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Biodegradation of phenol and benzoic acid in batch and sequencing batch reactors

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ABSTRACT

BIODEGRADATION OF PHENOL
AND BENZOIC ACID IN BATCH AND SEQUENCING BATCH REACTORS.

by
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Master of Science in Chemical Engineering, January 1992.
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A pure culture of *Pseudomonas aeruginosa* (ATCC 10145) was used for the biodegradation of phenol and benzoic acid. Two sets of small scale (shaker flask) experiments were originally performed: in the first set, phenol was the only carbon source present while in the second, benzoic acid was the sole carbon source. These experiments revealed the kinetics of benzoic acid degradation (they were found to be described by a Monod, non-inhibitory model), as well as of phenol biodegradation (they were found to be described by Andrews'inhibitory model) by *Pseudomonas aeruginosa*.

The kinetic expressions from the small scale experiments were used in predicting biodegradation in a larger scale. For the case of benzoic acid, batch as well as sequencing batch operation of a 4-liter reactor showed very good agreement between experimental data and model predictions. The ultimate objective was to study biodegradation of mixtures of phenol and benzoic acid, and to predict it from the kinetic expressions derived as described above. Assuming simultaneous, non-preferential biodegradation of the two substrates, a model was derived for both batch and sequencing batch reactor operation. Unfortunately, the model did not fit the data under these assumptions. Batch data showed a clear preference of *Pseudomonas*

under these assumptions. Batch data showed a clear preference of *Pseudomonas aeruginosa* for benzoic acid (diauxie phenomenon), and the two substrates were completely but sequentially mineralized. In SBR operation, the two substrates were simultaneously used but the model could not describe the data. It is assumed that in continuous operation the substrates are simultaneously used, but again, there is a certain preference for benzoic acid.

BIODEGRADATION OF PHENOL AND BENZOIC ACID IN BATCH AND SEQUENCING BATCH REACTORS

by
Stephane Reynaud

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1-INTRODUCTION

Continuous flow systems (CSTRs or chemostats) have been intensively used for many years for the biological treatment of wastes.

Nonetheless, the performance of many processes can be improved significantly by using controlled unsteady state operations (or periodic processes) (5).

Since Irvine and Davis described its operation in 1971 (12), the sequencing batch reactor has received considerable attention in the United States, West Germany and Japan.

Semibatch biological reactors (also called fill-and-draw, or sequencing batch reactors (SBR)) can cycle through five discrete periods: fill, react, settle, draw, and idle periods.

For the biological treatment of wastes, the advantages of the SBR over the continuous flow reactor include :

(1)- greater flexibility in handling a variable waste (13)

(2)- the possibility of having both anoxic and aerated periods for control of filamentous organisms (3).

(3)- much smaller volume for a single substrate degradation than a CSTR (2)

(4)- as it operates in a batch mode, the reaction products can be tested in the same container for their acceptability for discharge.

Y.F.Ko (19) and Y.S.Ko (20) examined the dinamics of a fill-and-draw reactor. The former studied the bacterial degradation of phenol by a pure culture, whereas the latter worked with mixed cultures. K.W.Wang (25) dealt with the biodegradation of phenol and 4-chlorophenol by a single culture (*Pseudomonas putida*). He found out that 4-chlorophenol can be degraded by this species only in the presence of phenol .

The purpose of the present research was to investigate if biodegradation of a mixture of two substances can be described by using data from experiments involving each one of the individual substances separately.

The two substrates used in this study are phenol and benzoic acid. Phenol widely used in industry, is a major pollutant. It is a troublesome contaminant in surface waters and it contributes to off flavors in drinking and food- processing waters. When phenol-containing water is chlorinated, toxic polychlorinated phenols can result. As a consequence, the EPA (Environmental Protection Agency) has set a water purification standard of less than 1 part per billion of phenols in surface waters (24). Benzoic acid was chosen because of its chemical structure similarity with phenol and because it was known to be biodegraded alone by different bacterial species (21). In this study, *Pseudomonas aeruginosa* was obtained from the American Type Culture Collection (ATCC 10145) and was used to degrade these two chemical compounds both separately and in mixtures. The intent was to use a SBR for treating the mixed waste.

2-LITERATURE REVIEW

Irvine and Busch described SBR operation in 1979 (13). Since then, a U.S. EPA demonstration study has shown that the SBR is an excellent alternative to conventional activated sludge treatment for municipal wastewater (14). Recently, results from bench-scale studies indicated that the SBR can provide substancial savings in energy and costs if it is used for biologically removing organic compounds found in hazardous waste instead of using an activated carbon process (16,22). A full-scale SBR study that would show similar cost and energy savings for biological treatment of hazardous wastes was co-funded by the New York State Energy Research and Development Authority and SECOS International, on the SECOS wastewater treatment site in Niagara Falls, NY. The construction of a 1900 m^3 SBR was completed in May 1984, and operation began 1 month later. TOC (total organic carbon) degradation averaged 76% and phenol degradation averaged 99% during the first month of operation (9).

Two SBR systems were investigated by Irvine et *al* (15) at Culver, Ind. during the summer 1984. The system with low organic loading , especially when operated for biological phosphorus removal, gave a slightly better quality. The more highly loaded system was more difficult to operate because of the tendancy for the system to be underaerated for several days. Both systems achieved a high degree of biological phosphorus removal.

The analysis of the full-scale SBR operation at Grundy Center, Iowa wastewater treatment plant was done by Irvine, Murthy, Arora, Copeman and Heidman (17). This 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 SBR at Culver, Ind.. Daily influent flow rates for the summer 1985 study, averaged roughly 2500 m^3 / day. This resulted in an average retention time of approximatively 26 hours (75% greater than the one used in Culver). The average organic loading for

the period was similar to that found at Culver. The most important conclusion from this study is that, after a rather difficult beginning, the wastewater treatment plant at Grundy Center functions well.

Y.F.Ko (19) and Y.S.Ko (20) found that an Andrews inhibitory model could describe biodegradation of phenol. The former, worked with a pure culture whereas the latter used mixed cultures. Both studied the dynamics of a fill-and-draw reactor. After solving the postulated models numerically, they tested their predictions experimentally using a 5 liter-fill-and-draw reactor with phenol as the sole carbon source. Experimental results were shown to match the model predictions very well.

K.W.Wang (25) worked with a pure culture (*Pseudomonas.putida*- ATCC 17514) growing on a mixed carbon source: phenol and 4-chlorophenol. His conclusions were that *Pseudomonas putida* can degrade phenol as the sole carbon source but cannot degrade 4-chlorophenol as sole carbon source. Moreover, 4CP can be degraded only in the presence of phenol but does not contribute to biomass growth. This seems to be due to enzyme induction by phenol. The assumed mathematical model was verified experimentally in a SBR.

For both transient and steady cycles, there was relatively good agreement between the model results and the experimental data.

The Bergey's manual (1) describes *Pseudomonas aeruginosa* as follows:

Ethymologically speaking, aeruginosa means full of copper rust or verdigris, hence green.

Two main colony types can be observed on common solid media. One is large, smooth, with flat edges and elevated center and the other is small, rough, convex. Variation of the large type to the small is easy to observe, but the reverse variation is extremely rare. A third colony type (mucous) often can be observed from respiratory and urinary tract secretions.

The optimum growth temperature is 37°C .

It can be isolated from soil and water (small colony type), or from clinical specimens such as wounds, burns and urinary tract infection (large colony type). *Pseudomonas aeruginosa* is a causative agent of "blue pus" and can be occasionally pathogenic for plants.

Extensive literature reviews of studies on phenol can be found in the previously reported theses (19, 20, 25), thus here, we concentrate on benzoates and mixtures of benzoates and phenol.

The research of Miguel d'Aquino et al (4) was directed to bacteria able to biodegrade high concentrations of phenol and benzoate, within 24 hours, even for the most extreme conditions found in industrial effluents. Two bacteria able to degrade phenol and benzoate were isolated from samples of natural waters. Biodegradation of the compounds under study was performed within 12 to 16 hrs even for concentrations above 200 mg/l and for conditions of pH and temperature found in industrial effluents. The strains were identified as species of the genera *Pseudomonas* and *Acinetobacter.* Growth rate and removal of the substrates under study were determined simultaneously. With phenol as the sole carbon source, strain *Pseudomonas* had a delay in the growth at 500 mg/l concentrations. Initial benzoate concentrations did not show any significant influence on the growth. The growth was significantly delayed not only with phenol at pH=5 and 9, but also with benzoate at pH=5. In a total of 100mg/l of both phenol and benzoate at 20°C and pH=7.4, the *Pseudomonas* species showed a biphasic growth pattern biodegrading 90% of the substrate in 14 hrs. The exponential phase of growth ended at 4.9×10^7 cells/ml. 5.6×10^7 cells/ml of pseudomonas were found at the end of the exponential phase with 100 mg/l of benzoate as the sole carbon source. In this case, the growth rate was 0.48 hr^{-1} for pseudomonas biodegrading 90% in 12 h.

Carol F.Feist et al (7) studied the regulation of tangential pathways in the metabolism of phenol and benzoate by *Pseudomonas putida*. They claimed that catechol occured as an intermediate in the metabolism of both benzoate and phenol by strains of *Pseudomonas putida*. During growth at the expense of benzoate, catechol undergoes ortho (1,2-oxygenase) cleavage and gets metabolized via the beta-ketoadipate pathway; during growth at the expense of phenol or cresol, the catechol or substituted catechols formed, are metabolized by a separate pathway following meta (2,3-oxygenase) cleavage of the aromatic ring of catechol. The conclusion is that the meta pathway serves as a general mechanism for catabolism of various alkyl derivatives of catechol derived from substituted phenolic compounds. The ortho pathway is more specific, and serves primarily in the catabolism of precursors of catechol and catechol itself.

W.J.Hickey and D.D.Focht (10) worked with *Pseudomonas aeruginosa* JB2 strain and their research elucidated new bacterial capabilities for halobenzoate degradation vis-a-vis the substrate range and the potential involvement of halobenzoate dioxygenases. *Pseudomonas aeruginosa* JB2 was isolated from a polychlorinated biphenyl-contaminated soil by enrichment culture containing 2-chlorobenzoate as the sole carbon source. Growth of strain JB2 cultures was routinely monitored by measuring the adsorbance at 546 nm with a Uvikon model 860 UV/VIS spectrophotometer. To define the range of substituted benzoates that strain JB2 used for growth , a 1% inoculum of a 2-CBa (2-chlorobenzoate)-grown culture was added to 100 ml of mineral salts medium (MSM) containing 500 micrograms of the target substrate/ml. Also tested as growth substrates for strain JB2 (at 100 ppm) were phenol; 2-,3-, and 4-chlorophenol; and 2,3-2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dichlorophenol. A substrate was determined to support growth of strain JB2 if, after a 20 day-incubation, the culture had a measurable increase in adsorbance.

Routine metabolite analysis was performed on a Hewlett-Packard model 5890 gas chromatograph (GC) fitted with a DB-5 megabore column and flame ionization detector.

The results were the following: strain JB2 readily used 2-CBa, 3-CBa, and 2,5-DCBa as growth substrates with doubling times of 3.3, 9.9 and 9.3 h respectively. In contrast, growth on 2,3-DCBa was much slower (t_d=31 h), although all growth substrates supported roughly the same maximum cell density. Strain JB2 also used 2,3,5-TCBa (t_d=14.2 h) as a sole carbon and energy source with complete stoichiometric chloride release.

In addition to the CBa's listed above, strain JB2 used a wide range of substituted benzoic acids as sole carbon sources including 2-bromo-, 2,5-dibromo-, 2-iodo-, 2-fluoro-, 4-fluoro-, 2-hydroxy-, 2,3-dihydroxy-, 2,5-dihydroxy-, 3,4-dihydroxy-, and 2-hydroxy-5-chloro-. The following substituted benzoates did not serve as growth substrates for strain JB2: 4-chloro-, 2,4-dichloro-, 2,6-dichloro-, 3,4-dichloro-, 3,5-dichloro-, 3-bromo-, 4-bromo-, 3-iodo-, 4-iodo-, 3-fluoro-, 2,4-dihydroxy-, 2,6-dihydroxy-,2-hydroxy-3-chloro and 2-dihydroxy-4-chloro-.

Where as phenol did serve as a growth substrate for strain JB2, no growth or chloride release occured with monochlorophenols or the dichlorophenols examined. DCBa's not used as growth substrates by strain JB2 were to varying degrees cometabolically dechlorinated. Mono CBa's or DCBa's were degraded in aerobic but not anaerobic, resting cell incubations.

Franck K. Higson and Dennis D.Focht (11) dealt with the degradation of 2-bromobenzoic acid by a strain of *Pseudomonas aeruginosa.* Bromobenzoates are formed in the cometabolism of polybromated biphenyls, applied extensively as flame retardants in the early seventies until they were banned because of their association with hepatic porphyric and teratogenic effects (18).

Reactions were started by injecting substrates as concentrated solutions of 100 ppm. The enrichment procedure generated a pure culture designated as 2BBZA that was able to grow on 2-bromobenzoate as the sole carbon source. The strain showed growth on a wide range of aromatic acids: 2-bromo-, 2-chloro-, 2-iodo-, 2-fluoro-, 3-bromo-, and 3-chlorobenzoates, 2,3-dichloro-, 2,5-dichloro-, and 2,5-dibromobenzoates, benzoate, and many others.

The specific growth rates of 2.BBZA on 2-bromo-, 2-chloro-, 2-iodo-, and 2-fluorobenzoates were reported for the exponential phase as 0.30, 0.30, 0.21, and 0.20/h respectively at initial concentration of substrate of 100 mg/l .

At 1g of substrate per liter, the cells reached a maximum turbidity of 0.47, 0.48, 0.46, and 0.43 respectively, at about 24 hours. 3-halobenzoates were initially poor substrates for the organism, but cells transferred serially, became acclimated to the new substrate such that growth rates of the chloro- and bromobenzoates at 1g/l were recorded at 0.20 and 0.27/h, respectively.

The preceding study was the first one to report the isolation of an organism from bromobenzoate enrichment. It was one of the few that metabolized halobenzoates carrying an ortho substituent and it showed a growth range wider than that of *Pseudomonas cepacia* (26) isolated from 2-chlorobenzoate enrichment of Moscow soil. The latter strain could not use 2-iodo or 3-chlorobenzoate, both of which supported the growth of 2-BBZA. *Pseudomonas putida* (6) also failed to mineralize 2-iodo or 3-chloro benzoate.

3-OBJECTIVES

The specific objectives of the present study were the following:

* Study the kinetics of phenol biodegradation by *Pseudomonas aeruginosa* (ATCC 10145) in small scale, shaker-flask experiments.

* Study the kinetics of benzoic acid biodegradation by *Pseudomonas aeruginosa* (ATCC 10145) in small scale, shaker-flask experiments.

* Use the data obtained from the two sets of experiments described above, to predict biodegradation of mixtures of phenol and benzoic acid by *Pseudomonas aeruginosa* (ATCC 10145). This prediction should be based on proper model equations.

* Validate the model described in the previous step with batch and SBR experiments with a 4-liter vessel.

* Optimize the SBR operation for treatment of mixed wastes.

4-DERIVATION OF THE MATHEMATICAL MODEL

4-1- Pure Culture Degrading a Single Substrate in a Batch System

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

$$\frac{db}{dt} = \mu(s)b - \mu_c b \qquad (1)$$

$$\frac{ds}{dt} = -\frac{1}{Y}\mu(s)b \qquad (2)$$

where b: biomass concentration

s: substrate concentration

t: time

μ specific growth rate of the species (1/h)

Y: yield coefficient of the species-on the pollutant; Y is assumed to be constant.

μ_c : specific rate of biomass consumption for maintenance purposes

$$\mu = \frac{\hat{\mu}s}{s+K+s^2/K_I}$$ for inhibitory kinetics (Andrews model)

$$\mu = \frac{\mu_m s}{K+s}$$ for non inhibitory kinetics (Monod model)

Under the assumption that is constant during the exponential phase of growth, and that maintenance requirements are not important, equation (1) gives after integration:

$$\ln\frac{b}{b_0}=\mu t \qquad (2a)$$

Therefore, a plot of ln (b/bo) (or lnb) vs time gives a straight line, the slope of which is μ.

Using equations (1) and (2), one can easily show that:

$$b - b_o = Y(s_o-s) \qquad (3)$$

where b_o: initial biomass concentration

s_o: initial substrate concentration

Using equation (3), one can determine Y as the slope of the line which can be interpolated in the b vs. s data.

4-2 Phenol and benzoic acid batch data (case of pure substrates) :

Assuming that maintenance requirements are negligible, and combining equations (1) and (3) for the case of inhibitory kinetics, one gets:

$$\frac{ds}{dt} = -\frac{1}{Y}\mu(s)b = -\frac{\hat{\mu}s}{s + K + s^2/K_I}\frac{1}{Y}[\,Y\,(s_o - s) + b_o\,]$$

or,

$$\frac{(K + s + s^2/K_I)\,Y}{\hat{\mu}\,[\,Y\,(s_o - s) + b_o\,]}\,ds = -\,dt$$

After integration between s_0 and s and 0 and t, one gets the following expression:

$$t = \frac{1}{\hat{\mu}}\ln\left[\frac{b_o + Y(s_o - s)}{b_o}\right] + \frac{1}{Y\hat{\mu}K_I}\{(Ys_o + b_o)\ln\left[\frac{b_o + Y(s_o - s)}{b_o}\right] - Y(s_o - s)\}$$
$$-\frac{YK}{\hat{\mu}}\frac{1}{Ys_o + b_o}\ln\left[\frac{sb_o}{(b_o + Y(s_o - s))\,s_o}\right] \qquad (4)$$

Since, as described later, the kinetics of phenol biodegradation were found to be inhibitory, equation (4) was used for predicting phenol biodegradation in batch experiments. The comparison between data and predictions can be seen in figures 4.1a through 4.10a.

Using equation (3), equation (4) can be rewritten as:

$$t = \frac{1}{\hat{\mu}} \frac{YK}{b_o + Ys_o} \ln \left[\frac{bYs_o}{(b + b_o + Ys_o)\, b_o}\right] + \frac{1}{\hat{\mu}} \ln \frac{b}{b_o} \left[1 + \frac{b_o + Ys_o}{YK_I}\right] - \frac{1}{\hat{\mu} YK_I} (b - b_o) \quad (5)$$

Equation (5) was used in Figures 4.1b through 4.10b for comparing biomass concentration data and model predictions.

For the case of benzoic acid, a non-inhibitory kinetics (Monod model) was found to describe biodegradation. In this case, equations (1) and (3) (when $\mu_c = 0$) yield:

$$t = \frac{1}{\mu_m} \frac{Y(K + s_o) + b_o}{s_o Y + b_o} \ln \frac{b_o + Y(s_o - s)}{b_o} - \frac{YK}{\mu_m (s_o Y + b_o)} \ln \frac{s}{s_o} \quad (6)$$

and,

$$t = \frac{1}{\mu_m} \frac{Y(K + s_o) + b_o}{s_o Y + b_o} \ln \frac{b}{b_o} + \frac{YK}{\mu_m (s_o Y + b_o)} \ln \frac{Ys_o}{Ys_o - (b - b_o)} \quad (7)$$

Equations (6) and (7) were used for predicting benzoic acid and biomass concentrations vs. time. Comparisons between predictions and actual data can be seen in figures 4.11 through 4.18 a and b.

4-3 Non-preferential Biodegradation of Two Substrates in a Batch Reactor

Assuming that the species exhibit no preference towards either of the substrates, a simple additive model can be used for describing the system as follows:

$$\frac{db}{dt} = (\mu_1 + \mu_2)\, b \qquad (8)$$

$$\frac{ds_1}{dt} = \frac{-\mu_1 b}{Y_1} \qquad (9)$$

$$\frac{ds_2}{dt} = \frac{-\mu_2 b}{Y_2} \qquad (10)$$

where s_1 : concentration of the first substrate

s_2: concentration of the second substrate

Y_i: yield coefficient of population b on the substrate i

(i=1 or 2)

μ_i= specific growth rate of the species on substrate i

4-4 Non-preferential Biodegradation of Two Substrates in a Sequencing Batch Reactor

If non-preferential use of two substrates is assumed, and a sequencing batch reactor is used, the equations describing the system at any instant of time are the following:

Overall mass-balance (assuming constant density)

$$\frac{dV}{dt} = Q_f - Q \qquad (11)$$

where,

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):

$$\frac{d(Vb)}{dt} = -Qb + \mu_1 bV + \mu_2 bV$$

$$\text{or } V\frac{db}{dt} + b\frac{dV}{dt} = -Qb + \mu_1 bV + \mu_2 bV$$

or, by using equation (11):

$$\frac{db}{dt} = -\frac{Q_f}{V} b + \mu_1 b + \mu_2 b \qquad (12)$$

Mass balance on the rate-limiting substrates

$$\frac{ds_1}{dt} = \frac{Q_f}{V}(s_{1f} - s_1) - \frac{\mu_1 b}{Y_1} \qquad (13)$$

$$\frac{ds_2}{dt} = \frac{Q_f}{V}(s_{2f} - s_2) - \frac{\mu_2 b}{Y_2} \qquad (14)$$

The symbols not specified before and used in the two previous equations are:

s_{1f} : concentration of toxic substrate 1 in the waste fed to the reactor.

s_{2f} : concentration of toxic substrate 2 in the waste fed to the reactor.

In equations (11) through (14), some terms become zero during some of the phases of SBR operation as it will become clear in the following sections.

As discussed later, it was found that phenol gets biodegraded following inhibitory kinetics (Andrews), while benzoic acid follows non-inhibitory kinetics (Monod). One can then write:

$$\mu_1 = \frac{\hat{\mu}_1 s_1}{s_1 + K_{S1} + s_1^2/K_{I1}} \quad \text{(phenol)}$$

$$\mu_2 = \frac{\mu_{m2} s_2}{K_2 + s_2} \quad \text{(benzoic acid)}$$

There is no settling or idle period in the present study.

The volume and volumetric flow rates appearing in equations (11) through (14) can be expressed as follows for the various phases:

(a) fill phase ($0 < t < t_1$):

$$Q = 0 \; ; \; V = V_o + Q_f t$$

(b) react phase ($t_1 < t < t_2$):

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

(c) draw phase ($t_2 < t < t_3$):

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

where V_{max} is the maximum working volume, ie., 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.

(a) fill phase: ($0 < t < t_1$)

$$\frac{db}{dt} = \frac{-bQ_f}{V_o + Q_f t} + (\mu_1 + \mu_2) b \qquad (15)$$

$$\frac{ds_1}{dt} = \frac{Q_f(s_{1f} - s_1)}{V_o + Q_f t} - \frac{\mu_1 b}{Y_1} \qquad (16)$$

$$\frac{ds_2}{dt} = \frac{Q_f(s_{2f} - s_2)}{V_o + Q_f t} - \frac{\mu_2 b}{Y_2} \qquad (17)$$

(b) react and draw phase ($t_1 < t < t_3$):

$$\frac{db}{dt} = \mu_1 b + \mu_2 b \qquad (18)$$

$$\frac{ds_1}{dt} = - \frac{\mu_1 b}{Y_1} \qquad (19)$$

$$\frac{ds_2}{dt} = -\frac{\mu_2 b}{Y_2} \qquad (20)$$

In the formulation of these equations, it has been assumed that the biodegradation occurs not only during the react phase, but also during the fill and draw phases.

Equations (15) through (20) were numerically integrated in order to predict the behavior of the system. These predictions were tested against experimental data as discussed in later sections.

5-EXPERIMENTAL APPARATUS

5-1 - Batch experiments

All experiments were carried out in 250 ml-flasks placed in a controlled environment incubator shaker (model # G25 New-Brunswick Scientific Co, INC. Edison, NJ, USA) at 30°C. No external aeration was provided but by shaking (250 rpm). It is speculated that enough oxygen from the flask head space was transfered in the liquid.

The acclimatization of the biomass was carried out in a G24 Environmental incubator shaker (New Brunswick Scientific Co, INC. Edison, NJ, USA) at 30°C and 250 rpm shaking.

5-2- Sequencing batch reactor experiments

All experiments were run at temperatures ranging from 29 to 31°C .

The reactor was a 15 cm ID, 5-liter Lucite cylindrical vessel with a removable lid. An effluent port was installed two liters above the bottom, with a solenoid valve to control the discharge of treated waste. Compressed air was passed through a filter before entering the reactor through a bottom diffuser. The volume of air was regulated by one needle-valve rotameter. To increase the contact efficiency between the air and the liquid medium, a porous diffuser stone was placed at the end of both air lines at the bottom of the reactor. Aeration and mechanical stirring (using a magnetic stirrer) provided the necessary agitation.

A microprocessor (Omron, sysmac-po sequence controller) controlled the system. 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 precisely by Ko (19).

5-3- Analytical equipment

* Spectra-physics High Performance Liquid Chromatograph System including a SP 8880 autosampler, a Spectra 200 programmable wavelength detector, a SP 8800 ternary HPLC pump and coupled with a PE NELSON 900 SERIES interface and a HP 3396A integrator linked to an IBM personal computer..

* column: lichrospher 60 RP-select B (5 mm) Merck in liChroCART 125-4.

* Varian DMS 200 UV-VISIBLE Spectrophotometer.

* Orion Model EA920 Expandable Ion Analyser for measuring pH values with Model 91-56 combination pH electrode.

* DO analyser: New Brunswick Scientific Co. INC. (Edison, New-Jersey, USA) model DO-50, using an O_2 electrode.

6-EXPERIMENTAL PROCEDURES

6-1 Choice of the growth medium

Excluding the carbon source, the composition of the defined medium used in this study is shown in Table 1 and follows the suggestions of Gaudi (8). It should be emphazised that there is little if any justification for medium formulations. In principle, the medium should provide the necessary nitrogen and carbon and should be able to act as a pH buffer for bacterial growth.

6-2 Analytical procedure

6-2.1 OD/biomass calibration curve

The growth of microbes was quantified by measuring the optical density (OD) of the liquor using a spectrophotometer at a wavelength of 540 nm with deionized water as the reference sample. The OD can be converted into biomass concentration using a calibration curve which was obtained earlier (23) and confirmed (25) for the analytical device used in this study. The calibration obtained for *Pseudomonas putida* is assumed valid for any microbial.

6-2.2 Substrate analysis

The instrument used for the substrate analysis was a Spectra-physics HPLC. Mobile phase A: 1% glacial acetic acid in pure methanol, mobile phase B: 1% glacial acetic acid in Mill-Q water. Ratio A:B was 55:45, run isocratically. The flow rate was 1 ml/min. The UV detector was set at 280 nm. The data were processed by PE Nelson chromatography software rev 5.10, interfaced with a 900 series PE Nelson.

Calibration curves were needed for phenol and benzoic acid before starting any analysis. 8 standard points ranging from 10 to 200 ppm were used for each calibration

curve. At the beginning of each HPLC run, standards close to the initial concentrations of substrate were tested to check the validity of the calibration curves. Right after the optical density was measured, the sample was filtered using a 0.22 mm millipore filter paper (GV type) for benzoic acid or the mixtures phenol-benzoic acid or a Nylaflo 0.2 mm nylon membrane filter for phenol only (because benzoic acid seemed to be retained inside the latter filter) in order to remove all suspended substances or bacteria cells being able to plug the chromatographic column. Then, a single drop of 6N HCl was added to the sample in order to kill all microbial life inside to prevent any further biodegradation and to shift the phenolics and benzoic acid to the unionized form. The prepared samples were then run immediately by HPLC.

