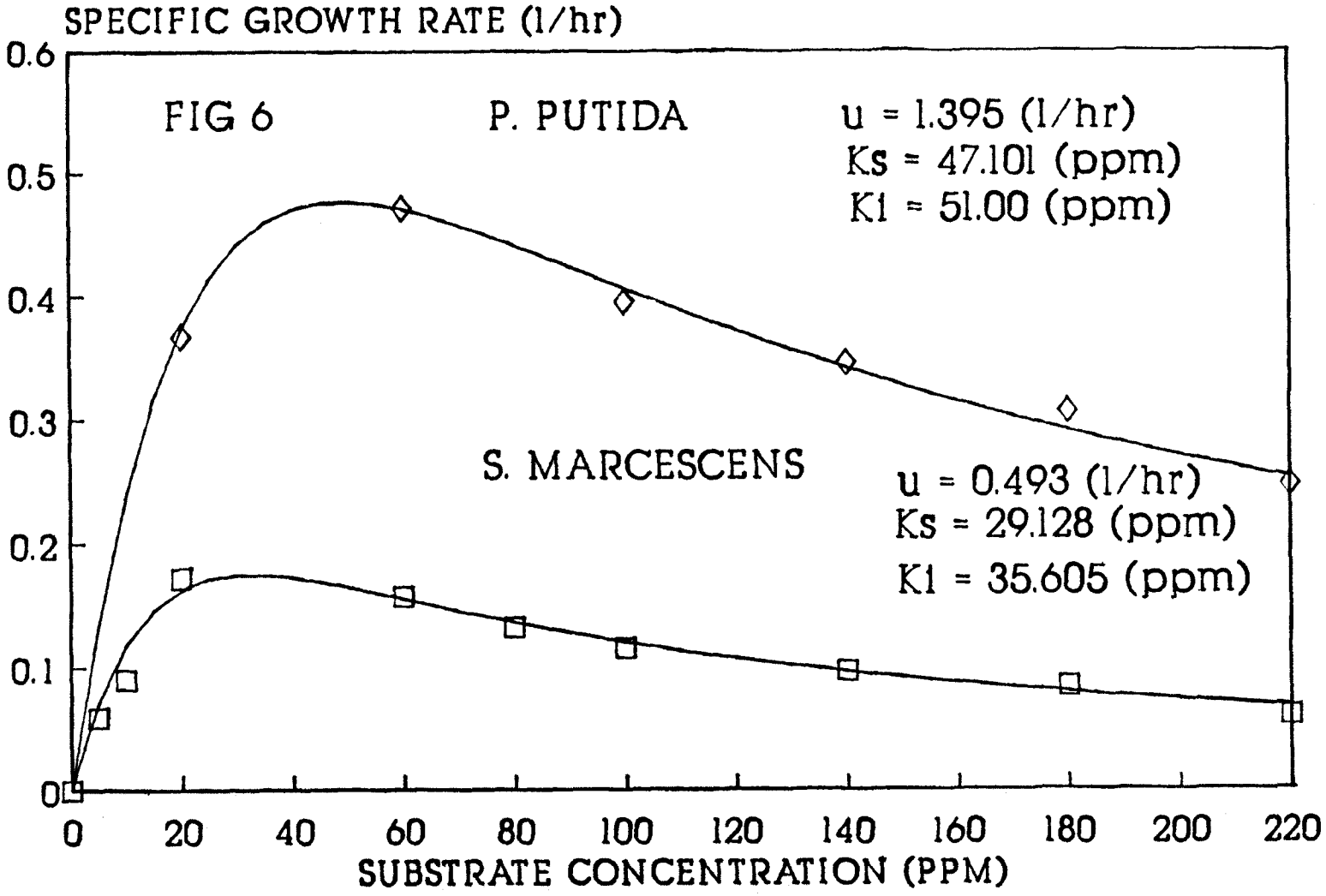

BIOMASS CONCENTRATION (PPM)
FIG 5-5
Y = 0.227
INITIAL PHENOL CONC : 80 PPM
6.5
5.5
4.5
3.5
2.5
1.5
55
57
59
61
63
65
67
69
71
73
75
77
79
PHENOL CONCENTRATION (PPM)

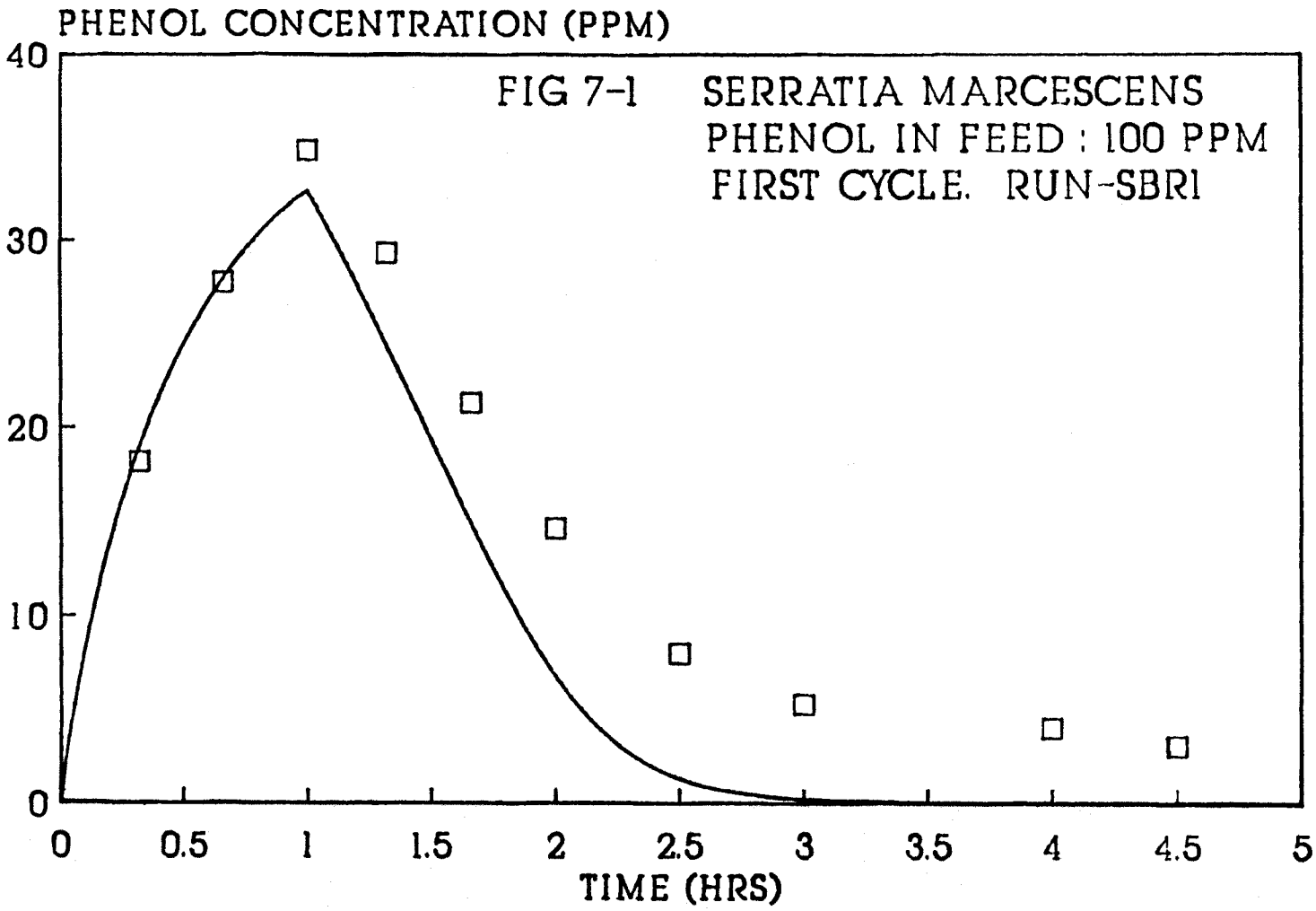

BIOMASS CONCENTRATION (PPM)
5.5
4.5
3.5
2.5
1.5
75
77
79
81
83
85
87
89
91
93
95
97
99
PHENOL CONCENTRATION (PPM)
FIG 5-6
Y = 0.224
INITIAL PHENOL CONC : 100 PPM

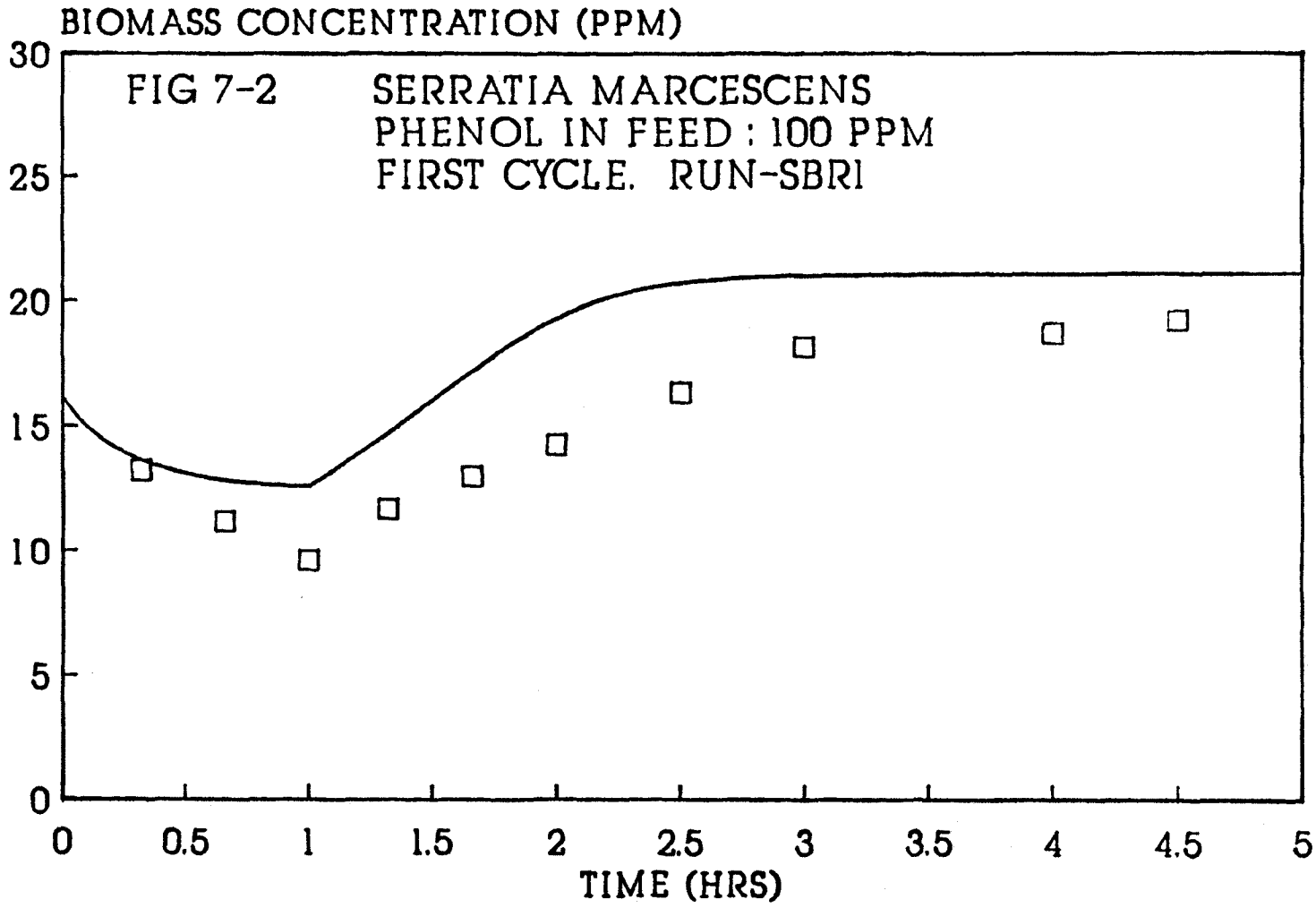

BIOMASS CONCENTRATION (PPM)
5
4.5
4
3.5
3
2.5
2
1.5
125
127
129
131
133
135
137
139
141
PHENOL CONCENTRATION (PPM)
FIG 5-7
Y = 0.244
INITIAL PHENOL CONC : 140 PPM

