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Microbial competition in the biodegradation of phenol

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ABSTRACT

Title of Thesis: Microbial Competition in the Biodegradation of Phenol

Mary Ellen Frank, Master of Science, 1989
Environmental Science (Toxics Option)

Thesis directed by: Dr Gordon A. Lewandowski
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Three phenol degraders were isolated from the mixed liquor of the Livingston, NJ municipal treatment plant. Shaker flask experiments using one of the degraders (a Pseudomonas sp.) resulted in the determination of its kinetic rate constants. The second phenol degrader (a Xanthamonas) lost its phenol degrading ability while being stored, and its kinetic rate constants could not be determined. The third phenol degrader (a Pseudomonas cepacia) formed flocs which interfered with the spectrophotometric determination of biomass and therefore its kinetic rate constants also could not be determined.

Mixed culture experiments were carried out using a the Pseudomonas sp. and a Pseudomonas putida which had been purchased from ATCC (# 31800). The kinetic rate constants for the P. putida had been previously determined in this laboratory. Computer modeling is presently being conducted to predict the kinetic behavior of the mixed culture, and compare the results to the experimental rates of phenol degradation.

MICROBIAL COMPETITION IN THE BIODEGRADATION OF PHENOL

by
Mary Ellen Frank

Thesis submitted to the Faculty of the Graduate School
of the
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in partial fulfillment of the requirements for the degree of
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1989

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I. INTRODUCTION

Microbial biodegradation of toxic organic chemicals is currently an area of much research activity. Possible applications of microbial biodegradation exist in fields such as water treatment and soil decontamination. Fundamental research in biodegradation is necessary in order to be able to evaluate its potential uses.

Of over 300 references on biodegradation which have been reviewed in previous studies (1,2) only 30 were found to contain kinetic data that is of use in waste water treatment plant design. The microbial populations or cultures that were used to produce the kinetic data, were not characterized or identified in many of the studies. The populations were often only described by their source, or referred to as unspecified mixed cultures. Without accurate characterizations of the cultures used no comparisons can be made of the kinetic results from different studies.

If microbial biodegradation is to be considered as a treatment alternative, fundamental research must be done to determine the degradation kinetics of accurately characterized cultures (both pure and mixed) for specific compounds. In this study, the kinetics of phenol degradation by pure cultures of bacteria, and by mixed cultures, were investigated.

Phenol was selected as the compound of interest because it is a ubiquitous environmental contaminant. Phenol has

been shown to be a mutagen and may also have long term effects on the liver and kidney (3). Phenol is produced during many industrial processes. Several examples are: coking operations, direct liquefication of coal, and polymeric resin production (4).

II. OBJECTIVES

The intent of this study was fundamental research to determine the kinetics of phenol degradation by accurately characterized, pure and mixed, bacterial cultures. The major objectives of the study were to:

(1) Isolate and identify phenol degrading bacteria from the Livingston, NJ municipal treatment plant's mixed liquor.

(2) Determine the kinetic rate constants for those phenol degraders.

(3) Compare the experimental rates of phenol degradation obtained with mixtures of individually identified phenol degraders, with a computer model that assumes pure and simple competition between degraders.

As it turned out, these objectives were modified to include in the mixed culture experiments a phenol degrader (Pseudomonas putida) purchased from American Type Culture Collection (ATCC 31800), for which pure culture rate constants had been previously determined (5).

III. PROCEDURES

Obtaining Mixed Liquor

Mixed liquor was removed from areation tanks at the Livingston, NJ municipal treatment plant on June 21 and August 2, 1988. (The Livingston plant treats 2.5 million gallons daily, more than 99% of which is of domestic origin.) The mixed liquor was transported back to the lab at New Jersey Institute of Technology in a half-filled, five gallon, screw top Nalgene container. At the lab, two-liter portions of the fresh mixed liquor were placed in six-liter, cylindrical, loosely covered, clear plastic batch reactors. New aquarium diffuser stones were used to bubble filtered air through the mixed liquor to provide areation and to keep the reactors well mixed. The reactors were maintained at room temperature.

Isolation of Phenol Degraders

The mixed liquor was shock-loaded to a phenol concentration of 100 parts per million (ppm). When the phenol concentration in the reactors dropped to less than 5 ppm (as determined by G.C. analysis), they were again shock loaded to 100 ppm phenol. This was repeated until the reactors had been shock-loaded five times.

Air stripping of phenol from the shock-loaded reactors was not a concern. It has been previously reported (6) that

theoretical calculations show that since water evaporates more quickly than phenol, phenol concentration would increase with time when the entering air is unsaturated. Experiments with an initial aqueous concentration of 100 ppm phenol exhibited a negligible concentration change over a two week period. (6)

The successive shock-loadings served to acclimate the indigenous microbial population to phenol and to eliminate any species that were not phenol-tolerant. Previous studies done in this laboratory have characterized the predominant genera in mixed liquor from the Livingston plant. This is illustrated in Table 1.

The phenol acclimated mixed culture was spread plated on nutrient agar to obtain isolated bacterial colonies. (Some phenol degrading molds and yeasts exist, but those are not of concern in this study.) Samples from all morphologically differentiable colonies were plated on nutrient agar to isolate pure cultures. Since there were many circular, convex, smooth edged whitish colonies on the mixed liquor spread plates, pure cultures were prepared from multiple colonies of this morphology in an attempt to isolate all phenol-tolerant species that may have been present. It is possible that different species may have been missed in the isolation process if they were similar in morphology.

The isolated phenol-tolerant bacteria were then tested for their ability to degrade phenol. To do this 125 mL

Erlenmeyer flasks with 50 mL of 80 ppm Gaudy's solution (see Table 2 (7) for Gaudy's formulation) were inoculated with 2 mL of a nutrient broth stock culture of the isolated species, to yield a primary culture. The flasks were then placed in a shaking water bath at 32 °C. After 24 hours, 2 mL of the primary culture was used to inoculate a similar flask containing Gaudy's solution to yield the secondary culture. After 24 hours, this procedure was repeated to yield a tertiary culture.

Phenol is the only carbon source in Gaudy's solution. Therefore, any microorganism capable of growing in pure culture in Gaudy's solution is a phenol degrader. In determining whether a species is a phenol degrader, it is necessary to go to a tertiary culture to assure that no carbon is left from the nutrient broth stock culture used to inoculate the primary culture, thus leaving phenol as the sole carbon source.

Attempts were made to use agar containing various concentrations of Gaudy's solution as a screening method for phenol degraders. This was done to try to directly isolate degraders, rather than isolating all tolerant species and then determining which were degraders. This attempt was not successful. While some known phenol degraders grew well on this agar, others grew very slowly or not at all. The reason for this is not known.

Identification of Degraders

Isolated phenol degraders were identified using the method illustrated in Figure 1. Morphological observations (to determine cell size and shape), and gram staining were first performed on the organisms. All of the phenol degraders isolated from the Livingston mixed liquor in this study, were gram negative bacilli (rods). Oxidase and catalase tests were the next steps. Oxidase positive bacteria were inoculated into Oxi-Ferm Tubes. Oxidase negative bacteria were innoculated into Enterotubes. (These diagnostic tubes are manufactured by Roche Diagnostic Systems.) Results from these diagnostic tubes were interpreted using scoring pads and code books, provided by Roche, to identify the bacteria. Additional confirmatory tests (such as growth on MacConkey's agar and sheep's blood agar, growth at 42 °C, odor, and diffusion of pigments into agar) were often necessary for positive identification. All identification procedures were done several times to confirm results.

Maintenance of Cultures

Each isolated phenol degrader was maintained in stock cultures in both nutrient broth and 80 ppm Gaudy's solution. The nutrient broth stocks were frequently returned to Gaudy's solution to assure that they did not lose their phenol degrading ability.

Preparation of Cultures for Kinetics Runs

To prepare a phenol degrader for a kinetics experiment, a nutrient broth culture was grown for 24 hours at room temperature. Successive primary, secondary, and tertiary cultures were inoculated in 125 mL flasks containing 50 mL of 80 ppm Gaudy's solution. These cultures were then grown for 24 hours in a shaking water bath at 32 °C. The tertiary cultures were spiked with Gaudy's solution to a phenol concentration of approximately 80 ppm for three days to assure phenol acclimation. After three days, the tertiary cultures were ready to be used in kinetic runs.

Performing Kinetics Runs

After phenol degraders had been isolated, identified, and acclimated, experiments to determine the kinetic parameters of their phenol degradation could be performed. 125 mL erlenmeyer flasks containing sterile 50 mL portions of Gaudy's solution, diluted to the desired initial phenol concentration, were prepared the day before a kinetics run. Approximately 12 hours before beginning a run, the tertiary culture which was to be used as the inoculum was spiked to a phenol concentration of about 80 ppm. This was done so that the culture would be in a period of exponential growth at the time that the kinetics run was started. This would hopefully reduce or eliminate the lag phase that was often seen at the beginning of the trials.

One hour before the kinetic runs were started, the following tasks were performed: the test flasks were placed in the 32 °C shaking water bath to bring them up to temperature; the spectrophotometer was turned on, warmed up, and zeroed; and the calibration of the gas chromatograph was checked. Other necessary supplies, such as sterile pipettes, data recording sheets, injection syringe, calculator, etc. were also set out so that all supplies would be available when they were needed later.

To begin the run, the test flask(s) were inoculated with the test organism(s) using sterile techniques. The amount of inoculum used was 2.0 to 3.0 mL, depending on the opacity of the tertiary culture. Immediately after inoculating a flask (and shaking it to distribute the bacteria), a sample was removed. These first samples, taken just after inoculation, are referred to as the zero-hour samples. Periodic samples were taken until the phenol supply was exhausted.

The optical density (for biomass determination) was obtained at 540 um in a Spectronic 70 spectrophotometer, using a 2.5 to 3.0 mL sample. Gas chromatography was used to determine the phenol concentration of each sample. At least 3 chromatographs were run on each sample, with an accuracy of +- 1 ppm.

Equipment Used for Kinetics Runs

*Gyrotory Water Bath Shaker

model G76, New Brunswick Scientific

temperature setting: 32 °C

shaker setting: 4

*Varian Gas Chromatograph

model 3700

with flame ionization detector

injection temperature: 210 °C

detector temperature: 250 °C

column temperaure: 140 °C

carrier gas: nitrogen at 40 cc/min

column: 6 ft. x 1/8 in. stainless steel packed column, with 10% SP2100 on 100/120 Supelcoport

*Hewlett Packard Electronic Integrator

model 3390A

integrator parameter settings:

zero: 0

attenuation 2: 5

chart speed: 1 cm/min

peak width: 0.04

threshold: 0

area rejection: 0

run time: 3 minutes

The integrator was calibrated using a standard 50 ppm phenol solution. The calibration was verified with standards before each kinetics experiment.

*Bausch and Lomb Spec 70

wavelength: 540 nm

blank: distilled water

Determination of Biomass Growth

Biomass growth during the kinetics runs was followed by measuring the absorbance (optical density) for each sample. The absorbance measurements were converted to biomass using a calibration curve, which was determined and confirmed during earlier studies in this lab (5,8). This calibration curve (shown in Figure 2) was prepared in the following manner. Pseudomonas putida was grown in nutrient broth for two days. It was then serially diluted and the optical density of each dilution measured using a spectrophotometer (wavelength = 540 nm). Cell mass was determined by placing a known volume of each dilution in pre-weighed aluminum dishes. These were placed in a drying oven for 24 hours to evaporate the water. To construct the calibration curve, biomass concentration (mg/L) was plotted vs. optical density for each dilution. It was determined and confirmed that 1 absorbance unit = 258.9 mg/L. In this study absorbance measurements were multiplied by 259 to yield biomass concentration in mg/L. The natural log of each biomass was also calculated.

Determination of Phenol Concentration

The phenol concentration of each sample removed during the kinetics runs was determined using gas chromatography. The instrumentation and settings used are described in the Equipment Section above. At least three chromatography runs were performed for each sample, and the average phenol concentration was determined. A sample chromatograph is included in Figure 3.

IV. KINETIC MODEL

A number of mathematical models exist for the kinetic behavior of microbial processes. A brief review of these can be found in (8). In the present study, we utilized an inhibitory (Andrews) model to describe the kinetics of phenol biodegradation:

$$u = \frac{u^* S}{K_m + S + S^2/K_I}$$

where: u is the specific growth rate,

u^*, K_m, and K_I are the kinetic rate constants, and

S is the substrate (phenol) concentration.

The specific growth rate (u) is the initial slope of the natural log of biomass concentration versus time. The yield coefficient (Y) is the slope of phenol concentration versus biomass concentration. These were determined experimentally in shaker flasks for each phenol degrader. Non-linear regression was then used to determine u^*, K_m, and K_I from specific growth rate (u) versus substrate concentration (S) data.

Equations (1-3) below, were then used to predict the kinetic behavior of a batch mixed culture, for which each of the pure culture growth parameters (subscripts 1 and 2) were previously determined:

$$\frac{dC_1}{dt} = u_1 C_1 \qquad (1)$$

$$\frac{dC_2}{dt} = u_2 C_2 \qquad (2)$$

$$\frac{dS}{dt} = -\frac{1}{Y_1} u_1 C_1 - \frac{1}{Y_2} u_2 C_2 \qquad (3)$$

where: C = biomass concentration.

V. RESULTS AND DISCUSSION

Phenol Degraders

Three phenol degraders were isolated. They were identified as Pseudomonas cepacia, Pseudomonas sp., and Group 2K-1 Pseudomonas-like (Xanthamonas).

Pseudomonas cepacia:

When plated on tryptic soy agar (TSA) the Pseudomonas cepacia formed smooth, circular colonies which produced a yellow-green pigment that diffused into the agar. When grown in 80 ppm Gaudy's solution, the liquid media also became yellow-green after a day or two. In preliminary kinetic trials this P. cepacia seemed to degrade phenol faster than the other two degraders. During the preliminary trials it was discovered that several hours into the trial, as the biomass increased, there always came a point where the P. cepacia would begin to form visible flocs floating in the media. These floating colonies made it impossible to accurately determine biomass using the spectrophotometer. Floc formation always occurred too rapidly to obtain enough data points for a determination of the specific growth rate. The initial inoculum was diluted in an attempt to solve this problem, but there were still too few data points before floc formation for the experiments to be useful. Without a reliable means to measure biomass, this phenol degrader could not be used in further studies.

Pseudomonas sp:

The Pseudomonas sp. when plated on TSA formed whitish, smooth (glistening surface), circular colonies, with a convex elevation. No pigments were produced either on TSA or in Gaudy's Solution. The cells are rod-shaped, approximately 2 um in length. This Pseudomonas sp. is gram negative, oxidase positive, catalase positive, doesn't have spores, is not acid fast, showed no hemolysis on Sheep's Blood Agar, is motile, does not grow on MacConkey's Agar, does not grow at 41 °C, and was positive for alkaline dextrose and alkaline xylose in Oxi/Ferm tubes. While many Pseudomonas species were ruled out in the identification process, we were not able to make a positive species identification. It is unlikely that this strain was a putida due to the lack of fluorescent pigments (9). Pure and mixed culture kinetic runs were performed using this organism.

Group 2K-1 Pseudomonas-Like (Xanthamonas):

This organism forms rod-shaped cells approximately 2-3 um long. It is gram negative, oxidase negative (or possibly very weakly positive), catalase positive, has no spores, is not acid fast, showed no hemolysis on Sheep's Blood Agar, did not grow on MacConkey's Agar, and did not grow at 41°C. When grown on TSA, the colonies were light yellow or peach in color, circular, with a smooth surface, and umbonate elevation. Some kinetic runs were performed using this degrader, but not all of the desired runs were completed.

Over Christmas holiday, cultures were stored in Gaudy's solution in the refrigerator. During this time the Xanthamonas lost its ability to degrade phenol. This problem emphasizes the importance of careful, continuous culture maintenance.

Pure Culture Experiments

Kinetic trials were run with Pseudomonas sp. at initial phenol concentrations of: 10, 20, 60, 100, 140, and 220 ppm. Three trials were run for each of the initial concentrations, except for 10 ppm for which two trials were run. The data collected are presented in Tables 3-A through 3-Q.

For each of the Pseudomonas sp. pure culture runs, specific growth rate and yield coefficient were determined. To determine specific growth rate (SGR), the natural log of the biomass concentration was plotted versus time. The initial slope (after the lag period, if any, ended) is the specific growth rate. Figures 4-A through 4-Q are the specific growth rate graphs. To determine yield coefficient (YC), biomass concentration was plotted versus phenol concentration. The slope is the yield coefficient. Figures 5-A through 5-Q are the yield coefficient graphs. The specific growth rates and yield coefficients for the 17 Pseudomonas sp. pure culture kinetic trials are summarized in Table 4. The specific growth rates were highest when the initial phenol concentration was 20 ppm. Figure 6

illustrates specific growth rates versus initial phenol concentrations.

Mixed Culture Experiments

Kinetic trials were run with a one-to-one mixed culture of *Pseudomonas sp.* and *Pseudomonas putida* (ATCC 31800). Pure culture kinetic parameters for the *Pseudomonas putida* (ATCC 31800) were previously determined by other investigators in this laboratory (5).

Initial phenol concentrations for the mixed culture were 15, 55, 80, 115, and 180 ppm. Three trials were run for each of the initial concentrations, except for 55 ppm for which two trials were run. The data collected for the mixed culture runs are presented in Tables 5-A through 5-N.

Computer modeling using the kinetic rate constants from the pure cultures of *Pseudomonas putida* (ATCC 31800) and *Pseudomonas sp.*, and assuming pure and simple competition, is being done presently. When these are complete they will be compared with the rates of phenol degradation obtained in the mixed culture trials.

VI. CONCLUSIONS AND COMMENTS

1. Three phenol degrading bacteria were isolated from mixed liquor obtained at the Livingston, NJ municipal treatment plant. The degraders were identified as a Pseudomonas cepacia, a Group 2K-1 Pseudomonas-Like (Xanthamonas), and a Pseudomonas sp..

2. Preliminary trials showed that the Pseudomonas cepacia formed flocs during phenol degradation. While this seemed to be a more efficient phenol degrader than the other two that were isolated, the problem of accurately measuring biomass spectrophotometrically caused by the floc formation, made it unsuitable for the kinetics experiments.

3. During storage the Xanthamonas lost its phenol degrading ability. This emphasizes the importance of continuous and diligent culture maintenance. The fact that this degrader formed colored colonies that were easily distinguishable from the other degraders when plated on TSA made it especially valuable and its loss more significant. Relative colony counts could have been done for plates inoculated at different times during the course of a mixed culture experiment. These relative counts would have provided a way to estimate the growth of each species over the course of each experiment. If other phenol degraders that form colored colonies can be isolated, they will prove useful in this type of study.

4. Pure culture kinetic experiments (shaker flask) were completed with the Pseudomonas sp.. Specific growth rates and yield coefficients were determined. Work is presently being done to use the kinetic rate constants in computer models to predict the kinetic behavior of a mixed culture of the Pseudomonas sp. and a Pseudomonas putida (ATCC 31800). The kinetic rate constants for the P. putida had been previously determined by another investigator in this laboratory. When this computer modeling is completed, the predictions that it yields will be compared against the phenol degradation rates that were obtained in the mixed culture experiments.

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APPENDIX A: TABLES

Table 1: Predominant Microbial Genera in Livington Mixed Liquor

Fresh	Phenol acclimated	2-chlorophenol acclim.
10^{10}bacteria/cm^3	10^9bacteria/cm^3	10^9bacteria/cm^3
Gram +/Gram - 1.3	0.5	0.3
Gram + rods (Bacillus)		
Gram + cocci (Micrococcus, Staphylococcus)		
Pseudomonas		
Acinetobacter		
Enterobacter		
Alcaligenes		
Serratia		
E. coli		
10^6yeast cells/cm^3	10^6yeast cells/cm^3	10^6yeast cells/cm^3
Canidida		
Cryptococcus		
Trichosporon		
Debaryomyces		
Penicillum		
Gliocladium		
Streptomyces		
Trichophyton		
10^5protozoa/cm^3	10^5protozoa/cm^3	10^5protozoa/cm^3
Epistylis		
Vorticella		
Paramecium		
Peranema		
Polychaos		
Carchesium		
(also rotifers and nemotodes)		

from: (6) "Biodegradation of Toxic Chemicals Using Commercial Preparations" G. Lewandowski et.al. Environmental Progress, volume 5, number 3, pp.212-217, August 1986.

