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Varuntanya, Chirasakdi Peter

THE USE OF PURE CULTURES AS A MEANS OF UNDERSTANDING THE PERFORMANCE OF A MIXED CULTURE IN THE BIODEGRADATION OF PHENOL

New Jersey Institute of Technology D.ENG.SC. 1986

"In the temple of science are many mansions, and various indeed are they that dwell therein and the motives that have led them thither. Many take to science out of a joyful sense of superior intellectual power; science is their own special sport to which they look for vivid experience and the satisfaction of ambition; many others are to be found in the temple who have offered the products of their brains on this altar for purely utilitarian purposes. Were an angel of the Lord to come and drive all the people belonging to these two categories out of the temple, the assemblage would be seriously depleted, but there would still be some men, of both present and past times, left inside. ....."

Albert Einstein
An excerpt from the address delivered at a celebration of Max Planck's sixtieth birthday (1918) before the Physical Society in Berlin. Published in Mein Weltbild, Amsterdam: Querido Verlag, 1934.

THE USE OF PURE CULTURES AS A MEANS OF UNDERSTANDING THE PERFORMANCE OF A MIXED CULTURE IN THE BIODEGRADATION OF PHENOL

by

Chirasakdi Peter Varuntanya

Dissertation submitted to the Faculty of the Graduate School
of the New Jersey Institute of Technology in partial
fulfilment of the requirements for the degree of
Doctor of Engineering Science
1986

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ABSTRACT

Title of Dissertation: The Use of Pure Cultures as a Means of Understanding the Performance of A Mixed Culture in the Biodegradation of Phenol

Chirasakdi Peter Varuntanya, Doctor of Engineering Science, 1986

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In an effort to gain a more fundamental understanding of the performance of mixed microbial cultures in the biodegradation of toxic organic chemicals, studies have been conducted using three phenol degrading species isolated from a municipal treatment plant. The rate of phenol degradation was investigated for each of the three pure phenol degrading species, and various combinations of the three species. A simple competitive model was used to predict the behavior of the mixed cultures by using the pure culture Monod rate constants. The model fit the growth data for total biomass very well, although (as with the pure culture experiments) the fit of the phenol degradation data was less accurate.

This dissertation is dedicated to
my beloved parents
Chongchit and Varun Varuntanya

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Above all, to his parents who have been not only supporting but also understanding, this work is dedicated to them.

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28-1	20 mg/lit
28-2	40 mg/lit
28-3	50 mg/lit
28-4	80 mg/lit
28-5	100 mg/lit
28-6	120 mg/lit
28-7	140 mg/lit
28-8	180 mg/lit

CHAPTER I

INTRODUCTION

Phenol and other phenolic compounds are common constituents of the aqueous effluents from such operations as polymeric resin production, oil refining, and coking plants. Phenols pose a serious environmental risk because of their acute toxicity and relative persistance to chemical and biological degradation, they are either toxic (reduced enzymatic activity) or lethal to fish at relatively low concentrations (5 to 25 mg/lit) and impart objectionable tastes to municipal drinking water at far lower concentrations.

One reason for selecting phenol for this study is that, as greater reliance on coal for energy and organic chemicals occurs, the production of phenolic wastes can be expected to increase significantly.

In natural habitats there are many driving forces that tend to increase the heterogeneity of a microbial community. The multitude of substrates, the variability of physical conditions, the compartmentalization of the habitat into individual niches, and the

transitory nature of natural aquatic systems with variable inputs and outputs all contribute toward the diversification of the community structure. The diversity of the microbial flora serves as a good environment for developing active cultures that can biodegrade synthetic organics in general. This could involve association, in which different types of microorganisms participate in sequential decomposition of the compound, or the genetic development of a single species that can use the target compound as its prime source of food and energy. For example, a great variety of phenols are presumably released in the process of biological degradation of lignins, various biocides, and synthetic detergents. The phenolics are further degraded by a wide range of soil microorganisms (4).

The large variety of microorganisms present in activated sludge systems, and the possible variety of species interactions due to this diversity, may hold the key to the effective treatment of phenolic compounds, most of which (particularly those that are chlorinated) are newly introduced into the biosphere and probably there was not enough time for the

evolution of specialized microbes capable of utilizing them as their only source of food and energy.

A kinetic description of these processes is needed in order to design processes and control their operation. It has become increasingly important that the kinetic formulas used in the design of biological waste treatment facilities and in prediction of their operational characteristics be based upon controlled studies of microbial behavior rather than empirical rules. This is particularly true of systems handling toxic compounds.

Most reactions in biological processes are autocatalytic, and it is usually assumed that the relationship between waste concentration and organism growth rate can be expressed when the biomass concentration is constant by a hyperbolic function as proposed by Monod (91, 92). However, kinetic modeling is made more difficult by the fact that these processes involve living, and evolving, organisms.

The metabolic activity of a microbial community depends in a complicated way on:

(a) the population diversity of the microbial community and shifts in that population.

(b) the physiological response of the individual microorganisms to a multisubstrate environment.

(c) the interactions between the various microbial species (126, 127).

Thus, the total metabolic activity of a microbial ecosystem is not necessarily the additive result of the physiological activities of the individual species responding to individual substrates.

From a thorough literature survey, it was concluded that despite the enormous amount of research effort spent on biological treatment processes and the rather extensive use of phenol biooxidation processes, surprisingly little work has been published on microbial growth kinetics for pure culture systems using phenol as the sole carbon source.

Therefore, the purpose of the present work was to elucidate the kinetics of a mixed microbial population by examining the kinetics of the pure species of which the mixed population is composed.

CHAPTER II

LITERATURE REVIEW

A. CASE STUDIES OF PHENOL BIODEGRADATION WITH PURE CULTURES AND MIXED CULTURES

As far back as 1909, Stormer (125) tested the effect on soil of a number of organic compounds, including toluene, xylene, phenol and p-cresol and claimed to have isolated organisms that could destroy these compounds.

Fowler, Ardern and Lochett (43) suspected the occurence of phenol-utilizing bacteria on sewage filter beds, because phenol produced in the decomposition of sewage proteins did not accumulate. They isolated from the sewage effluent a phenol degrading organism resembling B.helvolus.

Gupta (59) has studied quantitatively the disappearance of phenols and cresols from soils of diverse origin, and showed clearly the biological nature of the process.

Gray and Thornton (58) isolated many types of soil bacteria capable of destroying several aromatic compounds including phenol. Pure cultures of these organisms were capable of utilizing the compounds as sole source of organic carbon. In a study of the distribution of these microorganisms, they showed that they were most often found in arable soil, but rarely occured in unmanured or "wild" soil. From a study of their morphology and growth characteristics, the strains were classified into the following six families: Coccacae, Mycobacteriaciae, Bacteriaceae, Psedomonadaceae, Spirillaceae and Bacillaceae, respectively.

Mohlman (89) reported that phenol at low concentrations could be successfully treated by activated sludge. This was accomplished by diluting an industrial phenolic waste with domestic sewage and then treating the combined waste at a municipal treatment plant.

Evans (42) concluded from his experiments that phenol-decomposing bacteria do not grow anaerobically. He claimed that dissolved oxygen is essential to its decomposition.

Detailed studies of phenol decomposing species of Pseudomonas were reported by Davey and Turner (33), who emphasized the difficulty of identifying individual species by standard biochemical tests.

Kramer and Doetsch (76) surveyed the degradation of 55 phenolic compounds with a large variety of microorganisms. Most of the observations they reported have been repeatedly asserted by numerous workers since then. They concluded that the presence of nitro, amino or multiple hydroxyl groups reduced microbial attack. Unfortunately, they were not able to explain this behavior. Also, the ortho and para positions appear to be more susceptible to biological attack than the meta position, as demonstrated by the p-cresol/m-cresol or catechol/resorcinol pairs.

Spicer (120) performed experiments in a chemostat treating an inhibitory substrate, phenol and determined the criteria for stable steady states. He showed that an inhibition growth function predicts multiple steady states, one of which is unstable.

Graves (56) reported in a treatability study that

it was possible to completely oxidize 100 lb of phenolic compounds per day, at a flow rate of 70 gpm, in a refinery's sanitary sewage treatment unit.

Tabak, et. al. (130) investigated 104 aromatics. As observed by others (71), the formation of adaptive enzymes to metabolize phenol was indicated by a marked lag in oxygen uptake in respirometric tests.

Wase and Hough (140) found that biodegradation of phenol by the yeast Debaryomyces subclobosus was rapid below 1128 mg/lit. Catechol was formed as an intermediate.

Andrews (6, 7) presented a theoretical analysis of batch reactor and chemostat kinetics involving inhibitory substrates. He used an equation which is the analog of the Haldane equation (60) for enzyme kinetics. Andrews equation accounts for substrate inhibition, and relates the specific growth rate to exogeneous substrate concentration. He solved the resulting non-linear differential equations with a numerical integration technique.

Eckenfelder (39) used a Monod function to describe the removal of phenol in batch reactors by activated sludge, but concluded that phenol removal was inhibited by high initial concentrations.

Kostenbader and Flecksteiner (75) reported on the activated sludge treatment of a coke plant waste and noted that the reactor could oxidize up to 30 lb of phenol/day/ft^3 of reactor volume. They also noted that the system effluent contained less than 0.1 mg/lit of phenol which represented removal efficiencies greater than 99.9 percent.

Refinery wastewater treatability studies were reported by Short (116), and Volesky, et al. (137). Short reported the operating experiences of several refineries treating phenolic wastewater. In general, this investigator reported that the activated sludge process gave adequate treatment with effluent phenol concentrations always less than 1 mg/lit, while Volesky, et al. reported similar results for a pilot plant study.

Bayly and Wigmore (12) used mutant strains of P.

putida, strain U, which are deficient in enzymes of the degradative pathways of phenol, with particular reference to the metabolism of the products of ring-fission.

Jones,et. al. (65) examined the growth kinetics of a pure culture of bacteria (NCIB 8250) in two-stage continuous culture utilizing phenol as the sole carbon and energy source. These researchers utilized the Haldane function to relate specific growth rate to phenol concentration and noted the susceptibility of the systems to wash-out.

Pawlowsky and Howell (99, 100), and Pawlowsky, et al. (101) performed extensive studies on the kinetics of heterotrophic mixed culture growing in a chemostat on phenol under both the steady state and dynamic conditions. They reported that in all instances the modeling of phenol biodegradation required the use of an inhibition function. Two systems were studied, one in which cocci predominated (along with several predators), and another in which filamentous bacteria predominated (along with very few predators). They found that the microbial population (after an initial

sharp fall) did not decrease as much in the second system with increasing phenol concentration, since the filamentous bacterial forms are less affected by the inhibitory effect of phenol.

Pawlowsky, et. al. (101) also reported that multiple steady states with phenol were obtainable due to wall growth. However, rather than recommend a modeling approach to account for the wall growth, they simply recommended care in eliminating wall growth from chemostat experiments.

Adams (2) reported results of a treatability study conducted on a waste that had influent phenol concentrations of about 3300 mg/lit. It was determined that reactor detention times of 8 days yielded effluent concentrations less than 1 mg/lit in internal recycle reactors.

Radhakrishanan and Sinha Ray (106) performed studies with both pure cultures and heterogeneous populations metabolizing phenol, and modeled the results with Monod kinetics. They concluded that the

influent substrate concentration determined the substrate concentration in the reactor, which contradicts the theory of continuous culture for a chemostat. Also, for a pure culture of Bacillus cereus, they reported that the maximum specific growth rate observed in the chemostat was 0.620 hr^{-1}, while it was only 0.144 hr^{1} in a batch reactor. One could argue that in the continuous flow unit,as the dilution rate increased, faster growing mutants of B. cereus were being selected by the system, thus offering some explanation for the differences in maximum specific growth rate. However, a four-fold difference between batch and continuous flow results suggests that there may have been inconsistencies in the experimental protocol.

Hill and Robinson (63) studied the kinetics of degradation of phenol by a pure culture of Pseudomonas putida and utilized the Haldane function to model the results.

Substrate inhibition with phenol has also been reported by a number of workers (e.g. 16, 54, 70, 80, 99, 106, 108, 109, 110, 11, 115, 133). The values of

the kinetic parameters obtained and models proposed varied according to the nature of the microorganisms, temperature, pH, and other factors. Some also attributed changes in phenol conversion rate to wall growth, but, as mentioned previously, this occurence should probably be addressed at the experimental level.

Yang and Humphrey (141) performed an extensive study on phenol biodegradation using pure cultures of Pseudomonas putida (a bacterium), and Trichosporon cutaneum (a yeast). These workers also found that for both organisms it was necessary to use the Haldane inhibition function.

Lanouette (78) stated that in order to insure process stability and maintain proper conditions in the reactor, feed equalization basins should be provided. He also noted that loadings of 250 to 300 lb phenol/day /ft^3 could be successfully treated with removal effeciencies of 99.9 percent.

The studies by Lee and Scott (80) for breakdown of phenol by Pseudomonas putida in fluidized bed reactors obtained degradation rates of 20 to 200 mg/lit/hr. The

results also indicated substrate inhibition at phenol concentrations above 200 mg/lit. These results are consistent with those of Rozich and Gaudy (111).

Orhan and Tunay (95) presented a model of phenol biodegradation by activated sludge, in which the phenol was treated as a simple inhibitor. This modeling approach is both over-simplified and incorrect. It is incorrect because phenol does not inhibit biological processes in the same manner as an inorganic toxicant (e.g. a heavy metal). Rather, the phenol can also be utilized as a substrate. It is only when its concentration in the reactor increases, that it comes inhibitory to growth. Thus, the term "substrate inhibition" is applied. The reason the modeling approach is oversimplified is that, if phenol is mixed with more easily degradable substrates such as found in domestic sewage, mechanisms such as catabolite repression and/or inhibition could occur. The model presented by these workers could not account for such mechanisms.

Haller (61) performed several treatability studies on different phenolic compounds and concluded

that substantial preadaptation of the sludge to the phenolic compound would result in improved efficiencies for the treatment process.

Knackmuss and Hellwig (73) demonstrated that phenol is the growth substrate of Pseudomonas sp. B 13 (similar to P. palleronii) which appears to have a selectivity for highly phenol chlorinated phenolic substrate. They also made comparative studies on the enzyme of the catabolism of phenolic compounds.

Vela and Bolston (136) studied the effect of temperature on phenol degradation. They found that the effect of temperature was a very complex phenomenon and can not be described by a simple Arrhenius equation.

Holladay, et. al. (64) compared the performances of three types of reactors degrading phenol: stirred tank, fluidized bed, and packed bed. They concluded that the ordinary stirred tank process was the least desirable since it exhibited the lowest degradation rate and was observed to be unstable and subject to operational upset.

Stachowicz(121) performed studies on the oxygen uptake rate of activated sludges metabolizating phenol and concluded that sludges adapted for the longest times to phenol responded with the highest initial oxygen uptake rate.

Shivaramna, et. al. (115) found that yeast, Candida tropicalis is capable of degrading phenol up to a concentration of 2000 mg/lit with an initial biomass concentration of 440 mg/lit. About 90 % of the phenol was degraded in 24 hours at a feed concentration of 650 mg/lit, but with an increase in feed concentration, the rate of growth and degradation of phenol decreased. At a neutral pH, the presence of up to even 10,000 mg/lit ammonium chloride did not affect the degradation.

The kinetics of phenol degradation by activated sludge in batch reactors were studied by Beltrame,et. al. (13). These workers asserted that phenol degradation can be modeled with Monod growth kinetics. However, one of the figures in their work shows that the specific growth rate decreases with increasing initial phenol concentrations, suggesting inhibition kinetics.

Beltrame, et. al. (14) studied the biodegradation of phenol (up to 360 mg/lit) in a continuous stirred reactor using an activated sludge. They reported that, by using data from the continuous flow unit, phenol could be considered non-inhibitory and modeled with a Monod expression. They also studied the biodegradation of a mixture and 2,4-dichlorophenol (DCP) in a 9:1 carbon ratio. The tests were performed in a 3-litre continuous stirred tank reactor at 20^{o}C using activated sludge that was first acclimated to phenol. Inorganic nitrogen and phosphorus were added to the feed mixture so that the C:N:P weight ratio was 100:6:2. They reported that the phenol degraded according to a first-order equation

Phenol biodegradation kinetics were assessed in a completely mixed chemostat with a heterogeneous population by Neufeld and Valiknac (94). They concluded that the Monod model could adequately describe the kinetics. It should be noted that simply using a chemostat is not a definitive method to determine whether a substrate is inhibitory or non-inhibitory. It is possible to draw two different conclusions from wash-out experiments. For a non-

inhibitory waste, wash-out simply means that the growth rate was not high enough to replace organisms lost in the overflow; while for an inhibitory waste, washout can also occur when the system has gone past the peak in the specific growth rate curve. In view of this, the preferred technique of assessing the question of inhibition is either to run batch growth studies (98) or as, Yang and Humphrey (141) did, to combine continuous flow and batch data complete the growth curve.

Tomlins,et. al. (133) suggested the use of a storage model to describe phenol batch data with activated sludge. They utilized the ATP concentration in the sludge as a measure of active biomass. Although a novel conceptual approach was used in this study, the "modeling" study amounted to fitting equations with seven coefficients to batch data, and then assessing the "predictive" power of the model.

Barth and Dobbs (10) studied the biodegradation of 104 aromatic compounds at 25^{o}C in a batch reactor (Warburg respirometer) using bacteria acclimated to 300 mg/lit phenol. Among the 104 compounds tested were

phenol, benzyl alcohol, heterocyclics, benzoic and other acids, benzaldehydes and benzamides, and substituted benzenes. The phenolic ocmpounds studied included: phenol, catechol, resorcinol, quinol. With the phenolic compounds they observed a decrease in acclimation time with repeated exposure to the compound. They further concluded that there appeared to be a relationship between molecular structure and resistance to bacterial degradation. The relationship was apparently affected by the position of a group on the ring, the type of group, multiples of the same or different substituents, and the size and complexity of the substituent.

Walton and Dobbs (139) reported the use of surface application and underground injection of mutant strains of bacteria to combat a variety of spilled hazardous materials. Using a portable biological treatment system, they were able to degrade 30-40 mg/lit phenol to 0.1 mg/lit in approximately 26 days. They also reported an increased resistance to biodegradation with the addition of chlorine to the toxic molecules.

Luthy and Jones (82), and Luthy (83) studied biological oxidation of coking and coal gasification

wastewaters. The quenching of gaseous products after pyrolysis results in liquors with very high phenolic concentrations (400-3000 mg/lit). The yield coefficients for such liquors were characteristically low due to the presence of other inhibitors (thiocyanate and cyanide). They proposed first order kinetics for phenol degradations

Levenspiel (81) proposed some expressions for the specific growth rate which take into consideration product inhibition. However, other investigators (115-118) have considered only substrate inhibition for phenol degradation.

Baker and Mayfield (9) studied biological and non-biological degradation of phenol under aerobic and anaerobic conditions using clay loam soil collected in Waterloo County, Ontario.

Paris and Wolfe (96) tried to determine if a correlation exists between microbial degradation rates and the chemical structure of the carbon source. Using a single strain of bacteria, Pseudomonas putida strain U, they determined the second order rate constant of phenol degradation.

Kim, et. al. (70) and Kim and Armstrong (71, 72) performed an extensive study of phenol and methanol biodegradation kinetics using acclimated sludge in batch tests. They determined the effects of temperature, pH, salinity, and nutrients on the rate of degradation. These workers utilized a Monod expression in their mathematical analysis, and made several errors in integrating their governing differential equations. They consistently attempted to solve a system of two differential equations by not solving the system simultaneously. If they had attempted to solve these equations with the correct analytical approach, they would have discovered that because of the nonlinearities in these equations, a numerical integration procedure would be more appropriate. With inaccurate equations, the authors then proceeded to perform statistical manipulations of the equations to account for the deviation in experimental results, leaving one to question the values of their constants.

Singer, et. al. (118) studied the biological oxidation of coal gasification wastewater. Using a 22.5 litre activated sludge CSTR with solid recycle, operated with a 10-day hydraulic residence time and 20

day cell residence time, the phenol concentration was reduced from 560 mg/lit to 1.2 mg/lit. Also the COD and TOC of the wastewater were reduced 71% and 68% respectively. But despite the effectiveness of the biological treatment in removing phenol, the biologically-treated water was unacceptable for discharge to the aquatic environment or for reuse within the plant, and further treatment was required by physical-chemical methods.

Suidan, et. al. (128) studied the treatment of coke oven wastewater from a steel mill by a contact stabilization activated sludge process with powdered activated carbon, followed by denitrification in an anoxic (anaerobic) column packed with berl saddles. The coal conversion wastewater was treated at a concentration of 85-120 mg/lit.

Chian and Harris (24) studied phenol degradation in a pure chemostat of Pseudomonas putida and applied an elaborate noninhibitory model to the data.

Sokol and Howell (119) performed batch tests on phenol utilization by P. putida and found that a two-parameter inhibition model provided the best fit of the data.

B. PATHWAYS OF PHENOL METABOLISM

Initial studies on the metabolism of aromatics were carried out with a view to discovering intermediate compounds (42, 122). These studies led to the pathway shown in Figure 1. Additional studies concerning the adaptability and possible intermediates of activated sludge biodegradation of aromatic have been carried out (28, 51, 69, 84, 85, 86, 87)

Aromatic compounds prove to be quite resistant to biological breakdown, probably due to the large amount of energy required to break the benzene ring. Phenol has been used as a bactericide, and the ability of other chemicals to act as bactericides is often measured relative to phenol. Of 175 strains of fluorescent pseudomonads studied by Stanier, et al. (123), 11 were able to utilize phenol as a sole source of carbon and energy. These all belong to the species Pseudomonas putida. Nine of the 41 strains assigned to this group are nutritionally more versatile than the rest. Of these, strain 110 (ATCC 17484) was isolated by naphthalene enrichment and is therefore well-suited to consume aromatics.

Czekalowski and Skarzynski (31) studied an Achromobacter strain isolated from soil grown on a mineral salt medium containing phenolics or benzoic acid derivatives as the sole source of carbon. The strain did not breakdown unsubstituted aromatic hydrocarbons and their derivatives, but did split phenols and carboxy acids, an ability which was shown to be limited to mono-cyclic compounds.

Varga and Neujahr (135) also isolated 13 different strains of microorganisms among which were 6 bacteria, 3 yeasts, and 4 mycelial fungi able to grow on phenol. Based primarily on results utilizing strains of bacteria belonging to the genus Pseudomonas and Moraxella, they concluded that biodegradation generally followed the pathway of phenol to catechol to cis, cis-muconate.

The pathway shown in Figure 1 for the degradation of phenol is via oxidation to form β -ketoadipate (and eventually acetyl-CoA and succinate) which enter the TCA cycle (124). Oxygenases are used to both add hydroxyl groups and cleave intermediates. No reducing power (i.e. production of a reduced nucleotide which

can be oxidized by the respiratory transport chain to produce ATP) occurs, and the only source of ATP production comes through the TCA cycle where electrons are transferred to the respiratory transport chain. Pseudomonas putida has been shown to oxidize phenol by both NAD^+ dependent (see Figure 2) and independent pathways, preferring the dependent pathway (11, 23, 86). The preference of the dependent pathway is due to the production of an extra reduced nucleotide. The chief difference between Bayly's pathway and Stanier's pathway is found to be the site at which the benzene ring is split open. Stanier shows this occuring between the hydroxy groups whereas Bayly infers that it occurs to one side. This results in different metabolitic intermediates after catechol. Stanier indicates the consumption of $NADH_2$ to oxidize phenol whereas Bayly does not. Stanier's metabolitic products, succinate and acetyl-CoA, enter the TCA cycle directly and by the time each one has completed the cycle, 8 $NADH_2$, 3 $FADH_2$, and 2 GTP molecules are produced. With the consumption of one $NADH_2$ to produce catechol, and assuming each $NADH_2$ produces 3 ATP molecules and each $FADH_2$ produces 2 ATP molecules in the respiratory transport chain, yields a total of 29 high

energy phosphate molecules. Bayly's metabolitic products, acetaldehyde and pyruvate, are converted to acetyl-CoA, producing 2 $NADH_2$ molecules and consuming a high energy ATP molecule. Acetyl-CoA molecules complete the TCA cycle to produce 6 $NADH_2$, 2 $FADH_2$, and 2 GTP molecules. With the production of an $NADH_2$ molecule in the dependent pathway, a net of 32 high energy phosphate molecules are produced after completion of the respiratory transport chain. Of course, in reality, much of the succinate, pyruvate, etc., are syphoned off elsewhere and these theoretical yields are not achieved.