6-3 Acclimatization of culture on a given substrate

A stock culture was prepared by transfering one loop of dried biomass into BBL nutrient broth (BBL is a trademark of Becton Dickinson and company) and placed in the incubator for about 24 hours, then stored at $4^{o}C$ in the refrigerator. A loop of this broth culture was streaked on an agar plate (23 grams of nutrient agar dissolved in 1 liter distilled water , autoclaved and poured on a Petri dish) and incubated for about 24 hours to check the purity of the culture.

A 100 ppm solution of the given subtrate was prepared from a 1000 ppm substrate solution by diluting 1 ml in 99 ml of growth medium.

The primary culture was prepared by picking up a single colony from the agar plate and inoculating it into this solution

This solution was incubated in the shaker at $30^{o}C$ under agitation (250 rpm) until growth was observed.

The secondary culture was prepared by transfering 1 ml of this primary solution in a 100 ppm substrate solution and kept in the shaker for 24 hours.

The tertiary solution was prepared the same way.

The culture used for kinetic experiments was taken from this latter solution to make sure the species was properly acclimated on the chosen substrate, and that phenol or benzoic acid were the sole carbon sources.

All glassware, defined medium, and carbon source were autoclaved before usage at 121^{o}C under pressure to avoid contamination of the culture. The Lucite reactor used in SBR experiments was thoroughly washed with bleach diluted in water.

6-4 Determination of kinetic constants in batch experiments

The kinetic constants of a pure culture of *Pseudomonas aeruginosa* (ATCC 10145) were obtained from experiments at 30^{o}C using 250 ml-flasks and acclimated cultures from the tertiary solutions prepared earlier. At time intervals of 20 mins, a 3 ml-sample was taken from the culture. By UV spectrophotometry at a wavelength of 540 nm, its absorbance was read and a calibration curve gave the corresponding concentration of biomass in ppm (mg/l). Moreover, the substrate concentration of each sample was measured by HPLC. Semi-log plots of biomass concentration (ppm) vs.time (hour) were used to determine the slope of the straight line corresponding to the exponential specific growth rate at different substrate concentrations. The obtained slope is the specific growth rate which was then plotted vs. the average phenol concentration or the initial benzoic acid concentration during the exponential growth phase. In addition, the yield coefficients were also calculated by plotting the biomass concentration vs the substrate concentration. For phenol, Andrews parameters were obtained by regressing the data. Monod's constants in the case of benzoic acid were obtained by plotting the inverse of the specific growth rate vs the inverse of the initial benzoic acid concentration.

6-5 SBR experiments

Acclimated cultures in 100 ppm phenol and 100 ppm benzoic acid were prepared 24 hours before experiments. A portion of this biomass was diluted by defined medium so that 2 liters containing biomass at a predetermined concentration were available for starting SBR operation. Experiments were then run with benzoic acid only, and with both phenol and benzoic acid diluted in defined medium from 1000 ppm to the chosen substrate concentration.

The feed pump was calibrated in order to get 2 liters of liquid charged in the reactor in 30 mins during the feed phase.

Dissolved oxygen was monitored continuously during all three phases. pH was kept constant at 7.2 and dissolved oxygen ranged from 2 to 8 mg/l.

In the react phase, the feed pump was shut off and the reactor acted in batch mode for 1 hour.

At the end of the react phase, the decant solenoid valve was opened to completely discharge 2 liters of solution. After 10 mins of draw phase, the cycle started over again.

Samples were taken periodically for optical density, substrate concentration and pH readings.

A loop of solution was streaked on an agar plate during the experiment to determine whether contamination had occured. No contamination was observed.

7-RESULTS AND DISCUSSION

7-1 Kinetics of Phenol Biodegradation by *Pseudomonas aeruginosa* (ATCC 10145)

Batch runs in 250 ml flasks were performed at 9 different initial phenol concentrations ranging from 20 to 160 ppm. In these experiments, phenol was the sole carbon source. The data are shown in Table 2.1 and are plotted in Figures 1.1. According to theory, if phenol is the only rate-limiting substrate, the specific growth rate must remain constant through the exponential phase, leading to equation (2a). The data showed that μ is not really constant throughout the exponential growth phase. In fact, in most cases, it seems that the data fall on to two different lines as is shown in Figure 1.1 run 1.2, and run 1.9. In the latter case, it is shown that if all data are used, the slope (specific growth rate) is significantly different from what one would get if only the initial points were used. This discrepancy may be due to oxygen limitation. Oxygen is not supplied to the system, and it may become limiting after a certain point in time, something which makes the theory to breakdown. For this reason, it was decided to use only the initial points for getting the specific growth rate (μ). The slope, μ, is the specific growth rate at an average phenol concentration. The average is taken over the points used for interpolating the straight line. The μ versus phenol concentration data are shown on Table 3.1 and Figure 3.1. The data were regressed to an Andrews model and the following parameters were obtained $\hat{\mu}$ = 1.89 / h, K = 52.5 ppm, K_I=33.7 ppm. It should be mentioned that when the slope (μ) was attributed to the initial rather than the average phenol concentration, the regression program (for Andrews Kinetics) never converged. The biomass and phenol concentration data were also used (according to equation (3)) to predict the yield coefficient. The results are shown in Table 3.1 while two characteristic plots are shown in Figure 1.2. The kinetic constants

and the yield coefficients were used in order to predict all experimental data (shown in Table 3.1) according to equations (4) and (5). The results are shown in Figures 4.1 through 4.10. The agreement seems to be good, but the problems become severe as the time ellapses. Towards the end of the experiments, the model underpredicts the biomass concentration and overpredicts the phenol concentration. A possible reason can be the yield coefficient. It was assumed that the yield coefficient is constant, and its value was taken as the average from the different runs (Table 3.1). In fact, one can see the impact of Y by comparing Figures 4.8 and 4.9. These figures show the same data. The curves (predictions) in Figure 4.8 were drawn by using the value of Y measured in this experiment, while in Figure 4.9, the average (from all experiments) value of Y was used. It is clear that there is a much better agreement in Figure 4.8.

7-2 Batch results in the case of benzoic acid

The same procedure was followed in the case where benzoic acid was used as the sole carbon source. However, the specific growth rates were regressed as a function of the initial benzoic acid concentration rather than an average. The K_I found when an Andrews inhibitory model is assumed is very high (more than 2000 mg/l) which suggests that a Monod non-inhibitory model describes best *Pseudomonas aeruginosa* growth on benzoic acid. Therefore a plot of $1/\mu$ vs 1/s gives a straight line, the slope and intercept of which give μ_m and K. The data are shown in Tables 2.2, 3.2, 4.2 and 4.3 and in Figures 2.1, 2.2, 3.2 and 3.3. It should be mentioned that the correlation coefficient for data of Figure 3.3 is very poor.

The comparisons of biomass and benzoic acid concentrations vs time according to the Monod model and the experimental points give a good agreement on the whole range of substrate concentration . Using equations (6) and (7), model predictions for the data of Table 2.2 were made and are shown in Figures 4.11 through 4.18. It should be noted that although there are again some problems towards the end of the experiments,

the agreement between data and predictions for benzoic acid is much better when compared to the case of phenol.

7-3 Batch experiments using mixed substrates

In 250 ml-flasks, several experiments with mixed substrates were performed. Substrate and biomass concentrations vs time were plotted. The results are shown in Tables 5.1, 5.2 and 5.3 as well as in Figures 5.1.1 through 5.3.2.

When a mixture of 100 ppm phenol and 100 ppm benzoic acid was used (Table 5.1 and Figures 5.3.1 and 5.3.2) one can notice a preferential uptake of benzoic acid compared to phenol. Moreover, when the biomass concentration curve vs time is put in parallel with the substrate concentration vs time, one can see that during the second lag phase, phenol starts being significantly degraded while no biomass growth is observed. That observation tends to suggest that there is external release of biodegradative enzyme by the bacteria during benzoic acid degradation and that these enzymes, still present in the solution when all benzoic acid is depleted, biodegrade the other source of carbon present: phenol without any new release of enzyme associated with growth. After a while, these enzymes are not present any more, new ones are released and biomass growth associated with phenol degradation starts again. The same behavior is observed when benzoic acid and phenol are in the ratio 60/40 (Table 5.2 and Figures 5.2.1 and 5.2.2) or 40/60 (Table 5.3 and Figures 5.1.1 and 5.1.2). It should be added though that in the 40/60 and 60/40 cases, despite the preference for benzoic acid, both substrates are simultaneously used from the beginning of the experiment. The model of equations (8)-(10) cannot be used in this case. As shown in Figures 6.1.2, 6.2.2 and 6.3.2, the agreement is poor when this model is used.

When all benzoic acid is depleted, the agreement between experiment and model is always good as far as phenol concentration is concerned (because the phenol is the sole

carbon source for biomass growth) provided that the time axis is properly moved as has been done in Figures 6.1.1, 6.2.1, and 6.3.1.

As far as benzoic acid concentration is concerned, the model always underpredicts the concentration of this substrate which may mean that a part of the enzymes released for benzoic acid degradation are used for phenol degradation and therefore do not degrade benzoic acid as it is assumed by the non preferential uptake model.

7-4 Run in SBR when benzoic acid is used as the sole carbon source

Mixture acclimated culture was used in this SBR run with benzoic acid as the sole carbon source to check the validity of the Monod kinetics data found from batch experiments. The experiment was carried out until a steady cycle was reached. Four cycles were actually monitored. Data for cycle 3 are shown in Tables 6.1 and 6.2, and in Figures 7.1 and 7.2 From cycle 2 to cycle 4, good agreement was observed for both biomass and benzoic acid concentrations. However, after benzoic acid was depleted out , a decrease in biomass concentration was observed during cycles 3 and 4. This decrease was attributed to possible maintenance requirements. Therefore, Herbert's model where biomass is the only energy source for maintenance was assumed. A coefficient $\mu_c = 0.400$ was estimated according to these experimental data. When this coefficient was inserted in the state equations describing the system in a SBR, the model was completely off the experimental data, predicting a much smaller biomass concentration and a much larger substrate concentration for all the runs. Consequently, this model was not considered any further. Perhaps a value of μ_c equal to 0.10 or 0.15 would be more reasonable if this model should be considered any further.

7-5 Run in SBR when a mixture of phenol and benzoic acid was used as carbon sources

After the preceeding 4 cycles with benzoic acid only, a new stock of 80 ppm of both phenol and benzoic acid was used and the experiment was carried on for about 20 hours. The first four cycles and cycles 10 and 11 (steady cycles)were monitored. Data for cycles 4 and 10 are shown in Tables 7.1 and 7.2 and in Figures 8.1 through 9.2.

* A poor agreement between the model and the experimental points was observed for both biomass and phenol concentrations in both cycles.

* Benzoic acid concentration seems to be always underpredicted, more drastically at transient cycles than at steady cycle. The same trend was observed previously for batch experiments using mixed substrates. However, the discrepancy was less important here than in batch runs due to the fact that the continuous aspect of the system reduces the preferential uptake behavior present in batch systems when a mixture of substrates is used.

8-CONCLUSIONS AND RECOMMENDATIONS

* *Pseudomonas aeruginosa* (ATCC 10145) can degrade phenol and benzoic acid separately.

* The kinetic constants of *Pseudomonas aeruginosa*-phenol and *Pseudomonas aeruginosa*-benzoic acid have been evaluated from experiments.

* *Pseudomonas aeruginosa* degrades phenol according to an Andrews type of kinetics (inhibitory kinetics).

* *Pseudomonas aeruginosa* degrades benzoic acid according to a Monod type of kinetics (non-inhibitory kinetics).

* An apparent degradation of phenol by a benzoic acid induced enzyme seems to occur in the case of mixed substrates. This phenomenon may explain the fact that the actual concentrations of benzoic acid in that case are not in good agreement with the separate substrate degradation model proposed to describe the behavior of the mixed substrates system. However, this model describes with fair accuracy the behavior of the system during SBR runs.

* It would be recommended that the use of more detailed models be investigated; such models should include maintenance requirements and the way enzymes are used for degrading the mixture of two substrates.

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TABLE 1: PHENOL AND BENZOIC ACID DEFINED MEDIUM

AMMONIUM SULFATE	500 mg
MAGNESIUM SULFATE	100 mg
FERRIC CHLORIDE	0.5 mg
MANGANESE SULFATE	10 mg
Na_2HPO_4* 50 mM	600 ml
KH_2PO_4 * 50 mM	400 ml

*: The volumes are ajusted to reach a pH buffer of 7.2.

TABLE 2-1:OPTICAL DENSITY, BIOMASS CONC. AND PHENOL CONC. VS.TIME

RUN 1.1

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	21.22	0.047	12.85
0.433	18.97	0.051	13.94
0.767	16.58	0.057	15.58
1.150	13.75	0.065	17.77
1.533	10.55	0.074	20.23
1.883	7.48	0.083	22.69
2.300	2.73	0.094	25.70
2.650	0	0.102	27.89
3.000	0	0.102	27.89

RUN 1.2

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	33.45	0.036	9.84
0.250	33.55	0.036	9.84
0.750	31.90	0.036	9.84
1.330	32.10	0.036	9.84
2.167	24.74	0.036	9.84
2.490	20.51	0.041	11.21
2.750	16.22	0.046	12.58
3.000	10.41	0.069	18.86
3.250	4.78	0.086	23.51
3.500	0.22	0.110	30.07

TABLE 2.1 (CONT.)

RUN 1.3

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	45.07	0.047	12.85
0.367	43.88	0.049	13.40
0.700	43.22	0.052	14.22
1.034	40.88	0.058	15.72
1.367	37.82	0.068	18.59
1.700	33.34	0.084	22.96
2.050	27.97	0.096	26.24
2.384	21.74	0.114	31.17
2.784	9.39	0.158	43.19
2.951	2.91	0.157	42.92
3.484	0	0.178	48.66

RUN 1.4

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	41.40	0.041	11.21
0.333	41.55	0.043	11.76
0.583	40.69	0.043	11.76
0.833	39.55	0.040	10.94
1.100	37.70	0.037	10.12
1.417	35.93	0.040	10.94
1.667	33.92	0.046	12.58
2.083	27.67	0.056	15.31
2.417	22.00	0.072	19.68
2.750	13.21	0.091	24.88
3.083	3.09	0.109	29.80
3.417	0	0.146	39.91

TABLE 2.1 (CONT.)

RUN 1.5

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	50.33	0.032	8.75
0.333	50.11	0.033	9.02
0.750	49.09	0.036	9.84
1.000	47.96	0.040	10.94
1.334	45.46	0.047	12.85
1.684	41.45	0.059	16.13
1.917	36.84	0.068	18.59
2.234	31.93	0.080	21.87
2.500	25.54	0.097	26.52
2.751	16.72	0.120	32.81
3.000	6.47	0.142	38.82
3.250	0	0.170	46.48
3.500	0	0.178	48.66

RUN 1.6

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	60.84	0.037	10.12
0.500	59.34	0.039	10.66
0.884	58.97	0.040	10.94
1.217	56.63	0.045	12.30
1.600	53.78	0.056	15.04
1.950	48.46	0.068	18.59
2.217	44.36	0.080	21.87
2.450	38.87	0.092	25.15
2.700	31.91	0.109	29.80
2.950	23.94	0.131	35.81
3.200	13.53	0.155	42.37
3.450	0	0.198	54.13
3.700	0	0.214	58.50

TABLE 2.1 (CONT.)

RUN 1.7

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	79.32	0.047	12.85
0.333	77.88	0.047	12.85
0.666	79.49	0.047	12.85
1.000	78.35	0.049	13.40
1.333	74.96	0.054	14.76
1.666	72.68	0.064	17.50
2.083	65.94	0.081	22.14
2.333	62.27	0.091	24.88
2.583	55.83	0.105	28.71
2.833	48.02	0.123	33.63
3.100	37.80	0.143	39.09
3.333	25.87	0.171	46.75
3.700	1.20	0.228	62.33
4.050	0	0.257	70.26
4.400	0	0.258	70.53

TABLE 2.1 (CONT.)

RUN 1.8

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	143.86	0.034	9.30
0.333	140.76	0.034	9.30
0.683	140.12	0.036	9.84
1.017	136.65	0.038	10.39
1.433	138.33	0.039	10.66
1.783	135.94	0.041	11.21
2.167	134.02	0.051	13.94
2.417	131.19	0.053	14.49
2.667	126.04	0.060	16.40
2.917	123.36	0.062	16.95
3.267	118.00	0.073	19.96
3.600	108.50	0.087	23.78
3.950	103.18	0.099	27.06
4.267	97.05	0.110	30.07
4.600	89.54	0.123	33.63
4.917	81.15	0.136	37.18
5.267	73.66	0.154	42.10
5.600	63.12	0.170	46.48
5.950	53.08	0.187	51.12
6.283	43.58	0.205	56.04
6.583	34.75	0.225	61.51
6.933	18.03	0.238	65.07
7.250	10.20	0.267	72.99
7.617	0	0.306	83.66
7.917	0	0.325	88.85

TABLE 2.1 (CONT.)

RUN 1.9

TIME (HOURS)	PHENOL CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	161.82	0.033	9.02
0.283	160.08	0.033	9.02
0.583	160.03	0.034	9.30
0.833	163.53	0.033	9.30
1.083	157.35	0.033	9.02
1.333	159.72	0.034	9.30
1.667	154.02	0.035	9.57
1.950	159.61	0.036	9.84
2.250	162.17	0.036	9.84
2.583	151.25	0.039	10.66
2.917	153.25	0.043	11.76
3.267	149.64	0.046	12.58
3.584	151.63	0.054	14.76
3.917	145.07	0.059	16.13
4.250	141.08	0.068	18.59
4.584	133.47	0.076	20.78
4.934	128.09	0.092	25.15
5.284	116.98	0.112	30.62
5.584	103.01	0.134	36.63
5.918	93.26	0.160	43.74
6.251	77.30	0.194	53.04
6.584	63.64	0.230	62.88
6.951	45.02	0.266	72.72
7.268	28.01	0.303	82.83
7.651	1.92	0.362	98.96
8.035	0	0.378	103.34

TABLE 2-2: OPTICAL DENSITY, BIOMASS CONC. AND BENZOIC ACID CONC. VS. TIME

RUN 2.1

TIME (HOURS)	BENZOIC ACID CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	**	0.010	2.73
0.200	8.74	0.010	2.73
0.367	8.63	0.011	3.00
0.567	8.51	0.011	3.00
0.867	8.60	0.011	3.00
1.083	7.59	0.014	3.83
1.250	7.36	0.014	3.83
1.417	5.11	0.016	4.37
1.650	4.61	0.017	4.65
1.833	2.96	0.020	5.47
2.017	2.55	0.022	6.01
2.183	1.31	0.025	6.83
2.350	0	0.026	7.11
2.533	0	0.029	7.93
2.800	0	0.033	9.02

RUN 2.2

TIME (HOURS)	BENZOIC ACID CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	**	0.018	4.92
0.317	19.36	0.018	4.92
0.667	19.01	0.020	5.47
0.984	17.03	0.024	6.56
1.300	13.65	0.029	7.93
1.584	10.45	0.034	9.30
1.833	7.62	0.043	11.76
2.083	3.63	0.051	13.81
2.383	0	0.059	16.13
2.667	0	0.064	17.50
3.000	0	0.065	17.77

TABLE 2.2 (CONT.)

RUN 2.3

TIME (HOURS)	BENZOIC ACID CONCENTRATION (PPM)	OPTICAL DENSITY (PPM)	BIOMASS CONCENTRATION (PPM)
0	38.12	0.017	4.65
0.367	38.20	0.016	4.38
0.800	37.88	0.017	4.65
1.183	35.90	0.020	5.47
1.433	34.52	0.024	6.56
1.700	33.07	0.027	7.38
1.967	30.76	0.034	9.30
2.267	26.26	0.042	11.48
2.517	22.64	0.050	13.67
2.767	17.49	0.058	15.86
3.034	11.33	0.071	19.41
3.368	1.97	0.091	24.88
3.634	0	0.109	29.80

RUN 2.4

TIME (HOURS)	BENZOIC ACID CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	48.23	0.023	6.29
0.383	46.79	0.023	6.29
0.733	49.32	0.026	7.11
1.050	45.12	0.028	7.65
1.383	40.74	0.033	9.02
1.717	38.64	0.043	11.76
2.067	30.18	0.054	14.76
2.383	22.08	0.067	18.32
2.750	10.62	0.089	24.19
3.084	0	0.119	32.53
3.367	0	0.131	35.81

TABLE 2.2 (CONT.)

RUN 2.5

TIME (HOURS)	BENZOIC ACID CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	76.83	0.016	4.37
0.333	77.37	0.016	4.37
0.666	74.00	0.018	4.92
1.083	76.83	0.022	6.01
1.417	71.20	0.027	7.38
1.783	68.48	0.035	9.57
2.100	64.76	0.044	12.03
2.583	51.78	0.058	15.86
2.883	42.01	0.079	21.60
3.234	30.16	0.102	27.89
3.567	14.45	0.139	38.00
3.934	0	0.180	49.21

RUN 2.6

TIME (HOURS)	BENZOIC ACID CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	103.63	0.015	4.10
0.25	104.35	0.015	4.10
0.517	102.51	0.015	4.10
0.767	105.95	0.015	4.10
1.017	105.48	0.015	4.10
1.250	102.48	0.017	4.65
1.533	103.17	0.019	5.19
1.800	98.92	0.022	6.01
2.134	94.89	0.028	7.65
2.601	90.16	0.034	9.30
2.917	87.33	0.039	10.53
3.351	75.96	0.047	12.85
3.667	66.19	0.071	19.41
4.034	52.87	0.112	30.62
4.250	42.14	0.132	36.09
4.534	25.13	0.169	46.20
4.750	10.08	0.196	53.58
5.000	0	0.244	66.71

TABLE 2.2 (CONT.)

RUN 2.7

TIME(HOUR)	BENZOIC ACID CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	121.14	0.019	5.19
0.37	120.75	0.020	5.47
0.62	124.61	0.021	5.74
1.15	120.53	0.028	7.65
1.42	115.42	0.032	8.75
1.67	113.69	0.040	10.94
1.95	104.37	0.048	13.12
2.20	93.86	0.057	15.58
2.47	**	0.068	18.59
2.72	81.65	0.083	22.69
3.05	75.83	0.111	30.35
3.32	58.00	0.144	39.37
3.58	37.75	0.178	48.66
3.85	9.78	0.231	63.15
4.15	0	0.283	77.37
4.48	0	0.302	82.56

RUN 2.8

TIME (HOURS)	BENZOIC ACID CONCENTRATION (PPM)	OPTICAL DENSITY (UOD)	BIOMASS CONCENTRATION (PPM)
0	137.29	0.027	7.38
0.333	140.18	0.027	7.38
0.750	144.12	0.030	8.20
1.117	142.60	0.032	8.75
1.450	137.75	0.040	10.94
1.717	141.89	0.047	12.85
1.967	132.46	0.057	15.58
2.217	126.24	0.071	19.41
2.484	119.19	0.086	23.51
2.767	110.04	0.102	27.89
3.017	100.48	0.120	32.81
3.234	88.48	0.142	38.82
3.500	71.30	0.162	44.29
3.750	62.12	0.195	53.31
4.034	38.24	0.227	62.06
4.434	8.24	0.275	75.18
4.784	0	0.322	88.03
5.201	0	0.324	88.58

TABLE 3-1: SPECIFIC GROWTH RATE AND YIELD COEFFICIENT VS AVERAGE PHENOL CONCENTRATION

INITIAL PHENOL CONC.(PPM)	AVERAGE PHENOL CONC.(PPM)	SPECIFIC GROWTH RATE (1/HOUR)	YIELD COEFFICIENT	AVERAGE VALUE OF THE YIELD COEFFICIENT
18.97	9.49	0.3180	0.7356	
24.74	20.48	0.4211	0.7117	
35.93	31.80	0.5014	**	
40.88	31.31	0.5011	**	**0.6369**
49.09	40.51	0.5529	0.6929	
56.63	47.75	0.5838	0.7194	
78.35	63.19	0.5107	0.6825	
135.94	108.55	0.3821	0.4620	
159.61	146.54	0.3254	0.4543	

TABLE 3-2: SPECIFIC GROWTH RATE AND YIELD COEFFICIENT VS INITIAL BENZOIC ACID CONCENTRATION

INITIAL BENZOIC ACID CONC.(PPM)	SPECIFIC GROWTH RATE(1/HOUR)	YIELD COEFFICIENT	AVERAGE VALUE OF THE YIELD COEFFICIENT
8.60	0.5973	0.4944	
19.01	0.6495	0.5605	
35.90	0.6949	0.5581	
45.12	0.6969	0.5419	
74.00	0.6286	0.5441	**0.5405**
105.48	0.7102	0.5574	
120.53	0.6887	0.5299	
142.60	0.6667	0.5548	

TABLE 4-1: ANDREWS KINETICS PARAMETERS FOR PHENOL

$\hat{\mu}$ (1/hour)	K_s (ppm)	K_I (ppm)
1.90	52.50	33.70

TABLE 4-2: KINETICS PARAMETERS FOR BENZOIC ACID WHEN ANDREWS MODEL ASSUMED

$\hat{\mu}$ (1/hour)	K_s (ppm)	K_I (ppm)
0.72	1.70	2105.30

TABLE 4-3: KINETICS PARAMETERS FOR BENZOIC ACID WHEN MONOD'S MODEL ASSUMED

μ_m(1/hour)	K(ppm)
0.72	1.71

TABLE 5-1: EXPERIMENTAL RESULTS FOR BATCH SYSTEMS WITH 40:60 RATIO PHENOL:BENZOIC ACID

TIME(HOUR)	O.D(UOD)	BIOMASS CONC.(PPM)	PHENOL CONC.(PPM)	BENZOIC ACID CONC.(PPM)
0	0.040	10.94	44.28	63.88
0.33	0.042	11.48	43.89	64.13
0.66	0.045	12.30	42.65	63.02
1.00	0.050	13.67	41.18	61.12
1.33	0.058	15.86	39.64	57.81
1.66	0.069	18.86	38.89	54.56
2.00	0.083	22.69	37.25	48.60
2.35	0.101	27.61	33.41	38.37
2.66	0.121	33.08	32.55	29.48
3.00	0.166	45.38	29.21	14.18
3.33	0.193	52.16	26.72	1.17
3.66	0.217	59.32	22.03	0
4.00	0.225	61.51	15.11	0
4.33	0.240	65.61	4.79	0
4.66	0.257	70.26	0	0

