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ABSTRACT

KINETICS OF PHENOL BIODEGRADATION BY *Pseudomonas resinovorans* (ATCC 14235)

by

Susmita Guptapal,

Master of Science in Environmental Science,

January, 1992

Thesis directed by : Dr. Basil C. Baltzis _____

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The kinetics of phenol biodegradation were studied by using a pure culture of *Pseudomonas resinovorans* (ATCC 14235). Experiments were performed in shaker flasks and samples were analyzed for biomass content (based on optical density), and for phenol concentrations (based on HPLC measurements). The experimental data indicated that phenol is an inhibitory substrate at high concentrations. Specific growth rate data were fitted to an Andrews' expression with good success. The regressed kinetic constants were also used in predicting phenol biodegradation as a function of time. It was found that predicted and actual experimental values were close at least for the first part of each experiment. Deviations between experimental data and predicted values are believed to be due to oxygen limitation at high biomass concentrations. Oxygen was not monitored in detail during this study. Periodic plating indicated that the culture remained pure in all experiments.

KINETICS OF PHENOL BIODEGRADATION BY *PSEUDOMONAS RESINOVORANS* (ATCC 14235)

by

SUSMITA GUPTAPAL

Thesis submitted to the Faculty of Graduate Division of the

New Jersy Institute of Technology

in Partial Fulfilment of the Requirements for the Degree of

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January, 1992

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This thesis is dedicated to my mother

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I. INTRODUCTION

Phenol is a very common waste product of the pharmaceutical, fertilizer, polymeric resin and oil refining industries. Phenol is also the basic structural unit for a variety of commercial compounds, including many agricultural chemicals; it is also a common transformation product of many pesticides. According to RCRA (Resource Conservation Recovery Act) phenol is a `listed' hazardous waste. Phenol is toxic to fish and other aquatic species at concentrations as low as 5 ppm, and it gives objectionable taste and smell to the drinking water even in trace amounts. Therefore removal of phenol from the wastewater or from the natural environment is of major concern.

Among various methods, such as adsorption, ion-exchange, stabilization and other physical/ chemical treatment methods which have been studied, bioremediation is a preferred methodology. Though attention has been given to the kinetics of biodegradation of phenol, most prior studies deal with low concentrations of phenol in ppb and low ppm ranges [4, 7, 19, 21]. Furthermore, almost all prior kinetic studies report biodegradation of phenol by mixed, indigenous, unspecified cultures [2, 3, 4, 5, 8, 11, 18, 19, 21]. Moreover, very little concern has been given to the appropriateness of the various kinetic models employed.

In the present work, a detailed study of the kinetics of phenol biodegradation is described for a wide range of substrate concentrations (11 ppm to 170 ppm). It has been found in batch tests that an oxygen deficiency occurs at the higher phenol concentrations which may change the mechanism of enzymatic action. The biomass concentration was measured by optical density changes, and the phenol concentration was followed using HPLC. A single species, *Pseudomonas resinovorans* (ATCC 14235) was used in this work.

II.OBJECTIVES

The objectives of the present work are to identify a suitable bacterial species for biodegradation of phenol, mathematically describe the kinetics of growth of the species and phenol degradation, and verify the predicted model by shaker flask experiments.

This work can be viewed as the initial step in the degradation of a single substrate by a mixed culture.

The species studied was *Pseudomonas resinovorans* (ATCC 14235) which was chosen because of prior reports of phenol biodegradation [1]. Moreover most of the *pseudomonads* can easily adapt to a variety of environmental conditions and it was hoped that other phenol degrading *pseudomonads* would be found to coexist with *P. resinovorans*.

III. LITERATURE REVIEW

Because of the importance of phenol as an environmental pollutant, the biodegradation of this compound has been studied extensively. Most of the published studies have utilized *Pseudomonas putida, P. aeruginosa, Flavobacterium* and *Alcaligens* as phenol degrading species. Many reports have also been found on phenol degradation with mixed culture, either aerobically or anaerobically. No work has been reported yet on phenol biodegradation by *Pseudomonas resinovorans,* though it is very good user of phenol as sole carbon and energy source.

Bergey's manual (1984) [1] describes various characteristics of the family Pseudomonaceae, genus *Pseudomonas* and also different species of *Pseudomonas. Pseudomonas resinovorans* is thoroughly described in this manual. It has been reported that *P. resinovorans* are resin digesting, rod shaped bacteria of size 0.6 - 0.7 x 2.0 - 2.5 μm; they are motile by means of a polar flagellum; they produce fluorescent pigment; can not liquify gelatin; nitrate reduction is very weak; starch hydrolysis is weak; positive oxidase reaction. Optimum growth temperature is 28 - 30^{o}C, with no growth below 5^{o}C or above 42^{o}C. No acid produced from glucose, fructose, galactose etc. Growth occurs at the expense of colophony, canada balsam, or abietic acid. Phenol and napthalene can be used as sole carbon and energy sources for growth.

Jones and Alexander [11] have studied the kinetics of mineralization of phenol in lake water, at concentrations from 200 pg/ml to 5 μg/ml. The mineralization data were fit by nonlinear regression to equations for 14 kinetic models that describe patterns of biodegradation by nongrowing cells or by microorganisms growing on either the test chemical or other organic substrates. The kinetics of mineralization of phenol in water samples collected was best described by first order models for 0.5 ng of phenol per ml;

by Michaelis-Menten, logistic, and logarithmic models for 1.0 ng/ml, 2.0 ng/ml and 5.0 ng/ml to 1.0 μg/ml, respectively, if it is assumed that the mineralizing population uses phenol as the sole carbon source for growth. Under the test conditions, usually less than 10% of the phenol carbon that was metabolized was incorporated into microbial cells or retained by other particulate material in the water at substrate concentrations of 10 ng/ml or less, although the percentage increased at higher substrate concentrations. The mineralization of 2 ng of phenol/ml in water samples were best described by logistic or logarithmic models if the mineralizing bacteria were assumed to be growing on phenol. If the sample is incubated for 12 hours, the mineralization follows the zero order kinetics. A statistical F-test was used to compare the residual sums of squares for the best models at each phenol concentration. By the statistical test, the logarithmic model was found to be the best model at a concentration of 5000 ng of phenol per ml. The first order model was the best representation for both sets of models at 0.5 ng/ml. They have also found that the models based on considerations of the kinetics of biodegradation of organic compounds not supporting growth (i.e. 1 ng of phenol/ml of water) seem inappropriate to apply to the kinetics of biodegradation of compound that support microbial growth (i.e. 1 μg/ml of phenol substrate in the experiment). In these experiments they used the indigenous microbial population from the lake as the source of inoculum. Later they identified some species like *Pseudomonas aeruginosa, Aeromonas hydrophila, and Flavobacterium* as species which take part in phenol mineralization. They determined the growth rate of *P. aeruginosa* in phenol and found that the linear rate of biodegradation at low phenol concentration changes after 12 hours incubation of the microbial culture.

Rubin and Schmidt [19] also studied the growth of phenol mineralizing microorganisms present in samples of fresh water collected from lakes in New Jersey and New York. Sixty five percent or greater mineralization of phenol was considered a

positive test in the most probable number technique. Phenol mineralization rates were obtained by measuring the amount of exogenous phenol that disappeared from solution over time. Several species of actinomycetes, yeast, and several other unidentified bacteria were shown to metabolize phenol. Radiolabeled phenol was used, and mineralization was measured by radioisotope counting method. They reported an initial lag phase for both phenol mineralization and growth of phenol mineralizing microbes. After 18 hours incubation of the microbial culture, the growth rate slightly increased which indicated that mesotrophic organisms were responsible for mineralization. There were several indications that phenol is inhibitory in the ppm range. The phenol mineralizing rate and the number of microorganisms were greater at 10 ppm than 100 ppm. They reported a linear relationship between phenol concentration and phenol mineralization rate from 2 ppm to 10 ppm. At 100 ppm the degradation rate had dropped back down to that at 2.6 ppm. They also found that phenol mineralization rates were 6.3 times greater for bacteria than for fungi. They showed that the minimum amount of phenol required for the growth of mineralizing bacteria is 1 μg/ml. Later they found most of the phenol mineralizing bacteria were mesotrophic (grow best at 25-35^{o}C), though some can grow at higher temperature, and their growth followed Andrews' inhibitory model.

Chesney, Sollitti and Rubin [4] studied the fate of phenol in fresh water, and correlated the growth of microorganisms with phenol degradation, at phenol concentrations ranging from 1 ng/ml to 1 μg/ml. Approximately 20 % of the parent phenol was incorporated into the cell mass of the phenol degrading organisms. There was no apparent lag period before phenol utilization commenced, and incorporation was complete within 2 hours at all concentrations tested. A direct relationship was found between the initial phenol concentrations and both phenol mineralization and incorporation rates. At all concentrations, approximately 80 % of the initial phenol was

mineralized. The length of time for maximal amounts of phenol incorporation to occur decreased from 2 to 0.33 hours as the phenol concentration increased from 10 to 100 ng/ml. They measured the incorporation of phenol into the cell biomass using radiolabeled carbon. Mineralization was measured by disappearance of phenol from the water sample. They used indigenous microflora in fresh water and did not identify them.

Paris, Wolfe, Steen and Baughman [17] of EPA studied to find the effect of phenol molecular structure on bacterial transformation rates in pond and river samples. They tested a series of phenol compounds with *Pseudomonas putida* and found a correlation between microbial transformation rates and the van der Waal's radius of the compounds. They found that all phenols degraded by *P. putida* were converted into their corresponding catechols except p-hydrobenzoic acid, the product of p-acetylphenol. The correlation was also found to be valid for a mixed population. These results suggest that this approach may be useful for predicting the rate of transformation of xenobiotics by microorganisms in aquatic systems, as well as providing some insight into the details of the transformation at the mechanistic level.

Paris, Steen, Baughman, and Bernett [18] examined the kinetics and modelling of microbial degradation of organic compounds in natural waters. They did a shaker flask study with mixed culture, where bacterial levels and phenol concentrations were measured by plate counts and gas chromatography, respectively. They found that the microbial degradation for malathion, butoxy ethyl ester, and 2, 4-dichlorophenoxyacetic acid follow a second order model, i.e. the expression is as follows:

$$\frac{ds}{dt} = \frac{-\mu_m s}{K+s} \frac{b}{Y}$$ (where s is very small in comparison to K & K+s=K)

It was previously reported as a Monod model dependence, but later they found experimentally that the compound degradation rate was first order only in very low concentrations of substrate and the pseudo first order rate constants were proportional to bacterial concentrations. The reliability of second order rate constants for assessing microbial degradation kinetics in natural waters was confirmed by additional studies. They also mentioned that pH, microbial population, water temperature, and natural characteristics of xenobiotics will influence the transport and fate of chemicals in aquatic environments.

Folsom, Chapman, and Pritchard [7] studied the biodegradation kinetics of phenol, using a *Pseudomonas cepacia* G4 strain. The cells were grown in a stirred chemostat with 5mM phenol as the sole carbon source in a defined basal salts medium (BSM) at pH 7.5. Degradation rate assays were performed at room temperature, and biomass growth was measured by cell protein assay using the BCA method. The rates at which cells degraded were determined by monitoring changes in phenol concentration, using a modified colorimetric assay. Phenol concentrations were calculated by reference to a standard curve. Generally, phenol disappearance rates were calculated from six determinations over a 10 minute time period. Rates of phenol disappearance were calculated and reported as nanomoles per minute per milligram of protein. Inhibition of phenol degradation at high phenol concentrations was modeled by the Andrews expression. Phenol disappearance exhibited first order rate increases with phenol concentrations upto 50 μM and decreased in rate at higher concentrations. The experiments were conducted over 8 hours period during which the rate of phenol disappearance remained essentially constant (though a short lag period before the onset of phenol disappearance was noted after 8 hours of incubation). The apparent values of K_s, K_i and V_{max} for the best fit were reported as 8.5 μM, 454 μM, and 466 nmol/minute/mg of protein. They have reported another interesting observation that

the *P. cepacia* G4 grown on phenol as the sole carbon source produces catabolic enzymes required for both phenol and trichloroethylene degradation. Theoretically, inhibition is likely to be severe when both phenol and TCE are present, since their K_s values are almost the same, and experimental evidence bears this out. At equal concentration of phenol and TCE, a decrease of about 50% in the rate of phenol degradation was observed.

Biodegradation kinetics of substituted phenolics by electrolytic respirometry was reported by Brown, Grady and Tabak [2]. They used a bacterial culture of sewage origin to degrade phenol, chlorophenol, cresol, 2,4-dimethylphenol (2,4-DMP) and 2,4 dinitrophenol. The substrate utilization was monitored by the oxygen consumption using a sapromat electrolytic respirometer, interfaced with a microcomputer. In order for oxygen consumption to be a surrogate measurement from which the kinetics of biodegradation could be obtained, the concentration of the substrates were expressed as chemical oxygen demand (COD). Thus all mass-related parameters, such as K_s and K_i, are expressed as mg/l COD, and the yield (Y) has units of mass of biomass COD formed per unit of substrate COD removed. The experiments were conducted in batch mode at two different initial phenol concentrations (10 ppm and 100 ppm). During the experimental procedure the endogenous metabolism and biomass decay were neglected. They found that the biomass concentration for phenol in a batch reactor increased at a rate expressed by Monod kinetics, and oxygen was not found to be a limiting factor until 100 ppm. They also conducted the same experiment with both phenol and 2,4-DMP and found the shape of the curve of oxygen uptake was different than the experimental curve without 2,4-DMP. They reported that 4-chlorophenol and 2,4-dinitrophenol exhibited inhibition kinetics, but that phenol degradation was characterized by Monod kinetics. To evaluate the kinetics of biodegradation of different substrates they used spreadsheets and non linear curve fitting.

Speitel and DiGiano [21] determined microbial kinetic coefficients through measurement of initial rates by using radiolabeled phenol. They developed a new method for the measurement of Monod kinetic coefficients in a batch reactor, which was much faster and easier than chemostat studies. They also measured radioactivity of ^{14}C labeled biomass in a separate experiment. Experiments were conducted at $25^{o}C$ and a wide range of phenol concentrations (5 to 5000 μg/l) and kinetics were found to be of the Monod type. They also reported a long lag phase at all concentrations.

Biodegradation of p-nitrophenol(PNP) in an aqueous waste stream by immobilized bacteria was studied by Heitkamp, Camel, Reuter and Adams [8]. They identified three different *Pseudomonas* species from activated sludge for degradation of PNP as the sole source of carbon and energy. The species they isolated were ***P. flourescens, P. putida, and P. mendocina***. The identification was accomplished using the Biolog Identification System. They found the rate of degradation of PNP in a chemostat was higher using immobilized bacteria than that for the free cells. Also they have reported that the rates of biodegradation of PNP were different at different initial substrate concentrations, and followed Andrews kinetics, with a lag phase of 4 hours.

Byung, Chain, Cross and Cheng [12] have reported the use of adsorption, desorption and bioregeneration techniques in an anaerobic, granular activated carbon reactor for the removal of phenol. They used a two stage, pilot scale anaerobic GAC reactor. The results, obtained from running the reactor for 200 days indicated that the contribution of biogas production, adsorption, and biomass production were all important in the removal of phenol, among which biogas production was the most important.

Yang and Humphrey [22] studied the microbial degradation of phenol by pure and mixed cultures of *Pseudomonas putida* in batch systems. Their results indicated that it should be possible to achieve phenol removal from wastewater down to a level of 1 to 2 ppm. The kinetic behavior of the microbes were best fitted by the Andrews model.

Hill and Robinson [9] suggested substrate inhibition kinetics for phenol biodegradation by *Pseudomonas putida*. A pure culture was grown in both batch and continuous culture using phenol as the sole carbon source, and the Andrews model was found to best describe the kinetics. It was shown that wall growth exerts a significant effect on the biomass concentration and phenol conversion (both decreased with increasing amount of wall growth).

Schmidt, Scow and Alexander [20] studied the kinetics of simultaneous mineralization of p-nitrophenol(PNP), phenol, and glucose by a *Pseudomonas* species. The species did not mineralize PNP at a concentration of 10 ppb, but metabolized 50 ppb or higher concentration of PNP. The K_s values for three different chemicals are as follows:

	K_s(ppm)
PNP	1.1
Phenol	0.1
Glucose	0.25

The addition of glucose or phenol to the PNP changed the kinetics from a Monod equation to dual substrate and logistic models respectively. Thus the fitting of models to substrate depletion curves may lead to erroneous interpretation of data if the effects of a second substrate on population dynamics are not considered.

Molin and Nilsson [16] studied the degradation of phenol by *Pseudomonas putida* (ATCC 11172) in a continuous culture at different ratios of biofilm surface to culture

volume. *P. putida* was grown in a continuous culture with 50 ppm phenol as the only carbon and energy source. A culture without biofilm was compared with biofilm cultures of differing surface area/volume ratio. The biofilm did not significantly affect the maximal suspended cell concentration in the effluent, but it increased the maximal phenol reduction rate from 0.23 g/l/hr to 0.72 g/l/hr at the highest biofilm level (5.5 cm^2 of biofilm surface/ml of reactor volume). Though it was previously reported that wall growth or biofilm reduce the growth rate, this study revealed that biofilm enhanced the aerobic degradation of aromatic compounds. This might be explained by the biofilm; (i) catabolizing phenol at a higher rate; (ii) creating a physical diffusion barrier against toxic phenol concentration; or (iii) creating a reservoir of biomass not fully exploited for phenol reduction. They also reported pathways of phenol degradation and compared the kinetic parameters for phenol degradation by *Pseudomonas putida* with the previous studies of phenol mineralization by other *Pseudomonas species*. *P. putida* ATCC 11172 degraded phenol by *meta* cleavage pathway which was indicated by the accumulation of 2-HMA. 2-HMA produces yellow color visually discernible in the media and easily recorded by absorbance measurements at 375 nm even at concentration as low as 0.05 ppm. They reported that phenol has a potentially inhibitory effect on cell growth, i.e. at high concentration phenol causes substrate inhibition and follows Andrews kinetics. They compared K_s and K_i values for three species of *Pseudomonas*:

	K_s (mg/l)	K_i (mg/l)
P. putida (ATCC 11172)	3	500
P. putida (ATCC 17484)	> 1	100
P. putida (ATCC 17514).	2	500

Dwyer, Krumme, Boyd and Tiedje [6] have studied kinetics of phenol biodegradation by an immobilized methanogenic consortium. They reported that a phenol degrading

methanogenic enrichment was successfully immobilized in agar as shown by the stoichiometric conversion of phenol to CH_4 and CO_2. The enrichment contained members of three physiological groups necessary for the syntropic mineralization of phenol: a phenol oxidizing bacterium, Methanothrix, and an H_2-utilizing methanogen. The immobilization technique resulted in the cells being embedded in a long, thin agar strand that resembled spaghetti. Immobilization had three effects as shown by a comparative kinetic analysis of phenol degradation by free versus immobilized cells: (i) The maximum rate of phenol degradation was reduced from 14.8 to 10 μg per hour; (ii) the apparent K_m for overall reaction was reduced from 96 to 46 μg per ml, probably because of the retention of acetate, H_2, and CO_2 in the proximity of immobilized methanogens; and (iii) the cells were protected from substrate inhibition caused by high concentrations of phenol, which increased the apparent K_i value from 900 to 1,725 μg per ml. Estimates for the kinetic parameters K_m, K_i, and V_{max} were used in a modified substrate inhibition model that simulated rates of phenol degradation for given phenol concentrations. The simulated rates were in close agreement with experimentally derived rates over a wide range of phenol concentrations.

Boyd, Shelton, Berry and Tiedje [3] examined the anaerobic biodegradation of phenolic compounds in 10% anaerobic sewage sludge. Rates of degradation, mineralization, inhibition of methanogenesis, and intermediates in the degradative pathways were investigated during an 8 week incubation period. Substrate disappearance from 10% anaerobic sewage sludge was monitored by HPLC on a weekly basis, and GC/MS was used to confirm compound identity. Complete mineralization of the phenols in 10% sludge was monitored by measuring net CH_4 production as compared to unamended controls. They found that the time required for complete degradation of phenol was 2 weeks. In general, presence of Cl and NO_2

groups on phenols inhibited methane production. Elimination or transformation of these substituents was accompanied by increased methane production.

Anaerobic oxidation of toluene, phenol, and p-cresol by the dissimilatory iron reducing organism, GS-15 was studied by Lovely and Lonergan [14]. They reported in detail the growth and metabolism of GS-15 on phenol. The dissimilatory Fe(III) reducer, GS-15 is the first microorganism known to couple the oxidation of the aromatic compound to the reduction of Fe(III). The experiments showed that GS-15 obtained energy for growth by completely oxidizing phenol to CO_2 with reduction of Fe(III) as an electron acceptor. In order to test the ability of GS-15 to metabolize phenol, an inoculum of GS-15 was first grown on benzoate to which Fe(III) oxide was added. The culture was then incubated at $30^{o}C$ in the dark with radiolabeled phenol. GS-15 was found to metabolize phenol with concomitant reduction of Fe(III), and this metabolism was associated with cell growth. Low concentration of p-hydroxybenzoate accumulated and then were metabolized. After three cultures had metabolized an initial phenol concentration of 0.42mM, the ratio of phenol oxidized to Fe(II) produced was 29.1+/-1.3. This compared with 28 moles of Fe(II) theoretically reduced during oxidation of phenol to CO_2

Phenol growth kinetics using heterogeneous populations in a two stage continuous culture system was studied by Colvin and Rozich [5]. They determined the biokinetic constants of an acclimated heterogeneous population at phenol concentrations upto 500 ppm. They also studied the kinetics in batch method and compared them with the continuous culture system studies. For batch studies they collected growth data at different phenol concentrations, which was fitted with the Haldane model. They noticed that there was no degree of acclimation that could relieve the inhibitory effect of phenol. Also, the continuous flow data appeared to contradict the batch data, and

they suggested that phenol metabolism could be modeled using a noninhibitory growth function (such as the Monod equation), whereas the batch growth data clearly followed an inhibitory model. They suggested this discrepancy resulted from both ecological and reactor engineering differences. This disparity was not found in case of pure culture.

Lallai and Mura [13] studied the pH variation during phenol biodegradation in mixed cultures of microorganisms. The behavior of pH was investigated in two mixed microbial cultures growing aerobically in a batch reactor with phenol as the limiting substrate. A buffered synthetic medium was used, and in all experiments a variation in pH was observed. It was noted that the pH first decreased and then increased. The initial phenol concentrations used in the experiments ranged from 60 to 1000 ppm, for the first culture (Phenobac), and from 50 to 600 ppm for the second (Polybac). From this report, it emerges that the extent of the drop in pH depends upon the initial phenol concentration.

Biodegradation pathway of o-cresol by heterogeneous culture of phenol-acclimated activated sludge was studied by Masunaga, Urushigawa, Yonezawa [15]. Metabolic intermediates were analyzed by GC-MS. o-Cresol was first transformed into three dihydroxytoluenes. Among them 3-methylcatechol was the main route, and it was further degraded through at least two meta cleavage pathways, which indicated that various metabolisms with main and side pathways coexisted in the biodegradation process.

Control of catechol meta cleavage pathway in *Alcaligens eutrophus* was studied by Hughes and Bayly [10]. *Alcaligenes eutrophus* 335 (ATCC 17697) metabolizes phenol and p-cresol via a catechol meta cleavage pathway. Studies with mutant strains, each

defective in an enzyme of the pathway, showed that six enzymes are induced by the primary substrate. Studies with a putative polarity mutant defective in the expression of aldehyde dehydrogenase suggested that the structural genes encoding this and subsequent enzymes of the pathway exist in the same operon. From studies with mutant strains that constitutively synthesize catechol 2,3-oxygenase and subsequent enzymes, and from coordination of repression of these enzymes by p-toluate, benzoate, and acetate, it has been proposed the catechol 2,3-oxygenase structural gene is situated in this operon (2,3-oxygenase operon). Studies with regulatory mutant strains suggested that the 2,3-oxygenase operon is under negative control.

Many other papers reported on the pathways of phenol biodegradation. A few reports mentioned that the mechanism is exoenzymatic as well as endoenzymatic. Some of the reports also mentioned that the kinetic results are not exactly reproducible because of changes in the bacteria and sensitivity to various parameters. From a survey of many reports we can conclude that the history of the acclimatization of bacteria is another important factor to be considered during the growth kinetic studies in batch systems.

IV. MATHEMATICAL MODEL

Predicting the role of microorganisms in controlling the fate and concentration of xenobiotics in the natural environment requires a reliable mathematical description of the kinetics. For maximal use, the kinetic description should incorporate parameters that allow prediction of rates of degradation in various environments. Even if they are not highly accurate, relationships of this type would be of great use in predicting the behaviour of pollutants in the environment.

In most of the earlier studies reported in the literature, bacteria were cultured in a defined medium with very low concentrations of xenobiotics as the sole carbon source. The rate of biodegradation is described by the following expressions obtained by mass balance over the substrate (s) and the biomass :

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

$$\frac{db}{dt} = \mu\, b \qquad (2)$$

Where s = substrate concentrations; t = time; b = biomass concentrations; μ = specific growth rate; Y = yield coefficient.

Now the equation (2) can be written as

$$\frac{db}{b} = \mu\, dt \qquad (3)$$

Assuming constant growth rate (μ) during the exponential phase, intregration of eq.(3) gives :

$$\ln \frac{b}{b_0} = \mu\, t \qquad (4)$$

The growth rate μ can be expressed by Monod's model as,

$$\mu = \frac{\mu_m \ S}{K+s} \tag{5}$$

For Andrews inhibitory model this is given by:

$$\mu = \frac{\hat{\mu}\, s}{K + s + s^2/ K_i} \tag{6}$$

where μ_m is the maximal growth rate (per hour), K is the constant numerically equal to the xenobiotic concentration at which $\mu = 1/2\ \mu_m$; $\hat{\mu}$ = characteristic constant time^{-1}) and K_i = inhibition constant (mg/l).