C. MICROBIAL INTERACTIONS

When several populations of microorganisms inhabit a common environment they will almost invariably interact with one another. Attempts to explain the dynamic behavior of such systems must be based on knowledge of what interactions occur and on the kinetics of such interactions. Fredrickson (47) has classified microbial interactions into two main categories: direct and indirect. Direct interactions are those for which physical contact of individual organisms from the two different populations is a necessary part of the interaction. Indirect interactions are those which require the abiotic part of the environment as a necessary intermediary, while physical contact of the organisms is not required. Direct interactions are thus physical in nature, whereas indirect interactions are chemical in nature. He purposed further a scheme of naming binary interactions. In a system inhabitated by p different populations, there is a possibility of up to p!/2!(p-2)! binary interactions occurring.

Little is known about the fundamental characteristics of mixed microbial populations (basic dynamics, physiology, and ecology).

In the treatment of natural ecosystems, especially of waste disposal processes, the approach which is often used is to lump together the various species and treat them as a single functional population. However, this approach cannot handle quantitative and qualitative changes brought about by changes in the relative numbers of the species composing the functional population (45).

The adaptibility of mixed populations is a key to the study of the microbiology of natural environments. Understanding of the mechanisms underlying this adaptation could come from investigations of population dynamics and microbial interactions (17, 19, 25, 26, 45).

Although a number of interactions (both direct and indirect) are possible in a mixed population, the simplest (and the one considered in the present study) is pure and simple competition.

When two microbial populations are grown on the same rate limiting substrate, but do not otherwise affect each other, the interaction is considered to be pure and simple competition.

A more precise definition (46) of competition is given as follows:

Populations P_1 and P_2 compete for resource p if and only if:

(1) both P_1 and P_2 use, but do not necessarily require, ρ and

(2) resource ρ has a dynamical effect on at least one of the populations, and possibly on both of them.

Resource ρ has a dynamical effect on a population if its availability at any time has a significant effect on the net growth rate of that population.

When competition arises between two microbial populations, the question is whether one of the competitors will win, or if coexistence of the two populations is possible. It has been reported that (44) the effects of competition are more pronounced in a chemostat than in a batch culture, for the continual removal of cells with the overflow exerts a strong selective pressure on the system.

A number of investigators have examined this interaction, employing two organisms competing for a common growth-limiting substrate. Gause (55) was the first to study the dynamics of interactions between known microbial populations under laboratory conditions. He proposed the "competitive elimination principle", which generated considerable controversy.

Jost, et. al. (66) showed experimentally that in the competition of Escherichia coli and Azotobacter vinelandii for glucose, E. coli always won if only the two bacterial populations were present.

Alexander (3) studied symbiotic relationships between Rhodopseudomonas capsulatus and Azobacter vinelandii. The combination fixed three times more molecular nitrogen than did either pure culture.

Leal (79) studied both the pure and mixed culture behavior of Serratia marcescens and an unnamed yellow organism. In the mixture, S. marcescens predominated and was at the same concentration observed in pure culture. The concentration observed of the yellow organisms was about one-tenth of that obtained in pure culture.

Parker and Snyder (97) reported the mutual inhibition of Streptococcus salvarius and Veillonella alcalescens. Pure cultures grown in separate chemostats were fed to a mixed-culture vessel along with a feed of fresh medium. The dilution rate in this vessel was greater than the maximum specific growth rate of either species. There was clear evidence of interaction because each grew considerably more slowly in the mixture than in pure culture controls.

The review presented above gives only a very brief indication of a very large body of literature devoted to a very complex process (46).

D. MATHEMATICAL MODELS FOR BACTERIAL GROWTH

1. Introduction

Powell (105) suggested that the current specific growth rate of a population depends not only on the current state of the population's environment but also on the entire past history of environmental states seen by the population.

Volterra (138) suggested a similar approach by assuming that the specific growth rate of a population was a function of the population density.

Fredrickson (45) on the other hand argued that the specific growth rate is a function of the current state of the population only.

Dean and Hinshelwood (34) reviewed the various modes of kinetic behavior of bacterial growth that have been reported and some of the relationships that describe them.

Tsuchiya, et al. (134) summarized a number of the models of microbial growth that have been proposed and are useful for engineering purposes. They called "unstructured" those models which assume implicitly that either the state of a population is not changing in time or else that the specific growth rates are not appreciably affected by changes in the population's state. On the other hand, "structured" models are ones which do take into account changes in the population's internal state, and their effects on the specific growth rates of the species.

Eakman (38) modified the scheme proposed by Tsuchiya (134) for classifying the various growth moels. The first distinction is made between those models that account for interactions between the bacterial cells and the environment, and those models that do not do so. Models that neglect this interaction are unrealistic. A second division is made between **segregated** models which are based on individual cells and distributed models which consider the entire active biomass of the culture. Thirdly, the models can be **structured** or **unstructured**, as previously discussed. Finally, the models may be treated by either **stochastic**

methods(which consider a distribution of growth characteristics in the culture), or by deterministic methods(which consider the biomass to have constant properties).

2. Proposed Models

A number of mathematical models have been proposed for the kinetic behavior of microbial processes. Only a brief review is given here.

a. Curve Fitting Model

In representing the growth cycle mathematically, several approaches can be taken. Fitting the data to a polynomial expansion is one method used (36, 40).

$$C = a_0 + a_1 t + a_2 t^2 + ... + a_n t^n \qquad (1)$$

where C is the concentration of active biomass, t is time and the a_i's are polynomial coefficients. This equation has certain advantages due to the ease of curve fitting, but as pointed out by Edwards and Wilke (41), the fitted parameters have no physical significance, and the equation does not exhibit a steady-state. They proposed instead an equation of the form:

$$C = \frac{C_{max}}{1 + \exp[F(t)]} \qquad (2)$$

$$F(t) = a_0 + a_1 t + a_2 t^2 + ... + a_n t^n \qquad (3)$$

where C_{max} is the value of C at steady-state. They found that such an equation could fit batch fermentation data.

b. Unstructured Models for Growth

M'Kendrick and Pai (88) were the first workers to recognize that population growth changes the population's environment, and that this in turn changes the populations's growth rate. In batch reactors, this hypothesis leads to the dynamic logistic equation for bacterial growth:

$$\frac{dC}{dt} = kC(1 - C/C_c) \qquad (4)$$

where k is a constant.

Gaden (49) employed this expression in mathematically representing the growth rate in a

kinetic study of the conversion of glucose to 2-ketoglutonic acid by Pseudomonas. The integration of Equation 4 produces an S-shaped curve which is characteristic of batch cultures.

The basic equation for bacterial growth is given by Equation 5, if endogeneous respiration is neglected:

$$\frac{dC}{dt} = \mu C \qquad (5)$$

where μ is the specific growth rate and is a function of the system's environment, which is changing with time.

For the case where μ is constant, Equation (5) can be integrated to give the exponential growth law:

$$C = C_0 \exp(\mu t) \qquad (6)$$

where C_0 is the initial biomass concentration. A plot of $\ln(C/C_0)$ versus t is a straight line of slope μ. However, the specific growth rate, μ, is usually constant during only a short portion of the growth cycle, and is otherwise a function of substrate

concentration, toxic product concentration, and intracellular constitution.

It is usually assumed that, for a given organism and limiting substrate, the mass of bacterial cells produced per unit mass of nutrient utilized is a constant, under given environmental conditions. That is:

$$Y = \frac{\text{mass of organisms formed}}{\text{mass of limiting substrate utilized}}$$

$$= \text{constant} \qquad (7)$$

The factor Y is usually termed the yield coefficient.

This leads to the following relationship:

$$\frac{dC}{dt} = -Y \frac{dS}{dt} \qquad (8)$$

where S is the limiting substrate concentration.

For some biological processes, in which the micro-organisms are retained in the reactor for a long period of time, auto-oxidation or organism decay becomes important. Equation 5 can be modified to incorporate this as follows (27):

$$\frac{dC}{dt} = (\mu - K_d)C \qquad (9)$$

where K_d is the specific decay rate.

For the case where the exhaustion of a single essential substrate limits growth, Monod (91) applied the Michaelis-Menten model of enzyme kinetics to bacterial growth by using:

$$\mu = \frac{\mu_m S}{K_s + S} \qquad (10)$$

where S is the limiting substrate concentration, μ_m is the maximum specific growth rate, and K_s is the saturation constant (which is the concentration of limiting substrate when the specific growth rate is $\mu_m/2$).

Equations (5) and (10) can be combined as follows:

$$\frac{dC}{dt} = \frac{\mu_m S}{K_s + S} C \qquad (11)$$

Integrating Equation (8):

$$C - C_0 = Y (S_0 - S) \qquad (12)$$

where C_0 and S_0 are the initial biomass and substrate concentrations respectively.

If the biomass concentration is assumed to be constant, Equations (8) and (11) can be combined:

$$-\frac{dS}{dt} = \frac{k_1 S}{K_s + S} \qquad (13)$$

where $k_1 = \mu_m C_0/Y$. The assumption of constant biomass concentration can be a reasonable one if the change in substrate is small relative to C_0.

Equations (12) and (13) assume the yield coefficient to be constant. For pure cultures this is known to be true, except for cases where maintenance effects are important (45). In mixed cultures, Y is an average of the yield coefficients of each one of the individual populations, and it may vary with the microbial composition of the biomass.

Moser (93) modified the specific growth rate expression of the Monod equation as follows:

$$\mu = \frac{\mu_m}{1 + K_s S^{-X}} \qquad (14)$$

where X is a system constant which can be determined experimentally. The parameter X allows the expression to exhibit a greater variety of shapes. When X equals one, Equation (14) reduces to the Monod equation. This expression has been used for the description of waste treatment by heterogeneous populations (35, 63, 129).

Teissier (131, 132) expressed the specific growth rate as an exponential function:

$$\mu = \mu_m(1 - e^{-S/K_T}) \qquad (15)$$

where K_T is a constant.

Schulze (112, 113) has also applied this equation to data obtained from wastewater treatment plants, and concluded that it may be used to predict the performance of prototype activated sludge plants.

Garrett, et. al (50) , Eckenfelder (39), and Keshavan, et. al. (68) used a second order kinetic

model for microbial growth. In such a model, the rate of cell growth is considered to be directly proportional to the concentration of bacteria as well as substrate, i.e.,

$$\frac{dC}{dt} = KSC \qquad (16)$$

To unify the notation, Equation (16) can be written in terms of specific growth rate as (57):

$$\mu = KS \qquad (17)$$

This can be derived from the Monod expression when K_S is much greater than S.

Contois (30) assumed the specific growth rate to also be a function of cell concentration in proposing that:

$$\mu = \frac{\mu_m S}{BC + S} \qquad (18)$$

where B is a growth parameter that is constant under defined conditions, and C is population density.

Contois verified the applicability of the above equation by growing A. aerogenes in batch and continuous cultures. A non-linear decrease in the value of μ was observed with increasing values of the population density, C. The specific growth rate was dependent not only on the concentration of the limiting substrate but also on the population density of the growing bacteria.

Contois states that Monod's data were obtained from experiments in which specific growth rates were measured at the beginning of growth cycles of batch cultures with different concentrations of limiting substrate, and in which initial population densities were the same from culture to culture. Monod's results can be explained by Contois' function since, with population density essentially constant, Contois' expression reduces to Monod's.

Fujimoto (48) proposed a model which can be considered identical to Contois'. The model was verified with experimental data obtained with baker's yeast, alcohol yeast, and E. coli.

High substrate concentrations may inhibit growth and distort the metabolism of microorganisms. Enzyme kinetics provide a great variety of expressions to cope with the problem of excess substrate. These expressions are being applied to cultures of microorganisms, in the same way that the Michaelis-Menten expression was applied by Monod.

Andrews (6) in his study on methane producers found that the volatile acids which served as substrate were also inhibitory, which could not be modeled by the Monod method. He proposed that the Monod function be replaced by an expression accounting for inhibition of growth at high substrate concentration:

$$\mu = \frac{\mu_m S}{S + K_s + S^2/K_i} \qquad (19)$$

where K_i is an inhibition constant.

Jost, et al. (67) have proposed a "double saturation" model:

$$\mu = \frac{\mu_m S^2}{(K_1 + S)(K_2 + S)} \qquad (20)$$

Shehata and Marr (114) proposed the equation:

$$\mu = \frac{k_1 S}{K_1 + S} + \frac{k_2 S}{K_2 + S} \qquad (21)$$

where two parallel reactions are involved. This can be generalized by the addition of further terms of the same form.

c. Structured Models

Ramkrishna, et. al. (107) have proposed a series of models where the accumulation of toxic materials is taken into account by considering the biological reactions:

$$V + a_s S \longrightarrow 2V + a_t T \qquad (22)$$

$$V + T \longrightarrow N + (1 + a_{t1})T \qquad (23)$$

where V = active biomass

S = substrate

T = inhibitor

N = dead protoplasmic mass

a_s, a_t, a_{t1} = stoichiometric constants

To account for the lag phase, they have proposed a set of structured, distributed models. The active biomass is considered to be made up of two components which interact with their environment. In the proposed model, one component called G-mass refers to the nucleic acid, while the other component D-mass, refers to the rest of the active biomass.

Using dynamic programming techniques, Swanson (129) considered bacterial growth as an optimization process, with the amount of biomass produced maximized for a given amount of growth limiting substrate. He postulated that the growth rate is controlled by the amount of ribonucleic acid (RNA) produced by the organism. The resulting description fit all phases of the batch growth cycle.

Kono (74) has introduced the concepts of "critical concentration" and "coefficient of consumption activity" to develop a growth rate equation which corresponds to all phases of a batch culture, except the death phase. His model reduces to a set of four equations, each one of which represents one phase of the growth cycle, i.e., lag, acceleration, exponential and stationary phases. Using this model, Kono obtained excellent fits for batch fermentation data.

Shuler and Domach (117) used the term "population models" which can be constructed from ensembles of single-cell models, and contain both a high-level of structure and segregation. They used data for

Escherichia coli to ascertain the plausibility of their model with respect to control of DNA synthesis and ammonium ion assimilation. The models have the potential to make accurate predictions of transient responses, but are very complex and require a lot of data to regress.

3. Modeling of Competition between Two Species for a Single Rate-Limiting Substrate

Powell (104), and Pfennig and Jannasch (103) (among many investigators) have analyzed the situation when two populations grow in a chemostat and both have the same rate-limiting substrate. Growth is the only process occuring in either population, and in both it follows Monod's model. The model equations are:

$$\frac{dC_1}{dt} = -DC_1 + \mu_1 C_1 \tag{24}$$

$$\frac{dC_2}{dt} = -DC_2 + \mu_2 C_2 \tag{25}$$

$$\frac{dS}{dt} = D(S_f - S) - \frac{1}{Y_1}\mu_1 C_1 - \frac{1}{Y_2}\mu_2 C_2 \tag{26}$$

where

$$\mu_1 = \frac{\mu_{m1} S}{K_{s1} + S} \tag{27}$$

$$\mu_2 = \frac{\mu_{m2} S}{K_{s2} + S} \tag{28}$$

D is the dilution rate and subscripts 1 and 2 refer to the two populations, and S_f is the concentration of the rate-limiting substrate in the feed to the chemostat.

In a batch culture system, the corresponding equations would be:

$$\frac{dC_1}{dt} = \mu_1 C_1 \qquad (29)$$

$$\frac{dC_2}{dt} = \mu_2 C_2 \qquad (30)$$

$$\frac{dS}{dt} = -\frac{1}{Y_1}\mu_1 C_1 - \frac{1}{Y_2}\mu_2 C_2 \qquad (31)$$

Equations (29-31) were used in the present study to predict the behavior of a batch mixed culture, in which the pure culture growth parameters were previously determined.

CHAPTER III

MATERIALS AND METHODS

A. SLUDGE MORPHOLOGY AND ISOLATION PROCEDURES

Enriched cultures capable of completely biodegrading phenolic compounds have been obtained from the Passaic Valley Sewerage Commissioners (PVSC) wastewater treatment plant in Newark, New Jersey. Functional populations capable of metabolizing the target substrate were isolated and identified as shown in Figure 3.

The plant serves the heavily industrialized area of northern New Jersey. The influent flow is about 260 million gallons per day (mgd), of which 55% on a BOD basis is from industrial sources (18% by volume). The average influent phenol concentration is about 0.25 mg/lit (500 lb per day). The facility is designed to remove approximately 93% of the BOD and 94% of the TSS. The secondary treatment tanks utilize a pure oxygen activated sludge process. These tanks were the source of the mixed liquor used in the present study. At the time these samples were taken (February 1984 to August 1985) the PVSC plant had no primary clarifiers.

This plant was chosen as the source of the microorganisms because of its importance to northern New Jersey, and because of its history of long-term exposure to industrial chemicals.

Two liters of the primary effluent from the PVSC plant were aerated in a Lucite batch reactor with an aeration rate of 500 ml/min. Sufficient phenol stock solution was added to the reactor to bring the concentration to 100 mg/lit. After the concentration fell below the GC detection level (approximately 1 mg/lit), the reactor concentration was once again brought up to 100 mg/lit, and the procedure repeated two more times. After the third descent to 1 mg/lit, 10 ml of inoculum from the reactor were taken, diluted ten-fold times, and inoculated onto nutrient agar plates which were incubated for 24 hours at 37^{o}C. All microbial procedures were conducted under antiseptic conditions.

In this fashion, three batches of PVSC mixed liquor (obtained at different time of the year) were characterized after phenol acclimation (see Table 1). All colonies which seemed to be morphologically

different were isolated. These were streaked onto fresh nutrient agar plates and incubated at 37°C for another 24 hours. Successive isolations were made in order to insure that the final culture plate contained only a single organism. By this method 11 isolates from activated sludge were obtained. The isolated strains were maintained on agar plates and nutrient broth.

1. Species Identification

Figure 4 shows the specific procedure used in the present study, while Figure 5 indicates a generalized outline for bacterial identification. These two differ primarily in the use of diagnostic tubes as a labor saving device in the present study.

A sample of the purified colony was smeared onto a glass slide with a few drops of water, and allowed to air dry, followed by gram staining. The gram-stained slides were then observed under the microscope to determine positive or negative results, and the general shape of the bacteria.

Nearly all gram-positive bacteria are chemoheterotrophs, dependent on aerobic respiration or on fermentation for their supply of energy. Gram-positive bacterial colonies were grown on blood agar plates, subjected to catalase (indicating the presence of catalase enzyme) and coagulase tests (presence of coagulase enzyme), and inoculated onto dextrose tryptone agar (DTA) and phenolphthalein agar (for cocci only). These tests are used to characterize the enzyme system produced by the gram-positive organisms. The final test is the phosphatase test, performed only upon gram positive cocci. Gram-positive colonies were also inoculated into **Enterotubes** (Hoffmann-LaRoche, Inc., Nutley, NJ), and incubated for 24 hours at 37^{o}C. Each Enterotube contains 15 standard biochemical tests. The test performed are based upon the utilization of certain carbohydrate, nitrogen, and sulfur compounds. For each inoculum, test results were recorded and interpreted using Bergey's Manual (18).

It is difficult to give a succinct general account of gram-negative bacteria because these organisms are so diverse in both structural and functional respects. Furthermore, there can be several subdivisions based on

structural properties, such as the mode of energy-yielding metabolism. Most of the phenol-utilizing bacteria are gram-negative species (106).

Gram-negative bacteria were subjected to the oxidase test for the presence of cytochrome c. If the test was positive (presence of dark purple color), they were inoculated on **Oxiferm tubes** (Hoffmann-LaRoche, Inc., Nutley, NJ) and incubated for 18-24 hours at 37^{o}C, and the color changes coded. The same tube was then reincubated for another 24 hours, and once again color changes notes for confirmation. Each Oxiferm tube contains 9 biochemical tests. The results were recorded and the bacterial species was identified using code books (5, 29) supplied by Hoffmann-LaRoche. Additional confirmatory tests were usually required as outlined in the code books.

B. EXPERIMENTAL PROCEDURE

Stock cultures of each pure species were maintained by periodic subculture on Difco-Bacto nutrient. The primary culture was prepared by transferring a loop of stock culture to 10 ml of Difco nutrient broth and incubating at 37°C for 10-14 hours.

A secondary culture was prepared by transferring 2.5 ml of primary culture to 50 ml of defined medium solution containing phenol in various concentrations as the sole carbon source. These inoculated solutions were placed in 200 ml nephelometric shaker flasks on a rotary shaker (Model G-24, New Brunswick Scientific Company, New Brunswick, NJ) for 10-14 hours at 30°C (the rotation speeds were 200-300 rpm). There were no baffles. No air other than that transferred by shaking was provided. One more subculture (tertiary culture) was prepared in the same manner as the secondary culture (and from a secondary culture inoculum) to insure that the cells had fully adapted to growth on the phenol medium. Although some nutrient broth would be transferred from the primary to secondary culture, the sole source of carbon in the tertiary culture was

phenol. Figure 6 shows a diagram of the experimental procedure.

For mixed-culture experiments, a secondary culture was prepared by transferring 1.25 ml. of the primary culture of each pure species to 50 ml of defined medium solution (with phenol as a sole carbon source).

The decrease in phenol concentration in the tertiary cultures was measured by taking samples at various time intervals and storing them in the refrigerator at $5^{o}C$ for later GC analysis. Two further subcultures (quaternary and quinary) were inoculated in the same manner, and samples taken for phenol analysis.

Culture purity was verified by inoculating the samples of the subcultures onto nutrient agar plates. Colonies grown on the plates were then identified by the same procedures as mentioned earlier in this chapter. In addition, an independent confirmation was made by another experimentor in the same laboratory.

1. Formulation of Growth Media

Hill (62) suggested that Pseudomonas putida obtained from ATCC would be more efficiently cultured on tryptose phosphate broth rather than nutrient broth as advised by ATCC. But in the experiments performed here, nutrient broth was found to result in richer cultures.

In constructing a culture medium, several requirements had to be satisfied. First, the medium should have a well-defined carbon source. Second, it had to contain sufficient concentrations of essential minerals that would otherwise be rate limiting. Finally, it was essential that the medium could be easily prepared, and would be relatively inexpensive, because large quantities would be required.

Many formulations of medium solutions have been proposed (21, 27, 32, 52, 101, 102, 141) for which there is often little or no fundamental justification. The composition of the phenol defined medium solution used in the present study (Table 2) has been suggested by Gaudy (52), and appeared to be effective for phenol

biodegradation (52, 53, 108, 111). This was autoclaved prior to use.

The other type of culture medium used in the present study was a combination of phenol defined medium solution plus FSSAS (filtered supernatant solution of activated sludge). The purpose of this combined culture medium was to mimic the effect in a municipal wastewater treatment plant, involving both a domestic and a phenolic waste.

The FSSAS was prepared as followed: mixed liquor (activated sludge) from the PVSC plant was allowed to settle in a 2-liter batch reactor for half an hour. The supernatant solution was then filtered through 0.2 μm Metricel membrane filters (Gelman Sciences Inc., Ann Arbor, MI). This solution was then streaked onto plates to check for sterility. Since there was some growth, the filtered supernatant was autoclaved before being used. The sterile FSSAS solution was used to dilute the phenol defined medium solution (shown in Table 2) to the initial concentrations reported in Table 19.

2. Measurement of Optical Density

For all experiments, the course of growth of the microorganisms was determined by the optical density at 540 nm using a Spectronic 20 (Bausch & Lomb, Inc., Rochester, NY), and distilled water as the reference sample. Conversion of optical density to baterial cell count or biomass concentration was accomplished via calibration curves.

3. Determination of Number of Cells

The cell count was determined by transferring 10 ml of mixed liquor to a 25 ml sterilized glass bottle containing 5 grams of 5-millimeter diameter glass beads. The bottle was capped and shaken to disperse the organisms. 50 microliters were then withdrawn and transferred to a "Petroff-Hauser and Helber" counting chamber. Organisms were then counted in twenty grid squares. The total count of the 20 squares was averaged and divided by the volume of a square to give the number of organisms per cubic centimeter of solution.