TABLE 5-2: EXPERIMENTAL RESULTS FOR BATCH SYSTEM RUN WITH A RATIO 60:40 PHENOL:BENZOIC ACID

TIME(HOUR)	O.D.(UOD)	BIOMASS CONC.(PPM)	PHENOL CONC.(PPM)	BENZOIC ACID CONC.(PPM)
0	0.039	10.66	62.32	40.31
0.33	0.041	11.21	64.36	41.64
0.66	0.043	11.76	63.39	41.00
1.02	0.047	12.85	62.93	40.11
1.33	0.052	14.22	61.47	37.81
1.68	0.062	16.95	60.08	34.26
2.00	0.074	20.23	58.06	29.68
2.36	0.089	24.33	54.71	22.07
2.66	0.105	28.71	52.75	14.61
3.05	0.142	38.82	48.94	3.43
3.33	0.154	42.10	45.94	0
3.66	0.162	44.29	40.80	0
4.00	0.170	46.48	32.83	0
4.33	0.190	51.94	19.35	0
4.66	0.211	57.68	2.44	0
5.00	0.246	67.25	0	0

TABLE 5-3: EXPERIMENTAL RESULTS FOR BATCH RUN WITH A RATIO 50:50 PHENOL:BENZOIC ACID

TIME(HOUR)	O.D(UOD)	BIOMASS CONC.(PPM)	PHENOL CONC.(PPM)	BENZOIC ACID CONC.(PPM)
0	0.037	10.12	110.04	111.76
0.33	0.036	9.84	106.43	108.78
0.66	0.038	10.39	109.48	110.41
1	0.042	11.48	108.79	109.02
1.33	0.047	12.85	107.54	105.47
1.66	0.054	14.76	102.31	97.20
2	0.063	17.22	106.75	97.33
2.50	0.083	22.69	100.46	84.12
2.933	0.095	25.97	98.25	73.05
3.25	0.126	34.45	101.61	64.06
3.6	0.157	42.92	102.29	49.98
3.983	0.196	53.58	98.86	26.77
4.350	0.258	70.53	93.05	2.14
4.75	0.296	80.92	85.10	0
5.133	0.300	82.01	69.59	0
5.517	0.336	91.86	43.40	0
5.851	0.394	107.71	15.55	0
6.2	0.482	131.77	0	0

TABLE 6-1: OPERATING CONDITIONS OF SBR RUN WITH BENZOIC ACID ONLY

fill time	30 min
react time	1 hour
draw time	10 min
total cycle time	1 hour 40 min
phenol concentration in feed	0 ppm
benzoic acid concentration in feed	78.50 ppm
initial phenol concentration	0 ppm
initial benzoic acid concentration	0 ppm
initial biomass concentration	34.72 ppm
initial reactor volume	2 liter
volume after fill phase	4 liter
volume after draw-down phase	2 liter
feed flow rate	2 liter/hour

TABLE 6-2: EXPERIMENTAL RESULTS OF SBR RUN WITH BENZOIC ACID ONLY

CYCLE NO.	TIME (HOUR)	BIOMASS CONC. (PPM)	PHENOL CONC. (PPM)	BENZOIC ACID CONC. (PPM)	pH	TEMPER. (^{o}C)	DISSOLVED OXYGEN (PPM)
3	0	**	**	**	**	**	**
	0.22	33.08	0	16.66	**	**	**
	0.50	28.98	0	24.44	**	29.8	6.2
	0.75	36.63	0	13.52	**	29.8	5.9
	1.08	46.48	0	0	7.24	30.1	6.2
	1.33	47.57	0	0	**	30.1	6.9
	1.67	41.83	0	0	7.24	30.1	7.2

TABLE 7-1: OPERATING CONDITIONS FOR SBR RUN WITH PHENOL AND BENZOIC ACID

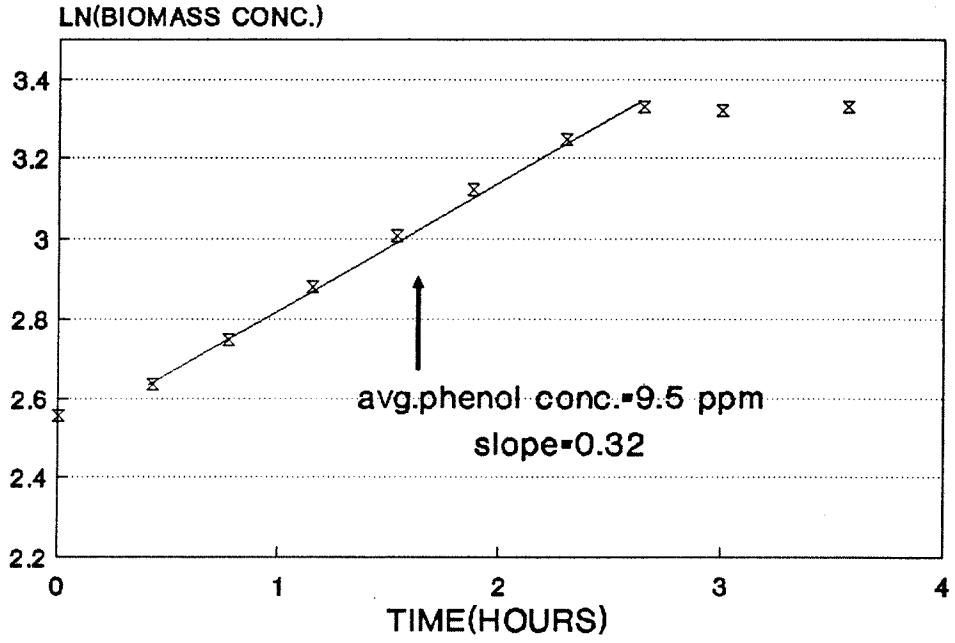
fill time	30 min
react time	1 hour
draw time	10 min
total cycle time	1 hour 40 min
phenol concentration in feed	0 ppm
benzoic acid concentration in feed	0 ppm
initial phenol concentration	88.77 ppm
initial benzoic acid concentration	78.48 ppm
initial biomass concentration	41.55 ppm
initial reactor volume	2 liter
volume after fill phase	4 liter
volume after draw down phase	2 liter
feed flow rate	2 liter/hour

TABLE 7-2: EXPERIMENTAL RESULTS OF SBR RUN WITH PHENOL AND BENZOIC ACID

CYCLE NO.	TIME (HOURS)	BIOMASS CONC. (PPM)	PHENOL CONC. (PPM)	BENZOIC ACID CONC. (PPM)	TEMP. (^{o}C)	pH	DISSOLVED OXYGEN (PPM)
4	0	98.14	0	0	29.6	7.21	5.5
	0.42	63.42	28.25	24.70	**	**	**
	0.74	85.02	18.15	10.97	29.6	**	1.6
	1.02	94.04	3.32	0.34	**	**	1.9
	1.37	103.07	0	0	29.7	**	5.1
	1.62	96.50	0	0	29.9	**	5.1
10	0	97.32	0	0	29.5	7.19	4.7
	0.35	88.58	15.03	12.47	30	**	0.5
	0.70	83.66	17.50	4.10	30	**	0.5
	1.02	103.07	1.13	0	30	**	1.6
	1.35	101.70	0	0	29.2	**	5.5
	1.67	101.15	0	0	29.6	7.20	5.5

SPECIFIC GROWTH RATES OF P.AERUGINOSA IN PHENOL

FIG.1.1: RUN 1.1 AT INITIAL PHENOL CONC. OF 19 PPM



RUN1.2 AT INITIAL PHENOL CONC. OF 24.7 PPM

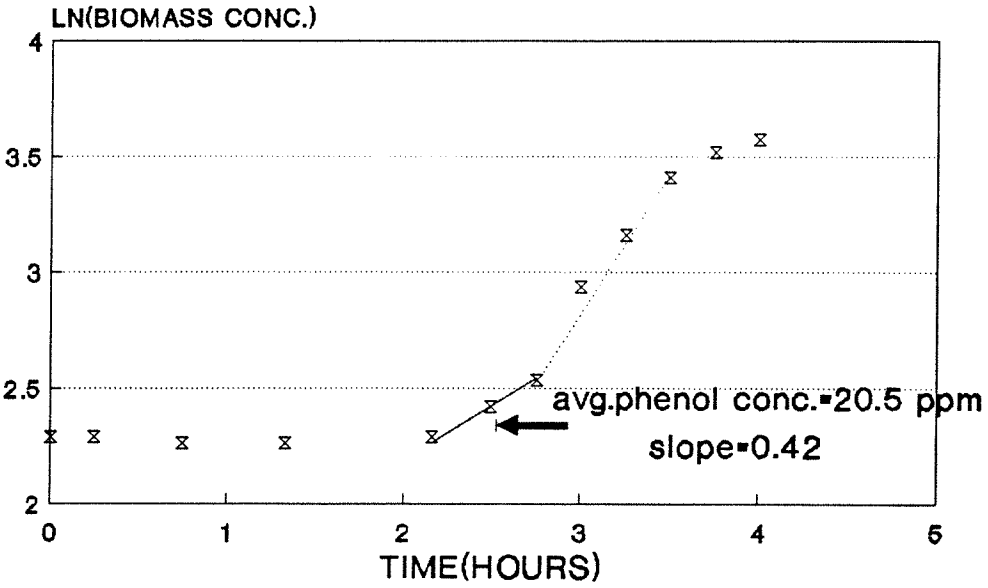
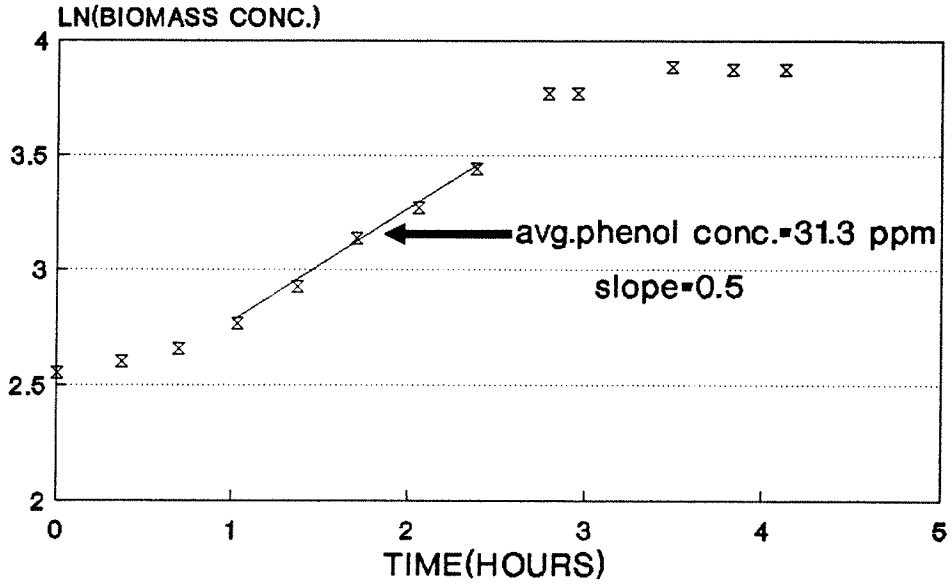


FIG.1.1(CONT.)

RUN1.3 AT INITIAL PHENOL CONC. OF 40.9 PPM



RUN1.4 AT INITIAL PHENOL CONC. OF 35.9 PPM

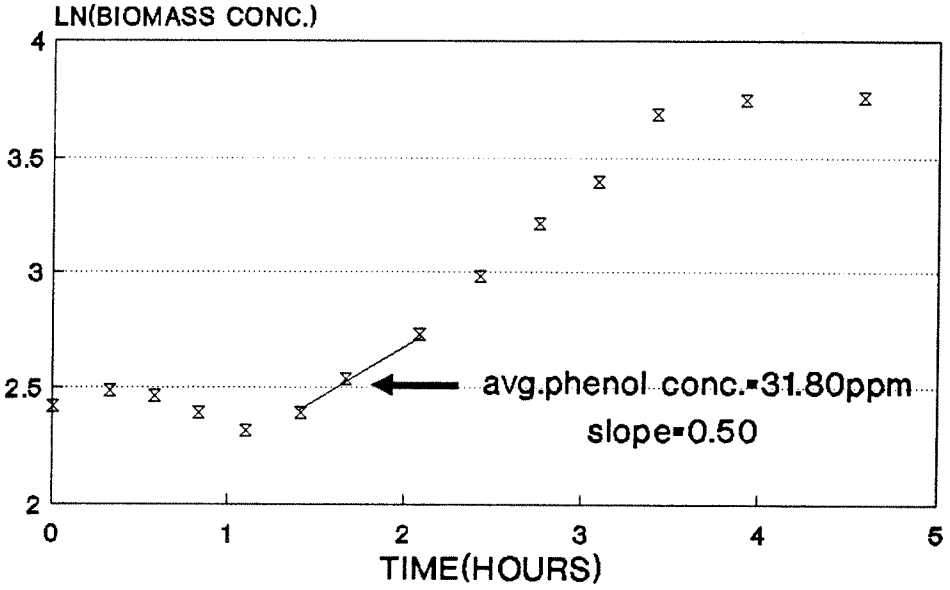
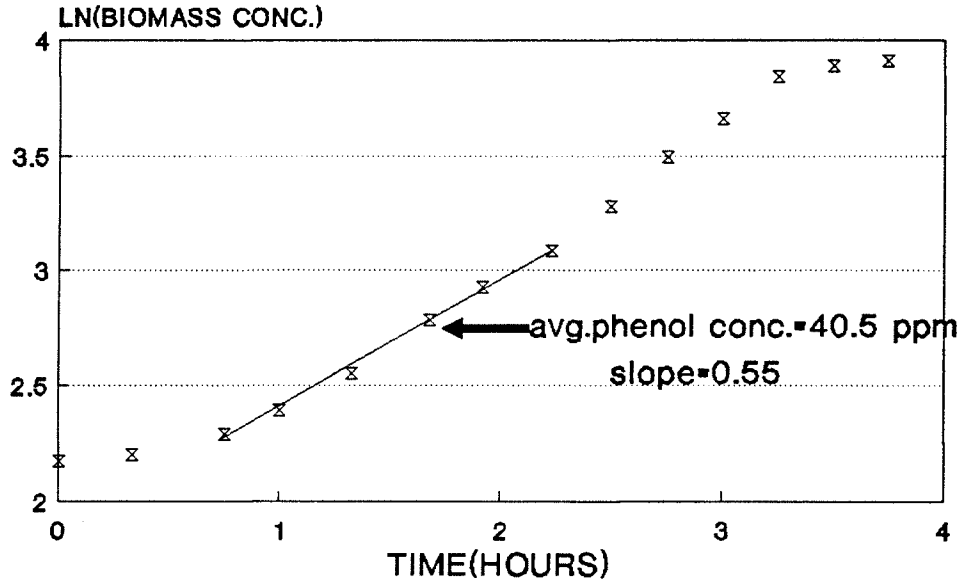


FIG.1.1 (CONT.)

RUN1.5 AT INITIAL PHENOL CONC. OF 49.1 PPM



RUN1.6 AT INITIAL PHENOL CONC. OF 56.6 PPM

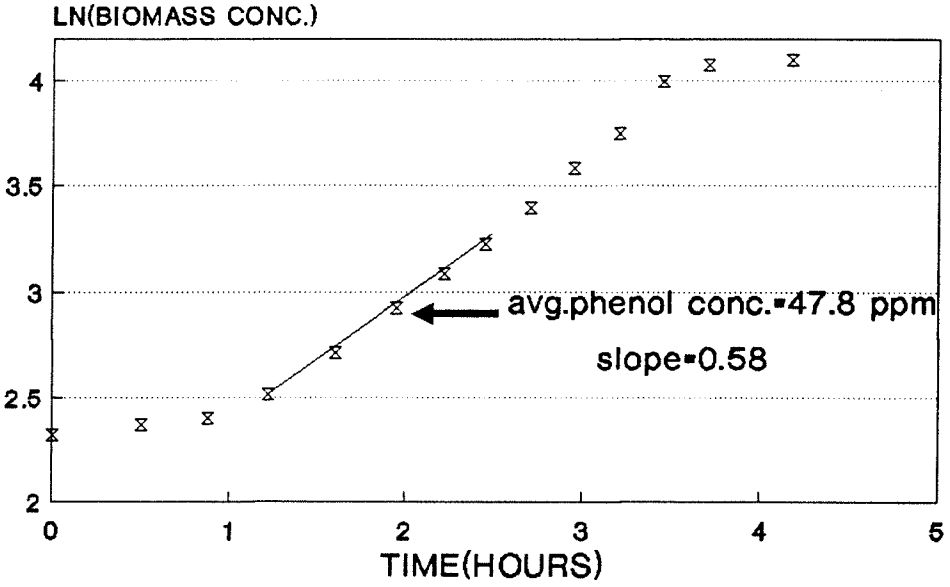
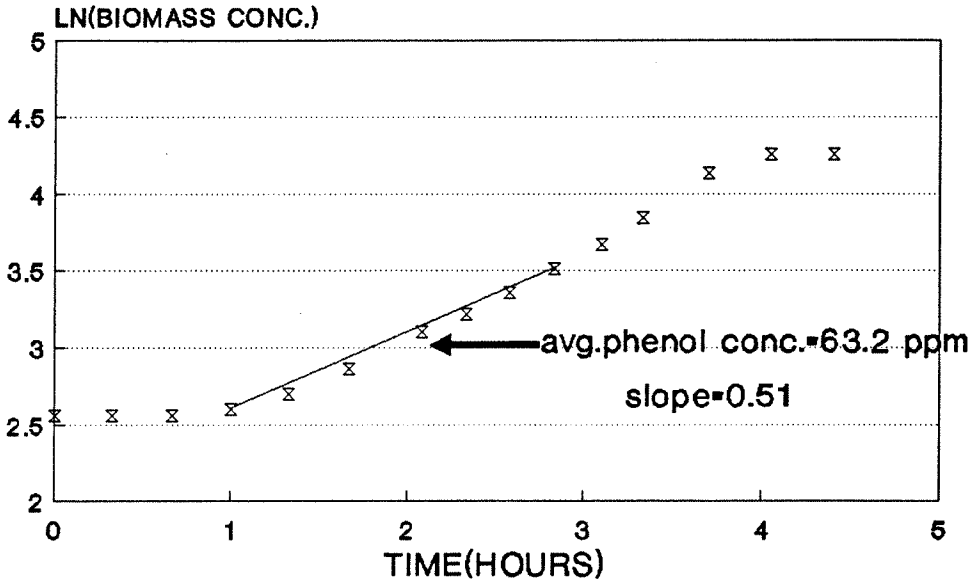
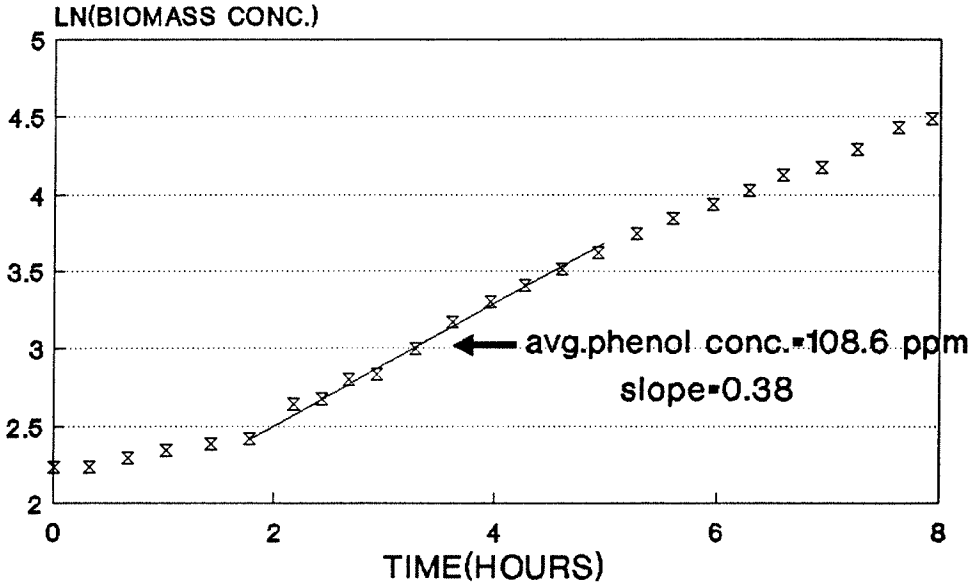


FIG.1.1 (CONT.)

RUN1.7 AT INITIAL PHENOL CONC. OF 78.4 PPM



RUN1.8 AT INITIAL PHENOL CONC. OF 136 PPM



RUN1.9 AT INITIAL PHENOL CONC. OF 160 PPM

FIG.1.1 (CONT.)

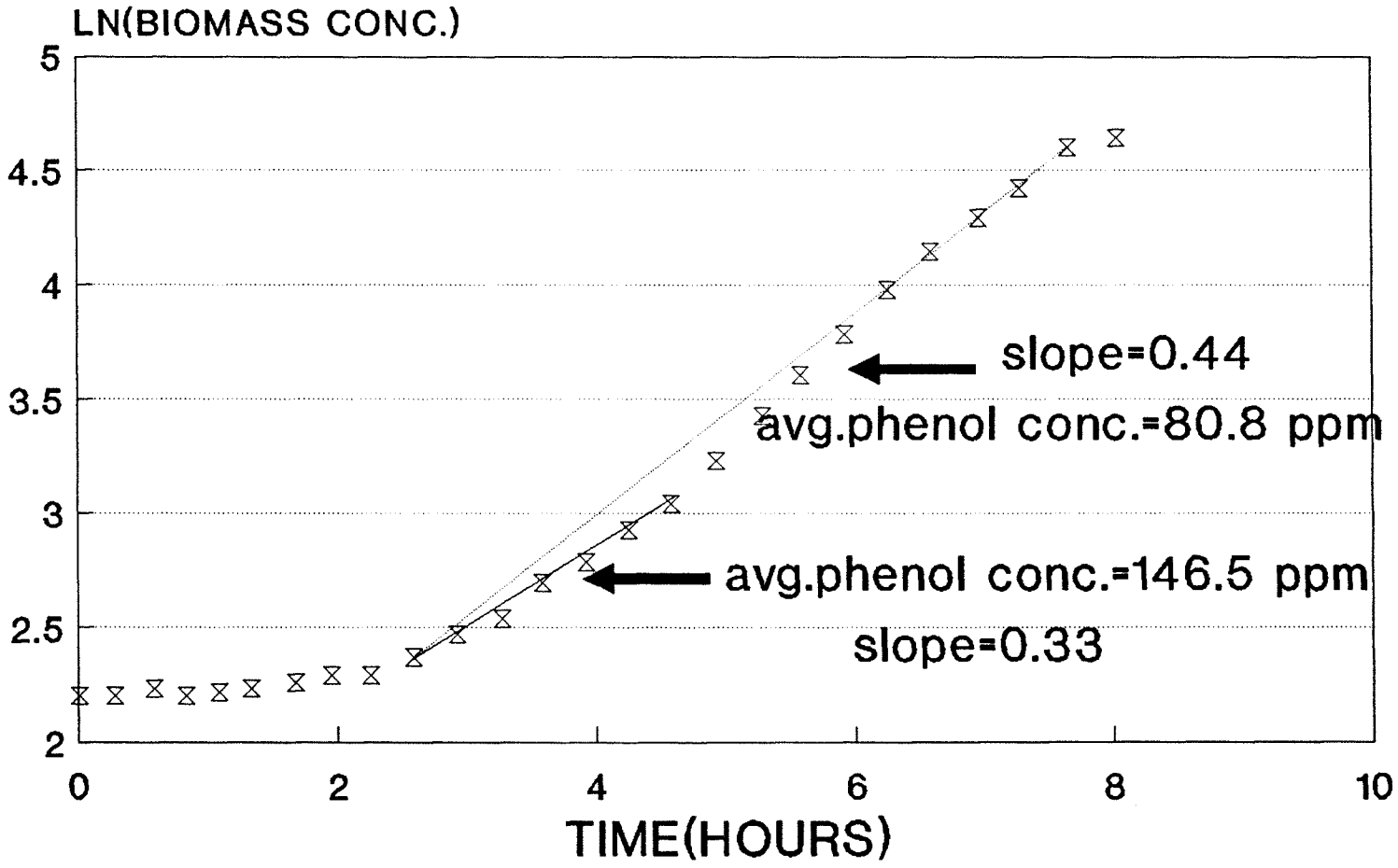
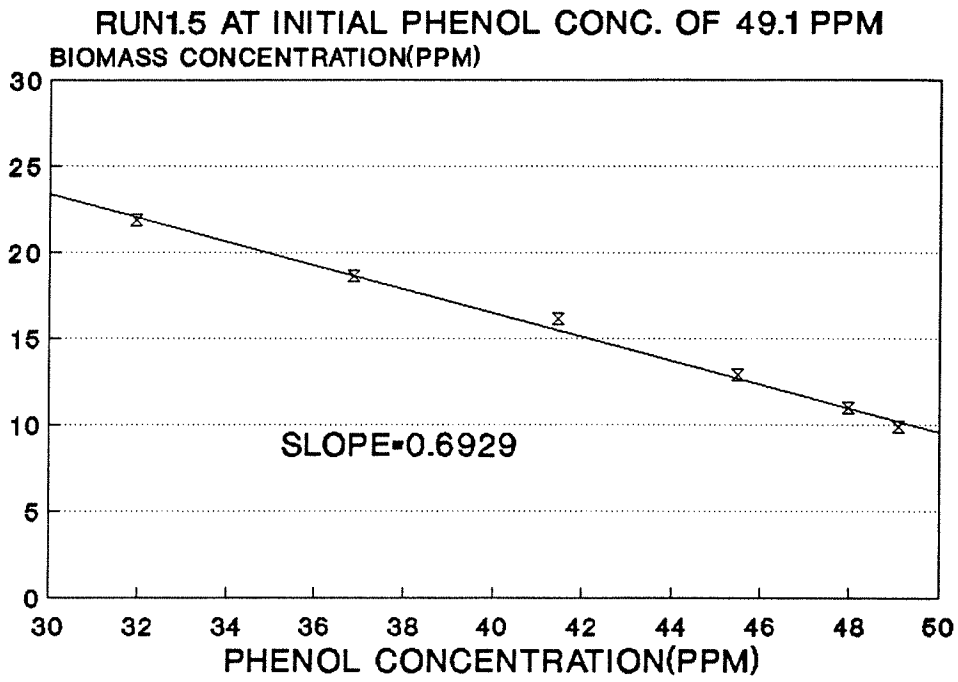