Table 2: Gaudy's Phenol Defined Medium Solution

Phenol	1000.0mg
Ammonium Sulfate	500.0mg
Magnesium Sulfate	100.0mg
Manganese Sulfate	10.0mg
Ferric Chloride	0.5mg
1.0 M Potassium Phosphate Buffer Solution (pH=7.2)	30.0mL
tap water	100.0mL
distilled water	to 1.0L

from: (7) "Exponential Growth in Systems Limited by Substrate Concentration". J.A.F. Gaudy et. al. Biotechnol. Bioeng. 15, 589 (1973)

Table 3-A:

Pseudomonas sp.
Initial phenol concentration: 10 ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.25	2.072	0.729	9.358
0.75	2.072	0.729	8.850
1.25	2.849	1.047	7.603
1.75	2.849	1.047	7.746
2.25	3.108	1.134	6.974
2.75	3.367	1.214	5.981
3.25	3.626	1.288	5.716
3.75	4.144	1.422	5.228
4.25	4.403	1.482	-
4.75	4.921	1.594	4.407
5.25	5.180	1.645	3.477
5.75	5.698	1.740	2.780
6.25	5.698	1.740	1.214
6.75	5.957	1.785	0.925

Table 3-B:

Pseudomonas sp.
Initial phenol concentration: 10 ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.50	2.590	0.952	8.883
1.00	2.590	0.952	9.091
1.50	2.849	1.047	7.546
2.00	-	-	7.436
2.50	2.849	1.047	7.471
3.00	2.849	1.047	6.673
3.50	3.108	1.134	5.957
4.00	3.626	1.288	5.026
4.50	3.885	1.357	4.985
5.00	4.144	1.422	4.288
5.50	4.144	1.422	3.690
6.00	4.403	1.482	2.447
6.50	4.403	1.482	1.217
7.00	4.403	1.482	0.309

Table 3-C:

Pseudomonas sp.
Initial phenol concentration: 20 ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	8.547	2.146	20.362
0.33	9.583	2.260	19.948
1.00	11.914	2.478	17.197
1.33	13.727	2.619	15.614
1.67	15.022	2.710	14.401
2.00	16.835	2.823	13.411
2.33	18.648	2.926	12.071
2.67	20.202	3.006	9.708
3.00	22.533	3.115	7.922
3.33	23.310	3.149	4.879
3.67	25.123	3.224	1.621
4.00	26.677	3.284	0.344

Table 3-D:

Pseudomonas sp.
Initial phenol concentration: 20 ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	9.324	2.233	20.088
0.33	9.842	2.287	19.424
0.67	10.360	2.338	17.743
1.00	12.173	2.499	-
1.33	13.468	2.600	16.343
1.67	14.763	2.692	15.888
2.00	16.576	2.808	14.595
2.33	18.389	2.912	12.315
2.67	20.202	3.006	10.589
3.00	22.274	3.103	8.238
3.33	23.310	3.149	6.352
3.67	26.159	3.264	3.904
4.00	27.972	3.331	1.847
4.33	30.044	3.403	0.000

Table 3-E:

Pseudomonas sp.
Initial phenol concentration: 20 ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	9.065	2.204	20.678
0.33	9.842	2.287	19.779
0.67	10.619	2.363	18.987
1.00	11.914	2.478	17.623
1.33	13.468	2.600	16.518
1.67	15.281	2.727	15.777
2.00	16.835	2.832	14.328
2.33	18.130	2.898	12.509
2.67	19.943	2.993	10.398
3.00	22.274	3.103	8.376
3.33	23.569	3.160	6.591
3.67	25.641	3.244	4.603
4.00	27.713	3.327	1.763
4.33	30.821	3.428	0.000

Table 3-F:

Pseudomonas sp.
Initial phenol concentration: 60 ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	10.878	2.387	62.620
0.50	12.173	2.499	60.317
1.00	13.468	2.600	58.505
1.50	15.022	2.710	-
2.00	18.389	2.912	57.757
2.50	19.943	2.993	57.569
3.00	22.792	3.126	51.712
3.50	25.123	3.224	47.210
4.00	28.490	3.350	45.117
4.50	28.490	3.350	41.137
5.00	30.821	3.428	40.058
6.00	35.224	3.562	31.832
6.50	39.109	3.666	28.879
7.00	41.958	3.737	24.437
7.50	45.325	3.814	22.779
8.00	-	-	17.710
8.50	53.613	3.982	10.688
9.00	60.347	4.100	6.160
9.50	65.268	4.179	0.879

Table 3-G:

Pseudomonas sp.
Initial phenol concentration: 60 ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	10.878	2.387	62.068
0.50	12.691	2.541	60.281
1.00	14.504	2.674	57.787
1.50	16.576	2.808	-
2.00	18.648	2.926	57.145
2.50	21.238	3.056	54.532
3.00	23.310	3.149	-
3.50	25.641	3.244	49.738
4.00	30.562	3.420	43.971
4.50	31.399	3.445	40.382
5.00	32.375	3.477	37.484
6.00	37.814	3.633	31.327
6.50	41.699	3.730	29.810
7.00	44.289	3.791	22.684
7.50	47.138	3.849	19.366
8.00	51.282	3.937	13.895
8.50	55.685	4.020	9.033
9.00	62.937	4.142	2.408
9.50	69.671	4.244	0.000

Table 3-H:

Pseudomonas sp.
Initial phenol concentration: 60 ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	11.396	2.433	62.402
0.50	12.173	2.499	61.599
1.00	14.245	2.656	57.768
1.50	16.058	2.776	55.075
2.00	18.389	2.912	53.123
2.50	20.979	3.044	50.631
3.00	23.310	3.149	-
3.50	25.123	3.224	46.645
4.00	29.008	3.368	42.074
4.50	30.562	3.420	41.679
5.00	32.893	3.493	38.580
6.00	39.109	3.666	30.000
6.50	43.253	3.767	27.739
7.00	47.138	3.853	21.385
7.50	51.023	3.932	18.829
8.00	55.685	4.020	8.527
8.50	60.865	4.109	3.947
9.00	63.973	4.158	0.000

Table 3-I:

Pseudomonas sp.
Initial phenol concentration: 100 ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	12.432	2.520	104.937
1.50	13.727	2.619	103.093
3.00	16.058	2.776	99.108
4.50	19.425	2.967	96.587
6.00	23.828	3.171	95.258
7.50	26.677	3.284	87.802
8.50	30.044	3.403	83.015
9.50	32.375	3.477	75.937
10.50	35.742	3.576	69.975
11.50	40.922	3.712	63.510
12.50	45.843	3.825	59.809
13.50	50.505	3.922	54.454
14.50	55.685	4.020	42.329
15.50	63.455	4.150	26.791
16.50	82.103	4.408	3.615

Table 3-J:

Pseudomonas sp.
Initial phenol concentration: 100 ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	11.137	2.410	102.692
1.50	14.245	2.656	100.438
3.00	17.353	2.854	-
4.50	21.756	3.080	88.554
6.00	25.641	3.244	78.028
7.50	30.821	3.428	70.391
8.50	34.447	3.539	64.203
9.50	38.332	3.646	58.386
10.50	43.512	3.773	51.498
11.50	47.915	3.869	40.596
12.50	54.908	4.006	32.605
13.50	64.750	4.171	17.893
14.50	79.513	4.376	0.000

Table 3-K:

Pseudomonas sp.
Initial phenol concentration: 100 ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	11.655	2.456	106.970
1.50	13.986	2.638	106.620
3.00	16.835	2.823	-
4.50	20.720	3.031	85.952
6.00	24.864	3.213	73.699
7.50	33.152	3.501	65.737
8.50	35.742	3.576	58.588
9.50	40.404	3.699	52.303
10.50	46.620	3.842	45.367
11.50	53.872	3.987	30.715
12.50	66.563	4.198	13.039
13.50	83.398	4.424	0.000

Table 3-L:

Pseudomonas sp.
Initial phenol concentration: 140 ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	10.619	2.363	139.695
1.50	12.950	2.561	-
3.00	15.002	2.710	138.487
4.50	18.130	2.898	-
6.00	22.015	3.092	122.993
7.50	25.123	3.224	117.815
8.50	28.479	3.359	-
9.50	31.857	3.461	113.490
10.50	35.742	3.576	105.887
11.50	39.627	3.680	89.699
12.50	42.217	3.730	88.187
13.50	49.210	3.896	77.499
14.50	53.095	3.972	71.209
15.50	59.311	4.083	58.671
16.50	67.858	4.217	34.023
17.50	79.513	4.376	8.887
18.50	91.427	4.516	0.653

Table 3-M:

Pseudomonas sp.
Initial phenol concentration: 140 ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	9.842	2.363	144.950
1.50	12.173	2.499	-
3.00	15.540	2.743	134.533
4.50	18.648	2.926	-
6.00	21.497	3.086	124.270
7.50	25.641	3.244	114.239
8.50	29.785	3.394	103.357
9.50	32.375	3.477	-
10.50	37.555	3.626	90.565
11.50	41.699	3.730	84.348
12.50	45.843	3.825	77.705
13.50	52.577	3.962	65.136
14.50	60.865	4.109	53.539
15.50	69.671	4.244	29.781
16.50	80.808	4.392	4.672
17.50	92.722	4.530	0.000

Table 3-N:

Pseudomonas sp.
Initial phenol concentration: 140 ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	10.360	2.338	139.850
1.50	12.432	2.520	136.078
3.00	16.058	2.779	130.404
4.50	20.979	3.044	128.873
6.00	25.382	3.234	121.010
7.50	31.598	3.453	116.825
8.50	36.001	3.584	101.383
9.50	45.843	3.825	92.945
10.50	49.469	3.901	88.430
11.50	53.872	3.987	76.434
12.50	58.016	4.061	57.759
13.50	66.045	4.190	47.025
14.50	70.187	4.251	23.077
15.50	79.772	4.379	1.009
16.50	88.319	4.481	0.000

Table 3-0:

Pseudomonas sp.
Initial phenol concentration: 220 ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	21.487	3.068	228.560
8.00	29.785	3.394	208.497
9.00	32.893	3.493	214.093
10.00	36.519	3.598	206.603
11.00	40.145	3.692	209.013
12.00	44.030	3.785	191.347
13.00	46.102	3.831	194.837
14.00	49.210	3.896	153.557
15.00	51.800	3.947	148.410
16.00	54.390	3.996	134.350
17.00	56.721	4.038	132.995
18.00	62.937	4.142	113.658
19.00	71.484	4.269	103.205
20.00	78.995	4.369	84.625
21.00	89.873	4.498	60.389
22.00	102.823	4.633	31.315
23.00	118.622	4.776	0.000

Table 3-P:

Pseudomonas sp.
Initial phenol concentration: 220 ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	20.461	3.019	227.305
8.00	29.526	3.385	209.653
9.00	32.375	3.477	204.407
10.00	36.519	3.598	199.113
11.00	38.591	3.653	188.773
12.00	42.735	3.755	-
13.00	43.512	3.773	178.690
14.00	46.620	3.842	159.950
15.00	48.951	3.891	148.500
16.00	52.577	3.962	130.243
17.00	58.275	4.065	111.667
18.00	61.124	4.113	105.460
19.00	68.376	4.225	91.087
20.00	76.923	4.343	70.928
21.00	85.470	4.448	49.480
22.00	98.938	4.594	17.327
23.00	114.219	4.738	0.000

Table 3-Q:

Pseudomonas sp.
Initial phenol concentration: 220 ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	13.727	2.619	221.870
8.00	31.857	3.461	187.807
9.00	34.188	3.532	183.963
10.00	38.591	3.653	179.057
11.00	42.217	3.743	174.527
12.00	44.030	3.784	-
13.00	48.433	3.880	126.223
14.00	50.246	3.912	124.397
15.00	53.354	3.977	117.088
16.00	56.203	4.029	104.983
17.00	61.383	4.117	86.077
18.00	68.635	4.229	85.863
19.00	73.815	4.302	66.113
20.00	83.657	4.427	52.028
21.00	91.168	4.513	26.962
22.00	105.413	4.658	1.114

Table 4: Summary of Specific Growth Rates and Yield Coefficients for the Pseudomonas sp. Pure Culture Kinetic Experiments.

Initial phenol conc.	Trial	S.G.R.	Y.C.
10 ppm	1	0.175300	0.591386
10 ppm	2	0.194600	0.565920
10 ppm	average	0.184950	0.578653
20 ppm	1	0.336561	1.157526
20 ppm	2	0.307067	1.028152
20 ppm	3	0.306610	1.097791
20 ppm	average	0.316746	1.094490
60 ppm	1	0.245400	0.960318
60 ppm	2	0.247967	1.116319
60 ppm	3	0.238967	0.879522
60 ppm	average	0.244111	0.985386
100 ppm	1	0.107470	0.657647
100 ppm	2	0.127355	0.641444
100 ppm	3	0.136424	0.631129
100 ppm	average	0.123783	0.643407
140 ppm	1	0.112319	0.535880
140 ppm	2	0.123894	0.550652
140 ppm	3	0.149026	0.600491
140 ppm	average	0.128413	0.562341
220 ppm	1	0.083965	0.380148
220 ppm	2	0.082509	0.364248
220 ppm	3	0.079629	0.364094
220 ppm	average	0.082034	0.369497

Table 5-A:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 15ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	1.813	0.595	12.656
2.25	3.108	1.134	10.199
3.50	4.403	1.482	8.855
4.50	4.662	1.539	8.825
5.50	-	-	9.034
8.50	4.662	1.539	-
11.00	5.689	1.740	7.361
12.00	6.475	1.868	5.902
13.00	7.252	1.981	3.738
14.00	8.288	2.115	3.185
15.00	9.324	2.233	1.768
16.00	10.878	2.387	0.417
17.00	10.878	2.387	0.000
18.00	10.619	2.363	0.000

Table 5-B:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 15ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	1.813	0.595	15.409
2.25	3.367	1.214	11.173
3.50	3.885	1.357	9.159
4.50	4.144	1.422	8.919
5.50	4.403	1.482	8.694
6.50	4.403	1.482	8.409
8.50	4.921	1.594	-
11.00	6.475	1.868	6.282
12.00	8.029	2.083	4.694
13.00	9.324	2.233	2.770
14.00	11.137	2.410	1.550
15.00	13.209	2.581	0.000
16.00	12.950	2.561	0.000
17.00	12.432	2.520	0.000
18.00	11.914	2.478	0.000

Table 5-C:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 15ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	2.590	0.952	12.116
2.25	3.626	1.288	10.315
3.50	4.144	1.422	10.019
4.50	4.662	1.539	9.574
5.50	5.183	1.645	8.814
6.50	5.698	1.740	8.394
8.50	6.734	1.907	8.142
11.00	7.252	1.981	7.177
12.00	8.547	2.146	4.733
13.00	8.806	2.175	4.189
14.00	9.384	2.233	3.124
15.00	10.619	2.363	1.789
16.00	11.914	2.478	0.635
17.00	13.468	2.600	0.000
18.00	12.950	2.561	0.000
19.00	12.173	2.499	0.000

Table 5-D:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 55ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.50	3.108	1.134	53.305
2.75	6.475	1.868	57.672
4.00	3.885	1.357	-
6.00	5.698	1.740	47.000
10.25	6.993	1.945	41.970
11.50	9.324	2.233	37.260
12.50	10.360	2.338	27.370
13.50	11.655	2.456	26.950
14.50	13.468	2.600	24.610
15.50	14.763	2.692	21.535
16.50	17.094	2.839	19.545
17.50	17.120	2.869	18.830
18.50	19.425	2.967	14.237
19.50	23.569	3.160	8.991
20.50	30.303	3.411	1.327
21.50	32.375	3.477	0.000
22.50	32.116	3.469	0.000
23.50	31.339	3.445	0.000

Table 5-E:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 55ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.50	2.331	0.846	55.879
2.75	4.144	1.422	50.490
4.00	4.403	1.482	48.046
6.00	6.216	1.827	43.151
10.25	10.619	2.363	41.389
11.50	10.619	2.363	39.465
12.50	11.655	2.456	37.062
13.50	12.432	2.320	24.256
14.50	13.727	2.619	21.318
15.50	16.058	2.776	16.583
16.50	18.130	2.898	10.672
17.50	21.499	3.068	4.001
18.50	25.461	3.244	0.782
19.50	27.713	3.322	0.000
20.50	27.972	3.331	0.000
21.50	27.193	3.303	0.000

Table 5-F:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 80ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	3.626	1.214	81.505
3.00	5.689	1.740	75.051
6.00	7.252	1.981	71.355
9.00	9.324	2.233	63.525
13.25	12.432	2.520	53.138
16.00	17.612	2.869	45.992
18.50	17.612	2.869	45.919
20.50	20.202	3.006	29.477
22.50	23.310	3.149	28.333
25.00	29.526	3.385	25.435
27.00	33.929	3.524	22.188
29.50	42.217	3.743	9.591
31.00	50.505	3.922	3.807
32.00	54.649	4.001	0.000
33.00	51.541	3.942	0.000
34.00	50.505	3.922	0.000

Table 5-G:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 80ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.00	3.367	1.214	78.575
3.00	4.921	1.594	73.235
6.00	6.734	1.907	71.432
9.00	8.029	2.083	60.937
13.25	11.137	2.410	56.876
16.00	13.727	2.619	49.590
18.50	18.130	2.898	47.249
20.50	20.202	3.006	31.374
22.50	25.641	3.244	26.464
25.00	29.785	3.394	22.824
27.00	35.224	3.562	15.510
29.50	44.289	3.791	5.333
31.00	51.800	3.947	0.000
32.00	51.023	3.932	0.000
33.00	49.469	3.901	0.000

Table 5-H:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 80ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
1.25	4.403	1.482	85.788
3.00	5.180	1.645	76.557
6.00	7.252	1.981	70.514
9.00	9.065	2.204	60.940
13.25	13.468	2.600	-
16.00	17.353	2.835	41.091
18.50	20.202	3.006	32.752
20.50	24.605	3.203	29.340
22.50	26.936	3.293	24.268
25.00	34.965	3.554	19.841
27.00	44.807	3.802	13.053
29.50	58.275	4.065	0.000
31.00	55.685	4.020	0.000
32.00	54.390	3.996	0.000
33.00	52.318	3.957	0.000

Table 5-I:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 115ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
1.00	4.403	1.482	110.270
3.50	5.180	1.645	105.413
6.50	6.734	1.907	97.455
9.50	7.511	2.016	80.674
14.00	10.619	2.363	74.580
16.75	11.914	2.478	74.159
19.00	12.201	2.502	74.369
23.00	15.540	2.743	68.661
28.00	22.792	3.126	57.156
31.00	26.677	3.284	43.079
36.00	42.735	3.755	19.750
39.00	54.649	4.001	0.000
40.00	54.908	4.006	0.000
42.00	52.577	3.962	0.000

Table 5-J:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 115ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
1.00	4.921	1.594	115.923
3.50	5.698	1.740	112.223
6.50	6.993	1.945	97.884
9.50	9.065	2.204	89.000
14.00	13.727	2.619	75.191
16.75	16.317	2.792	71.979
19.00	18.648	2.926	68.509
23.00	25.123	3.224	62.217
28.00	42.735	3.755	31.821
31.00	79.772	4.379	0.294
36.00	67.858	4.217	0.000
37.00	65.268	4.179	0.000

Table 5-K:

Pseudomonas putida(ATCC 31800) and Psuedomonas sp.
Initial phenol concentration: 115ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.50	3.885	1.357	118.735
3.50	5.698	1.740	102.285
6.50	6.743	1.907	103.345
9.50	8.547	2.146	94.712
14.00	13.727	2.619	84.727
16.75	15.022	2.710	74.875
19.00	18.130	2.898	72.792
23.00	20.720	2.031	63.118
28.00	27.713	3.322	58.184
31.00	33.929	3.524	50.222
36.00	53.095	3.972	22.360
39.00	62.678	4.138	2.007
40.00	66.304	4.194	0.000
41.00	64.491	4.167	0.000

Table 5-L:

Pseudomonas putida(ATCC 31800) and Psuedomonas sp.
Initial phenol concentration: 180ppm
Trial 1

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
1.25	4.921	1.594	183.245
4.00	6.216	1.827	165.430
7.25	7.252	1.981	155.420
10.25	8.547	2.146	153.465
15.00	13.209	2.581	138.245
17.50	16.058	2.776	132.990
19.50	19.425	2.967	129.710
24.00	26.159	3.264	122.810
28.50	35.487	3.669	110.323
31.50	45.325	3.814	98.918
36.00	103.082	4.636	18.571
37.00	113.960	4.736	0.000
38.00	109.557	4.696	0.000
39.00	108.003	4.682	0.000

Table 5-M:

Pseudomonas putida(ATCC 31800) and Psuedomonas sp.
Initial phenol concentration: 180ppm
Trial 2

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
0.75	4.403	1.482	181.650
4.00	5.180	1.645	165.770
7.25	6.734	1.907	160.130
10.25	9.065	2.204	143.755
15.00	12.432	2.520	132.945
17.50	15.799	2.760	125.710
19.50	19.425	2.967	125.960
24.00	26.418	3.274	121.885
28.50	36.778	3.605	99.130
31.50	48.174	3.875	92.325
36.00	113.690	4.736	8.205
37.00	119.399	4.782	0.000
38.00	118.363	4.774	0.000
39.00	112.147	4.720	0.000

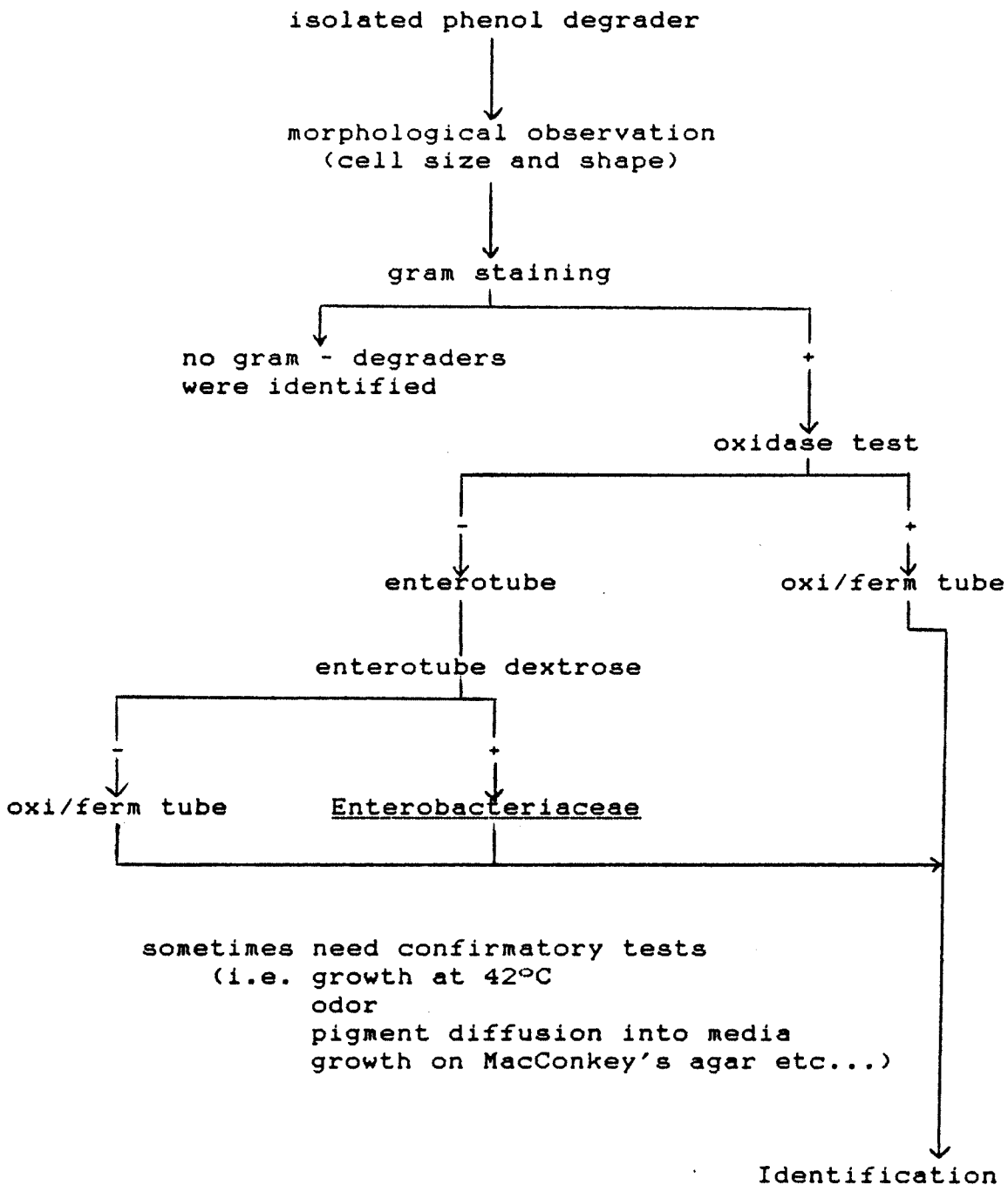
Table 5-N:

Pseudomonas putida(ATCC 31800) and Pseudomonas sp.
Initial phenol concentration: 180ppm
Trial 3

time(hrs)	biomass(ppm)	ln biomass	phenol conc.(ppm)
1.25	4.662	1.539	173.275
4.00	5.598	1.740	173.190
7.25	6.993	1.945	-
10.25	8.547	2.146	153.815
15.00	11.655	2.256	144.075
17.50	13.727	2.619	134.697
19.50	17.353	2.845	114.750
24.00	23.310	3.149	105.315
28.50	31.857	3.461	94.590
31.50	39.886	3.686	-
36.00	66.822	4.202	63.635
37.00	80.808	4.392	36.985
38.00	101.010	4.615	2.101
39.00	110.593	4.706	0.000
40.00	110.075	4.701	0.000