In order to determine the calibration curve for cell count vs. optical density, S. liquefaciens was grown in standard nutrient broth and incubated for 12 hours. 0.5 ml of the culture were withdrawn using a sterilized pipet, and diluted with sterile distilled water. The optical densities of the diluted samples were determined, and the bacterial cells counted by the above procedure. The result for 5 dilutions is shown in Table 4 and Figure 7, along with the regressed calibration equation.

4. Determination of Biomass Concentration

Once again, S. liquefaciens was grown in a standard nutrient broth, harvested towards the end of the logarithmic growth phase,and serially diluted. The turbidty of all these dilutions was determined spectrophotometrically, and ranged from 0.03 to 1.0 optical density units.

For the dry weight determination of cell mass (8, 15, 22), each 150 ml of diluted culture was divided into three samples. Each sample was then poured into a separate 50 ml capacity round bottom polyethylene

centrifuge tube. The tubes were centrifuged in a Sorvall Refrigerated Centrifuge Model RC 2-B (E. I. Du Pont de Nemours, Wilmington, DE) at a speed of 10,000 RPM for 10 minutes. A pellet of condensed biomass would adhere to the bottom of the tube, while the supernatant was clear. The pellets were removed and resuspended in 10 ml of 0.85 % sodium chloride solution using a Vortex Jr. Mixer (Scientific Industries, Bohemia, NY). The suspensions were recentrifuged at 10,000 RPM for ten minutes, the biomass pellets removed, and the saline solution wash followed by centrifugation was repeated once more. The supernatant was carefully poured off, and the biomass pellets allowed to dry within the centrifuge tubes for 24 hours. The air-dried pellets were then removed from the tube and placed in pre-tared weighing dishes, and oven dried to constant weight at 100^0 C. (Note: Both the refrigerated centrifuge and vortex mixer were loaned, courtesy of Rutgers-Newark.)

The replicate dry pellet weights thus obtained proved to be proportional to the optical densities of the diluted culture up to a reading of 0.6 O.D. units (Figure 8), and thus are consistent with the Beer-Lambert Law.

One O.D. unit was found to be equivalent to 260 mg/lit of cell mass. Beyond an O.D. of 0.6, the calibration was no longer linear due to flocculation of the cells.

5. Phenol Analysis

Initial phenol concentrations (S_0) were determined gravimetrically. Thereafter, phenol concentrations in the culture fluid were measured by gas chromatography (Tracor Model 560, Tracor Inc., Austin, TX), equipped with a flame ionization detector, autoinjector, and packed column (6' x 1/8" SS, 10% SP2100 on Supelcoport). Oven temperature was 145 ^{o}C, and the phenol peak retention time was 2 min at a nitrogen flow rate of 40 cc/min. Samples of the culture fluid were injected three consecutive times after cells had been removed by centrifugation. Copper sulfate (100 mg/lit) was added to the samples as a biocide. The phenol concentration was determined on a Hewlett-Packard Model 3390A integrator by comparing the peak height with calibration curves made with standard solutions. Thymol was added to the samples as an internal standard in order to increase the accuracy of the analytical technique. The accuracy of the GC analysis was approximately $\pm$ 1.0 mg/lit. with an average standard deviation between samples of $\pm$ 3.0 mg/lit.

Because the samples were normally stored in the refrigerator prior to analysis, sample preservation experiments were performed initially. Various samples were taken and divided in two. One of the two portions was analyzed immediately for phenol. The other portion was refrigerated for 1 month and then analysed by the same method. The results showed no difference in phenol concentration, which indicates that the GC measurements accurately reflected the original reactor contents.

CHAPTER IV

RESULTS AND DISCUSSION

A. ISOLATION AND IDENTIFICATION OF PHENOL-ACCLIMATED BACTERIAL SPECIES FROM PVSC MIXED LIQUOR

The results obtained in the present work are shown in Table 3 under the column entitled "Investigator III". This represents a comprehensive list of the results from Table 1. Table 3 compares the results of the present work to those obtained previously in the same laboratory by two other investigators. Some differences in dominant species are noted, but in general the results are very similar. The discrepancies may be due to the variability between batches, or the variability between investigators utilizing fairly complex and somewhat subjective tests.

It should also be noted that the naming of bacteria on the basis of biochemical and morphological tests is not always straight-forward. For example, Group 2K pseudomonas-like bacteria are also referred to as Xanthomonas, and Acinetobacter anitratus is also

called Achromobacter anitratum. Therefore, apparent differences in bacterial lists may only be due to different choices in nomenclature.

For gram-positive bacteria, morphologically the colonies were flat, dull, cream colored with scalloped margins. By using Bergey's Manual (18) and the results of the individual biochemical tests, these rod-shaped bacteria were identified as Bacillus cereus (Experimentor III, Table 3).

Each of the isolated bacterial species under Experimentor III was tested for its ability to degrade phenol over a two-week period. Only three of the eleven species listed consumed phenol: Serratia liquefaciens, Klebsiella pneumoniae, and Pseudomonas putida. The presence of other dominant species in the phenol-acclimated liquor is apparently a result of their utilization of metabolic products from the primary phenol degraders.

Two of the the three phenol degrading species were also obtained from commercial sources[(Klebsiella pneumoniae from ATCC (#11778), and Pseudomonas putida

from Carolina Biological (#15-5265)]. However, neither of the commercially obtained species were able to degrade phenol in the laboratory. This indicates that there are strains of the same species which have very different responses to a given carbon source. This may be the result of attenuation of the enzymatic response of a microbe due to prolonged culturing on a relatively passive substrate (e.g. glucose).

Klebsiella pneumoniae, Serratia liquefaciens, and Pseudomonas putida are all gram-negative rod bacteria. The colors and the shapes of the colonies of these three species are similar, which makes it impossible to use the colony-count technique for relative numbers in mixed culture. Nevertheless, for each seeding, the number of cells inoculated in the shaker flasks was counted. Moreover, toward the end of each experiment, the organisms were checked for their purity by exactly the same techniques as used in the process of identification of the organisms when first isolated.

B. EXPERIMENTS USING THE THREE PURE PHENOL-DEGRADING BACTERIAL SPECIES

In this series of experiments the phenol concentration was varied from 20 to 180 mg/lit to determine its effect on the growth rates of the three pure phenol-degrading organisms. Results are shown in Tables 6 to 11 and Figures 9 to 14

The initial slope of the growth curves on the semi-log plots (Figures 9 to 11) represents the specific growth rate (μ) at the initial phenol concentration. These values are recorded in Tables 12 to 14, and plotted in Figures 15 to 17. The graphs of μ versus S_o show a hyperbolic shape that is well-described by the Monod model. A non-linear regression program (Appendix 1) was used to find the Monod parameters μ_m (maximum specific growth rate) and K_s (the half-saturation constant). These results are summarized in Table 15.

The curves drawn on Figures 15 to 17 reflect the "best-fit" Monod parameters

For each pure culture, the set of phenol degradation experiments was repeated in triplicate.

Figures 9 to 14 also show that growth levelled off at about the same time that the phenol was depleted (after about 6 to 12 hours), which is consistent with the fact that phenol is the rate-limiting substrate.

Monod's model generally fits the experimental growth data very well (Figures 15 to 17). (However, it should be noted that in a mathematical sense, although a model may be chosen on the basis of its curve-fitting ability, it must be recognized that such an approach does not provide proof of the theoretical validity of such a model.)

Inhibition was not detected in the range of concentrations studied (20-180 mg/lit), although Gaudy and co-workers (115-118) found inhibition with an unspecified heterogeneous population in continuous flow reactors at phenol concentrations greater than about 100 to 150 mg/lit.

The data shown for the specific growth rates, and phenol degradation rates, were obtained on the

quaternary cultures, which had already been acclimated to phenol in two previous transfers. (In addition to the data reported here, experiments on the quinary subcultures were also performed in order to confirm the results.) In most experiments, there was no evidence of a lag phase after phenol addition.

After, the quinary subculture, the reactor contents were cultured on agar plates to confirm that they were still pure cultures.

The kinetic data reported in this work were all taken before wall growth became visible. Previous investigators [e.g. Pawlowsky (104)] have shown that wall growth can affect the kinetics of substrate removal.

Calculations based on the Monod parameters (μ_m and K_s) indicate that the specific growth rates of the pure cultures on phenol decreased in the order *K. pneumoniae*, *S. liquefaciens*, *P. putida* for phenol concentrations above 2.5 mg/lit. Below 2.5 mg/lit, the order decreased from *K. pneumoniae*, to *P. putida*, to *S. liquefaciens*.

For phenol degradation by P. putida, Hill (63) reported the following Monod parameters at initial phenol concentrations up to 250 mg/lit: μ_m = 0.159 hr^{-1} and K_s = 11.8 mg/lit. This agrees very closely with the average parameters reported in this study for P. putida (μ_m = 0.158 hr^{-1}, and K_s = 15.3 mg/lit).

The yield coefficients for the three organisms are shown in Figures 18 to 20, and are summarized in Tables 16 to 18. Some of the plots are clearly non-linear (see e.g. Figures 18-1, 18-2, 18-3, 18-5, 20-1, 20-2). The reason for this is not known.

These yield coefficients were used, along with the values of μ_m and K_s, to draw the predicted curves for phenol degradation shown in Figures 12 to 14. These fits are not quite as good as those for biomass growth.

For the experiments in which the FSSAS (Filtered Supernatant Solution of Activated Sludge) was used, both lag phase and diauxic phenomena were observed at phenol concentrations greater than 40 mg/lit (Figure 21 and Table 19).

The inflection point of the diauxic growth curve can be considered as the point at which the first substrate is completely depleted and the second substrate begins to be utilized. This phenomenon was first reported by Monod (95). The first phase is probably a result of the metabolism of carbon sources in the FSSAS solution, whereas the next phase reflects the metabolism of phenol. By comparing the growth curves (e.g. Figure 21-3) with the phenol degradation curve (Figure 22-3) it appears that degradation of phenol accelerates in the region where the first growth phase ends and the second begins. However, uncertainties in the data (e.g. the definition of S_o) make it impossible to draw a direct comparison between the specific growth rate during the second phase with that obtained on phenol as sole carbon source.

C. RESULTS WITH MIXED CULTURES

Mixtures of two of the pure species were cultured in triplicate in the same way as they were in the pure culture experiments. At the start of the experiments, the number of cells of each species varied from 5×10^9 cells to 8×10^9 cells/cm^3. However, cell counts of individual bacteria during the mixed culture experiments could not be made (all three are gram-negative); and furthermore, colony counts could not be mode (all three produce round, cream-colored colonies with scalloped edges). Since there could be a large number of colonies on an individual growth plate, biochemical differentiation was considered to be too time consuming and was not attempted. However, random colonies were checked at the end of each experiment to confirm the presence of both species.

The results of these experiments are given in Tables 20 and 25 and Figures 23 to 28.

There were three cases which were studied, they were the mixed cultures of: (1) S. liquefaciens and K. pneumoniae, (2) K. pneumoniae and P. putida, and (3) P.

putida and S. liquefaciens. The initial concentrations of phenol were approximately the same as in the pure-culture experiments. For the pure culture experiments, 5 ml of primary culture were used to inoculate the secondary culture; whereas for the mixed-culture experiments, 2.5 ml of each of the pure primary cultures were used. Once again, the data shown for growth and phenol degradation were obtained using the quaternary culture.

A mathematical model for simple competition in batch reactors was written to describe the kinetic behavior of the mixed cultures (Equations 27 to 29). This model assumes that each organism independently uses the same food source, and the kinetic parameters were all obtained from the pure cultures.

Figures 23 to 28 compare the results of the kinetic model simulation (solid lines) with the experimental data. The kinetic model uses average values at $_m$ and K_s from the pure culture data.

The simple competitive model appears to agree very well with the experimental growth data, although (as

with the pure culture results) not quite as well with the phenol degradation data. This is not entirely unexpected, since the Monod parameters were obtained directly from growth data, while the predicted curves for phenol degradation require additional theoretical assumptions (for example, that the change in biomass concentration is linearly dependent on substrate removal). In addition, there may be other interactions between the species that the simple competitive model does not take into account (such as secondary metabolite utilization, or inhibition). These additional interactions should continue to be explored in an effort to improve and expand the kinetic model.

CONCLUSIONS

1. Eleven dominant bacterial species were isolated from a phenol-acclimated mixed liquor obtained originally from the Passaic Valley Sewerage Commissioners waste water treatment plant in Newark, NJ. However, of these eleven species, only three (*S. liquefaciens*, *K. pneumoniae*, and *P. putida*) were able to degrade phenol. Therefore, the remaining eight species must have survived by utilizing the metabolic products of the three primary phenol degraders.

2. Regarding the three primary phenol degraders,when two of these (*K. pneumoniae* and *P. putida*) were purchased from commercial suppliers, they could not degrade phenol, which underlines the importance of the strain as well as the species.

3. Monod's expression fits the pure culture growth data very well, although the phenol degradation rates could only be fit approximately from the growth parameters.

4. Using the kinetic rate parameters from the single species experiments, a simple competitive model was tested for phenol utilization by any two of the three primary phenol degraders. This model was able to predict the rate of total biomass growth very well. However as with the pure culture experiments, the fit of the phenol degradation data was less accurate.

CHAPTER VI

RECOMMENDATIONS

1. Colony-count techniques are needed in this type of research. The three pure species which were isolated and identified are all gram-negative bacteria and have the same rod-shape. Their colonies also look very much the same on the TSA growth medium used in the laboratory. More specific biochemical tests should be conducted in order to distinquish different bacterial colonies, e.g. by using growth media that will color the colonies differently, or which utilize differentiating antibiotics.

2. Continuous culture studies should be performed, using different dilution rates, to better determine the kinetics and competitive effects.

3. In order to obtain more reliable data for μ and S_o in the low substrate concentration range (0 to 20 mg/lit of phenol), a more sophisticated experimental technique must be used. One method is the so-called "phenolstat" technique of Yang and Humphrey (153) where data for μ and S_o are collected in a batch reactor that

maintains a constant phenol concentration. Although this method will allow the experimentor to obtain growth data on phenol at low concentrations, the experimental set-up is rather complex, and one cannot expect to produce the quantity of data that can be produced with the batch growth study technique used in the present study.

NOMENCLATURE

a_i	:	Polynomial coefficients of the equation (1)
a_s	:	Coefficient of the equation (22)
a_t	:	Coefficient of the equations (22), (23)
C	:	Concentration of biomass, mg/lit
C_c	:	Concentration of the biomass (carrying capacity), mg/lit appearing in equation (4)
C_o	:	Concentration of initial biomass, mg/lit
C_{max}	:	Maximum concentration of biomass, mg/lit appearing in equation (2)
D	:	Dilution rate, hr^{-1} appearing in equations (24-26)
k	:	Constant of the equation (4)
k_1	:	Constant of the equations (13), (21)
k_2	:	Constant of the equation (21)
k_d	:	Specific decay rate, hr^{-1}
K	:	Constant of the equations (16), (17)
K_1	:	Constant of the equations (20), (21)
K_2	:	Constant of the equations (20), (21)
K_i	:	Inhibition constant, mg/lit
K_s	:	Kinetic parameter, mg/lit (called the "Saturation Constant" in Monod expression)
K_T	:	Teissier constant, mg/lit
N	:	Dead protoplasmic mass

P_1	:	Population 1
P_2	:	Population 2
S	:	Concentration of substrate, mg/lit
t	:	time, hr
T	:	Inhibitor, equations (22), (23)
V	:	Active Biomass, equations (22), (23)
X	:	Moser's constant
Y	:	Yield coefficient

Greek Letters

μ	:	Specific growth rate, hr^{-1}
μ_m	:	Kinetic parameter (called the "Maximum Specific Growth Rate", in the Monod expression)
ρ	:	Resource competed for by P_1 and P_2

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TABLE 1

DOMINANT BACTERIAL SPECIES IN
PHENOL-ACCLIMATED PVSC MIXED LIQUOR (I)

Feb 1984*	Dec 1984
Bacillus cereus	Aeromonas hydrophilia
Enterobacter cloacae	Escherichia coli
Escherichia coli	Klebsiella pneumoniae
Pseudomonas putida	Pseudomonas putida
Pseudomonas sp.	Serratia liquefaciens

June 1985	Aug 1985
Acinetobacter lwoffii	Bacillus cereus
Bacillus cereus	Enterobacter cloacae
Pseudomonas cepacia	Klebsiella pneumoniae
Pseudomonas fluorescens	Pseudomonas sp.
Serratia liquefaciens	

*Date of Mixed Liquor Sample from PVSC

TABLE 2

PHENOL DEFINED MEDIUM SOLUTION

(Source: Gaudy, 1973)

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

TABLE 3

DOMINANT BACTERIAL SPECIES IN PHENOL-ACCLIMATED PVSC MIXED LIQUOR (II)

INVESTIGATOR I (17)

Achromobacter species biotype 2
Acinetobacter lwoffii
Bacillus
Enterobacter agglomerans
Enterobacter gergoviae
Escherichia coli
Micrococcus
Moraxella species
Group M-4 Moraxella-like
Pseudomonas cepacia
Pseudomonas fluorescens
Pseudomonas maltophilia
Pseudomonas species
Group 2K-1 Pseudomonas-like
Serratia marcescens
Staphylococcus

INVESTIGATOR II (1)

Acinetobacter anitratus
Acinetobacter lwoffii
Alcaligenes faecalis
Bacillus
Enterobacter agglomerans
Micrococcus
Providencia stuarti
Pseudomonas aeruginosa
Pseudomonas capacia
Pseudomonas fluorescens
2K-1 Pseudomonas-like
5E-1 Pseudomonas-like

Table 3 (continued)

INVESTIGATOR III (present work)

Acinetobacter lwoffii
Aeromonas hydrophilia
Bacillus cereus
Enterobacter cloacae
Escherichia coli
Klebsiella pneumoniae
Pseudomonas cepacia
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas sp.
Serratia liquefaciens

TABLE 4

CALIBRATION TABLE FOR THE DETERMINATION OF
BACTERIAL CELL COUNT AS A FUNCTION OF OPTICAL DENSITY

Optical Density, UOD	Cell Count, 10^{10} cells/ml.
0.192	0.120
0.264	0.348
0.325	0.356
0.531	1.040

TABLE 5

CALIBRATION TABLE FOR THE DETERMINATION
BIOMASS CONCENTRATION AS A FUNCTION OF OPTICAL DENSITY

Optical Density, UOD	Biomass Concentration, mg/lit
0.03	5.0
0.08	12.0
0.10	28.0
0.16	39.0
0.21	45.0
0.33	92.0
0.45	112.0
0.56	150.0
0.75	275.0
0.90	475.0
1.00	650.0

TABLE 6-1

BIOMASS CONCENTRATION vs. TIME
(*S. liquefaciens*, Run: VLB 045)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	14.8	15.0	16.0	13.7
1.2	17.1	18.9	20.7	18.9
2.4	19.2	22.9	25.9	25.4
3.6	20.8	26.6	30.8	32.9
4.8	21.9	29.4	34.4	40.2
6.0	22.7	31.3	36.6	45.5
7.2	23.1	32.0	37.5	48.0
8.4	23.4	32.4	37.9	48.8
9.6		32.6	38.1	49.1
10.8		32.7	38.1	49.1

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	10.9	9.6	8.5	7.2
1.2	15.0	12.4	11.4	13.1
2.4	21.0	20.2	18.1	15.4
3.6	28.5	27.1	26.8	25.1
4.8	37.4	38.4	34.1	33.2
6.0	46.0	47.6	48.8	51.2
7.2	51.7	57.6	61.5	66.1
8.4	54.0	61.5	68.3	81.3
9.6	54.7	62.5	70.2	86.2
10.8	54.9	62.7	70.5	86.2

TABLE 6-2

BIOMASS CONCENTRATION vs. TIME
(*S. liquefaciens*,Run: VLB 045-A)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	13.6	13.0	14.0	13.2
1.0	15.2	16.1	18.1	17.6
2.0	17.0	20.2	23.3	23.1
3.0	19.8	25.4	29.7	30.9
4.0	22.0	31.1	38.1	40.3
5.0	24.6	39.0	48.9	53.5
6.0	24.7	39.4	48.8	53.5
7.0	24.6	39.5	48.8	53.7
8.0		39.5	48.5	53.7
9.0		39.6	48.5	53.8

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	12.1	13.0	10.8	10.4
1.0	16.3	18.4	15.4	14.5
2.0	22.0	25.8	21.3	20.7
3.0	29.6	36.7	29.8	28.8
4.0	40.2	51.6	41.9	40.5
5.0	54.2	73.1	58.8	56.9
6.0	54.8	73.5	82.0	80.1
7.0	54.7	73.5	82.1	81.3
8.0	54.7	73.5	82.1	89.4
9.0	54.9	73.7	82.1	91.3

TABLE 6-3

BIOMASS CONCENTRATION vs. TIME
(*S. liquefaciens*,Run: VLB 045-B)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	14.0	13.8	12.0	13.1
1.0	15.9	16.2	14.9	16.6
2.0	18.2	19.1	18.8	21.2
3.0	20.5	22.4	23.4	26.8
4.0	23.5	26.8	29.3	34.3
5.0	24.5	30.8	36.4	43.7
6.0	24.5	36.3	45.6	55.8
7.0	24.6	36.7	45.7	55.6
8.0	24.6	36.7	45.7	55.7
9.0	24.7	36.7	45.7	55.7
10.0	24.7	36.5	45.8	55.8

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	14.2	11.0	9.8	9.5
1.0	18.3	14.7	13.3	12.9
2.0	23.9	19.4	18.0	17.6
3.0	30.9	25.6	24.5	24.2
4.0	40.0	34.1	32.8	32.9
5.0	51.9	45.6	44.8	44.7
6.0	51.7	59.8	60.9	60.8
7.0	51.7	79.7	60.9	82.7
8.0	51.8	89.6	72.5	93.8
9.0	51.8	90.7	79.7	101.7
10.0	51.7	90.9	80.1	102.8

TABLE 7-1

BIOMASS CONCENTRATION vs. TIME
(*K. pneumoniae*, Run: VLB 049)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	12.9	10.4	10.9	9.8
1.2	15.5	14.8	14.9	14.3
2.4	18.2	18.1	20.1	22.6
3.6	20.8	22.8	26.3	28.2
4.8	23.0	27.8	33.1	40.3
6.0	25.5	32.0	39.4	52.4
7.2	27.1	36.8	44.3	62.8
8.4	27.2	38.7	47.2	67.3
9.6	27.6	40.2	48.7	71.0
10.8	27.7	40.2	48.7	71.7
12.0	27.7	40.3	48.8	71.8

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	6.7	8.3	7.5	8.3
1.2	10.3	12.7	11.7	13.2
2.4	15.0	19.4	18.2	21.0
3.6	22.8	29.3	28.0	33.2
4.8	32.0	43.4	42.4	51.8
6.0	45.8	61.6	62.6	78.8
7.2	61.2	81.8	86.9	112.3
8.4	74.0	96.2	107.4	140.0
9.6	81.6	100.4	114.8	147.4
10.8	83.7	101.3	116.0	148.0
12.0	84.1	101.3	116.1	148.0

TABLE 7-2

BIOMASS CONCENTRATION vs. TIME
(*K. pneumoniae*, Run: VLB 049-A)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	11.8	10.0	10.2	10.6
1.0	14.4	12.7	13.8	15.4
2.0	17.6	16.1	18.7	22.3
3.0	21.5	20.4	25.2	32.3
4.0	26.3	25.8	34.1	46.8
6.0	26.4	41.6	46.8	76.3
7.0	26.5	41.7	46.9	76.5
8.0	26.5	41.8	46.9	76.7

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	8.4	6.6	8.2	7.0
1.0	11.6	9.7	12.7	10.7
2.0	16.4	14.4	19.3	16.1
3.0	23.4	22.4	29.4	24.5
4.0	32.4	41.2	45.7	37.1
6.0	65.3	73.8	106.4	85.2
7.0	88.5	109.7	167.1	132.5
8.0	125.5	165.8	254.8	200.1
9.0	130.1	172.1	260.7	231.5
10.0	131.2	175.0	270.1	241.7
12.0	131.2	175.0	271.2	242.6

TABLE 7-3

BIOMASS CONCENTRATION vs. TIME
(*K. pneumoniae*, Run: VLB 049-B)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	11.8	10.8	11.2	8.4
1.0	13.2	13.2	14.2	11.5
2.0	15.6	16.5	19.9	21.5
3.0	18.1	20.5	26.4	29.5
4.0	20.6	25.4	35.1	40.6
6.0	27.4	39.9	48.1	75.3
7.0	27.5	41.3	48.2	75.6
8.0	27.5	41.4	48.3	75.7
9.0	27.6	41.4	48.3	75.7