FIG.1.2: YIELD COEFFICIENTS IN PHENOL



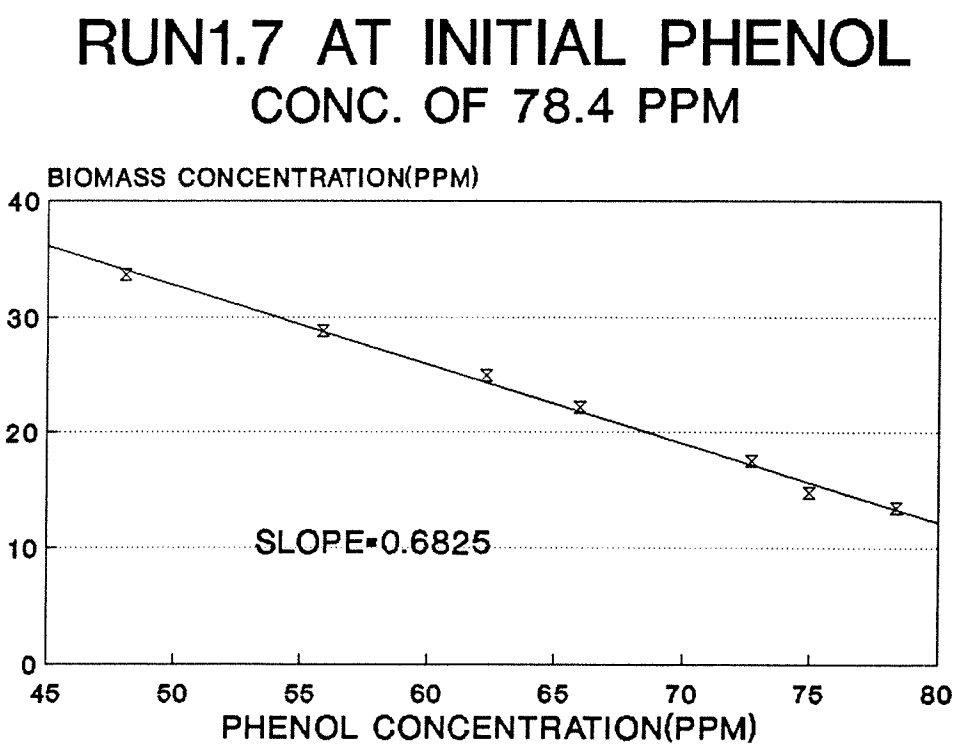
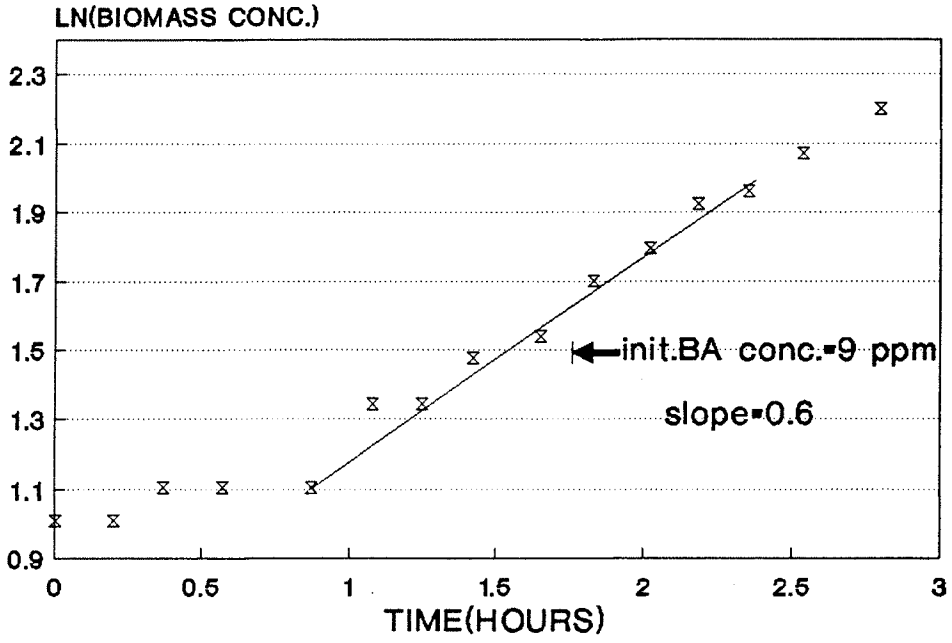


FIG.2.1: SPECIFIC GROWTH RATES OF P.AERUGINOSA IN BA

RUN2.1 AT INITIAL BENZOIC ACID CONC. OF 9 PPM



RUN2.2 AT INITIAL BENZOIC ACID CONC. OF 19 PPM

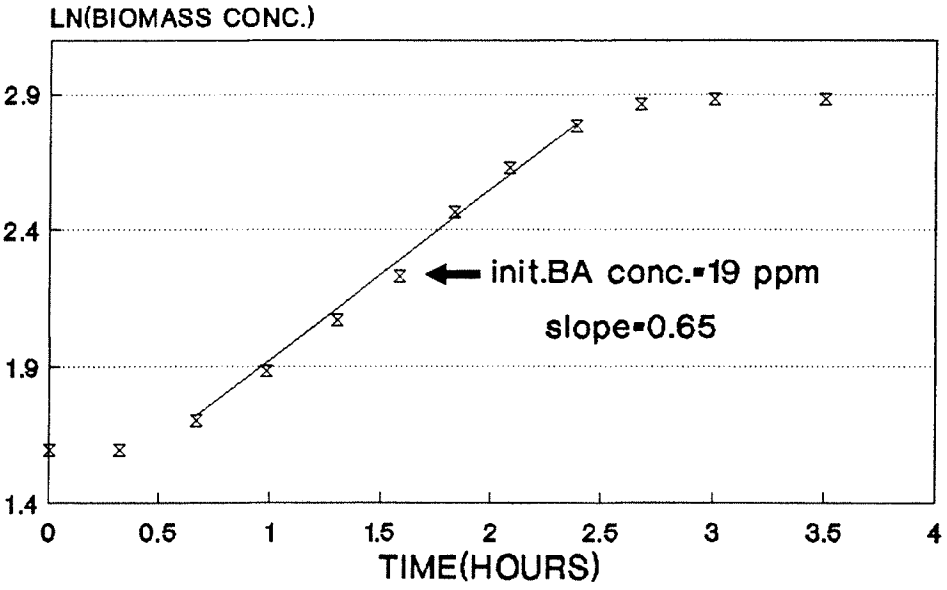
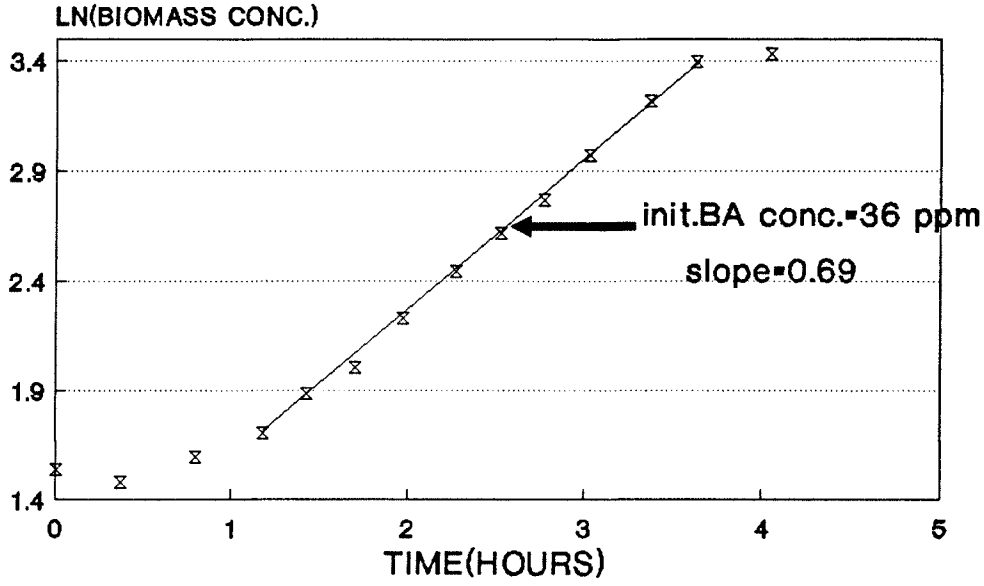


FIG2.1 (CONT.)

RUN2.3 AT INITIAL BENZOIC ACID CONC. OF 36 PPM



RUN2.4 AT INITIAL BENZOIC ACID CONC. OF 45.1 PPM

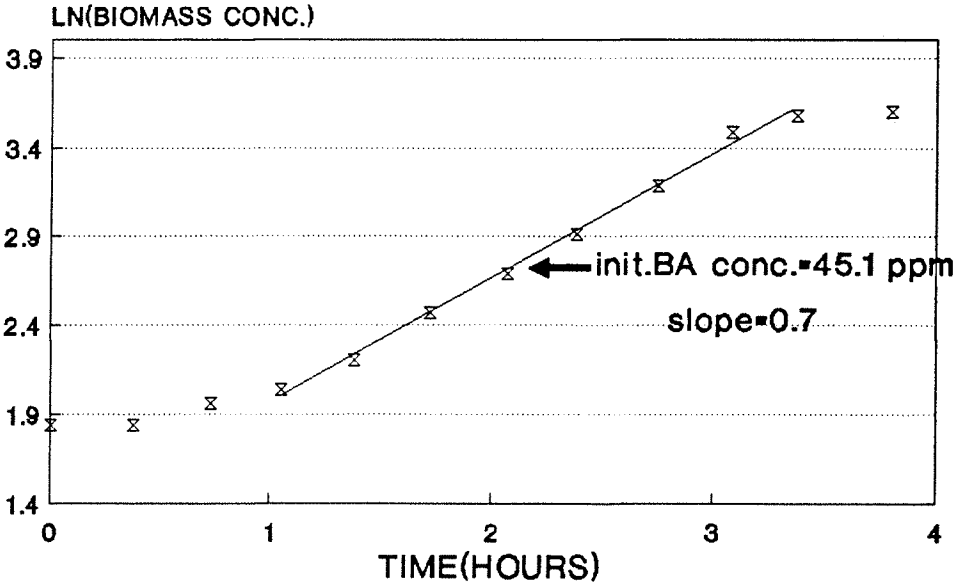
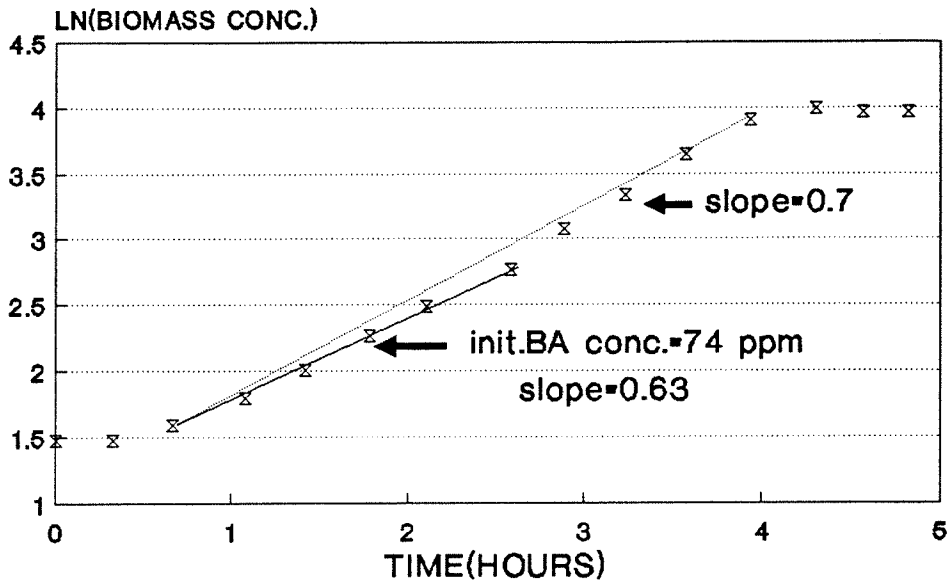
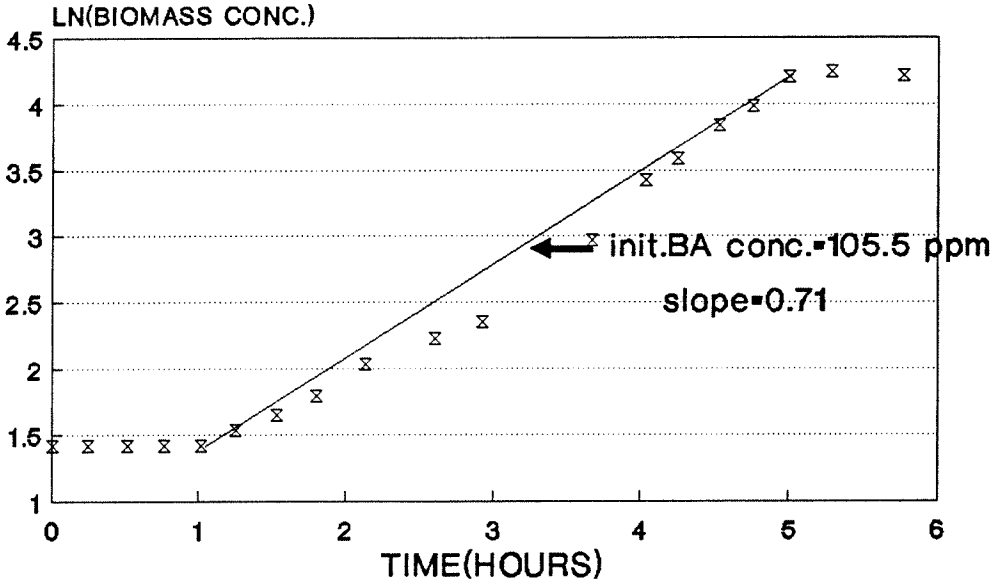


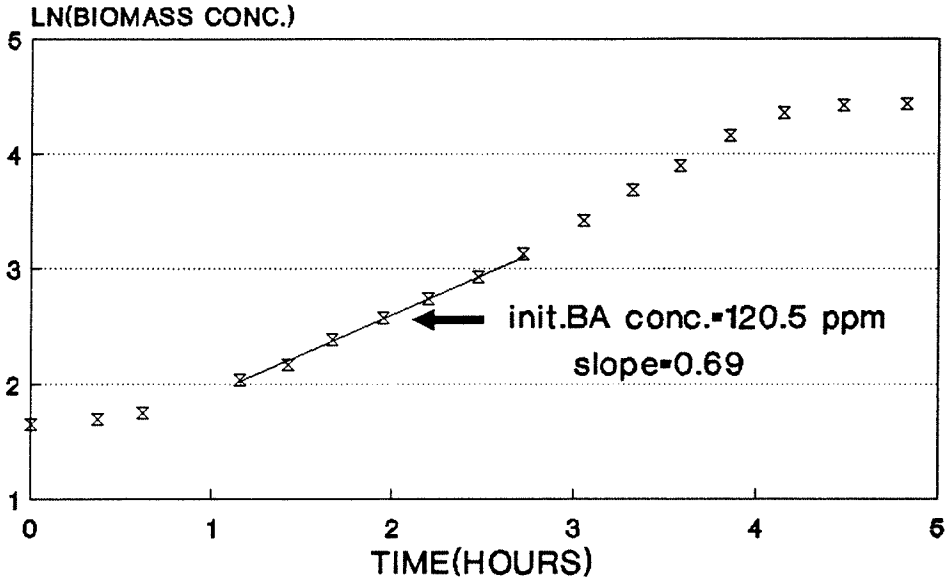
FIG2.1 (CONT.)


RUN2.5 AT INITIAL BENZOIC ACID CONC. OF 74.0 PPM
LN(BIOMASS CONC.)
4.5
4
3.5
3
2.5
2
1.5
1
0
1
2
3
4
5
TIME(HOURS)
slope=0.7
init.BA conc.=74 ppm
slope=0.63


RUN2.6 AT INITIAL BENZOIC ACID CONC. OF 105.5 PPM
LN(BIOMASS CONC.)
4.5
4
3.5
3
2.5
2
1.5
1
0
1
2
3
4
5
6
TIME(HOURS)
init.BA conc.=105.5 ppm
slope=0.71

RUN2.7 AT INITIAL BENZOIC ACID CONC. OF 120.5 PPM

FIG.2.1 (CONT.)



RUN2.8 AT INITIAL BENZOIC ACID CONC. OF 142.6 PPM

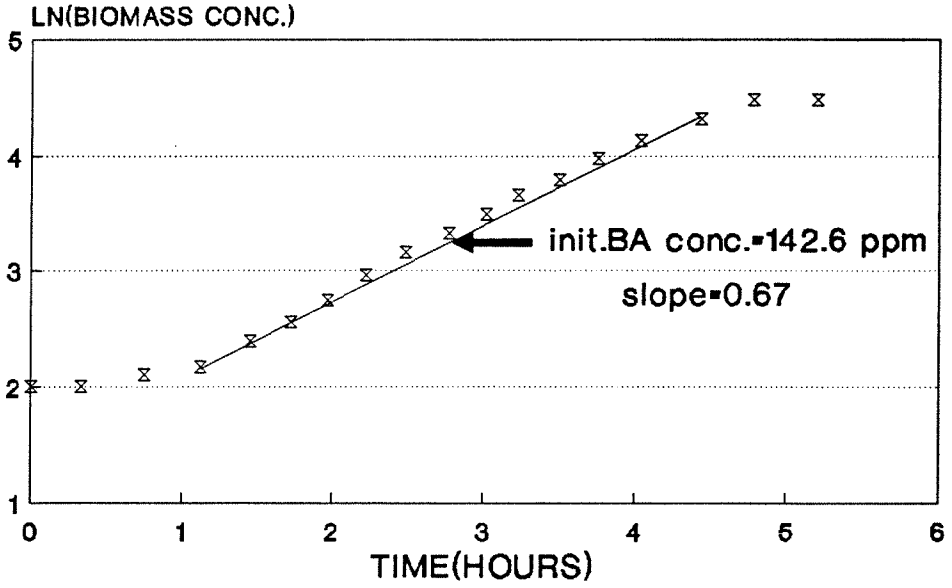
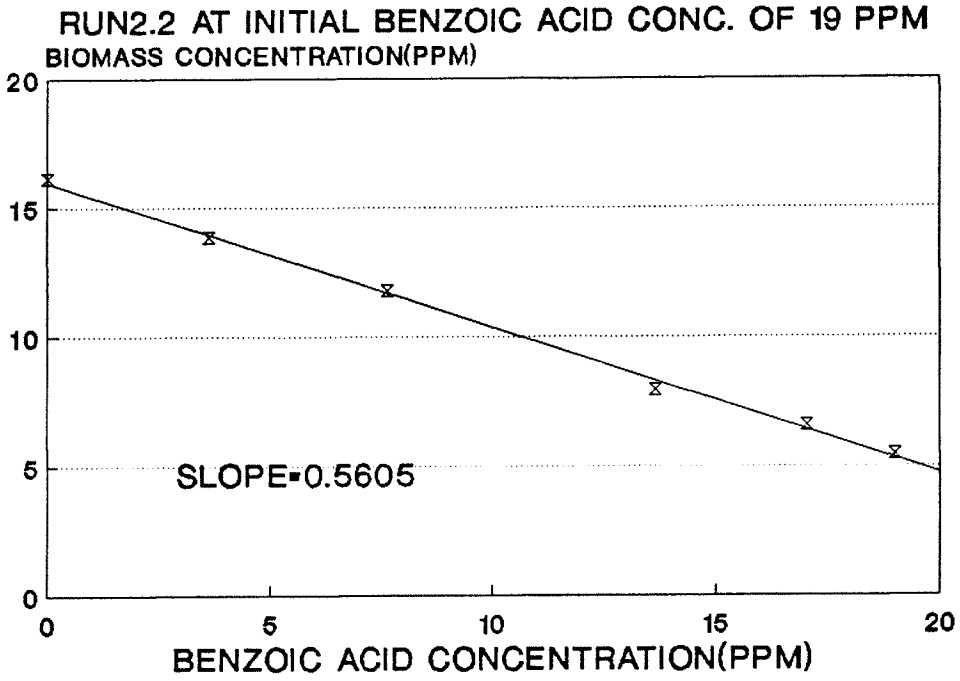


FIG 2.2: YIELD COEFFICIENT FOR BENZOIC ACID



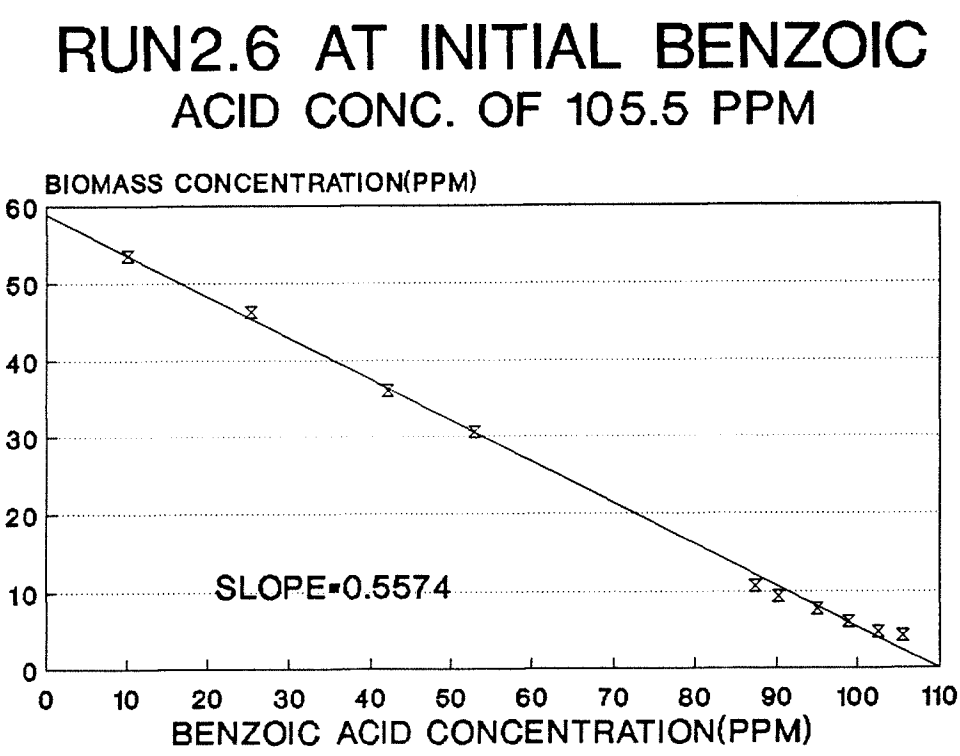


FIG.3.1: SPECIFIC GROWTH RATE VS AVERAGE PHENOL CONC.FOR ANDREWS MODEL

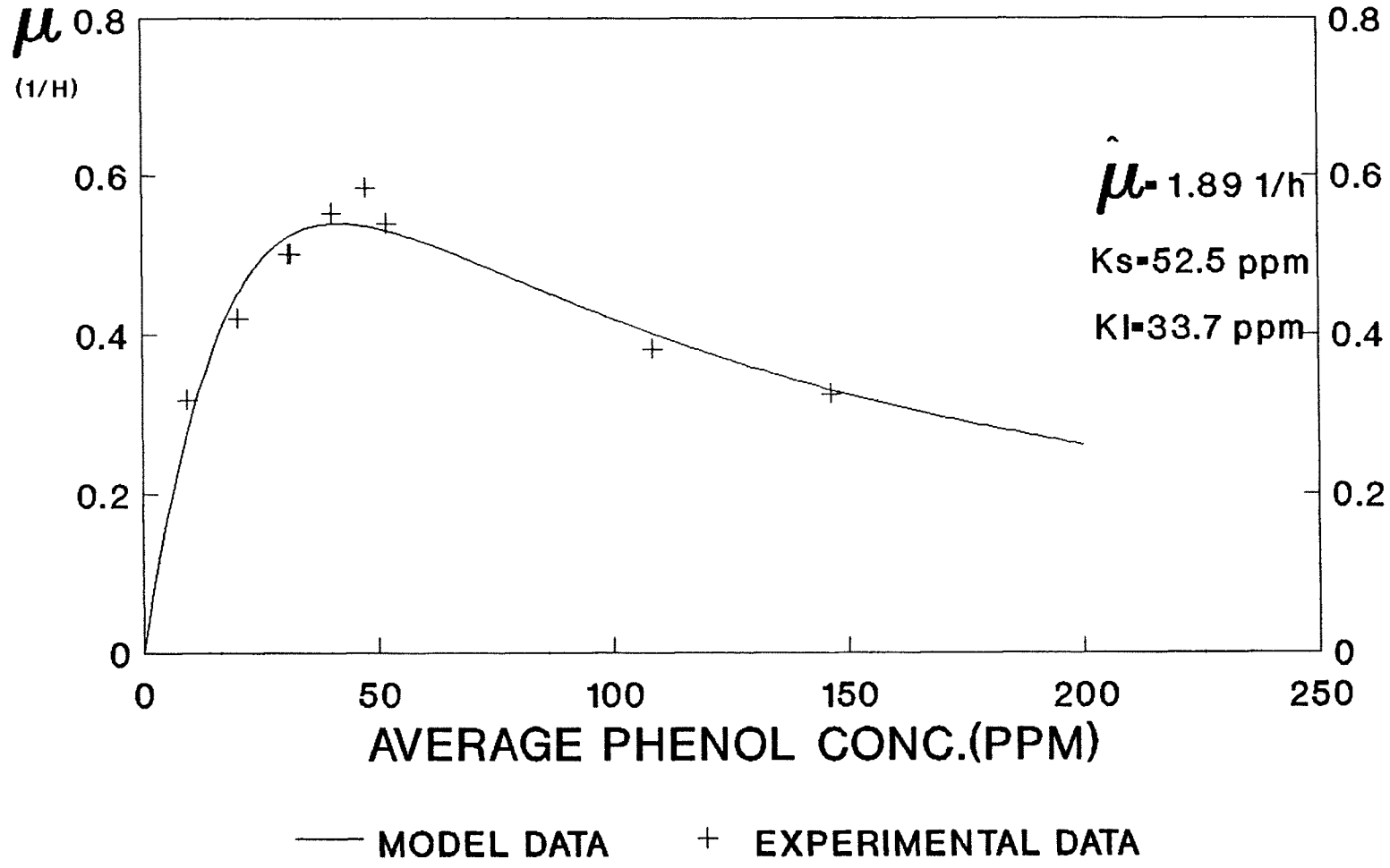
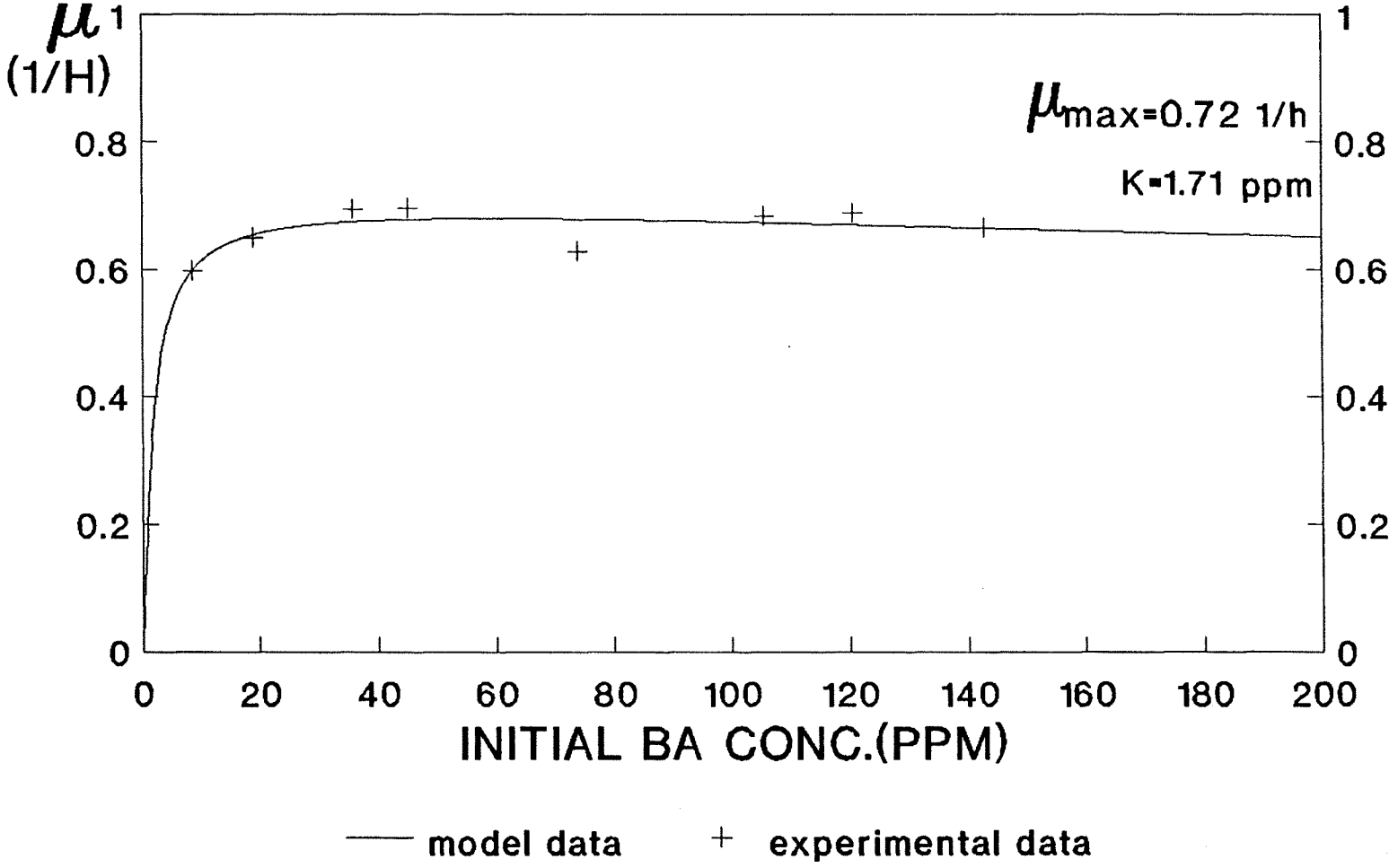