APPENDIX B: FIGURES

Figure 1: Identification of Phenol Degraders



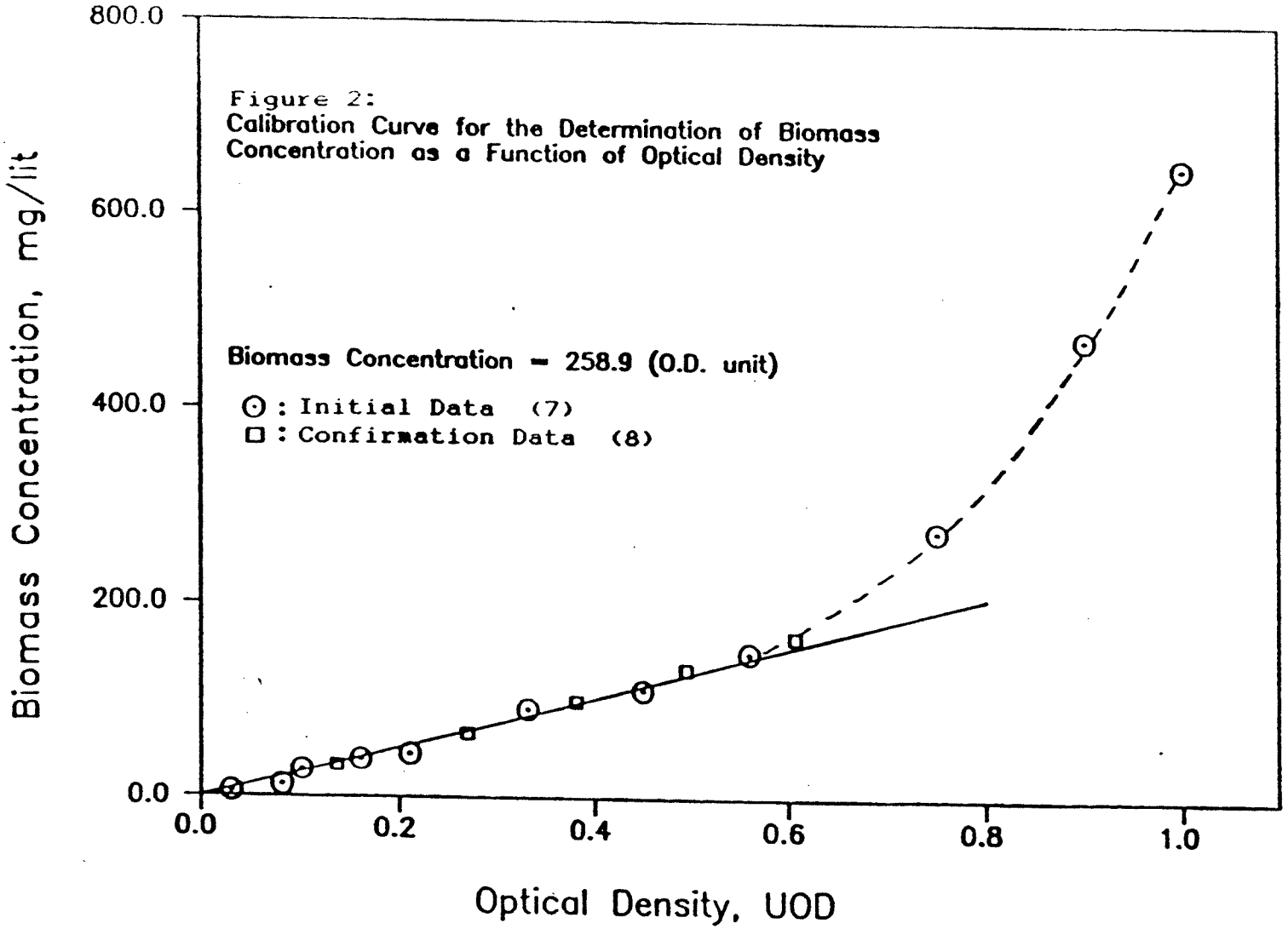
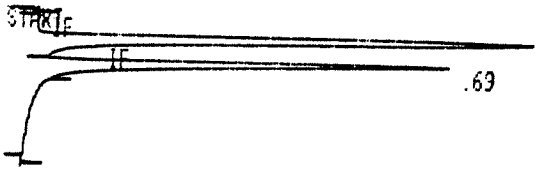


Figure 2:
Calibration Curve for the Determination of Biomass Concentration as a Function of Optical Density
Biomass Concentration = 258.9 (O.D. unit)
⊙ : Initial Data (7)
□ : Confirmation Data (8)
Biomass Concentration, mg/lit
800.0
600.0
400.0
200.0
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Optical Density, UOD

Figure 3: Sample Chromatograph.
(The first peak is the water peak.
The phenol peak is at 0.69 minutes.)

```
METH  6 @

LIST: METH @

RUN PRMTRS
ZERO   =  0
ATT 2↑ =  5
CHT SP =  1.0
PK WD  =  0.04
THRSH  =  0
AR REJ =  0

RPRT OPTNS
  2. RF UNC PKS=      1.0000E+00
  3. MUL FACTOR=      1.0000E+00
  4. PK HEIGHT MODE       NO
  5. EXTEND RT            NO
  6. RPRT UNC PKS         NO

TIME TBL
   0.10 INTG # =  9
   0.90 INTG # = -9
   3.00 STOP

CALIB TBL
ESTD                        CALIB RUNS  1
REF % RTW:  -0.30           % RTW:   3.00


LIST: ZERO =  0,-0.7

LIST: ZERO =  0,-0.6

LIST: ZERO =  0,-0.4
```




```
STOP


RUN #  461

ESTD
   RT          AREA TYPE  CAL#        AMOUNT
  0.69       941780   BB    1R         9.778

TOTAL AREA=      941780
MUL FACTOR= 1.0000E+00
```

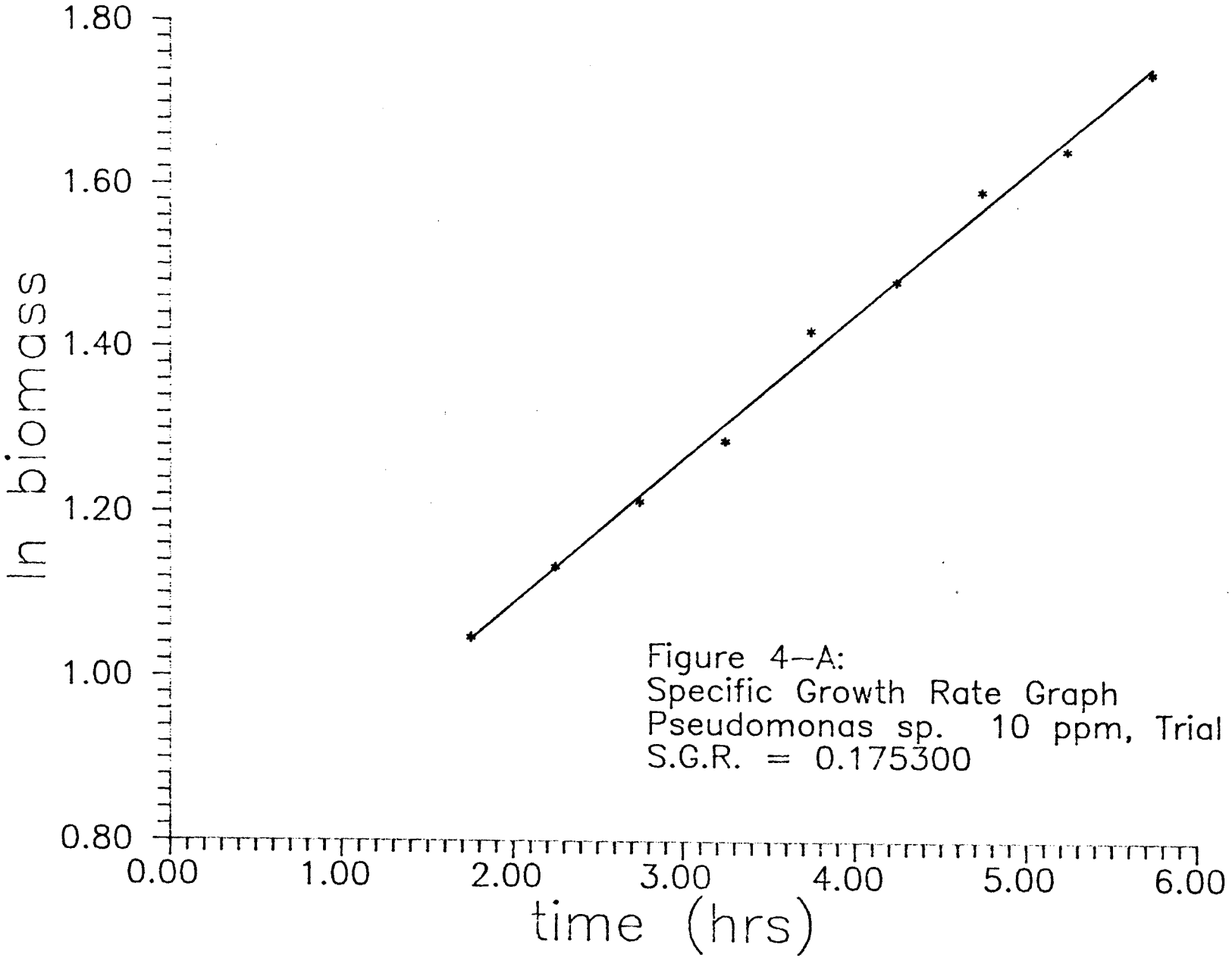


Figure 4–A:
Specific Growth Rate Graph
Pseudomonas sp. 10 ppm, Trial 1
S.G.R. = 0.175300

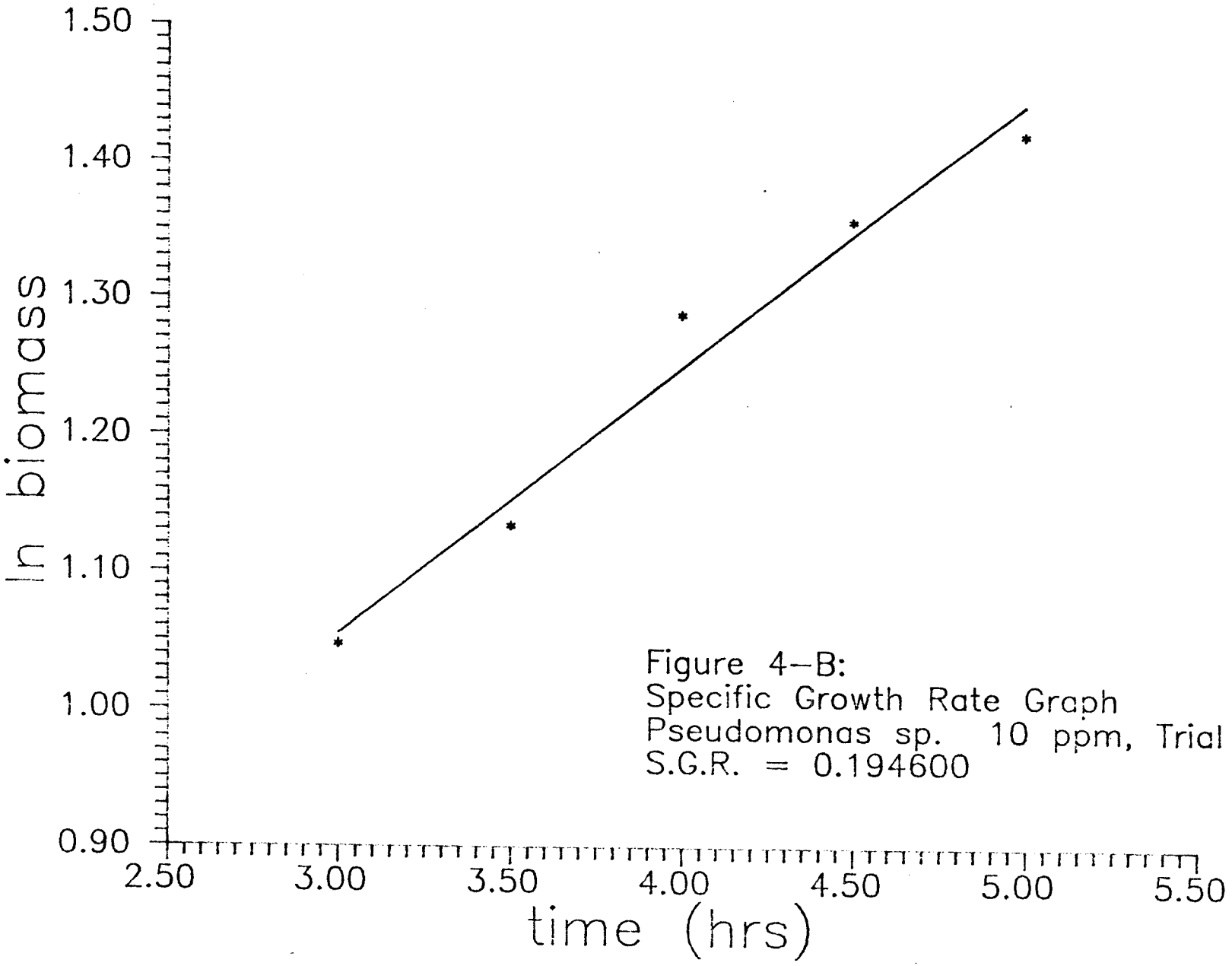


Figure 4–B:
Specific Growth Rate Graph
Pseudomonas sp. 10 ppm, Trial 2
S.G.R. = 0.194600

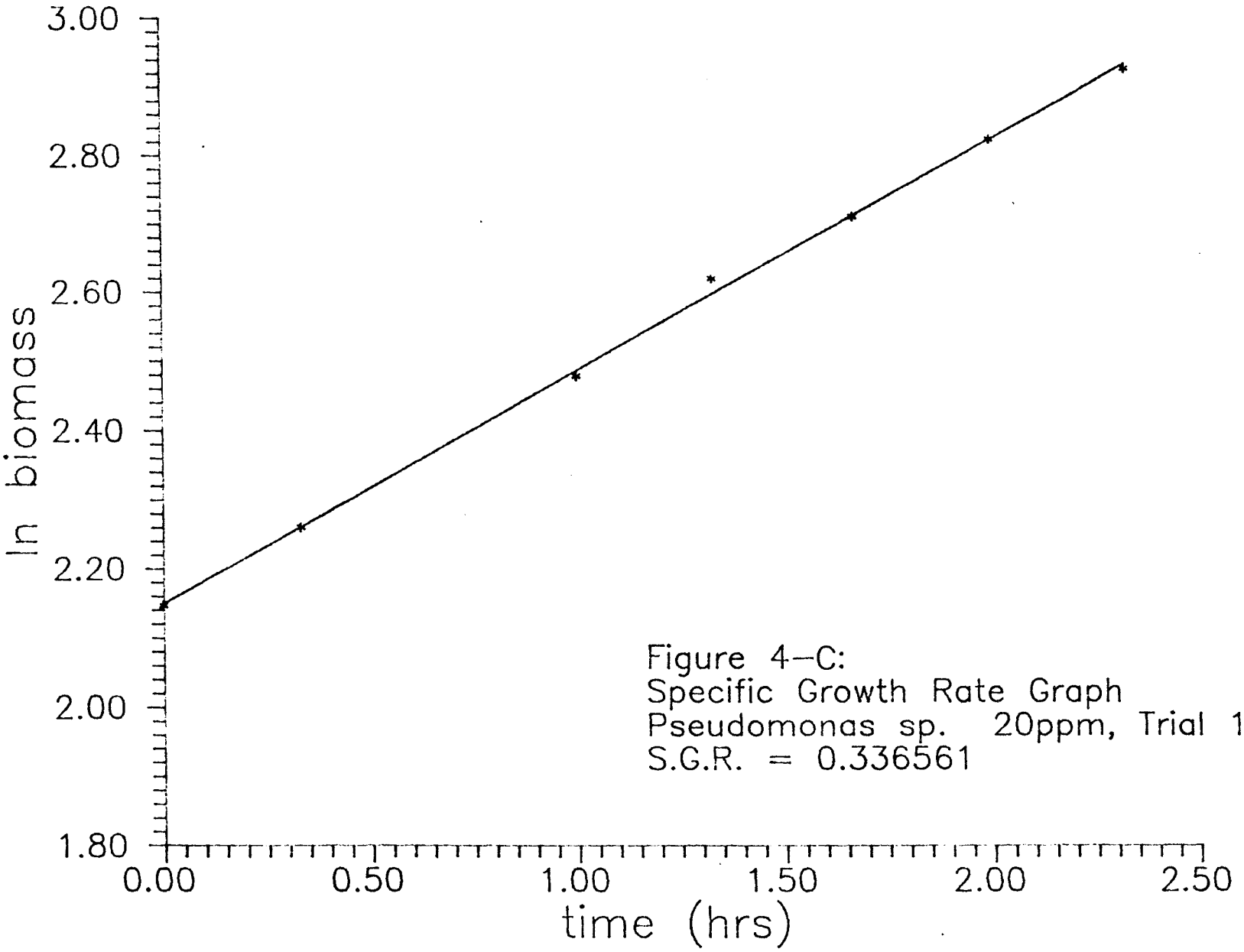


Figure 4–C:
Specific Growth Rate Graph
Pseudomonas sp. 20ppm, Trial 1
S.G.R. = 0.336561

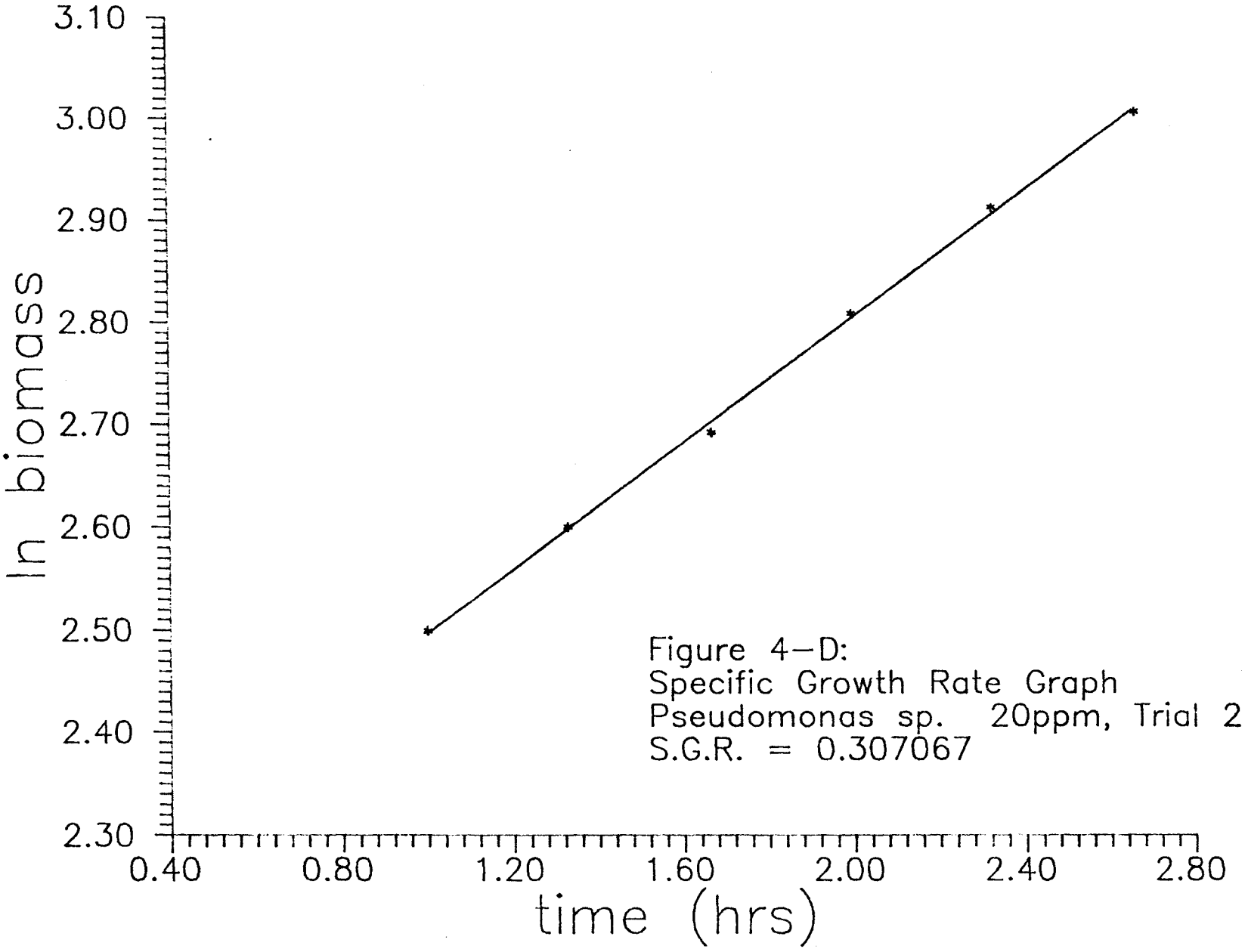


Figure 4–D:
Specific Growth Rate Graph
Pseudomonas sp. 20ppm, Trial 2
S.G.R. = 0.307067

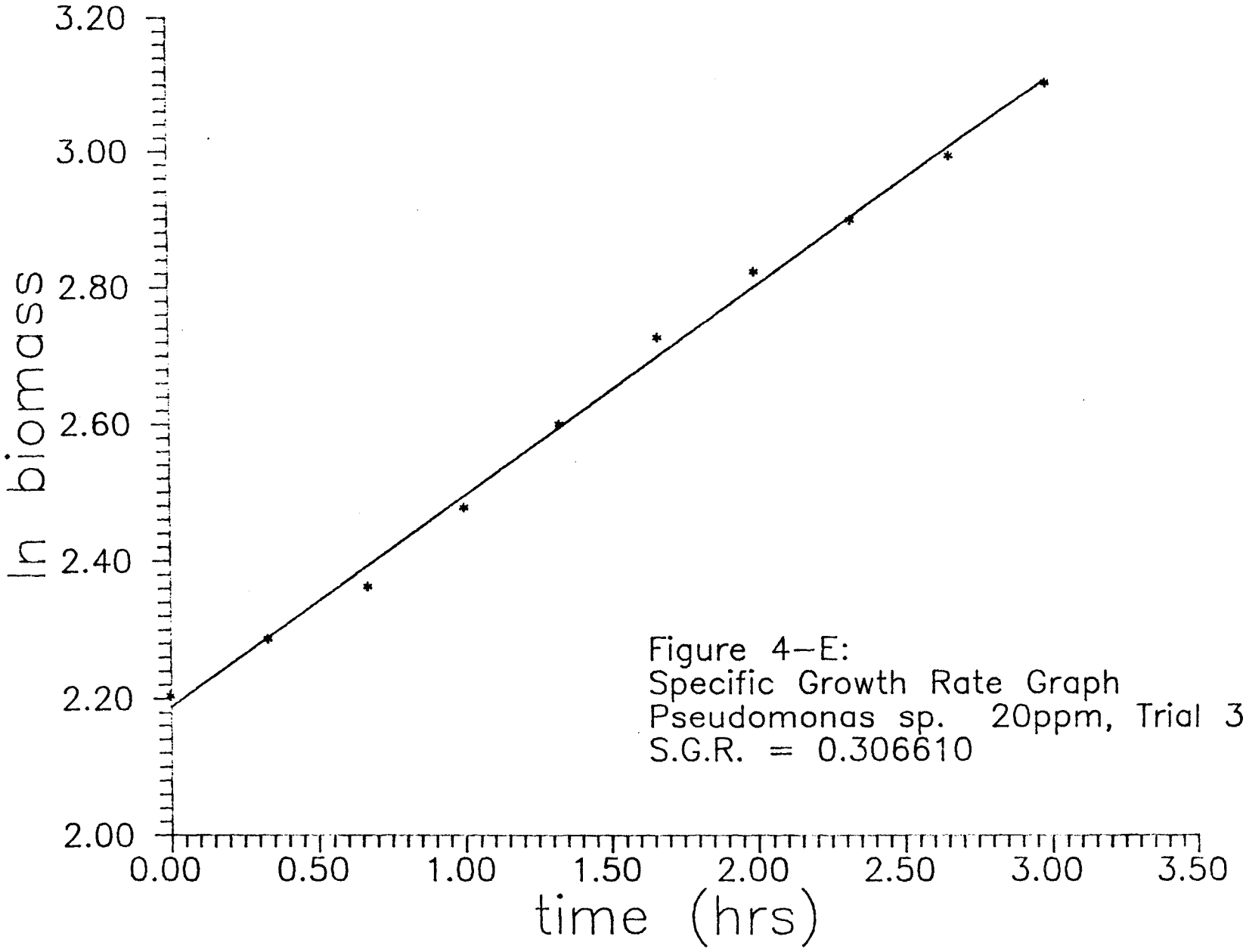


Figure 4–E:
Specific Growth Rate Graph
Pseudomonas sp. 20ppm, Trial 3
S.G.R. = 0.306610