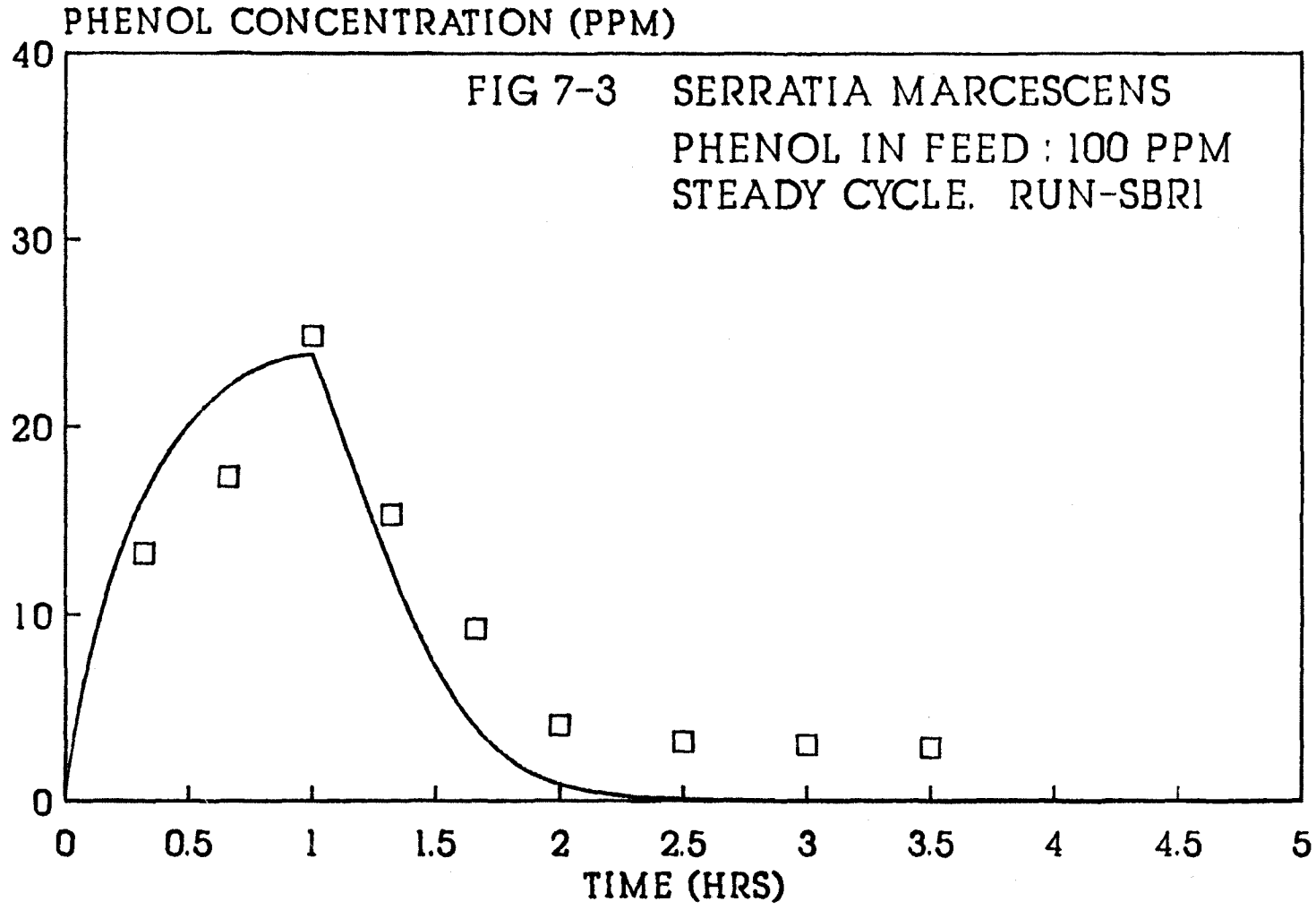

BIOMASS CONCENTRATION (PPM)
4.5
4
3.5
3
2.5
2
1.5
165
167
169
171
173
175
177
179
PHENOL CONCENTRATION (PPM)
FIG 5-8
Y = 0.265
INITIAL PHENOL CONC : 180 PPM

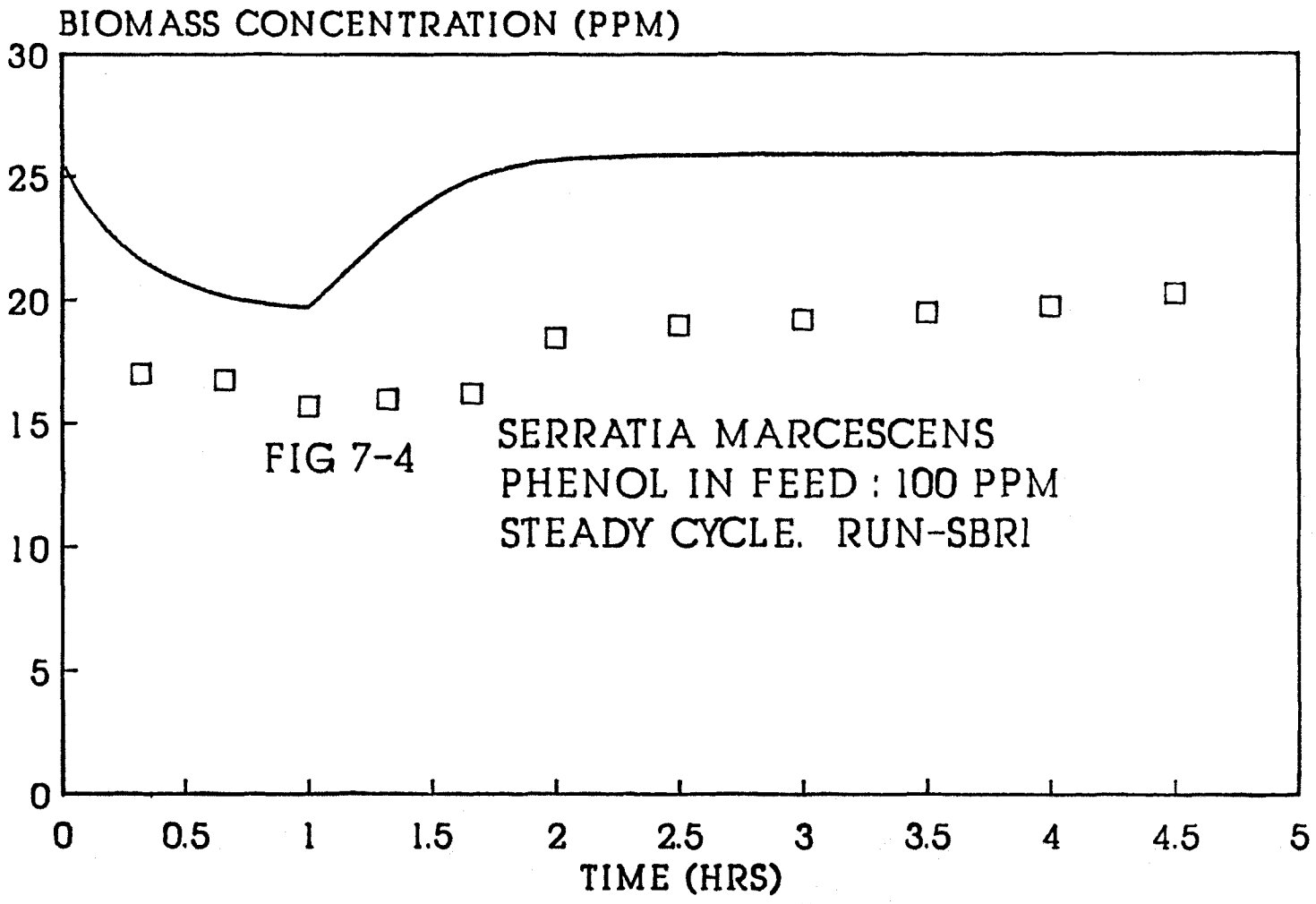

BIOMASS CONCENTRATION (PPM)
3.5
3
2.5
2
1.5
FIG 5-9
Y = 0.215
INITIAL PHENOL CONC : 220 PPM
210
211
212
213
214
215
216
217
218
219
220
PHENOL CONCENTRATION (PPM)

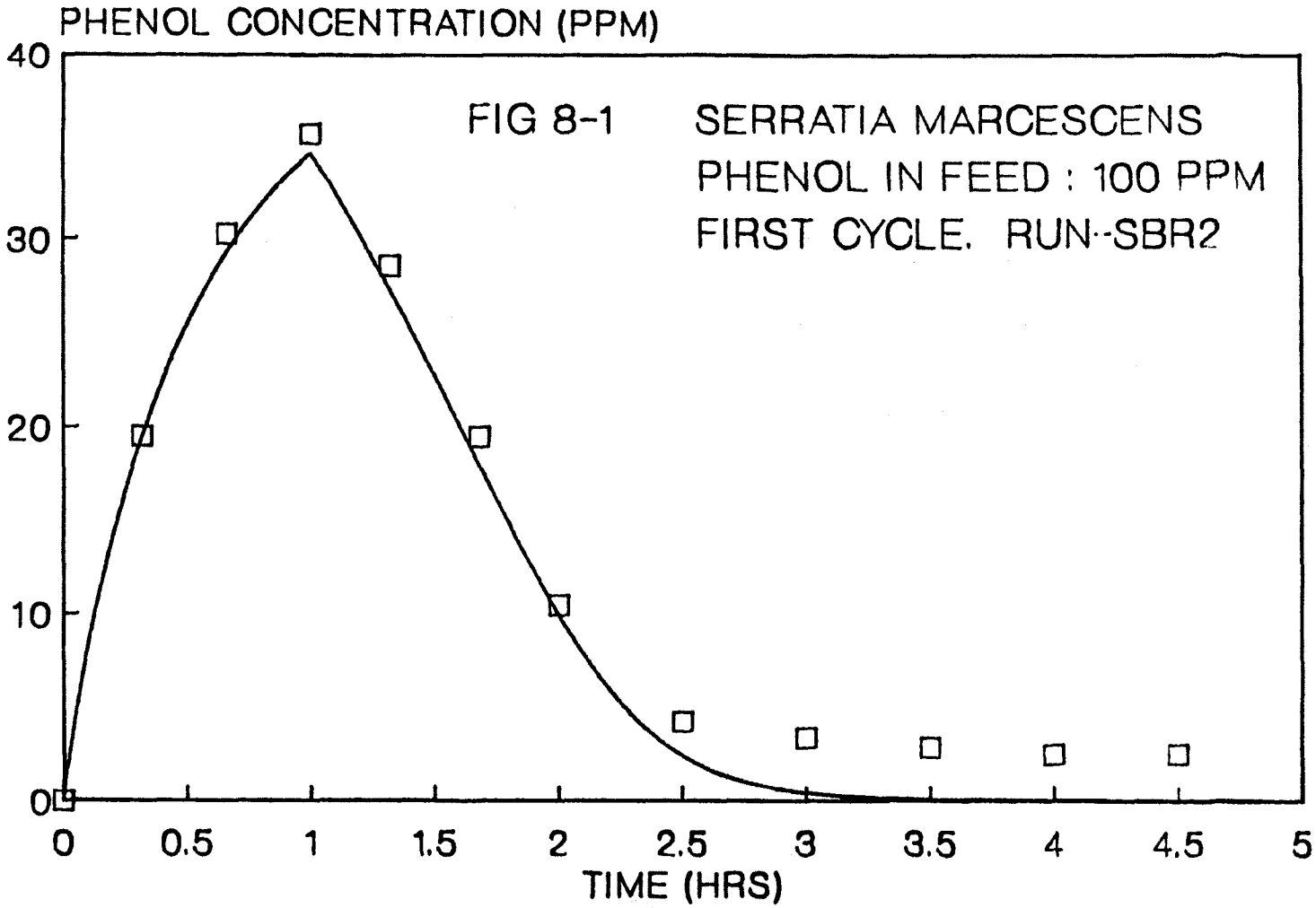

SPECIFIC GROWTH RATE (1/hr)
0.6
0.5
0.4
0.3
0.2
0.1
0
FIG 6
P. PUTIDA
u = 1.395 (1/hr)
Ks = 47.101 (ppm)
Ki = 51.00 (ppm)
S. MARCESCENS
u = 0.493 (1/hr)
Ks = 29.128 (ppm)
Ki = 35.605 (ppm)
0
20
40
60
80
100
120
140
160
180
200
220
SUBSTRATE CONCENTRATION (PPM)


PHENOL CONCENTRATION (PPM)
40
30
20
10
0
FIG 7-1 SERRATIA MARCESCENS
PHENOL IN FEED : 100 PPM
FIRST CYCLE. RUN-SBR1
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)


BIOMASS CONCENTRATION (PPM)
30
25
20
15
10
5
0
FIG 7-2
SERRATIA MARCESCENS
PHENOL IN FEED : 100 PPM
FIRST CYCLE. RUN-SBR1
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)


PHENOL CONCENTRATION (PPM)
40
30
20
10
0
FIG 7-3 SERRATIA MARCESCENS
PHENOL IN FEED : 100 PPM
STEADY CYCLE. RUN-SBR1
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)


BIOMASS CONCENTRATION (PPM)
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)
FIG 7-4
SERRATIA MARCESCENS
PHENOL IN FEED : 100 PPM
STEADY CYCLE. RUN-SBR1


PHENOL CONCENTRATION (PPM)
40
30
20
10
0
FIG 8-1
SERRATIA MARCESCENS
PHENOL IN FEED : 100 PPM
FIRST CYCLE. RUN--SBR2
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)