Here we see from the eq.(5), the maximum growth rate (μ_m) is directly obtained in Monod's model whereas, in case of Andrew's model, maximum growth rate is expressed as follows:

$$\mu_m = \frac{\mu}{1 + 2\sqrt{\frac{K}{K_i}}} \tag{7}$$

The growth of the microbes occurs over three phases: i) lag phase, ii) log phase, and iii) stationary phase. After that, lysis of biomass starts. During the log phase the growth rate (μ) is considered to be constant. Therefore, in the experiments conducted in the present study, the normal logarithm of the biomass concentration during the log phase was plotted against time, and the slope of the straight line was taken as μ. Y (yield coefficient) was assumed constant and was calculated by the slope of the straight line of biomass concentration versus substrate concentration.

V. EXPERIMENTAL APPARATUS

All experiments were conducted in a constant temperature shaker apparatus at $28^{o}C$, using 250 ml Erlenmayer flasks (with cotton plugs) on a rotary shaker (Model G-24 New Brunswick Scientific Company, New Brunswick, NJ).

Analytical Equipment:

For measuring growth of biomass, UV-VIS Spectroscopy (Model Varian DMS 200) was used at wavelength 540 nm. Biomass was measured by optical density (Absorbance %) with reference to distilled water.

Analysis of phenol concentration was determined by High Pressure Liquid Chromatography (HPLC).

HPLC model:	Waters 600 E System Controller Millipore.
Detector:	Waters 484 E millipore
Sofware used:	Nelson Interface
Printer used:	Epson
Monitor used:	IBM

HPLC Column:

Serial no. 0605901 Catalog no. 70090

Packing Material: ECONOSPHERE C8 5U COLUMN

Length: 150 mm ID: 4.6mm

Particle size: 5 u OD: 1/4 in.

HPLC Running Conditions:

Mobile phase A: Methanol (with 1% Acetic acid)

Mobile phase B: HPLC Water (with 1% Acetic acid)

Ratio of A and B: 55 : 45

Flow rate: 1ml/min

Run time: 4 minutes

Detector: UV @ 280nm

Temperature: Ambient

Pressure: 1750-1800 psig

Autosampler Model: Waters 715 Ultra Wisp Sampling Processor

DO and pH meter : Orion Research, model 701A.

VI. EXPERIMENTAL PROCEDURE:

Stock Culture:

The pure dried culture of *Pseudomonas resinovorans* was recieved from American Type Culture center (ATCC 14235). A stock culture of *P. resinovorans* was made by transferring 1 loop of dried biomass to BBL nutrient broth in several autoclaved culture tubes, and incubating them at 28-30^{o}C for 24 hours. The cultures were stored in a refrigerator at 4^{o}C (as it has been reported that the species can not grow below 5^{o}C but remains viable).

Experimental Culture:

In order to acclimatize the species in phenol medium, primary, secondary, tertiary, quaternary and quinary cultures were made. The primary culture was prepared by inoculating 1 loop of stock culture in 100 ppm of phenol medium (10 cc of 1000 ppm phenol stock solution, in 90 cc growth medium) though when it was measured by HPLC, actually it was 97 ppm. This medium was taken in a 250 ml Erlenmayer flask, plugged with cotton and incubated at 28^{o}C in a shaker at 250 rpm. After one day when sufficient growth was found, a secondary culture was made in the same way by transferring 1 cc (1 loop takes a longer time for sufficient biomass growth in phenol medium) of primary culture in 100 ppm phenol medium and incubated in a shaker for 24 hours. Then tertiary and subsequent cultures were made from the secondary culture in the same procedure in order to ensure that the culture is fully adapted to utilize phenol as sole carbon source. It has been found experimentally that *P. resinovorans* requires 5 days to completely acclimatize to 100 ppm phenol.

Experimental Conditions:

It is already reported in Bergey's manual [1] that the optimum temperature for growth of *P. resinovorans* is 28-30^{o}C. From the previous studies [23]), 250 rpm of the rotary shaker was found to be most suitable for the growth of *Pseudomonads*. Therefore temperature and speed of the shaker were already defined. In addition the most suitable pH of the growth medium has been reported as 7.2 [23].

The present study has shown that (as expected) the specific growth rate of this species does not vary much with the initial biomass concentration. Although very low initial biomass concentrations (OD= 0.002-0.008) take a long time to consume the phenol, and resulted in longer lag times, more consistent results were obtained than those at high initial biomass concentrations (O.D. =0.054-0.076). No lag phase was found at high initial biomass concentration. Also the dissolved oxygen (DO) at high initial phenol concentrations decreases as the biomass increases in the shaker flask, and this may inhibit the growth rate. To minimize these problems, the optimum initial biomass concentration was chosen at 6.5 ppm (OD = 0.024 to 0.030).

Calibration of OD vs Biomass Concentration:

Growth of the bacterial species was determined by measuring the optical density of the experimental cultures in a UV-VIS spectrophotometer (model Varian DMS 200) at a wavelength of 540 nm. Distilled water was used as the reference sample in the spectrometer. In order to convert the optical density (OD) to biomass concentration, a calibration curve was required.

The culture was grown in an Erlenmayer flask in 100 ppm phenol medium until it reached the maximum OD (at the end of the logarithmic phase). Then the culture was serially diluted as 1/10, 2/10, 3/10, 4/10, 5/10 and so on. The turbidity of each dilution was measured by UV-VIS spectrophotometer in reference to distilled water.

The dry weight of cell mass for the original(undiluted) culture was determined from the average of three 20 ml samples. After centrifuging the culture, the supernatant was decanted, the biomass washed thoroughly with distilled water to remove the water soluble salts, and centrifuged again. The process was repeated several times, and then the washed biomass was placed in three weighed aluminium dishes and dried in an oven at 95^{o}C for 24 hours. After 24 hours the dishes were taken out from the oven and placed in a dessicator until they came to room temperature. Then the three dishes with dried biomass were reweighed. Thus the weight and the concentration of the 20 ml of biomass of the original culture can be obtained. The concentrations of the serially diluted samples were determined by dividing the dry weight value by the dilution volume. One weighed aluminium dish was used as control. The calibration curve of OD vs biomass concentrations is shown in the Figure 1.

Formulation of Growth Medium:

Many papers have proposed different formulations of growth medium using phenol as sole carbon source. Modified Gaudy's growth medium [24] was used in this work. The medium was minimal medium, composed of carbon, nitrogen, magnesium, manganese and phosphate. Carbon source used in this experiment was phenol. Ammonium sulfate, magnesium sulphate, manganous sulfate and sodium phosphate provided the other components of the growth medium. Vitamins and other growth factors were not used in the growth medium. 0.05 micromole buffer (sodium phosphate, dibasic and monobasic) was used in the media, and the pH was maintained at 7.2. The components of the growth medium are given in Table 2.

Sampling Procedure:

During experiments the inoculum was transferred from the culture on the fifth day to different concentrations of phenol (eg. 11, 24, 38, 48, 62, 92, 104, 131, 155, 170 ppm

of phenol) in 250 ml Erlenmayer flasks and mixed them well in the medium and plugged them with cotton. The inoculated cultures were placed on a rotary shaker at $28^{o}C$ and 250 rpm. At every 20 or 30 minute interval, a small amount of sample was taken by disposable pipets in the sampling bottles, then measured for biomass concentration and phenol concentration.

Biomass Growth:

In order to measure the growth rate of the species in a specific concentration of phenol medium, the optical density of the samples was measured at a fixed interval. In this experiment, the samples were taken in 20 minute interval for 11 ppm, 24 ppm, 38 ppm, 48 ppm, 62 ppm, 92 ppm phenol media, every 30 minutes for 104 ppm, 131 ppm, 155 ppm & and every hour for 170 ppm phenol concentration. The growth rates were slower at high concentrations and enough data points could be obtained during the degradation of phenol. For lower concentration of phenol, degradation occurs faster, therefore in order to get enough data, one has to take samples more frequently. Though many other methods (like colony counting or cell counting) have been reported for biomass measurement, in this experiment the optical density was used because it is faster and easier. Analytical error of the UV-VIS spectrometer is +/- 2%.

For all experiments in this study, in order to avoid a significant reduction in volume caused by taking large amount of samples from the flasks, 3 ml samples were taken each time (the minimum volume required for measuring optical density is 2.5 ml). Disposable pipets were used to avoid contamination of the experimental culture, which was streaked periodically on agar plates to test for contamination. The ODs of biomass in different phenol concetrations are given in Tables 3 (3.1 to 3.10).

During the OD measurement, the cuvettes were rinsed thoroughly with distilled water after every reading and wiped with tissue. Dry and clean cuvettes were used for each

sample reading. After finishing the experiments, the cuvettes were cleaned with chromerge to remove all particles from the inner side of the walls of cuvette.

Susbstrate Analysis:

HPLC with autosampler was used to analyze the phenol concentration of the sample. After measuring the OD by spectrophotometer, the samples were prepared for HPLC. 1.5 ml samples were filtered through a 0.45 micrometer millipore filter in the HPLC sampling tubes. In order to make the sample acidic (since the HPLC column requires acid medium and the sample pH was 7.2) 0.002 ml of 1 molar hydrochloric acid was added to the sample, mixed in a stirrer for a while, and placed in the autosampler tray. For phenol analysis, methanol with 1% acetic acid and HPLC grade water with 1% acetic acid were used as mobile phases A and B, respectively. Each sample was injected 2 or 3 times to confirm the results. The run time for each sample was 4 minutes. After finishing the experiment, the HPLC column was washed with pure methanol and HPLC grade water for 40 minutes. The instrumental error for the HPLC model is +/-1 ppm. The results of phenol degradation in different concentrations are given in Table 3.

Measurement of Dissolved Oxygen (DO) and pH:

During the experiment, DO and pH were measured for every sample by means of a calibrated DO probe and pH meter. It was found that pH remained more or less unchanged at all phenol concentrations. However for higher concentrations (above 100 ppm) of phenol, as the biomass concentration increased, DO was found to decrease from 8 to 6 ppm.

Determination of Andrews Parameters:

The growth parameters of *Pseudomonas resinovorans* were determined from the batch experiments conducted in shaker flasks. The inoculum was taken from phenol acclimated culture and exposed to ten different concentrations (11, 24, 38, 48, 62, 92, 104, 131, 155 and 170 ppm) of phenol growth media. The optical density in each experiment was measured every 20 or 30 minutes. These Optical density values were then converted into biomass concentration using the calibration curve (Fig.1). Semi-log plots of biomass concentration (ppm) versus. time (hr) were used to obtain the slope for each of the ten different initial phenol concentrations (as the sample figures are shown in 2.1 and 2.2). The slope of these plots is specific growth rate (μ). The yield coefficient (Y) was determined by plotting the biomass concentration versus phenol concentration as shown in the figures 3.1 and 3.2. Finally the specific growth rates (μ) versus phenol concentrations data were used to obtain the Andrews parameters by nonlinear regression.

VII. RESULTS AND DISCUSSION

The present study emphasized the kinetics of the growth of *Pseudomonas resinovorans*, as well as the rate of biodegradation of phenol, in shaker flask studies. Kinetics of biomass growth were measured by measuring optical density every 20 minutes for 11 to 92 ppm phenol, every 30 minutes for 104, 131 and 155 ppm phenol and every hour for 170 ppm phenol. At the same time after measuring optical density, the sample was filtered and acidified and then 2 ml of it was taken for measuring phenol concentration by HPLC. Before starting the experiments, the species was acclimated in 100 ppm phenol for five days. When the five days' acclimated species was added to the experimental cultures, it was found that at 11 ppm of phenol medium, there was no lag phase during the growth of the biomass. This was also checked from the HPLC result as the phenol concentration reduced from 11 to 6 ppm during the first 30 minutes. But in all other concentrations ranging from 24 to 170 ppm, a lag phase of 30 minutes to 2 hours was found before growth started.

Specific growth rates were measured from the slope of the plot of natural logarithm of biomass concentration in the exponential phase of growth versus time (Fig 3.1 to 4.2). Sometimes it was very confusing which point should be taken as a starting point for calculating specific growth rates. Data from high phenol concentrations indicate that initially (for about 2.5 to 3 hours) growth occurs at a certain rate while later, this rate changes abruptly to a higher value. Moreover it was also noticed that till 92 ppm, the growth of the species was more consistent than at 104, 131 or higher concentrations. This might be due to the acclimation of the species at 97 ppm. Here, for our experiments we calculated the specific growth rates in two ways, as given in tables 4.a and 4.b. In table 4a, the specific growth rates were calculated by taking all points in the exponential growth up to, but not including, the point at which the phenol concentration went to zero. Data for this calculation are named as data set # 1. These

specific growth rates were matched with the corresponding average phenol concentrations (average of the initial and the final phenol concentrations) and regressed for Andrews constants by nonlinear regression (Fig. 7.1). In Table 4b the specific growth rates were calculated by taking all points in the exponential growth phase up to and including the point at which the phenol concentration reached zero and considered as data set # 2. These data were then regressed using the initial phenol concentration (Fig. 7.2). Both sets of data were best fitted with the Andrews model, although the kinetic parameters differ. The kinetic parameters for the data set # 1 (Table 4a) are $\hat{\mu}$= 0.996, K_s = 8.95, K_i = 74.6, μ_{max} as calculated by using these three parameters is 0.588 and the absolute error percent is 2.59% whereas the parameters for the data set # 2 (Table 4b) are 0.947, 13.1, 152, 0.597 and 2.95% respectively. Therefore we can see that $\hat{\mu}$, and μ_{max} do not vary much but K_s and K_i vary widely. In both cases the error % was higher(10%) at 155 ppm while all other points were within the range of 0.33% to 3.5% error.

Now fixing the two sets of Andrews parameters, equation (1) and (2) of page 16 can be integrated and biodegradation at different initial phenol concentrations can be predicted. This was done for all ten concentrations used in the experiments and the results were compared to the actual experimental data (Fig. 8 and 9).

As can be seen from Fig 8.1a to 9.10b, for initial phenol concentrations up to 62 ppm, the experimental data points are pretty close to the predicted values, until the phenol concentrations reach zero. After that the biomass still increased in concentration for about the next 20 to 40 minutes, beyond the predicted values. Perhaps stored energy or nutrients were utilized by the cells to divide or grow. At the higher initial concentrations, particularly at 92, 104, 131, 155 ppm, it was found that when the biomass concentrations reach 15 to 20 ppm, the growth rates became much faster than the predicted values. Similar observations were made in the case of substrate vs time where the phenol concentration went down in a steady fashion, though the

experimental points at lower phenol concentrations are slightly above the predicted values which might be due to instrument errors. The accuracy of the HPLC is +/- 1 ppm.

Data set #2 using the initial phenol concentration (Fig.8.1b to 8.10b) show that actual growth rates at 11 to 170 ppm are slower than the predicted values, especially at higher concentrations like 155 or 170 ppm. In all experiments, biomass continued to increase after disappearance of phenol. Dissolved oxygen concentration did not vary much at initial phenol concentrations of 24 and 48 ppm (Tables 3.2 and 3.4), but at 131 ppm (Table 3.8) there was a decrease in DO from 8.3 to 6.3 ppm. This may have affected growth at the higher initial phenol concentrations, such as 104, 131, 155, and 170.10 ppm, although DO was not measured in all cases.

For substrate vs time, data set #1 (Table 4a; Fig. 9.1a to 9.10a) give better agreement with the predicted values than the data set #2 (Table 4b; Fig. 9.1b to 9.10b). There are a number of factors which may cause a variation in the results. Injection volume, dilution of the sample with acid, pressure of the HPLC during analysis, light intensity of the detector may cause errors in the experimental data. In addition, although batch experiments are faster and easier than continuous flow, earlier reports of Speitel et al [21], pointed out the potential for erroneous estimates of kinetic coefficients in batch experiments because of a lag phase (which underestimates μ_{max}). In addition, Colvin and Rozich [5] mentioned that cells growing at higher concentrations of phenol have a tendency for wall growth, and biomass tends to flocculate. During our experiments, though the culture flasks were shaken continuously at 250 rpm, still wall growth was noticed after 2 to 3 hours, especially at higher concentrations, which also might be the cause of experimental errors. Another cause of experimental error at higher phenol concentrations (ranging from 104 to 170 ppm) might be the acclimation of the species at 97 ppm, as it was reported earlier, history of the species is very important for kinetic studies.

Pseudomonads are often used by biodegradation researchers because the genus is very versatile, easily adaptable to different carbon and nitrogen sources, and most of the species can grow at room temperature (25 to 30^{O} C). Reviewing the literature on biodegradation of phenol by Pseudomonads, we got the maximum value of K_i = 100 ppm for *Pseudomonas putida* (ATCC 17514), [16] whereas for *P. resinovorans* (ATCC 14235) K_i = 151.88 ppm (Table 4b) which indicates its inhibition by phenol may be less than other Pseudomonads. Values of Andrews parameters for *P. resinovorans* (ATCC 14235) are given in Tables 4a and 4b.

VIII. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

* It has been experimentally verified that *Pseudomonas resinovorans* can grow luxuriously in phenol media by using phenol as sole carbon source. Kinetic studies have shown that phenol degradation follows the Andrews inhibition model. The Andrews parameters for specific growth rate using data set # 1 (Table 4a) are $\hat{\mu}$ = 0.95, K_s = 13.1, K_i = 152, μ_{max} = 0.597 while for the data set # 2 (Table 4b) the Andrew's parameters are $\hat{\mu}$ = 0.996, K_s = 8.95, K_i = 74.61 and μ_{max} = 0.588.

* Since the species is strictly aerobic, the growth rate at high phenol concentrations (above 50 ppm) might be affected due to oxygen deficiency.

* Using the parameters of set #1, one can predict that μ_{max}= 0.588 and it occurs at 26 ppm; from Table 4a, it is found that these predictions are close to the experimentally measured value (μ_{max} = 0.581 at 28 ppm). Similarly for data set #2, the predicted μ_{max} = 0.597 hr^{-1} at 45 ppm which is also close to the experimental μ_{max} = 0.598 hr^{-1} at 48 ppm (as given in Table 4b). The predicted μ_{max} was calculated by using the Andrews parameters for data set #1 (Table 4a) and data set #2 (Table 4b) in equation 7 (page 17).

Recommendations

During the experiments, one has to monitor dissolved oxygen continuously to avoid oxygen defficiency at high concentration, which is very difficult in a shaker flask. Instead, one can do the experiment in larger volume in batch methods with continuous monitoring of dissolved oxygen.

To compare the results at higher concentrations of phenol, one should acclimate the species at higher phenol concentration before starting experiments.

If one can find another species with different colored colonies, capable of biodegrading phenol, whose specific growth rate curve can cross that of *P. resinovorans*, then it could be used to verify models for coexistence of two species competing for phenol as their carbon source.

In a further extention of this work, one can model mixed populations with mixed substrates, which would be more applicable to actual waste treatment problems.

APPENDIX I

General characteristics of Pseudomonas resinovorans

Cell diameter	0.6 - 0.7 μm
Cell length	2.0 - 2.5 μm
Cell shape	rod
Number of flagella	1, polar
Motility	+
Flourescent pigment	+
Optimum growth temp.	28 - 30^{o}C
Oxydase reaction	+
Growth factor	-
Denitrification	-
Nitrate reduction	weak
Hydrolysis of starch	weak
Acidification of glucose fructose, galactose, lactose, xylose, maltose	-
Growth occurs in expense of colophony, Canada balsam, Phenol, napthalene, m-cresol, salicylic acid	+

APPENDIX II

Presumptive Identification of *Pseudomonas resinovorans*

Pseudomonas genus is **glucose non fermentive gram negative rod** shaped bacteria. Many of them are pathogenic in nature. To test the species of *Pseudomonas* one can use many laboratory or commercial methods.

Two tubes of oxidative-fermentive (OF) glucose are inoculated with the culture species. One of them is then sealed with petroleum and both tubes are incubated at 30-35^{o}C. A fermentive organism will produce acid in the unsealed (aerobic) tube as well as in the sealed (anaerobic) tube. However the *Pseudomonas* being strictly aerobic organisms, cannot acidify glucose in the sealed tube. This is a good method to separate all *Pseudomonas* species from the fermentive gram negative rods. *Pseudomonas resinovorans* can not acidify glucose even in an open tube. This property can be used to distinguish the species from other glucose oxidizer *Pseudomonas* species.

*P. resinovorans*shows positive response to the Cytochrome oxydase test (but this should be remembered that McConey agar can not be used for the test as it gives a false negative result). It also gives flourescent pigment at 30 - 35^{o}C and the species can grow in resin medium which can isolate the species from other *Pseudomonas* species.

REFERENCES

[1] Bergey, D.H. "Bergey's manual of Systematic Bacteriology". 1: 158-199; 1984.

[2] Brown, S.C., C.P. Lesley, Jr., H.H. Tabak, "Biodegradation Kinetics of Substituted Phenolics : Demonstration of Protocol Based on Electrolytic Respirometry", Water Research; 24: 853-861; 1990.

[3] Boyd, Stephen A., Shelton, D.R., Berry, D. "Anaerobic Biodegradation of Phenolic Compounds in Digested Sludge", Applied & Environmental Microbiology; 46: 50-54; July, 1983.

[4] Chesney, R.H., Solliti, P., Rubin, H.E. "Incorporation of Phenol Carbon at Trace Concentration by Phenol Mineralizing Microorganisms in Fresh Water"; Applied & Env. Microbiology, 49: 15-18; January, 1985.

[5] Colvin, R.J., Rozich A.R. "Phenol Growth Kinetics of Heterogeneous Populations In a Two-Stage Continuous Culture System"; Journal Water Pollution Control Federation; 58: 326-332; April, 1986.

[6] Dwyer, D.F., Krumme, M.L., Boyd, S.A. "Kinetics of Phenol Biodegradation by an Immobilized Methanogenic Consortium"; Applied & Env. Microbiology; 52: 345-351; August, 1986.

[7] Folsom, B.R., Chapman, P.H., Pritchard, P.H. "Phenol & TCE Degradation by *Pseudomonas cepacia G4:* Kinetics & Interactions Between Substrates", Applied & Env. Microbiology; 56: 1279-1285; May, 1990.

[8] Heitkamp, M.A., Camel, V., Reuter, T.J., Adams, W.J. "Biodegradation of p-Nitrophenol in an Aqueous Waste stream by Immobilized Bacteria", Applied & Env. Microbiology; 56: 2967-2973; October, 1990.

[9] Hill, G.A., Robinson, C.W. "Substrate Inhibition Kinetics on Phenol Degradation by Pseudomonas putida", Biotech. Bioeng.; 17: 1599-1615; 1975.

[10] Hughes, E.J.L., & Bayly, R.C. "Control of Catechol meta-Cleavage Pathway in Alcaligenes eutrophus"; J. of Bacteriology; 154: 1363-1370; June, 1983.

[11] Jones, S.H. & Alexander, M. "Kinetics of Mineralization of Phenols in Lake Water"; Applied & Env. Microbiology; 51: 891-897; May, 1986.

[12] Kim, B.R., Chian, E.S.K., Wendall, H.C., Cheng, S. "Adsorption, Desorption, and Bioregeneration in an Anaerobic, Granular Activated carbon Reactor for the Removal of Phenol"; J. of Water Pollution Control Federation; 58: 35-40; January, 1986.

[13] Lallai, A., and Mura, G. "pH Variation During Phenol Biodegradation in Mixed Cultures of Microorganisms"; Water Research; 23: 1335-1337; 1989.

[14] Lovely, D.R., and Lonergan, D.J. "Anaerobic Oxidation of toluene, Phenol, and p-Cresol by the Dissimilatory Iron-Reducing Organism, GS-15"; Applied and Env. Microbiology; 56: 1858-1863; June, 1990.

[15] Masunaga, S., Urushigawa, Y., and Yonezawa, Y. "Biodegradation Pathway of o-Cresol by Heterogeneous Culture, (phenol acclimated activated sludge)"; Water research; 20: 477-484; 1986.

[16] Molin, G., and Robinson, C.W. "Degradation of Phenol by *Pseudomonas putida* ATCC 11172 in Continuous Culture at Different Ratios of Biofilm Surface to Culture Volume"; Applied & Env. Microbiology; 50: 946-950; October, 1985.

[17] Paris, D.F., Wolfe, N.L., Steen, W.C., and Baughman, G.L. "Effect of Phenol Molecular Structure on Bacterial Transformation Rate Constants in Pond & River Samples"; Applied & Env. Microbiology; 45: 1153-1155; 1983.

[18] Paris, D.F., Steen, W.C., Baughman, G.L. and Barnett, J.T., Jr. "Second-order Model to Predict Microbial degradation of Organic Compounds in Natural Waters"; Applied & Env. Microbiology; 41: 603-609; March, 1991.

[19] Rubin, Howard E. and Schmidt, S. "Growth of Phenol Mineralizing Microorganisms in Fresh Water" Applied and Environmental Microbiology, 49:11-14; January, 1985.

[20] Schmidt, S.K., Scow, K.M., and Alexander, M. "Kinetics of p-Nitrophenol Mineralization by a *Psedomonas* species: Effects of Secondary Substrates"; 53: 2617-2623; Nov.,1987.

[21] Speitel, G.E., Jr., and DiGiano, F.A. "Determination of Microbial Kinetic Coefficients Through Measurement of Initial Rates by radiochemical Techniques"; Water Research, 22: 829-835; 1988.

[22] Yang, R.D., and Humphrey, A.E. "Dynamic and Steady State Studies of Phenol Biodegradation in Pure & Mixed Culture"; Biotech. Bioeng.; 27: 1211-1235; 1975.

[23] Ko, Y.S. ; 1990 "Biodegradation of phenol by two competing microbial species in a sequencing batch reactor"; MS Thesis, Dept. of Chemical Eng., NJIT.

[24] Gaudy, Jr. A.F., M. Ramanathan & B.S. Rao, Kinetics Behaviour of Heterogeneous Populations in Completely Mixed Reactors, Biotech. Bioeng.; 9:387; 1967.