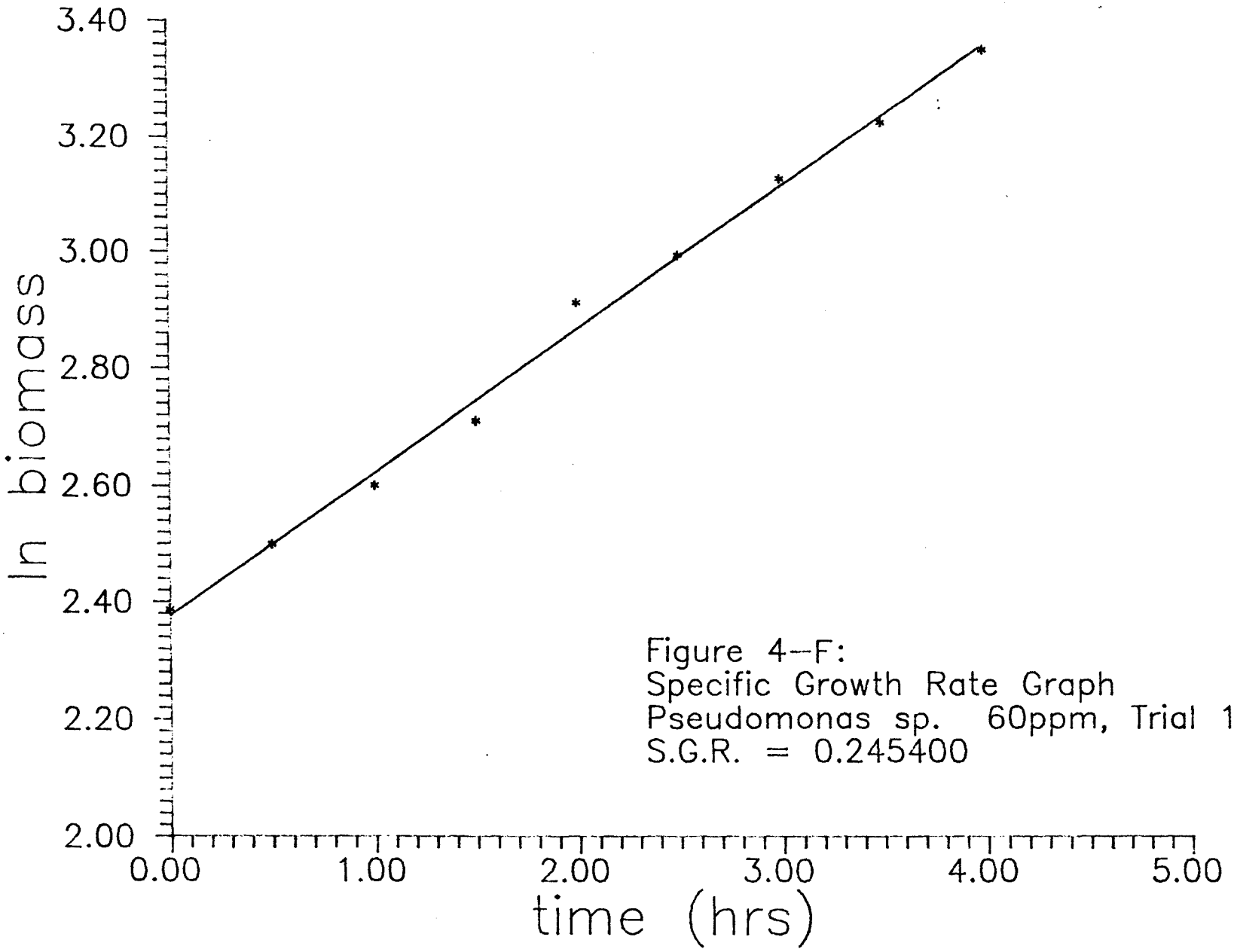


Figure 4--F:
Specific Growth Rate Graph
Pseudomonas sp. 60ppm, Trial 1
S.G.R. = 0.245400

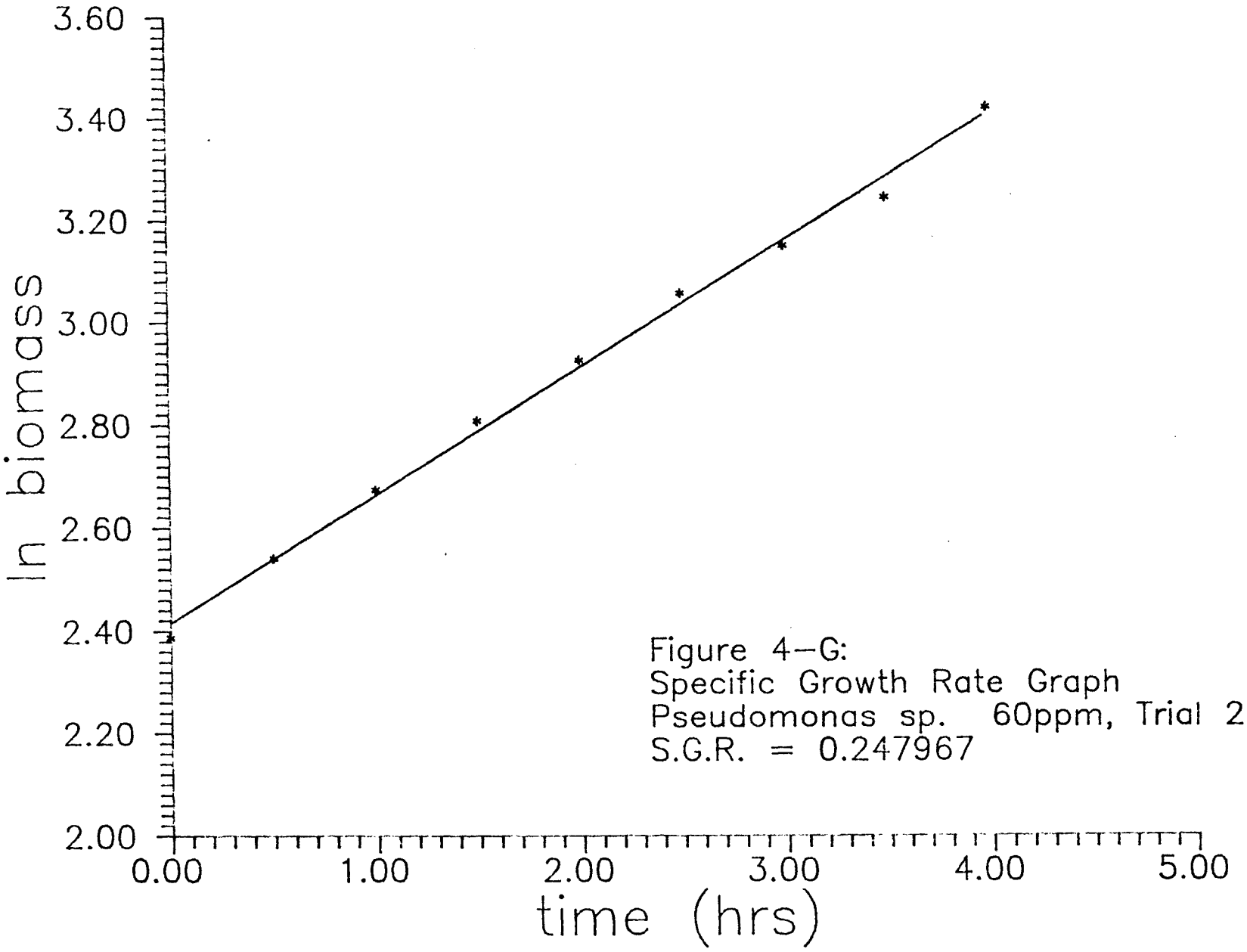


Figure 4–G:
Specific Growth Rate Graph
Pseudomonas sp. 60ppm, Trial 2
S.G.R. = 0.247967

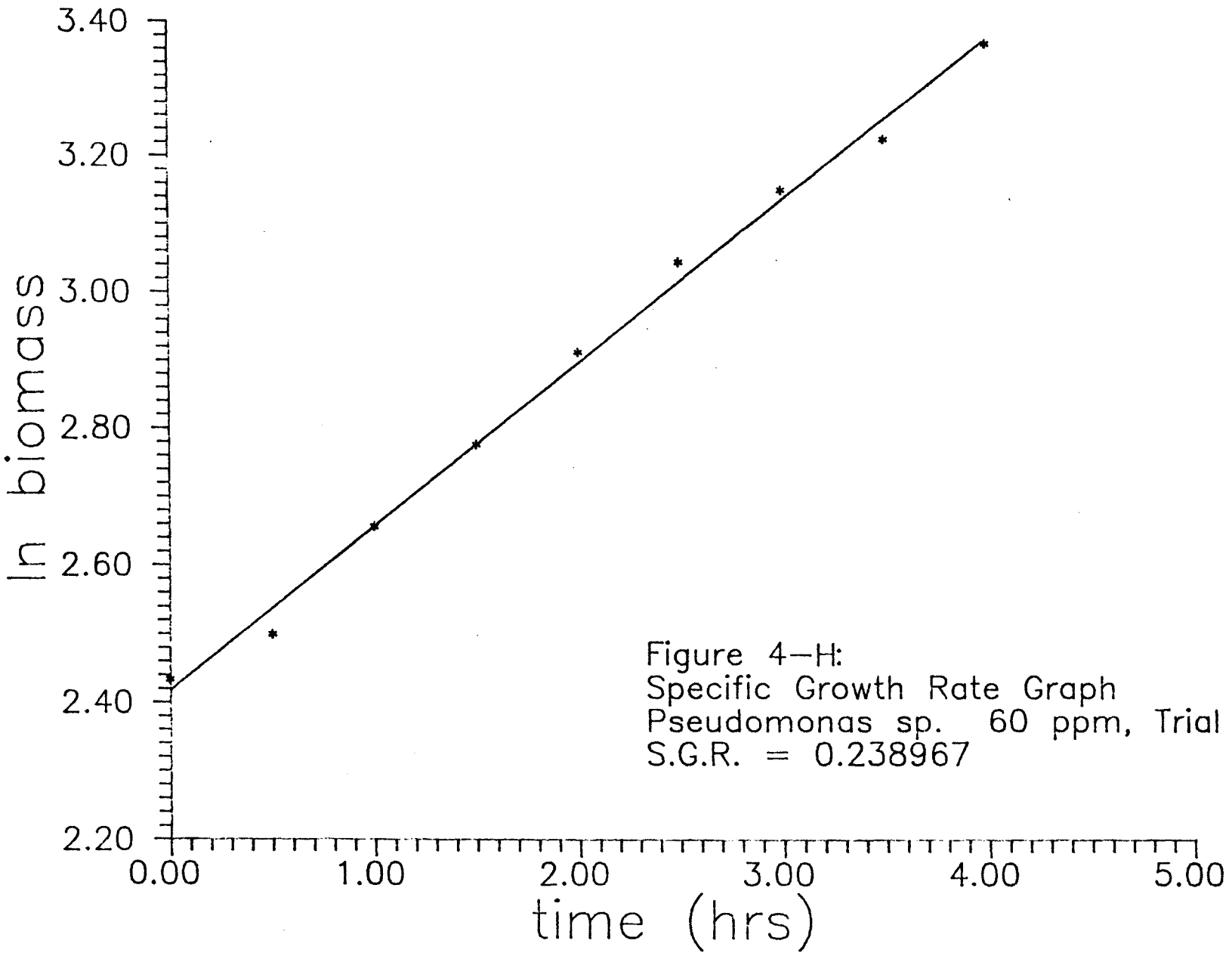


Figure 4—H:
Specific Growth Rate Graph
Pseudomonas sp. 60 ppm, Trial 3
S.G.R. = 0.238967

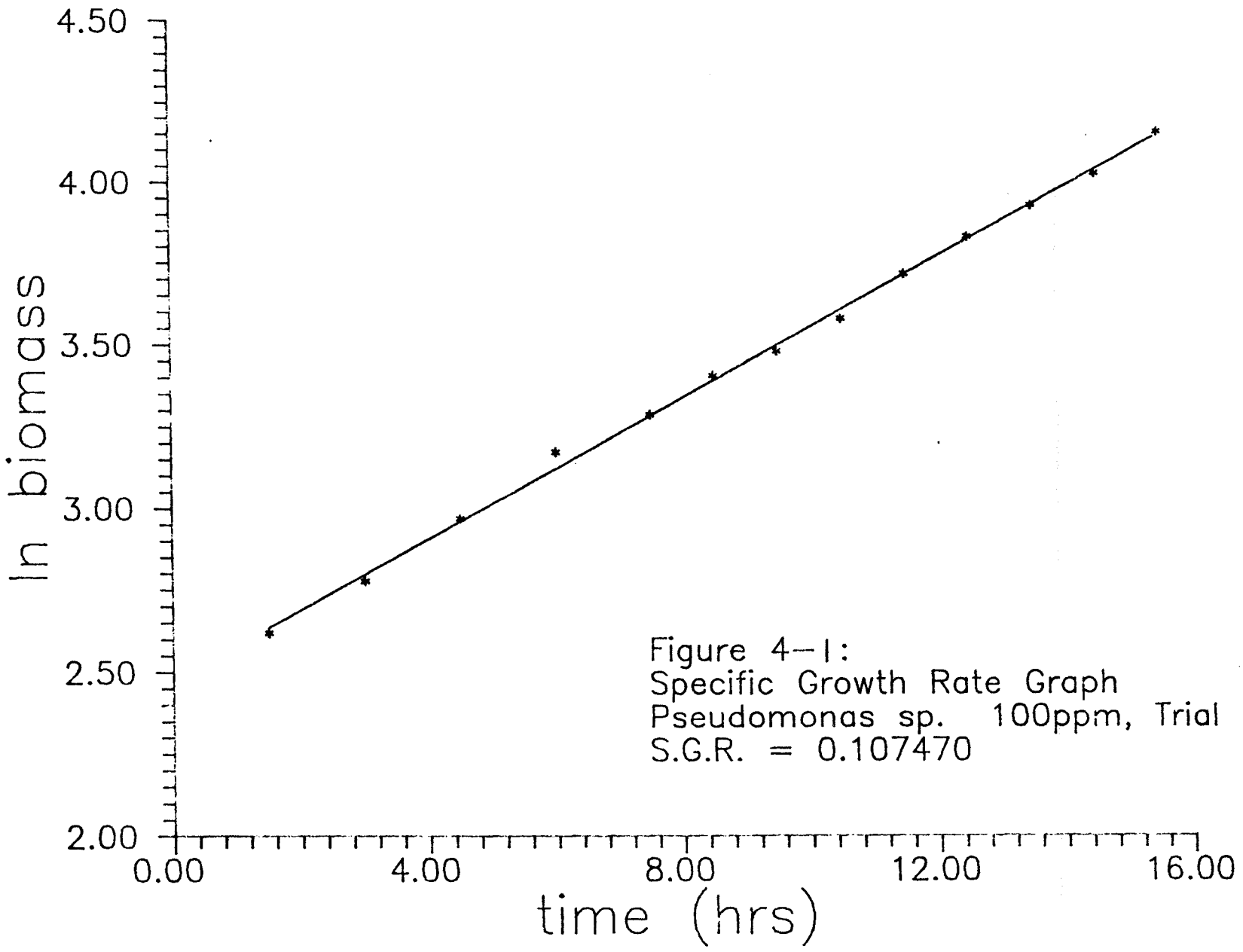


Figure 4–1:
Specific Growth Rate Graph
Pseudomonas sp. 100ppm, Trial 1
S.G.R. = 0.107470

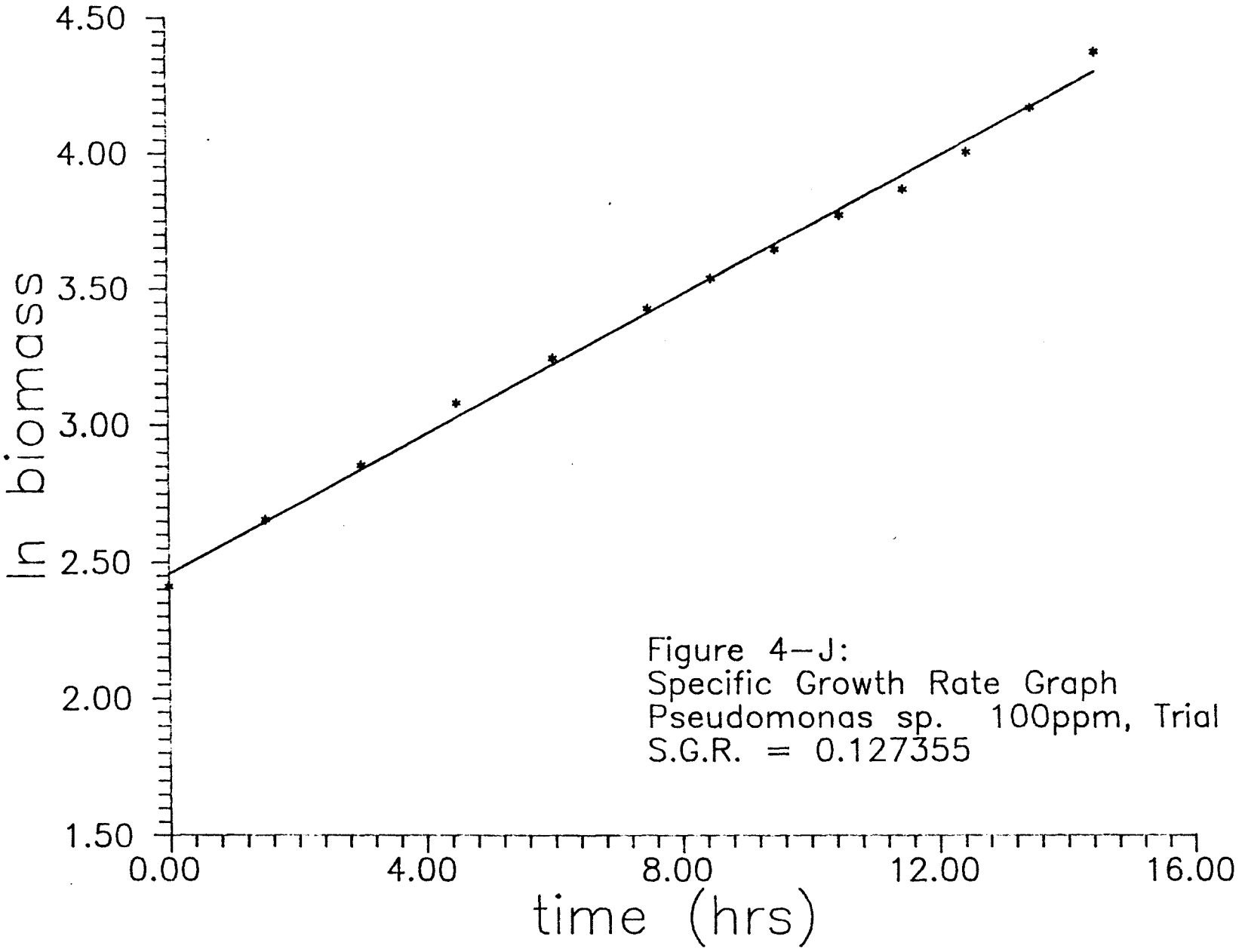


Figure 4–J:
Specific Growth Rate Graph
Pseudomonas sp. 100ppm, Trial 2
S.G.R. = 0.127355

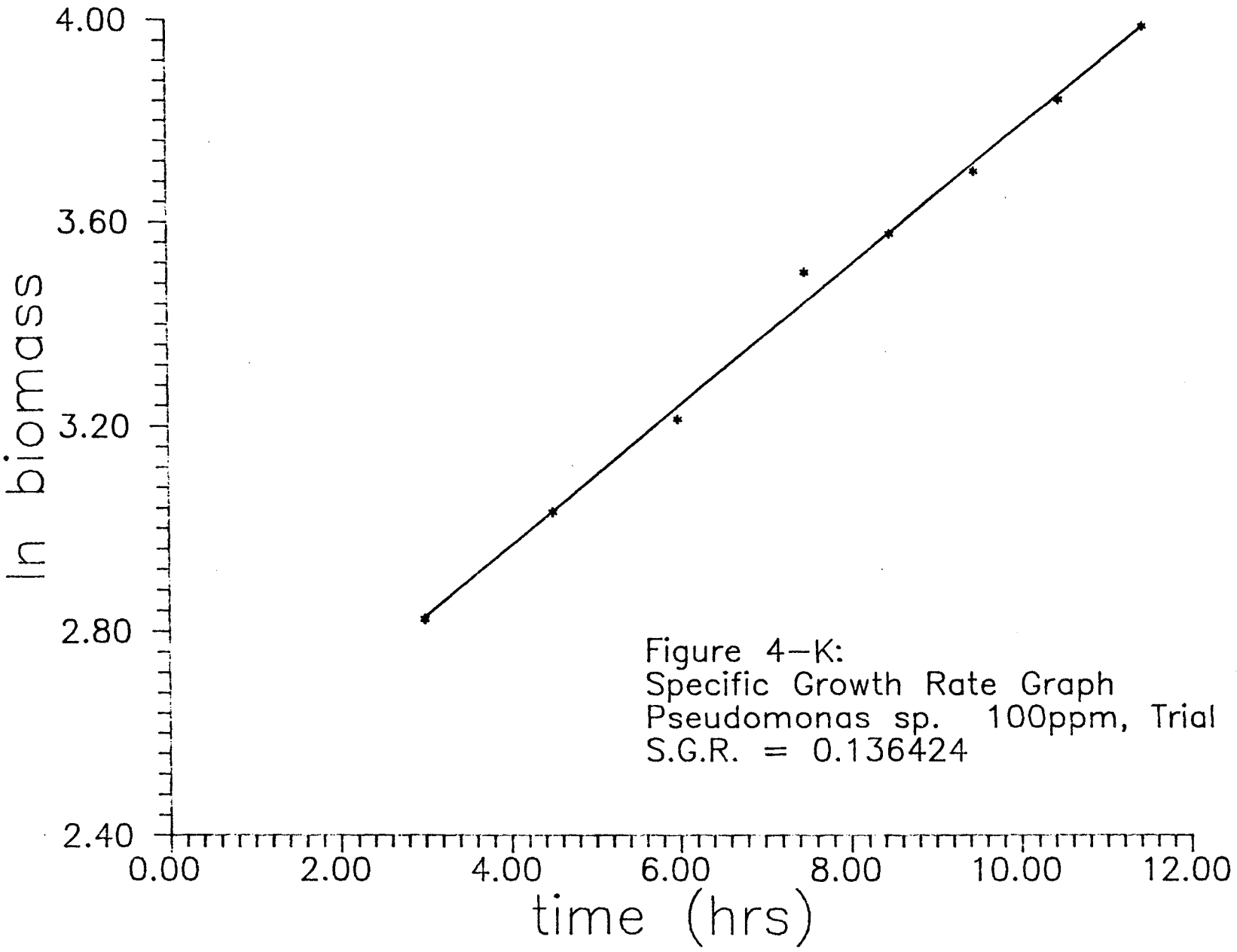


Figure 4–K:
Specific Growth Rate Graph
Pseudomonas sp. 100ppm, Trial 3
S.G.R. = 0.136424