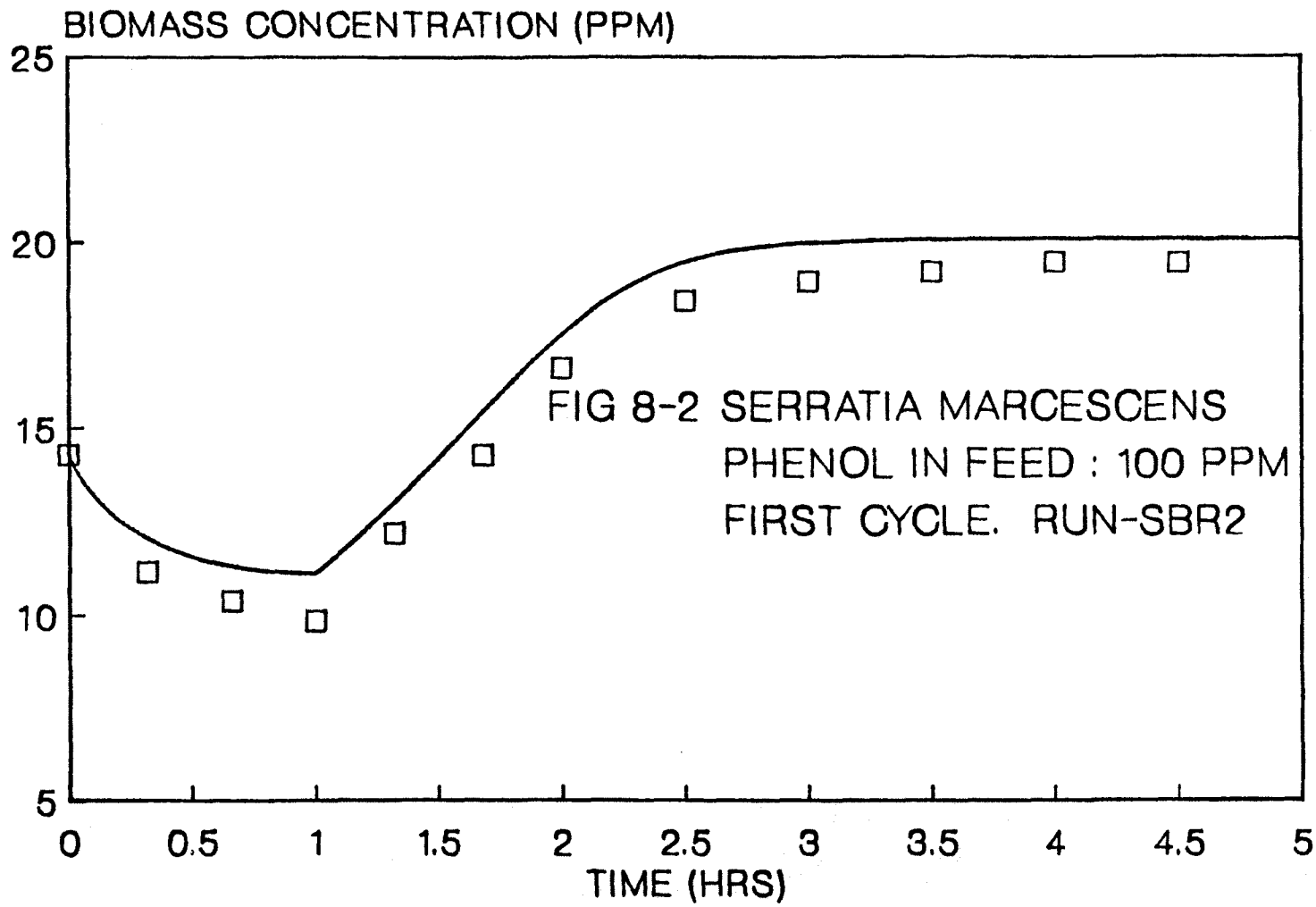
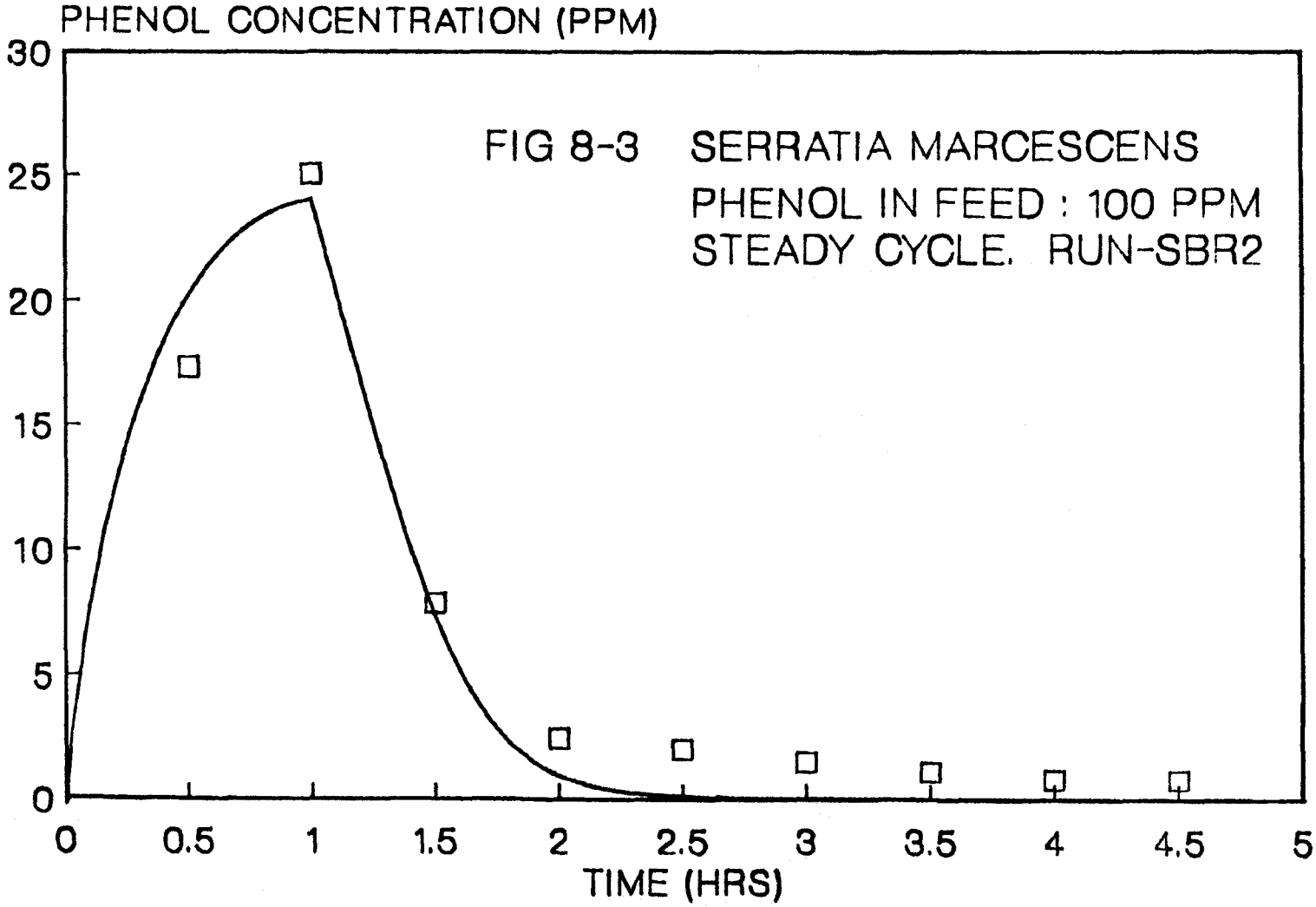
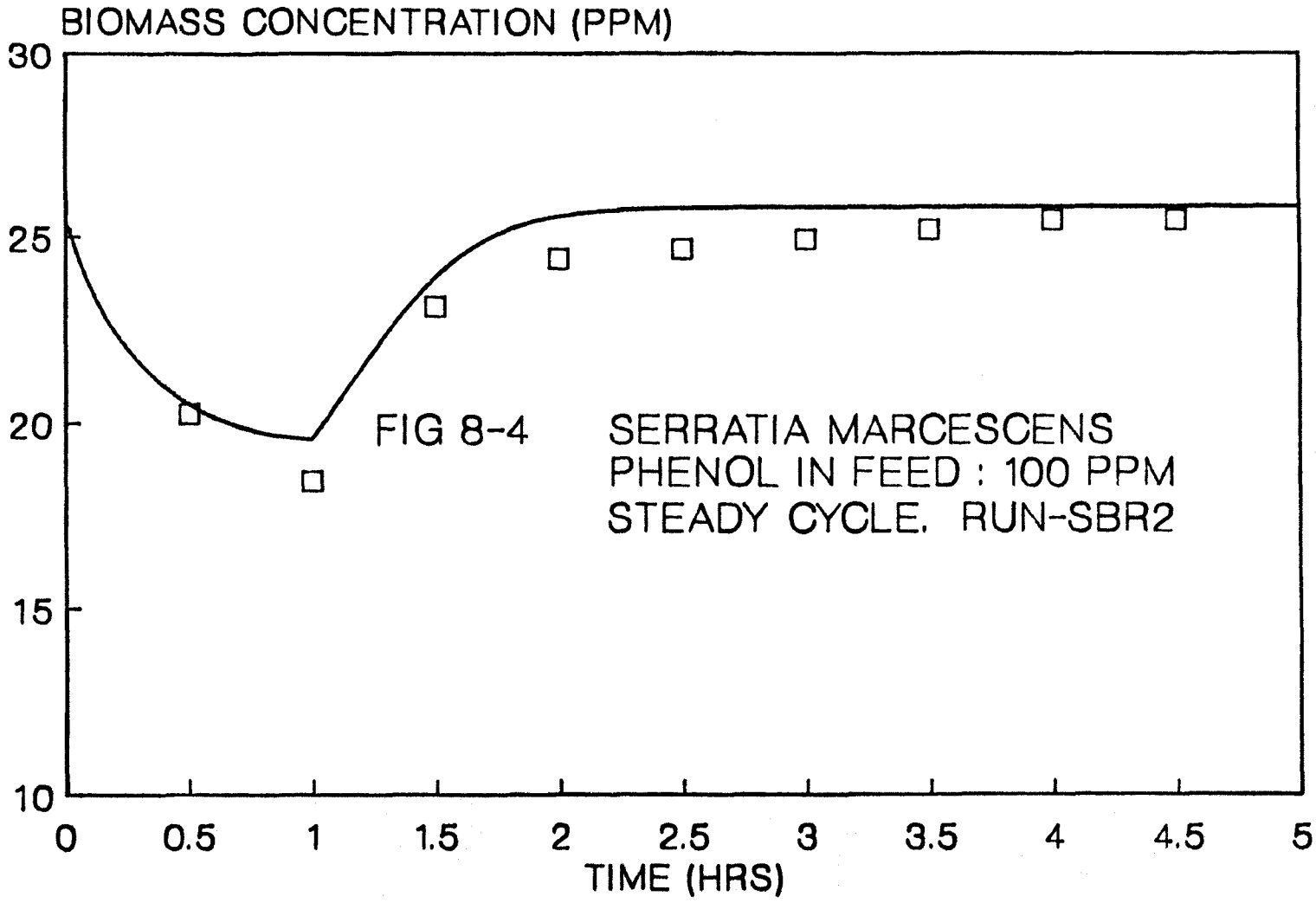
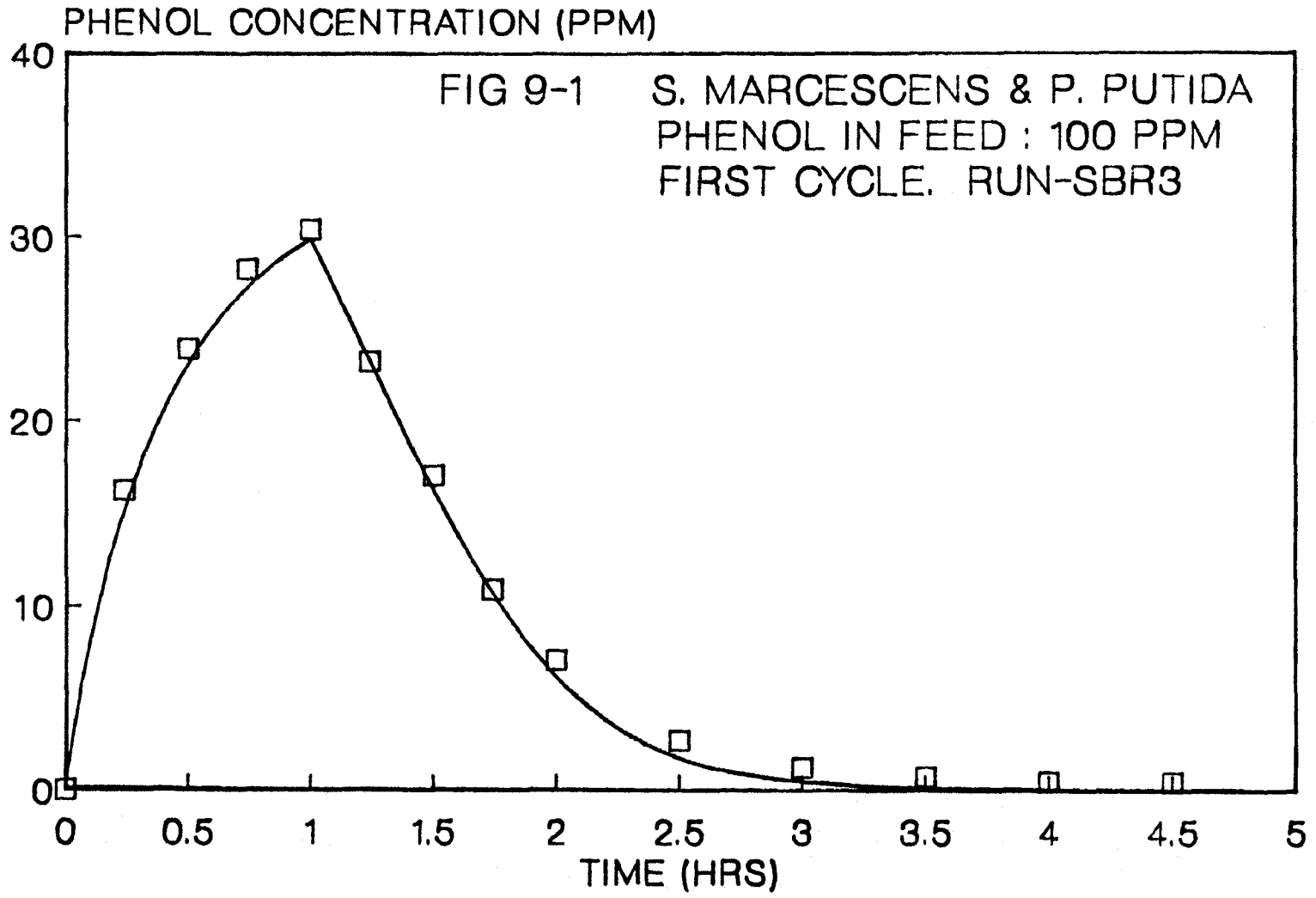
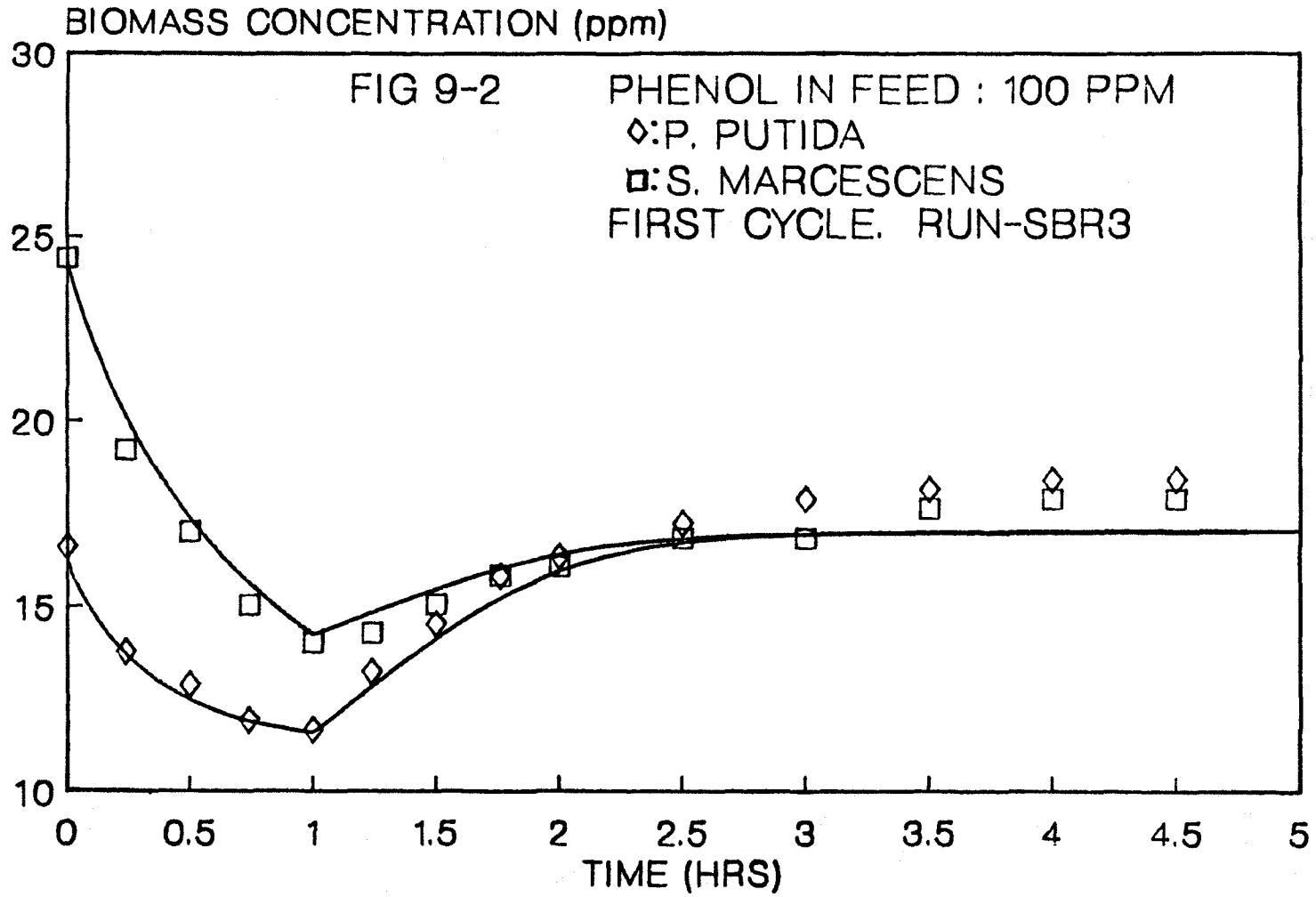