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	8.6	7.7	9.1	8.5
1.0	12.1	10.7	12.5	11.7
2.0	17.1	14.7	19.4	15.9
3.0	24.1	20.2	25.0	22.1
4.0	33.5	28.4	35.1	30.9
6.0	67.0	58.2	69.2	57.4
7.0	94.4	78.4	78.5	78.4
8.0	129.7	105.4	95.7	108.4
9.0	180.1	151.4	135.1	154.1
10.0	190.6	175.4	165.4	206.2
12.0	190.7	175.6	166.2	206.9

TABLE 8-1

BIOMASS CONCENTRATION vs. TIME
(*P. putida*,Run: VLB 051)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	17.0	18.4	18.6	18.9
1.2	18.9	21.2	21.4	21.3
2.4	20.9	24.2	25.1	26.4
3.6	22.9	27.6	28.9	31.0
4.8	24.7	31.2	33.1	36.4
6.0	26.4	34.8	37.4	42.4
7.2	27.6	38.3	41.8	48.4
8.4	28.9	41.2	45.9	56.7
9.6	29.7	43.5	49.3	63.0
10.8	30.1	44.8	50.4	69.0
12.0				72.2

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	18.4	24.4	18.0	20.0
1.2	21.1	28.5	22.6	23.3
2.4	26.0	35.2	26.2	26.1
3.6	29.2	41.8	30.1	34.5
4.8	36.5	49.2	34.1	41.9
6.0	43.0	58.2	45.1	50.9
7.2	50.4	68.4	51.8	60.2
8.4	58.7	79.7	62.6	71.9
9.6	67.1	91.2	72.8	85.6
10.8	76.4	102.1	86.4	103.1
12.0	82.1	101.8	98.2	118.8

TABLE 8-2

BIOMASS CONCENTRATION vs. TIME
(*P. putida*,Run: VLB 051-A)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	15.5	14.2	17.5	18.8
1.0	17.0	15.5	20.1	21.5
2.0	19.5	19.1	24.4	26.4
3.0	22.5	22.4	28.1	31.1
4.0	25.1	26.8	33.1	36.4
6.0	32.1	35.8	44.4	52.2
7.0	36.6	40.2	52.4	52.6
8.0	36.6	40.2	52.6	63.4
9.0	36.6	40.6	52.6	73.9
10.0	36.6	40.6	52.9	73.9

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	15.8	14.2	14.0	15.3
1.0	18.4	17.4	15.6	17.2
2.0	22.4	20.6	19.5	21.3
3.0	26.2	25.5	24.0	26.4
4.0	31.4	29.3	29.1	31.3
6.0	43.6	40.6	41.1	44.4
7.0	55.2	51.2	48.1	52.3
8.0	64.6	61.1	56.2	62.9
9.0	77.2	72.1	67.1	74.7
10.0	92.1	86.1	80.1	89.2
12.0	92.9	91.1	91.2	115.2

TABLE 8-3

BIOMASS CONCENTRATION vs. TIME
(*P. putida*,Run: VLB 051-B)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	16.1	17.4	16.9	14.2
1.0	17.1	19.2	18.4	16.2
2.0	18.9	21.9	21.1	18.1
3.0	19.1	24.1	24.3	21.3
4.0	20.9	26.6	26.1	25.1
5.0	24.1	33.1	34.2	31.6
6.0	24.5	37.1	38.2	36.5
7.0	26.5	41.3	44.1	41.2
8.0	27.9	46.2	50.7	46.8
9.0	28.1	49.1	51.2	50.1
10.0	29.1	50.2	51.9	53.5

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	12.6	10.7	11.2	12.3
1.0	14.4	12.2	12.1	14.6
2.0	15.9	13.9	15.2	16.2
3.0	18.1	16.1	15.9	19.1
4.0	21.0	17.5	19.1	21.1
5.0	24.9	21.1	22.6	24.6
6.0	25.5	20.6	25.2	29.1
7.0	31.5	29.1	29.4	32.5
8.0	39.1	31.9	33.9	38.3
9.0	42.9	37.7	39.1	42.9
10.0	49.1	44.2	44.5	46.9
12.0	65.1	54.1	56.2	64.4

TABLE 9-1

PHENOL CONCENTRATION vs. TIME
(*S. liquefaciens*,Run: VLB 045)

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	20	40	50	80
0	20.0	40.0	50.0	80.0
1.2	17.8	34.3	44.4	74.6
2.4	14.5	26.9	37.8	60.2
3.6	6.4	17.8	28.5	40.0
4.8	0	6.0	14.3	17.6
6.0		0	2.5	0
7.2			0	

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	100	120	140	180
0	100.0	120.0	140.0	180.0
1.2	92.5	110.1	135.1	171.2
2.4	85.0	108.0	121.4	164.2
3.6	72.5	92.1	106.8	157.5
4.8	50.6	72.3	83.1	130.7
6.0	18.1	50.4	63.0	103.7
7.2	0	29.1	43.8	68.6
8.4		10.8	22.7	46.4
9.6		0	9.8	14.7
10.8			0	0

TABLE 9-2

PHENOL CONCENTRATION vs. TIME
(*S. liquefaciens*, Run: VLB 045-A)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	18.3	36.4	46.5	74.0
2.0	15.2	28.2	41.1	61.8
3.0	9.8	21.1	31.4	45.1
4.0	2.2	10.8	21.2	15.2
5.0	0	3.6	9.7	6.2
6.0		0	0.2	0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	98.2	107.8	131.3	165.5
2.0	86.1	100.0	118.4	152.3
3.0	73.0	89.5	96.6	142.3
4.0	56.2	69.8	81.1	124.1
5.0	19.2	47.1	59.5	101.5
6.0	5.0	25.3	40.2	55.2
7.0	0	9.4	22.1	41.7
8.0		0	5.2	9.4
9.0			0	0

TABLE 9-3

PHENOL CONCENTRATION vs. TIME
(*S. liquefaciens*, Run: VLB 045-B)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	18.1	36.1	46.1	74.8
2.0	14.2	27.3	40.2	61.2
3.0	4.9	20.4	32.1	49.6
4.0	1.2	9.9	14.2	21.3
5.0	0	0	1.3	9.4
6.0			0	0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	90.1	107.2	132.1	168.3
2.0	82.3	100.5	120.2	155.1
3.0	68.3	92.3	93.2	143.2
4.0	45.1	66.5	76.1	127.3
5.0	9.4	46.2	54.3	94.1
6.0	1.2	21.1	40.4	66.4
7.0	0	8.8	16.4	41.2
8.0		5.2	5.1	15.0
9.0		0	0	2.5

TABLE 10-1

PHENOL CONCENTRATION vs. TIME
(K. pneumoniae, Run: VLB 049)

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	20	40	50	80
0	20.0	40.0	50.0	80.0
1.2	18.2	37.7	47.3	75.6
2.4	16.3	30.0	45.3	69.4
3.6	11.8	22.2	34.4	56.0
4.8	5.7	16.0	24.0	28.4
6.0	3.1	9.0	10.0	11.4
7.2	0	1.0	0	0

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	100	120	140	180
0	100.0	120.0	140.0	180.0
1.2	97.5	115.8	136.8	174.4
2.4	92.4	108.0	128.4	167.8
3.6	85.0	99.4	117.9	157.5
4.8	77.0	84.4	104.1	132.9
6.0	59.5	60.8	77.4	103.5
7.2	34.3	36.9	51.4	60.8
8.4	25.5	11.3	31.5	26.6
9.6	0	0	12.2	11.3
10.8			0	0

TABLE 10-2

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae*, Run: VLB 049-A)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	18.6	38.1	47.9	72.0
2.0	12.4	31.2	45.1	68.2
3.0	3.5	15.4	25.2	54.2
4.0	0.1	4.4	13.2	29.1
6.0		0.3	0.2	8.1
7.0			0	1.2
8.0				0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	95.0	117.0	134.2	176.5
2.0	83.4	102.3	119.2	156.9
3.0	73.2	93.1	102.3	148.2
4.0	73.2	84.2	95.1	132.1
6.0	58.2	65.4	72.1	101.2
7.0	35.6	39.2	59.4	68.5
8.0	29.1	10.8	31.9	38.2
9.0	1.4	0.3	16.4	11.2
10.0	0		0	0

TABLE 10-3

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae*, Run: VLB 049-B)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	19.9	39.1	46.3	77.3
2.0	16.4	32.1	45.2	65.4
3.0	12.4	26.1	32.1	54.2
4.0	7.2	19.2	26.1	23.1
6.0	5.2	2.4	14.2	12.1
7.0	0	0	1.2	2.4
8.0			0	0.1

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	95.3	116.4	133.2	175.3
2.0	92.1	102.2	123.5	165.2
3.0	82.1	94.5	114.5	132.5
4.0	79.6	85.3	108.6	129.7
6.0	58.3	60.4	75.6	103.2
7.0	37.5	39.8	54.5	64.3
8.0	29.4	15.4	35.4	29.4
9.0	3.2	4.2	9.2	10.4
10.0	0.7	0	0.3	0

TABLE 11-1

PHENOL CONCENTRATION vs. TIME
(*P.putida*, Run: VLB 051)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.2	18.6	37.9	48.2	77.0
2.4	16.7	34.2	44.6	65.6
3.6	14.9	28.6	41.5	60.0
4.8	10.9	25.4	31.5	51.6
6.0	4.8	15.0	22.0	39.6
7.2	0	6.7	15.1	35.2
8.4		0	8.4	24.0
9.6			0	10.8
10.8				0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.2	97.8	117.9	138.2	176.4
2.4	88.0	111.0	133.0	171.0
3.6	82.8	102.1	126.3	164.2
4.8	78.8	90.0	105.4	156.1
6.0	69.0	75.8	104.3	135.4
7.2	51.0	81.8	96.3	117.0
8.4	43.8	39.0	81.6	103.5
9.6	22.8	20.2	58.1	82.8
10.8	6.5	1.0	28.8	44.1
12.0	0	0	0	9.9

TABLE 11-2

PHENOL CONCENTRATION vs. TIME
(*P. putida*, Run: VLB 051-A)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	19.1	38.1	49.1	77.9
2.0	13.2	35.2	44.5	64.2
3.0	10.1	29.3	40.2	59.2
4.0	9.2	21.4	31.2	41.2
6.0	2.3	13.2	13.2	32.2
7.0	0	5.4	4.2	23.1
8.0		1.2	0.2	13.2
9.0		0.1	0	2.6
10.0				0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	98.2	114.2	138.2	173.2
2.0	91.2	112.3	134.2	169.4
3.0	83.2	105.1	128.2	160.1
4.0	79.3	92.2	107.3	158.2
6.0	69.3	75.4	102.2	132.1
7.0	52.3	53.8	95.6	115.6
8.0	45.4	41.2	84.2	103.2
9.0	26.7	29.5	59.3	83.4
10.0	6.5	3.4	23.4	43.1
12.0	0	0	2.3	4.4

TABLE 11-3

PHENOL CONCENTRATION vs. TIME
(*P. putida*,Run: VLB 051-B)

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	19.0	38.1	46.7	77.5
2.0	16.5	36.4	43.4	64.3
3.0	13.4	23.4	40.3	51.2
4.0	10.2	21.4	31.4	41.3
5.0	7.5	16.4	22.8	32.9
6.0	2.3	4.3	13.4	24.3
7.0	0	1.1	7.6	13.2
8.0		0	0.6	8.3
9.0				2.3

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	98.1	118.1	139.1	177.4
2.0	89.3	112.3	135.3	173.9
3.0	84.3	105.9	125.4	169.0
4.0	78.3	93.2	110.2	159.3
5.0	71.2	84.3	103.2	134.5
6.0	57.5	68.2	93.2	123.4
7.0	52.1	39.2	84.2	110.6
8.0	40.2	29.3	59.3	95.3
9.0	21.3	18.3	28.3	49.6
10.0	4.3	2.3	9.9	12.1
12.0	0	0.2	1.2	3.2

TABLE 12

SPECIFIC GROWTH RATE vs.
INITIAL PHENOL CONCENTRATIONS FOR
Serratia liquefaciens

S_o (mg/lit)	μ (hr^{-1})		
	VLB 045	VLB 045-A	VLB 045-B
20	0.128	0.121	0.129
40	0.186	0.220	0.160
50	0.240	0.250	0.221
80	0.323	0.280	0.240
100	0.366	0.300	0.259
120	0.319	0.345	0.282
140	0.279	0.338	0.303
180	0.322	0.340	0.309

TABLE 13

SPECIFIC GROWTH RATE vs.
INITIAL PHENOL CONCENTRATIONS FOR
Klebsiella pneumoniae

S_o (mg/lit)	μ (hr^{-1})		
	VLB 049	VLB 049-A	VLB 049-B
20	0.143	0.200	0.140
40	0.206	0.237	0.217
50	0.266	0.302	0.285
80	0.366	0.371	0.322
100	0.331	0.338	0.343
120	0.393	0.403	0.331
140	0.369	0.430	0.338
180	0.357	0.418	0.319

TABLE 14

SPECIFIC GROWTH RATE vs.
INITIAL PHENOL CONCENTRATIONS FOR
Pseudomonas putida

S_o (mg/lit)	μ (hr^{-1})		
	VLB 051	VLB 051-A	VLB 051-B
20	0.089	0.122	0.060
40	0.100	0.151	0.112
50	0.117	0.157	0.121
80	0.104	0.171	0.132
100	0.086	0.176	0.135
120	0.105	0.180	0.140
140	0.104	0.174	0.136
180	0.104	0.176	0.138

TABLE 15-1

MONOD PARAMETERS
FOR THREE PURE PHENOL-DEGRADING SPECIES

RUN	μ_{max} (hr^{-1})	K_s (mg/lit)	σ^2 x 10^{-3}	S.D. x 10^{-2}
Serratia liquefaciens				
VLB 045	0.401	42.4	1.312	3.307
VLB 045-A	0.440	42.9	0.228	1.400
VLB 045-B	0.387	45.5	0.185	1.260
Klebsiella pneumoniae				
VLB 049	0.473	39.3	0.876	2.701
VLB 049-A	0.515	37.1	0.513	2.336
VLB 049-B	0.412	30.2	0.413	2.407
Pseudomonas putida				
VLB 051	0.117	11.0	0.066	0.742
VLB 051-A	0.193	11.3	0.012	0.328
VLB 051-B	0.165	23.6	0.085	0.853

TABLE 15-2

AVERAGE MONOD PARAMETERS
FOR THREE PURE PHENOL-DEGRADING SPECIES

PURE SPECIES	μ_{max} (hr^{-1})	K_s (mg/lit)
S. liquefaciens	0.409	43.6
K. pneumoniae	0.467	35.5
P. putida	0.158	15.3

TABLE 16

YIELD COEFFICIENTS vs. INITIAL PHENOL CONCENTRATIONS FOR *Serratia liquefaciens*

	Yield Coefficient		
S_o, mg/lit	VLB 045	VLB 045-A	VLB 045-B
20	0.420	0.494	0.466
40	0.436	0.670	0.419
50	0.596	0.736	0.561
80	0.353	0.489	0.467
100	0.652	0.425	0.374
120	0.556	0.551	0.655
140	0.518	0.569	0.480
180	0.518	0.482	0.530

TABLE 17

YIELD COEFFICIENTS vs. INITIAL PHENOL CONCENTRATIONS FOR *Klebsiella pneumoniae*

S_o, mg/lit	Yield Coefficient		
	VLB 049	VLB 049-A	VLB 049-B
20	0.646	0.634	0.796
40	0.669	0.718	0.744
50	0.527	0.672	0.758
80	0.604	0.847	0.846
100	0.896	1.349	1.760
120	0.882	1.475	1.272
140	0.895	2.186	1.029
180	0.855	1.391	0.935

TABLE 18

YIELD COEFFICIENTS vs. INITIAL PHENOL CONCENTRATIONS FOR *Pseudomonas putida*

S_o (mg/lit)	Yield Coefficient		
	VLB 051	VLB 051-A	VLB 051-B
20	0.496	1.005	0.494
40	0.545	0.683	0.624
50	0.527	0.640	0.653
80	0.642	0.693	0.453
100	0.620	0.806	0.447
120	0.599	0.622	0.289
140	0.554	0.573	0.276
180	0.586	0.564	0.238

TABLE 19-1

BIOMASS CONCENTRATION vs. TIME
IN THE FSSAS SOLUTION
(*K. pneumoniae*: Run VLB 047)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	10.0	11.4	11.5	9.8
1.0	15.3	14.5	13.6	10.7
3.0	20.4	18.6	14.1	17.4
5.5	42.2	32.1	24.3	17.9
6.5	43.4	48.8	43.6	26.4
8.5	44.2	53.6	70.6	78.9
10.5		53.6	71.7	101.3

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	4.7	8.4	6.6	8.2
1.0	6.8	8.0	9.2	9.7
3.0	15.8	11.5	11.8	14.7
5.5	20.1	14.8	20.7	12.5
6.5	34.2	21.8	26.4	14.7
8.5	95.6	49.7	45.6	29.7
10.5		49.1	48.6	58.7

TABLE 19-2

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae*, Run: VLB 047)

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	16.8	31.1	50.0	80.0
3.0	9.3	14.7	49.6	79.8
5.5	0.7	1.3	41.9	79.6
6.5		0.9	27.2	69.5
8.5			1.8	44.4
10.5				3.7

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	99.8	119.4	137.5	179.8
3.0	99.6	111.5	137.7	179.5
5.5	99.6	104.3	90.7	180.1
6.5	60.7	55.1	52.4	142.0
8.5	10.3	3.1	6.7	68.2
10.5		0.0	1.3	16.8

TABLE 19-3

BIOMASS CONCENTRATION vs. TIME
IN THE FSSAS SOLUTION
(*K. pneumoniae*: Run VLB 048)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	13.0	10.3	10.9	9.8
2.2	14.7	12.6	12.4	10.3
3.2	20.1	17.0	13.4	16.8
5.2	42.3	27.1	25.8	18.6
7.7	36.1	43.6	40.2	29.7
9.0	38.4	48.8	59.3	64.5
11.0	40.8	49.5	61.9	79.7

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	6.7	8.2	7.5	8.2
2.2	7.7	8.2	9.0	9.8
3.2	15.5	11.4	13.2	14.7
5.2	12.9	14.7	20.4	12.6
7.7	19.6	23.5	25.0	15.5
9.0	33.8	47.0	39.2	29.4
11.0	75.3	47.5	43.6	66.3

TABLE 19-4

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae*, Run: VLB 048)

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	20	40	50	80
0	20.0	40.0	50.0	80.0
2.2	19.4	33.2	50.0	78.8
3.2	15.7	15.2	49.3	76.4
5.2	9.7	8.5	45.3	72.3
7.7	5.6	3.6	32.5	64.2
9.0	0.2	0	19.6	45.3
11.0			4.2	21.7
13.0				5.2

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	100	120	140	180
0	100.0	120.0	140.0	180.0
2.2	96.7	119.5	135.7	176.3
3.2	89.4	104.3	113.2	169.7
5.2	87.8	95.4	94.3	130.2
7.7	63.8	74.8	61.2	120.1
9.0	42.9	60.2	30.2	103.2
11.0	29.3	38.4	20.3	98.2
13.0	9.2	10.2	8.4	50.3

TABLE 20-1

BIOMASS CONCENTRATION vs. TIME
(*K. pneumoniae* + *P. putida*)
(Run: VLB 070)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	40.0	38.0	36.4	34.3
1.0	46.0	45.3	45.7	44.2
2.0	50.8	56.2	56.9	54.8
3.0	52.7	61.4	63.8	67.3
4.0	54.4	66.7	68.4	79.0
5.0	54.3	65.4	70.6	89.7
7.0	54.3	65.4	70.5	90.3
8.0	54.3	65.4	70.5	90.1
9.0			68.9	90.2

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	30.6	28.4	26.6	24.2
1.0	38.2	34.1	34.3	32.1
2.0	50.6	41.1	43.2	40.8
3.0	67.0	54.3	51.3	55.7
4.0	82.4	72.2	72.4	74.3
5.0	92.6	90.2	92.2	101.2
7.0	100.8	106.2	111.5	130.2
8.0	110.2	120.1	134.2	161.3
9.0	113.7	120.2	134.2	161.4
10.0	113.4	120.4	132.0	161.4

TABLE 20-2

BIOMASS CONCENTRATION vs. TIME
(K. pneumoniae + P. putida)
(Run: VLB 070-A)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	34.2	35.4	32.1	34.6
1.0	35.4	36.4	34.2	36.4
2.0	36.8	35.3	35.4	38.6
3.0	37.6	36.4	36.4	39.6
4.0	39.2	38.6	39.2	41.2
5.0	42.4	42.3	42.8	42.2
6.0	42.3	47.7	47.4	48.4
7.0	42.4	48.5	48.4	53.7
8.0		48.7	48.6	54.5
9.0			48.7	54.6

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	29.1	35.2	32.1	26.8
1.0	34.7	36.7	37.4	32.2
2.0	48.9	41.7	44.3	44.2
3.0	58.4	53.2	54.2	57.5
4.0	71.3	74.2	74.3	73.2
5.0	93.2	92.1	93.2	91.1
6.0	112.3	112.3	104.2	120.1
7.0	123.4	123.1	123.1	142.5
8.0	132.4	142.7	142.9	153.2
9.0	133.0	145.1	153.2	162.1
11.0	134.1	146.2	155.4	169.8

TABLE 20-3

BIOMASS CONCENTRATION vs. TIME
(*K. pneumoniae* + *P. putida*)
(Run: VLB 070-B)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	32.4	36.4	34.5	23.5
1.0	34.5	38.6	35.4	29.3
2.0	36.5	42.3	49.3	36.4
3.0	43.4	53.2	55.6	41.2
4.0	54.4	64.3	65.3	54.3
5.0	65.7	73.2	72.1	64.5
6.0	66.1	75.2	85.3	83.2
7.0	66.8	76.1	86.0	92.1
8.0		76.3	86.6	97.2
10.0				98.0
12.0				98.2

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	24.3	25.3	29.4	29.4
1.0	29.4	28.6	32.7	38.4
2.0	32.8	34.2	38.6	41.2
3.0	41.3	48.5	46.5	49.7
4.0	52.1	53.8	53.2	56.4
5.0	69.7	67.4	65.2	71.2
6.0	81.3	79.5	77.7	84.9
7.0	100.2	91.4	89.4	98.2
8.0	112.5	112.2	98.4	105.8
10.0	132.4	130.4	112.0	121.4
12.0	135.3	139.5	124.5	130.0

TABLE 21-1

BIOMASS CONCENTRATION vs. TIME
(*K. pneumoniae* + *S. liquefaciens*)
(Run: VLB 071)

	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit)			
TIME, hr.	20	40	50	80
0	18.0	24.3	12.1	10.0
1.0	20.4	30.1	14.5	12.8
2.0	24.2	34.2	17.0	15.3
3.0	24.9	41.9	22.1	22.2
4.0	27.4	44.1	28.5	30.6
5.0	28.6	46.2	31.9	32.4
6.0	29.9	48.2	35.7	39.8
7.0	30.0	48.2	38.4	50.1
8.0	30.0	49.9	35.4	51.1
9.0	28.5	46.3	38.5	52.3
10.0	28.5	46.3	38.5	54.2

	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit)			
TIME, hr.	100	120	140	180
0	14.2	8.4	10.0	10.0
1.0	19.5	15.2	12.2	13.2
2.0	20.3	17.5	18.4	18.4
3.0	36.2	21.1	28.4	27.8
4.0	46.5	32.9	33.1	44.2
5.0	55.4	38.6	44.1	55.3
6.0	60.0	55.0	62.4	67.0
7.0	64.5	61.2	77.5	93.4
8.0	68.5	74.3	92.4	116.3
9.0	74.6	79.7	92.5	118.0
10.0	73.9			

TABLE 21-2

BIOMASS CONCENTRATION vs. TIME
(*K. pneumoniae* + *S. liquefaciens*)
(Run: VLB 071-A)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	32.1	31.2	30.2	29.5
1.0	35.2	32.4	32.5	31.3
2.0	37.5	34.3	34.3	35.4
3.0	39.3	38.5	38.6	39.7
4.0	43.2	42.1	41.3	45.6
5.0	49.6	46.1	45.6	51.7
6.0	50.2	48.1	50.5	58.4
7.0	50.9	48.6	58.7	64.3
8.0	51.1	48.9	59.8	74.2
9.0			60.1	85.9
10.0			60.4	86.1
12.0				87.0