Table 1

Data of Optical Density vs Biomass Concentration for Calibration Curve

# of readings	Optical Density	Biomass Concentration
1.	0.000	0
2.	0.087	21.2
3.	0.167	42.4
4.	0.244	63.6
5.	0.320	84.8
6.	0.399	106.0
7	0.473	127.2
8	0.544	148.4

Slope = 273.3827 ; Correlation = 0.999

Table 2

Defined Growth Medium Composition

Phenol Stock Solution (1000 ppm)	
Phenol	1000 mg
Distilled Water	1000 ml
Ingredients of Growth medium Solution (pH 7.2)	
Ammonium Sulfate	500 mg
Magnesium Sulfate	100 mg
Ferric Chloride	0.5 mg
Manganous Sulfate	10 mg
Phosphate Buffer (pH 7.2)	1000 ml
Ingredients of Phosphate Buffer Solution (pH 7.2)	1000 ml
50 mM Sodium Phosphate Dibasic (Na_2HPO_4)	190 ml
50 mM Potassium Phosphate Monobasic (KH_2PO_4)	

Table 3

Experimental Data

[No. = Number of readings; Time = Time of measurement in hours; O.D.= Optical density; B = Biomass concentration (ppm); S= Phenol concentration (ppm); μ_a & μ_b = Slopes of ln biomass vs time (Specific growth rate) to their corresponding phenol concentrations. μ_a corresponds to the initial phenol concentration, μ_b corresponds to average phenol concentrations calculated by using all but the last values (in bold face); Y_a & Y_b = Yield coefficient corresponding to μ_a & μ_b.]

N.B. Here all the data which were actually used for calculating Specific Growth Rates are shown in bold face in the following tables.

Table 3.1: Initial Phenol Conc.= 11.08 PPM

Average Phenol Conc.= 5.79 PPM

No.	Time	O.D.	B	S
1	**0.000**	**0.034**	**9.295**	**11.08**
2	**0.500**	**0.038**	**10.389**	**6.36**
3	**0.8333**	**0.044**	**12.029**	**3.79**
4	**1.1667**	**0.053**	**14.489**	**0.50**
5	**1.4999**	**0.064**	**17.496**	**0.00**
6	1.8333	0.066	18.043	0.00
7	2.1667	0.066	18.043	0.00

μ_a = 0.377 (0.977); μ_b = 0.429 (0.982); Y_a = 0.487 (0.962); Y_b = 0.659 (0.911); pH = 7.2 to 7.16

Table 3.2: Initial Phenol Conc. = 23.55 PPM
Average Phenol Conc. = 12.46 PPM

No.	Time	O.D.	B	DO	S
1	0.000	0.033	9.022	8.78	23.58
2	**1.000**	**0.034**	**9.295**	**8.76**	**23.55**
3	**1.333**	**0.036**	**9.842**	**8.26**	**22.03**
4	**1.667**	**0.043**	**11.755**	**8.15**	**19.47**
5	**2.000**	**0.051**	**13.943**	**8.04**	**14.69**
6	**2.333**	**0.065**	**17.770**	**7.96**	**9.58**
7	**2.667**	**0.082**	**22.417**	**7.84**	**1.36**
8	**3.000**	**0.096**	**26.245**	**7.91**	**0.00**
9	3.333	0.098	26.792	8.56	0.00
10	4.000	0.096	26.245	8.64	0.00

μ_a = 0.544 (0.986); μ_b = 0.554 (0.991); Y_a = 0.600 (0.998); Y_b = 670 (0.989);
pH = 7.2 to 7.1

Table 3.3: Initial Phenol Conc. = 37.70 PPM
Average Phenol Conc. = 19.42 PPM

No.	Time	O.D.	B	S
1	0.000	0.035	9.568	41.43
2	**0.500**	**0.039**	**10.662**	**37.70**
3	**0.833**	**0.043**	**11.755**	**35.40**
4	**1.167**	**0.051**	**13.943**	**31.68**
5	**1.499**	**0.061**	**16.676**	**26.23**
6	**1.833**	**0.073**	**19.957**	**19.23**
7	**2.167**	**0.096**	**26.245**	**9.50**
8	**2.499**	**0.117**	**31.986**	**1.13**
9	**2.833**	**0.140**	**38.274**	**0.00**
10	3.167	0.140	38.274	0.00

μ_a = 0.564 (0.993); μ_b = 0.572 (0.995); Y_a = 0.574 (0.998); Y_b = 0.654 (0.981);
pH = 7.21 to 7.16

Table 3.4: Initial Phenol Conc. = 48.27 PPM

Average Phenol Conc. = 27.80 PPM

No.	Time	O.D.	B	DO	S
1	0.000	0.032	8.748	8.75	48.47
2	1.000	0.031	8.475	8.78	48.86
3	**1.333**	**0.032**	**8.748**	**8.71**	**48.27**
4	**1.667**	**0.036**	**9.842**	**8.48**	**45.91**
5	**2.000**	**0.041**	**11.209**	**8.26**	**43.12**
6	**2.333**	**0.052**	**14.216**	**8.12**	**39.78**
7	**2.667**	**0.064**	**17.497**	**8.01**	**32.86**
8	**3.000**	**0.079**	**21.597**	**7.86**	**27.94**
9	**3.333**	**0.096**	**26.245**	**7.59**	**20.62**
10	**3.667**	**0.119**	**32.533**	**7.24**	**7.33**
11	**4.000**	**0.153**	**41.828**	**8.32**	**0.00**
12	4.333	0.154	42.101	8.70	0.00

μ_a = 0.581 (0.996); μ_b = 0.598 (0.997); Y_a = 0.602 (0.997); Y_b = 653 (0.994);

pH = 7.27 to 7.22

Table 3.5: Initial Phenol Conc. = 62.45 PPM

Average Phenol Conc. = 31.46 PPM

No.	Time	O.D.	B	S
1	0.000	0.030	8.201	62.55
2	1.000	0.032	8.748	62.51
3	**1.333**	**0.034**	**9.295**	**62.45**
4	**1.667**	**0.039**	**10.662**	**61.10**
5	**2.000**	**0.048**	**13.122**	**56.14**
6	**2.333**	**0.057**	**15.583**	**52.02**
7	**2.667**	**0.068**	**18.590**	**47.99**
8	**3.000**	**0.080**	**21.871**	**39.86**
9	**3.333**	**0.096**	**26.245**	**31.12**
10	**3.667**	**0.126**	**34.446**	**18.24**
11	**4.000**	**0.163**	**44.561**	**0.47**
12	**4.333**	**0.187**	**51.123**	**0.00**
13	4.667	0.189	51.669	0.00
14	5.000	0.193	52.763	0.00

μ_a= 0.576 (0.996); μ_b= 0.580 (0.997); Y_a= 0.559 (0.999); Y_b= 0.607 (0.992);

pH= 7.27 to 7.22

Table 3.6: Initial Phenol Conc. = 91.98 PPM
Average Phenol Conc. = 47.40 PPM

No.	Time	O.D.	B	S
1	0.000	0.030	8.201	93.73
2	1.000	0.031	8.475	93.65
3	**1.333**	**0.033**	**9.022**	**91.98**
4	**1.667**	**0.037**	**10.115**	**89.82**
5	**2.000**	**0.044**	**12.029**	**85.48**
6	**2.333**	**0.052**	**14.216**	**82.67**
7	**2.667**	**0.059**	**16.130**	**78.39**
8	**3.000**	**0.070**	**19.137**	**72.12**
9	**3.333**	**0.081**	**22.144**	**66.08**
10	**3.667**	**0.109**	**29.799**	**56.41**
11	**4.000**	**0.132**	**36.087**	**42.95**
12	**4.333**	**0.163**	**44.561**	**26.88**
13	**4.667**	**0.193**	**52.763**	**2.82**
14	**5.000**	**0.239**	**65.338**	**0.00**
15	5.333	0.249	68.072	0.00

μ_a = 0.541 (0.996); μ_b = 0.550 (0.996); Y_a = 0.514 (0.996); Y_b = 0.557 (0.991); pH = 7.23 to 7.16

Table 3.7: Initial Phenol Conc. = 103.54 PPM
Average Phenol Conc. = 57.03 PPM

No.	Time	O.D.	B	S
1	0.000	0.029	7.928	105.31
2	1.000	0.029	7.928	104.40
3	**1.500**	**0.031**	**8.475**	**103.54**
4	**2.000**	**0.037**	**10.115**	**100.23**
5	**2.500**	**0.046**	**12.576**	**96.53**
6	**3.000**	**0.057**	**15.583**	**91.30**
7	**3.500**	**0.075**	**20.504**	**83.43**
8	**4.000**	**0.103**	**28.158**	**72.06**
9	**4.500**	**0.146**	**39.914**	**54.10**
10	**5.000**	**0.200**	**54.677**	**9.65**
11	**5.500**	**0.289**	**79.008**	**0.00**
12	6.000	0.289	79.008	0.00

μ_a=0.538 (0.993); μ_b=0.543 (0.995); Y_a= 0.511 (0.988); Y_a= 0.511 (0.988); Y_b=0.541 (0.992); pH = 7.26 to 7.15

Table 3.8: Initial Phenol Conc. = 130.58 PPM
Average Phenol Conc. = 69.30 PPM

No	Time	O.D.	B	DO	S
1	0.000	0.029	7.928	8.27	130.82
2	**1.500**	**0.029**	**7.928**	**8.33**	**130.58**
3	**2.000**	**0.033**	**9.022**	**8.26**	**129.14**
4	**2.500**	**0.037**	**10.115**	**8.09**	**125.22**
5	**3.000**	**0.049**	**13.396**	**7.96**	**121.66**
6	**3.500**	**0.055**	**15.036**	**7.90**	**115.96**
7	**4.000**	**0.074**	**20.230**	**7.78**	**108.10**
8	**4.500**	**0.101**	**27.612**	**7.63**	**96.49**
9	**5.000**	**0.143**	**39.094**	**7.51**	**77.71**
10	**5.500**	**0.212**	**57.957**	**7.01**	**49.16**
11	**6.000**	**0.299**	**81.741**	**6.53**	**8.02**
12	**6.500**	**0.345**	**94.317**	**5.84**	**0.00**

μ_a = 0.524 (0.984); μ_b = 0.531 (0.988); Y_a = 0.609 (0.999); Y_b = 0.638 (0.998);
pH = 7.23 to 7.11

Table 3.9: Initial Phenol Conc. = 154.92 PPM
Average Phenol Conc. = 88.60 PPM

No	Time	O.D.	B	S
1	0.000	0.027	7.381	155.34
2	**1.500**	**0.027**	**7.381**	**154.92**
3	**2.000**	**0.029**	**7.928**	**153.44**
4	**2.500**	**0.032**	**8.748**	**150.55**
5	**3.000**	**0.035**	**9.568**	**146.51**
6	**3.500**	**0.042**	**11.482**	**142.86**
7	**4.000**	**0.055**	**15.036**	**136.88**
8	**4.500**	**0.063**	**17.223**	**128.26**
9	**5.000**	**0.087**	**23.784**	**113.41**
10	**5.500**	**0.120**	**32.806**	**93.28**
11	**6.000**	**0.167**	**45.655**	**65.35**
12	**6.500**	**0.229**	**62.605**	**22.27**

$\mu_a = \mu_b = 0.434$ (0.976); $Y_a = Y_b = 0.423$ (0.999); pH = 7.21 to 7.14

Table 3.10: Initial Phenol Conc.= 170.10 PPM
Average Phenol Conc. = 91.26 PPM

No	Time	O.D.	B	S
1	0.000	0.033	9.022	175.58
2	1.000	0.032	8.748	175.12
3	2.000	0.033	9.022	174.22
4	**3.000**	**0.042**	**11.482**	**170.10**
5	**4.000**	**0.049**	**13.396**	**164.45**
6	**5.000**	**0.067**	**18.317**	**150.22**
7	**6.000**	**0.101**	**27.612**	**137.50**
8	**7.000**	**0.164**	**44.835**	**93.49**
9	**8.000**	**0.254**	**69.439**	**42.41**
10	**9.000**	**0.391**	**106.893**	**12.41**

$\mu_a = \mu_b = 0.389$ (0.992); $Y_a = Y_b = 0.557$ (0.982); pH = 7.26 to 7.12

Table 4

Data For Andrews Kinetic Parameters of *P.resinovorans*

Table 4.1: Data set #1; Specific Growth Rate & Yield Coefficient vs Average Phenol Concentration for *Pseudomonas resinovorans* (ATCC 14235)

S_o	S_{av}	μ	Y	Y_{av}	Parameters
11.08	5.79	0.377	0.487	0.544	$\hat{\mu} = 0.996$ $K_s = 8.95$ $K_i = 74.61$ $\mu_{max} = 0.588$
23.55	12.46	0.544	0.600		
37.70	19.42	0.564	0.574		
48.27	27.80	0.581	0.602		
62.45	31.46	0.576	0.559		
91.98	47.40	0.541	0.514		
103.54	56.60	0.538	0.511		
130.58	69.30	0.524	0.609		
154.92	88.60	0.434	0.423		
170.10	91.26	0.389	0.556		

* Here the values of at S = 0 are not included.

* S_o = Initial Phenol Concentration (ppm)

S_{av} = Average Phenol Concentration (ppm)

μ = Specific growth rate at a particular Phenol conc.

Y = Yield coefficient at that Phenol conc.

Y_{av} = Average Yield coefficient for 10 different conc.

Parameters = Andrews kinetic parameters from non linear regression.

Table 4.2 : Data set #2; Specific Growth Rate & Yield Coefficient vs Initial Phenol Concentration for *Pseudomonas resinovorans*(ATCC 14235)

S_0	μ	Y	Y_{av}	Parameters
11.08	0.429	0.659		
23.55	0.554	0.670		
37.70	0.572	0.654		$\hat{\mu}$=0.947
48.27	0.598	0.653		K_s = 13.06
62.51	0.580	0.607	0.596	K_i = 151.88
91.98	0.550	0.557		μ_{max} = 0.597
103.54	0.543	0.541		
130.58	0.531	0.638		
154.92	0.434	0.423		
170.10	0.389	0.556		

* Here the values of μ at S = 0 are included for each Conc.

* Notations are same as above

Table 5

EXPERIMENTAL OBSERVATION

for *Serratia marcesens* (ATCC 17991).

Table 5.a:

Observation of Growth of *Serratia marcesens* in 50 ppm Phenol Growth medium.

Date	Time	Inoculum	O.D.	# of colony	S (ppm)
5/12	8:30 A.M.	Stock	0.717	87	Broth *1
5/12	9:00 P.M.	Primary	0.037	22	50.42
5/13	10.00 P.M.	Primary	0.039	28	50.51
5/13	10.30 P.M.	Secondary	0.010	8	50.77
5/14	9:30 A.M.	Secondary	0.007	11	50.61
5/14	9:40 A.M.	Tertiary*2	0.005	7	49.21
5/15	8:00 A.M.	Tertiary	0.001	1	50.01

Broth*1 = BBL nutrient broth.

*2 = Since OD in secondary culture was very low, so more inoculum (10 cc) were added in Tertiary culture to increase OD of the culture. Primary and secondary cultures were made with 2 cc inoculum.

Colonies were made in pour plate method.

Table 5.b:

Study of Primary Culture of *Serratia marcesens* with Higher Inoculum Concentration.

Date	Time	Inoculum	O.D.	# of colony	S (ppm)
5/15	8:30 A.M.	Primary	0.127	134	20.98
5/15	10:30 P.M.	Primary	0.108	128	20.46
5/16	8:00 P.M.	Primary	0.096	104	20.87
5/16	9:30 P.M.	Primary	0.076	84	20.97
5/17	8:30 P.M.	Primary	0.038	55	20.11
5/20	8:30 P.M.	Primary	0.008	7	20.85

* Here 10 cc of inoculum from stock was added to 100 cc of Phenol medium. Colonies were tested in Pour Plate method.

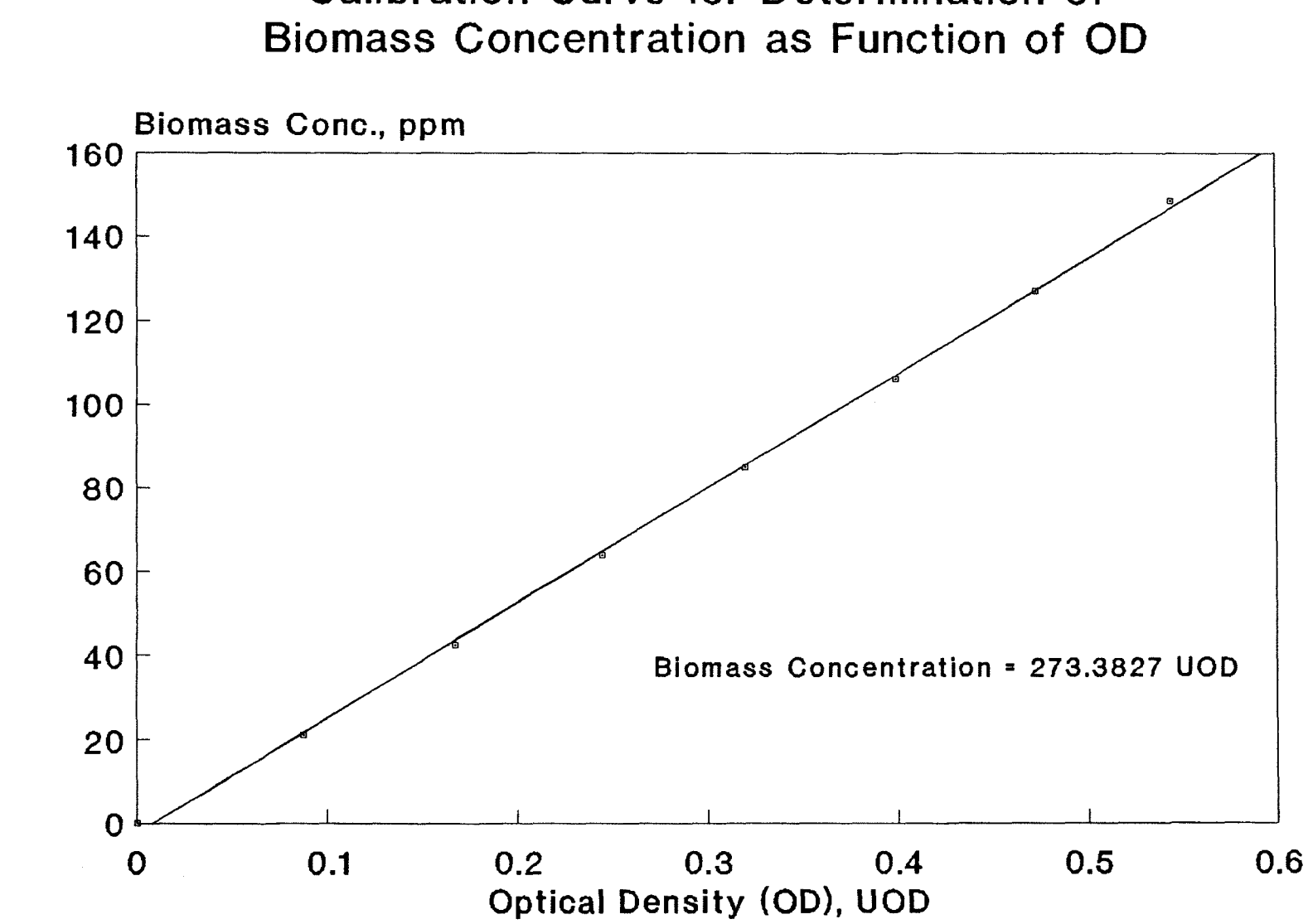
Calibration Curve for Determination of
Biomass Concentration as Function of OD
Biomass Conc., ppm
160
140
120
100
80
60
40
20
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Optical Density (OD), UOD
Biomass Concentration = 273.3827 UOD

Fig 1

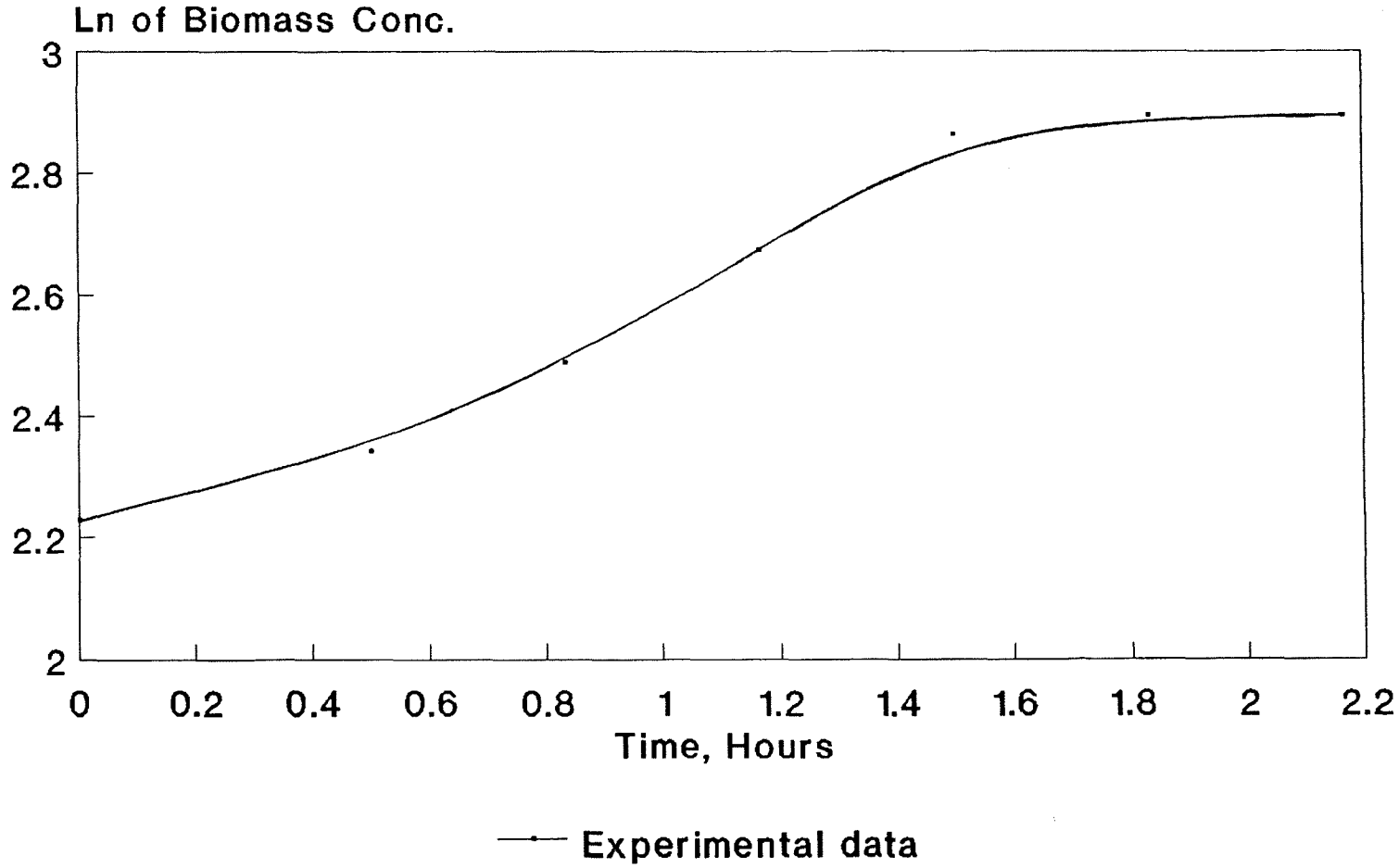
Ln of Biomass Concentration vs Time
Initial Phenol Concentration 11 ppm
Ln of Biomass Conc.
3
2.8
2.6
2.4
2.2
2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
Time, Hours
Experimental data

Fig 2.1

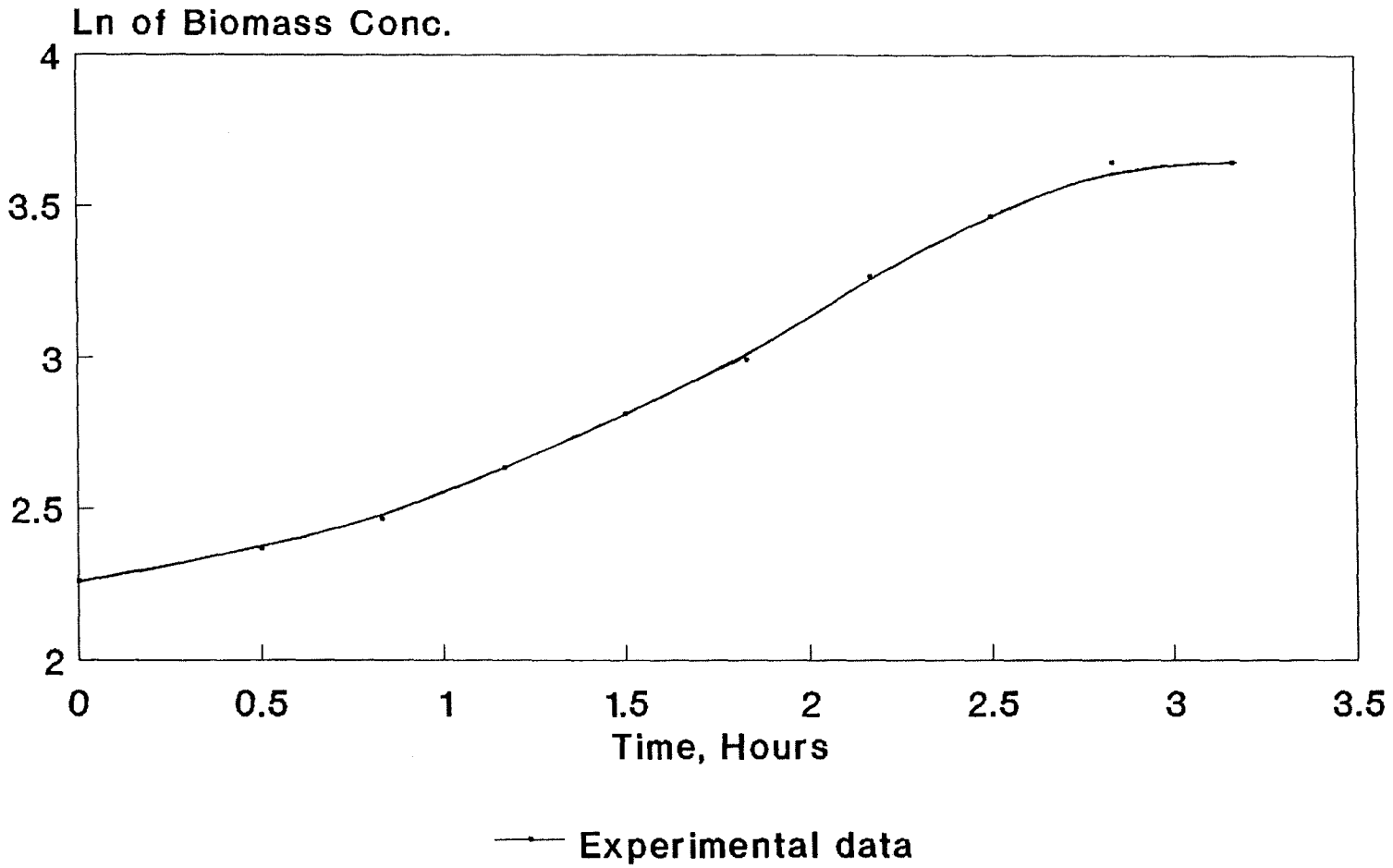
Ln of Biomass Concentration vs Time
Initial Phenol Concentration 38 ppm
Ln of Biomass Conc.
4
3.5
3
2.5
2
0
0.5
1
1.5
2
2.5
3
3.5
Time, Hours
Experimental data