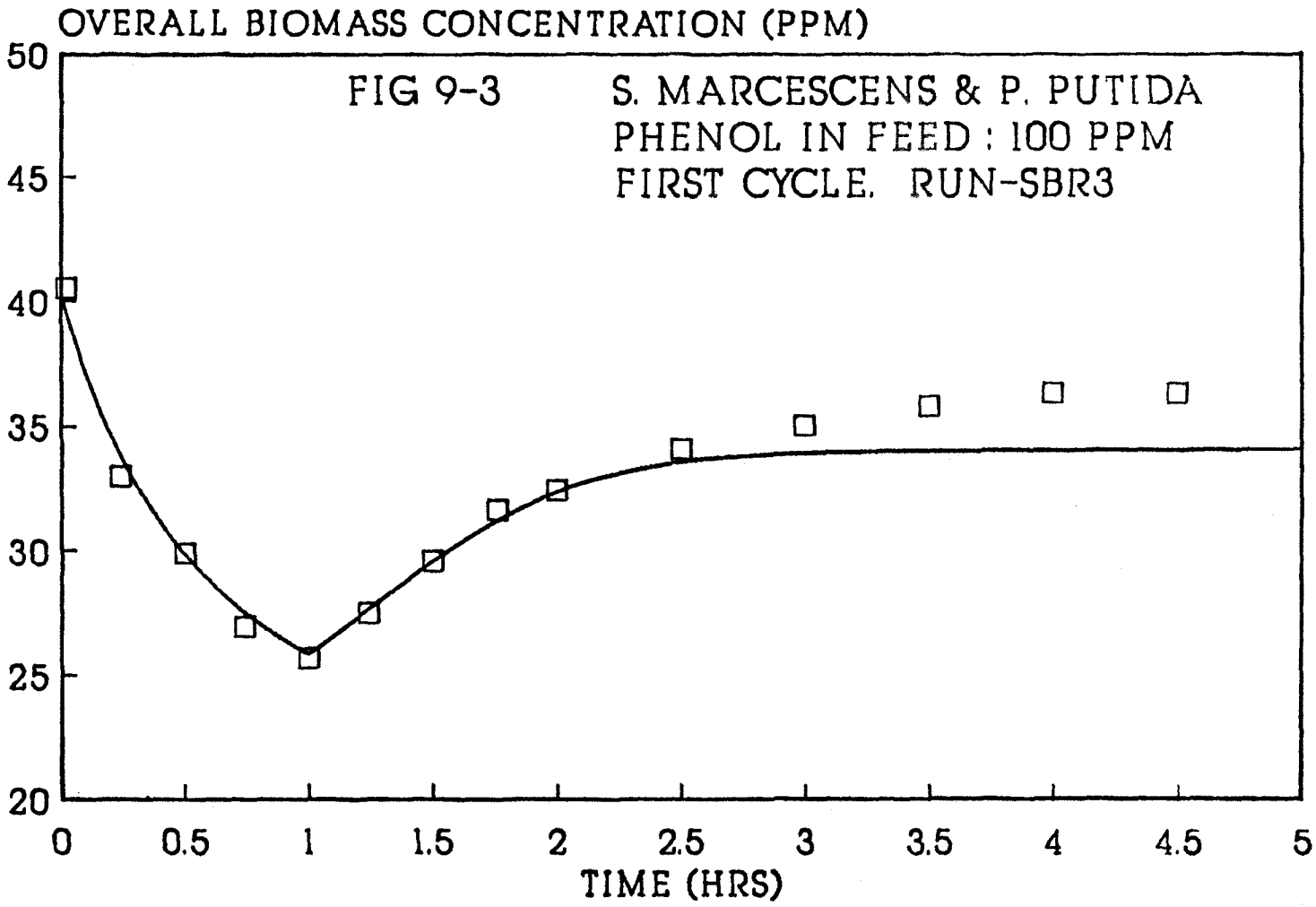
FIG 8-2 SERRATIA MARCESCENS
PHENOL IN FEED : 100 PPM
FIRST CYCLE. RUN-SBR2

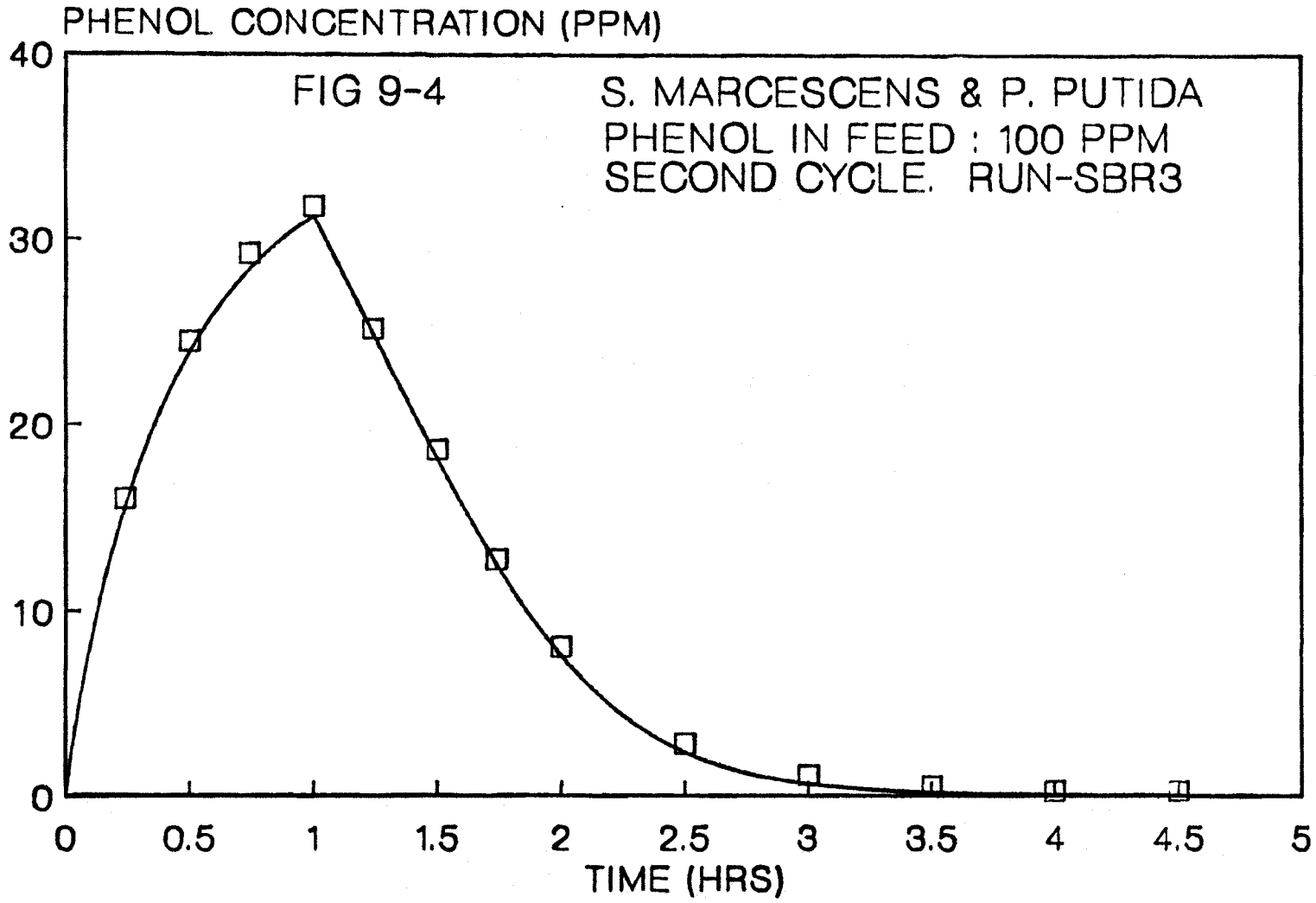

PHENOL CONCENTRATION (PPM)
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)
FIG 8-3 SERRATIA MARCESCENS
PHENOL IN FEED : 100 PPM
STEADY CYCLE. RUN-SBR2

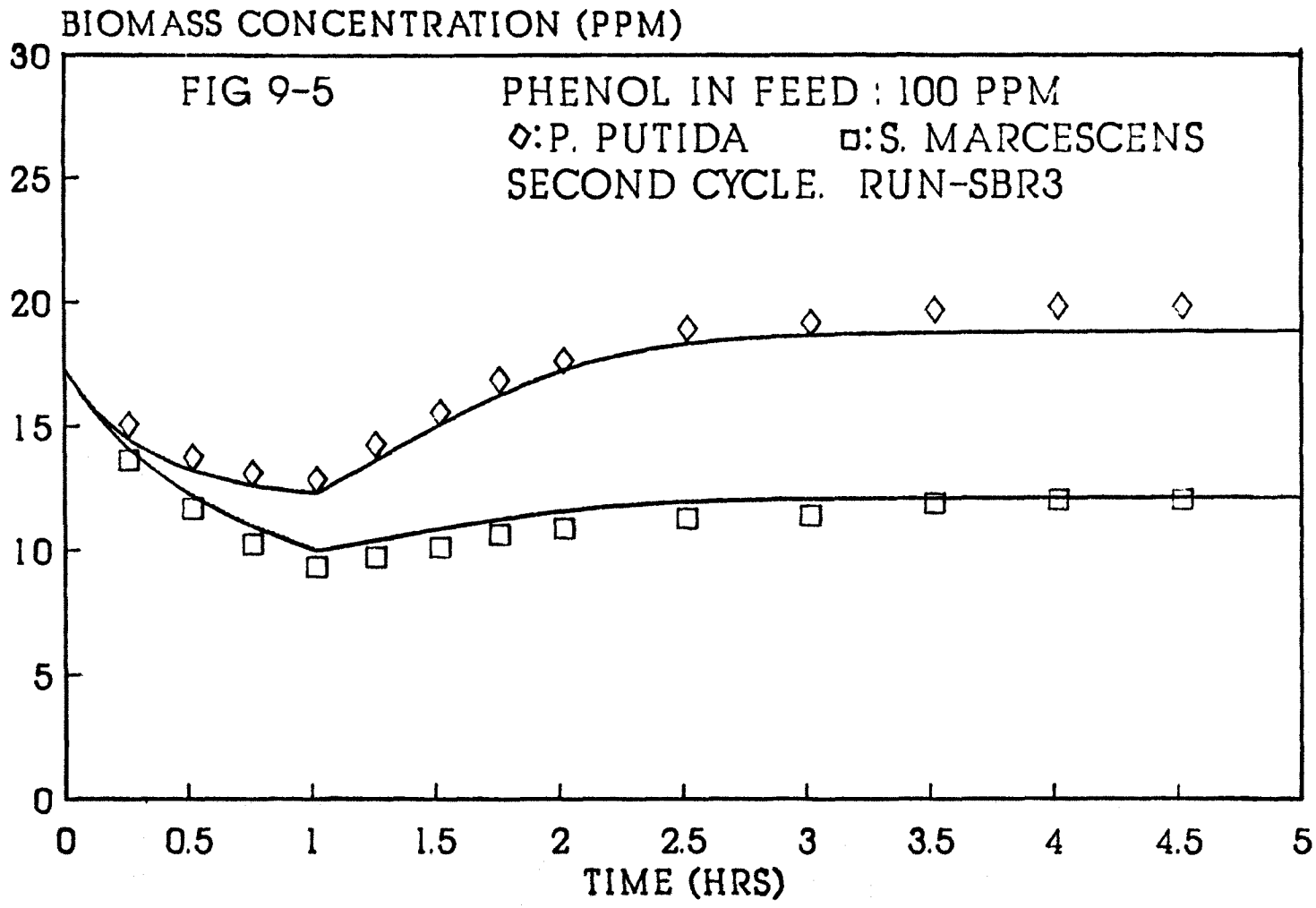

BIOMASS CONCENTRATION (PPM)
30
25
20
15
10
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)
FIG 8-4
SERRATIA MARCESCENS
PHENOL IN FEED : 100 PPM
STEADY CYCLE. RUN-SBR2