FIG.3.2: SPECIFIC GROWTH RATE VS INITIAL BENZOIC ACID CONC. FOR MONOD'S MODEL PARAMETERS



BA:benzoic acid

FIG.3.3 INVERSE OF SPECIFIC GROWTH RATE VS. INVERSE OF INITIAL BENZOIC ACID CONC. FOR MONOD MODEL PARAMETERS

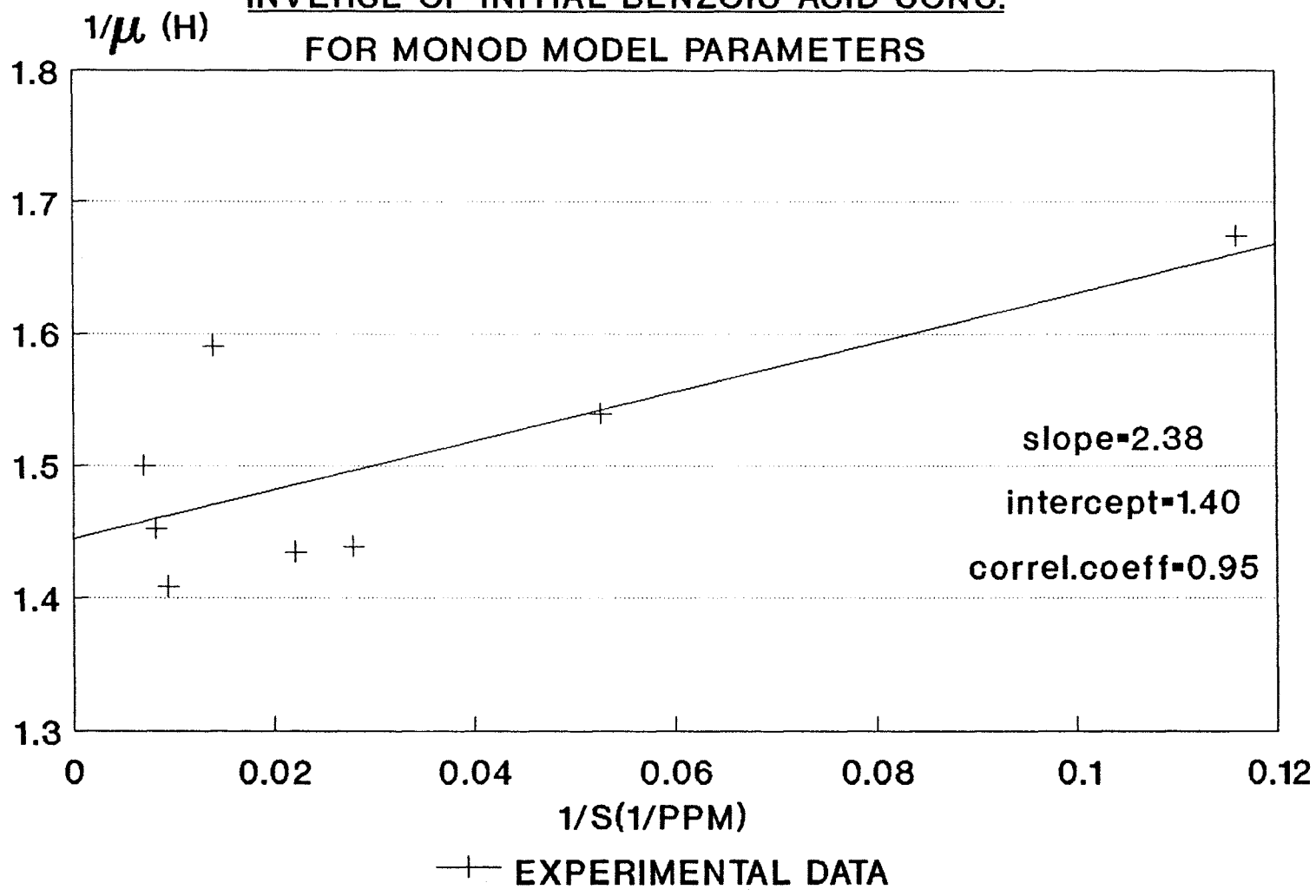
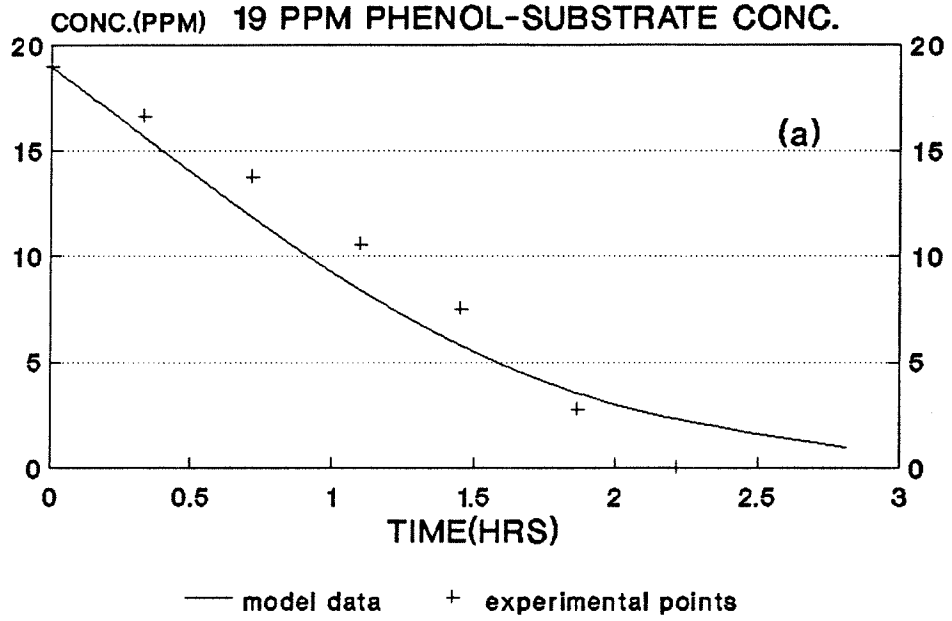


FIG.4.1: PHENOL AND BIOMASS CONC. VS.TIME
(model and experimental data comparison)



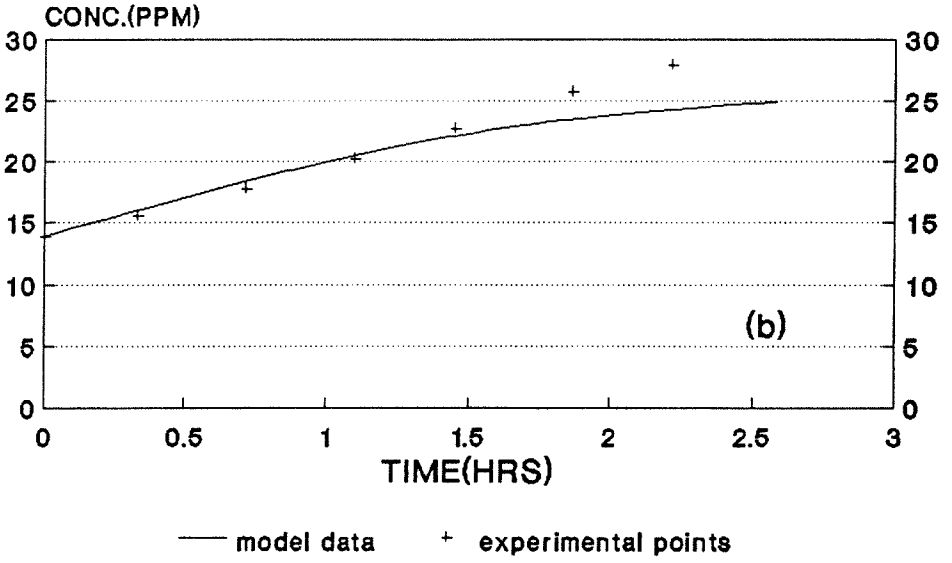
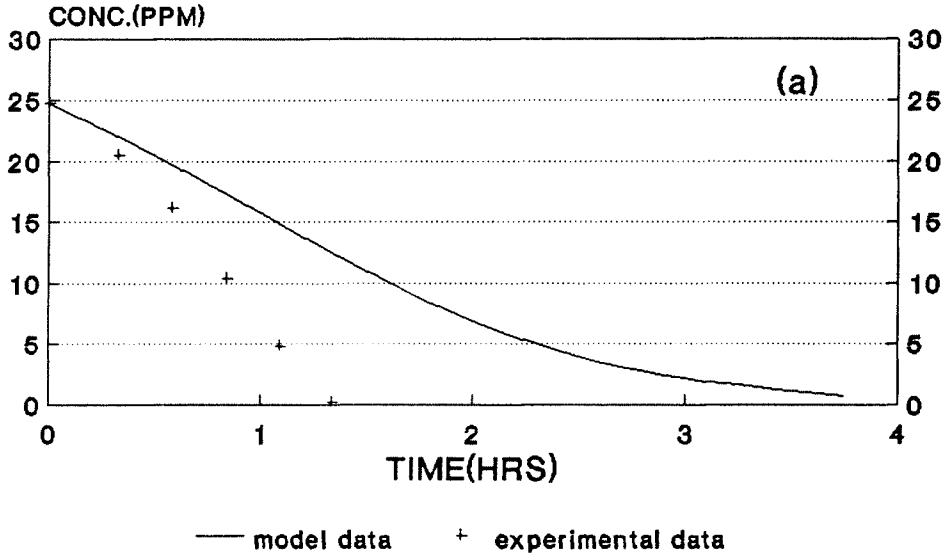


FIG.4.2: 24.8 PPM PHENOL
SUBSTRATE CONC.



24.8 PPM PHENOL
BIOMASS CONC.

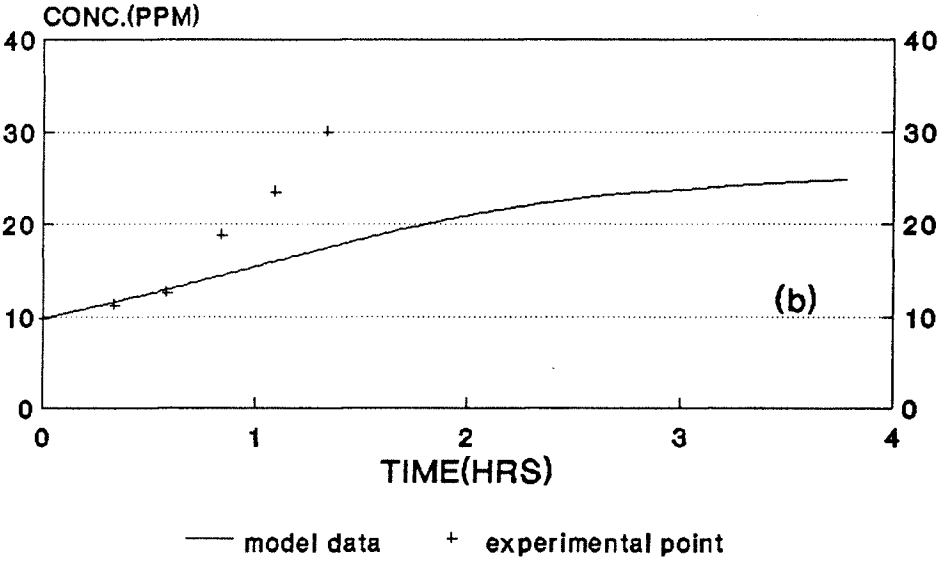
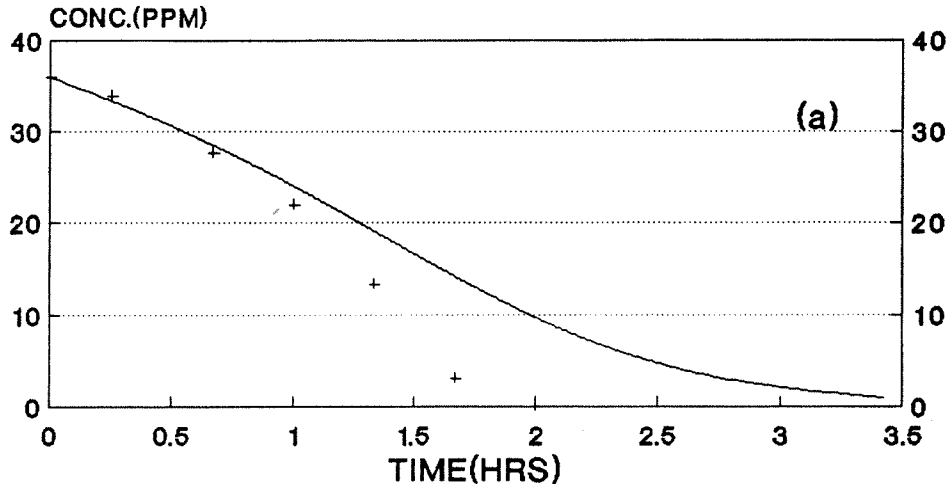


FIG.4.3:

36 PPM PHENOL
SUBSTRATE CONC.



36 PPM PHENOL
BIOMASS CONC.

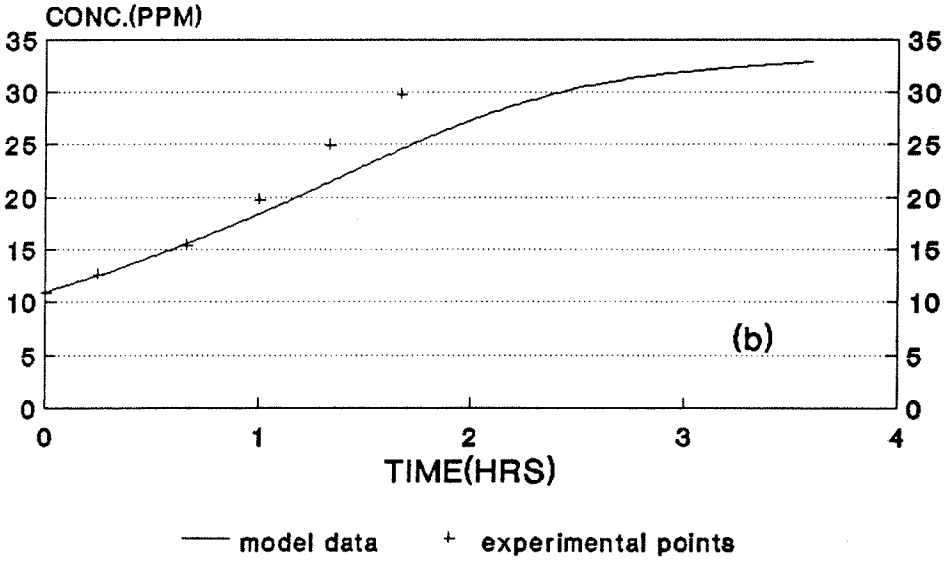
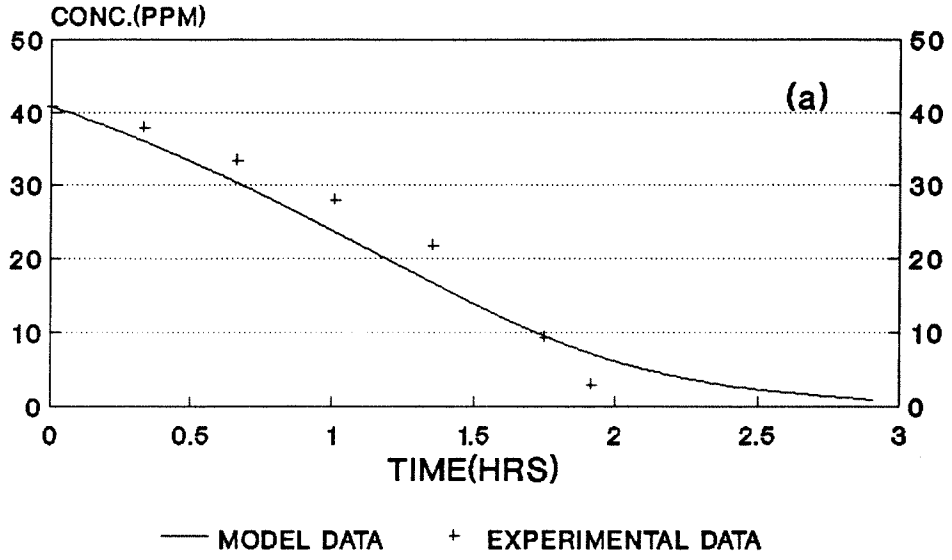


FIG.4.4: 40.9 PPM PHENOL
SUBSTRATE CONC.



40.9 PPM PHENOL
BIOMASS CONC.

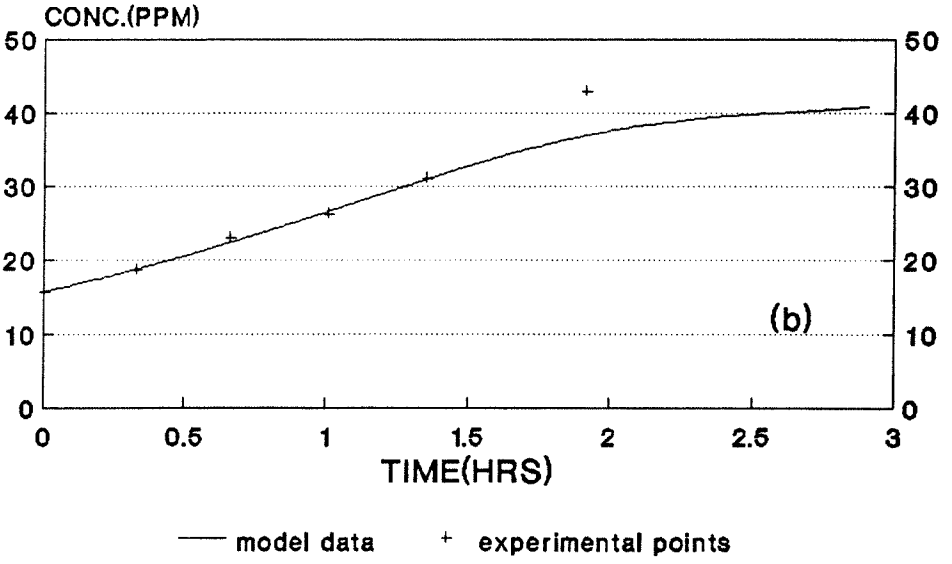
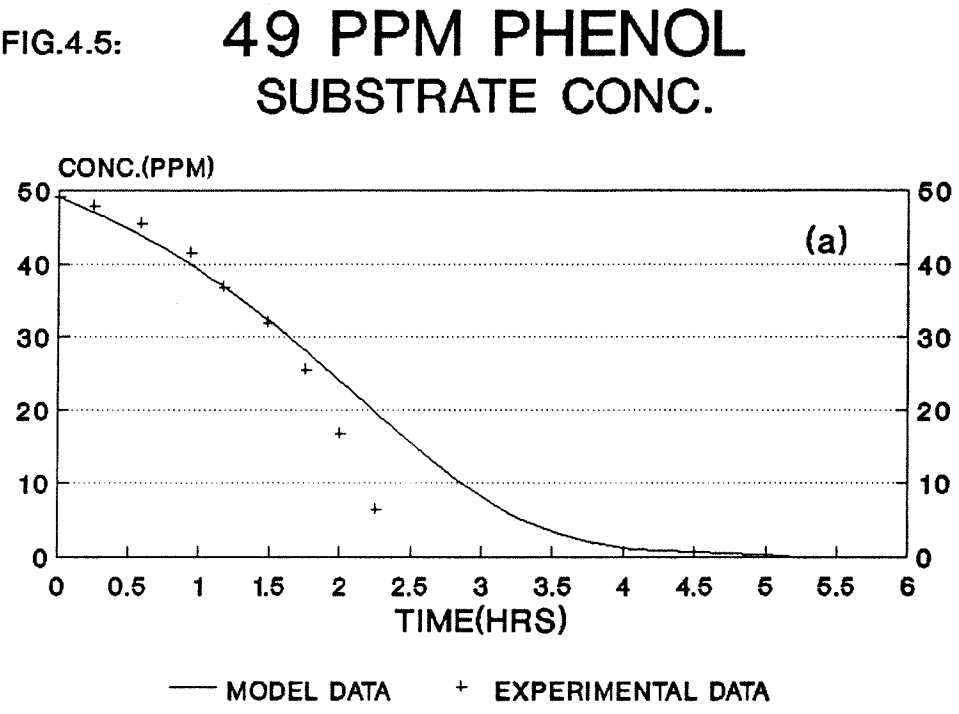


FIG.4.5:


49 PPM PHENOL
SUBSTRATE CONC.
CONC.(PPM)
50
40
30
20
10
0
(a)
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6
TIME(HRS)
MODEL DATA
EXPERIMENTAL DATA

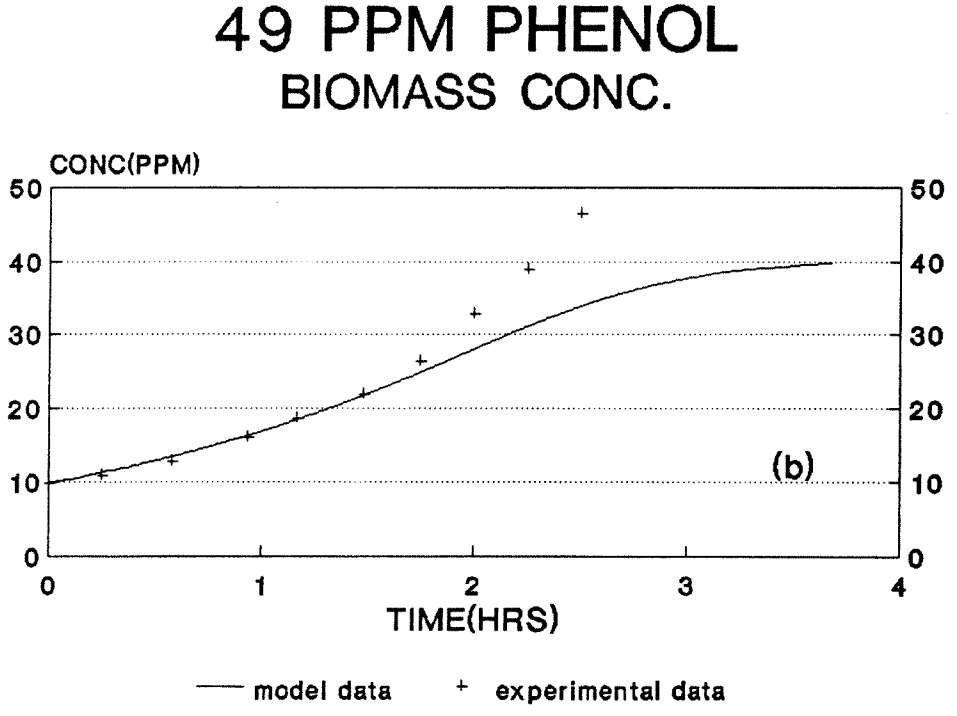
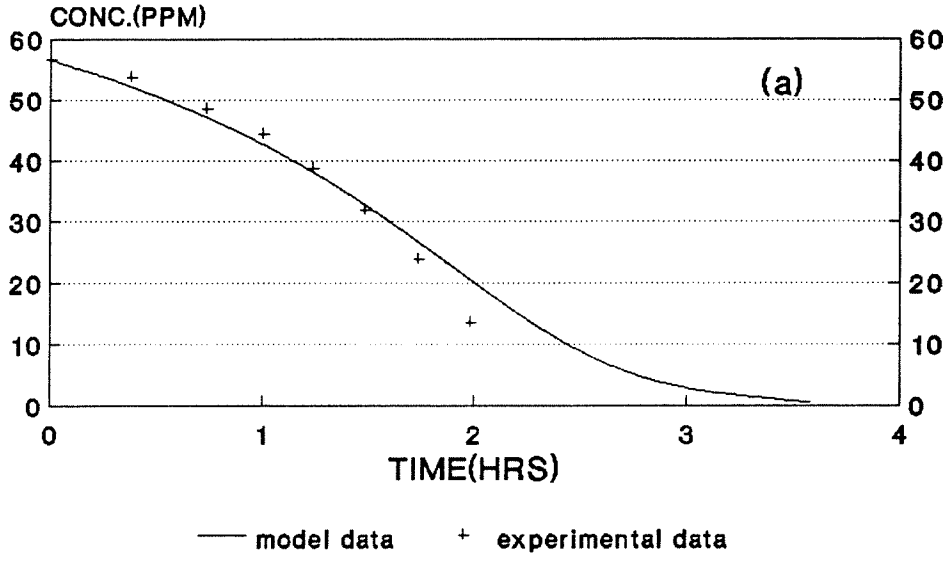

49 PPM PHENOL
BIOMASS CONC.
CONC(PPM)
50
40
30
20
10
0
(b)
0 1 2 3 4
TIME(HRS)
model data
experimental data

FIG.4.6: 56.6 PPM PHENOL
SUBSTRATE CONC.