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	19.7	18.2	20.5	29.6
1.0	29.4	26.4	28.7	32.6
2.0	34.5	32.1	32.8	38.4
3.0	36.4	34.2	39.4	41.2
4.0	46.4	43.2	43.4	45.7
5.0	54.3	54.3	54.3	54.6
6.0	60.2	59.6	61.2	62.3
7.0	68.8	65.4	74.2	72.4
8.0	76.3	75.3	87.2	86.2
9.0	76.3	87.2	92.0	96.3
10.0	76.4	87.3	103.4	110.3
12.0		87.3	103.2	120.6

TABLE 21-3

BIOMASS CONCENTRATION vs. TIME
(*K. pneumoniae* + *S. liquefaciens*)
(Run: VLB 071-B)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	21.3	23.4	22.5	19.6
1.0	28.5	28.7	29.5	22.6
2.0	32.6	32.8	32.5	35.3
3.0	39.5	40.1	42.1	42.9
4.0	47.4	49.4	45.3	49.6
5.0	58.6	56.3	52.4	55.4
6.0	58.5	69.2	67.2	63.4
7.0		69.3	67.9	63.7
8.0			67.4	63.2

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	15.9	19.3	20.0	30.4
1.0	19.5	29.8	27.6	37.5
2.0	23.4	32.6	36.4	41.6
3.0	32.9	41.3	45.3	48.6
4.0	42.1	48.6	50.4	52.4
5.0	54.3	54.3	52.3	60.0
6.0	60.0	65.3	62.1	67.8
7.0	72.1	72.9	73.5	78.8
8.0	72.5	83.2	89.8	89.8
9.0		83.2	92.3	98.6
10.0		83.6	92.6	110.2
12.0		84.1	93.1	111.1

TABLE 22-1

BIOMASS CONCENTRATION vs. TIME
(*P. putida* + *S. liquefaciens*)
(Run: VLB 072)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	20.0	24.0	20.0	20.0
1.0	24.0	25.3	22.5	24.7
2.0	24.0	28.8	28.9	40.1
3.4	28.4	34.9	31.5	51.1
5.0	29.8	35.6	42.5	52.3
6.0	29.7	40.7	43.8	53.6
7.0	29.5	40.0	47.8	58.8
8.0	29.5	40.0	45.6	60.7
8.6	29.6	39.8	45.6	61.5
10.0	29.4	39.7	43.8	61.5

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	18.0	18.1	16.0	16.2
1.0	22.1	18.9	19.1	17.4
2.0	39.8	21.4	23.2	24.4
3.4	62.0	49.6	32.3	35.4
5.0	66.4	54.6	50.2	56.7
6.0	67.2	70.0	60.1	70.1
7.0	68.1	74.3	71.3	86.9
8.0	69.5	75.1	80.0	95.6
8.6	69.3	75.4	79.8	103.2
10.0	69.4	72.4	79.6	102.8

TABLE 22-2

BIOMASS CONCENTRATION vs. TIME
(*P. putida* + *S. liquefaciens*)
(Run: VLB 072-A)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	21.3	23.4	25.3	23.2
1.0	26.3	28.3	29.3	28.7
2.0	34.1	34.5	35.4	34.2
3.0	39.4	40.9	41.2	39.7
5.0	43.2	45.4	44.5	45.6
6.0	43.5	49.5	50.8	50.3
7.0	43.5	50.0	58.6	58.5
8.0		50.1	58.6	63.4
9.0		49.9	58.9	63.6
10.0				63.9

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	21.3	20.4	27.4	20.1
1.0	28.4	27.5	39.1	27.4
2.0	31.4	36.4	42.4	32.4
3.0	42.4	40.1	48.5	39.6
5.0	49.6	48.6	52.1	47.8
6.0	54.3	59.3	60.6	57.4
7.0	68.1	69.5	73.1	64.1
8.0	88.4	76.5	86.5	84.3
9.0	94.3	85.6	93.3	95.4
10.0	104.2	94.5	104.2	104.5
12.0	120.3	119.6	121.6	118.6

TABLE 22-3

BIOMASS CONCENTRATION vs. TIME
(*P. putida* + *S. liquefaciens*)
(Run: VLB 072-B)

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 20	40	50	80
0	21.4	24.5	24.3	22.4
1.0	24.5	25.4	28.6	29.4
2.0	32.1	32.1	34.6	37.5
3.0	38.6	38.4	41.2	46.2
5.0	42.0	42.3	43.9	52.5
6.0	41.9	54.2	56.2	67.9
7.0	42.1	55.0	67.2	73.2
8.0	42.3	55.1	67.8	84.3
9.0		55.2	-	85.5
10.0			68.1	85.6
12.0				86.6

TIME, hr.	BIOMASS CONCENTRATION, mg/lit (at different initial phenol concentrations, mg/lit) 100	120	140	180
0	19.3	19.3	21.3	23.2
1.0	22.3	21.4	24.5	28.5
2.0	28.4	29.4	29.4	31.2
3.0	35.4	38.6	34.6	39.6
5.0	54.3	54.5	50.3	54.3
6.0	62.2	64.3	63.1	65.4
7.0	68.1	74.1	71.3	76.5
8.0	72.1	78.9	83.2	86.6
9.0	72.4	79.0	95.8	93.4
10.0	72.6	79.1	96.1	93.9
12.0	73.0	80.0	96.2	94.1

TABLE 23-1

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae* + *P. putida*)
(Run: VLB 070)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	12.0	29.6	34.1	64.1
2.0	5.8	19.2	26.1	53.6
3.0	4.1	9.7	15.0	31.5
4.0	1.8	4.1	5.2	19.8
5.0	0	0	0.4	4.7
6.0			0	0

TIME,hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	83.2	112.4	128.4	175.2
2.0	70.2	80.3	114.3	170.4
3.0	59.7	60.0	99.6	137.2
4.0	40.6	34.5	70.0	94.3
5.0	19.8	6.2	37.4	67.5
6.0	2.2	0	20.4	25.6
8.0			4.2	8.5
9.0				1.4

TABLE 23-2

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae* + *P. putida*)
(Run: VLB 070-A)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	15.4	32.1	42.1	66.4
2.0	9.4	20.5	29.7	57.2
3.0	2.1	9.8	20.4	41.0
4.0	0	0.5	8.9	18.4
5.0		0	1.2	10.2
6.0			0	5.4
7.0				0.5

TIME,hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	91.5	109.5	123.2	170.4
2.0	84.3	90.5	110.7	164.5
3.0	65.4	78.7	102.1	141.3
4.0	40.6	54.6	95.4	120.4
5.0	20.2	39.2	73.2	103.2
6.0	9.5	24.1	53.2	90.6
7.0	4.2	12.3	32.3	68.4
8.0	0.2	1.2	13.7	43.1
9.0			2.3	13.7
11.0			0.1	1.1

TABLE 23-3

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae* + *P. putida*)
(Run: VLB 070-B)

	PHENOL CONCENTRATION, mg/lit			
TIME, hr.	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	13.2	31.2	40.2	69.5
2.0	8.5	21.2	31.0	54.3
3.0	3.1	11.2	20.3	35.3
4.0	0.3	2.0	9.4	20.3
5.0	0	0	1.2	6.3
6.0			0	0.3

	PHENOL CONCENTRATION, mg/lit			
TIME,hr.	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	85.4	109.5	131.1	169.5
2.0	72.2	92.8	118.5	160.2
3.0	65.4	75.5	99.5	143.7
4.0	53.2	54.4	86.5	123.8
5.0	32.1	30.6	63.3	103.2
6.0	12.3	19.4	43.2	84.2
8.0	8.5	7.9	25.8	67.3
10.0	0.6	3.7	3.8	31.2
12.0		0.4	1.2	5.2

TABLE 24-1

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae* + *S. liquefaciens*)
(Run: VLB 071)

PHENOL CONCENTRATION, mg/lit				
TIME, hr.	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	18.1	34.2	41.7	73.2
2.0	14.1	20.0	-	63.6
3.0	8.4	14.9	35.2	49.6
4.0	4.4	7.6	26.2	41.0
5.0	1.9	2.6	21.2	32.1
6.0	1.1	0	13.6	18.2
7.0	0		6.5	14.5
8.0			3.8	8.6
9.0			0	3.0

PHENOL CONCENTRATION, mg/lit				
TIME, hr.	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	90.0	112.1	137.1	174.2
2.0	77.2	101.1	130.1	160.0
3.0	58.1	90.0	120.4	150.2
4.0	40.2	76.5	98.2	123.0
5.0	20.3	67.5	70.3	103.4
6.0	9.7	41.4	42.1	66.5
7.0	0.7	24.5	20.1	32.5
8.0		8.6	12.1	0.6
9.0			8.4	0

TABLE 24-2

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae* + *S. liquefaciens*)
(Run: VLB 071-A)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	16.4	33.2	43.5	72.9
2.0	12.1	29.2	39.3	64.9
3.0	9.6	14.3	32.1	51.3
4.0	2.8	8.5	27.6	44.3
5.0	0	0.5	10.3	23.4
6.0			3.4	13.7
7.0			0.8	6.3
8.0				1.8
9.0				0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	91.7	110.5	134.6	172.6
2.0	82.3	103.3	126.7	161.2
3.0	61.2	93.3	113.6	148.6
4.0	41.3	81.6	99.3	124.4
5.0	22.2	62.4	75.4	107.5
6.0	11.2	43.6	53.5	86.5
7.0	6.4	15.7	36.3	64.3
8.0	0	9.4	13.3	35.8
9.0		0.3	7.3	19.3
10.0			0	3.2
12.0				0

TABLE 24-3

PHENOL CONCENTRATION vs. TIME
(*K. pneumoniae* + *S. liquefaciens*)
(Run: VLB 071-B)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	17.4	33.6	40.4	74.9
2.0	11.7	21.5	37.4	62.9
3.0	9.3	13.2	33.4	50.3
4.0	3.2	8.9	22.3	44.0
5.0	0.3	1.4	11.2	32.4
6.0		0	3.3	19.4
7.0			0	5.7
8.0				0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	93.9	113.6	130.3	172.7
2.0	73.2	108.5	128.3	162.6
3.0	53.5	86.8	117.5	148.6
4.0	38.6	74.3	95.4	119.9
5.0	17.6	67.8	76.4	98.6
6.0	7.5	46.4	46.4	64.3
7.0	0	27.4	22.3	38.6
8.0		3.2	12.5	6.6
9.0		0	0	0

TABLE 25-1

PHENOL CONCENTRATION vs. TIME
(*P. putida* + *S. liquefaciens*)
(Run: VLB 072)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	13.8	34.4	39.8	68.2
2.0	9.9	25.6	33.2	57.6
3.4	5.6	14.0	24.1	40.0
5.0	0.9	6.1	9.3	19.7
6.0	0	0	4.4	8.6
7.0			0	0.6
8.0				0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	90.0	116.4	135.1	175.2
2.0	75.5	100.1	123.3	160.1
3.4	58.6	74.4	105.3	135.5
5.0	35.1	44.9	70.0	89.8
6.0	16.0	25.1	45.4	45.6
7.0	6.5	15.2	19.2	22.1
8.0	0	7.9	2.4	0
8.6		3.0		

TABLE 25-2

PHENOL CONCENTRATION vs. TIME
(*P. putida* + *S. liquefaciens*)
(Run: VLB 072-A)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	14.2	35.8	38.5	69.8
2.0	10.4	24.5	32.4	59.5
3.0	6.5	12.1	29.2	41.3
5.0	0	7.9	11.2	20.4
6.0		0	7.3	11.4
7.0			0	1.9
8.0				0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	90.2	112.3	138.5	174.7
2.0	72.4	102.7	121.3	159.7
3.0	55.3	85.4	96.8	132.5
5.0	37.8	56.4	78.6	104.3
6.0	19.4	28.5	58.3	95.4
7.0	9.3	18.4	23.5	68.4
8.0	0	9.4	4.5	29.4
9.0		1.2	0.2	1.6

TABLE 25-3

PHENOL CONCENTRATION vs. TIME
(*P. putida* + *S. liquefaciens*)
(Run: VLB 072-B)

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	20	40	50	80
0	20.0	40.0	50.0	80.0
1.0	15.7	32.4	41.2	70.4
2.0	8.4	24.3	36.4	59.5
3.4	3.2	12.5	29.4	42.4
5.0	0	5.4	10.4	18.4
6.0		0	4.9	3.9
7.0			0	0.6
8.0				0

TIME, hr.	PHENOL CONCENTRATION, mg/lit			
	100	120	140	180
0	100.0	120.0	140.0	180.0
1.0	95.3	114.3	132.9	173.4
2.0	75.6	109.4	127.4	160.3
3.4	59.4	81.4	109.5	141.4
5.0	39.7	48.5	81.4	88.4
6.0	13.4	28.5	49.5	46.4
7.0	5.6	15.3	21.4	20.4
8.0	0	0	5.6	4.3
9.0			0.5	0

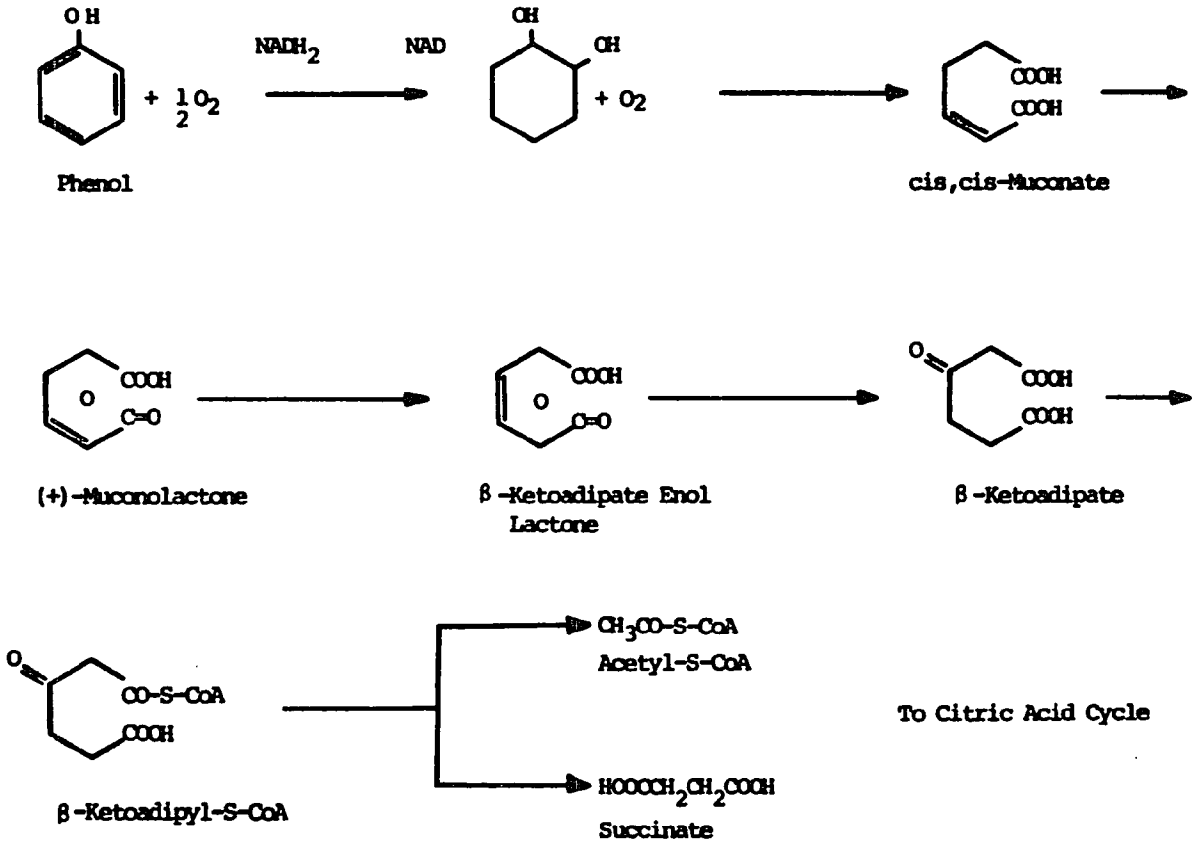


Figure 1

Stanier's Metabolic Pathway for Phenol Degradation

(Source: Stanier, 1948)

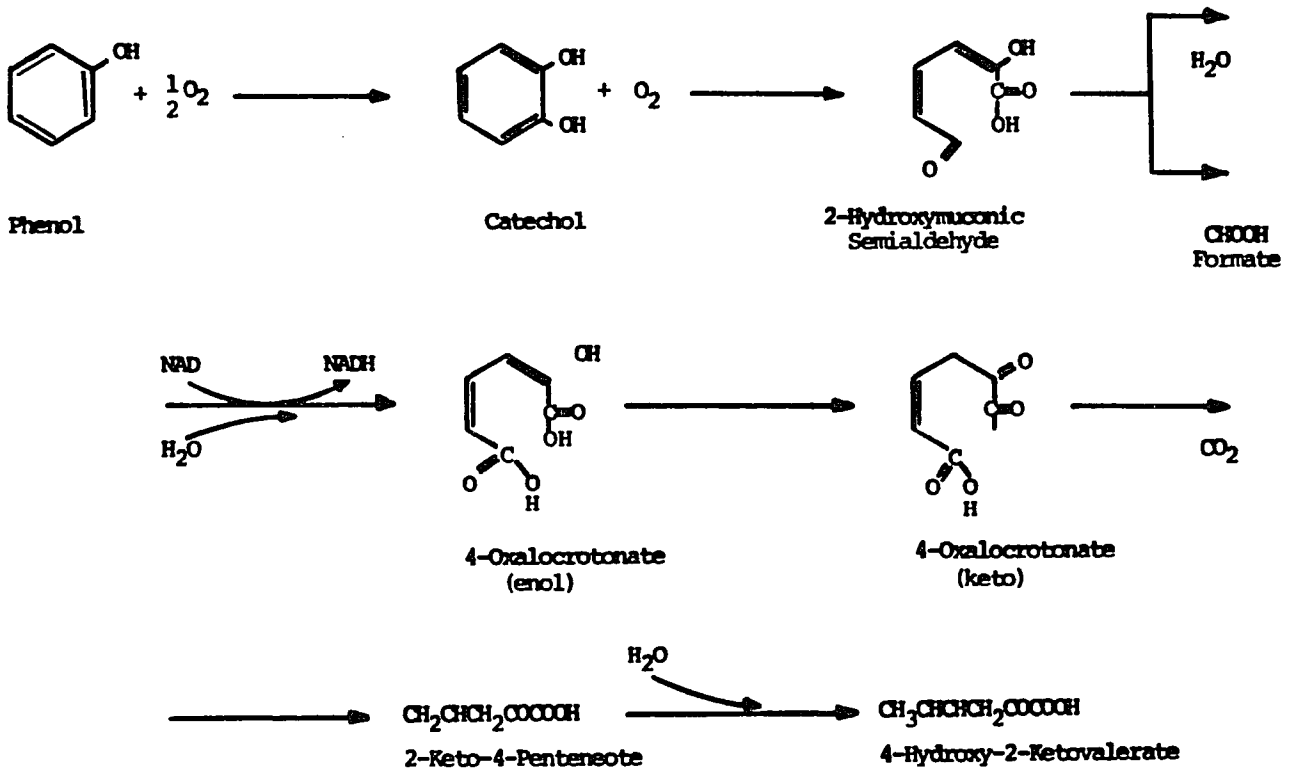


Figure 2

Bayly's Metabolic Pathway for Phenol Degradation

(Source: Bayly, 1966)