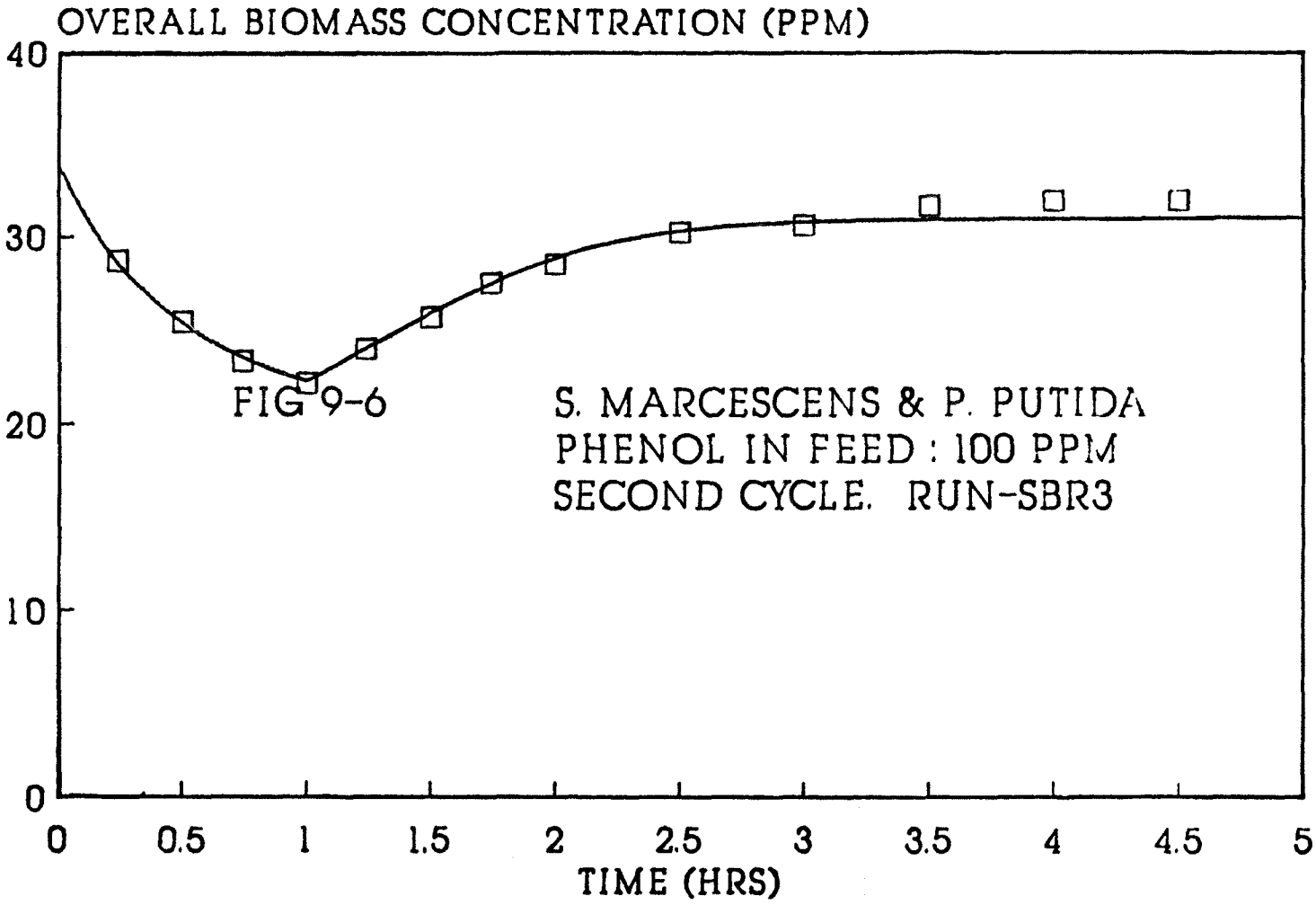

PHENOL CONCENTRATION (PPM)
FIG 9-1 S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
FIRST CYCLE. RUN-SBR3
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)

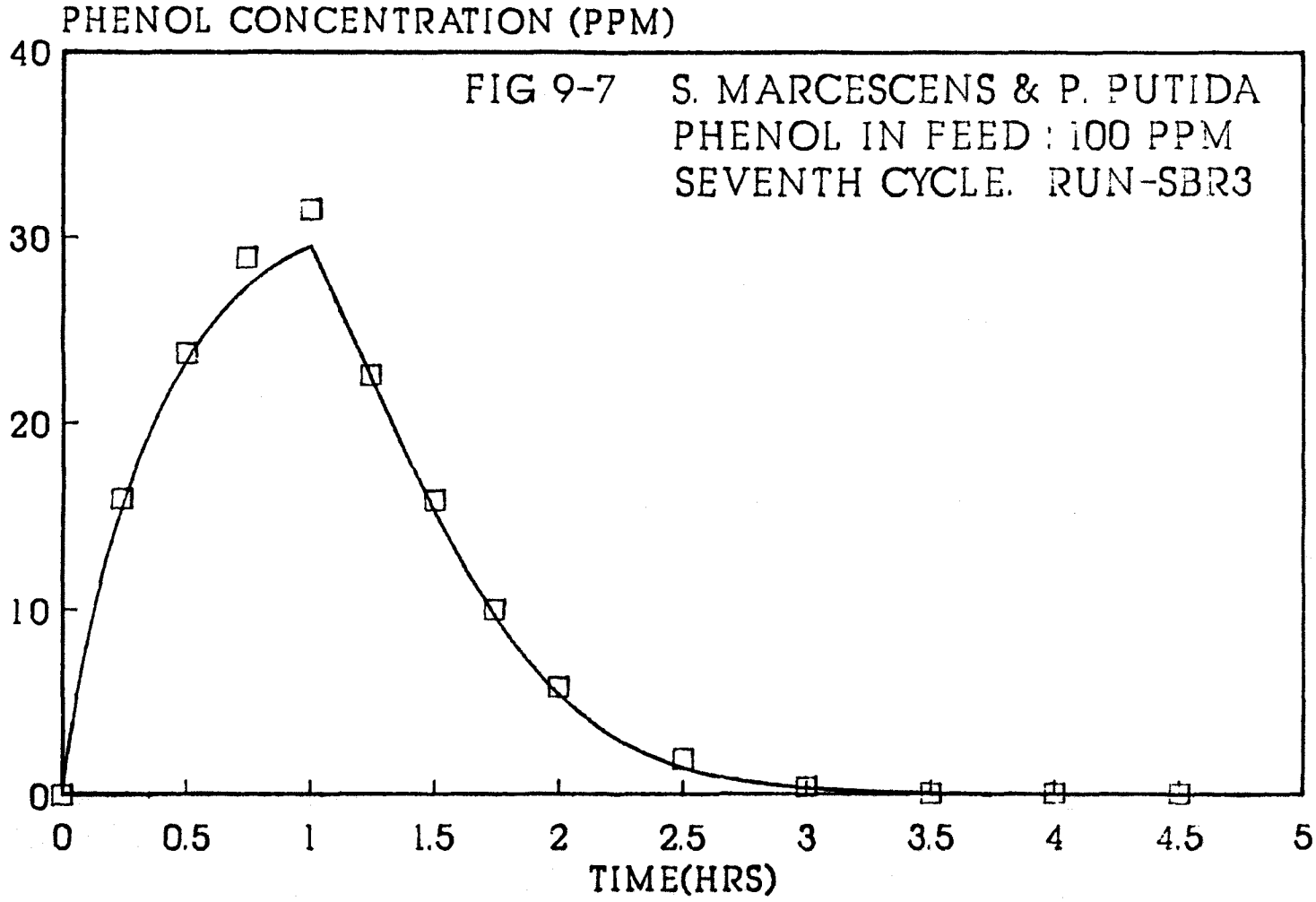

BIOMASS CONCENTRATION (ppm)
30
25
20
15
10
FIG 9-2
PHENOL IN FEED : 100 PPM
◇:P. PUTIDA
□:S. MARCESCENS
FIRST CYCLE. RUN-SBR3
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)

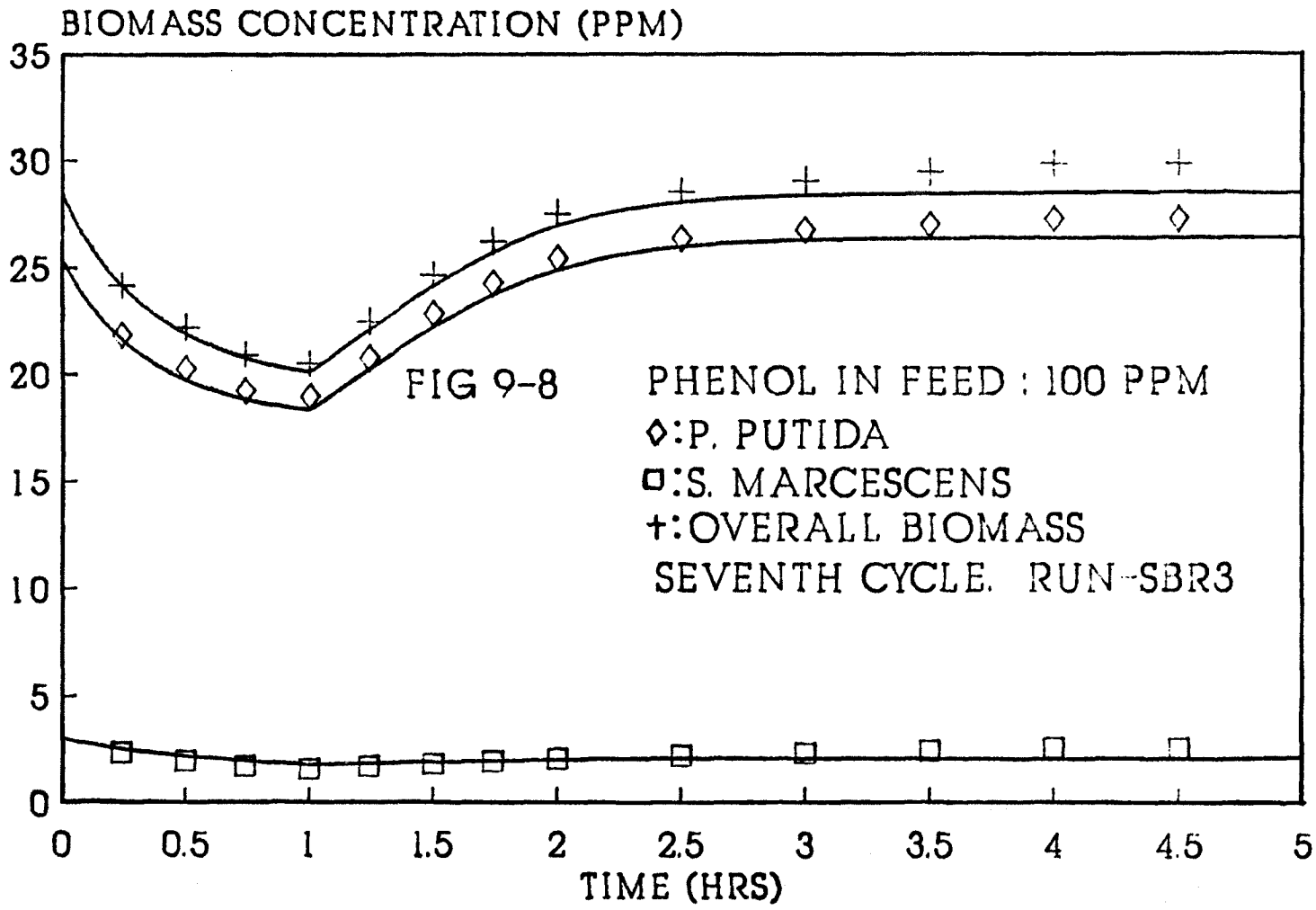

OVERALL BIOMASS CONCENTRATION (PPM)
FIG 9-3
S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
FIRST CYCLE. RUN-SBR3
50
45
40
35
30
25
20
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)