56.6 PPM PHENOL
BIOMASS CONC.

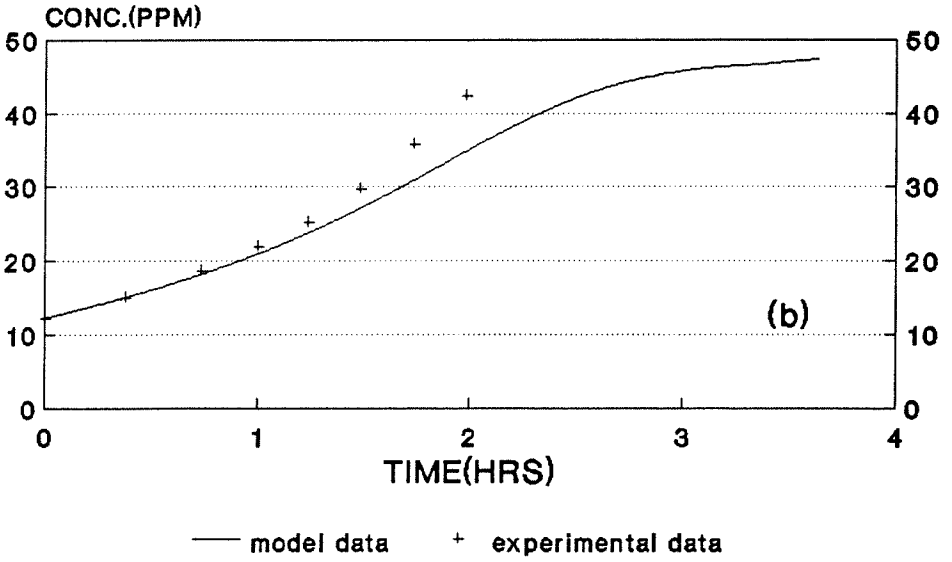
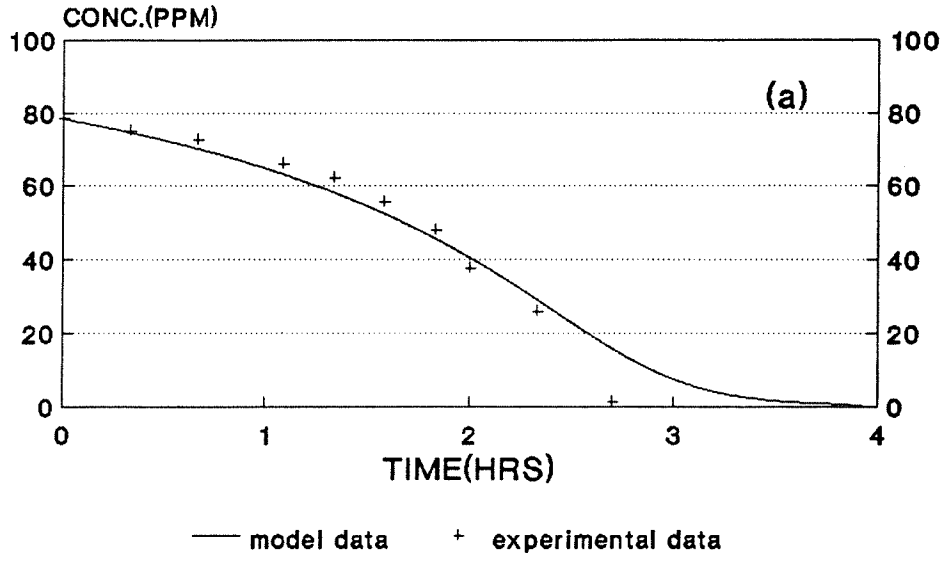


FIG.4.7: 78.4 PPM PHENOL
SUBSTRATE CONC.



78.4 PPM PHENOL
BIOMASS CONC.

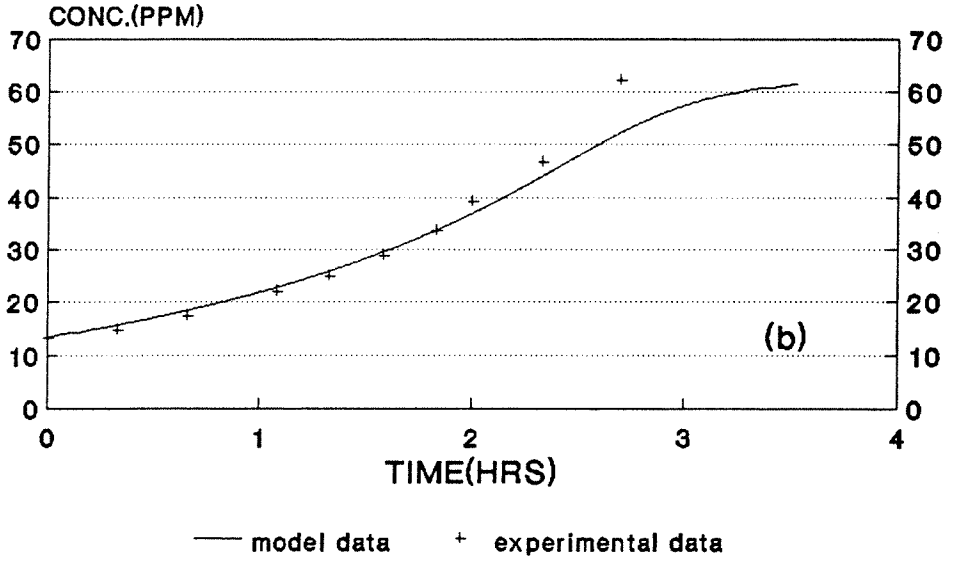
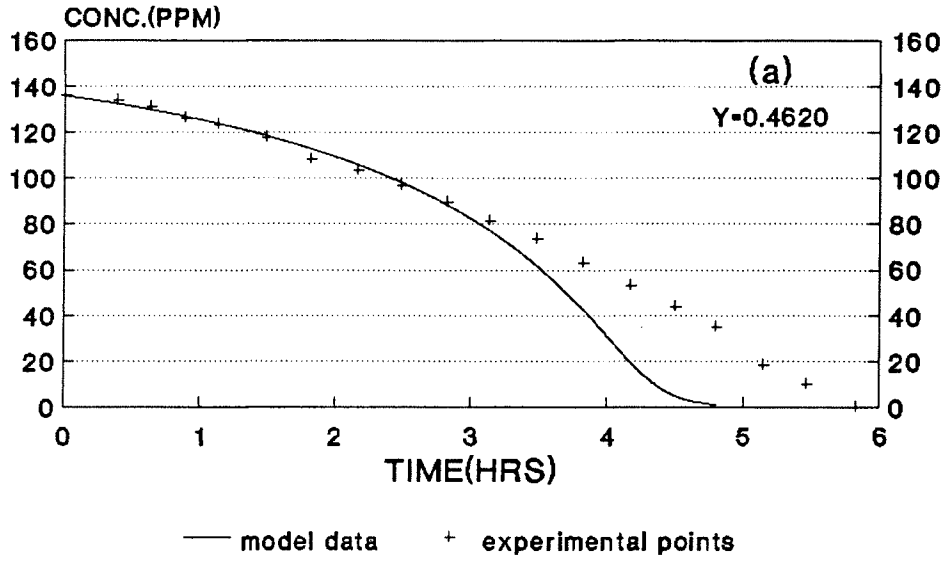


FIG.4.8: 136 PPM PHENOL
SUBSTRATE CONC.



136 PPM PHENOL
BIOMASS CONC.

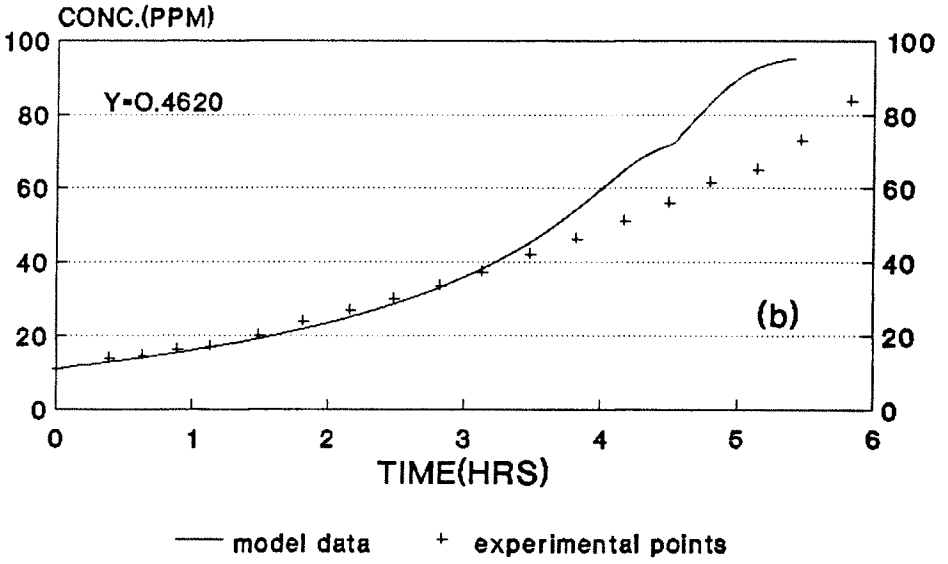
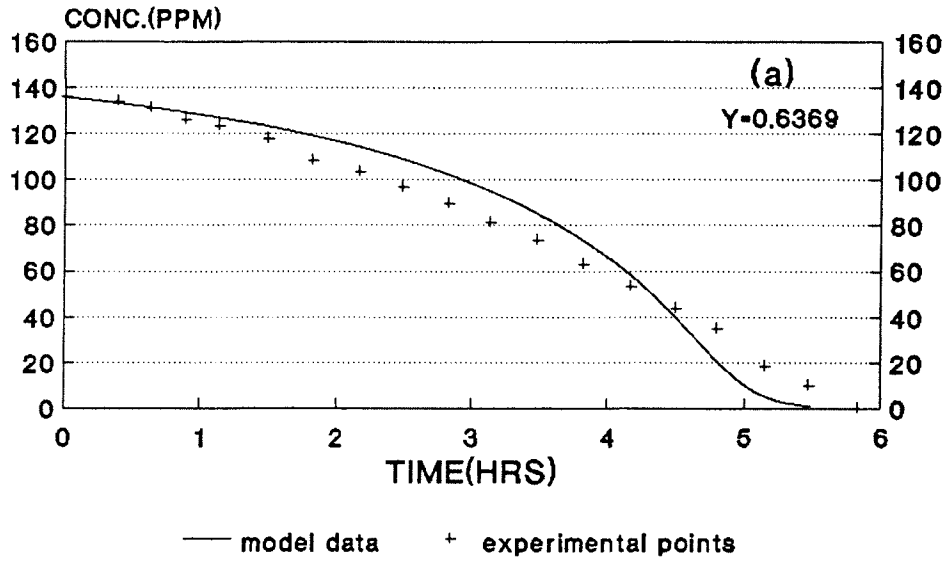


FIG.4.9: 136 PPM PHENOL
SUBSTRATE CONC.



136 PPM PHENOL
BIOMASS CONC.

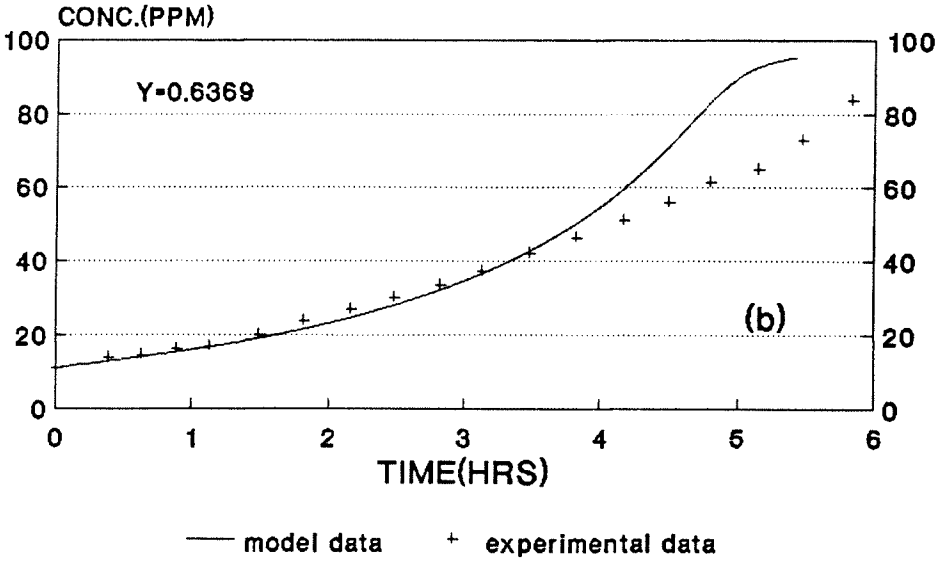
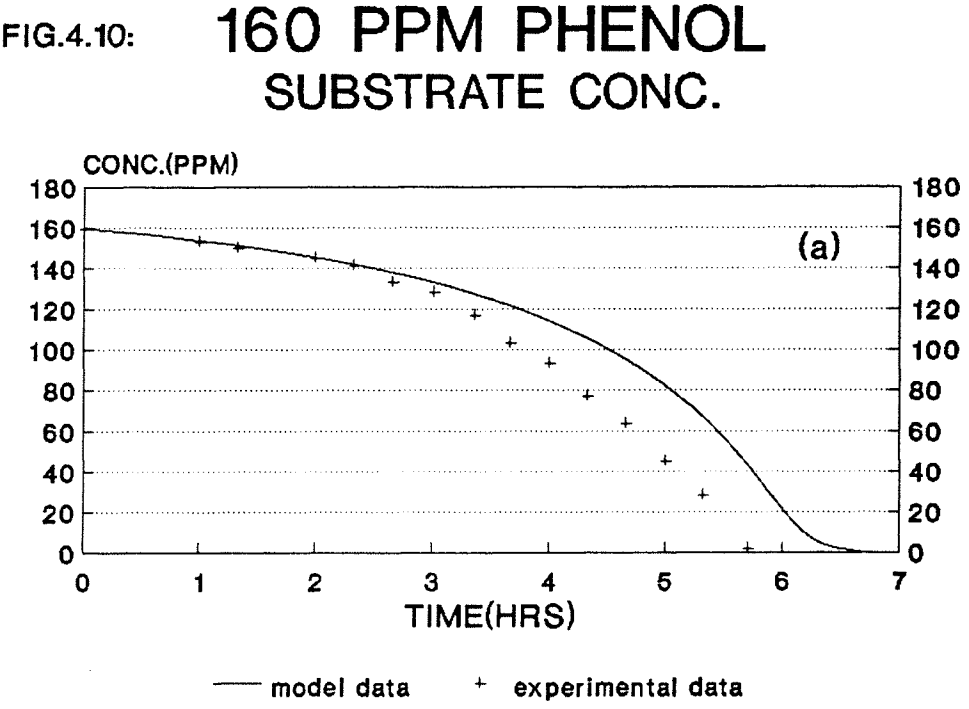
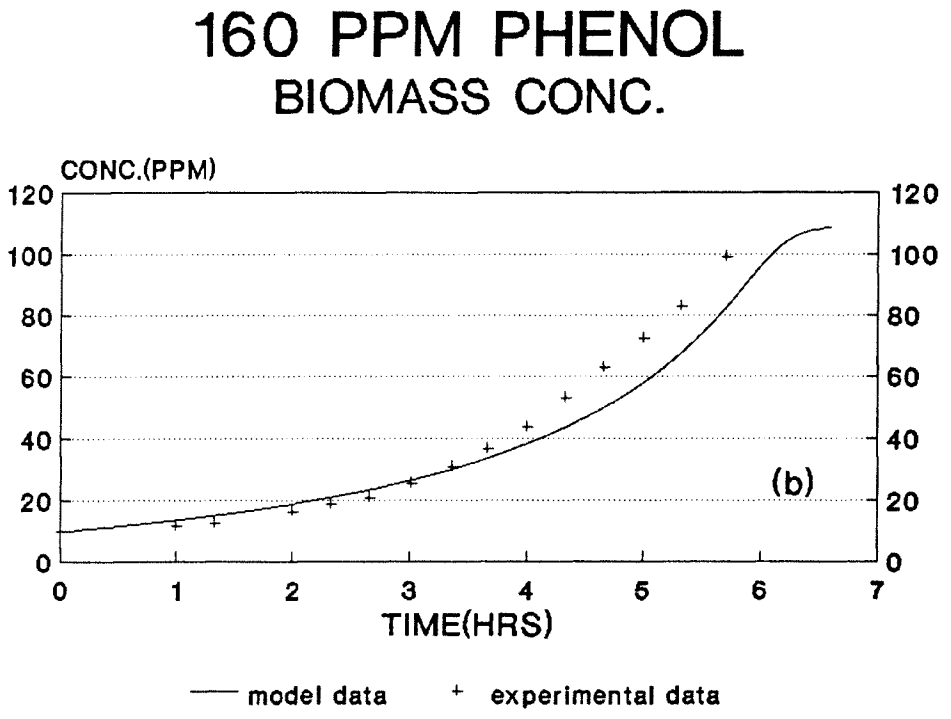


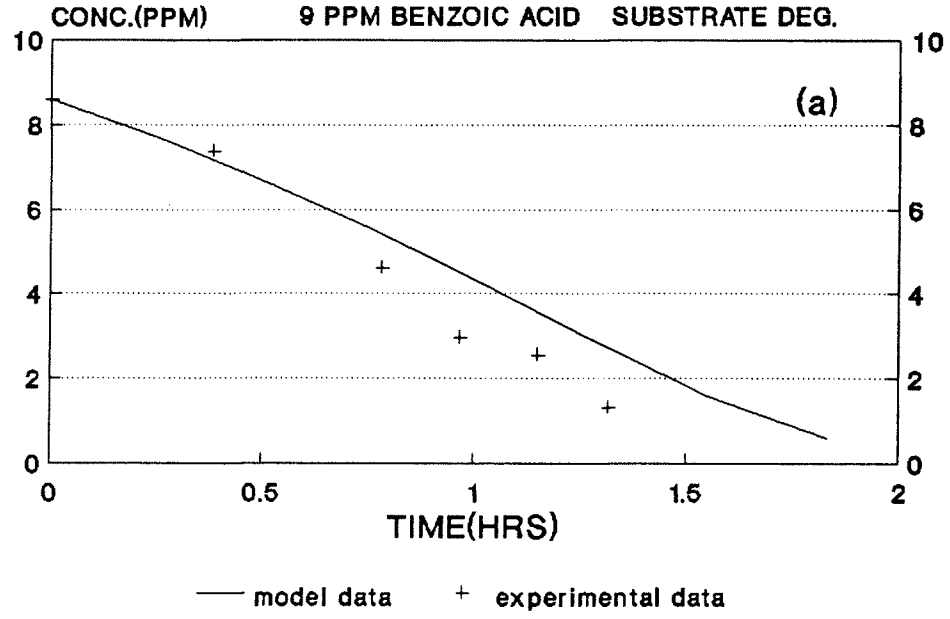
FIG.4.10:


160 PPM PHENOL
SUBSTRATE CONC.
CONC.(PPM)
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
TIME(HRS)
(a)
model data
experimental data


160 PPM PHENOL
BIOMASS CONC.
CONC.(PPM)
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
TIME(HRS)
(b)
model data
experimental data

BENZOIC ACID AND BIOMASS CONC. VS. TIME

fig.4.11: (model and experimental data comparison)



9 PPM BENZOIC ACID

BIOMASS CONC.

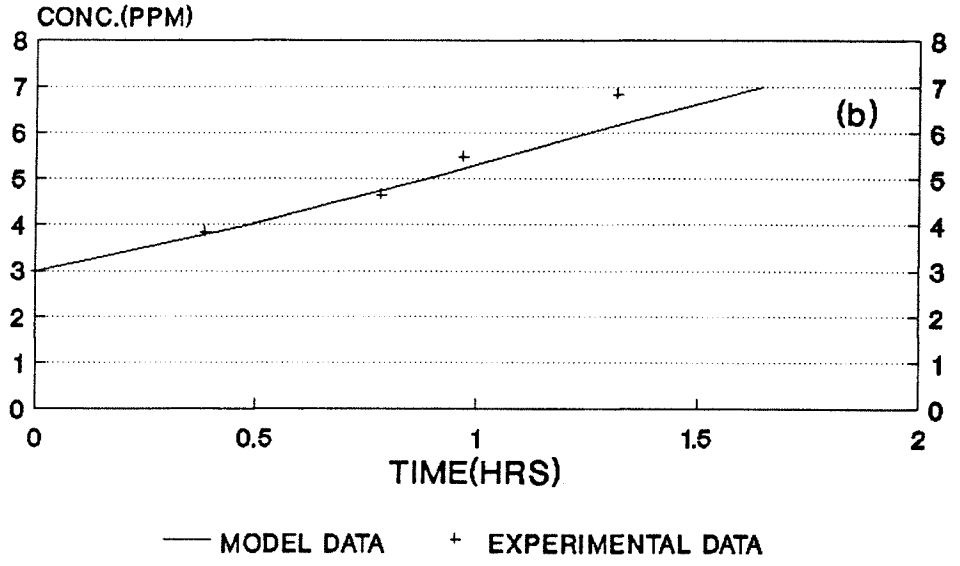
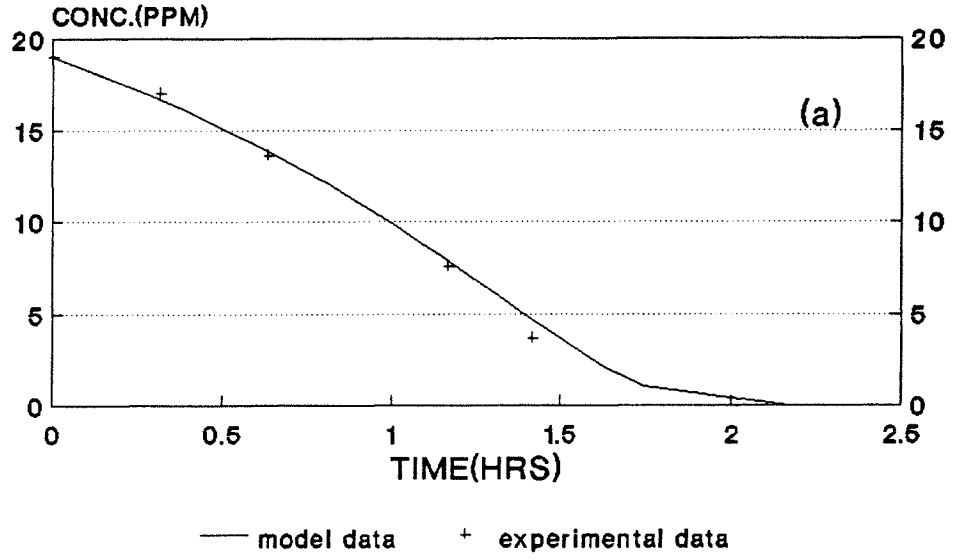


FIG.4.12: 19 PPM BENZOIC ACID
SUBSTRATE CONC.



19 PPM BENZOIC ACID
BIOMASS CONC.

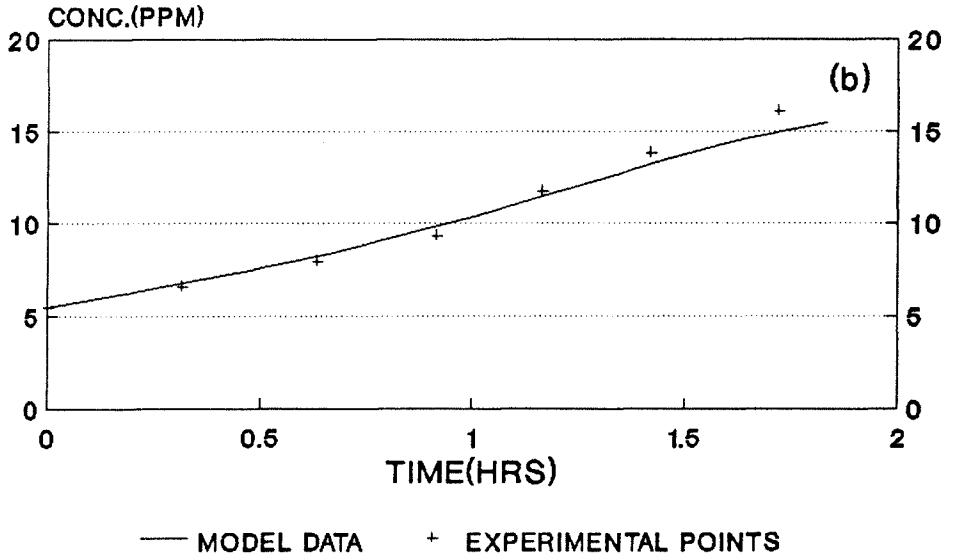
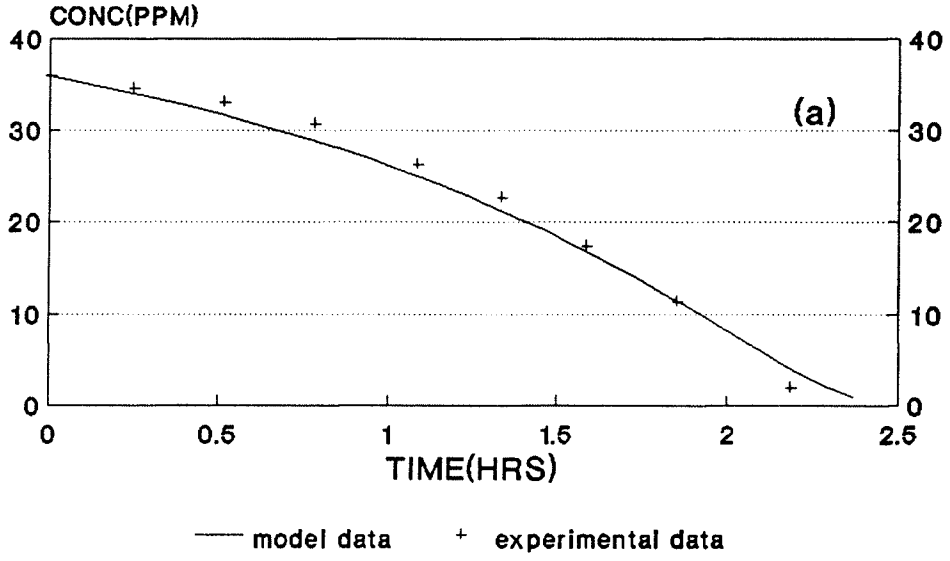


FIG.4.13: 36 PPM BENZOIC ACID
SUBSTRATE CONC.