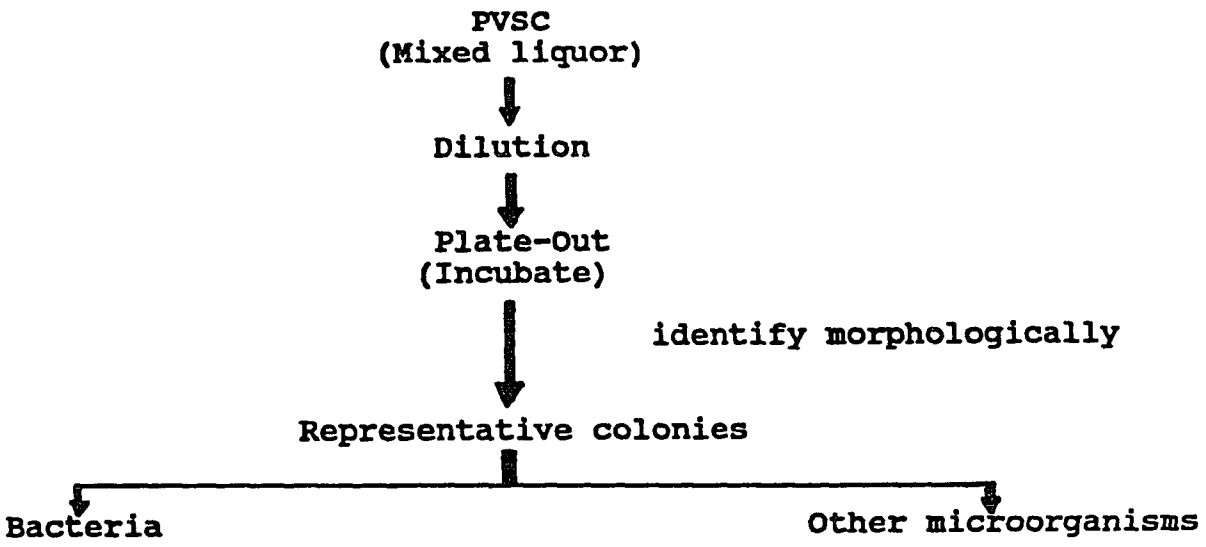


Figure 3

Isolation of Dominant Phenol-Acclimated Species

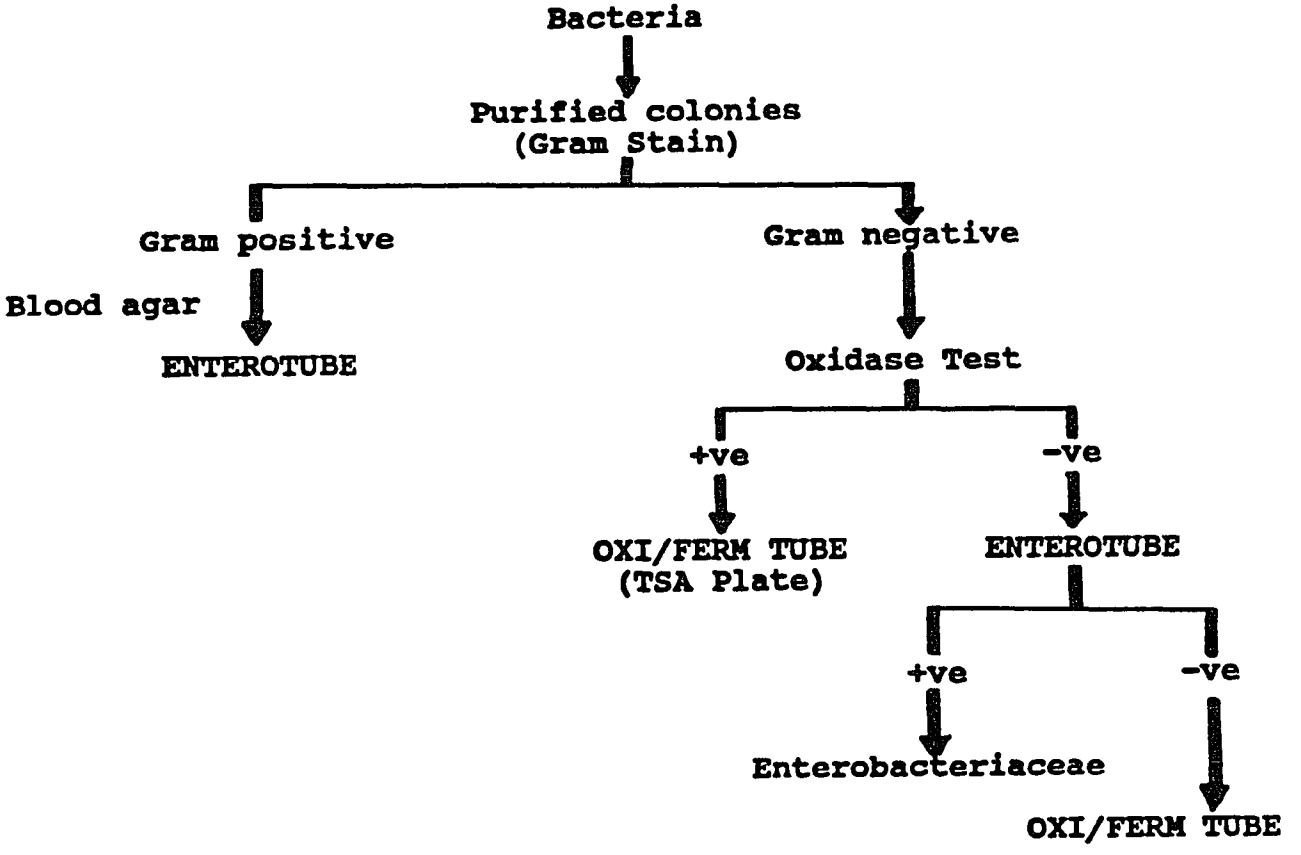


Figure 4

Identification of
Dominant Phenol-Acclimated Bacteria

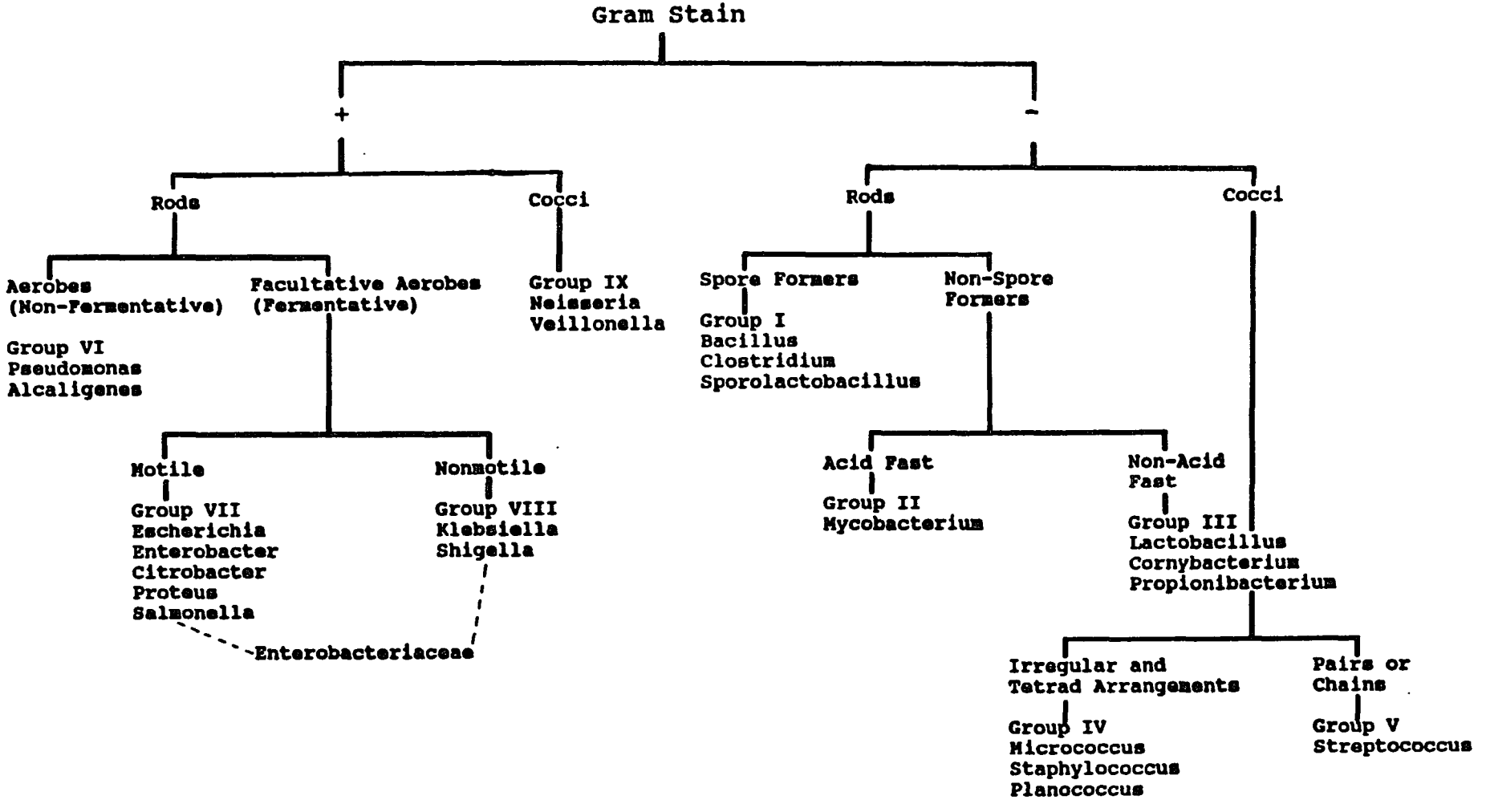


Figure 5

Separation Outline for the Most Common Bacterial Genera

(Source: Benson, 1979)

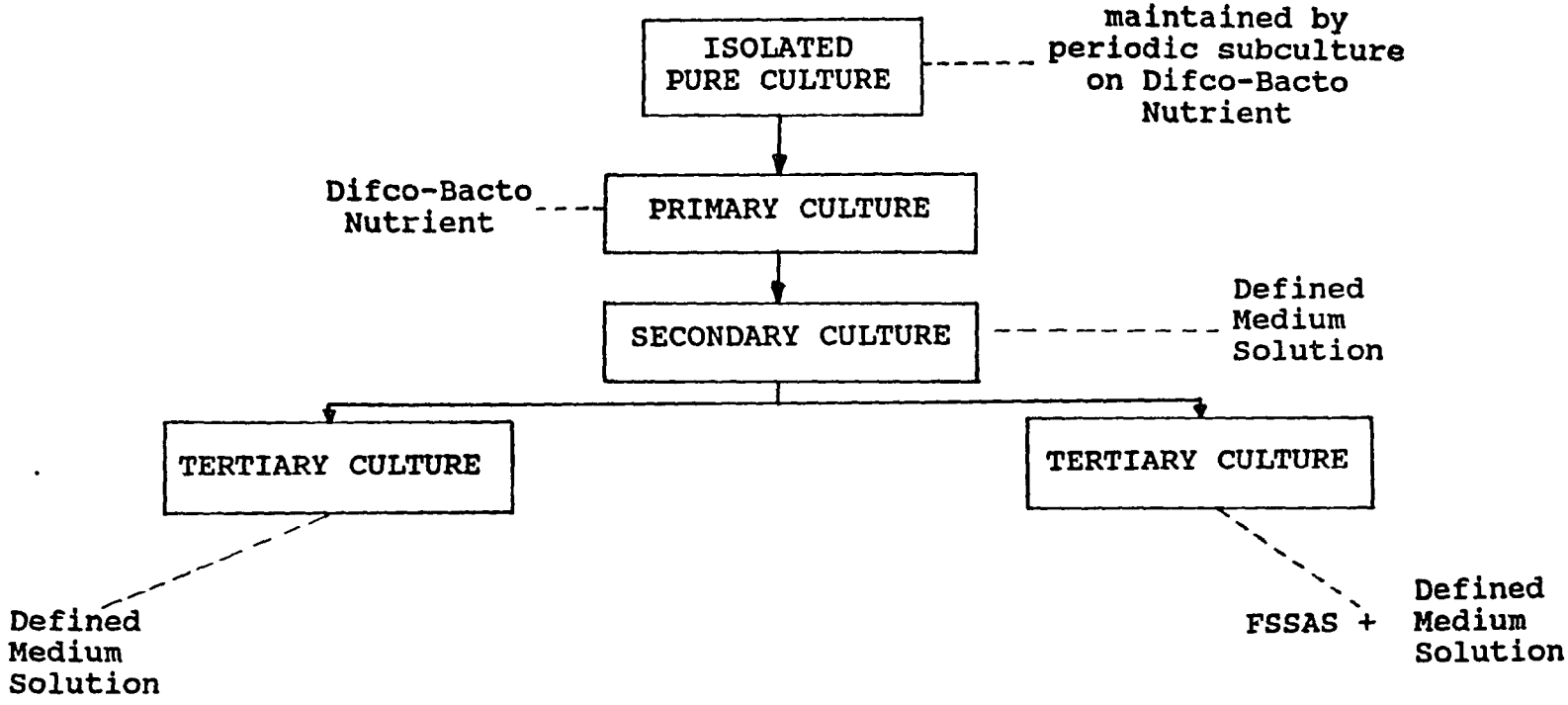


Figure 6

Diagram of the Experimental Procedure

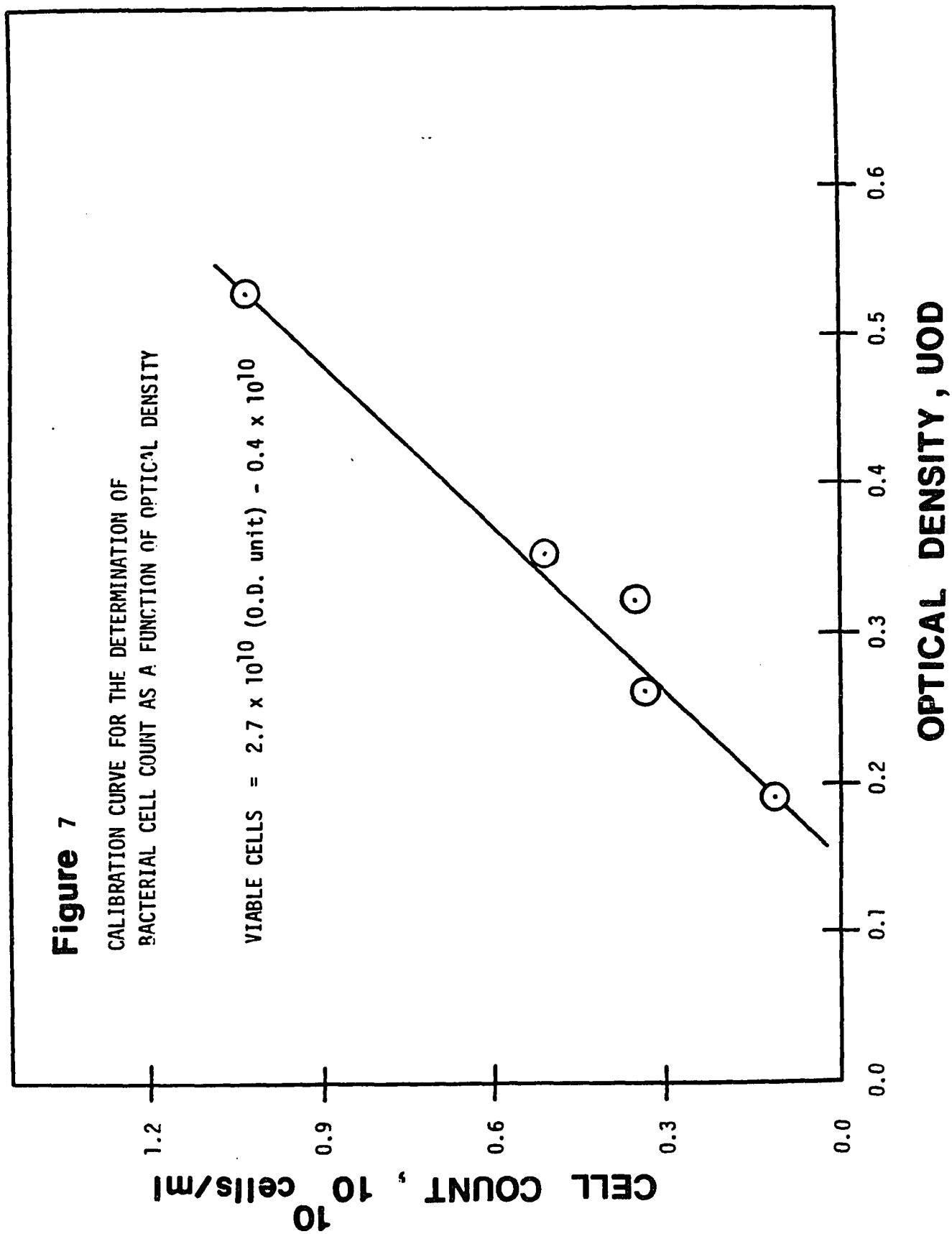


Figure 7
CALIBRATION CURVE FOR THE DETERMINATION OF
BACTERIAL CELL COUNT AS A FUNCTION OF OPTICAL DENSITY
VIABLE CELLS = 2.7 x 10^10 (O.D. unit) - 0.4 x 10^10
CELL COUNT, 10^10 cells/ml
1.2
0.9
0.6
0.3
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
OPTICAL DENSITY, UOD

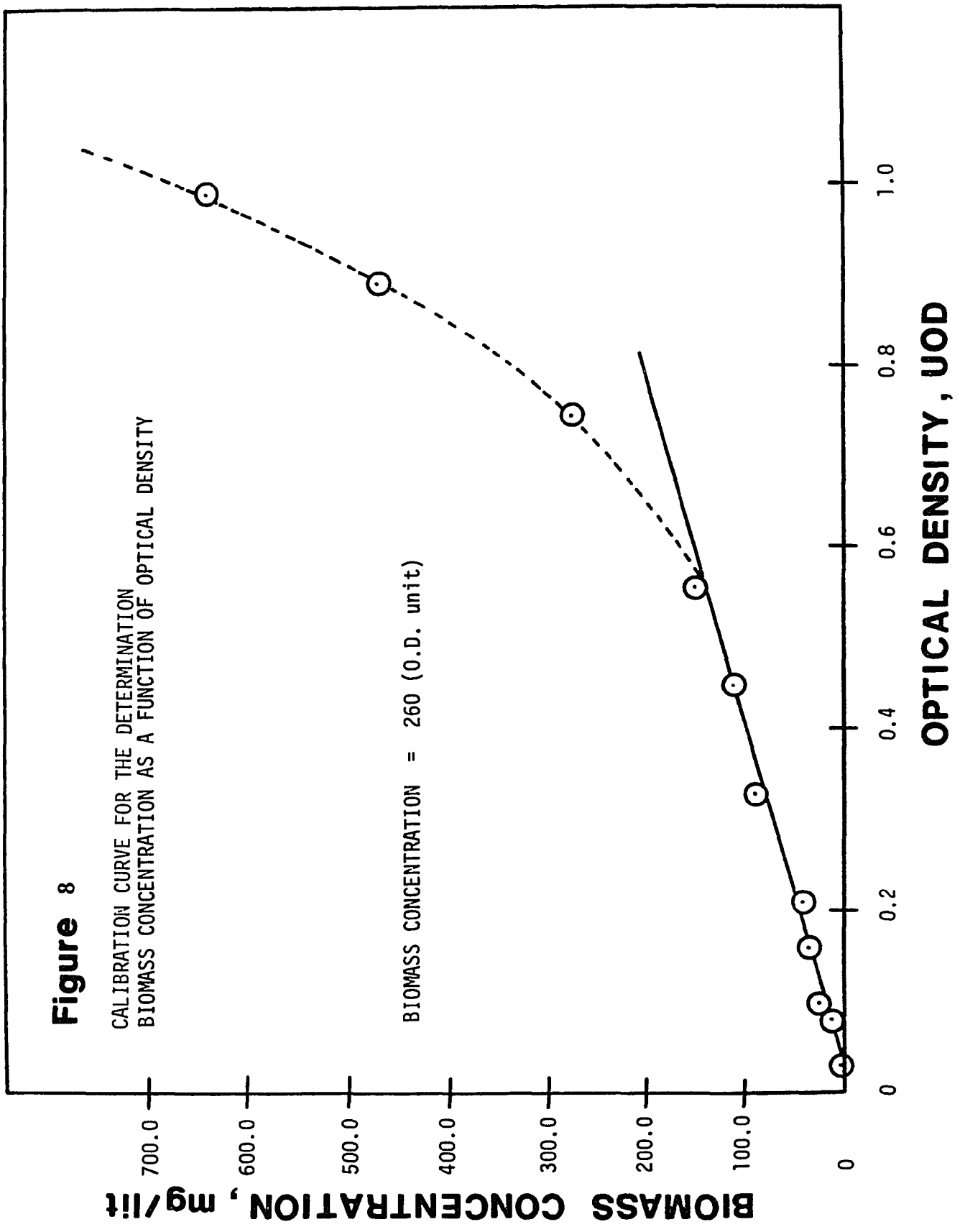


Figure 8
CALIBRATION CURVE FOR THE DETERMINATION
BIOMASS CONCENTRATION AS A FUNCTION OF OPTICAL DENSITY
BIOMASS CONCENTRATION = 260 (O.D. unit)
BIOMASS CONCENTRATION, mg/lit
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0
0
0.2
0.4
0.6
0.8
1.0
OPTICAL DENSITY, UOD

Figure 9

Growth Curves of Serratia liquefaciens at the following Initial Phenol Concentrations (Semi-log plots) (Run VLB 045-1 to VLB 045-8)

9-1	20 mg/lit
9-2	40 mg/lit
9-3	50 mg/lit
9-4	80 mg/lit
9-5	100 mg/lit
9-6	120 mg/lit
9-7	140 mg/lit
9-8	180 mg/lit

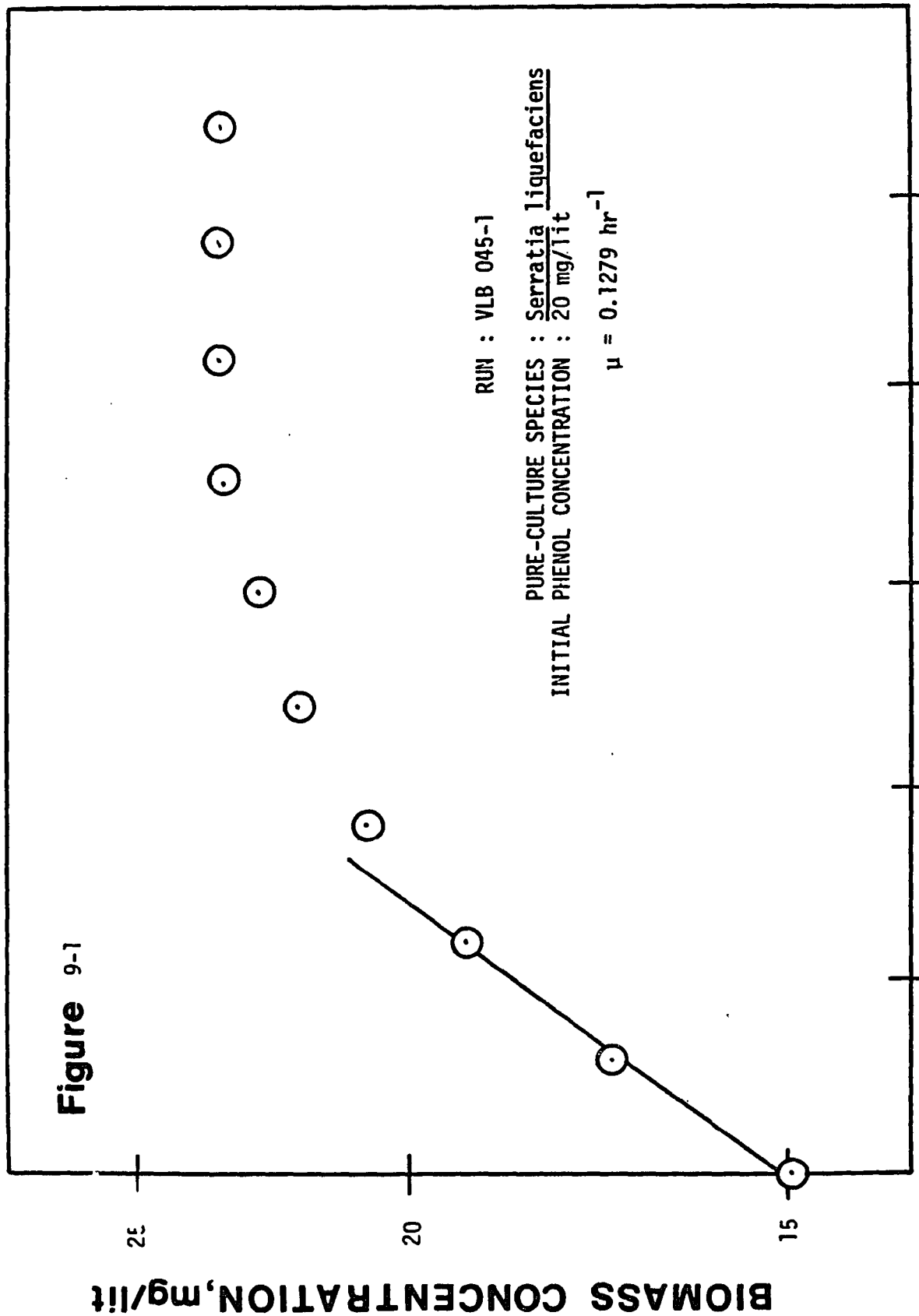

RUN : VLB 045-1
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 20 mg/lit
μ = 0.1279 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
15
20
25
0
2
4
6
8
10
TIME, hr.

Figure 9-1

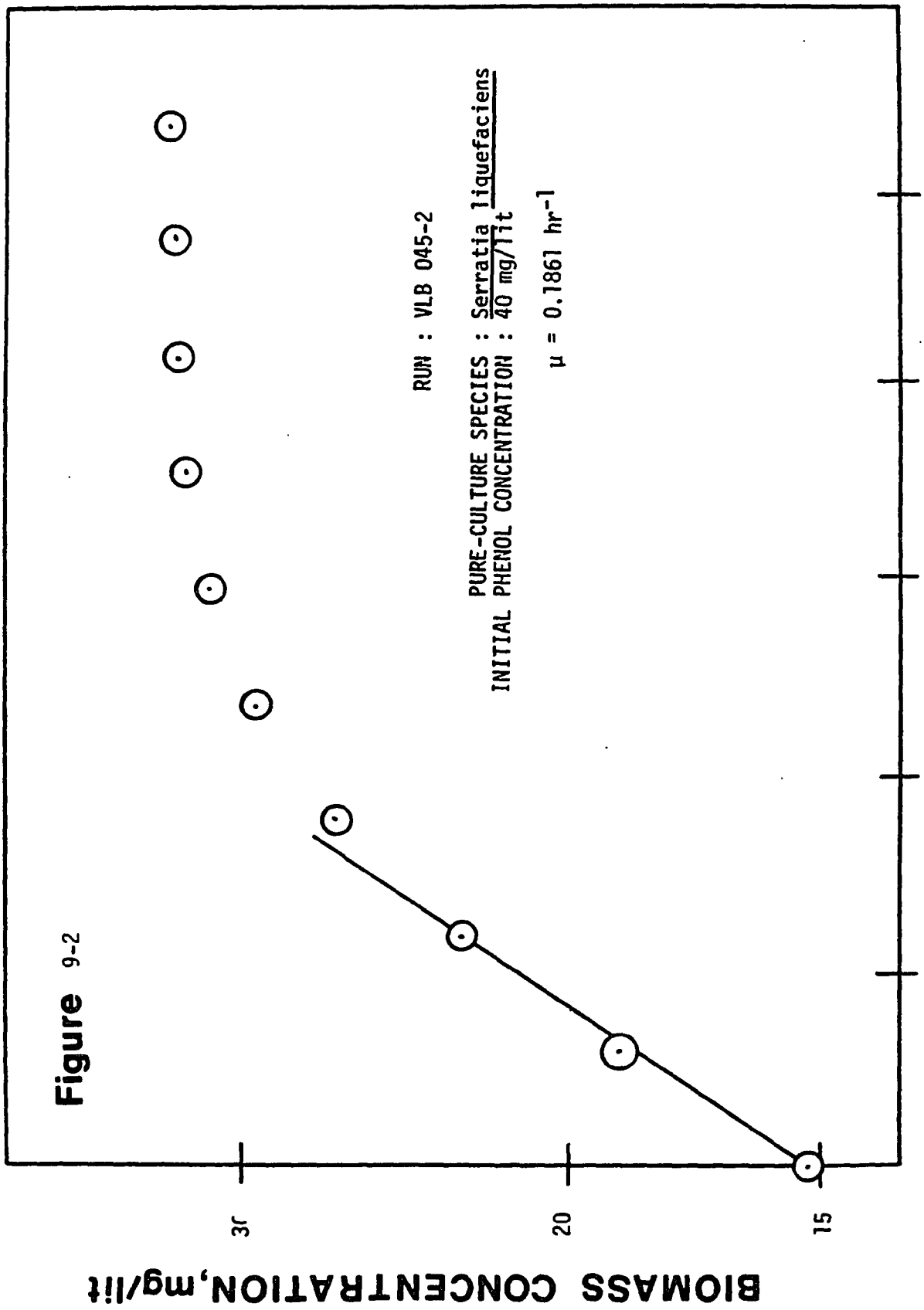

RUN : VLB 045-2
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 40 mg/lit
μ = 0.1861 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
15
20
25
0
2
4
6
8
10
TIME, hr.