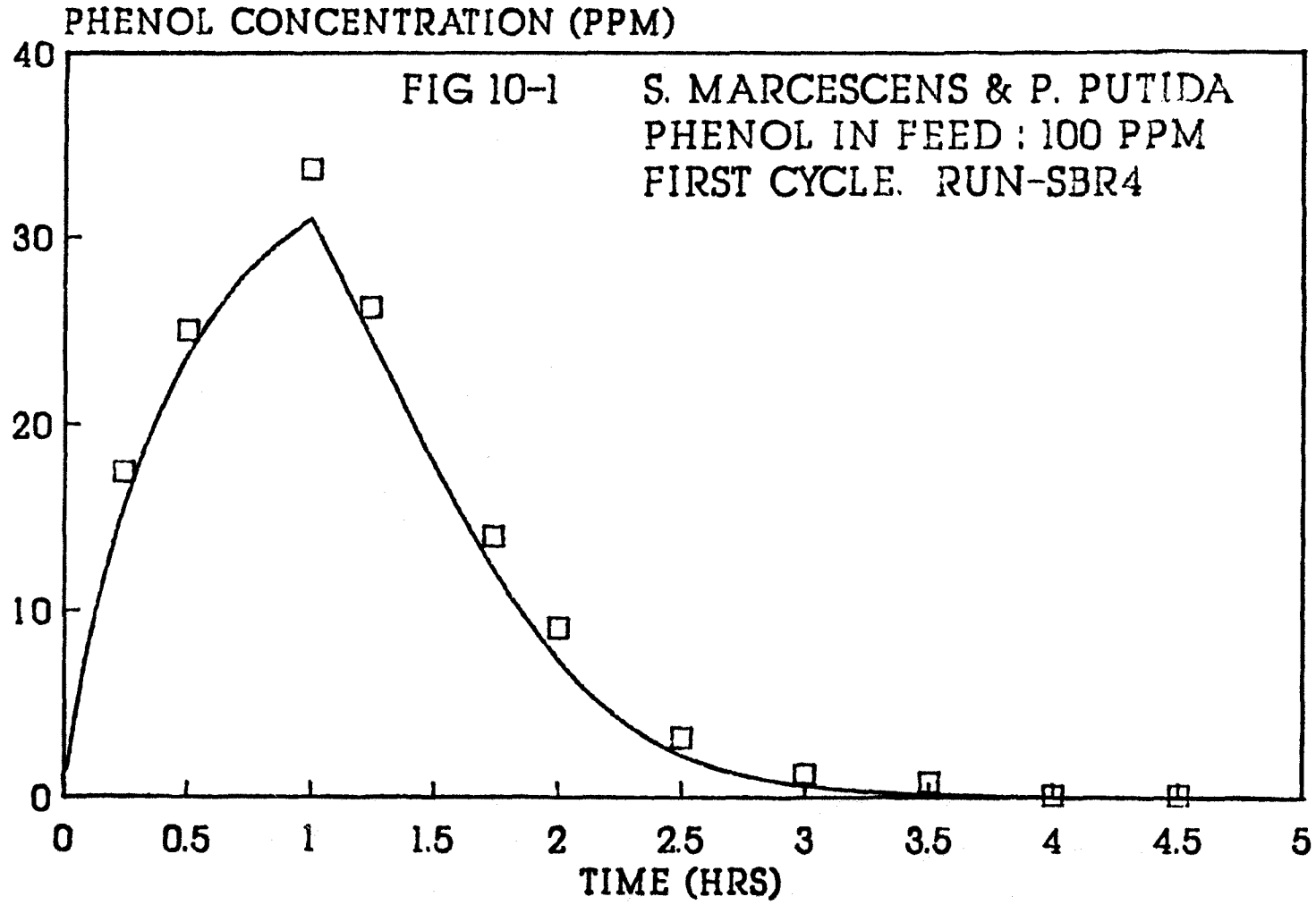

PHENOL CONCENTRATION (PPM)
FIG 9-4
S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
SECOND CYCLE. RUN-SBR3
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)

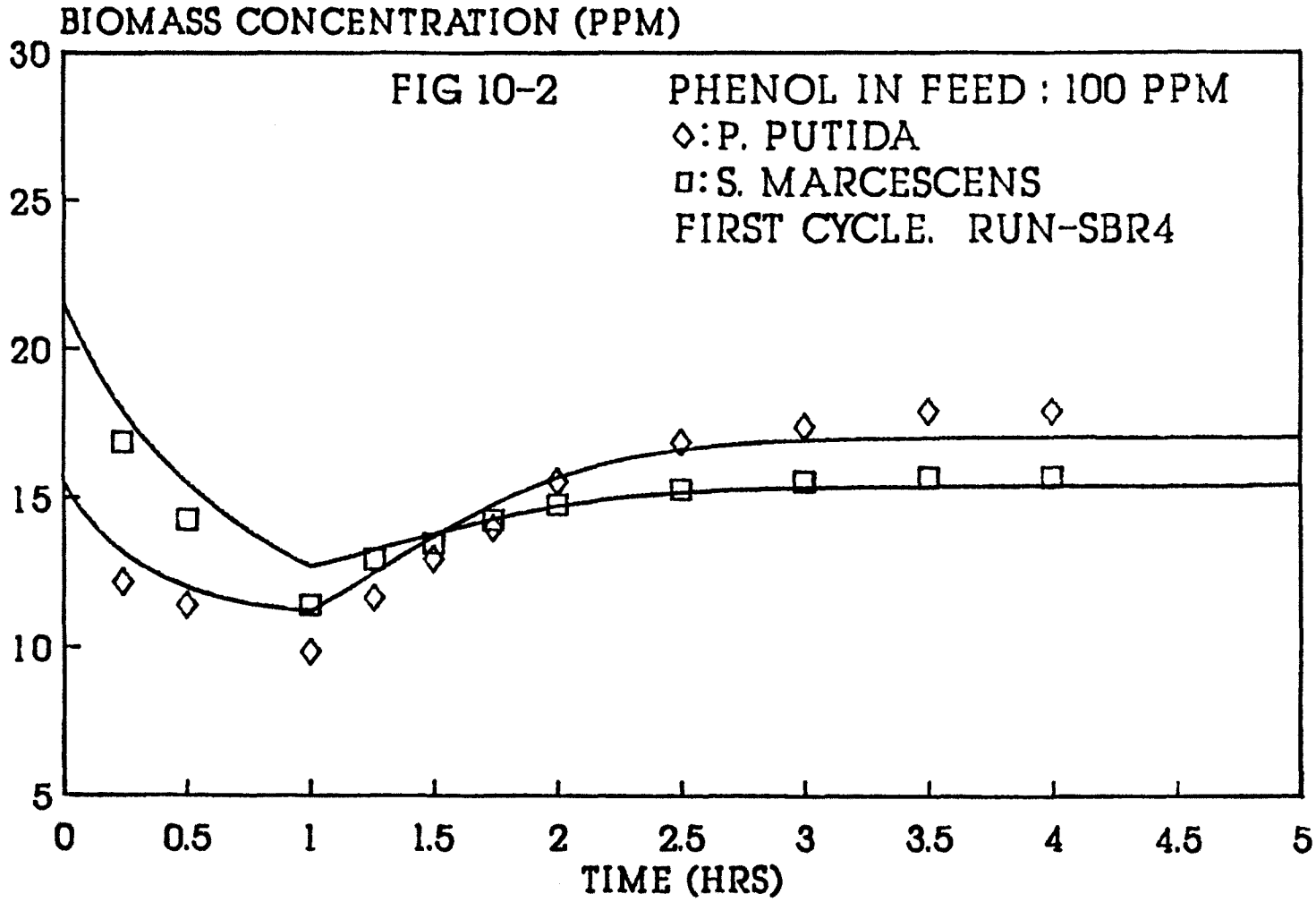

BIOMASS CONCENTRATION (PPM)
FIG 9-5
PHENOL IN FEED : 100 PPM
◇:P. PUTIDA
□:S. MARCESCENS
SECOND CYCLE. RUN-SBR3
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)

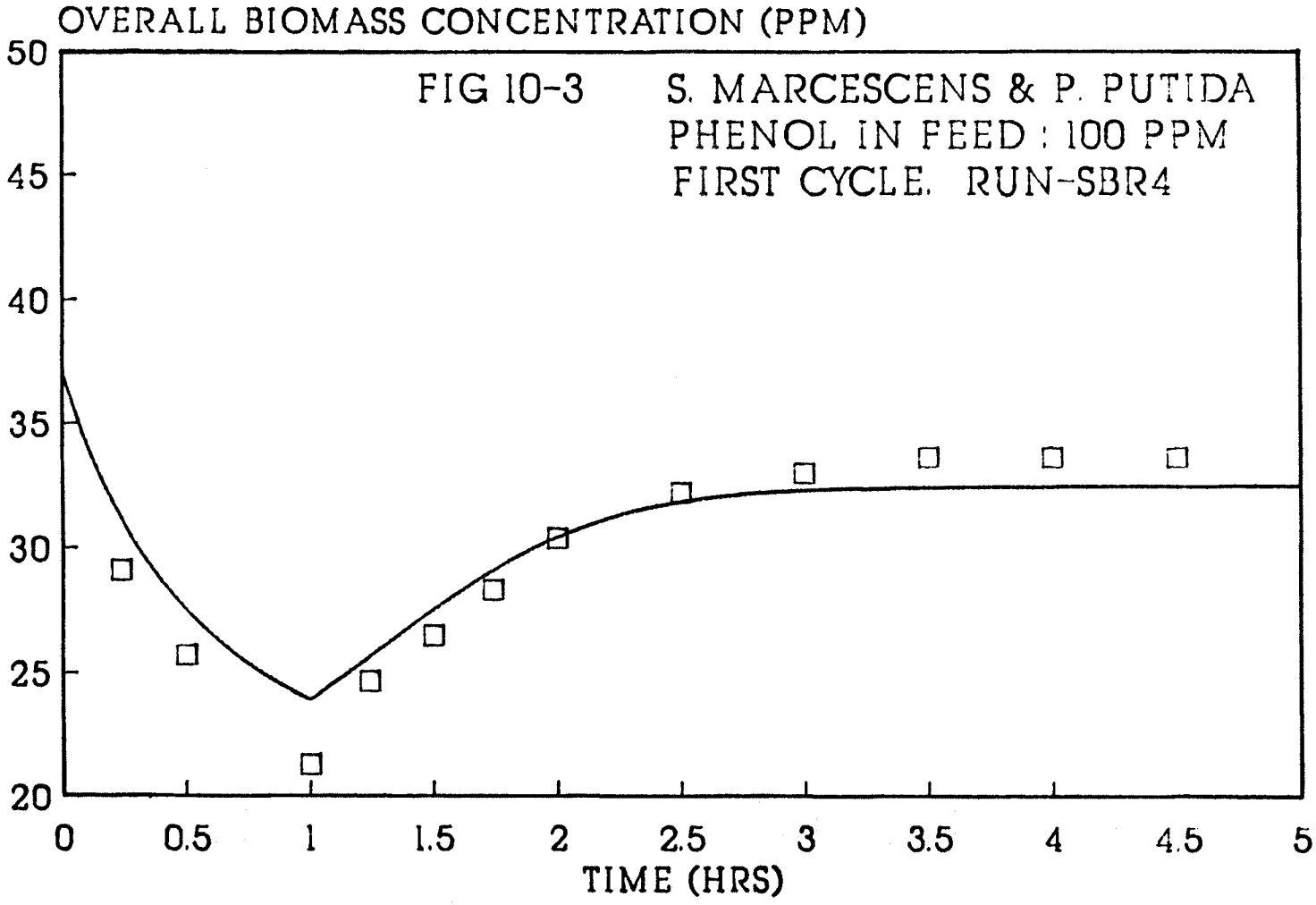

OVERALL BIOMASS CONCENTRATION (PPM)
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)
FIG 9-6
S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
SECOND CYCLE. RUN-SBR3

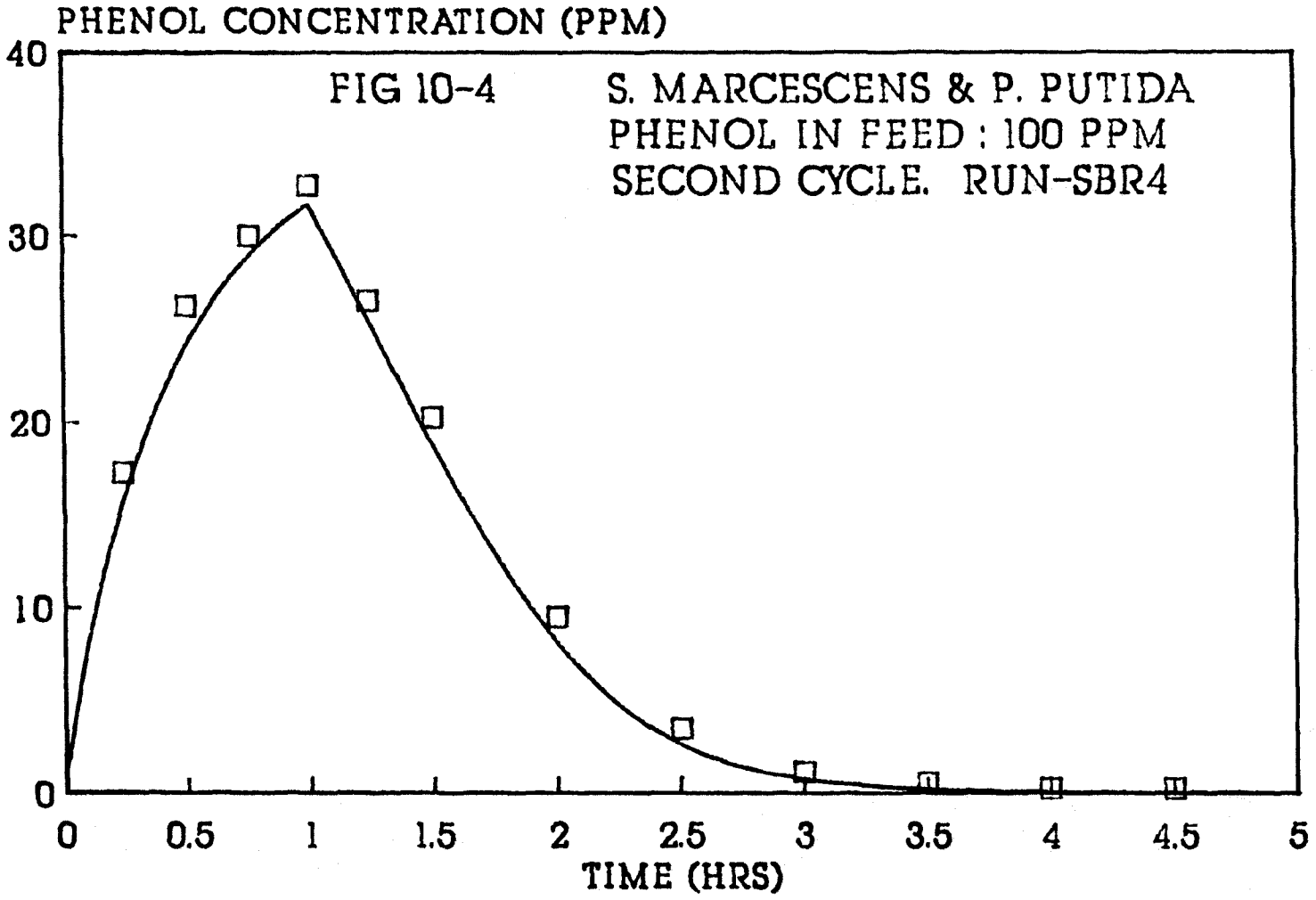

PHENOL CONCENTRATION (PPM)
40
30
20
10
0
FIG 9-7 S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
SEVENTH CYCLE. RUN-SBR3
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME(HRS)