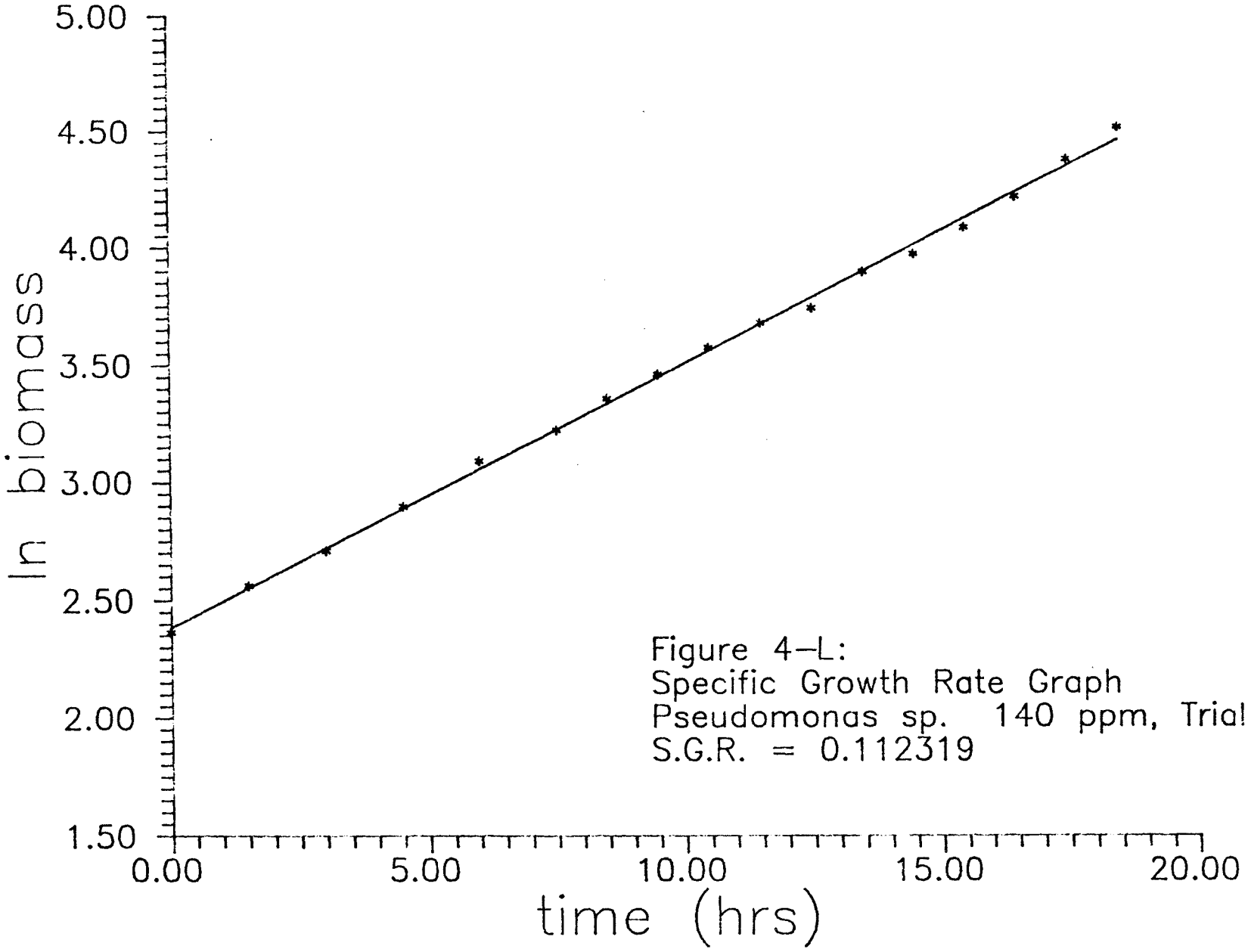


Figure 4–L:
Specific Growth Rate Graph
Pseudomonas sp. 140 ppm, Trial 1
S.G.R. = 0.112319

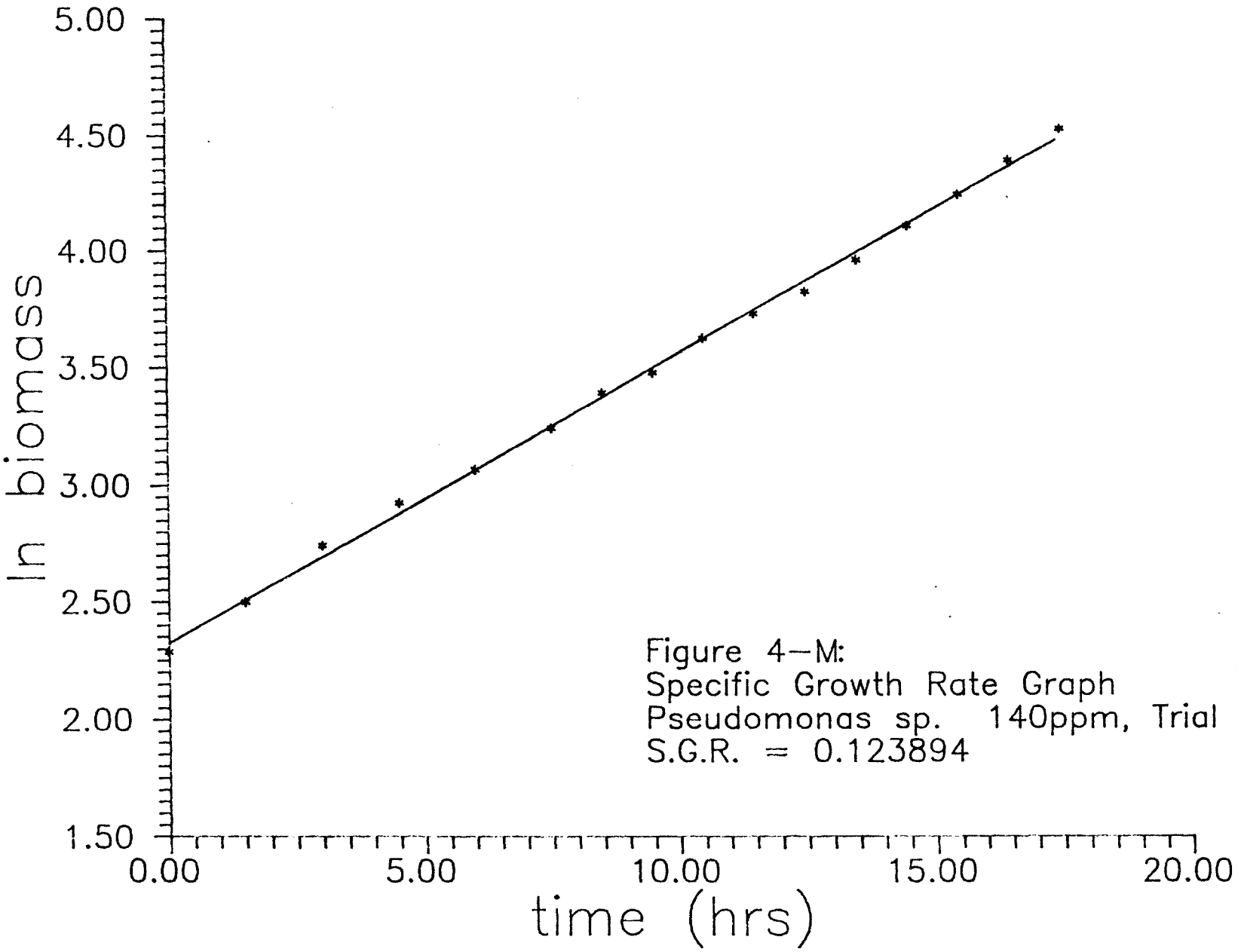


Figure 4—M:
Specific Growth Rate Graph
Pseudomonas sp. 140ppm, Trial 2
S.G.R. = 0.123894

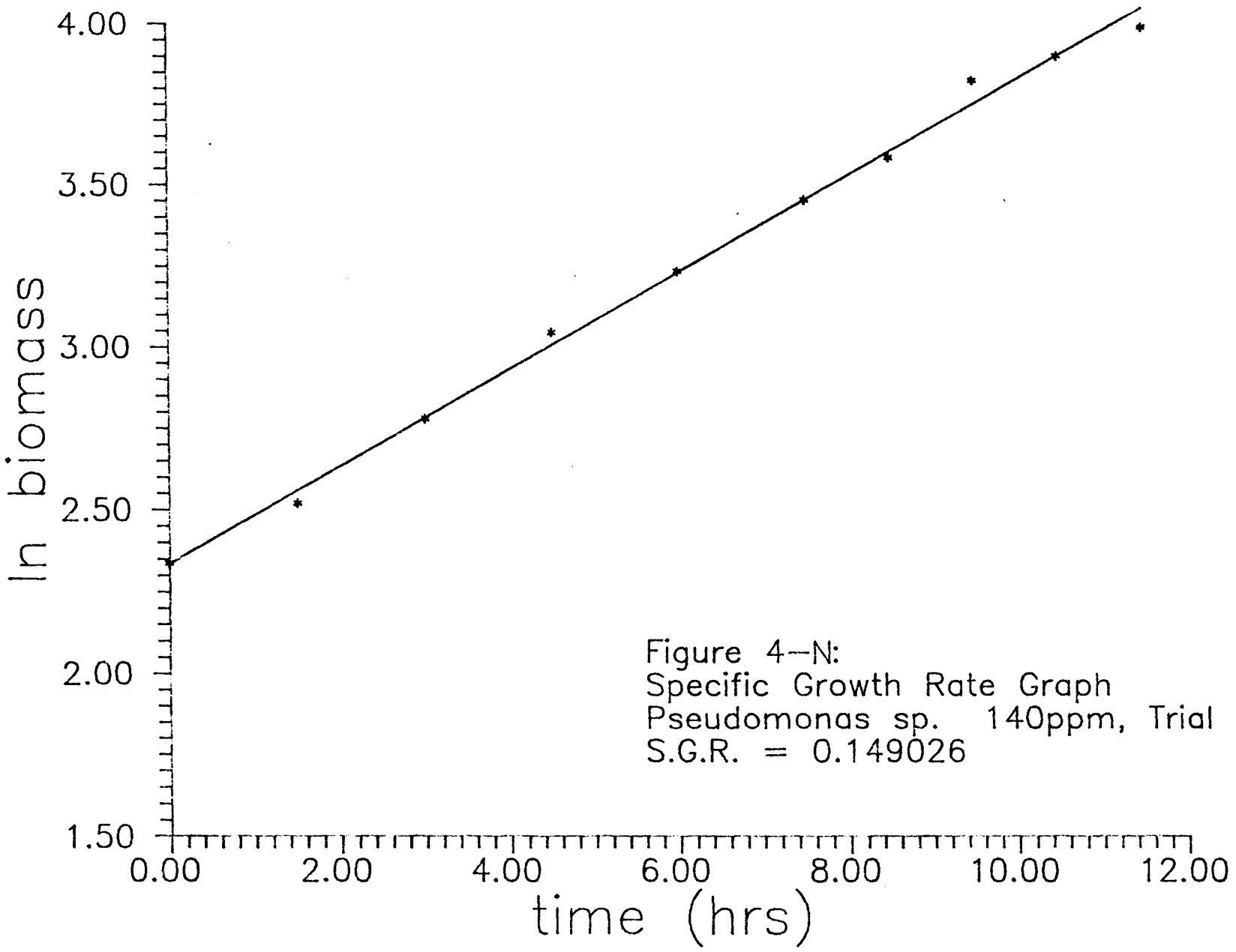


Figure 4--N:
Specific Growth Rate Graph
Pseudomonas sp. 140ppm, Trial 3
S.G.R. = 0.149026

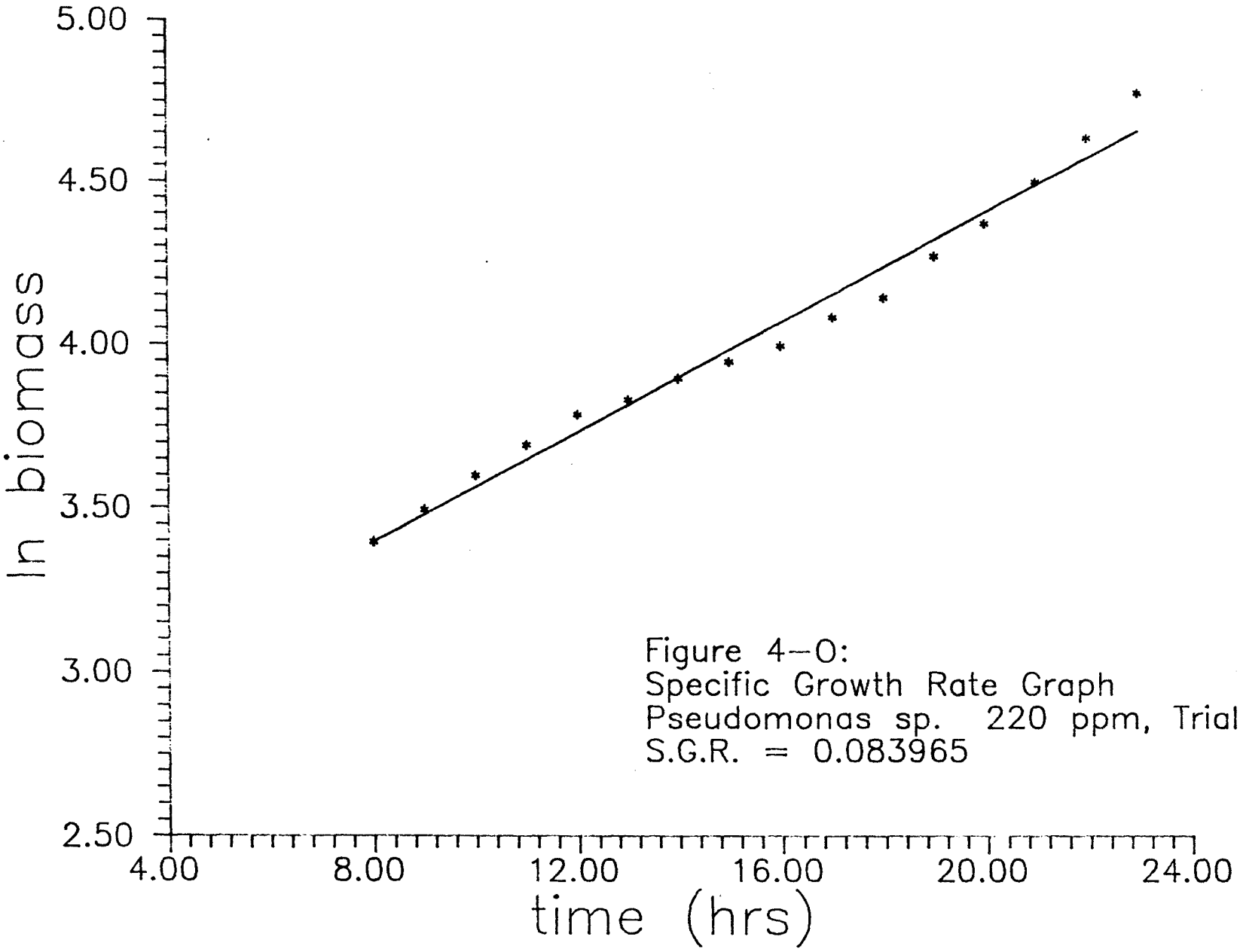


Figure 4–O:
Specific Growth Rate Graph
Pseudomonas sp. 220 ppm, Trial 1
S.G.R. = 0.083965

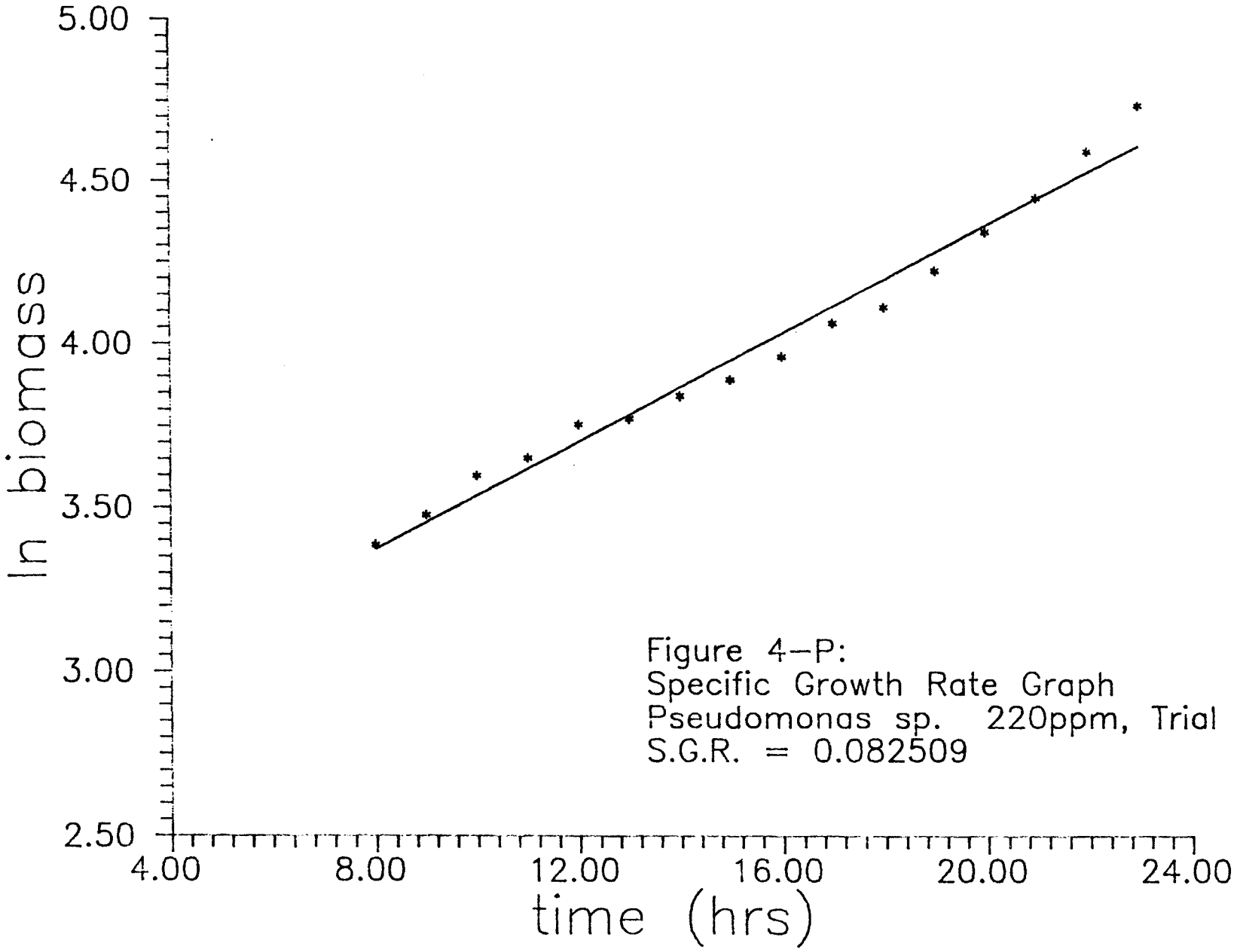


Figure 4–P:
Specific Growth Rate Graph
Pseudomonas sp. 220ppm, Trial 2
S.G.R. = 0.082509

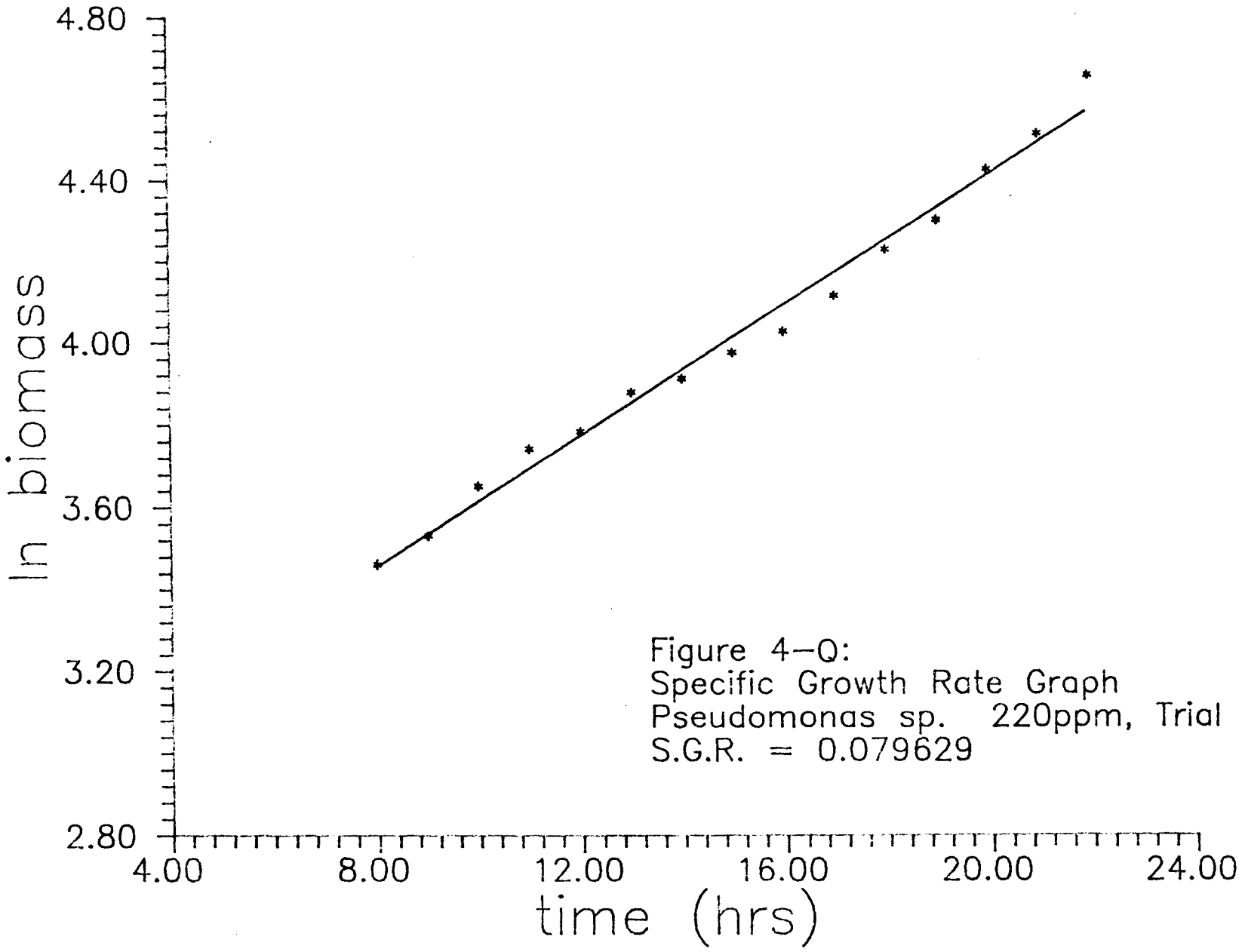


Figure 4–Q:
Specific Growth Rate Graph
Pseudomonas sp. 220ppm, Trial 3
S.G.R. = 0.079629