Figure 9-2

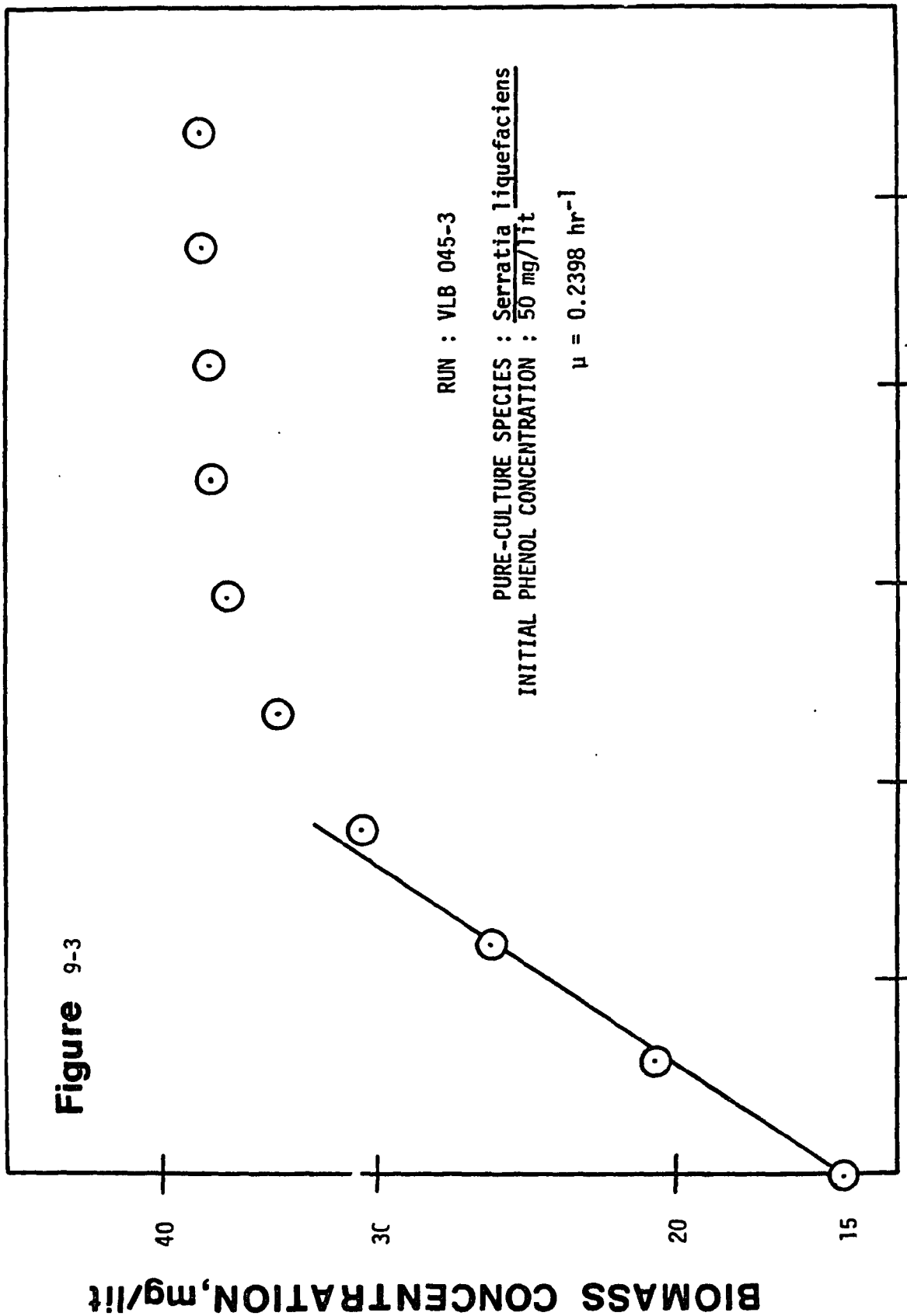

RUN : VLB 045-3
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 50 mg/lit
μ = 0.2398 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
TIME, hr.
15
20
30
40
0
2
4
6
8
10

Figure 9-3

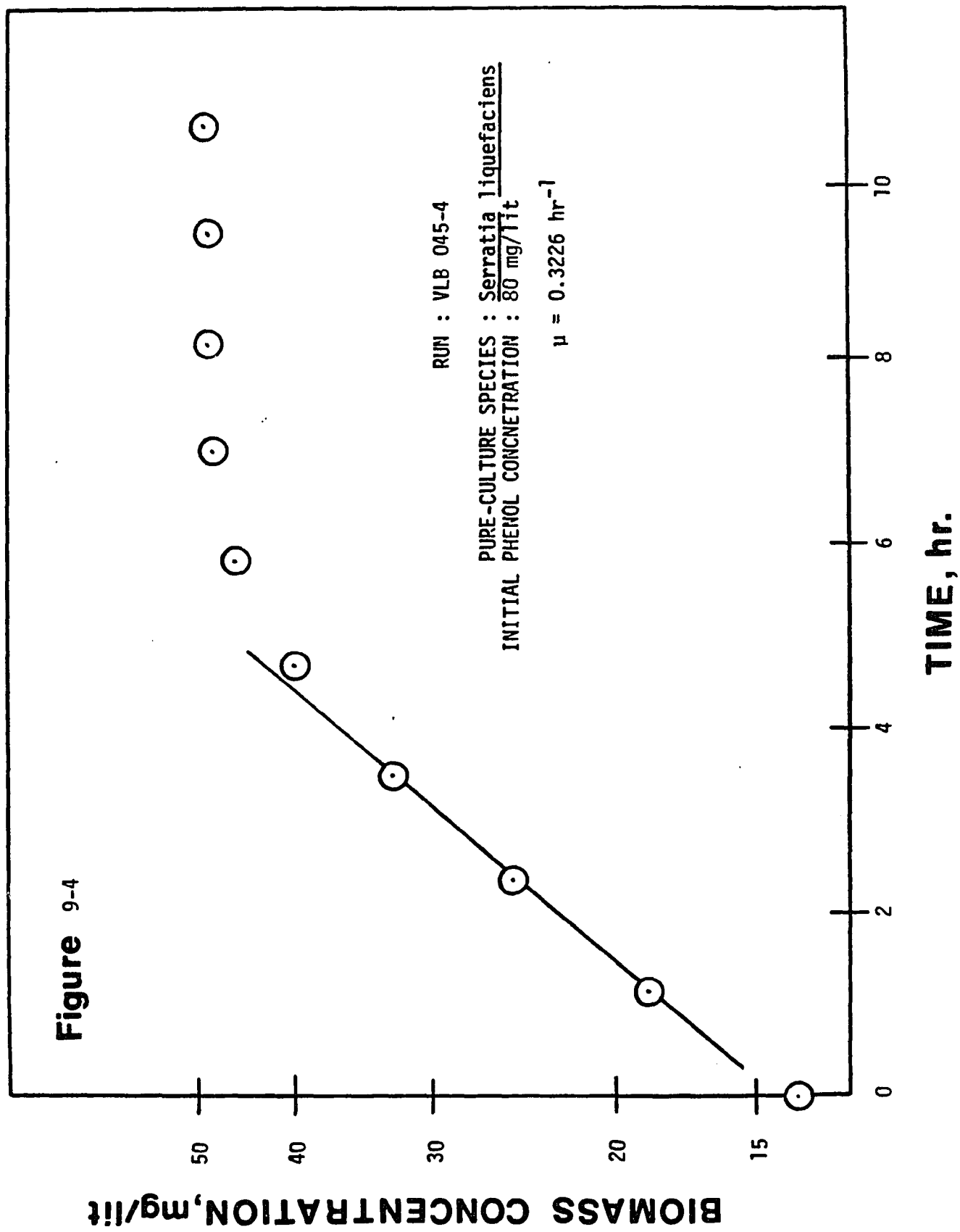

BIOMASS CONCENTRATION, mg/lit
50
40
30
20
15
0
2
4
6
8
10
TIME, hr.
RUN : VLB 045-4
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCNETRATION : 80 mg/lit
μ = 0.3226 hr⁻¹

Figure 9-4

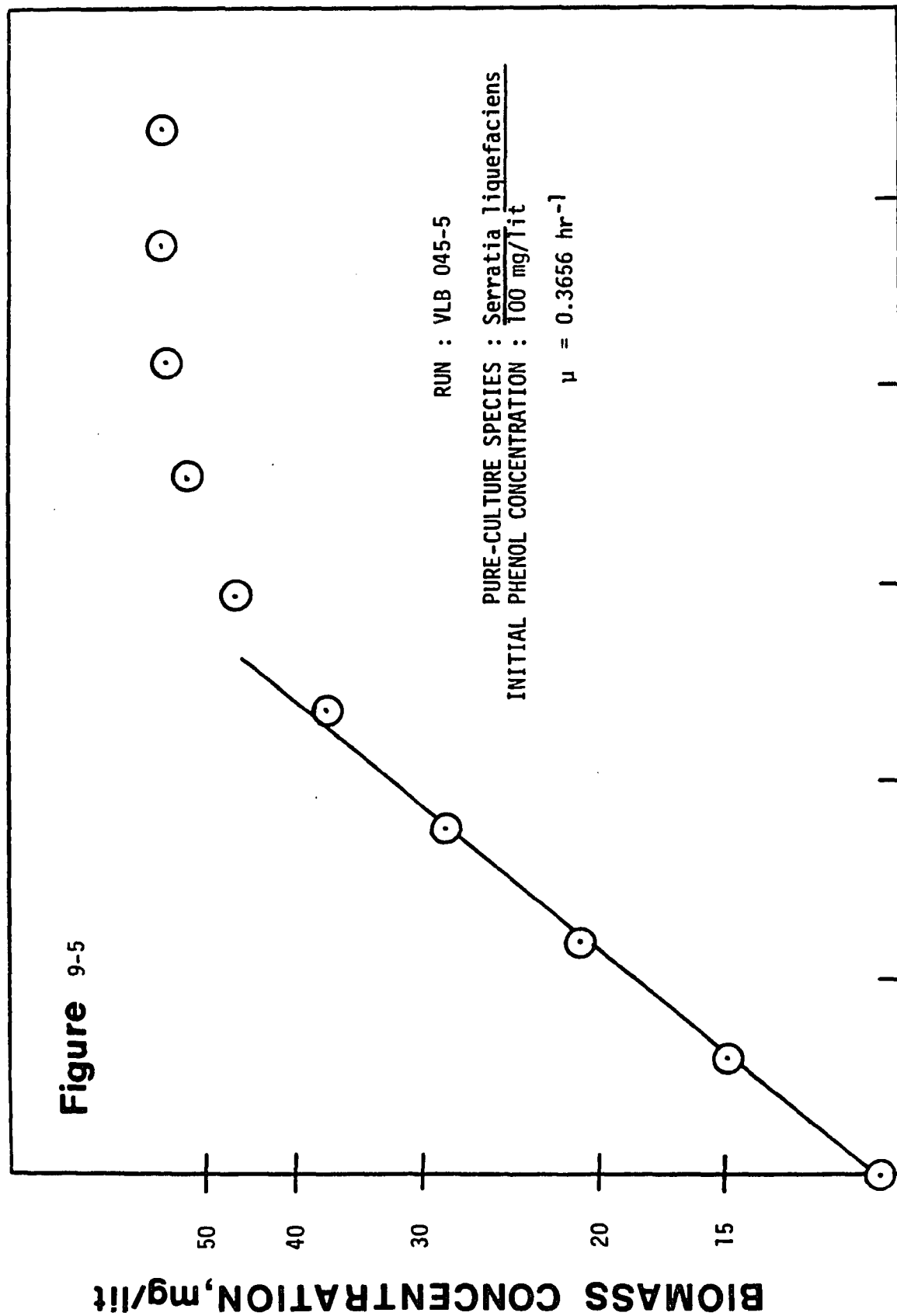

RUN : VLB 045-5
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 100 mg/lit
μ = 0.3656 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
15
20
30
40
50
0
2
4
6
8
10
TIME, hr.

Figure 9-5

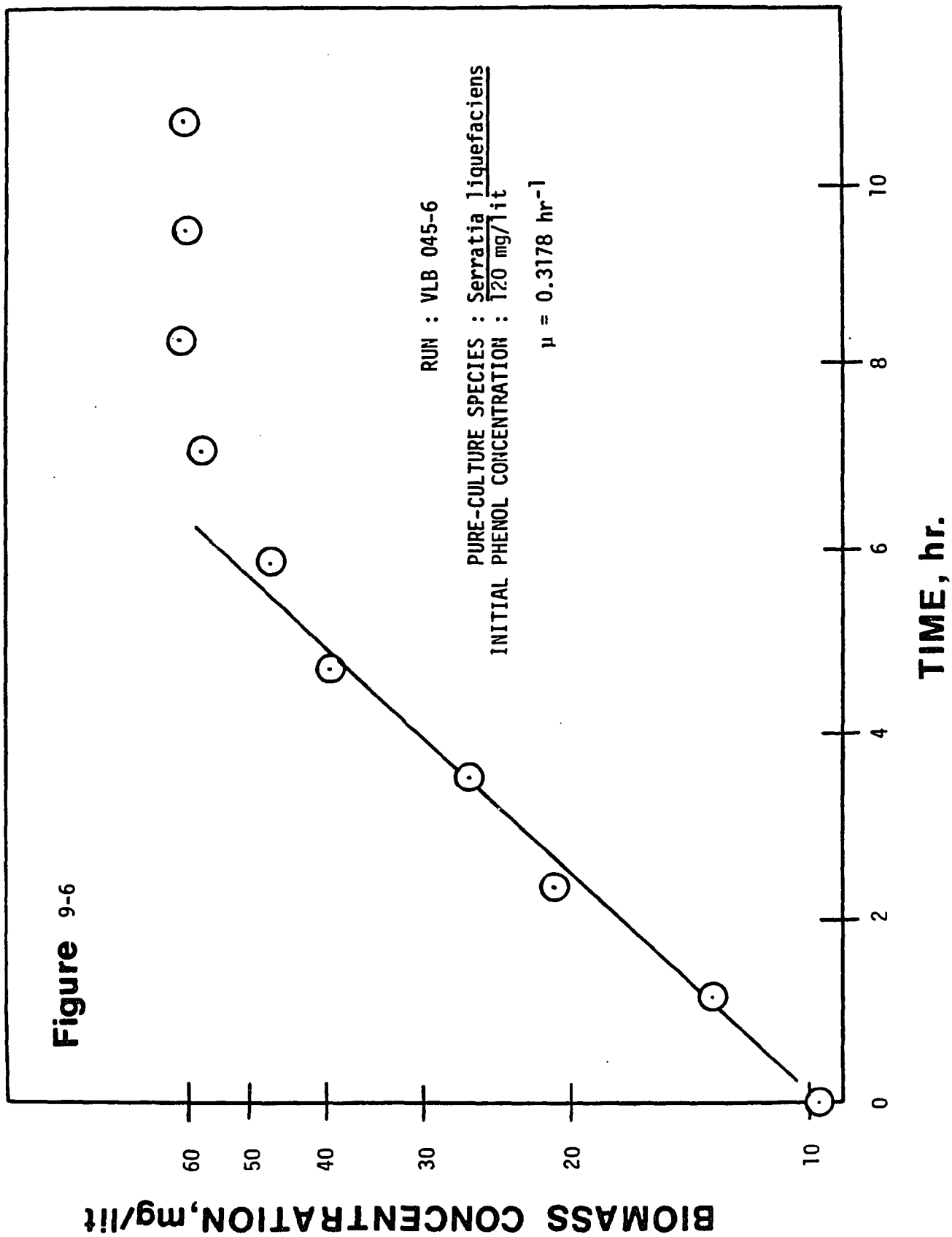

BIOMASS CONCENTRATION, mg/lit
10
20
30
40
50
60
0
2
4
6
8
10
TIME, hr.
RUN : VLB 045-6
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 120 mg/lit
μ = 0.3178 hr⁻¹

Figure 9-6

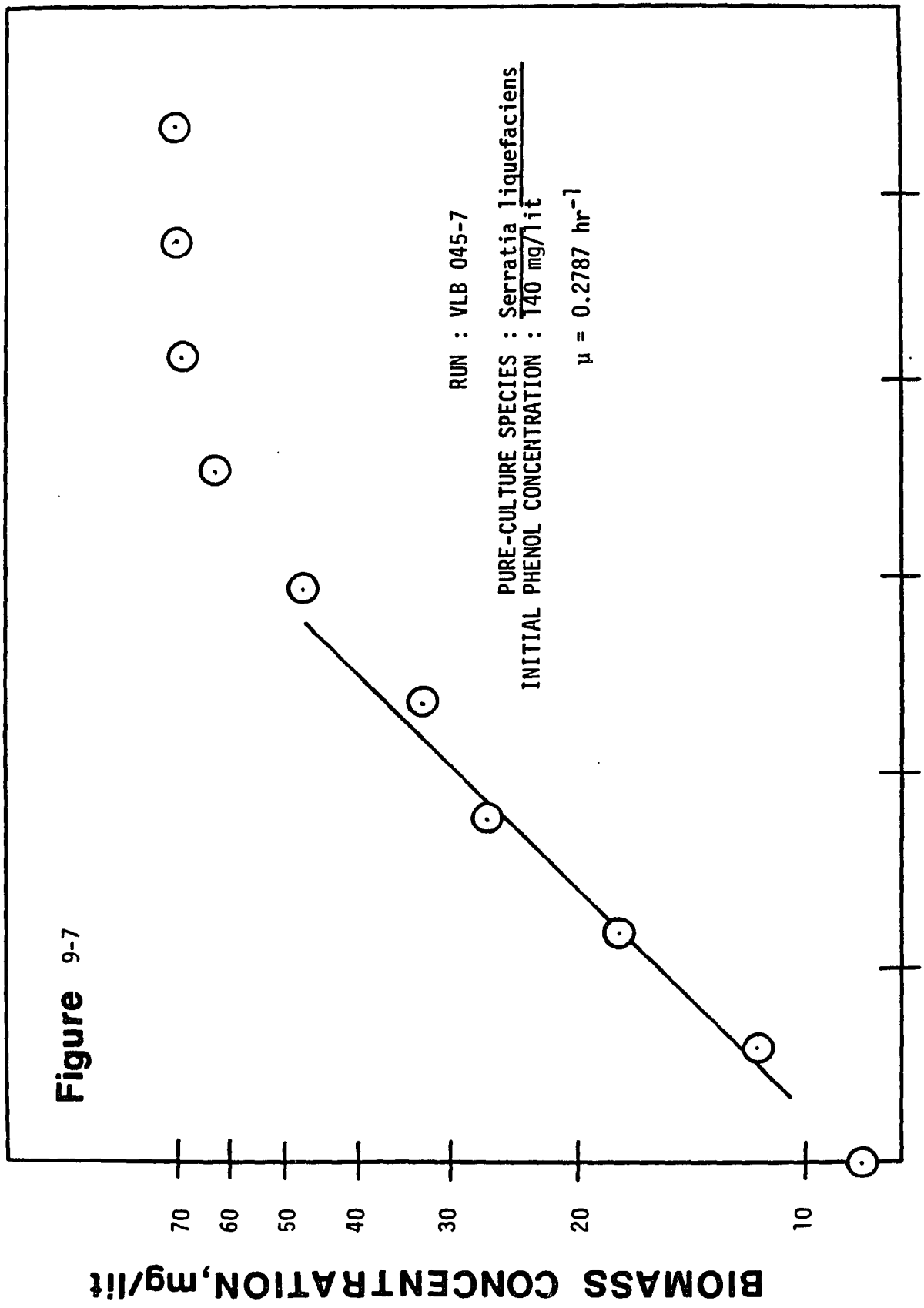

BIOMASS CONCENTRATION, mg/lit
70
60
50
40
30
20
10
0
2
4
6
8
10
TIME, hr.
RUN : VLB 045-7
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 140 mg/lit
μ = 0.2787 hr⁻¹

Figure 9-7

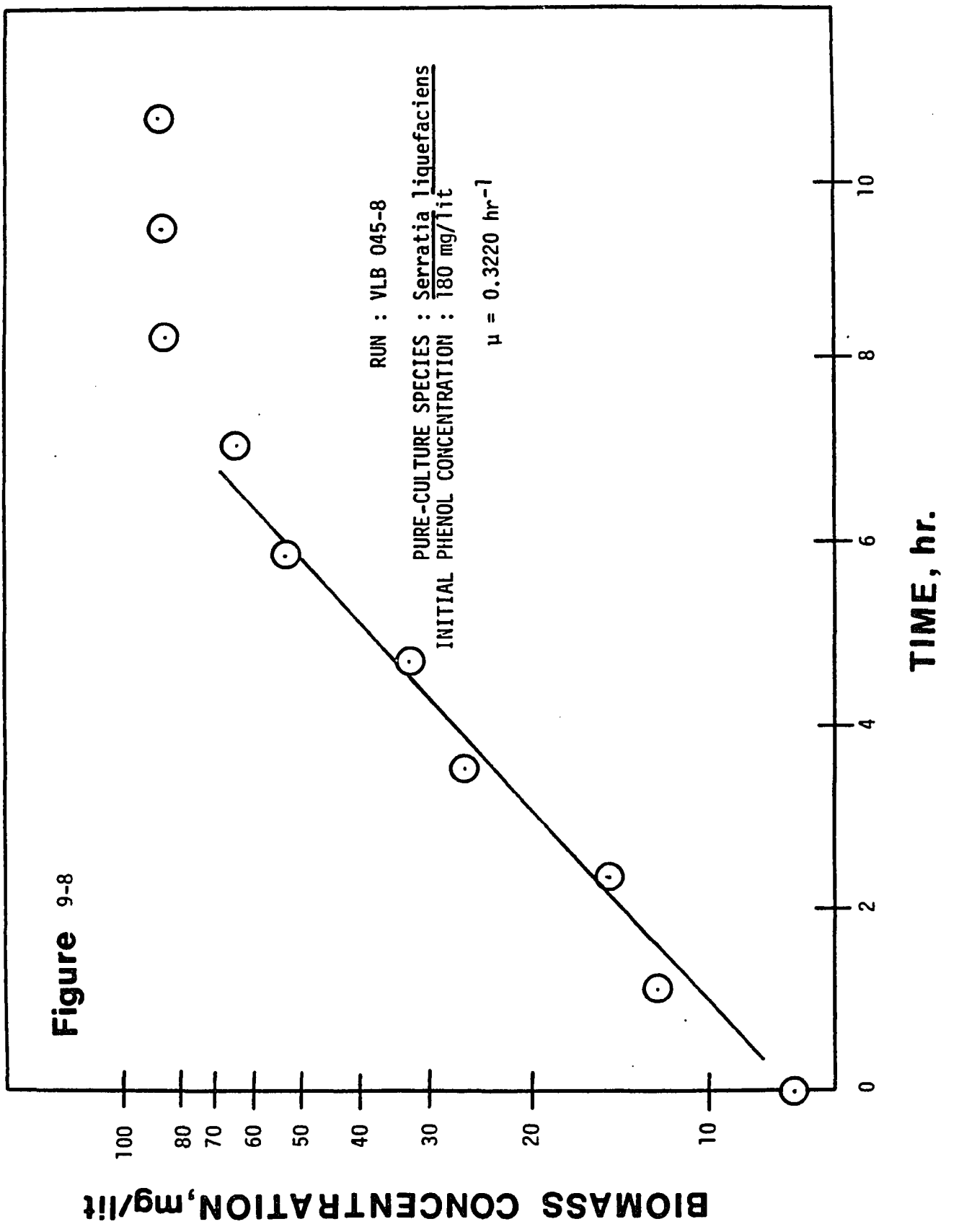

BIOMASS CONCENTRATION, mg/lit
100
80
70
60
50
40
30
20
10
0
2
4
6
8
10
TIME, hr.
RUN : VLB 045-8
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 180 mg/lit
μ = 0.3220 hr⁻¹