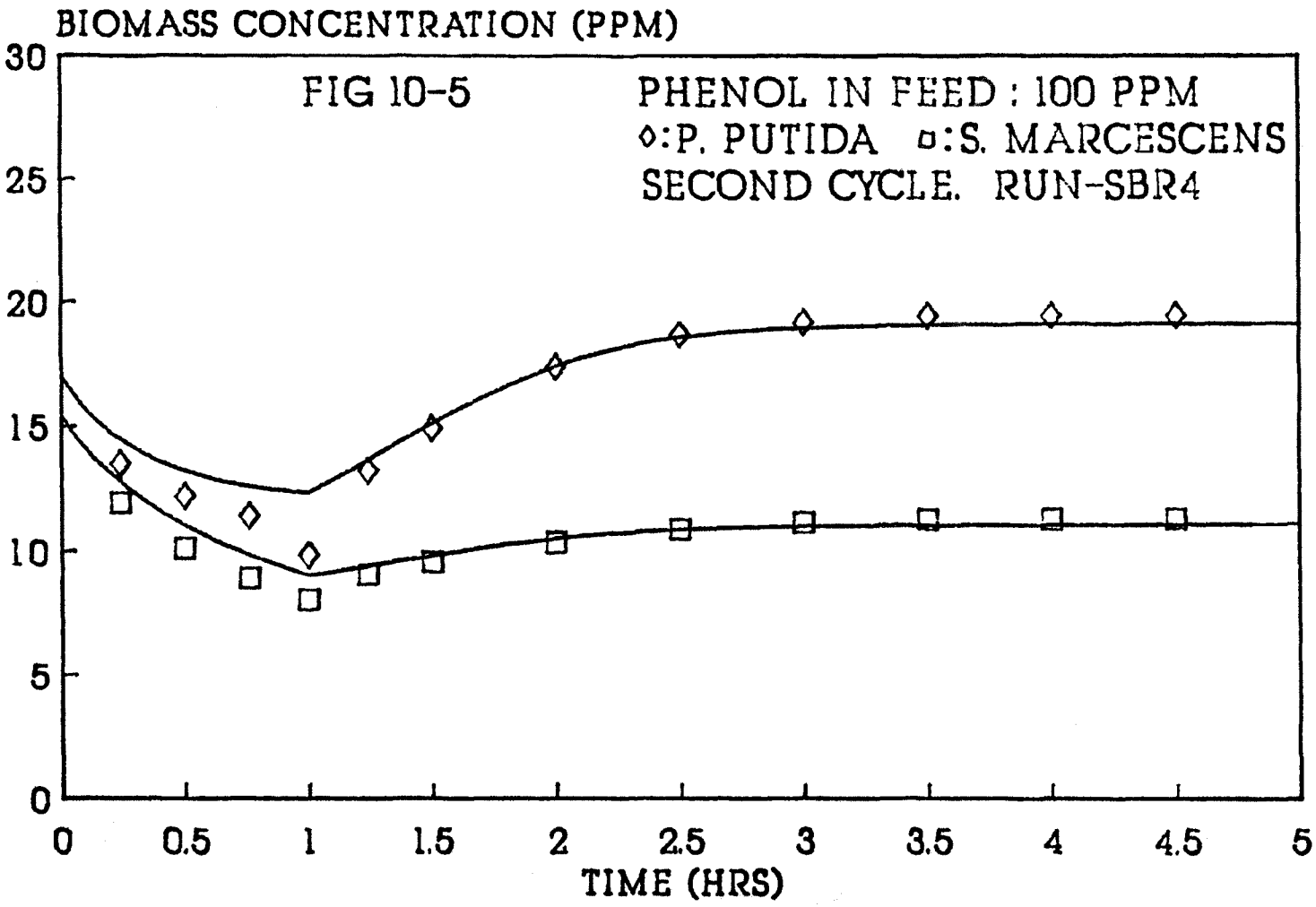

BIOMASS CONCENTRATION (PPM)
35
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)
FIG 9-8
PHENOL IN FEED : 100 PPM
◊:P. PUTIDA
□:S. MARCESCENS
+:OVERALL BIOMASS
SEVENTH CYCLE. RUN-SBR3

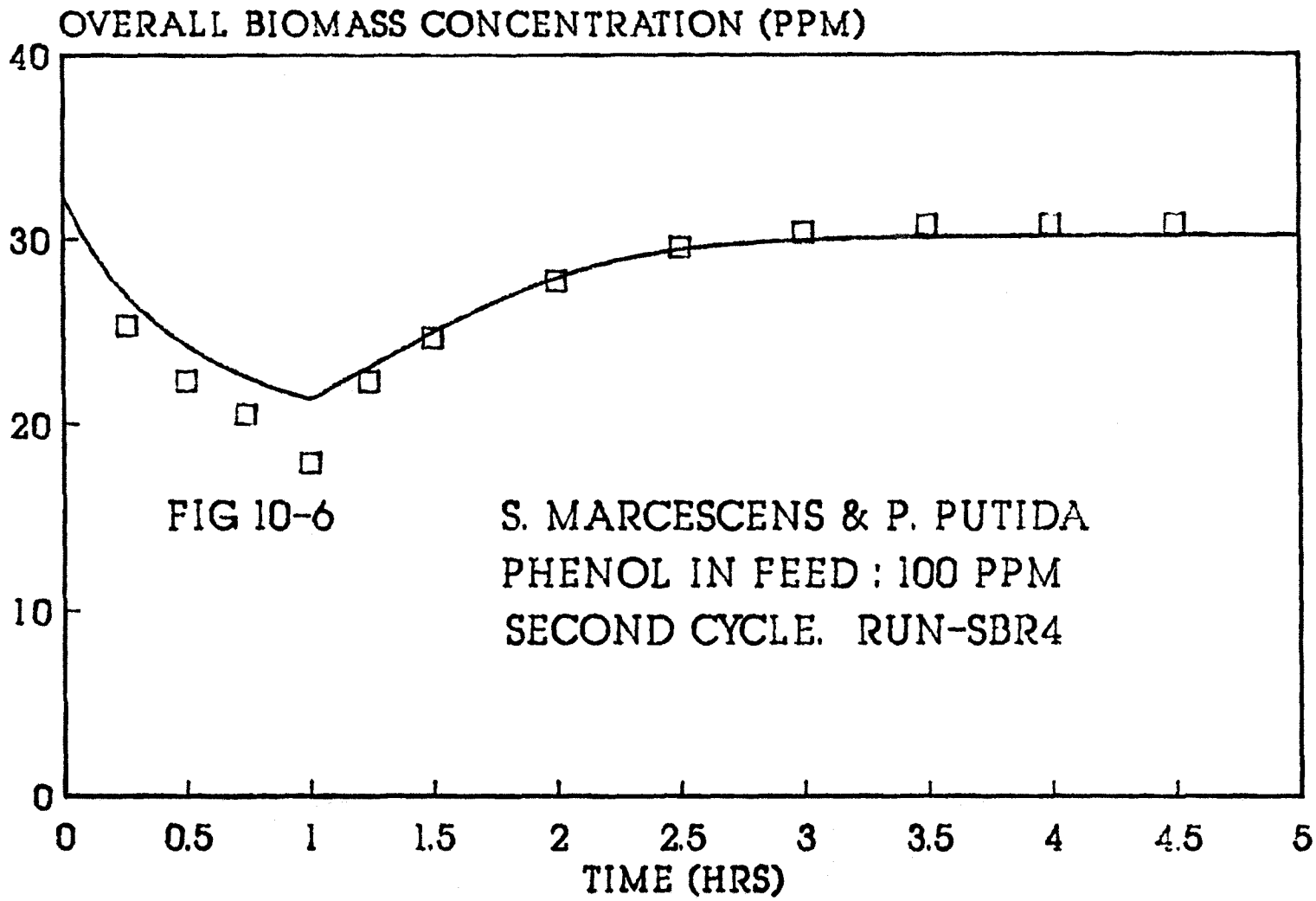

PHENOL CONCENTRATION (PPM)
40
30
20
10
0
FIG 10-1
S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
FIRST CYCLE. RUN-SBR4
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)

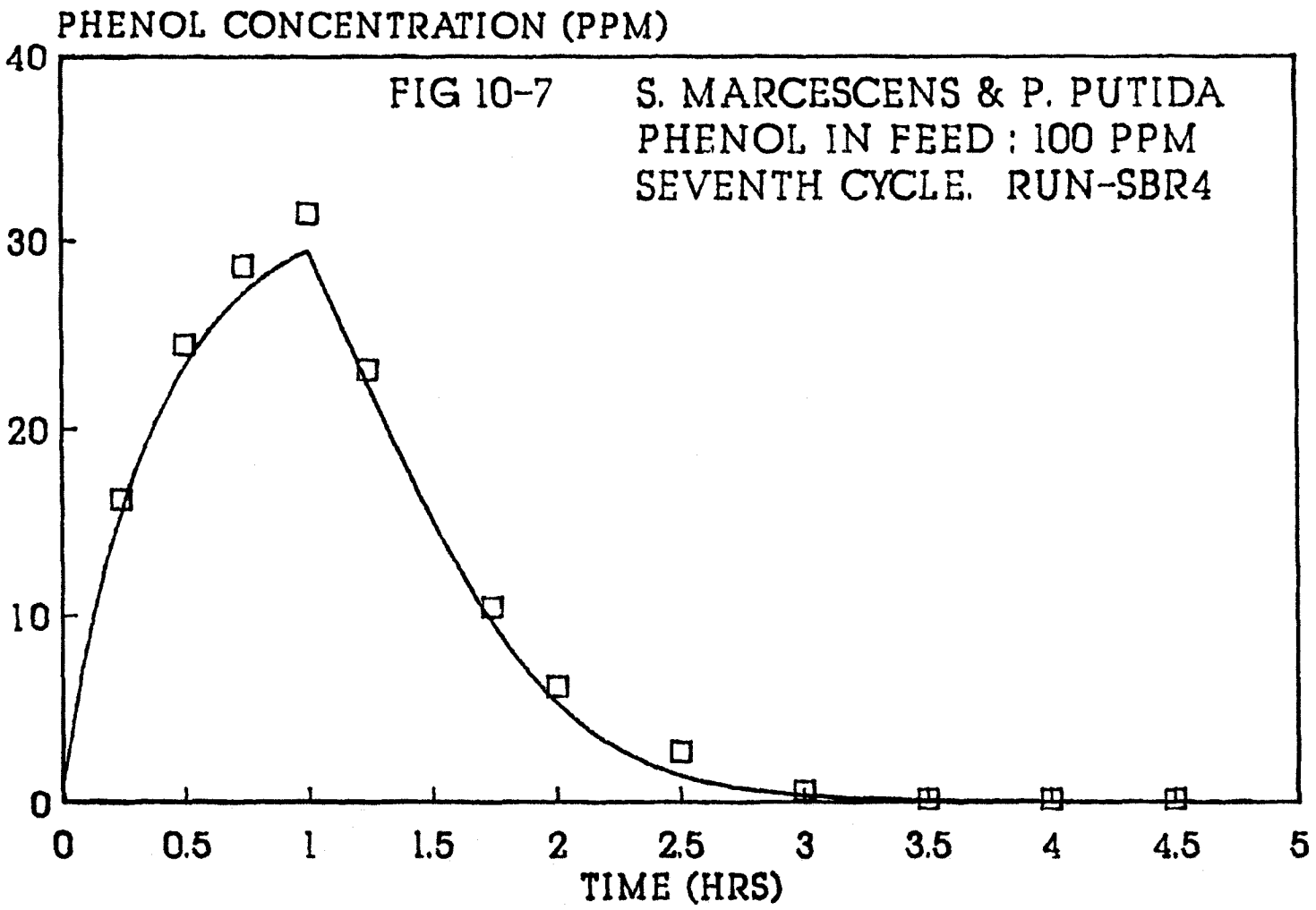

BIOMASS CONCENTRATION (PPM)
30
25
20
15
10
5
FIG 10-2
PHENOL IN FEED : 100 PPM
◇: P. PUTIDA
□: S. MARCESCENS
FIRST CYCLE. RUN-SBR4
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)

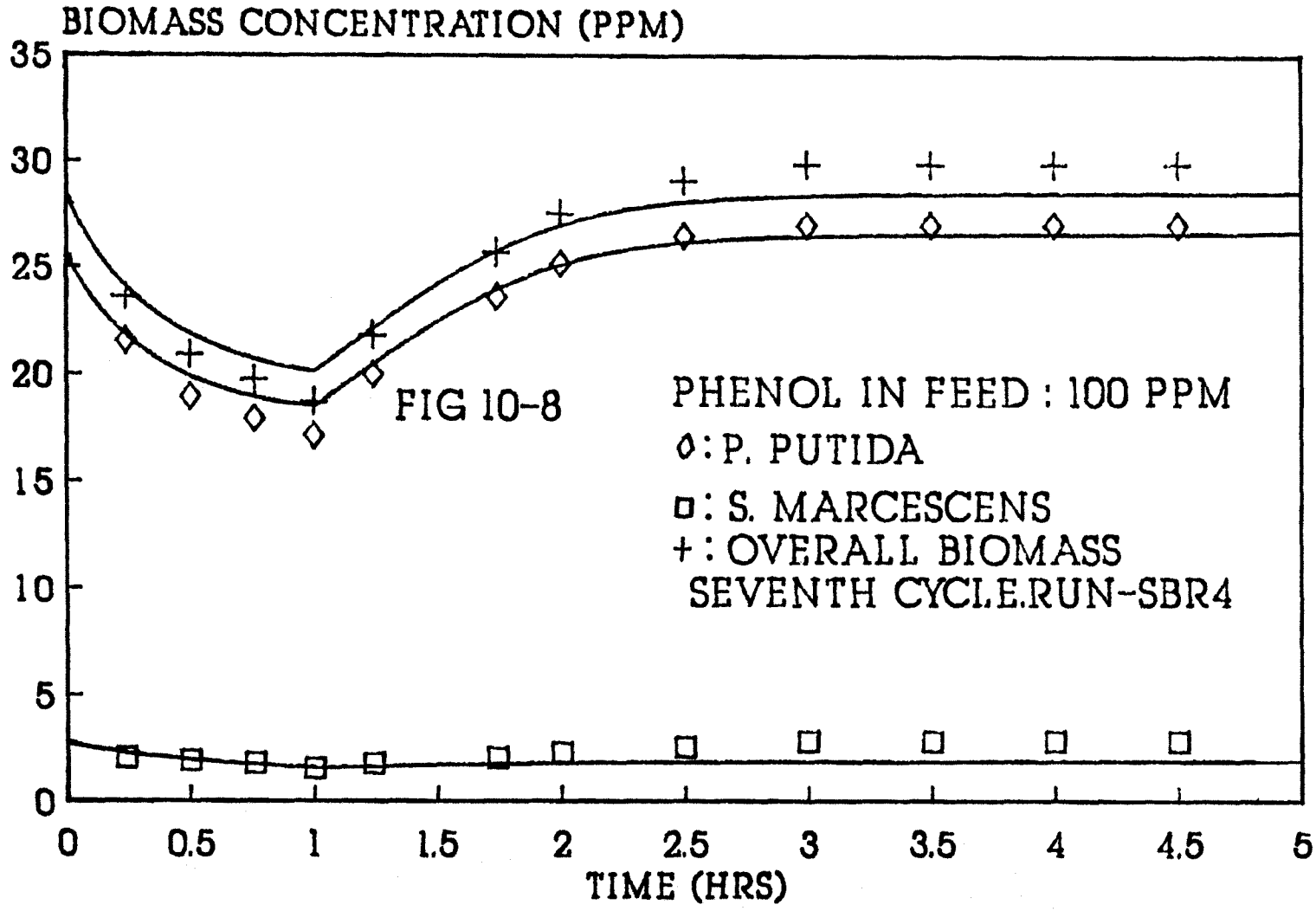

OVERALL BIOMASS CONCENTRATION (PPM)
50
45
40
35
30
25
20
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)
FIG 10-3 S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
FIRST CYCLE. RUN-SBR4