36 PPM BENZOIC ACID
BIOMASS CONC.

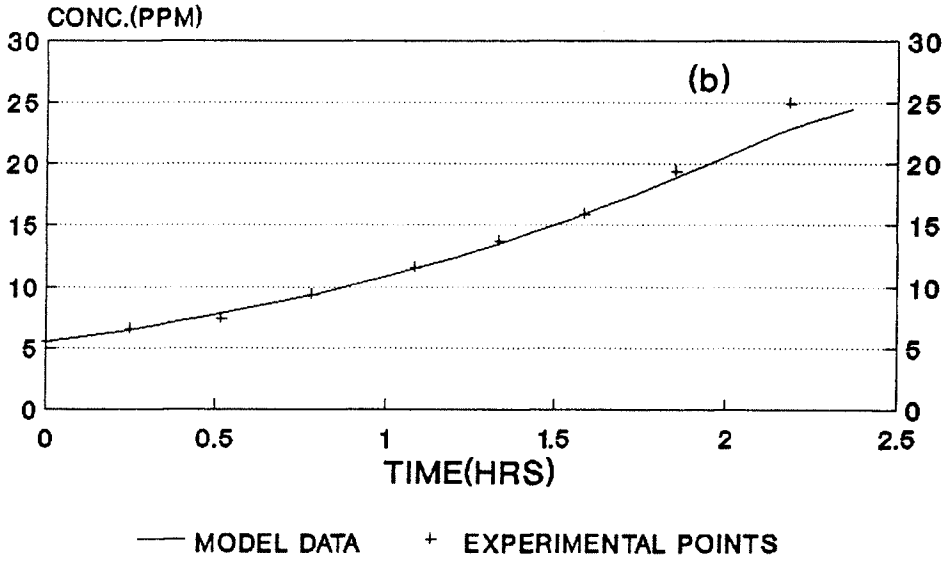
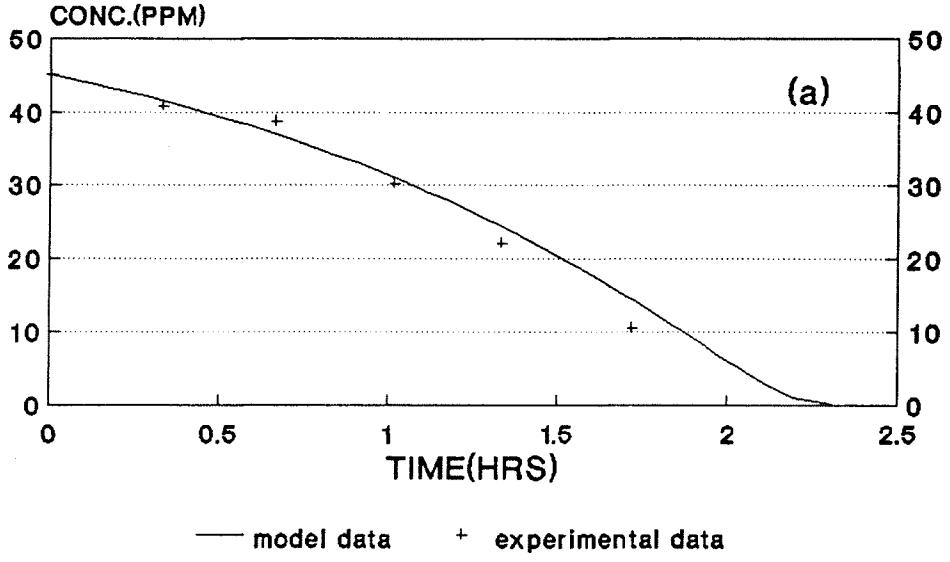


FIG.4.14: 45 PPM BENZOIC ACID
SUBSTRATE CONC.



45 PPM BENZOIC ACID
BIOMASS CONC.

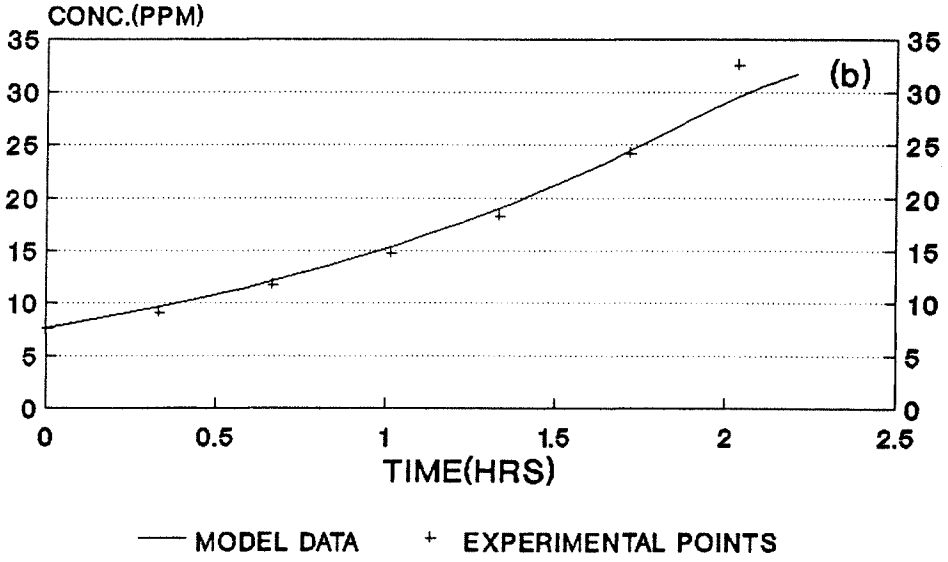
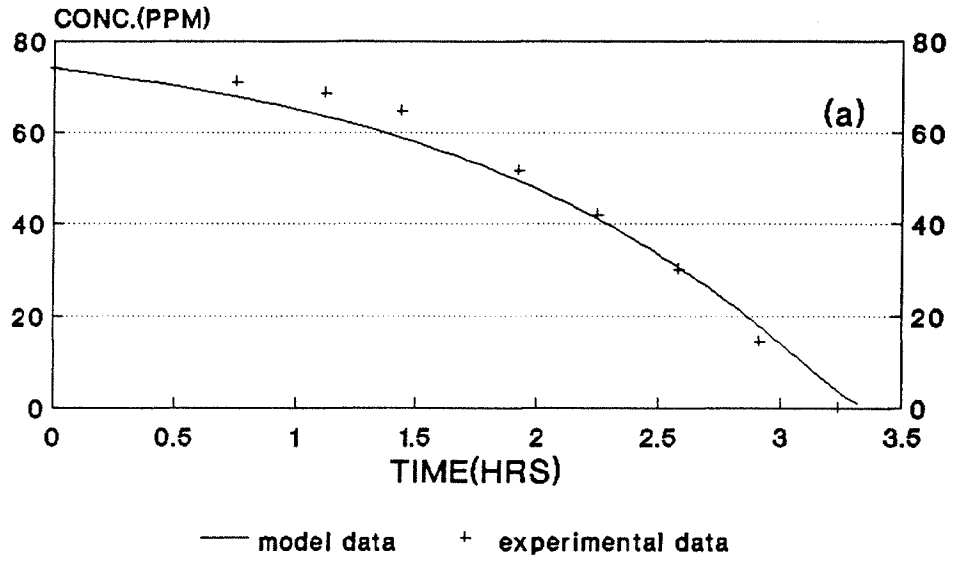
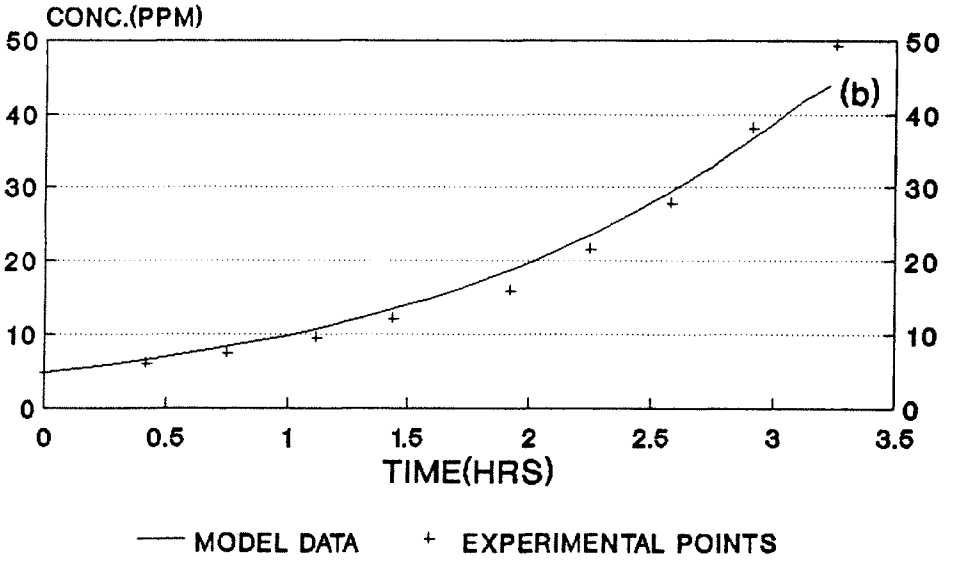


FIG.4.15: 74 PPM BENZOIC ACID
SUBSTRATE CONC.

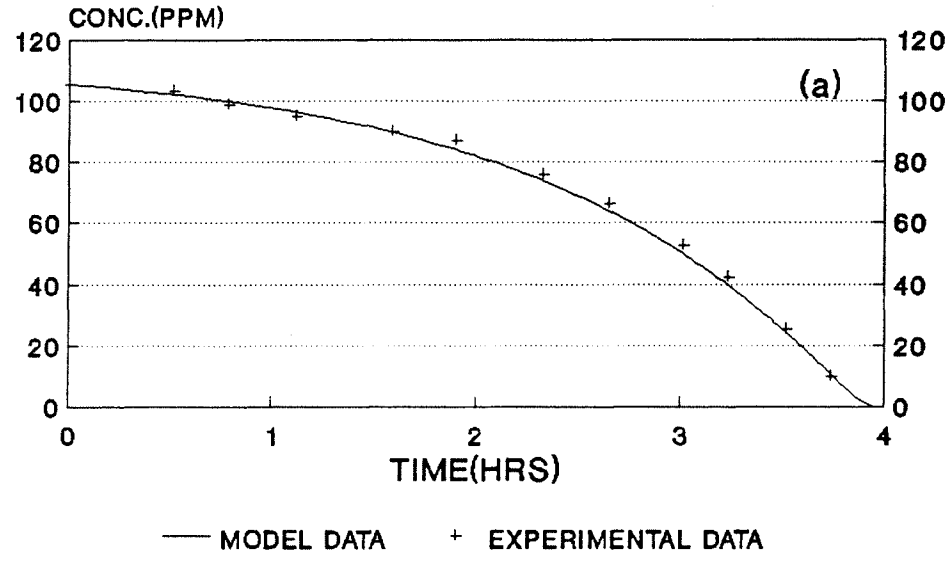


74 PPM BENZOIC ACID
BIOMASS CONC.



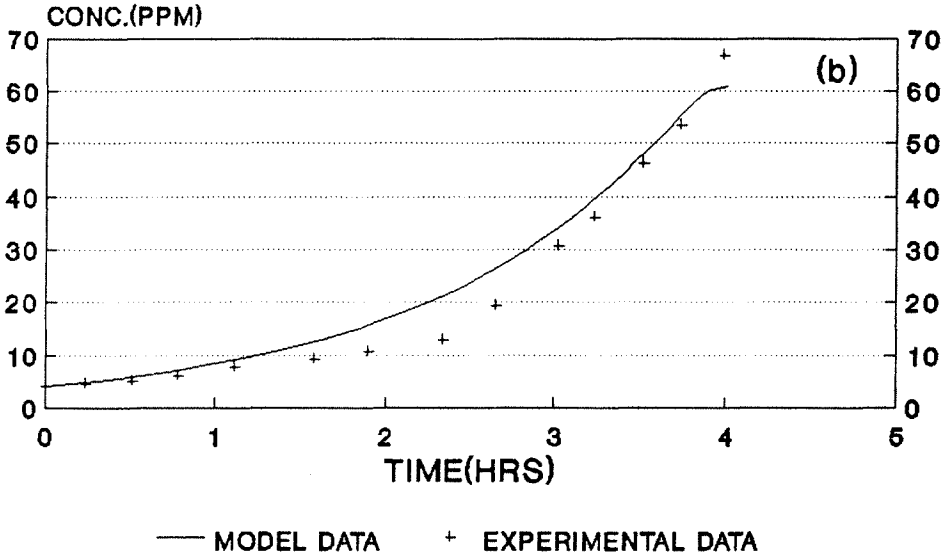
105.5 PPM BENZOIC ACID

FIG.4.16: SUBSTRATE CONC.



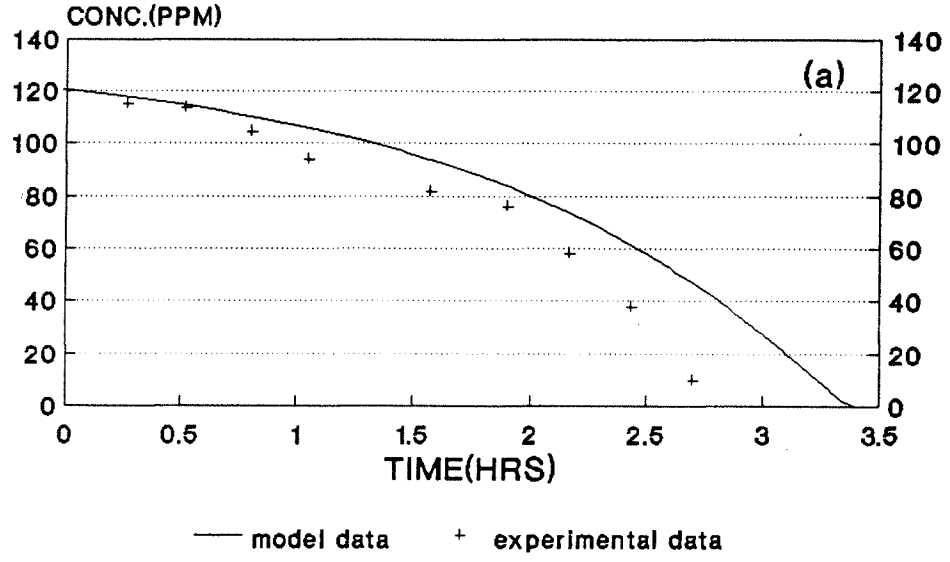
105.5 PPM BENZOIC ACID

BIOMASS CONC.



120 PPM BENZOIC ACID

FIG.4.17: SUBSTRATE CONC.



120 PPM BENZOIC ACID

BIOMASS CONC.

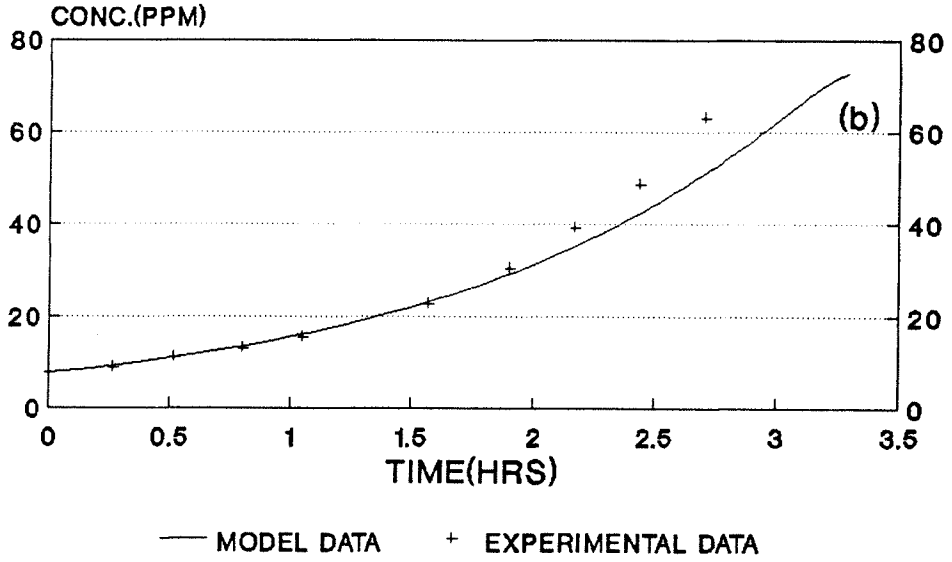
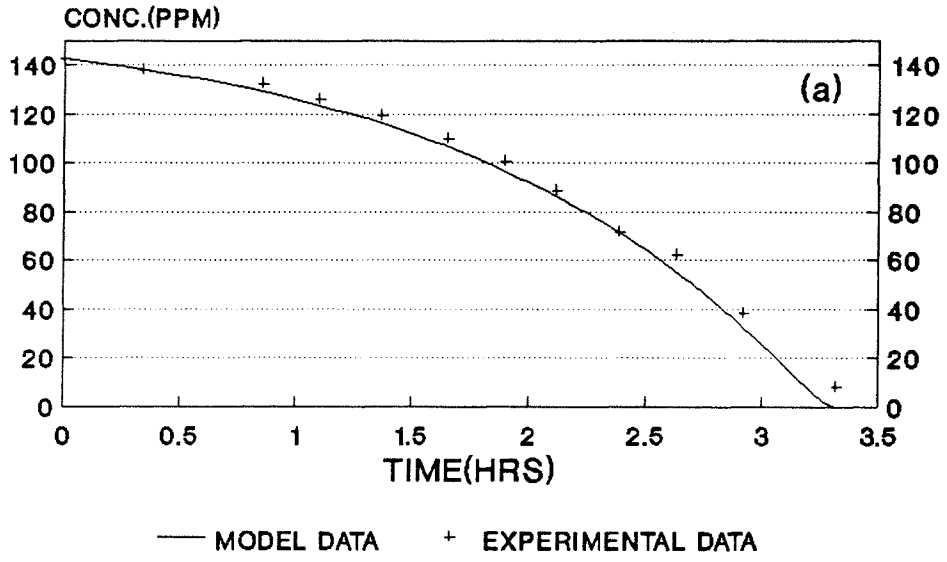


FIG.4.18: 143 PPM BENZOIC ACID
SUBSTRATE CONC.



143 PPM BENZOIC ACID
BIOMASS CONC.

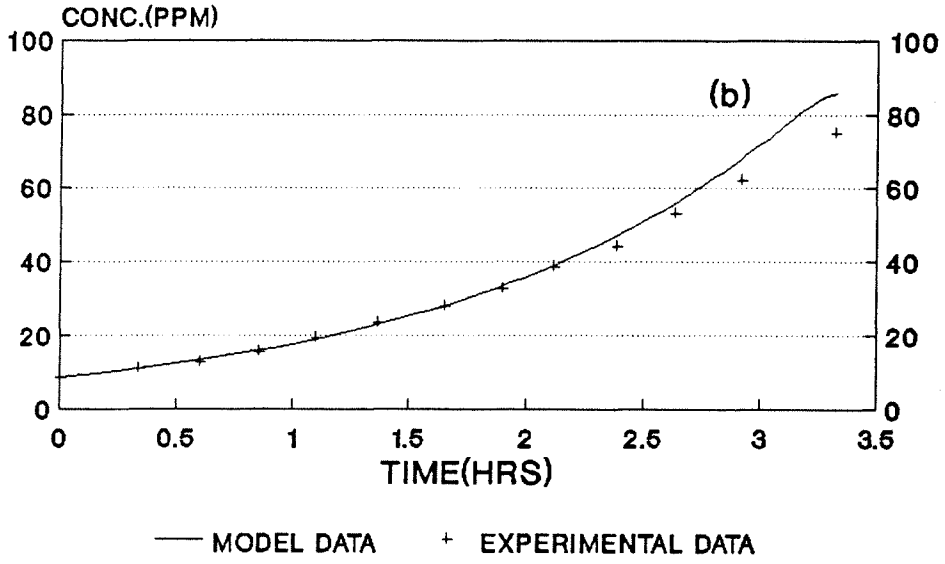
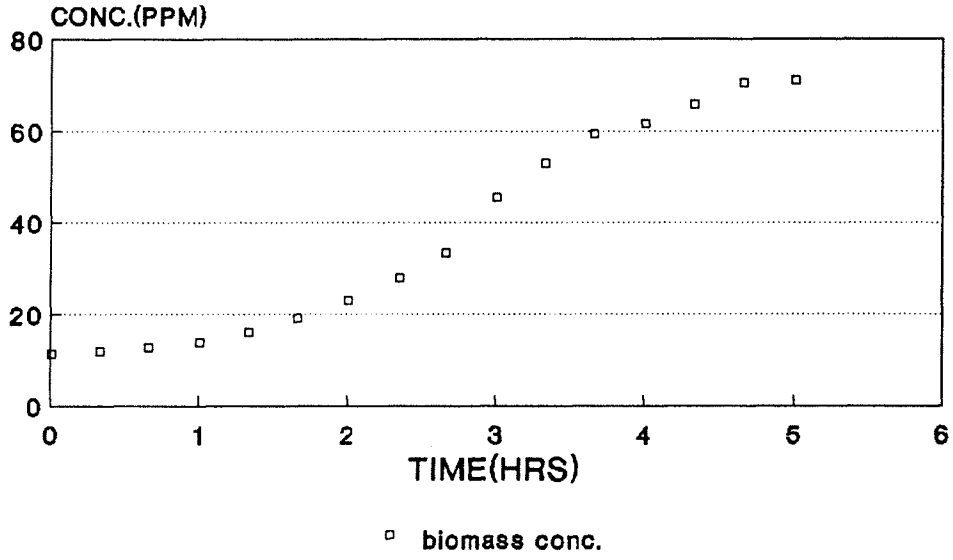
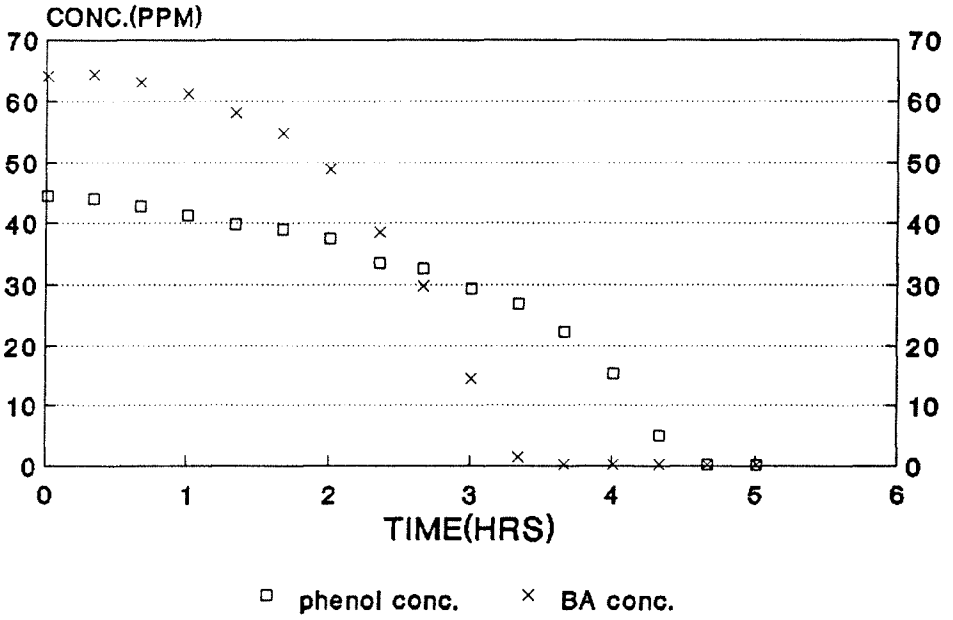