Fig 2.2

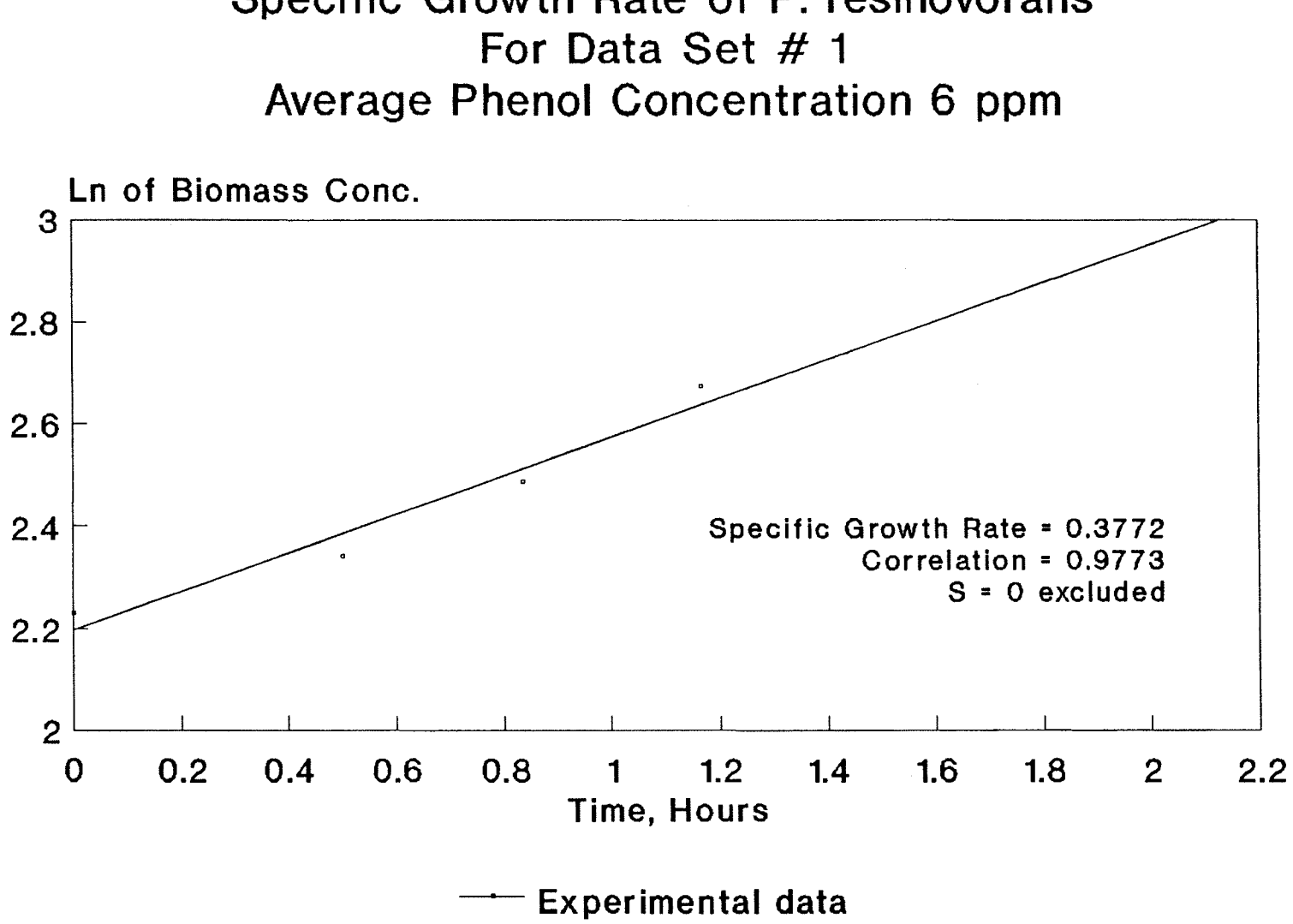
Specific Growth Rate of P. resinovorans
For Data Set # 1
Average Phenol Concentration 6 ppm
Ln of Biomass Conc.
3
2.8
2.6
2.4
2.2
2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
Time, Hours
Specific Growth Rate = 0.3772
Correlation = 0.9773
S = 0 excluded
Experimental data

Fig 3.1

Specific Growth Rate of P. resinovorans
For Data Set # 1
Average Phenol Concentration 19 ppm

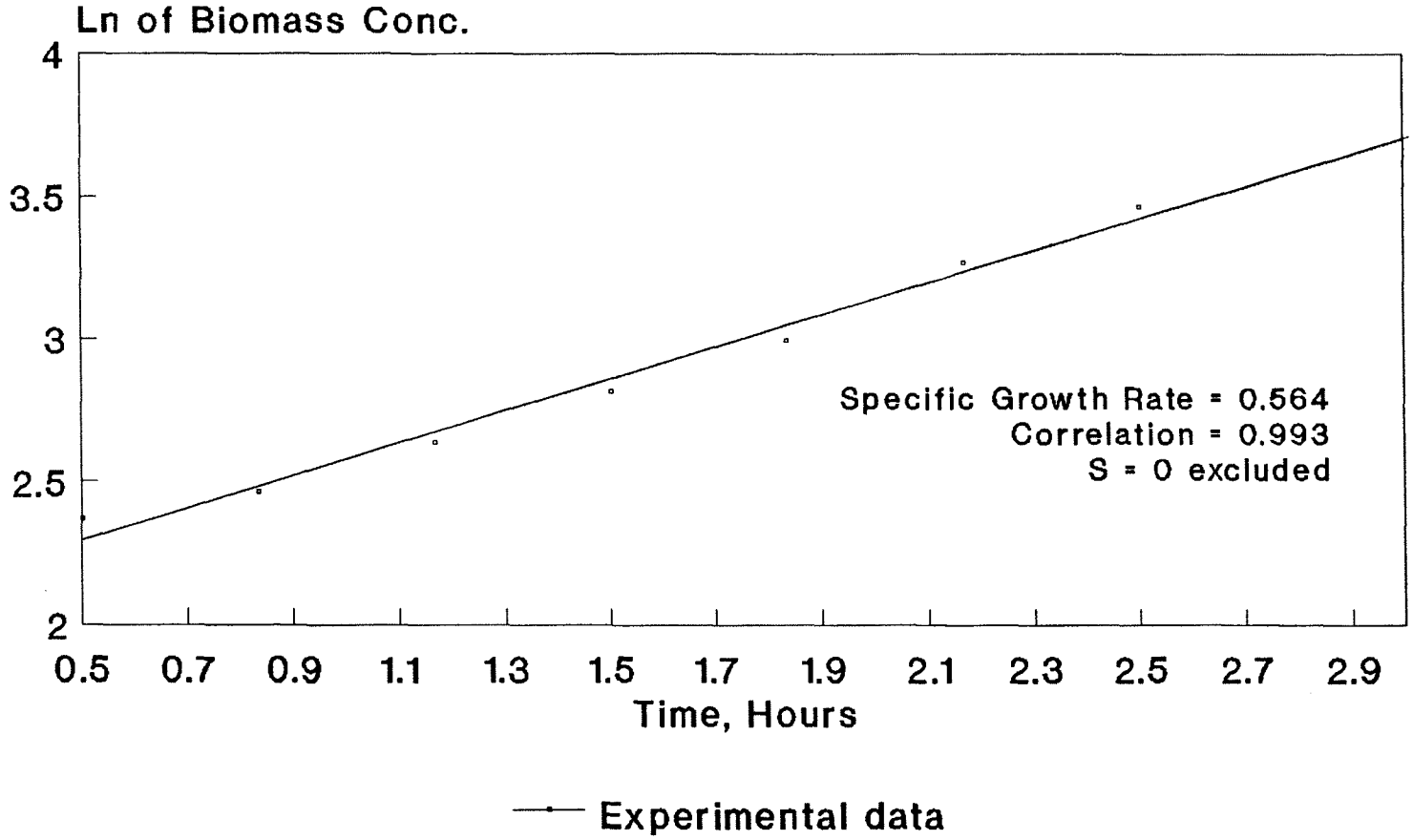

Fig 3.2

Specific Growth Rate of P. resinovorans For Data Set # 2 Initial Phenol Concentration 11 ppm

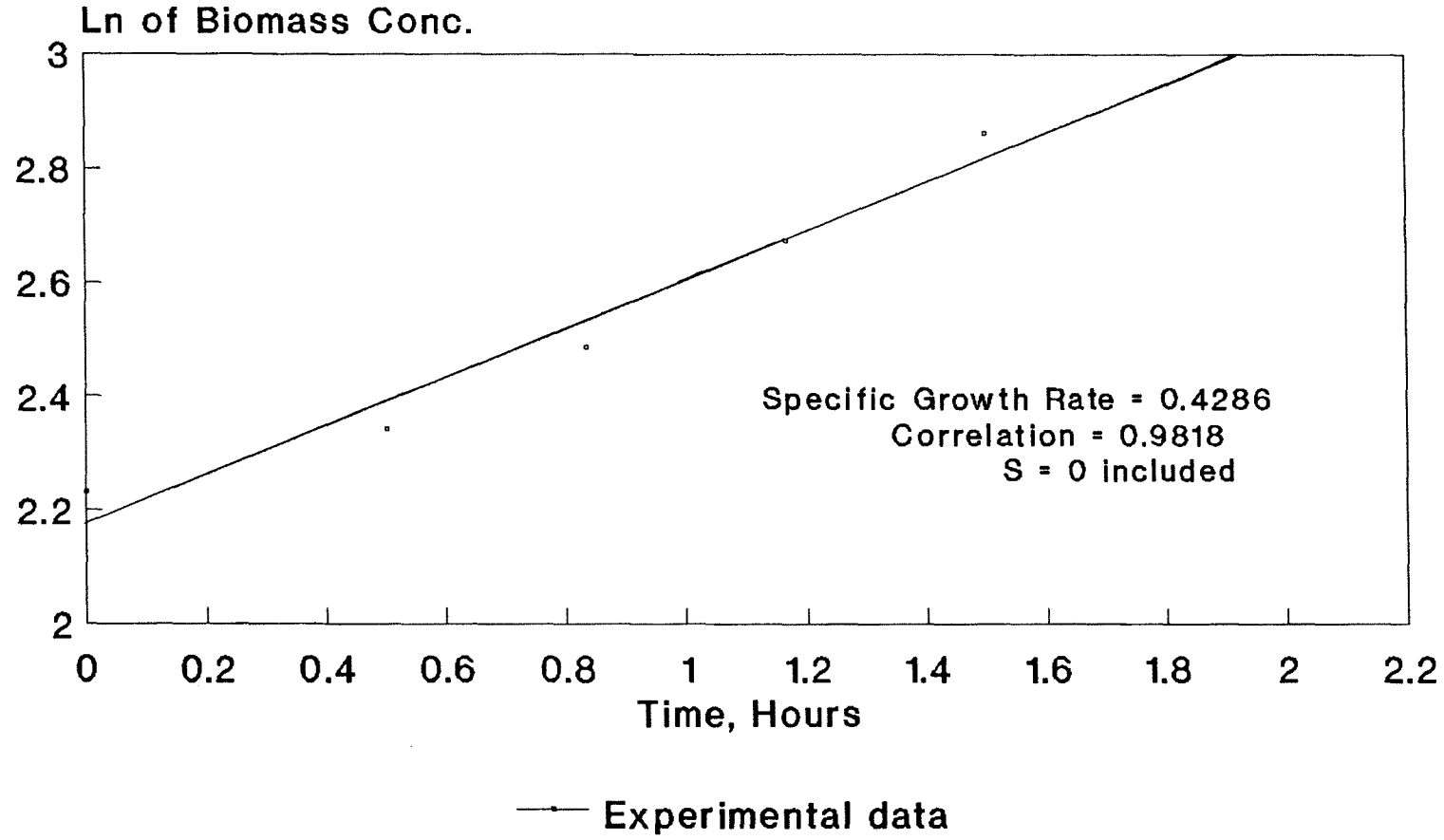

Fig 4.1

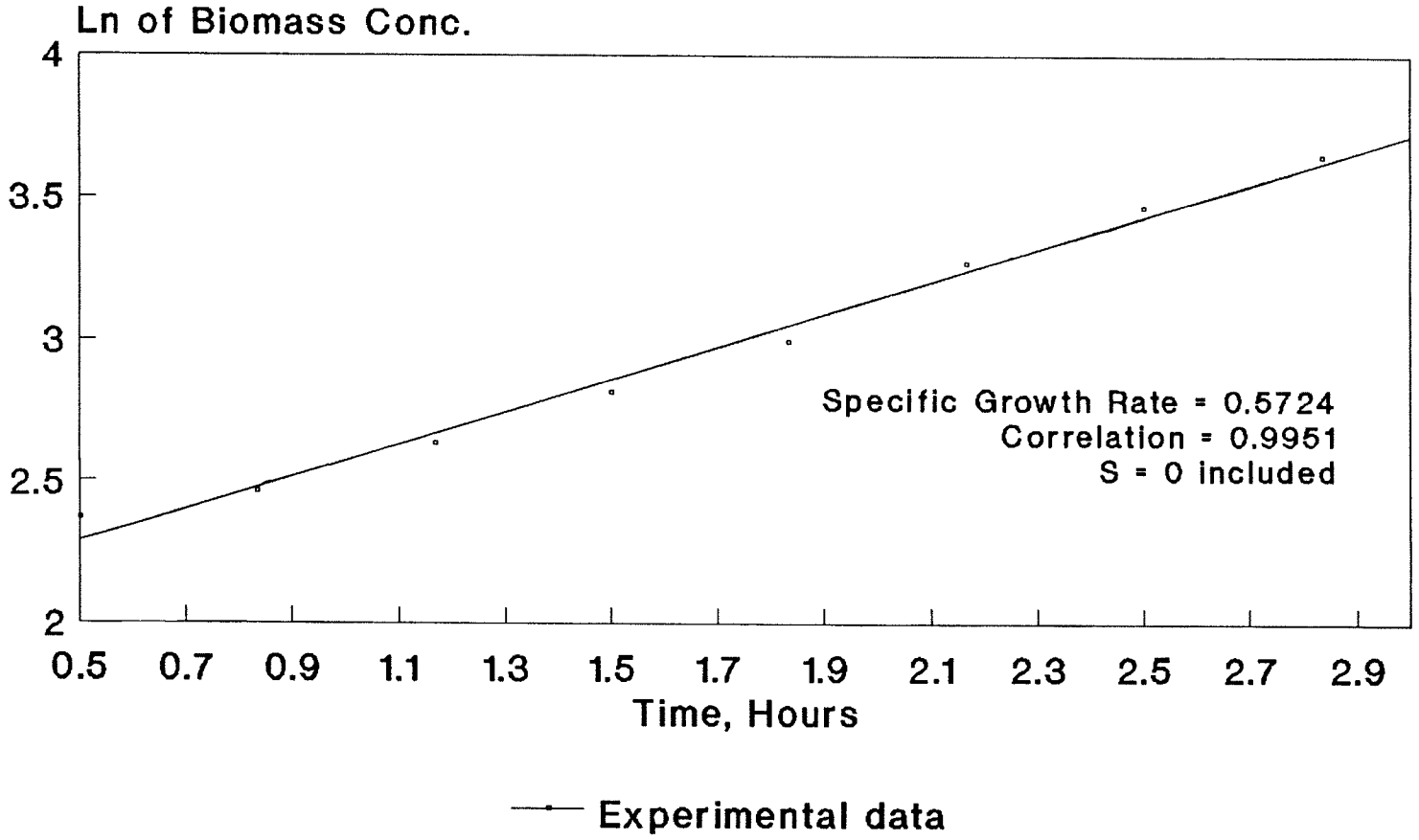
Specific Growth Rate of P. resinovorans
For Data Set # 2
Initial Phenol Concentration 38 ppm
Ln of Biomass Conc.
4
3.5
3
2.5
2
0.5
0.7
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9
Time, Hours
Specific Growth Rate = 0.5724
Correlation = 0.9951
S = 0 included
Experimental data

Fig 4.2

Yield Coefficient of P. resinovorans
For Data Set # 1
Average Phenol Concentration 6 ppm

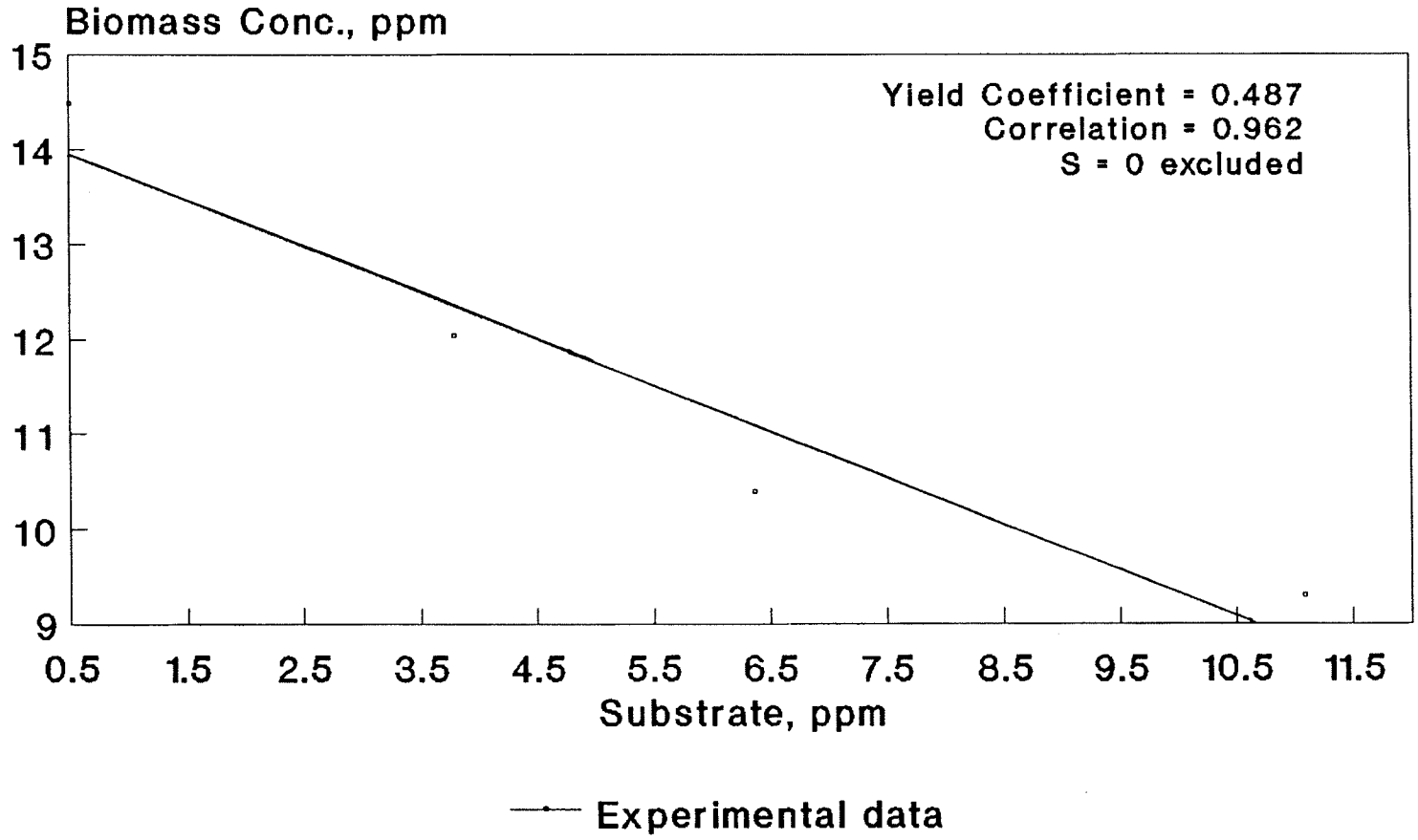

Fig 5.1

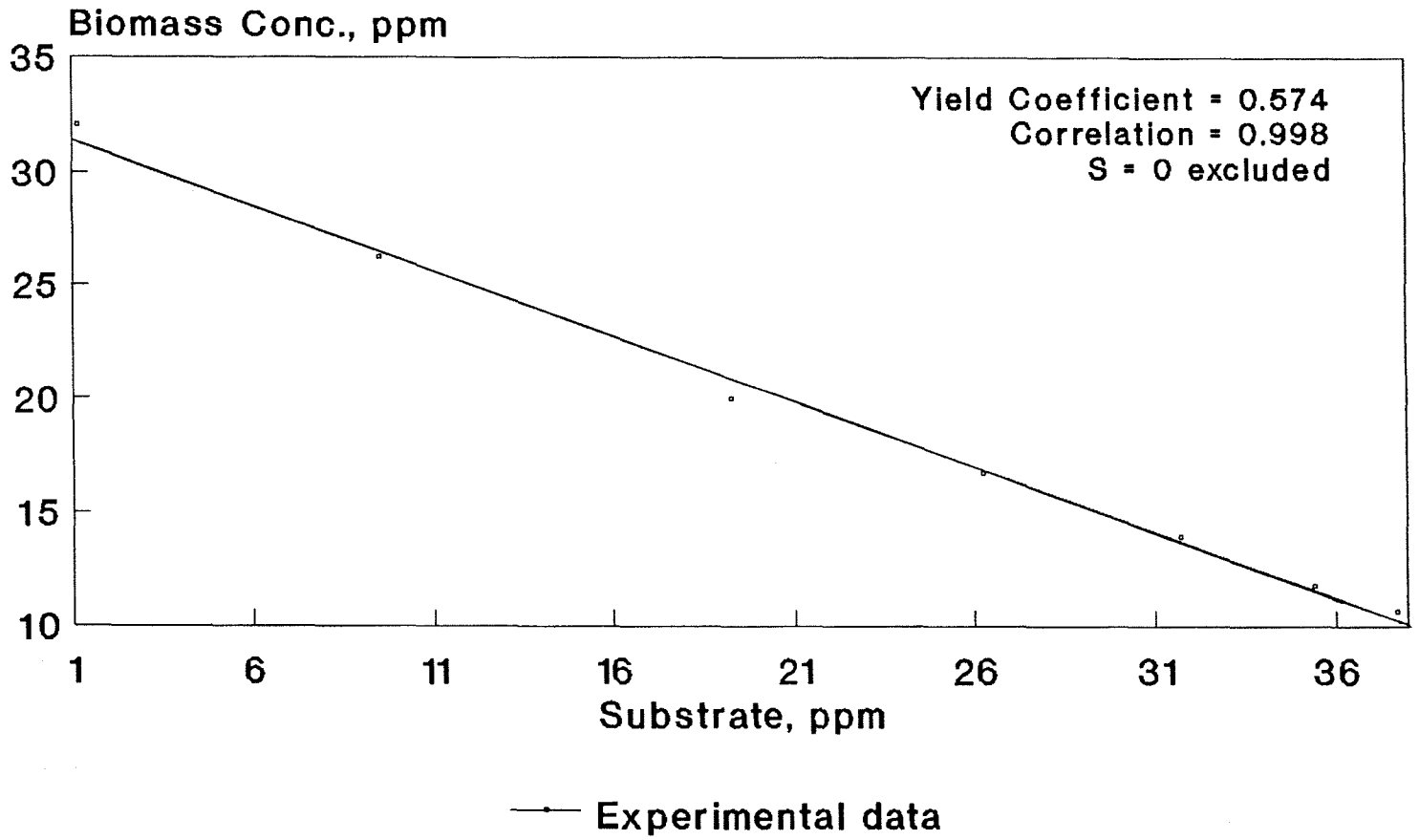
Yield Coefficient of P. resinovorans
For Data Set # 1
Average Phenol Concentration 19 ppm
Biomass Conc., ppm
35
30
25
20
15
10
1
6
11
16
21
26
31
36
Substrate, ppm
Yield Coefficient = 0.574
Correlation = 0.998
S = 0 excluded
Experimental data

Fig 5.2

Yield Coefficient of P. resinovorans
For Data Set # 2
Initial Phenol Concentration 11 ppm