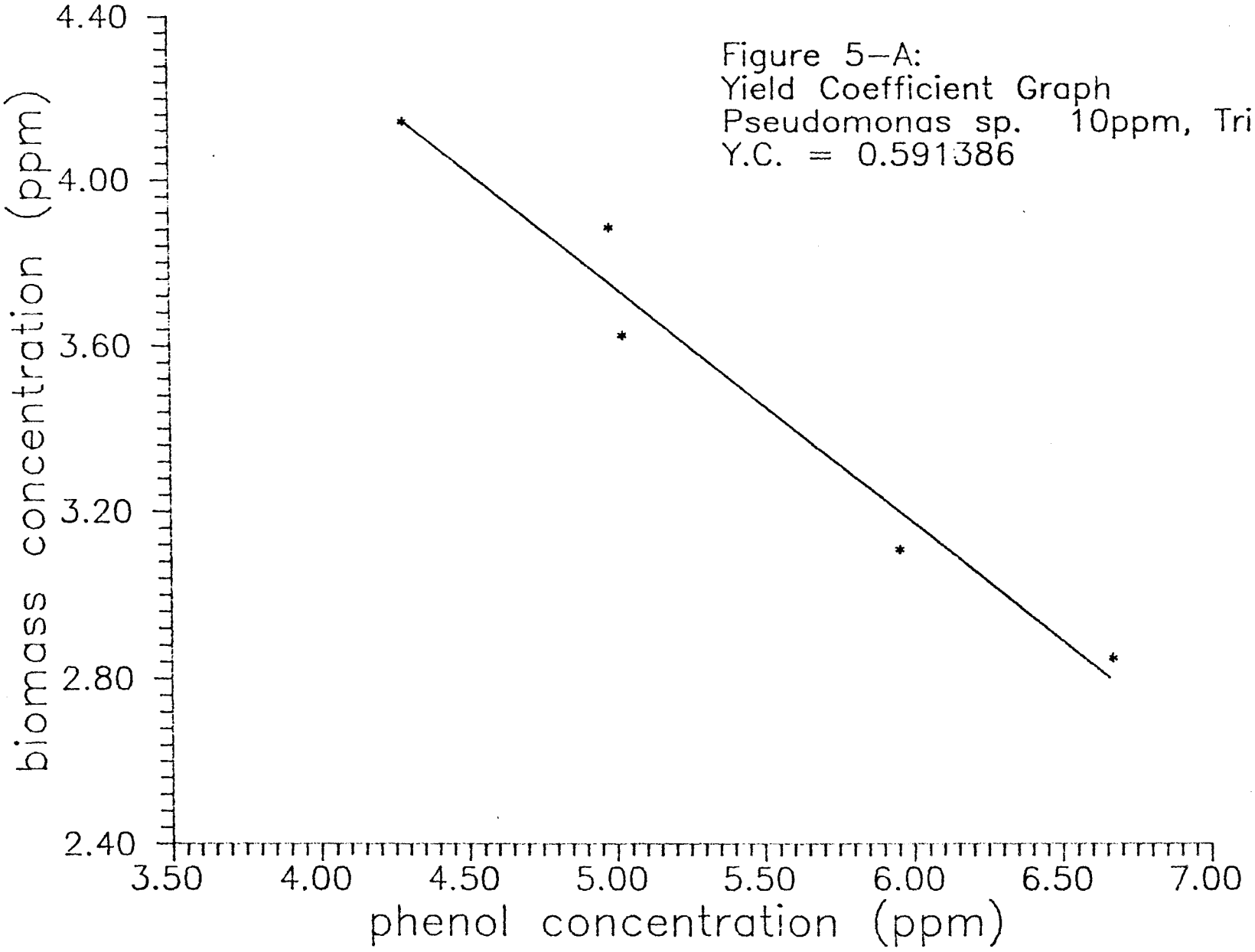


Figure 5–A:
Yield Coefficient Graph
Pseudomonas sp. 10ppm, Trial 1
Y.C. = 0.591386

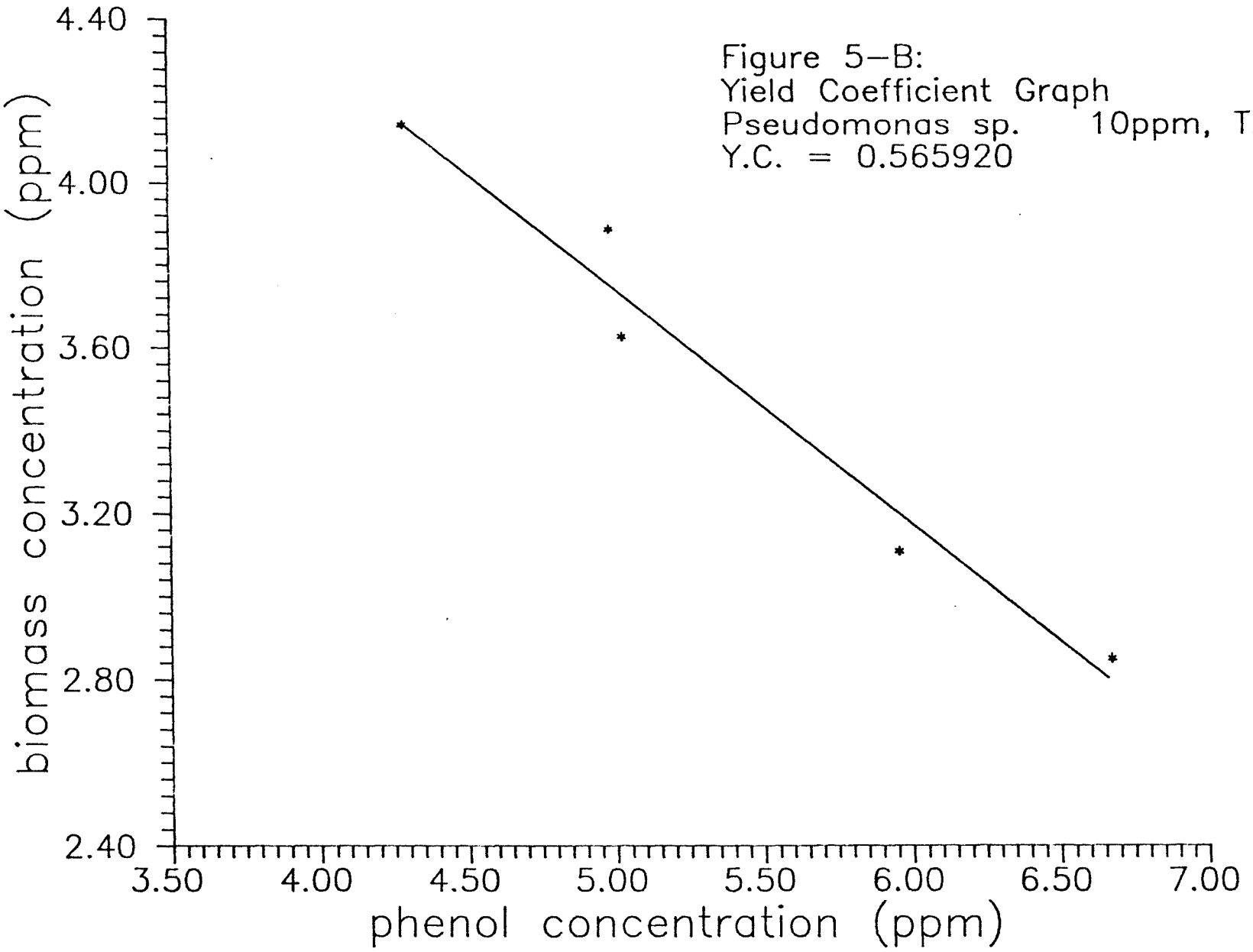


Figure 5–B:
Yield Coefficient Graph
Pseudomonas sp. 10ppm, Trial 2
Y.C. = 0.565920

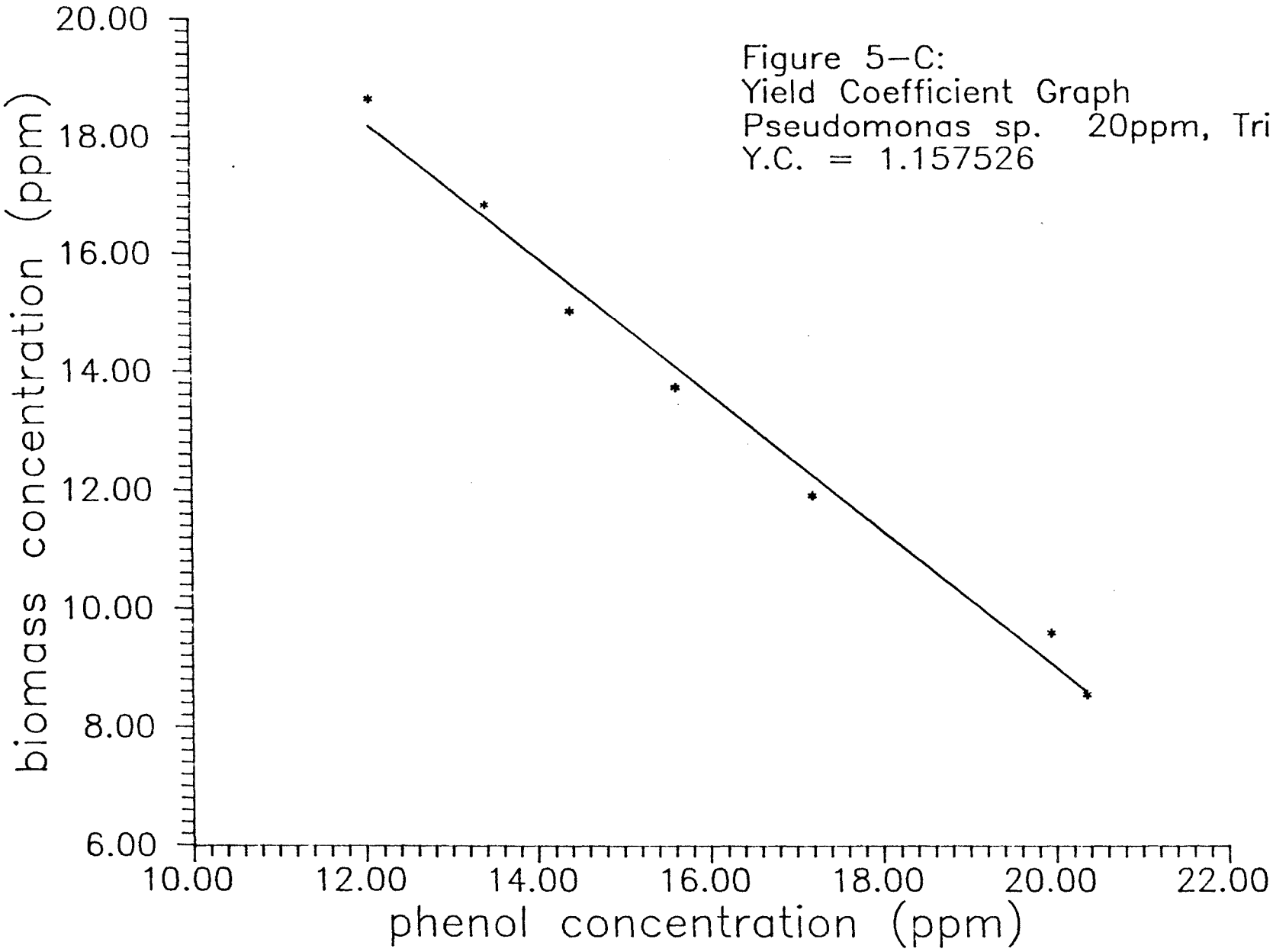


Figure 5–C:
Yield Coefficient Graph
Pseudomonas sp. 20ppm, Trial 1
Y.C. = 1.157526

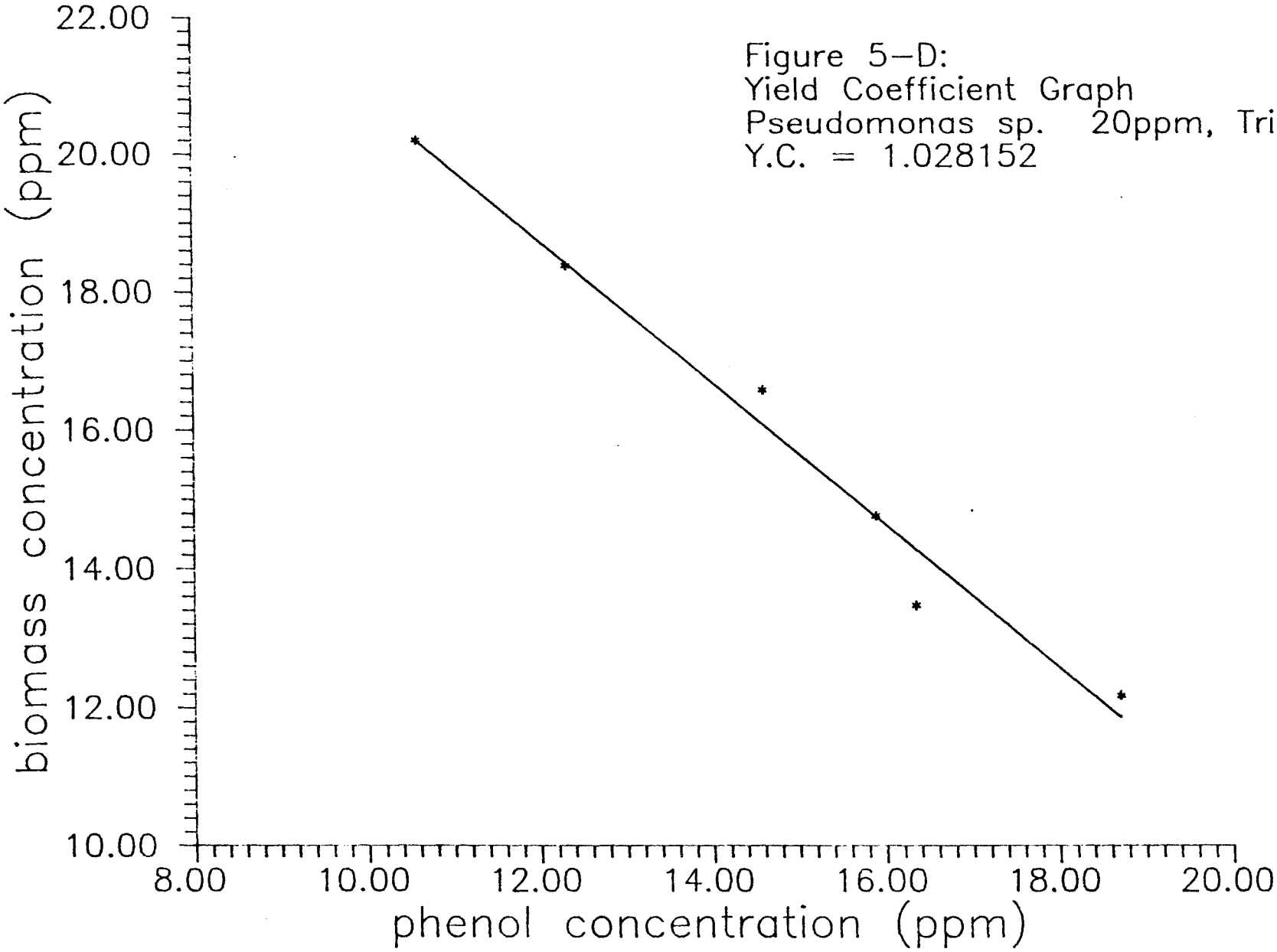


Figure 5–D:
Yield Coefficient Graph
Pseudomonas sp. 20ppm, Trial 2
Y.C. = 1.028152

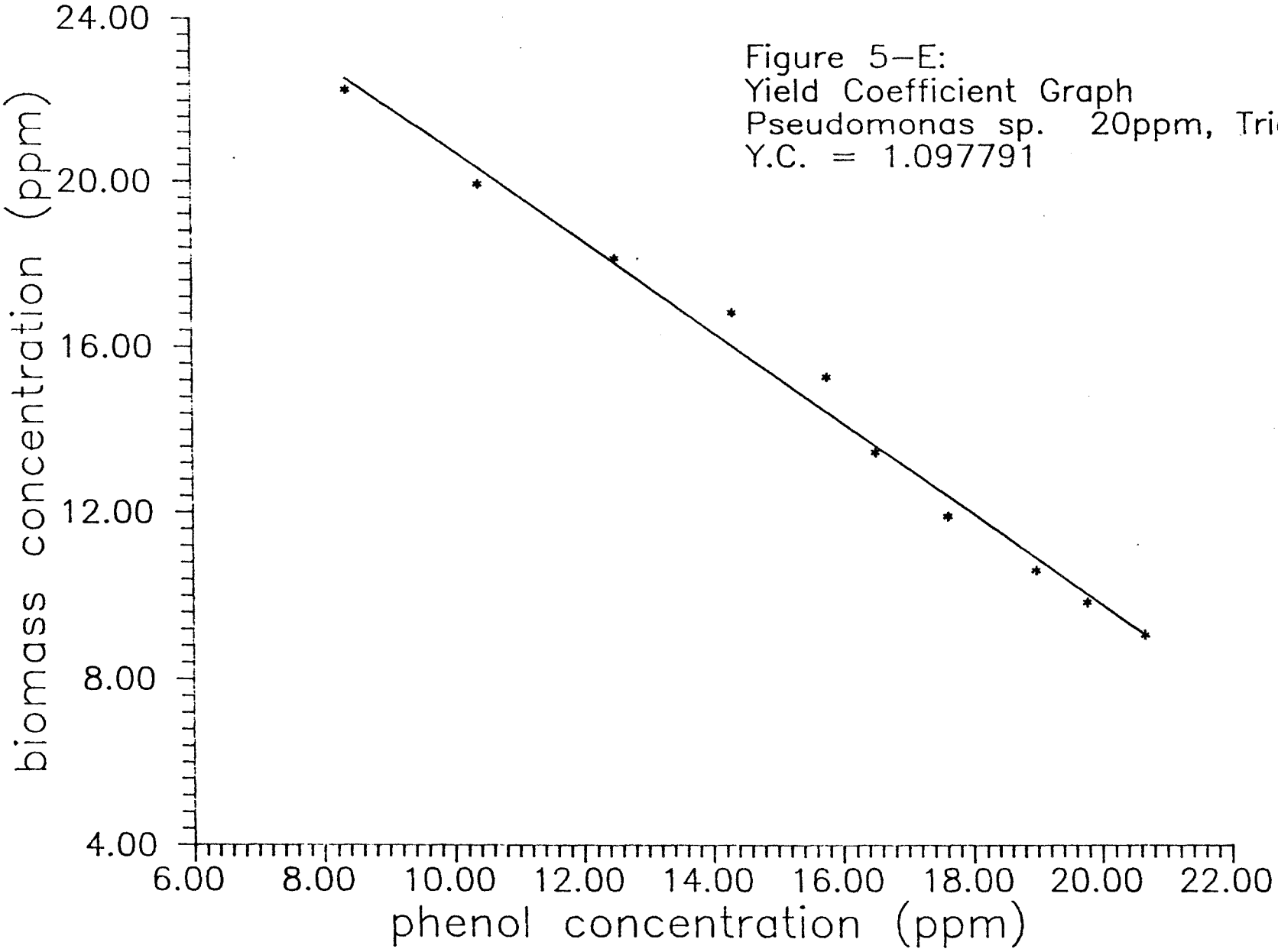


Figure 5–E:
Yield Coefficient Graph
Pseudomonas sp. 20ppm, Trial 3
Y.C. = 1.097791

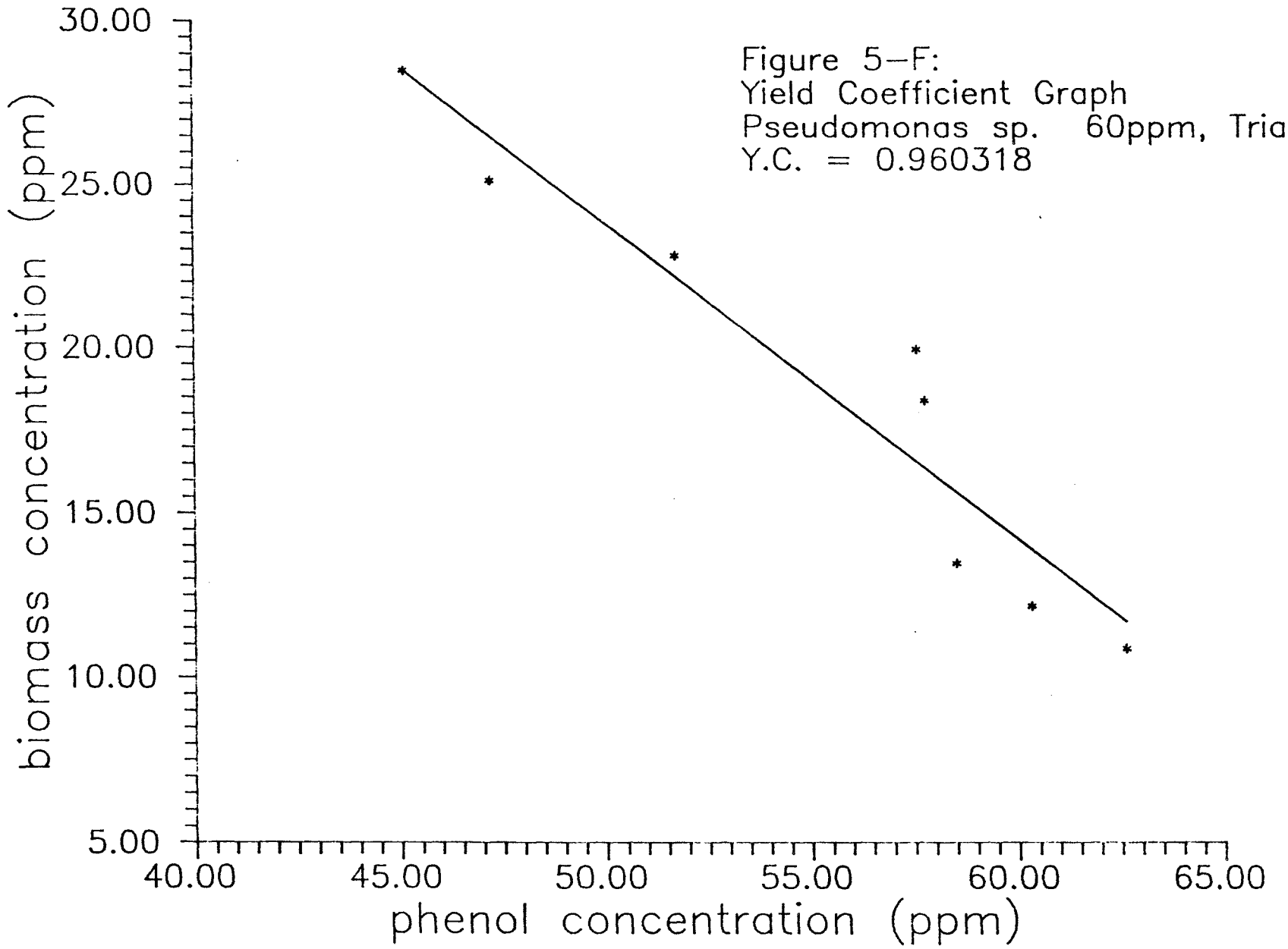


Figure 5–F:
Yield Coefficient Graph
Pseudomonas sp. 60ppm, Trial 1
Y.C. = 0.960318