FIG.5.1.1 BIOMASS GROWTH IN 40PPM PHENOL-60 PPM BA



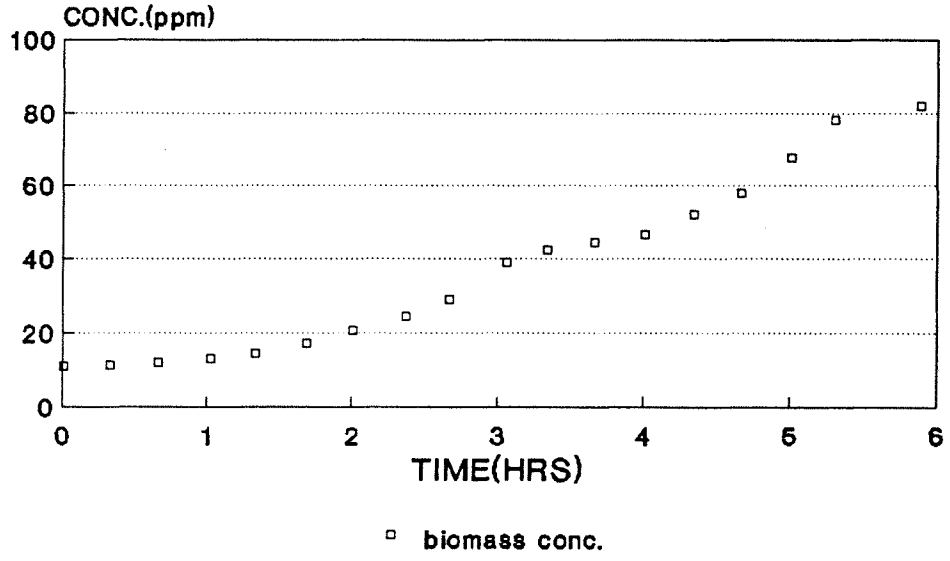
BA:benzoic acid

FIG.5.1.2 SUBSTRATE DEGRADATION BY P.AERUGINOSA IN 40 PPM PHENOL-60 PPM BA



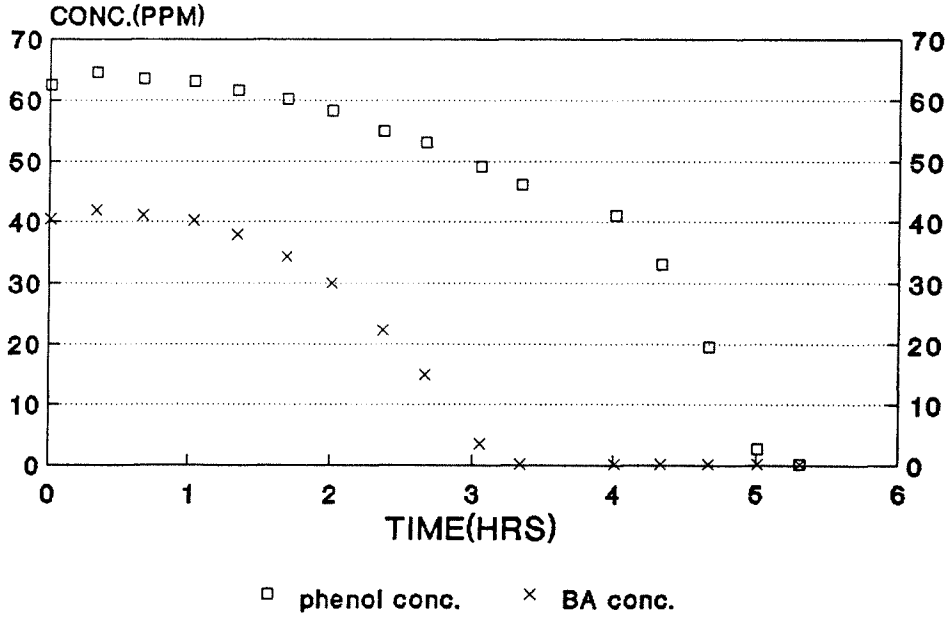
BA:benzoic acid

FIG.5.2.1 BIOMASS GROWTH IN 60 PPM PHENOL-40 PPM BA



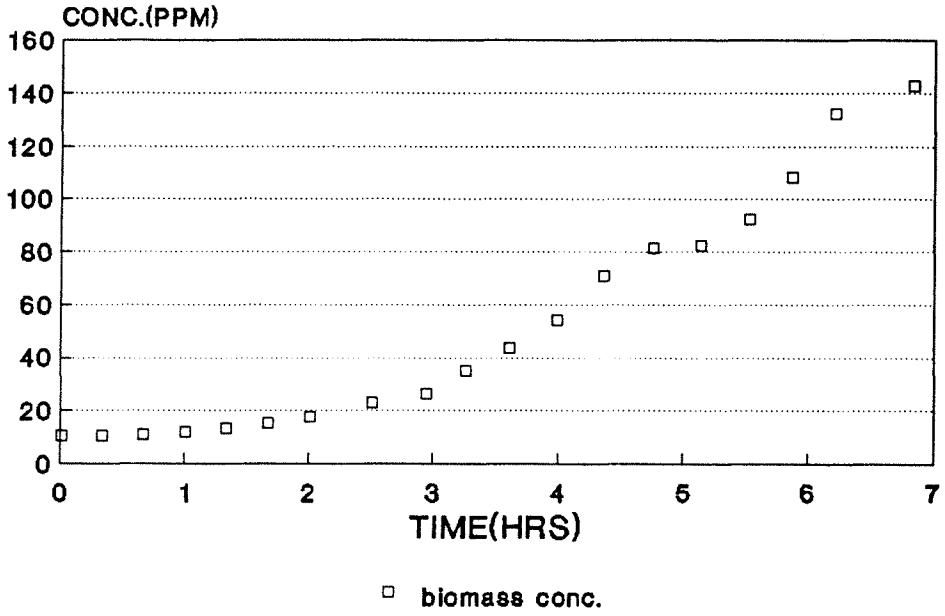
BA:benzoic acid

FIG.5.2.2 SUBSTRATE DEGRADATION BY P.AERUGINOSA IN 60 PPM PHENOL-40 PPM BA



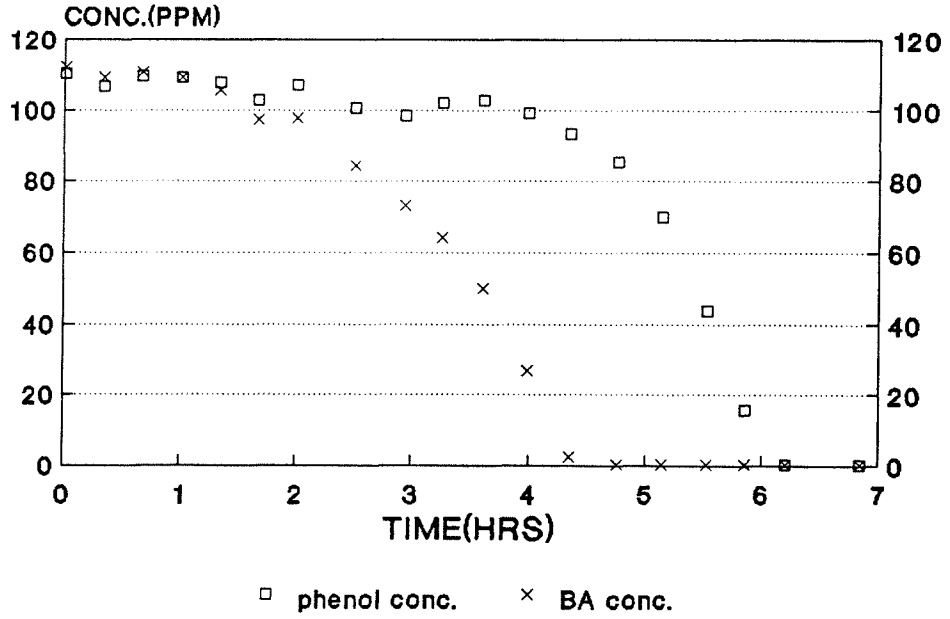
BA:benzoic acid

FIG.5.3.1 BIOMASS GROWTH IN 100 PPM PHENOL AND 100 PPM BA



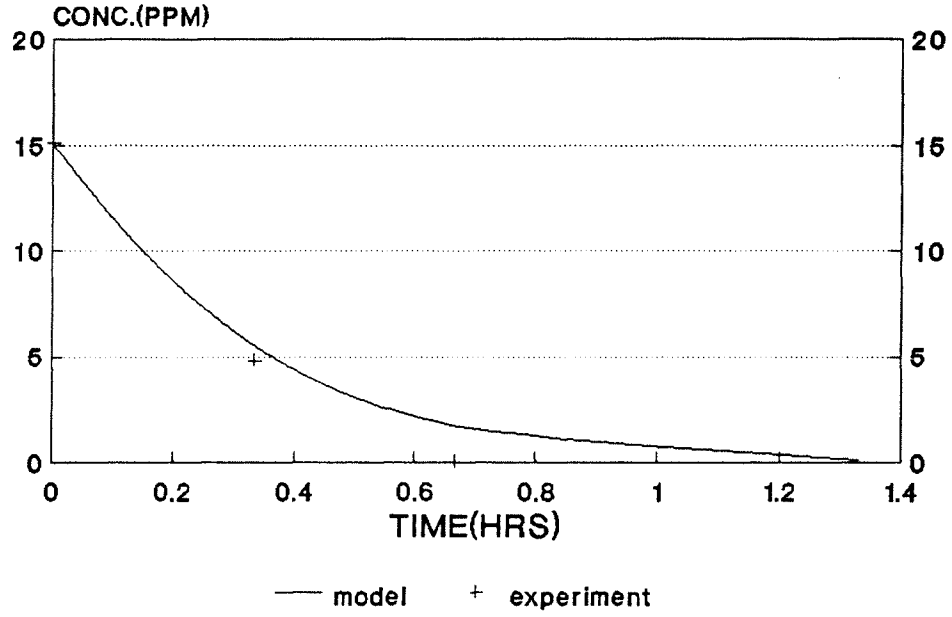
BA:benzoic acid

FIG.5.3.2 SUBSTRATE DEGRADATION BY P.AERUGINOSA IN 100 PPM PHENOL-100 PPM BA



BA:benzoic acid

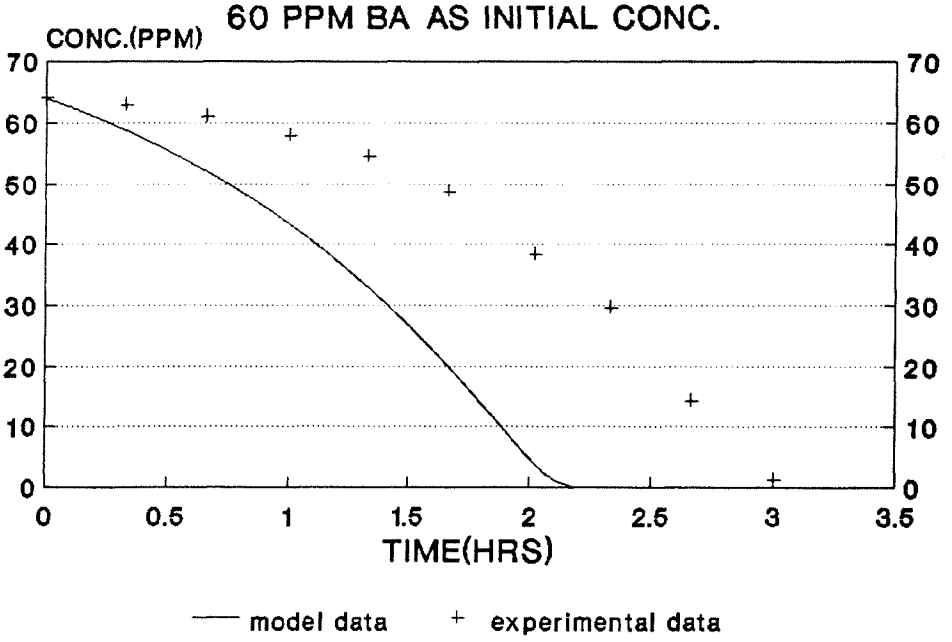
FIG.6.1.1:PHENOL DEGRADATION* IN 40 PPM PHENOL AND 60 PPM BA AS INITIAL CONC.



BA:benzoic acid

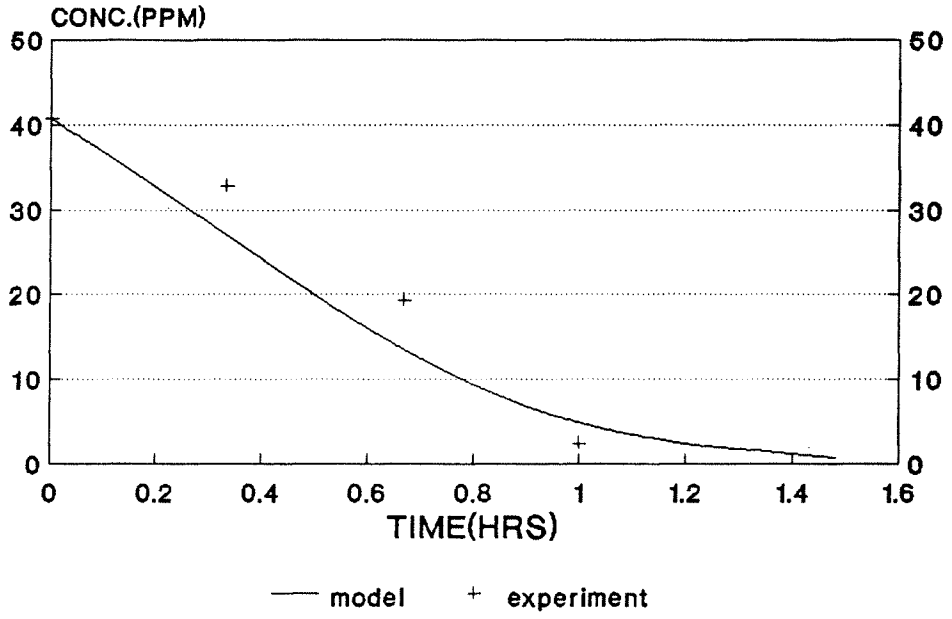
* when all benzoic acid is depleted out

FIG.6.1.2:DEGRADATION IN 40 PPM PHENOL AND 60 PPM BA AS INITIAL CONC.



BA:benzoic acid

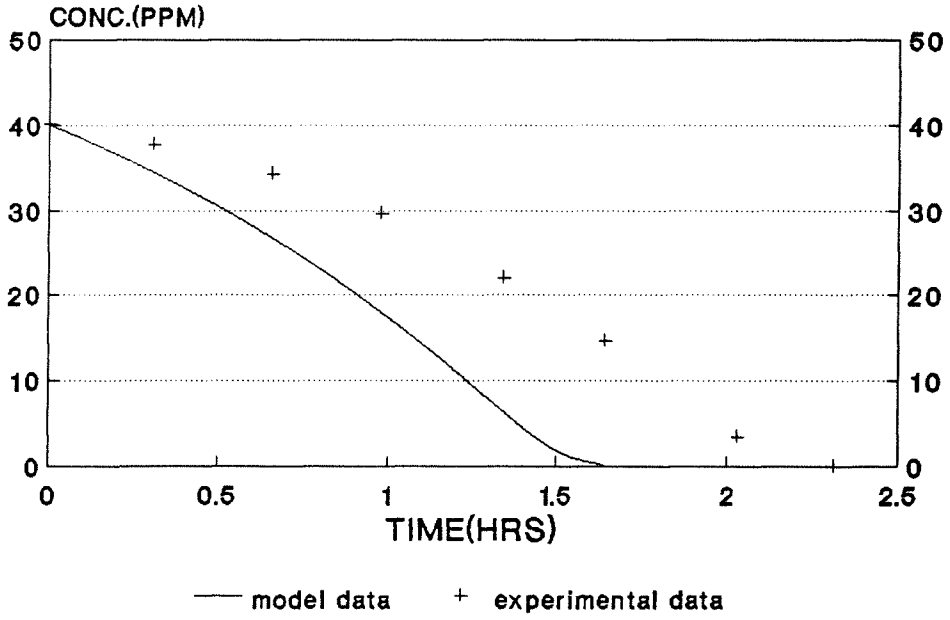
FIG.6.2.1: PHENOL DEGRADATION* IN 60 PPM PHENOL AND 40 PPM BA AS INITIAL CONC.



*:when all benzoic acid is depleted out

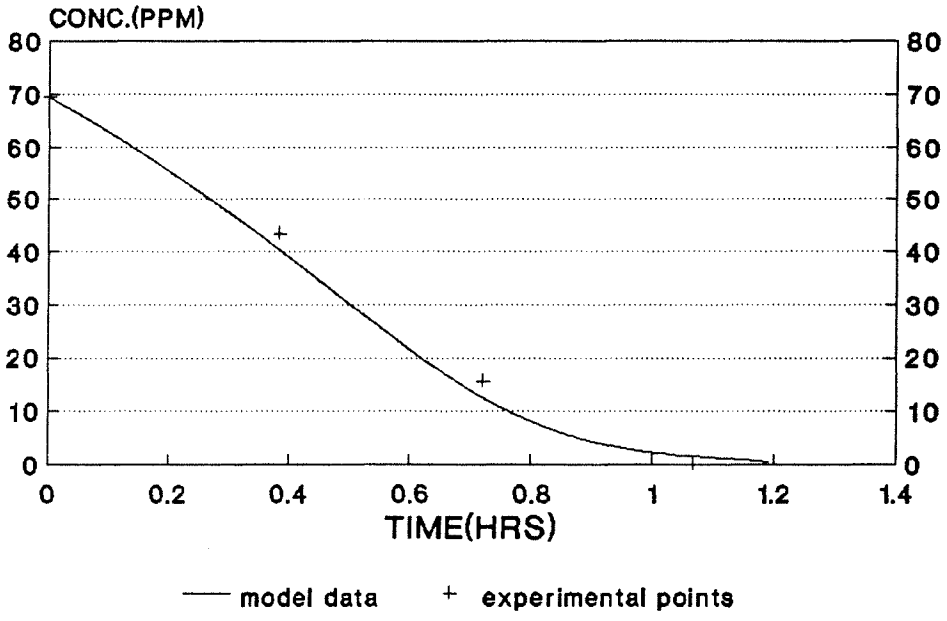
BA:benzoic acid

FIG.6.2.2:BA DEGRADATION IN 60 PPM PHENOL AND 40 PPM BA AS INITIAL CONC.



BA:benzoic acid

FIG.6.3.1 PHENOL DEGRADATION *IN 100 PPM PHENOL-100 PPM BA AS INITIAL CONC.



* after all BA is depleted out

FIG.6.3.2 BENZOIC ACID DEGRADATION IN 100 PPM PHENOL-100 PPM BA

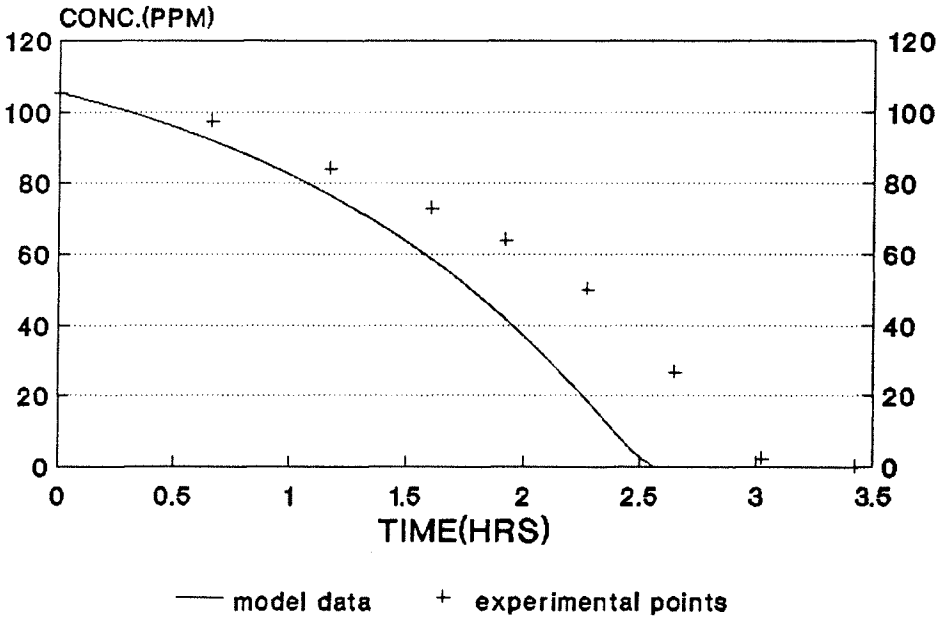
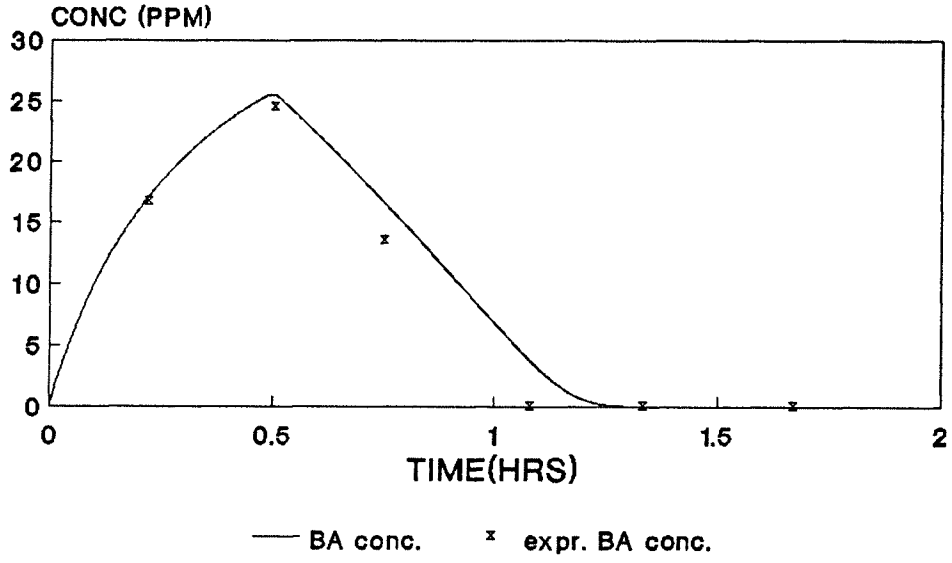
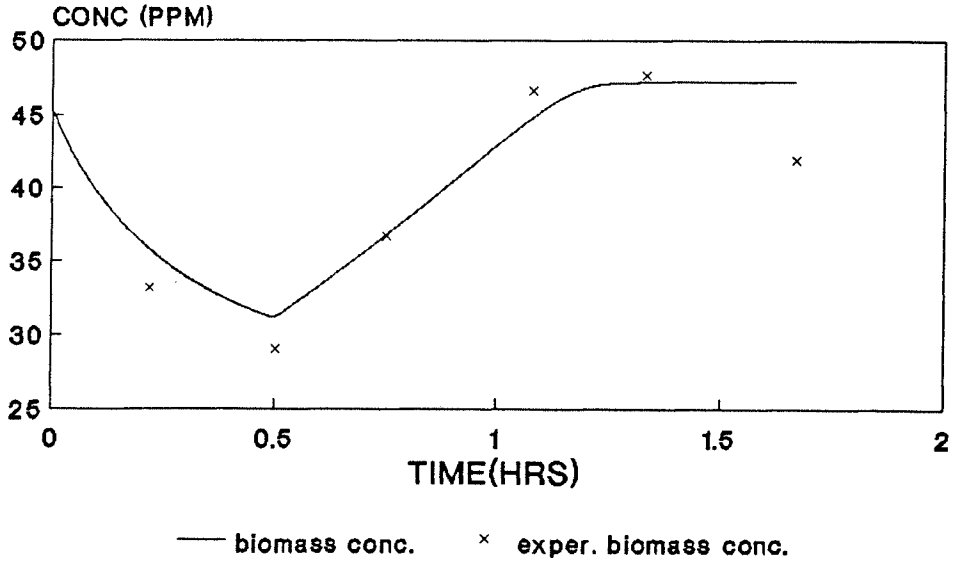


FIG 7.1 SBR RUN2 CYCLE 3
BENZOIC ACID ONLY



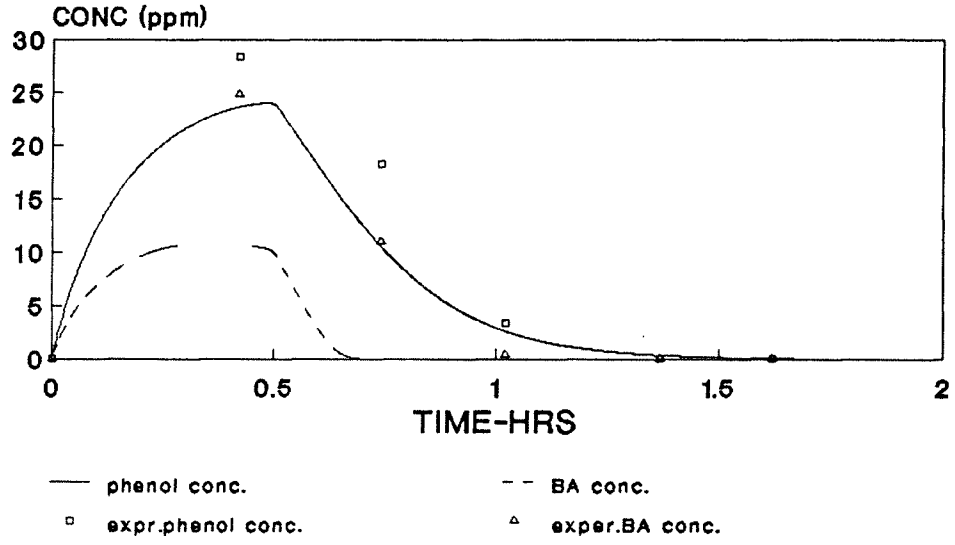
BA: benzoic acid

FIG. 7.2 SBR RUN2 CYCLE 3
BENZOIC ACID ONLY



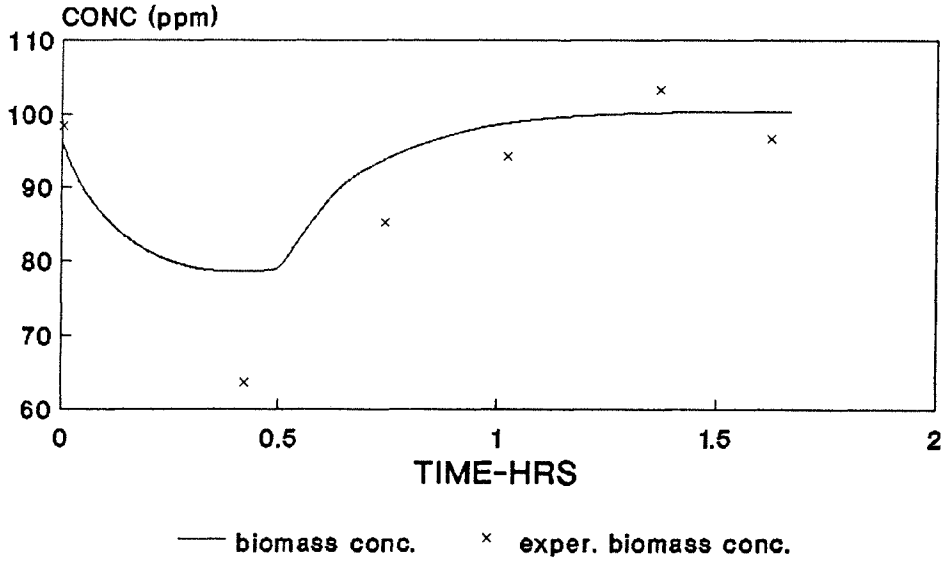
BA: benzoic acid

FIG.8.1 SBR RUN2 CYCLE 4
phenol-benzoic acid



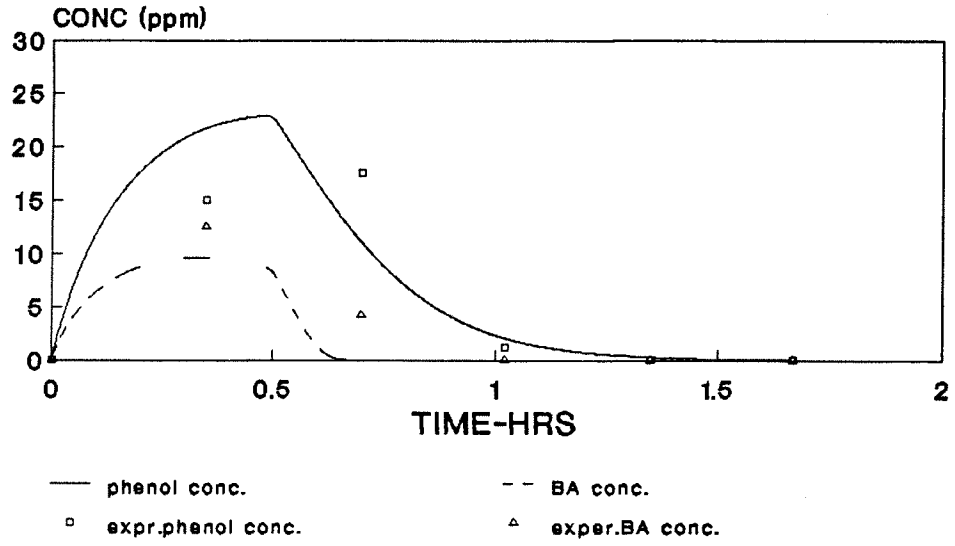
BA: benzoic acid

FIG.8.2 SBR RUN2 CYCLE 4
phenol-benzoic acid



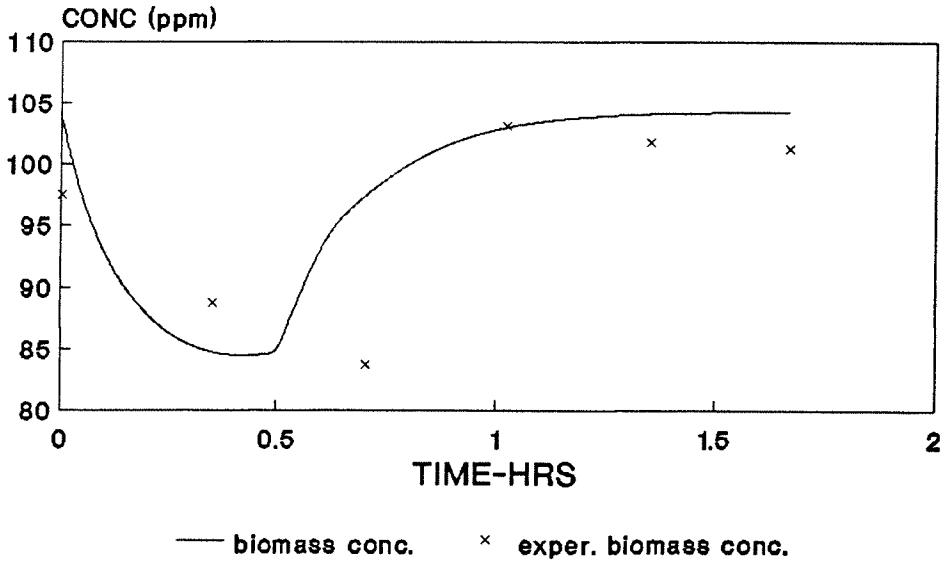
BA: benzoic acid

FIG.9.1 SBR RUN2 CYCLE 10
phenol-benzoic acid



BA: benzoic acid

FIG.9.2 SBR RUN2 CYCLE 10
phenol-benzoic acid



BA: benzoic acid