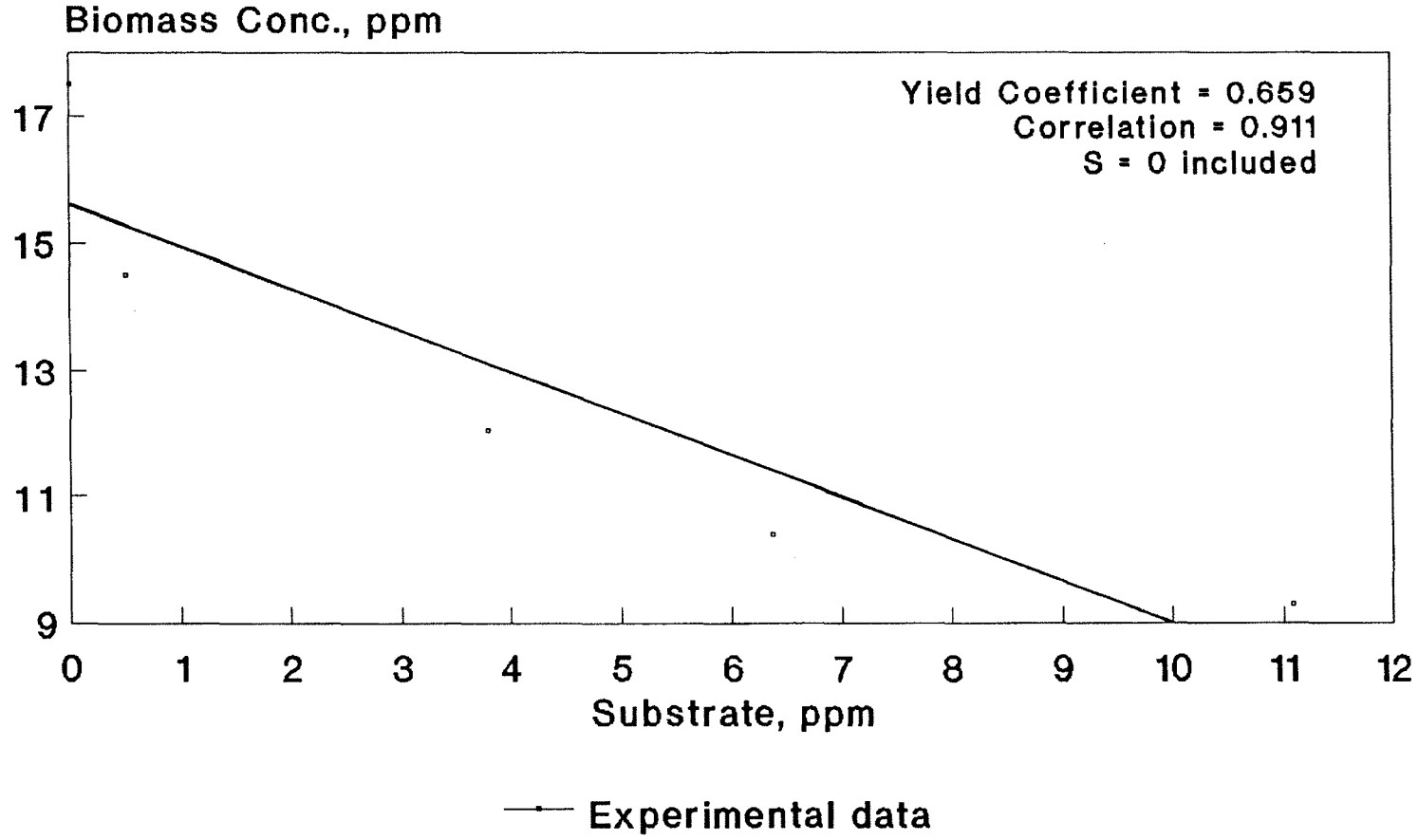

Fig 6.1

Yield Coefficient of P. resinovorans
For Data Set # 2
Initial Phenol Concentration 38 ppm

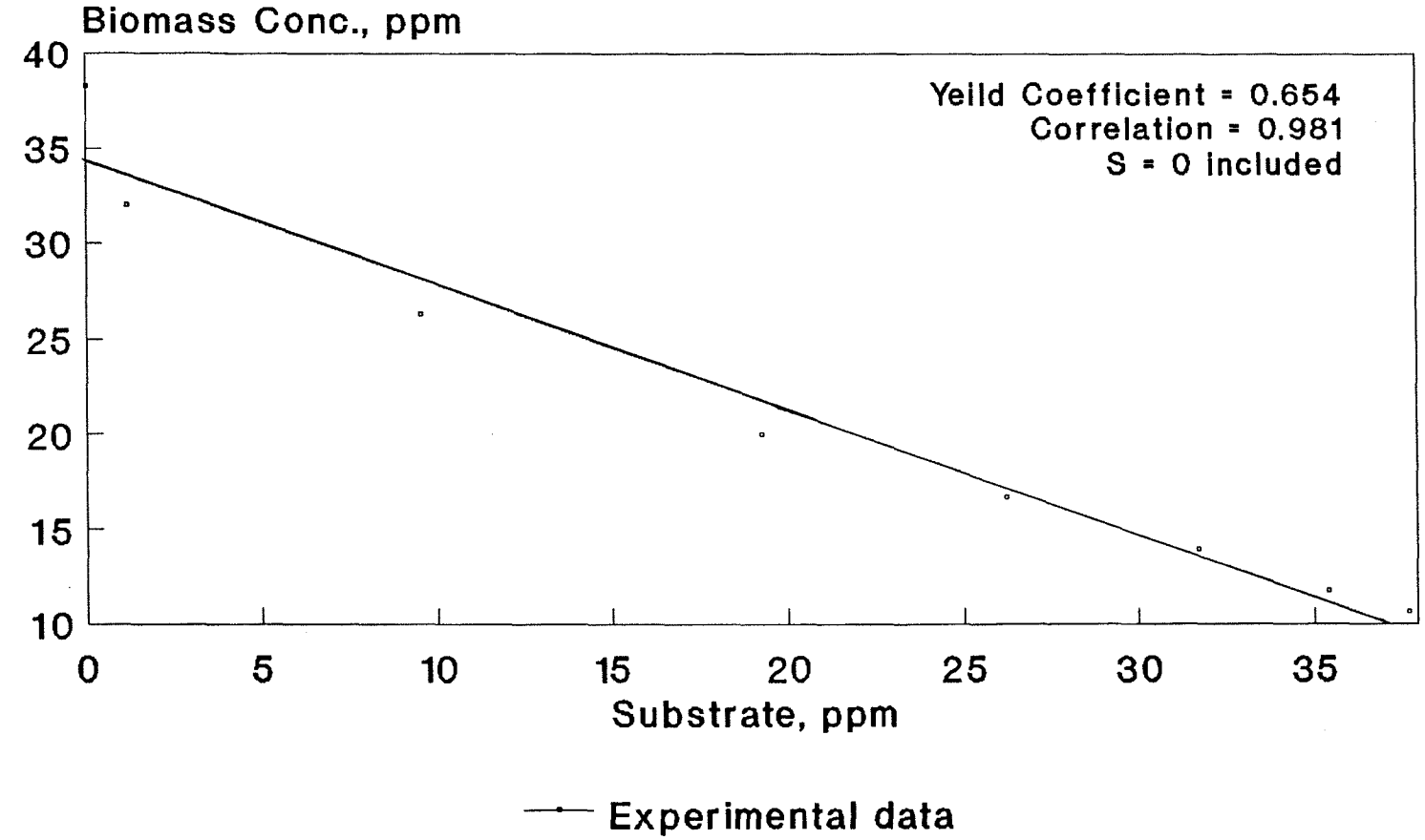

Fig 6.2

Experimental & Predicted Specific Growth Rate versus Corresponding Average Phenol Concentrations Based On Data Set # 1

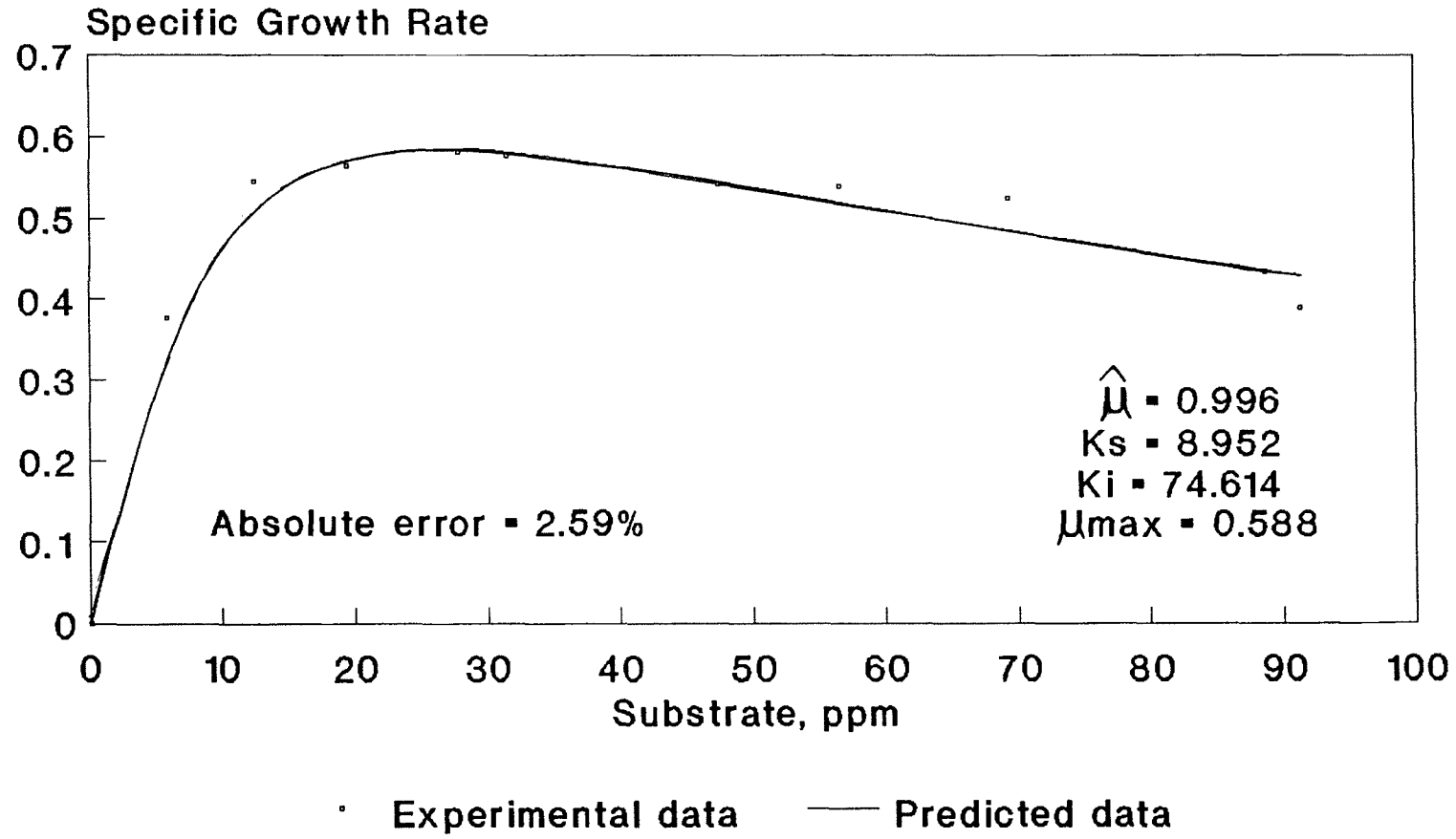

Fig 7.1

Experimental & Predicted Specific Growth Rate versus Corresponding Initial Phenol Concentrations Based On Data Set # 2