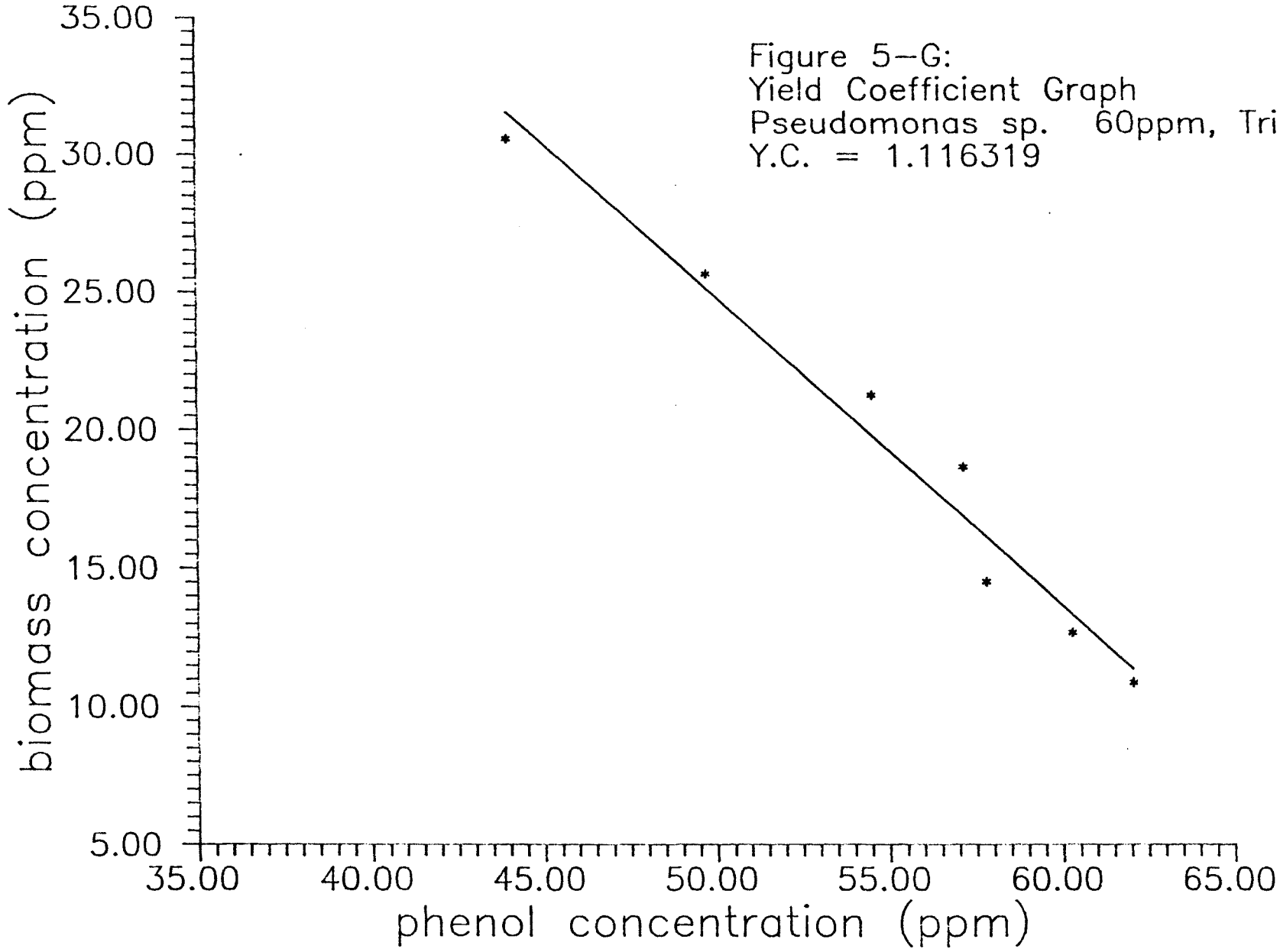


Figure 5–G:
Yield Coefficient Graph
Pseudomonas sp. 60ppm, Trial 2
Y.C. = 1.116319

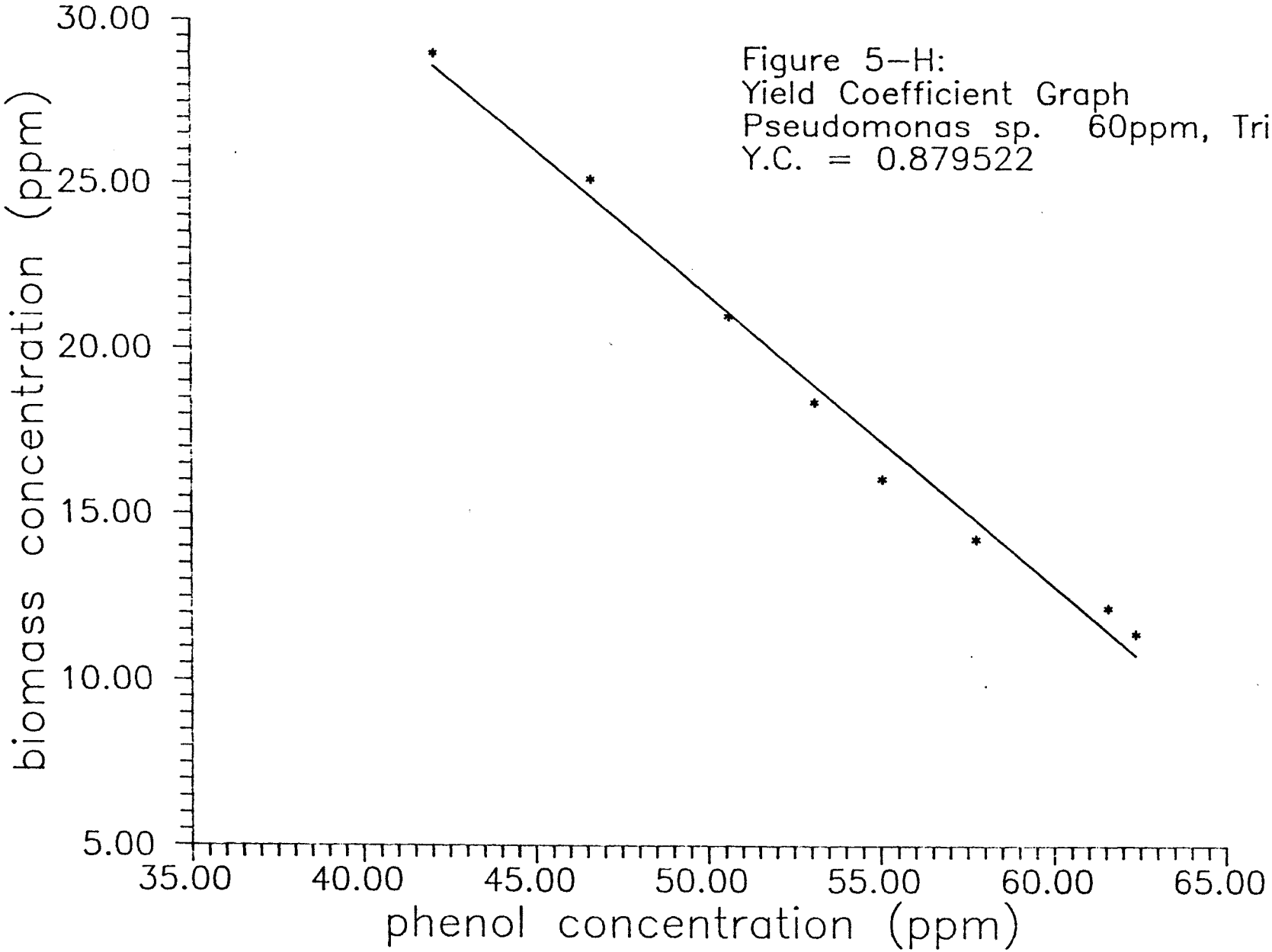


Figure 5–H:
Yield Coefficient Graph
Pseudomonas sp. 60ppm, Trial 3
Y.C. = 0.879522

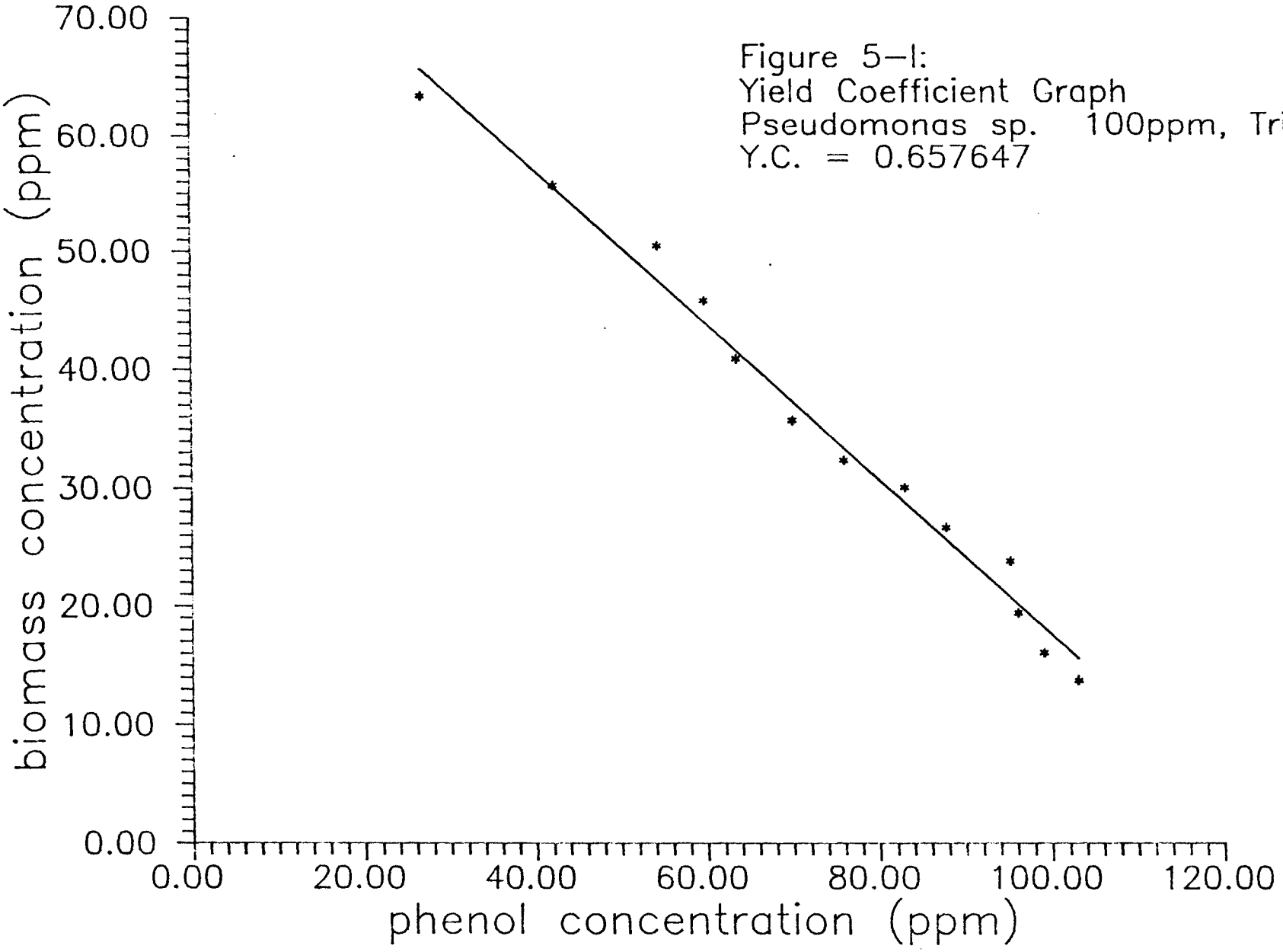


Figure 5–I:
Yield Coefficient Graph
Pseudomonas sp. 100ppm, Trial 1
Y.C. = 0.657647

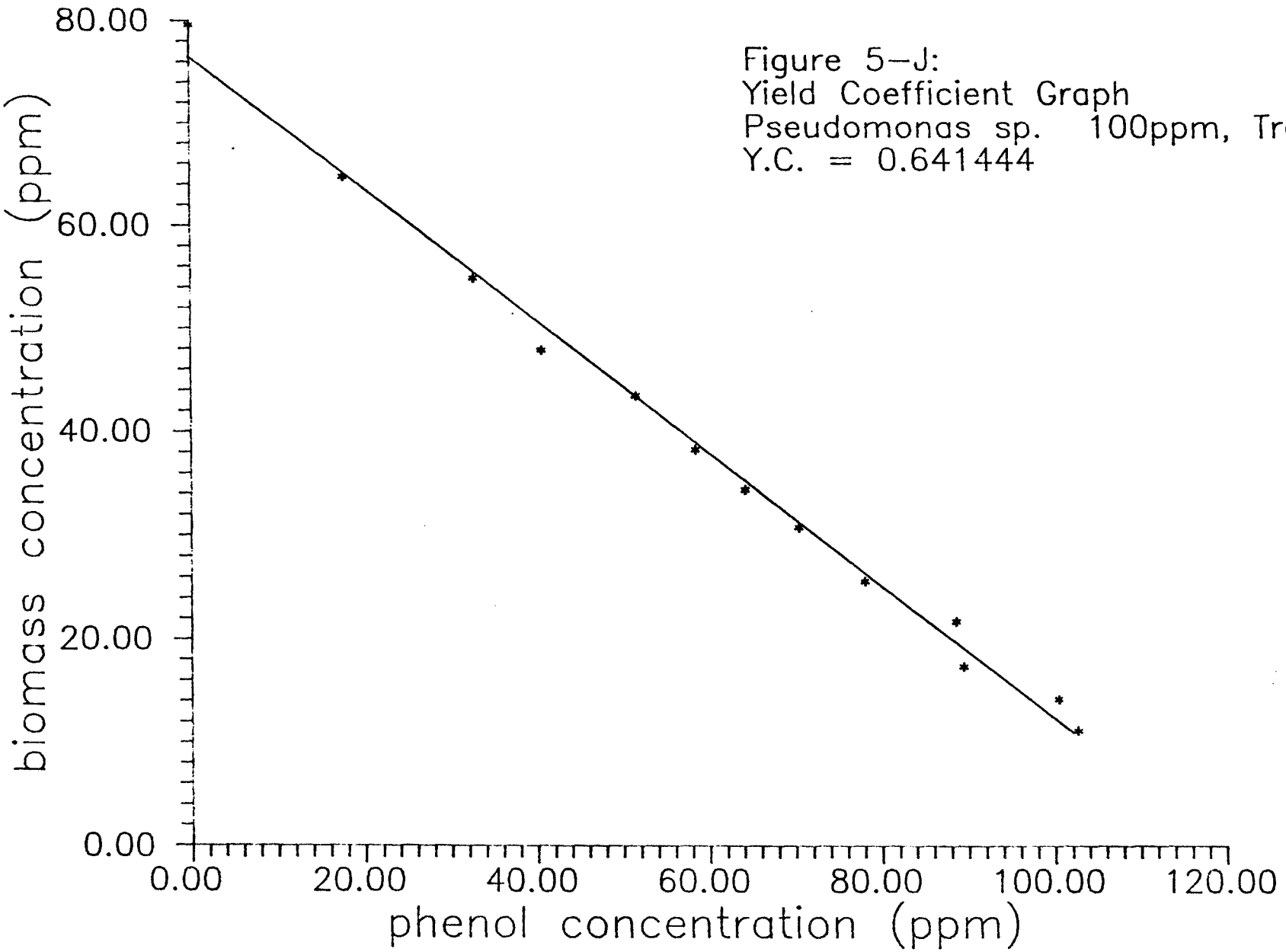


Figure 5–J:
Yield Coefficient Graph
Pseudomonas sp. 100ppm, Trail 2
Y.C. = 0.641444

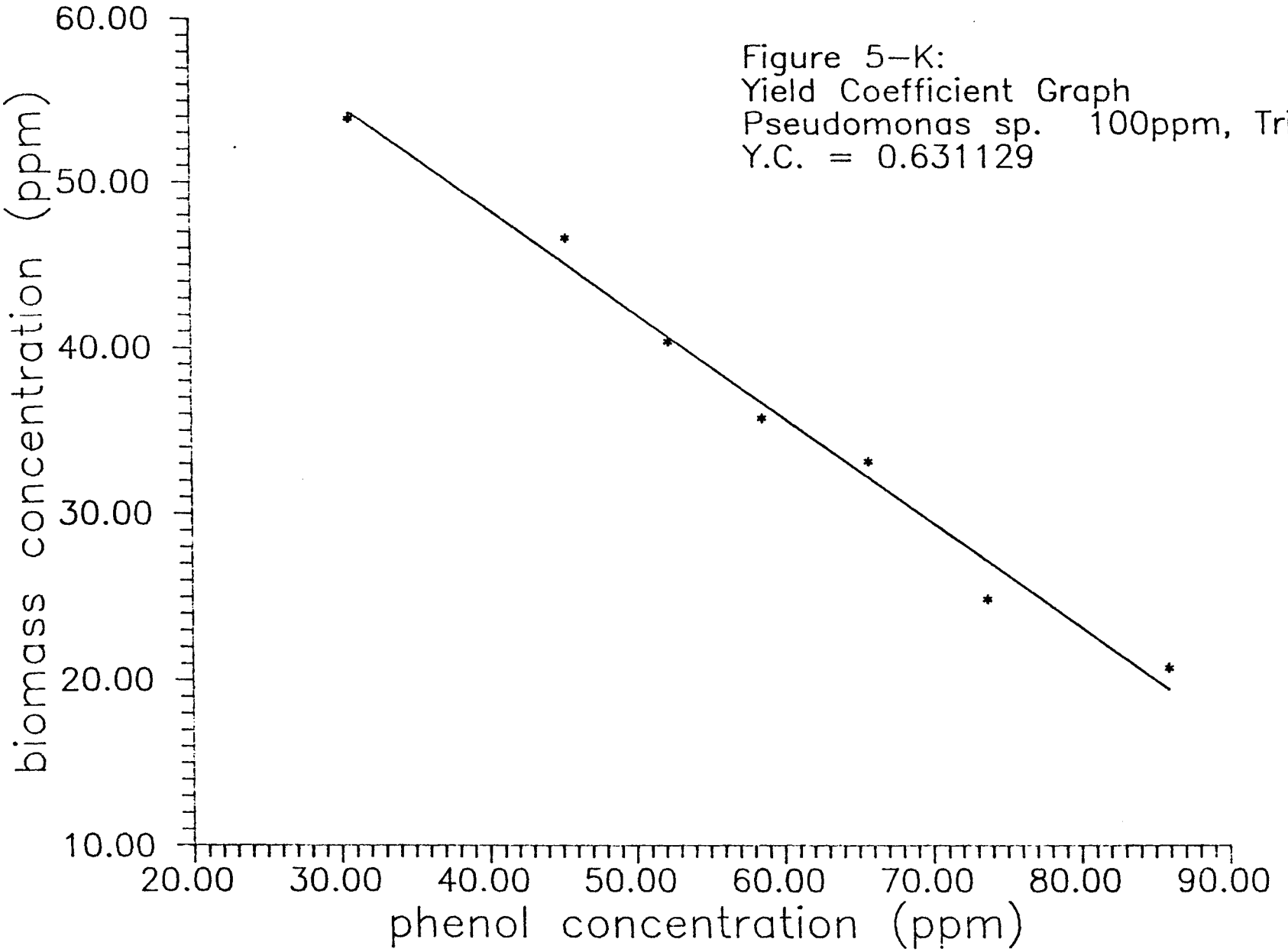


Figure 5–K:
Yield Coefficient Graph
Pseudomonas sp. 100ppm, Trial 3
Y.C. = 0.631129

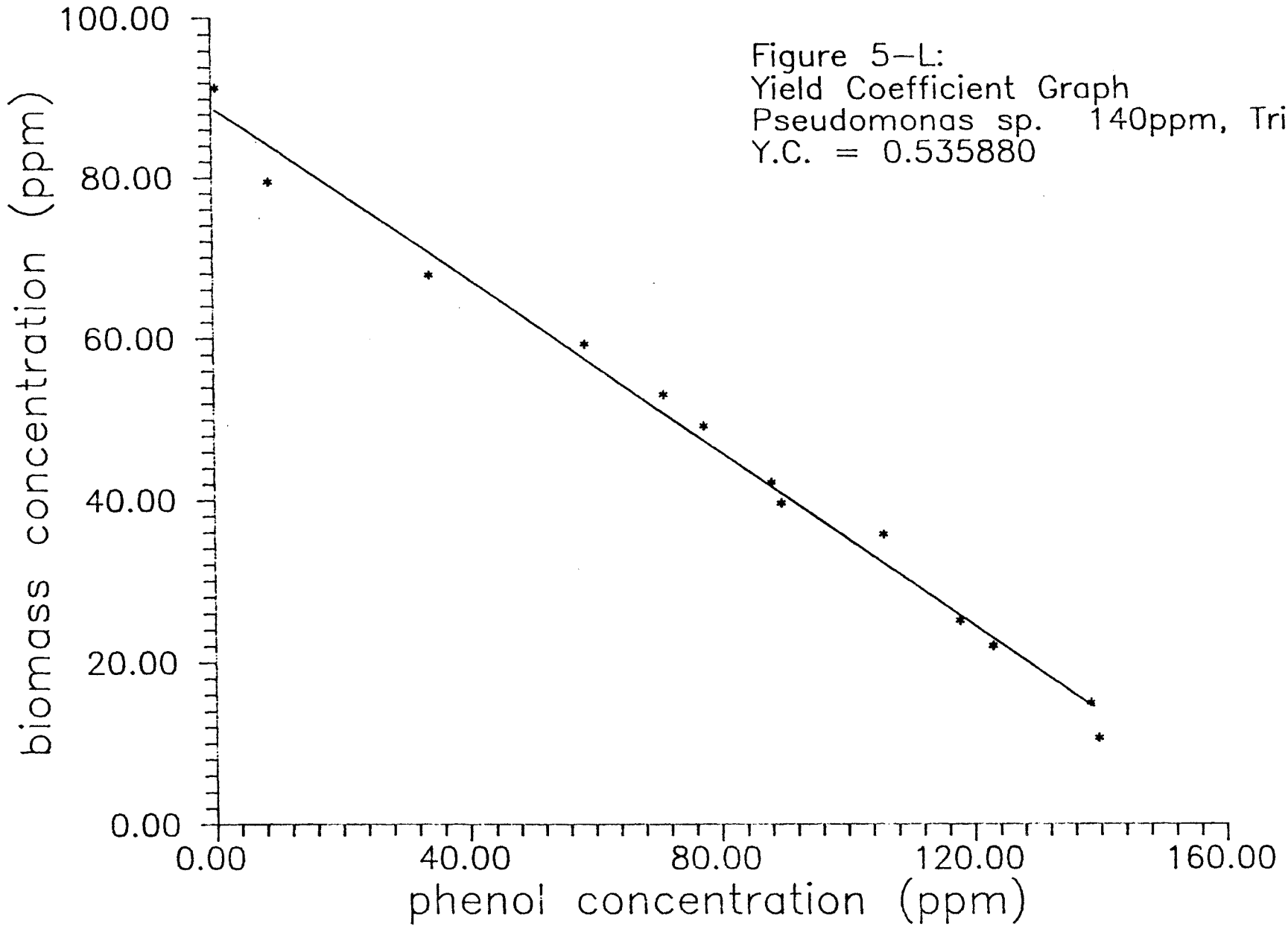


Figure 5–L:
Yield Coefficient Graph
Pseudomonas sp. 140ppm, Trial 1
Y.C. = 0.535880