PHENOL CONCENTRATION (PPM)
40
30
20
10
0
FIG 10-4
S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
SECOND CYCLE. RUN-SBR4
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)


BIOMASS CONCENTRATION (PPM)
FIG 10-5
PHENOL IN FEED : 100 PPM
◊:P. PUTIDA ▫:S. MARCESCENS
SECOND CYCLE. RUN-SBR4
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)


OVERALL BIOMASS CONCENTRATION (PPM)
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)
FIG 10-6
S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
SECOND CYCLE. RUN-SBR4


PHENOL CONCENTRATION (PPM)
FIG 10-7
S. MARCESCENS & P. PUTIDA
PHENOL IN FEED : 100 PPM
SEVENTH CYCLE. RUN-SBR4
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)


BIOMASS CONCENTRATION (PPM)
35
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (HRS)
FIG 10-8
PHENOL IN FEED : 100 PPM
◊: P. PUTIDA
□: S. MARCESCENS
+: OVERALL BIOMASS
SEVENTH CYCLE.RUN-SBR4

APPENDIX A

INTRODUCTION TO THE SYXMAS-PO SEQUENCE CONTROLLER

Programming of this sequence controller can be done in the same easy manner as an electronic calculator by merely depressing the appropriate keys in step sequence.

SYSMAC-PO automatically shecks key input errors during programming through the keyboard and alerts the operator by a buzzer upon detection of any program error.

Details of programming the sequence controller can be referred to the User's Manual [21]. The following example is the programming procedure used in SBR experiments (with two mixed culture) of this study, in which " @ " represents " depress " followed by the name of a certain key in { } and a brief explanation in ( ).

- Turn the " Program Console Switch " to " ON "
- STEP 1 : (FEED PHASE)
  - @ { SET STEP }, @ { 0 }, @ { 1 } (set step number " 01 ")
  - @ { INS }, @ { 9 } (set operation code " 9 ", which is the timer function)
  - @ { 1 }, @ { , } (set value of " DATA-1 ", which means " 1 hr " for fill)
  - @ { 0 }, @ { 0 } (set value of " DATA-2 ", which ,in conjunction with DATA-1 ,means 1hr, o minute for feed)
  - @ { OUT } (set output functions)

@ { ON }, @ { OFF }, @ { OFF }, @ { OFF }, @ { ON },
@ { OFF }, @ { OFF }, @ { OFF } (OUTPUT-1 " ON " means open the feed reservoir solenoid valve. OUTPUT-5 " ON " means turn on the feed pump)

* @ { R/W } (write the set program of STEP1 into RAM)

o STEP 2 : (REACT PHASE)

* @ { 0 }, @ { 2 } (set step number " 02 ")
* @ { INS }, @ { 9 } (same as STEP 1)
* @ { 3 }, @ { , } (set " DATA-1 " " 3 hrs ")
* @ { 5 }, @ { 3 } (set " DATA-2 " " 53 min " which in conjunction with DATA-1, means 3 hrs, 53 minutes for react)
* @ { R/W } (write the set programe of STEP 2 into RAM)

o STEP 3 : (DRAW PHASE)

* @ { 0 }, @ { 3 }
* @ { INS }, @ { 9 }
* @ { 0 }, @ { 7 }
* @ { 0 }, @ { 0 }
* @ { OUT }
* @ {OFF}, @ {OFF}, @ {OFF}, @ {OFF}, @ {OFF}, @ {OFF}, @ {OFF}, @ {ON} (OUT-PUT8 " ON " means open the draw-out solenoid valve)
* { R/W }

o STEP 4 : (REPEAT THE CYCLE)

* @ { 0 }, @ { 4 }
* @ { INS }, @ { 8 } (set operation code " 8 " , which is the function of " REPEAT ")
* @ { 0 }, @ { 7 } (set " DATA-1 " which is required for the " REPEAT " function. " 07 " means repeat the cycle 7 times which was more than enough for one experiment)
* @ { 0 }, @ { 1 } (set STEP NUMBER [01] to go to)
* @ { R/W }

APPENDIX B

COMPUTER PROGRAMS FOR SOLVING THE MATHEMATICAL MODEL

```
C*****************************************************************
C  THIS PROGRAM WAS USED TO GET THEORETICAL CONCENTRATION PROFILE*
C  OF BIOMASS & SUBSTRATE IN SBR.  4TH ORDER RUNGE KUTTA WAS ADA-*
C  PTED TO THIS PROGRAM.                                         *
C*****************************************************************

      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
      OPEN (6,FILE='OP.OUT',STATUS='NEW)
      DATA DEL,S1,S3,GA,GB/.5,.2,.0223,.92355,1.3229/
      DATA H,UO,N/0.002,0.0,100/
      DATA W,PI,ETA/0.6184,0.3534,1.1/
      XAO=15.56/(0.286*47.101)
      XBO=21.68/(0.286*47.101)
      UF=2.1231
      U=UO
      XA=XAO
      XB=XBO
      BETA=13.95
      DO 30 I=1,7
      AL=1.
      T=0.001
```

```
C     FILL PHASE
   40 CALL RUNGE (ETA,W,PI,H,T,U,XA,XB,S1,DEL,BETA,UF,AL,GA,GB)
      B1=47.101*0.286*XA
      B2=47.101*0.286*XB
      S=47.101*U
      T1=T-0.001
      T2=10.*T1
      B3=B1+B2
      WRITE(6,101)T2,B1,B2,B3
  101 FORMAT(4(5X,F9.5))
      IF(T.LE.(1.-DEL)*S1) THEN
      GO TO 40
      END IF
C     REACT PHASE
      AL=0.
   50 CALL RUNGE(ETA,W,PI,H,T,U,XA,XB,S1,DEL,BETA,UF,AL,GA,GB)
      B1=47.101*0.286*XA
      B2=47.101*0.286*XB
      S=U*47.101
      T1=T-0.001
      T2=10.*T1
      B3=B1+B2
      WRITE(6,102)T2,B1,B2,B3
 102  FORMAT(4(5X,F9.5))
      IF(T.LE.(1.-DEL)*(1.-S3)) THEN
```

```
      GO TO 50
      END IF
C     DRAW PHASE
   60 CALL RUNGE(ETA,W,PI,H,T,U,XA,XB,S1,DEL,BETA,UF,AL,GA,GB)
      B1=47.101*0.286*XA
      B2=47.101*0.286*XB
      S=47.101*U
      T1=T-0.001
      T2=10.*T1
      B3=B1+B2
      WRITE(6,103)T2,B1,B2,B3
  103 FORMAT(4(5X,F9.5))
      IF(T.LE.(1-DEL)) THEN
      GO TO 60
      END IF
   30 CONTINUE
      STOP
      END

C     INTEGRATION FOR OBTAINING CONCENTRATION PROFILE BY USING 4TH
C     ORDER RUNGE KUTTA METHOD

      SUBROUTINE RUNGE (ETA,W,PI,H,T,U,XA,XB,S1,DEL,BETA,UF,AL,GA,GB)
      IMPLICIT REAL*8 (A-H,O-Z)
      F1(T,U,XA,XB)=AL*(UF-U)/(S1*DEL+T)+
```

```
&(XA*U*BETA)/(1.+U+GA*U**2)-
&(XB*U*BETA*PI*ETA/(W+U+GB*U**2)
 F2(T,U,XA,XB)=-(AL*XA*(XB**0))/(S1*DEL+T)+
&(XA*U*BETA*(XB**0))/(1.+U+GA*U**2)
 F3(T,U,XA,XB)=-(AL*XB*(XA**0))/(S1*DEL+T)+
&(XB*U*BETA*PI*(XA**0))/(W+U+GB*U**2)
 AK1=H*F1(T,U,XA,XB)
 BK1=H*F2(T,U,XA,XB)
 CK1=H*F3(T,U,XA,XB)
 AK2=H*F1(T+H/2.,U+AK1/2.,XA+BK1/2.,XB+CK1/2.)
 BK2=H*F2(T+H/2.,U+AK1/2.,XA+BK1/2.,XB+CK1/2.)
 CK2=H*F3(T+H/2.,U+AK1/2.,XA+BK1/2.,XB+CK1/2.)
 AK3=H*F1(T+H/2.,U+AK2/2.,XA+BK2/2.,XB+CK2/2.)
 BK3=H*F2(T+H/2.,U+AK2/2.,XA+BK2/2.,XB+CK2/2.)
 CK3=H*F3(T+H/2.,U+AK2/2.,XA+BK2/2.,XB+CK2/2.)
 AK4=H*F1(T+H,U+AK3,XA+BK3,XB+CK3)
 BK4=H*F2(T+H,U+AK3,XA+BK3,XB+CK3)
 CK4=H*F3(T+H,U+AK3,XA+BK3,XB+CK3)
 U=U+(AK1+2.*AK2+2.*AK3+AK4)/6.
 XA=XA+(BK1+2.*BK2+2.*BK3+BK4)/6.
 XB=XB+(CK1+2.*CK2+2.*CK3+CK4)/6.
 T=T+H
 RETURN
 END
```

```
C*****************************************************************
C THIS PROGRAM WAS TO GET CONCENTRAION PROFILE OF S. MARCESCENS  *
C*****************************************************************

      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
      OPEN (4,FILE='OP1.OUT',STATUS='NEW)
      DATA DEL,S1,S3,GA/.5,.2,.0223,1.2904/
      DATA H,UO,N/0.001,0.0,100/
      XO=1.3403
      UF=2.4076
      U=UO
      X=XO
      BETA=13.95
      DO 130 I=1,7
      AL=1.
      T=0.001
C     FILL PHASE
   50 CALL RUNGE (H,T,U,X,S1,DEL,BETA,UF,AL,GA)
      B=29.128*0.26*X
      S=29.128*U
      T1=T-0.001
      T2=10.*T1
      WRITE(4,181)T2,B,S
  181 FORMAT(3(5X,F9.5))
      IF(T.LE.(1.-DEL)*S1) THEN
```

```
      GO TO 50
      END IF
C     REACT PHASE
      AL=0.
   70 CALL RUNGE(H,T,U,X,S1,DEL,BETA,UF,AL,GA)
      B1=29.128*0.26*XA
      S=U*29.128
      T1=T-0.001
      T2=10.*T1
      WRITE(4,182)T2,B,S
  182 FORMAT(3(5X,F9.5))
      IF(T.LE.(1.-DEL)*(1.-S3)) THEN
      GO TO 70
      END IF
C     DRAW PHASE
   80 CALL RUNGE(H,T,U,X,S1,DEL,BETA,UF,AL,GA)
      B1=29.128*0.26*XA
      S=29.128*U
      T1=T-0.001
      T2=10.*T1
      WRITE(4,183)T2,B,S
  183 FORMAT(3(5X,F9.5))
      IF(T.LE.(1-DEL)) THEN
      GO TO 80
      END IF
```

```
  130 CONTINUE
      STOP
      END

C     INTEGRATION TO SOLVE THE MASS BALANCE ON SUBSTRATE &
C     SUBSTRATE

      SUBROUTINE RUNGE (H,T,U,X,S1,DEL,BETA,UF,AL,GA)
      IMPLICIT REAL*8 (A-H,O-Z)
      F1(T,U,X)=AL*(UF-U)/(S1*DEL+T)+
     &(X*U*BETA)/(1.+U+GA*U**2)
      F2(T,U,X)=-(AL*X*)/(S1*DEL+T)+
     &(X*U*BETA)/(1.+U+GA*U**2)
      AK1=H*F1(T,U,X)
      BK1=H*F2(T,U,X)
      AK2=H*F1(T+H/2.,U+AK1/2.,X+BK1/2.)
      BK2=H*F2(T+H/2.,U+AK1/2.,X+BK1/2.)
      AK3=H*F1(T+H/2.,U+AK2/2.,X+BK2/2.)
      BK3=H*F2(T+H/2.,U+AK2/2.,X+BK2/2.)
      AK4=H*F1(T+H,U+AK3,X+BK3,)
      BK4=H*F2(T+H,U+AK3,X+BK3)
      U=U+(AK1+2.*AK2+2.*AK3+AK4)/6.
      X=X+(BK1+2.*BK2+2.*BK3+BK4)/6.
      T=T+H
      RETURN
```

```
END
```