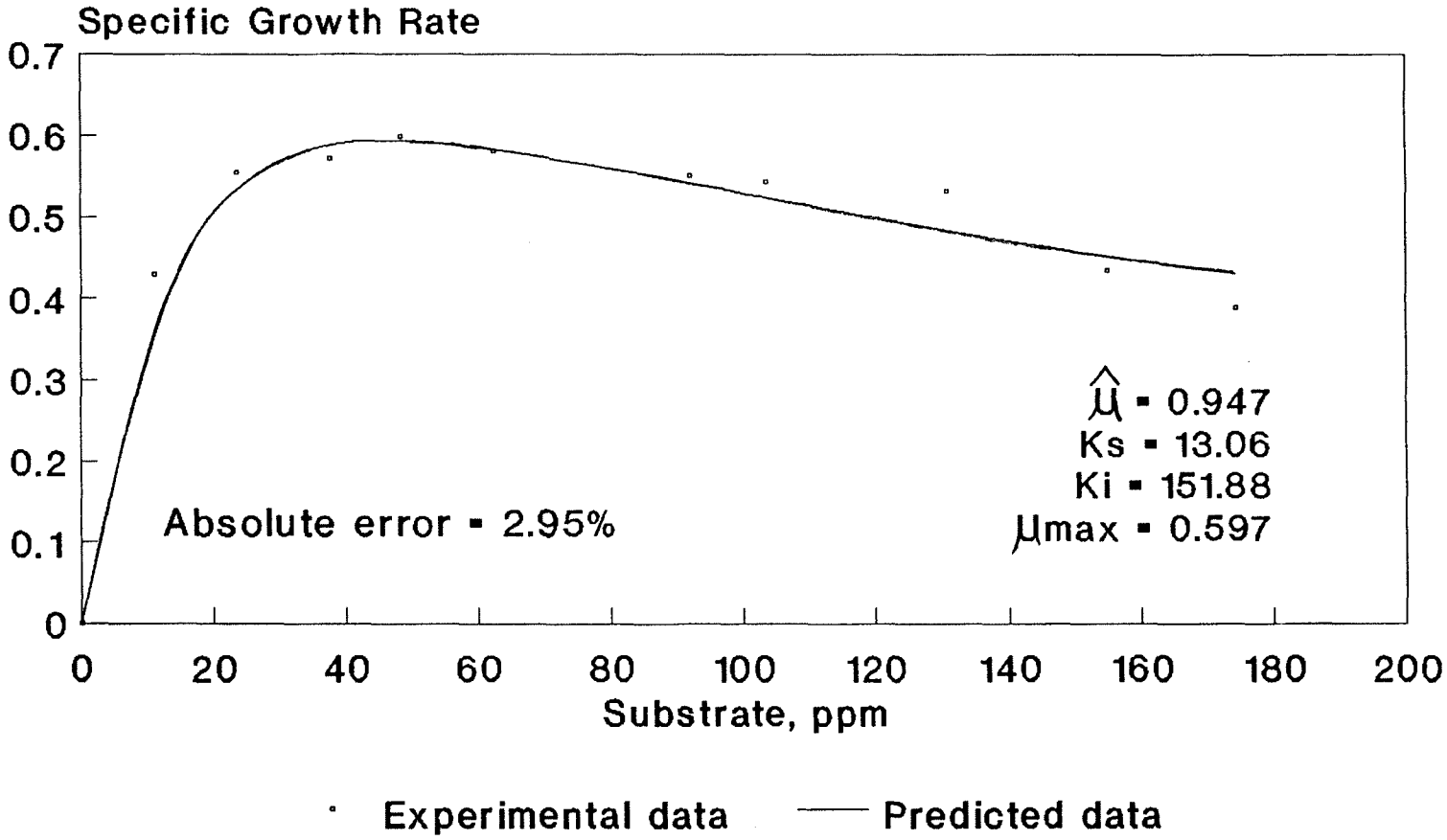

Fig 7.2

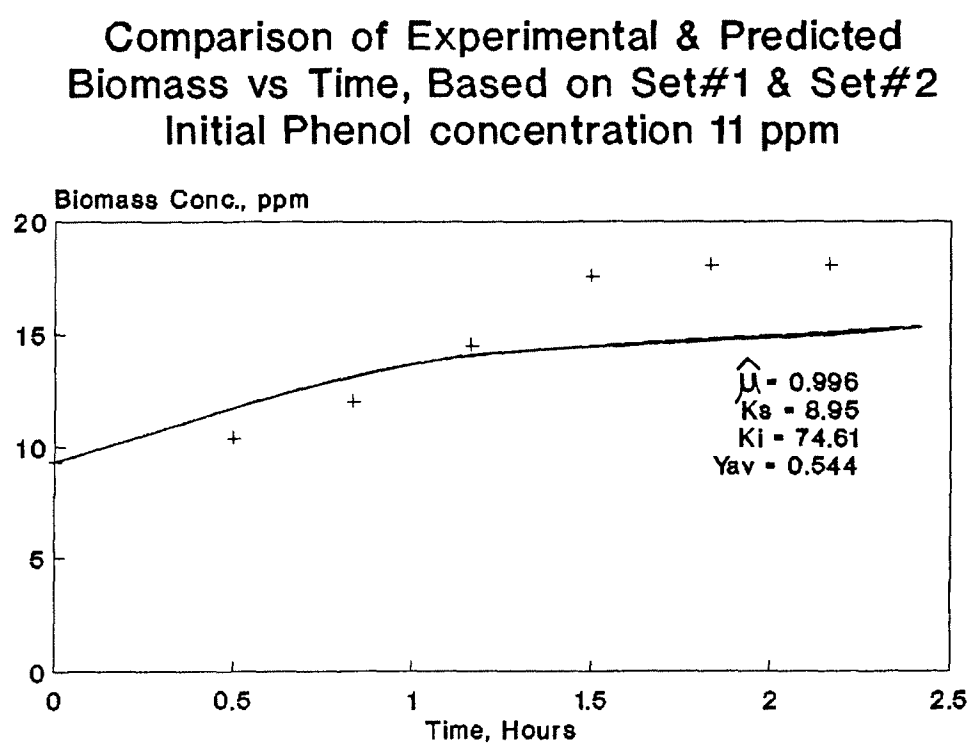

Fig 8.1a: data set #1

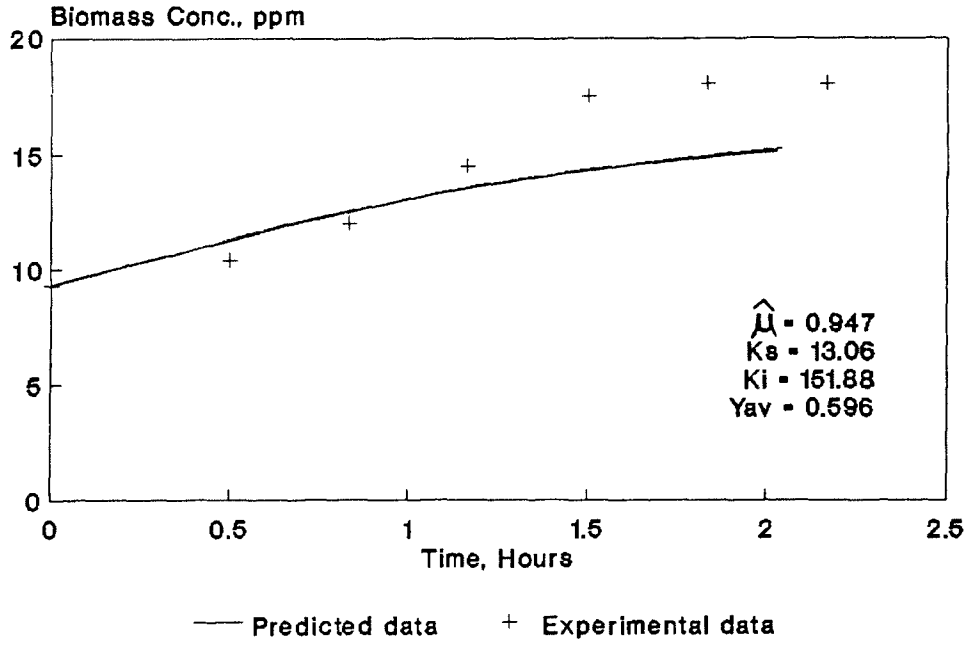

Fig 8.1b: data set # 2

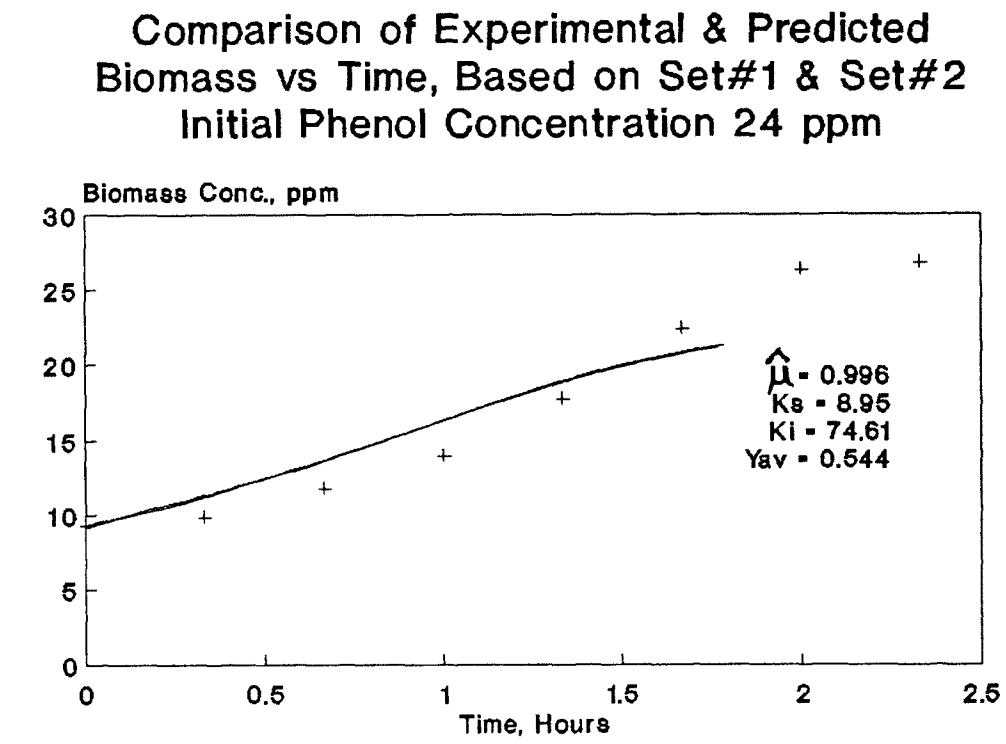

Fig 8.2a: data set #1

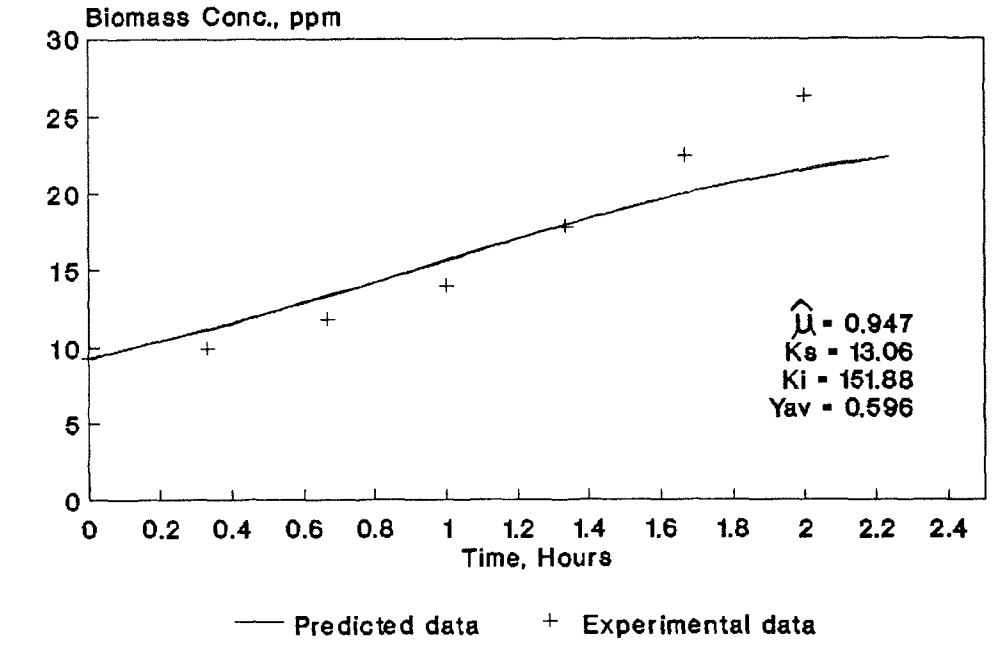

Fig 8.2b: data set # 2

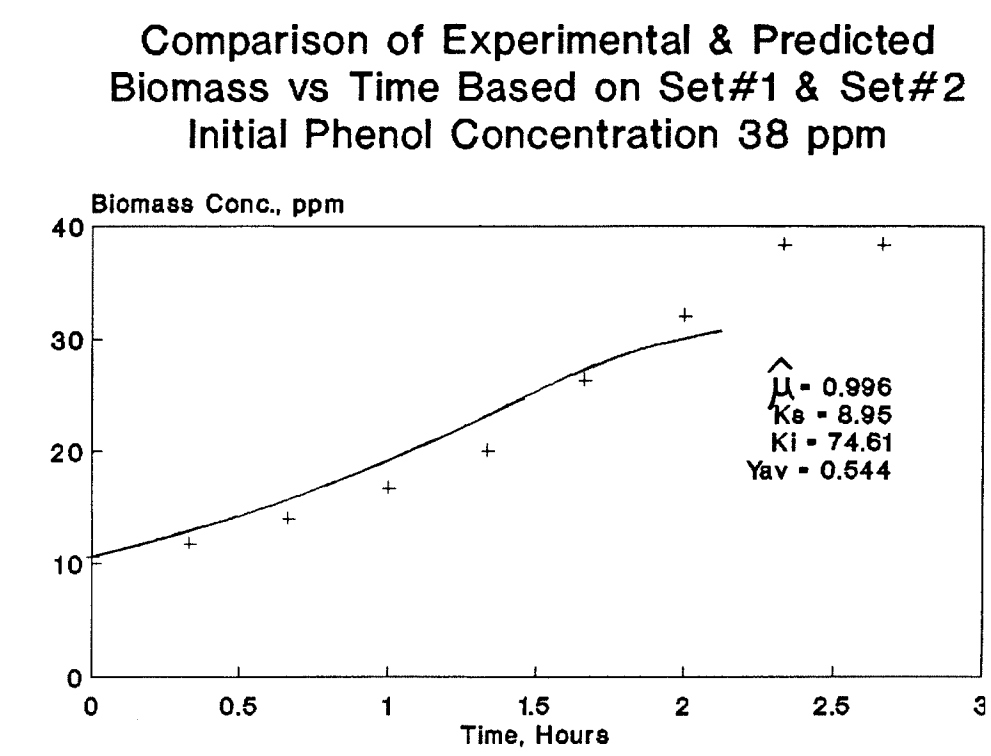

Fig 8.3a: data set # 1

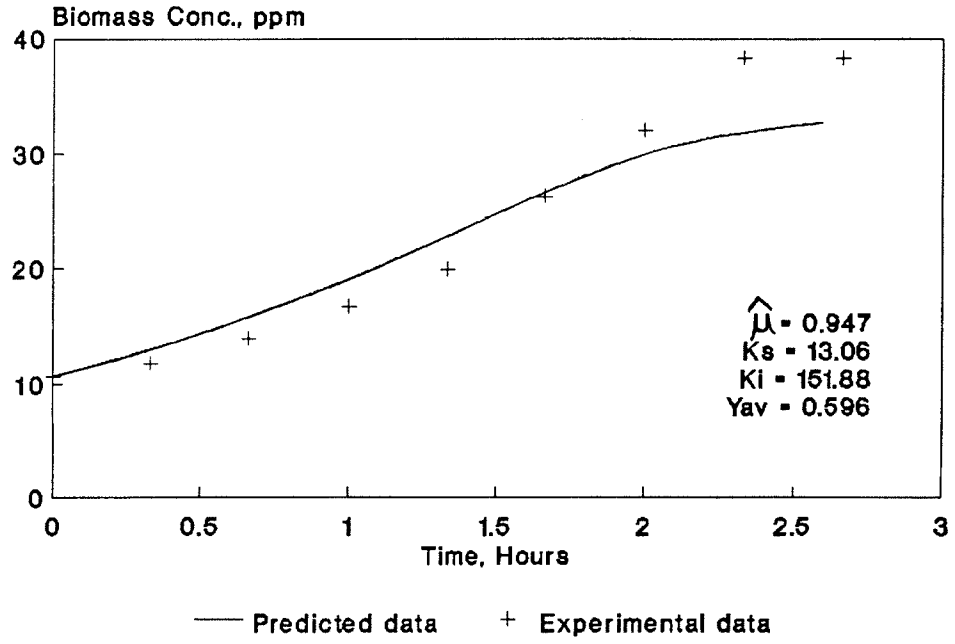

Fig 8.3b: data set # 2

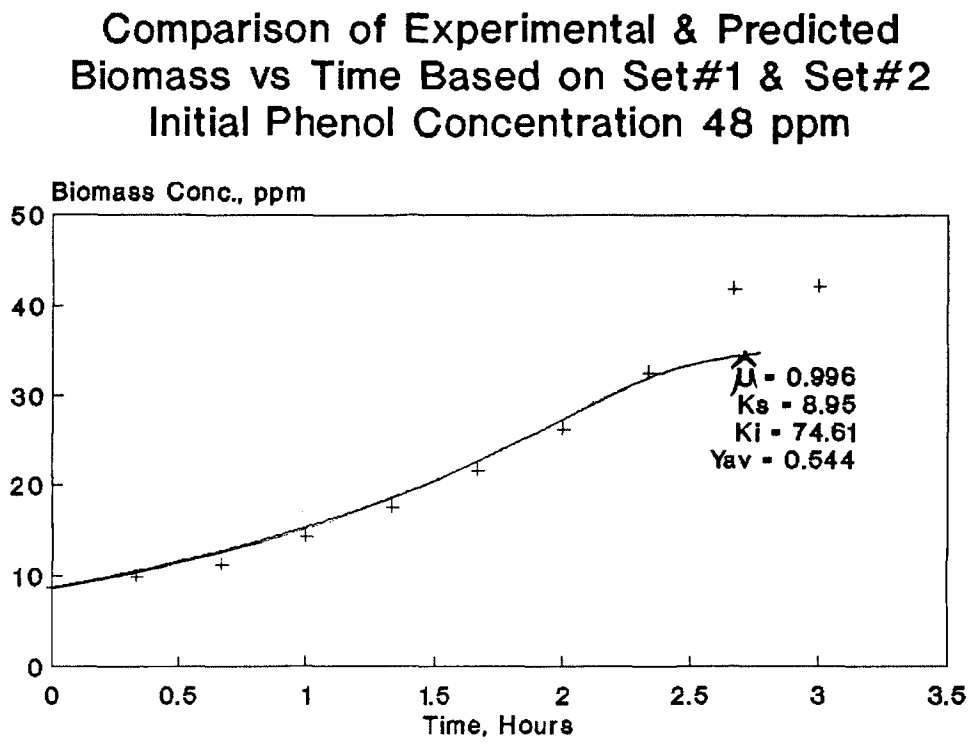

Fig 8.4a: data set # 1

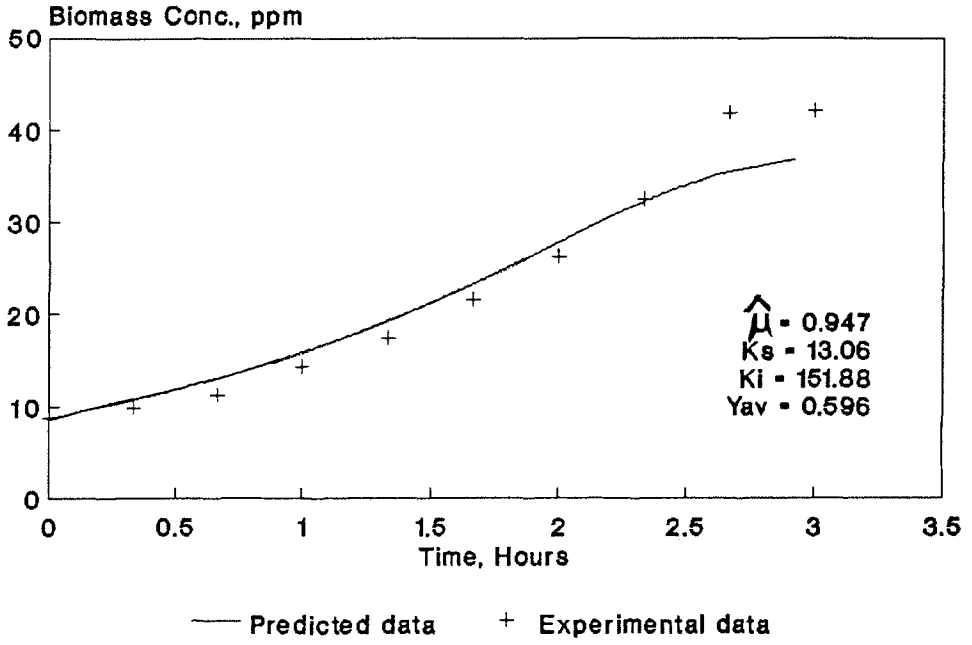

Fig 8.4b: data set # 2

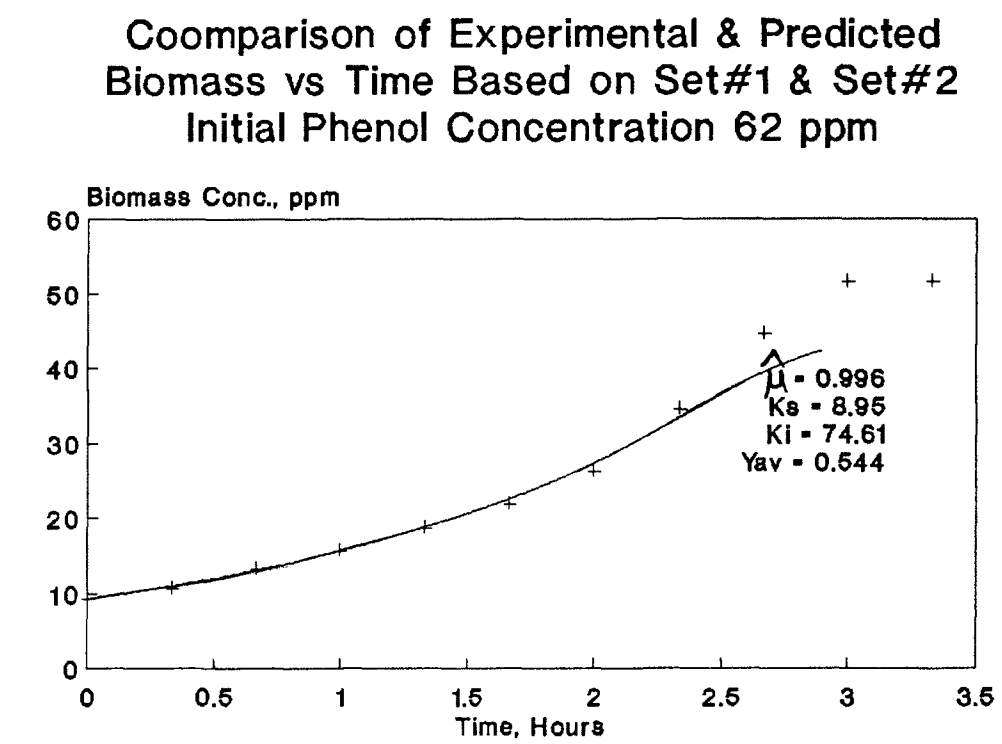

Fig 8.5a: data set # 1

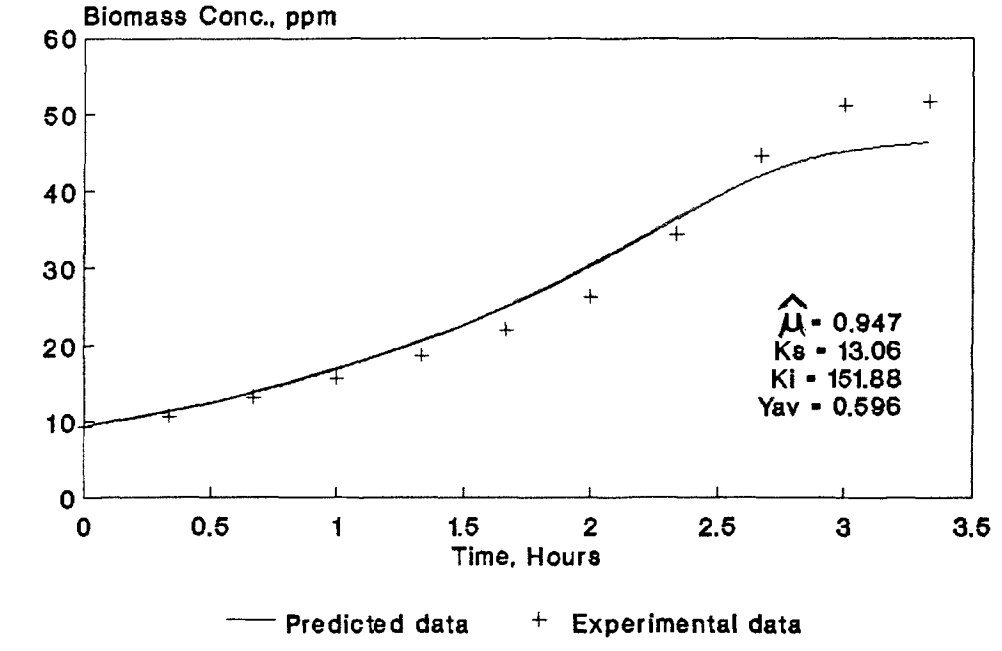

Fig 8.5b: data set # 2

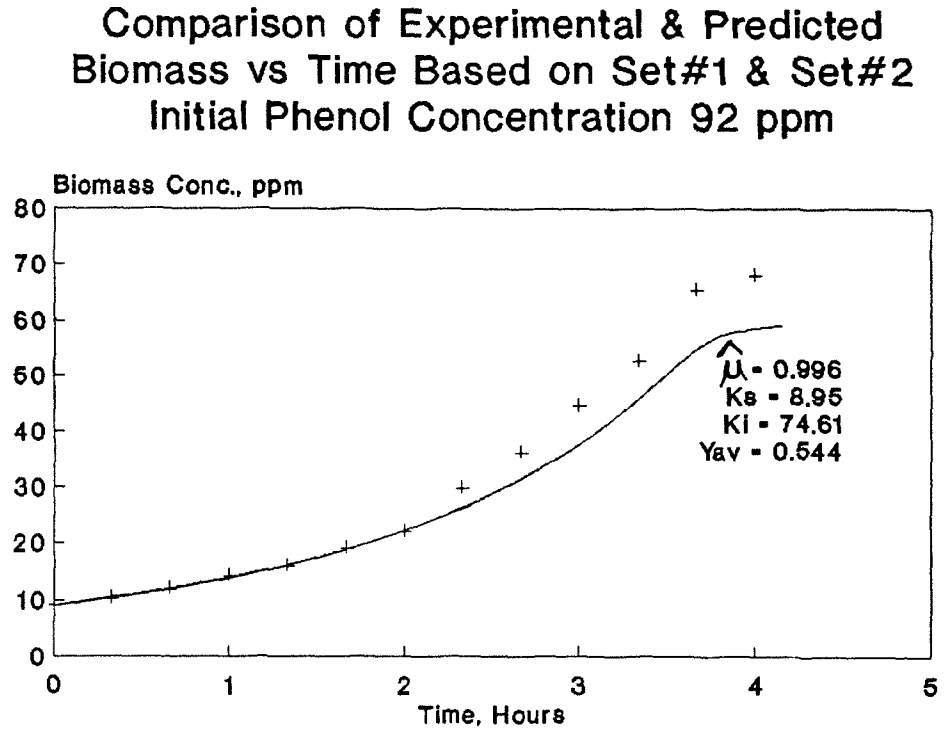

Fig 8.6a: data set # 1

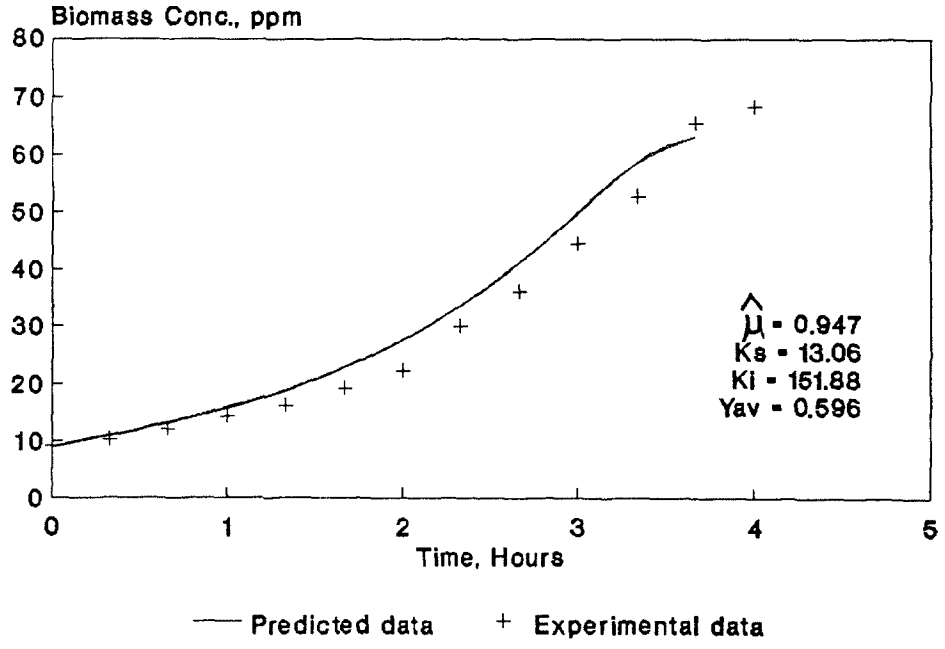

Fig 8.6b: data set # 2

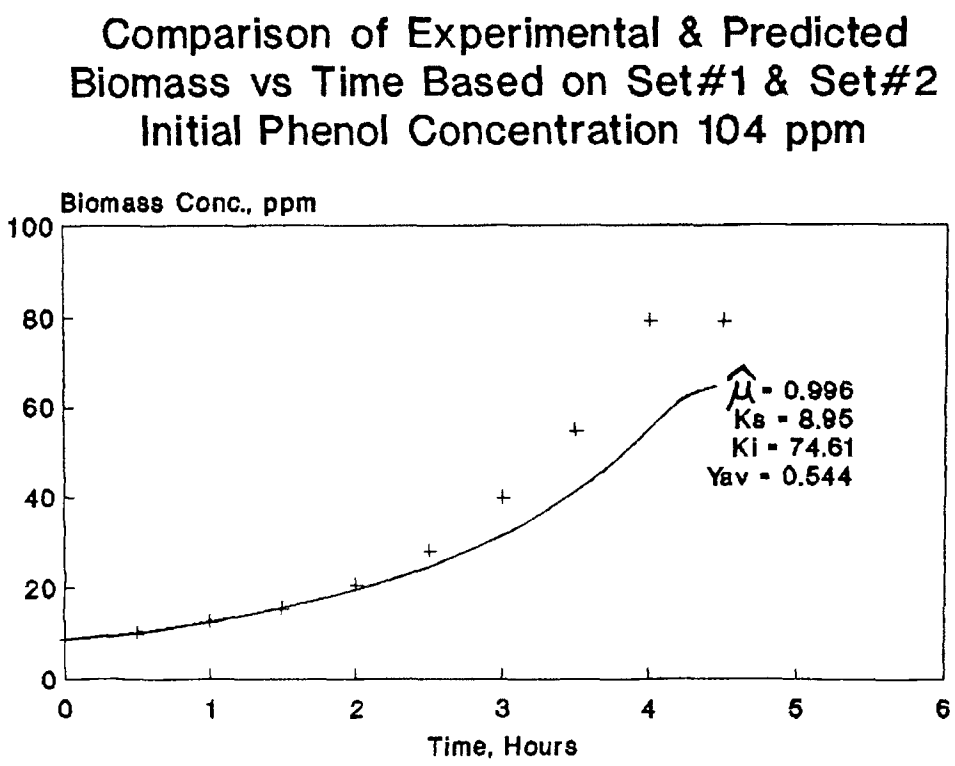

Fig 8.7a: data set # 1

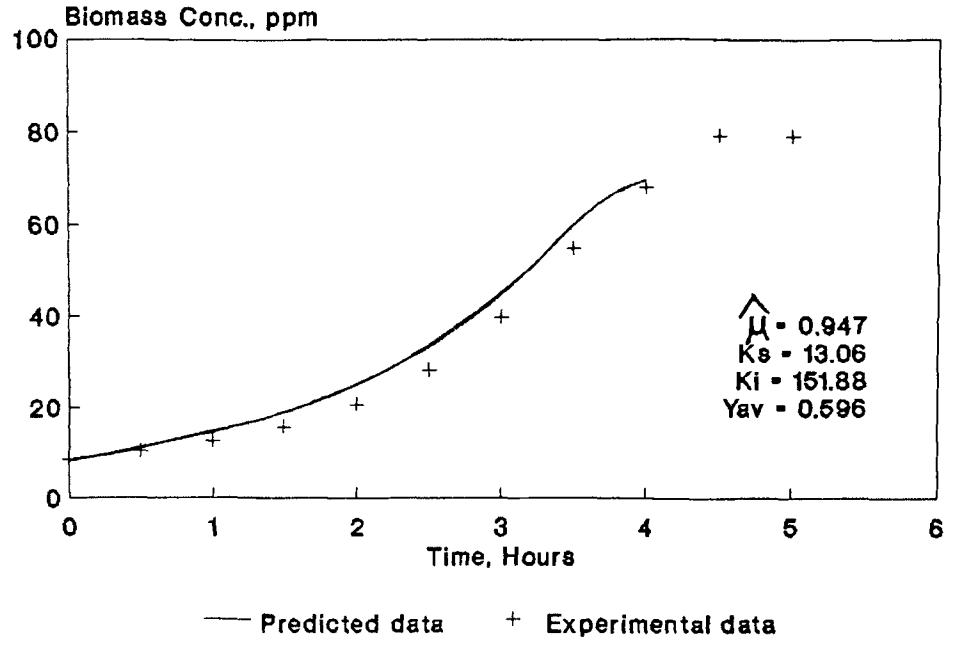