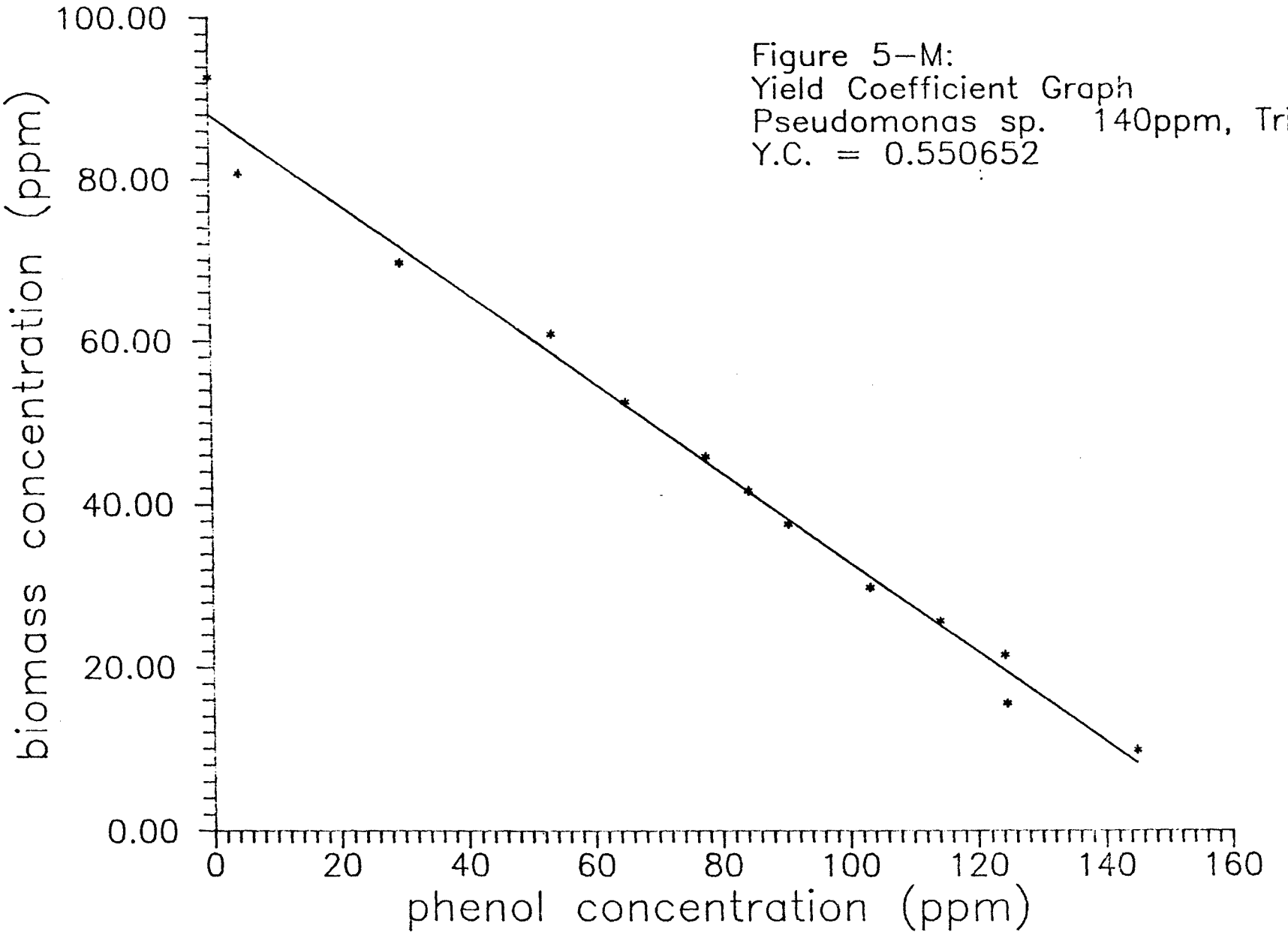


Figure 5–M:
Yield Coefficient Graph
Pseudomonas sp. 140ppm, Trial 2
Y.C. = 0.550652

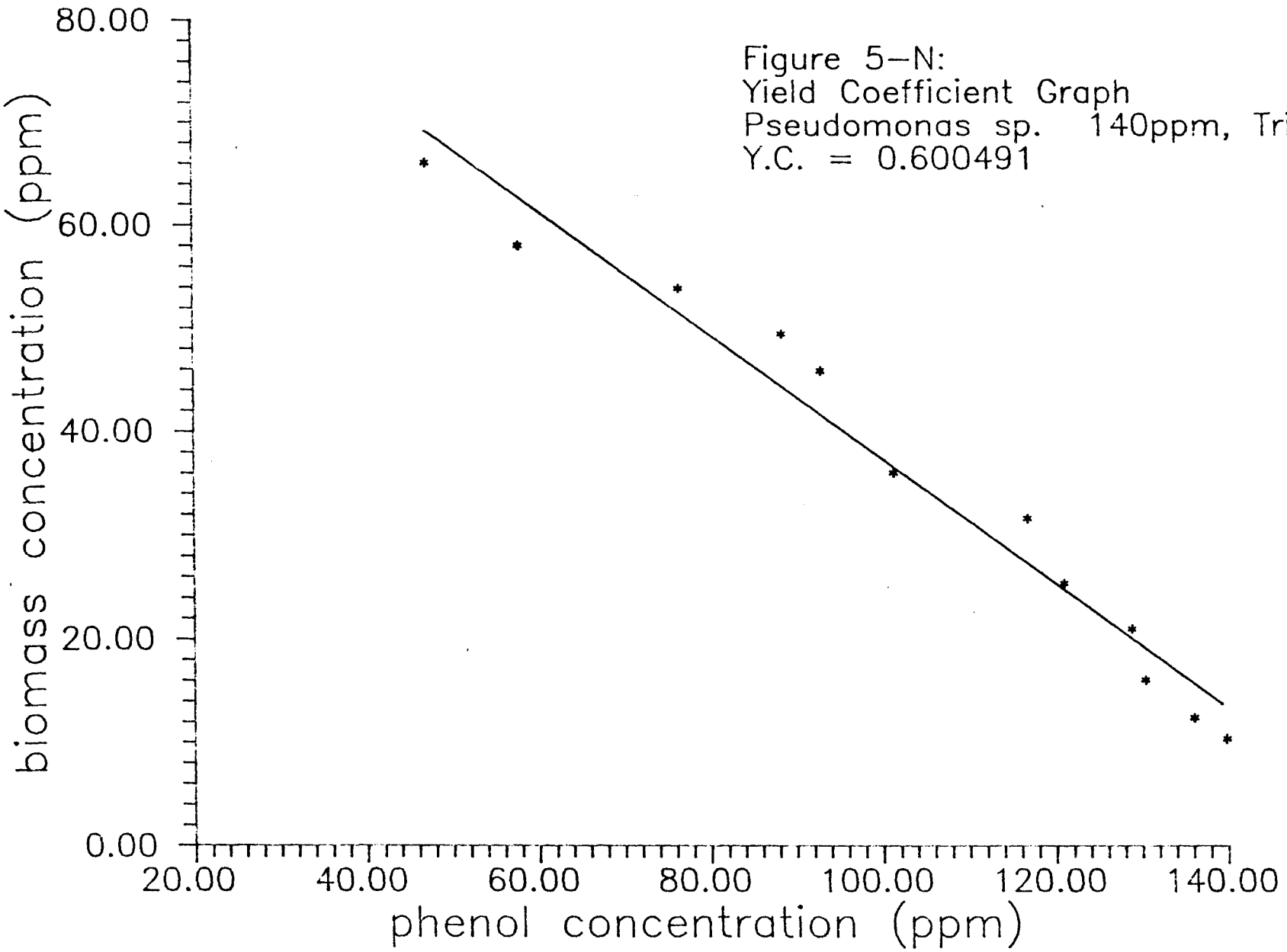


Figure 5–N:
Yield Coefficient Graph
Pseudomonas sp. 140ppm, Trial 3
Y.C. = 0.600491

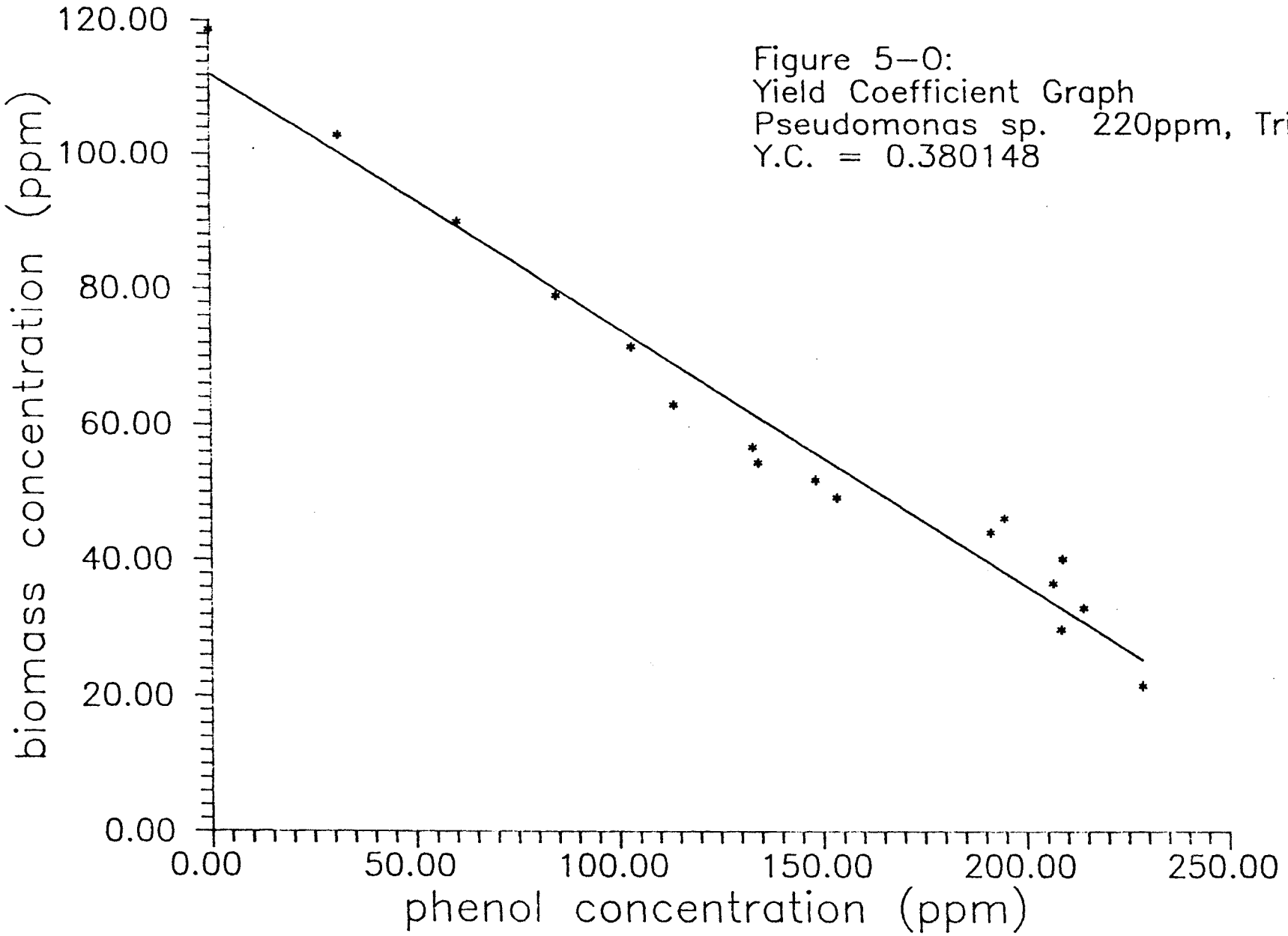


Figure 5–0:
Yield Coefficient Graph
Pseudomonas sp. 220ppm, Trial 1
Y.C. = 0.380148

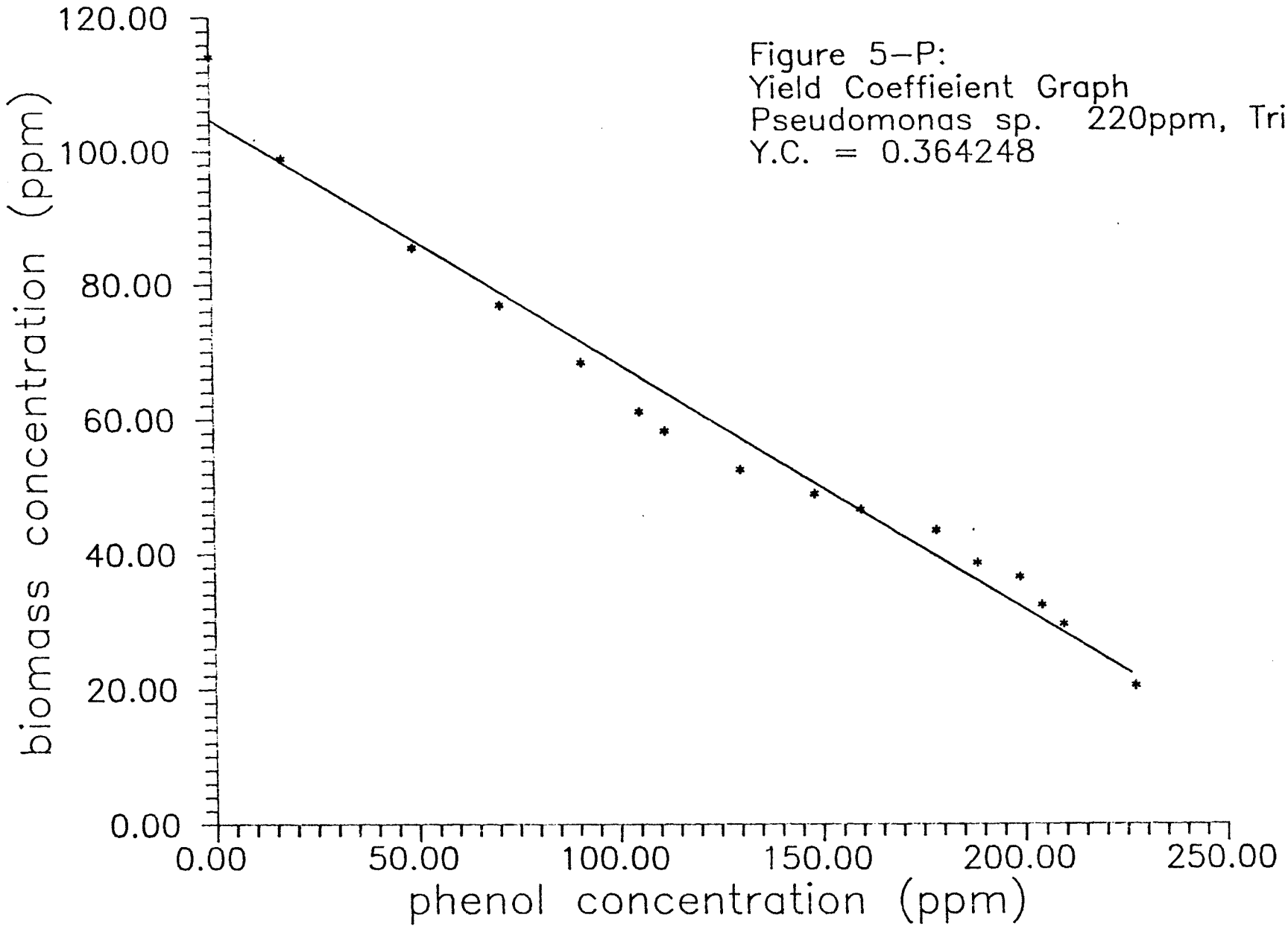
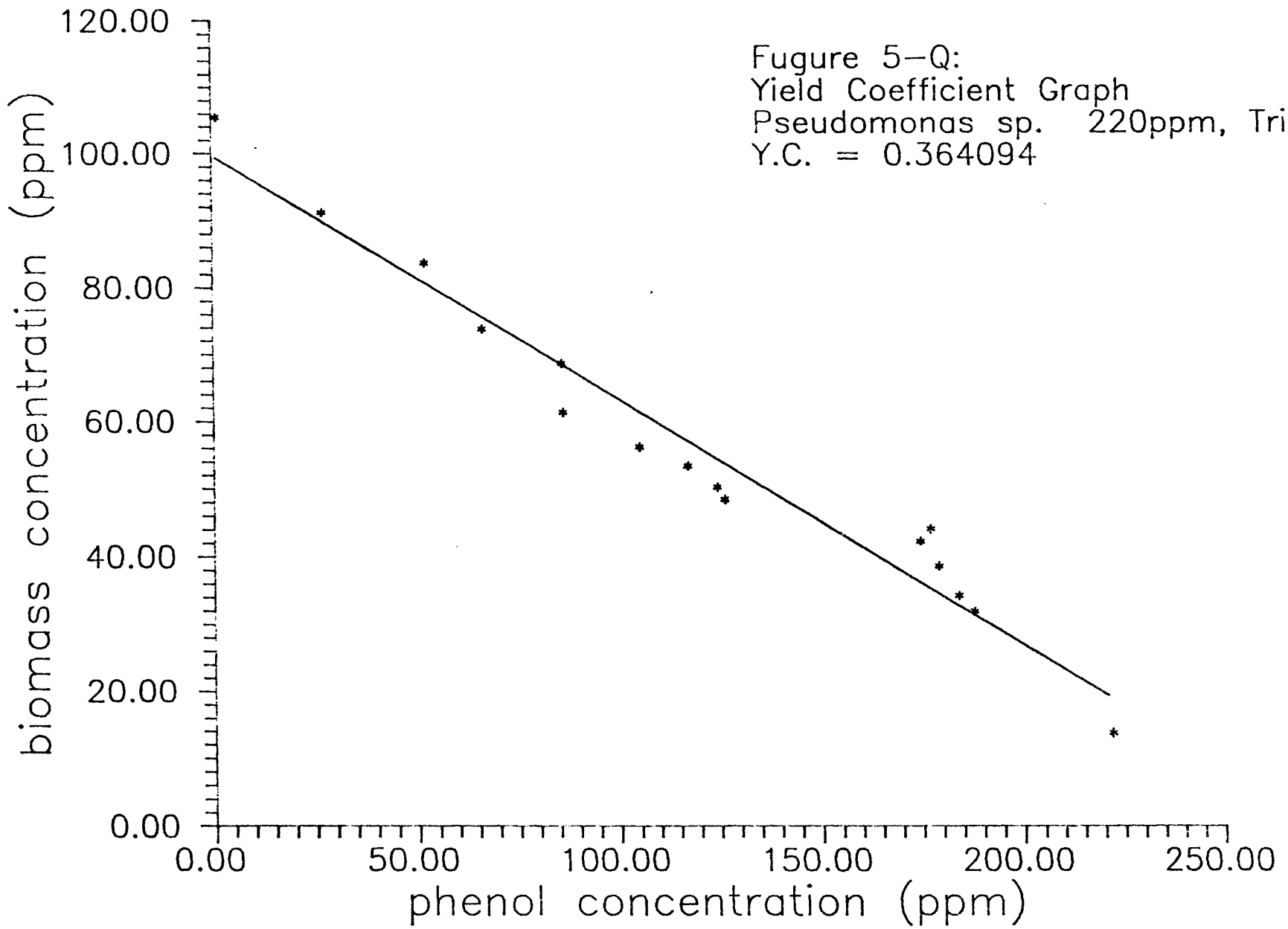


Figure 5–P:
Yield Coeffieient Graph
Pseudomonas sp. 220ppm, Trial 2
Y.C. = 0.364248



Fugure 5–Q:
Yield Coefficient Graph
Pseudomonas sp. 220ppm, Trial 3
Y.C. = 0.364094

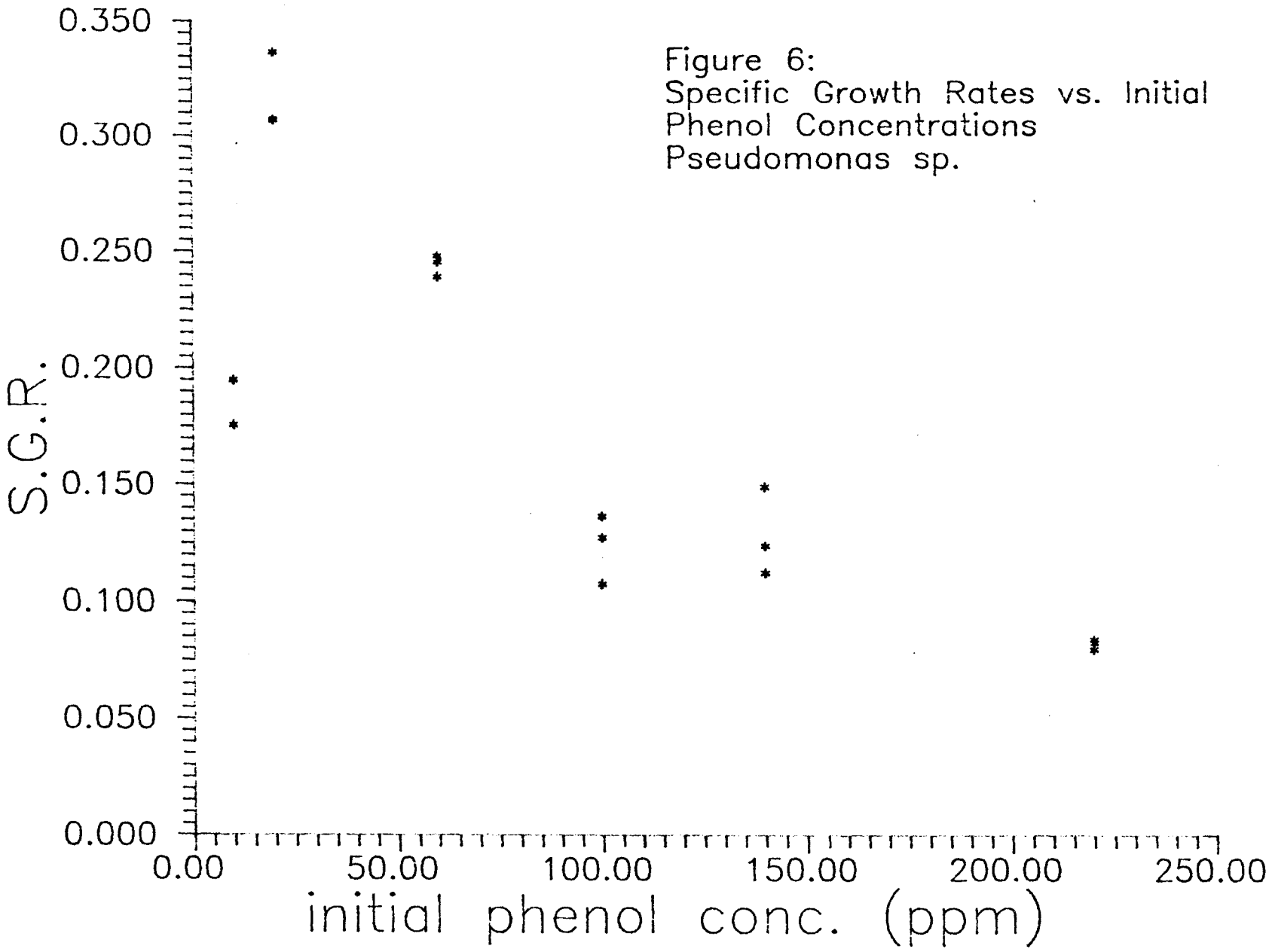


Figure 6:
Specific Growth Rates vs. Initial Phenol Concentrations
Pseudomonas sp.