Fig 8.7b: data set # 2

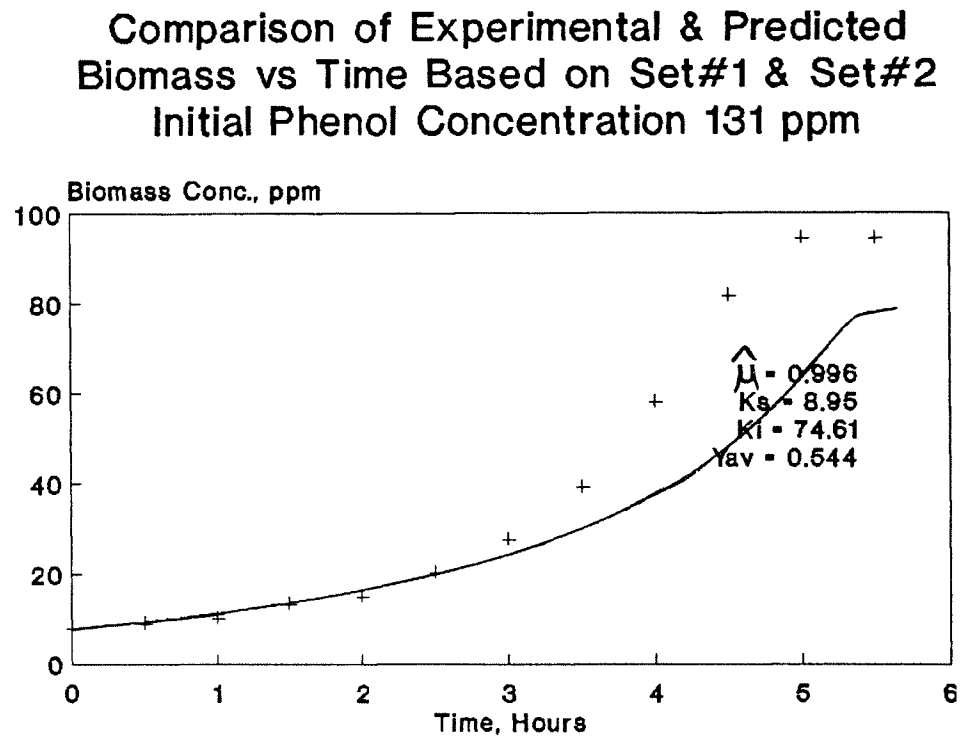

Fig 8.8a: data set # 1

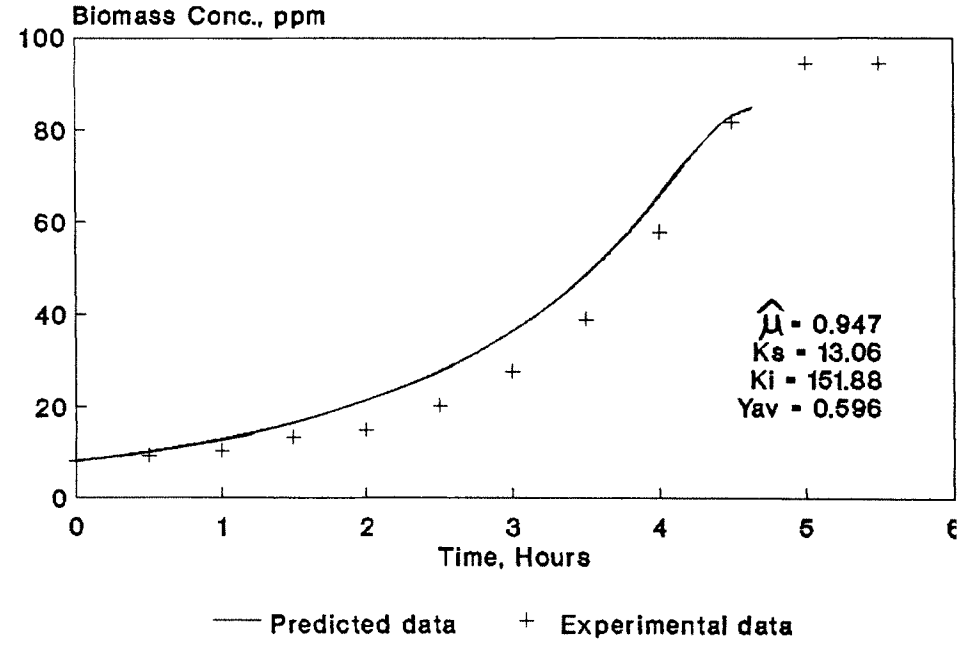

Fig 8.8b: data set # 2

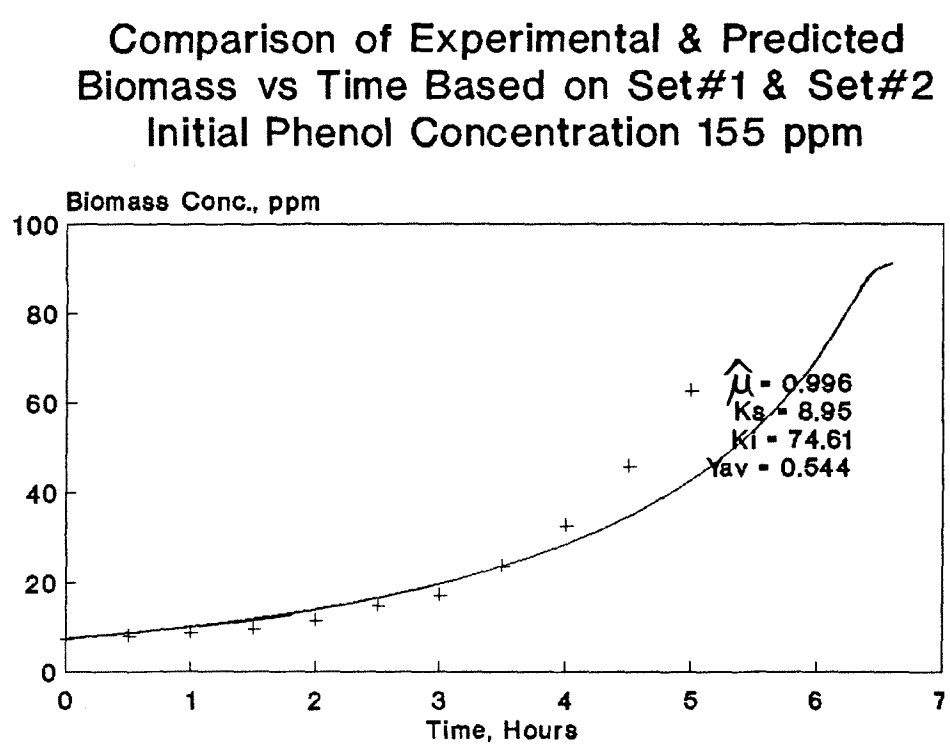

Fig 8.9a: data set # 1

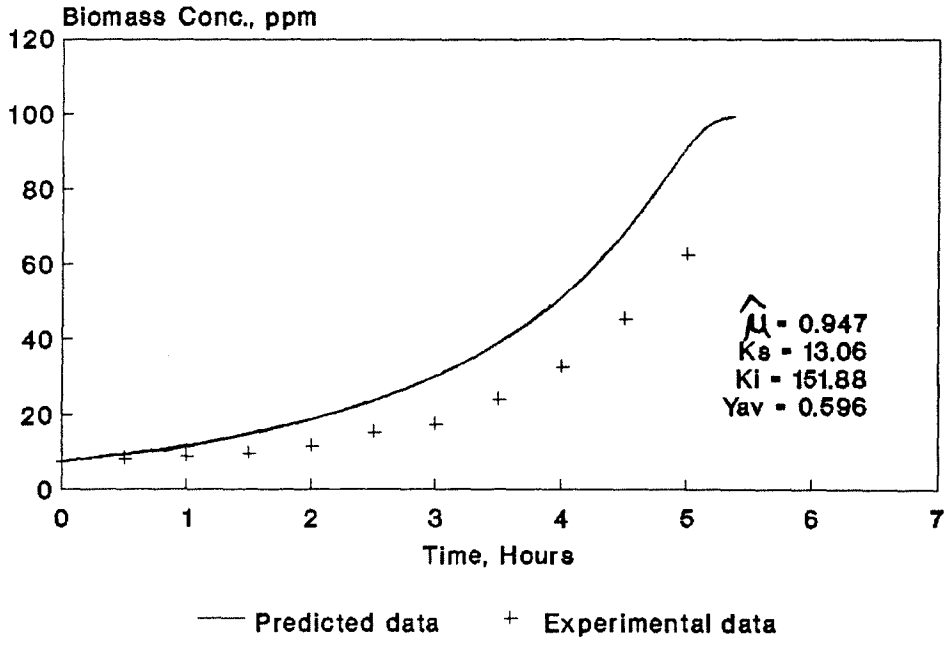

Fig 8.9b: data set # 2

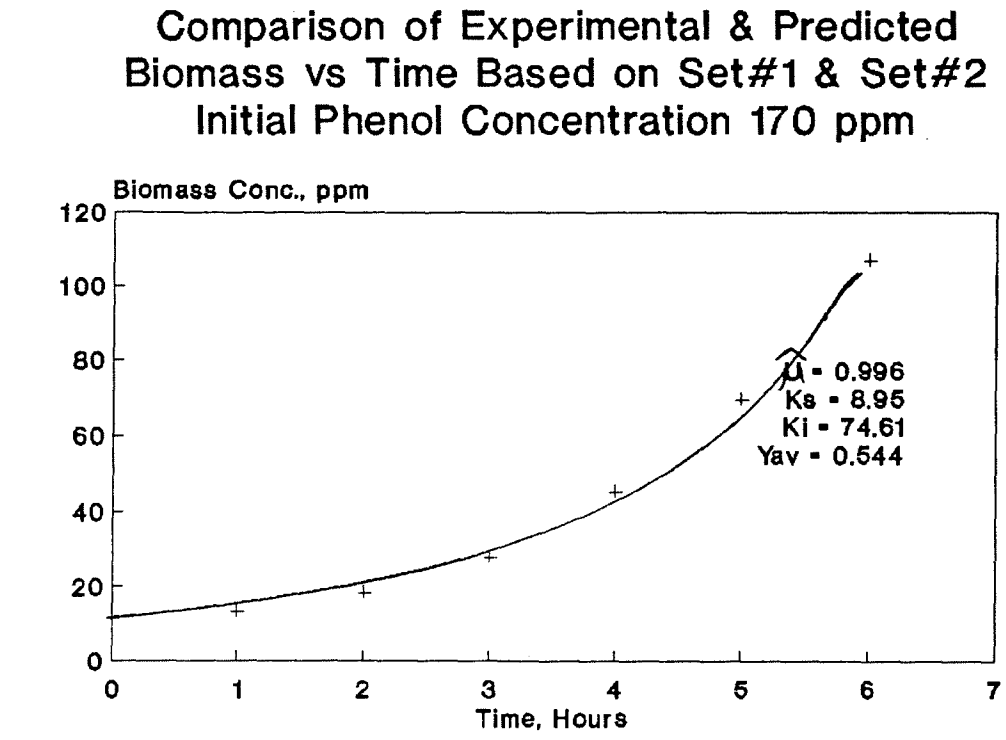

Fig 8.10a: data set # 1

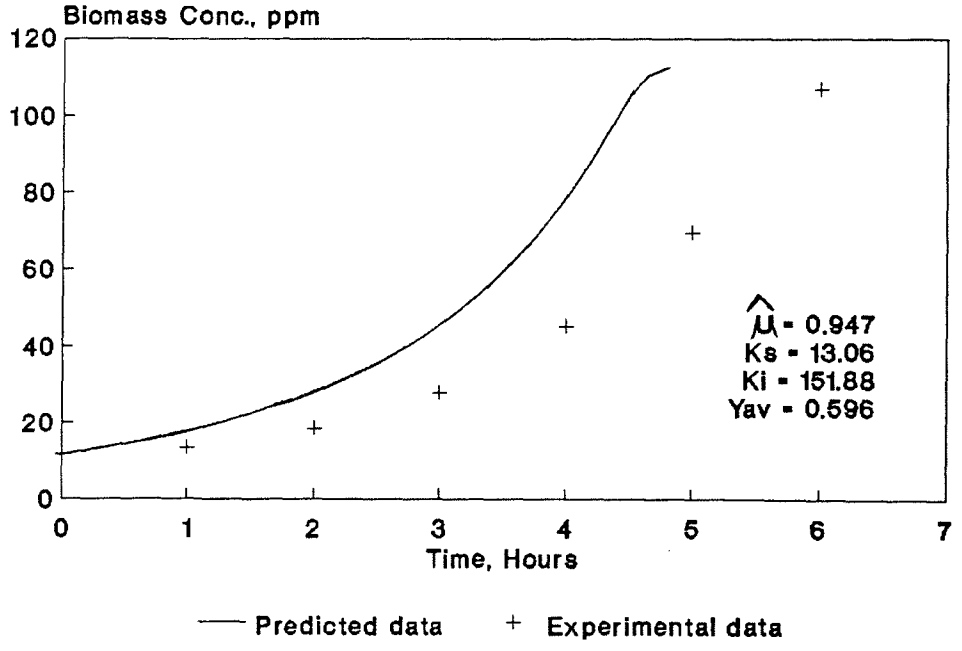

Fig 8.10b: data set # 2

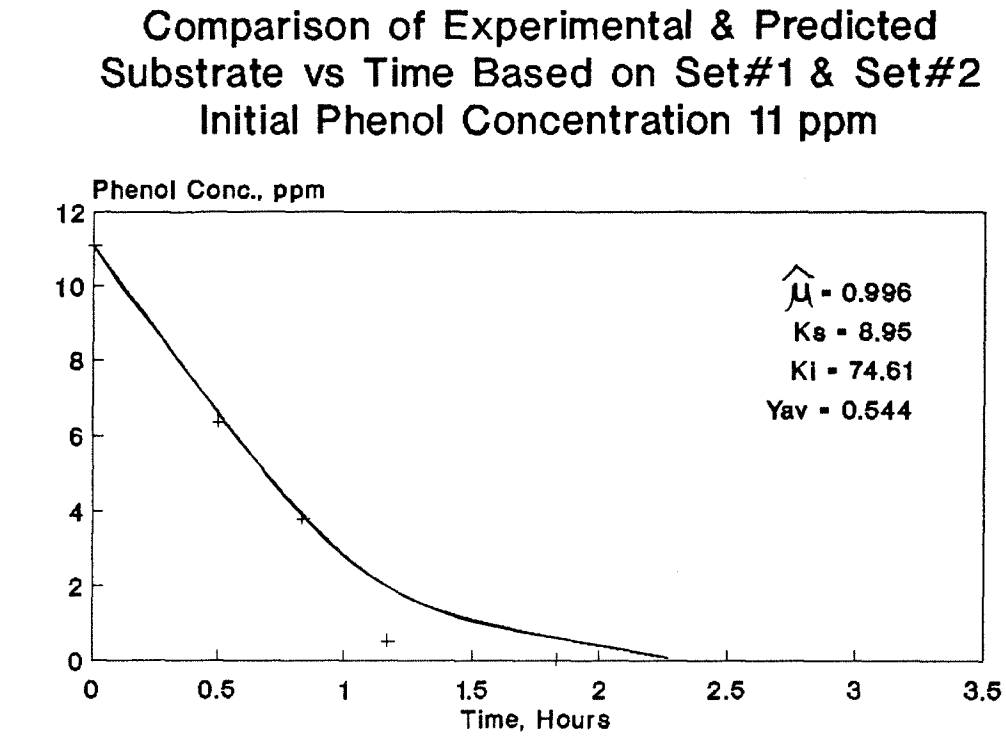

Fig 9.1a: data set # 1

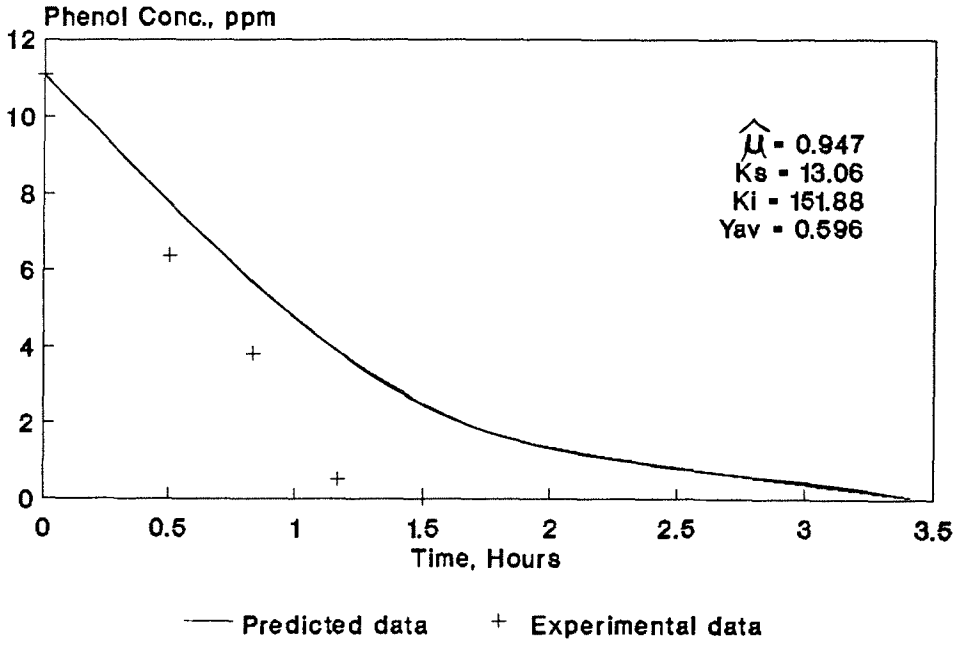

Fig 9.1b: data set # 2

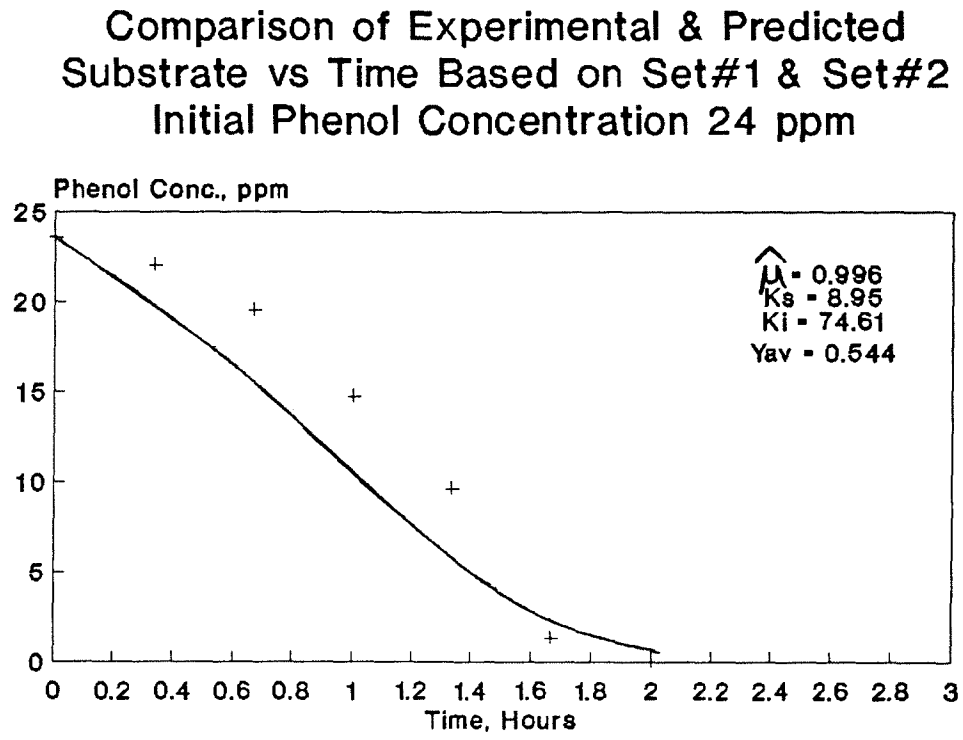

Fig 9.2a: data set # 1

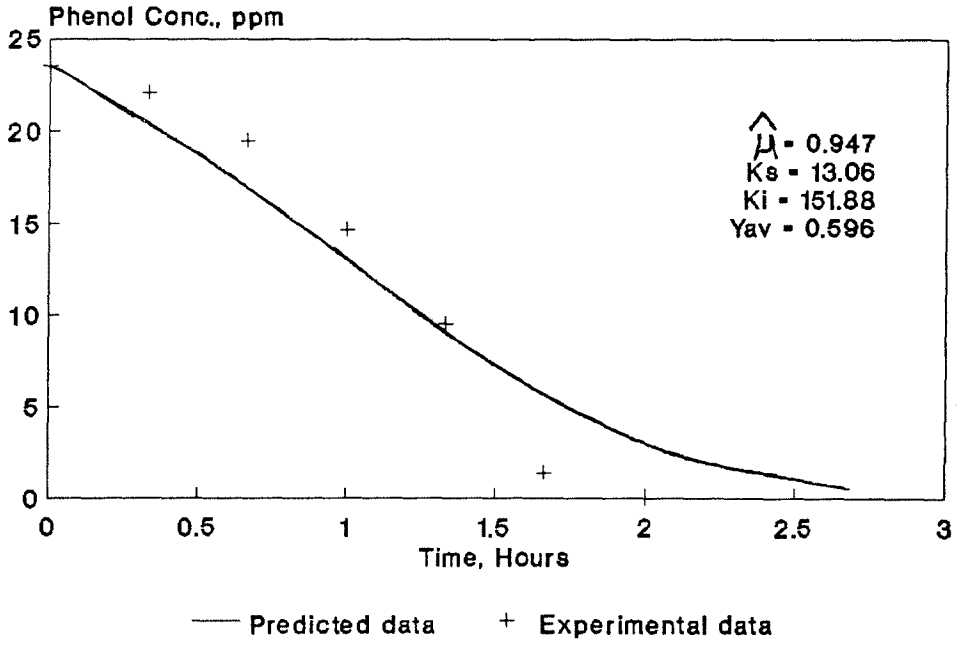

Fig 9.2b: data set # 2

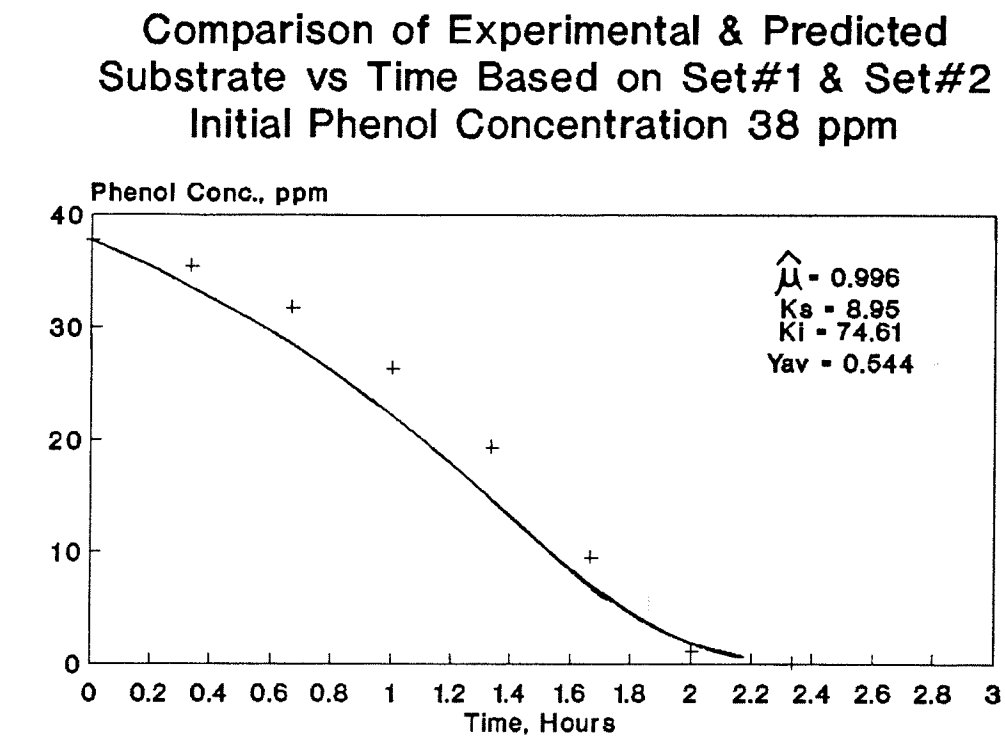

Fig 9.3a: data set # 1

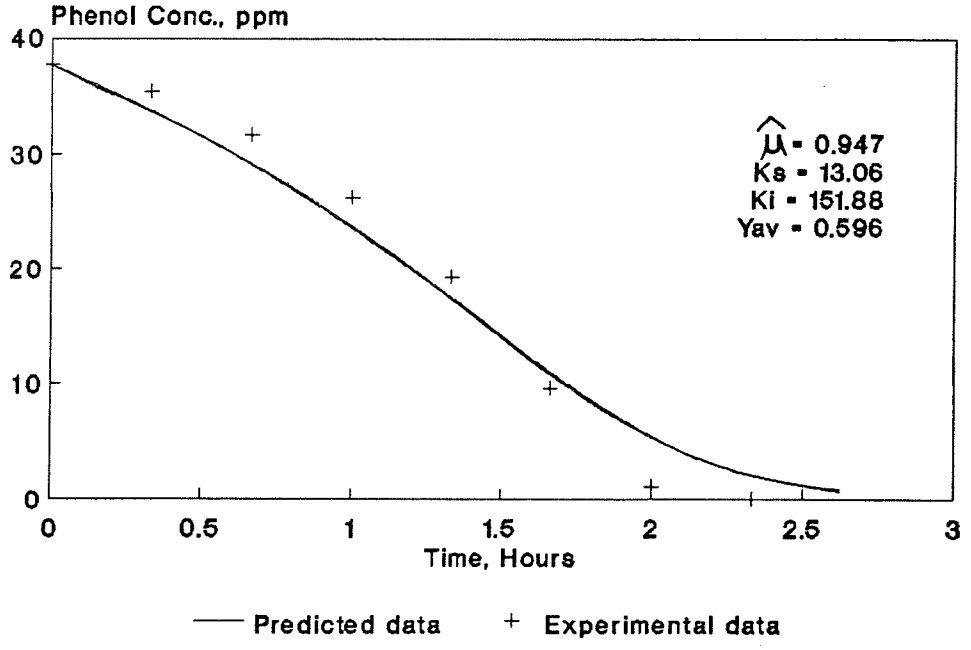

Fig 9.3b: data set # 2

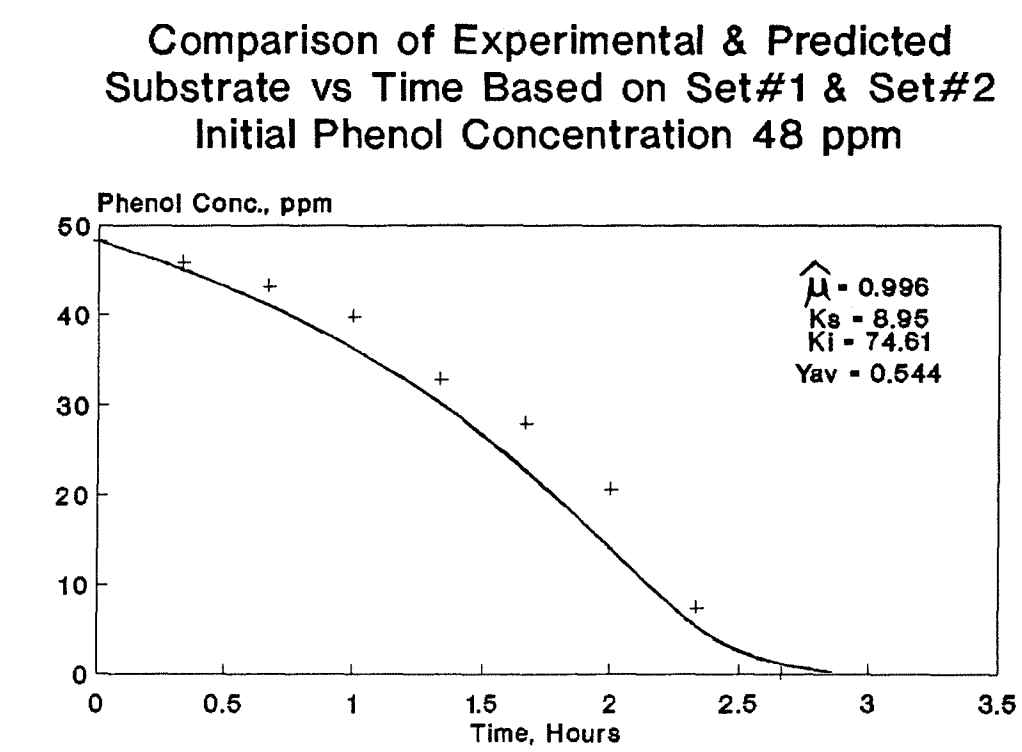

Fig 9.4a: data set #1

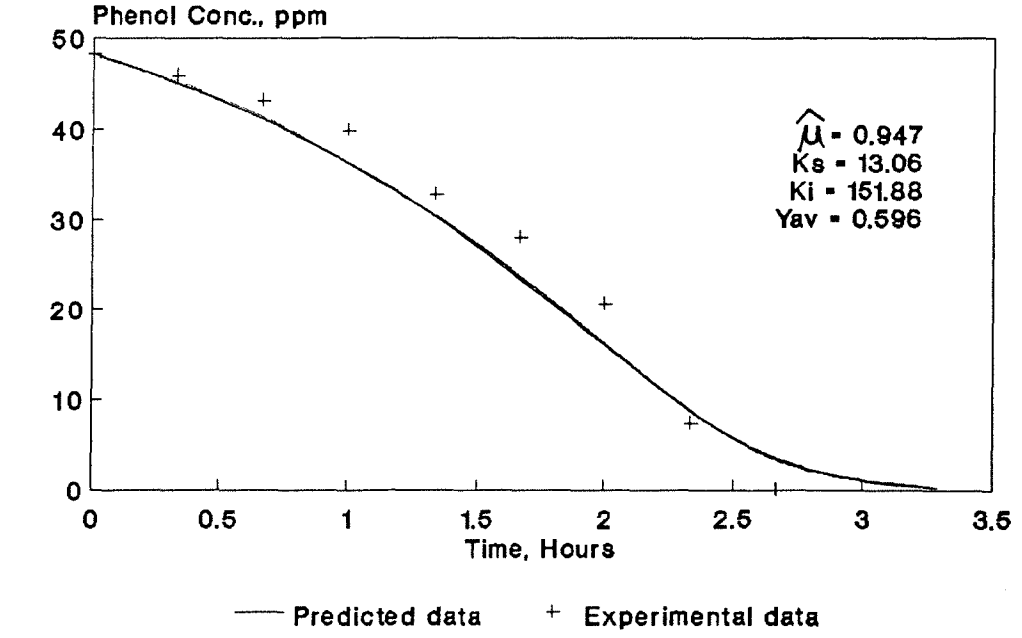

Fig 9.4b: data set # 2

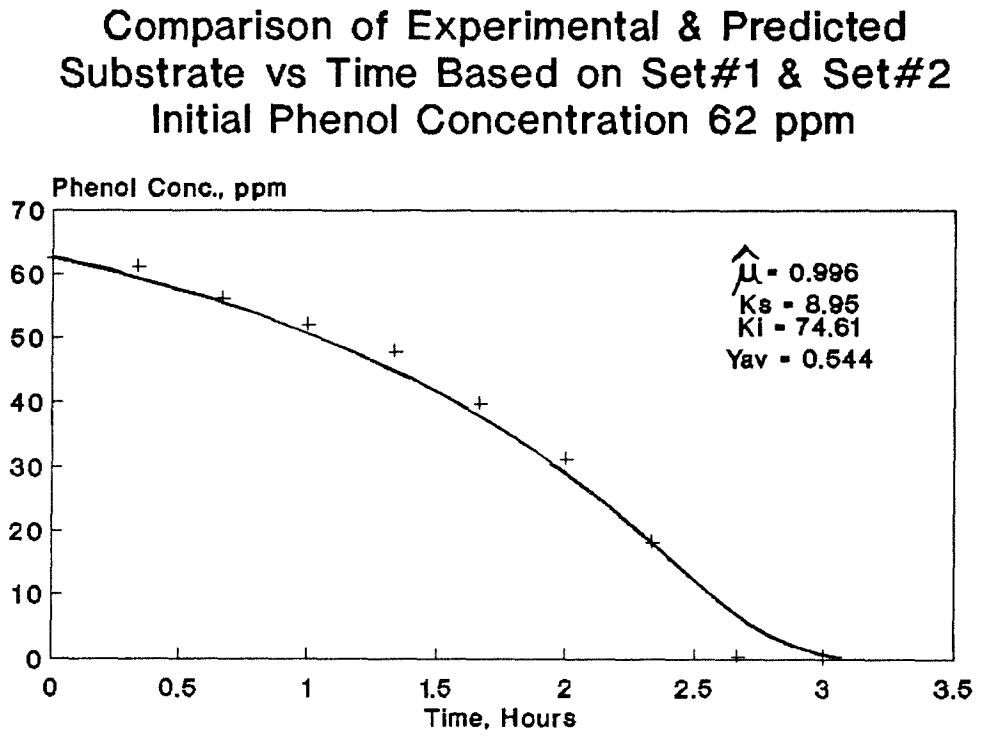

Fig 9.5a: data set # 1

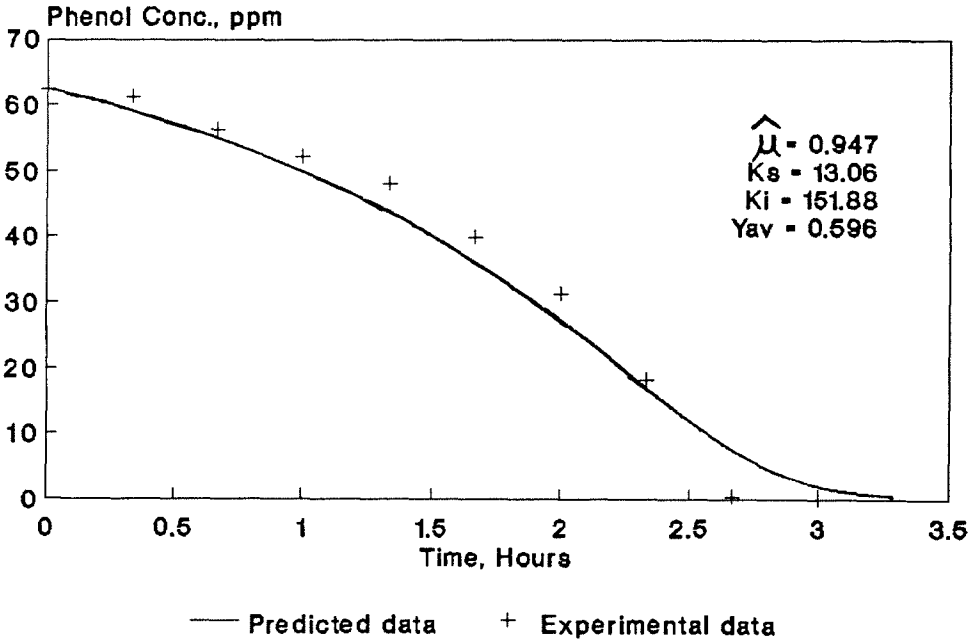

Fig 9.5b: data set # 2

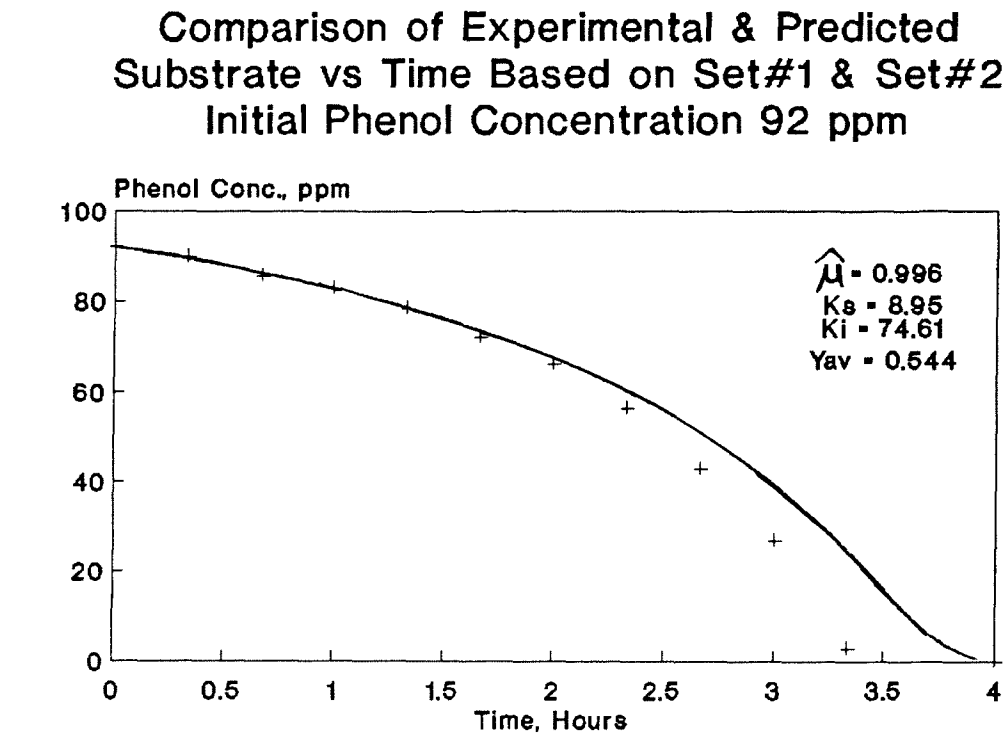

Fig 9.6a: data set # 1

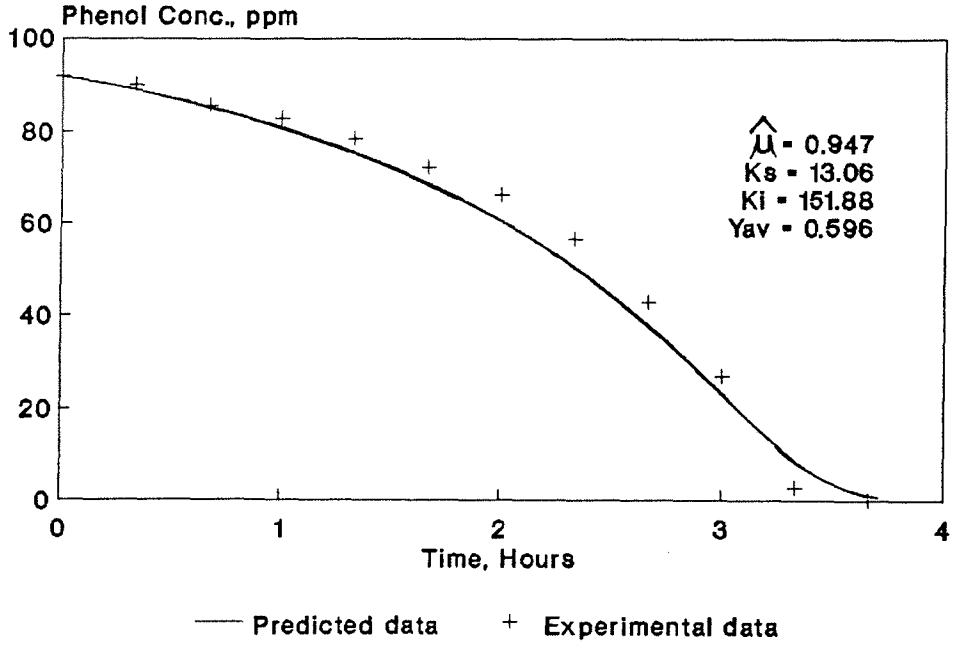

Fig 9.6b: data set # 2

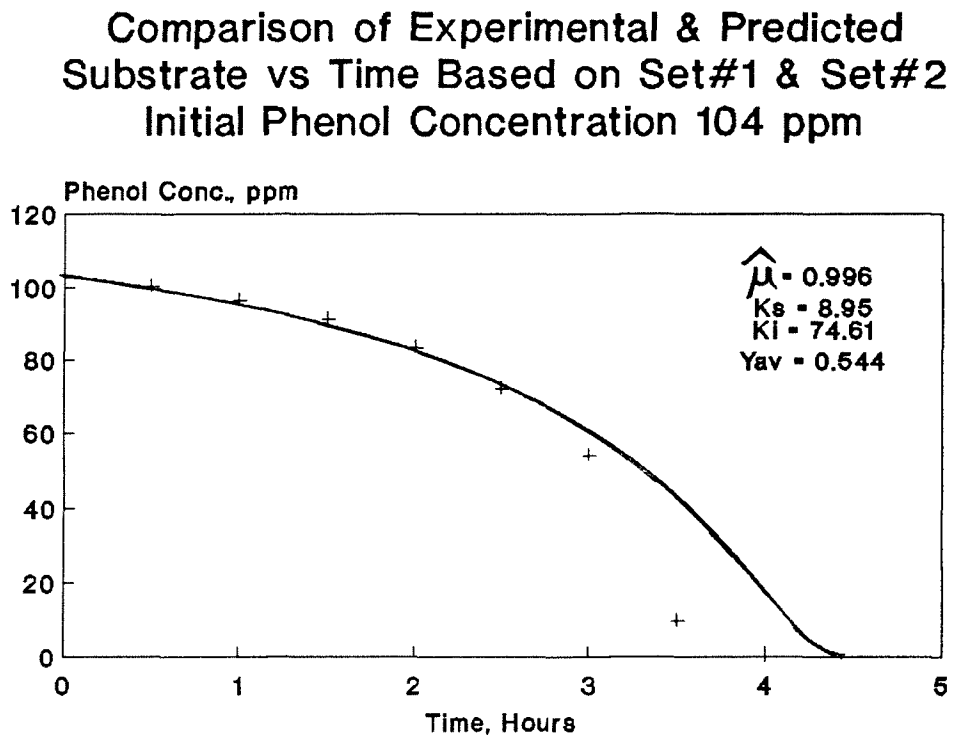

Fig 9.7a: data set #1

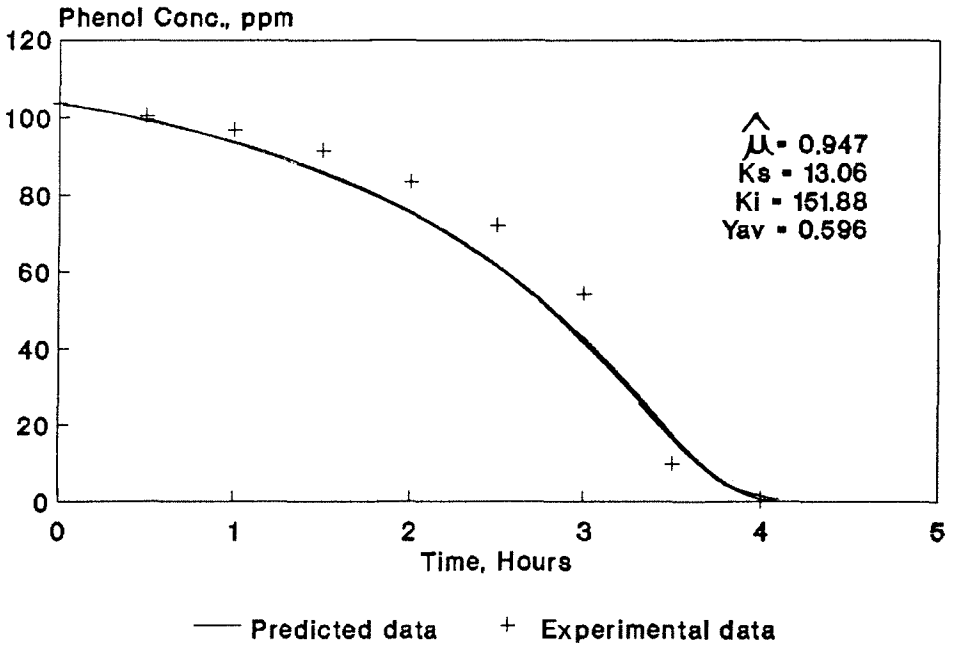

Fig 9.7b: data set # 2

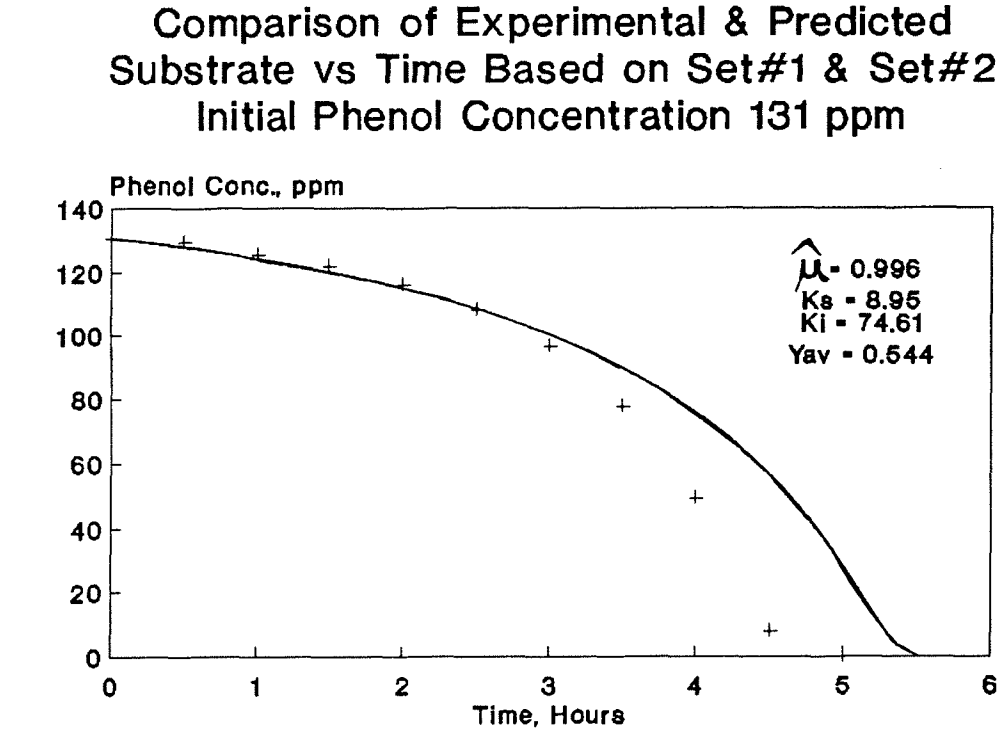

Fig 9.8a: data set # 1

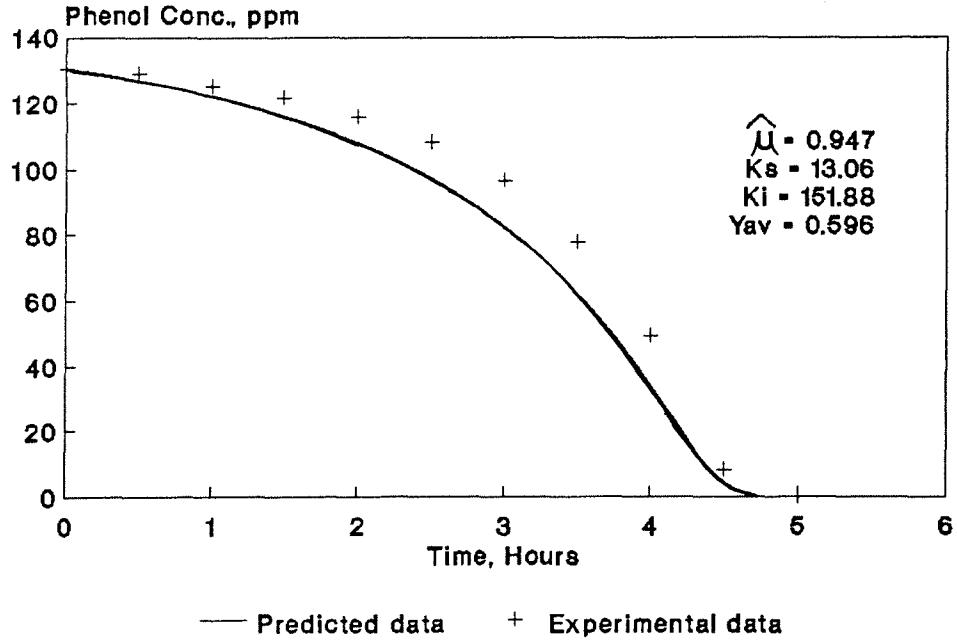

Fig 9.8b: data set # 2

Comparison of Experimental & Predicted Substrate vs Time Based on Set#1 & Set#2 Initial Phenol Concentration 155 ppm

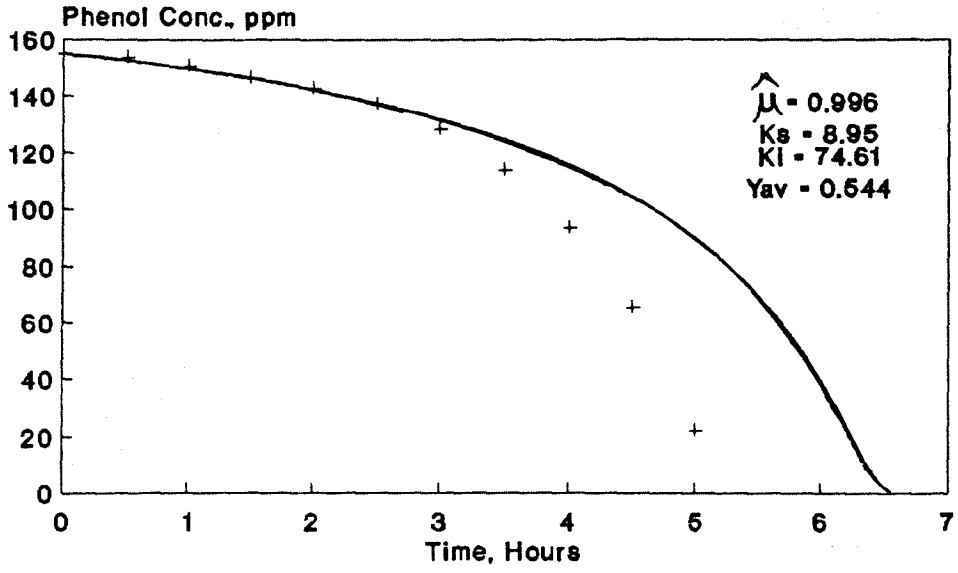

Fig 9.9a: data set # 1

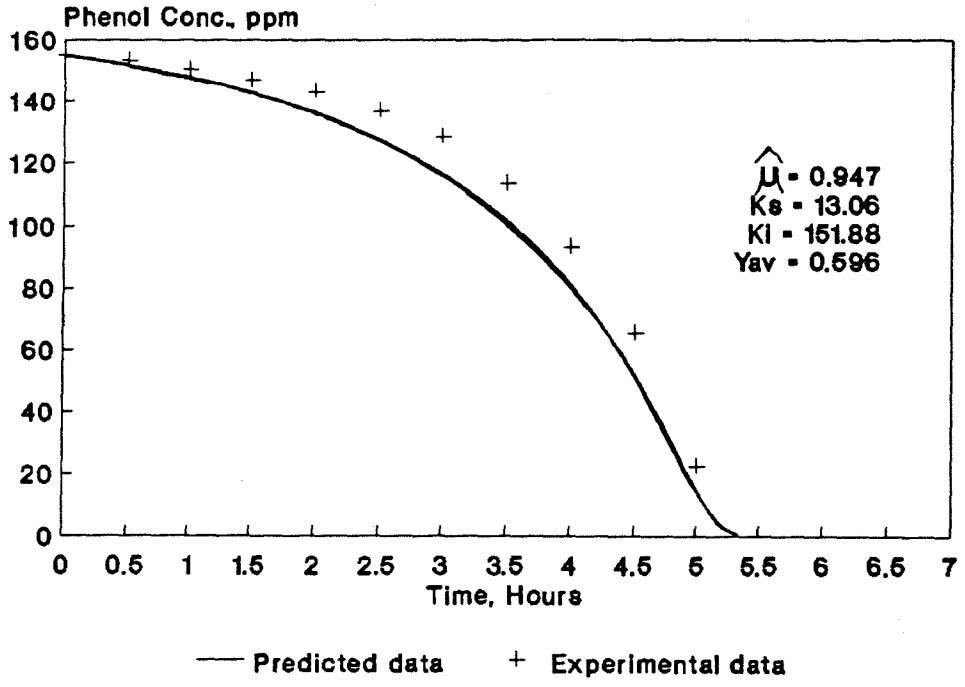

Fig 9.9b: data set # 2

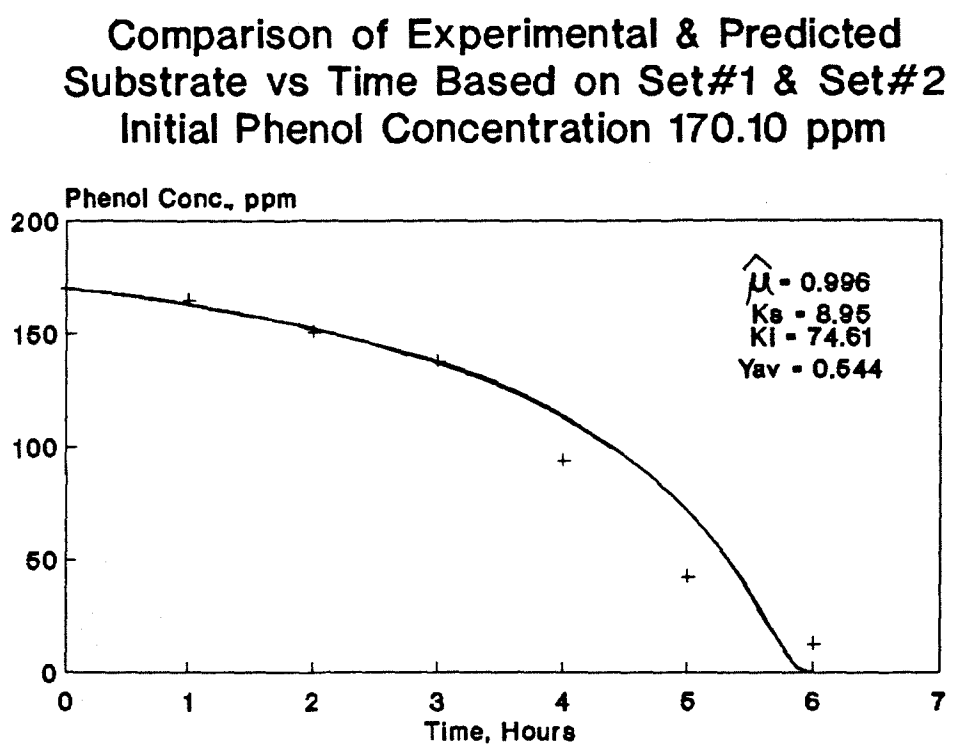

Fig 9.10a: data set # 1

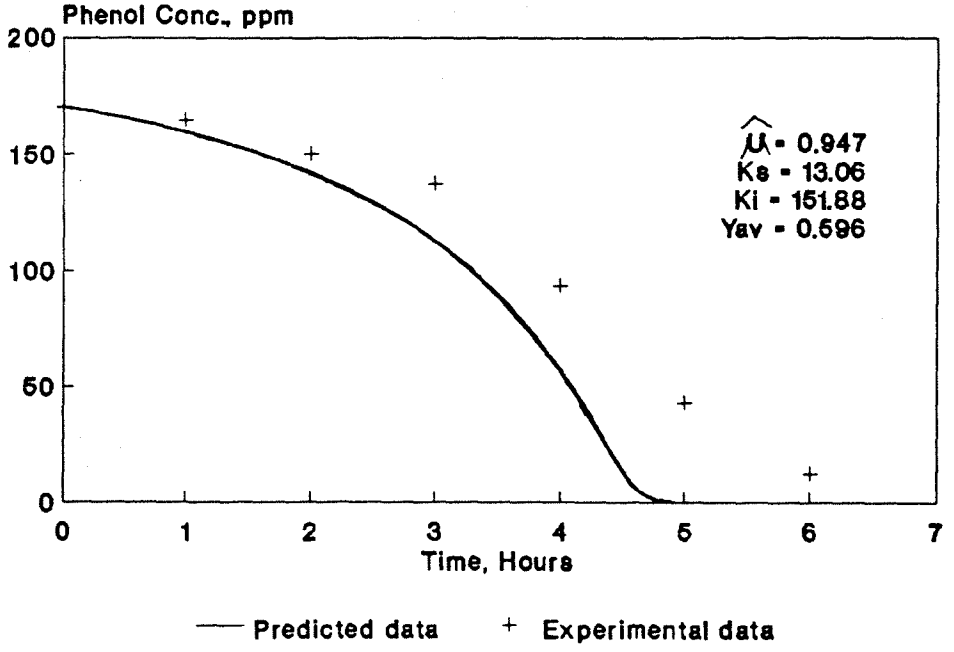

Fig 9.10b: data set # 2