Figure 9-8

Figure 10

Growth Curves of *Klebsiella pneumoniae* at the following Initial Phenol Concentrations (Semi-log plots) (Run VLB 049-1 to VLB 049-8)

10-1	20 mg/lit
10-2	40 mg/lit
10-3	50 mg/lit
10-4	80 mg/lit
10-5	100 mg/lit
10-6	120 mg/lit
10-7	140 mg/lit
10-8	180 mg/lit

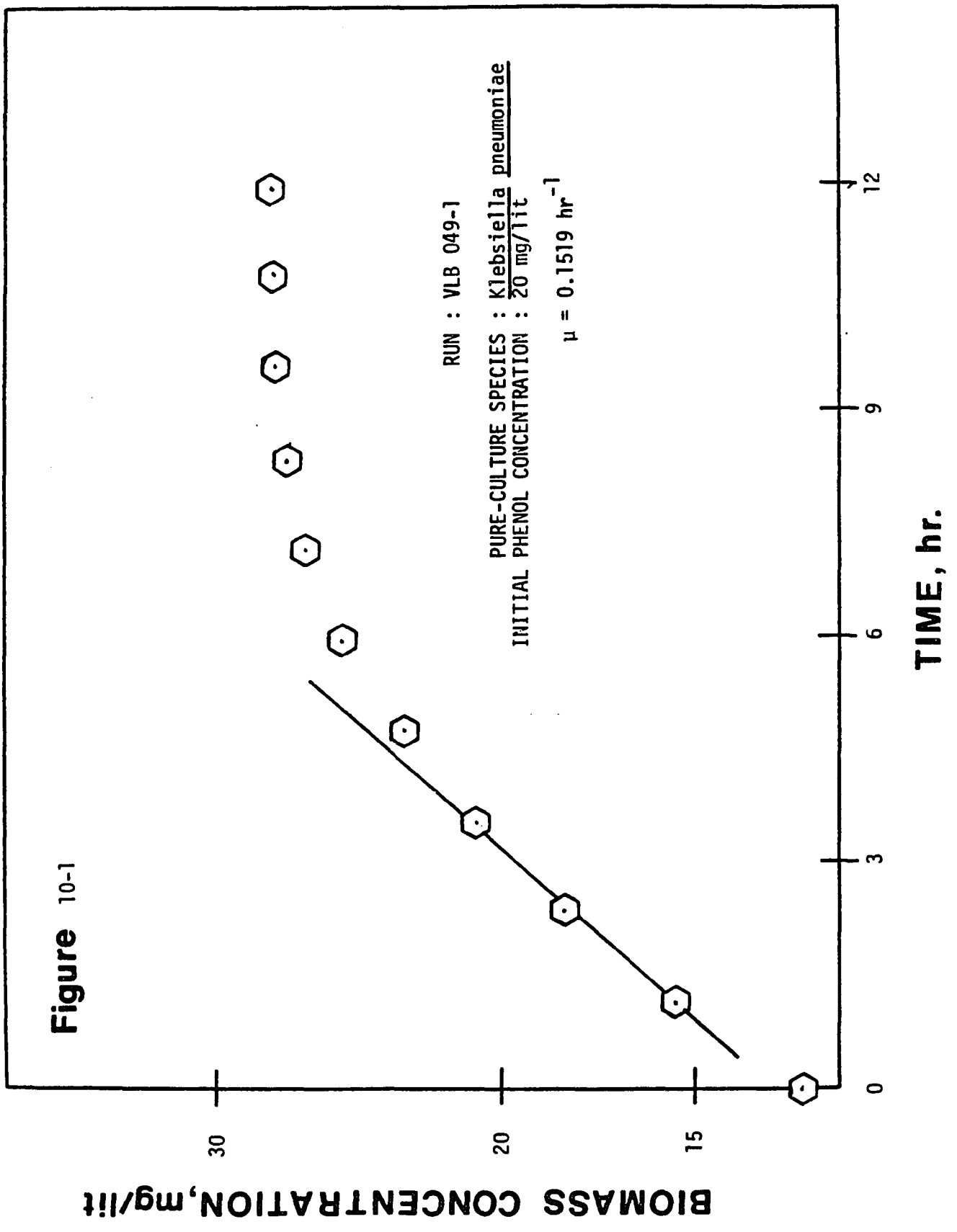

BIOMASS CONCENTRATION, mg/lit
30
20
15
0
3
6
9
12
TIME, hr.
RUN : VLB 049-1
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20 mg/lit
μ = 0.1519 hr⁻¹

Figure 10-1

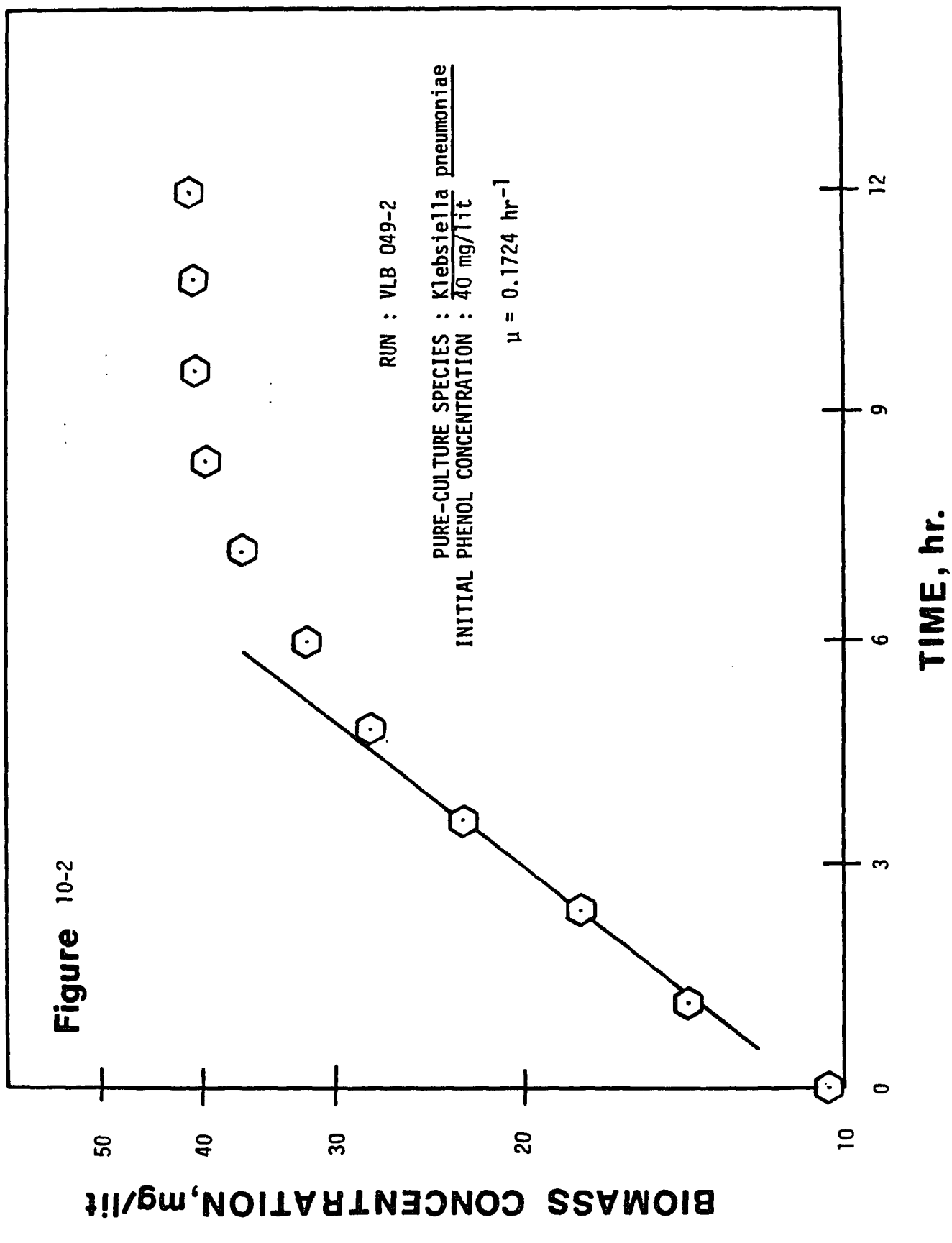

RUN : VLB 049-2
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 40 mg/lit
μ = 0.1724 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
TIME, hr.
10
20
30
40
50
0
3
6
9
12

Figure 10-2

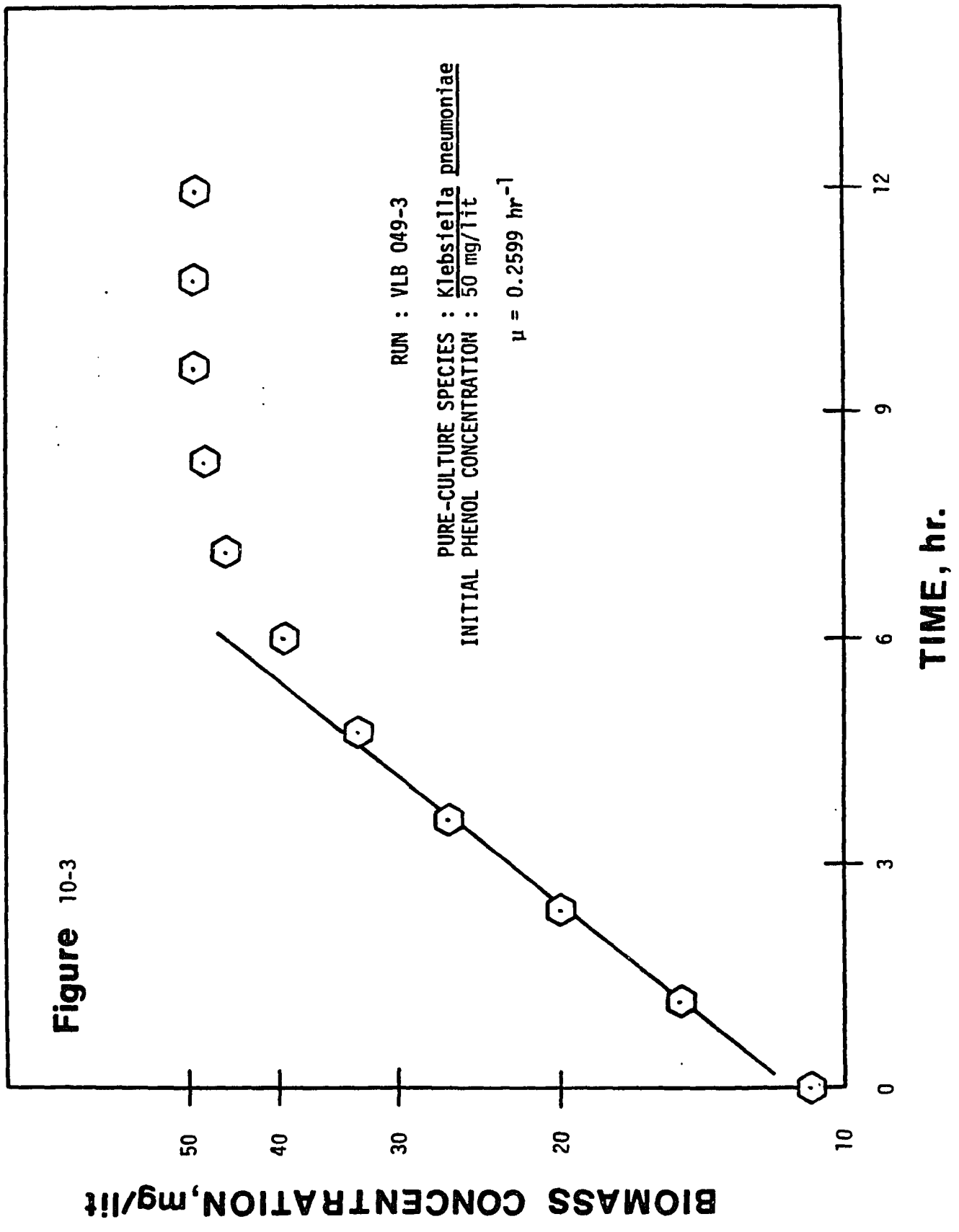

BIOMASS CONCENTRATION, mg/lit
50
40
30
20
10
0
3
6
9
12
TIME, hr.
RUN : VLB 049-3
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 50 mg/lit
μ = 0.2599 hr⁻¹

Figure 10-3

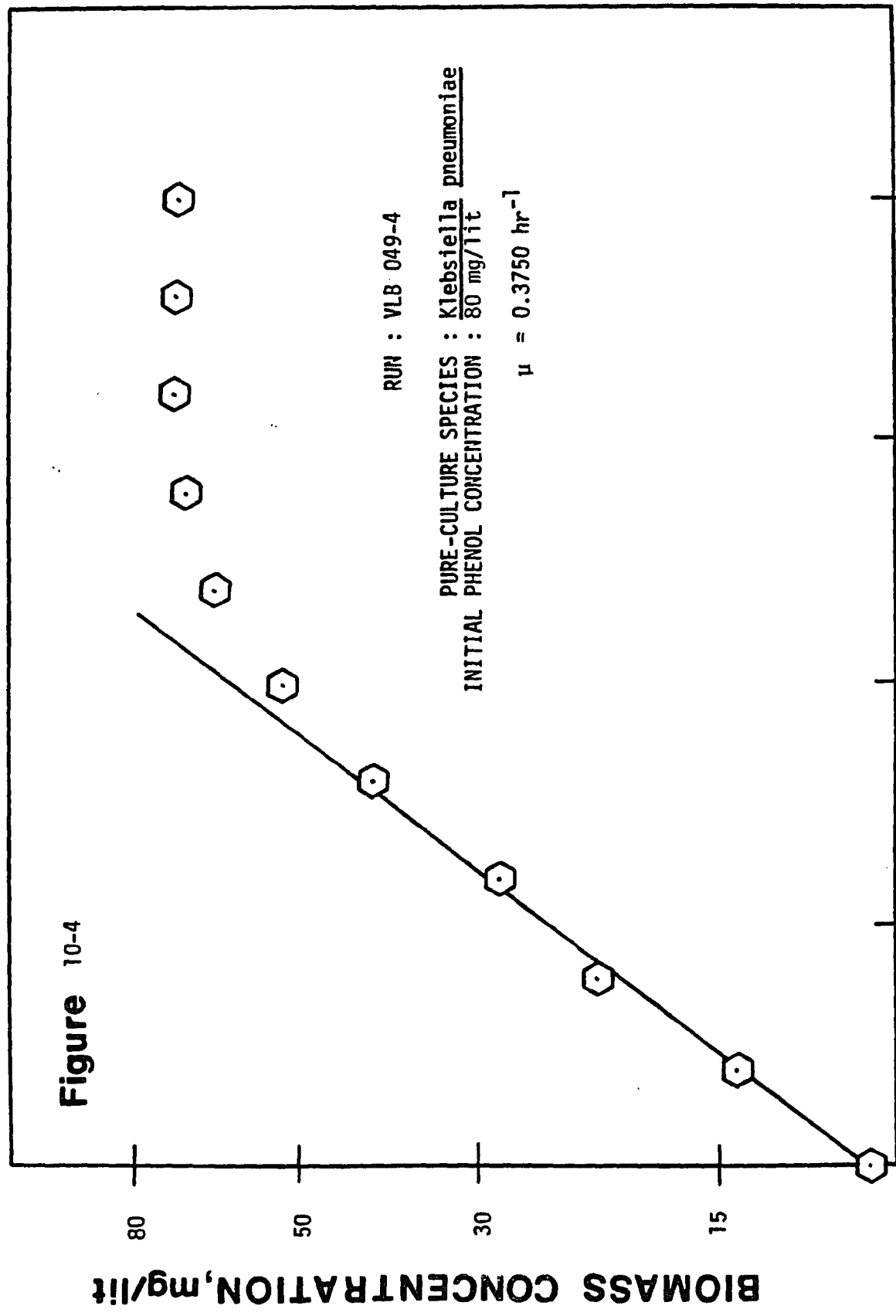

BIOMASS CONCENTRATION, mg/lit
80
50
30
15
0
3
6
9
12
TIME, hr.
RUN : VLB 049-4
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 80 mg/lit
μ = 0.3750 hr⁻¹

Figure 10-4

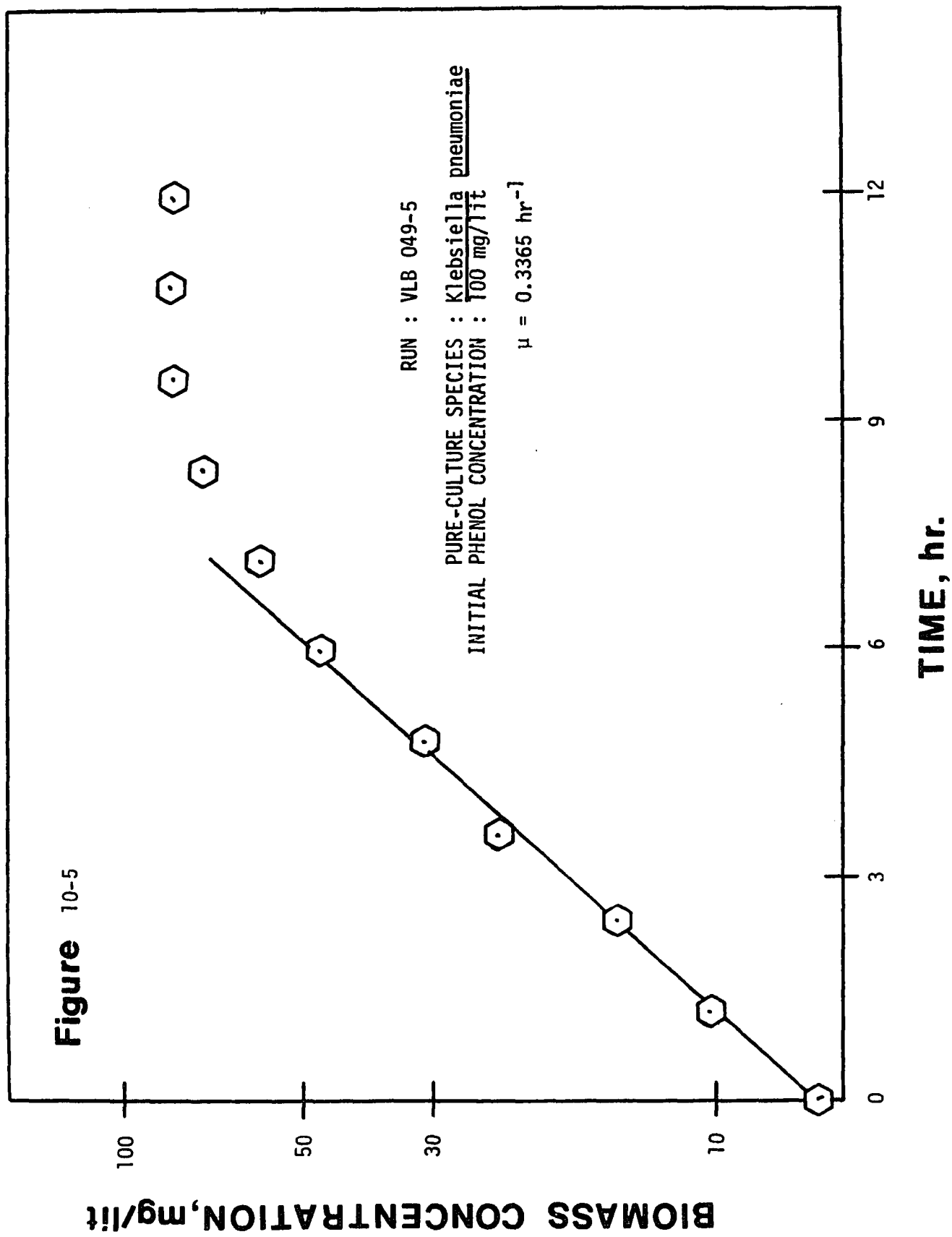

BIOMASS CONCENTRATION, mg/lit
100
50
30
10
0
3
6
9
12
TIME, hr.
RUN : VLB 049-5
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 100 mg/lit
μ = 0.3365 hr⁻¹

Figure 10-5

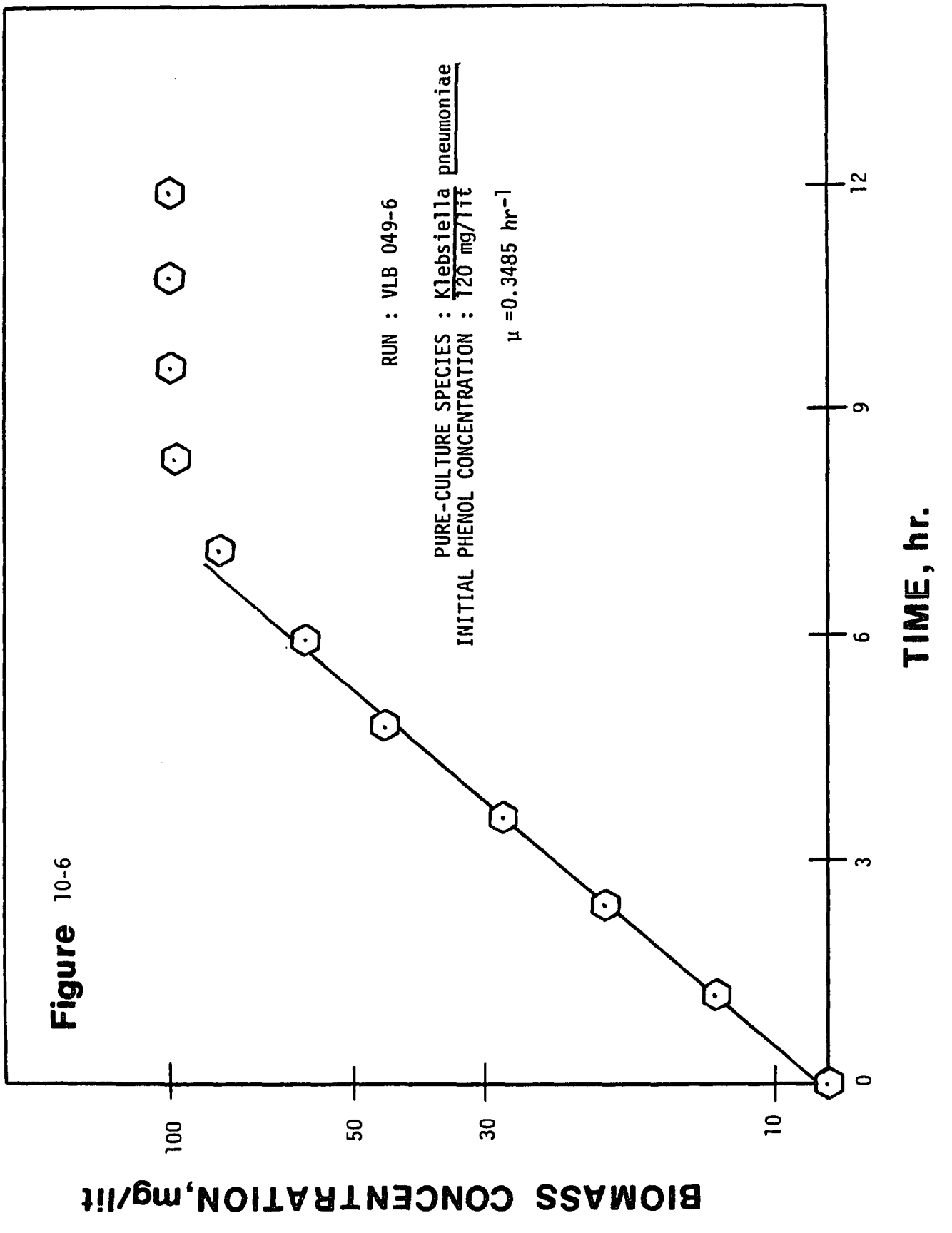

BIOMASS CONCENTRATION, mg/lit
100
50
30
10
0
3
6
9
12
TIME, hr.
RUN : VLB 049-6
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 120 mg/lit
μ =0.3485 hr⁻¹

Figure 10-6

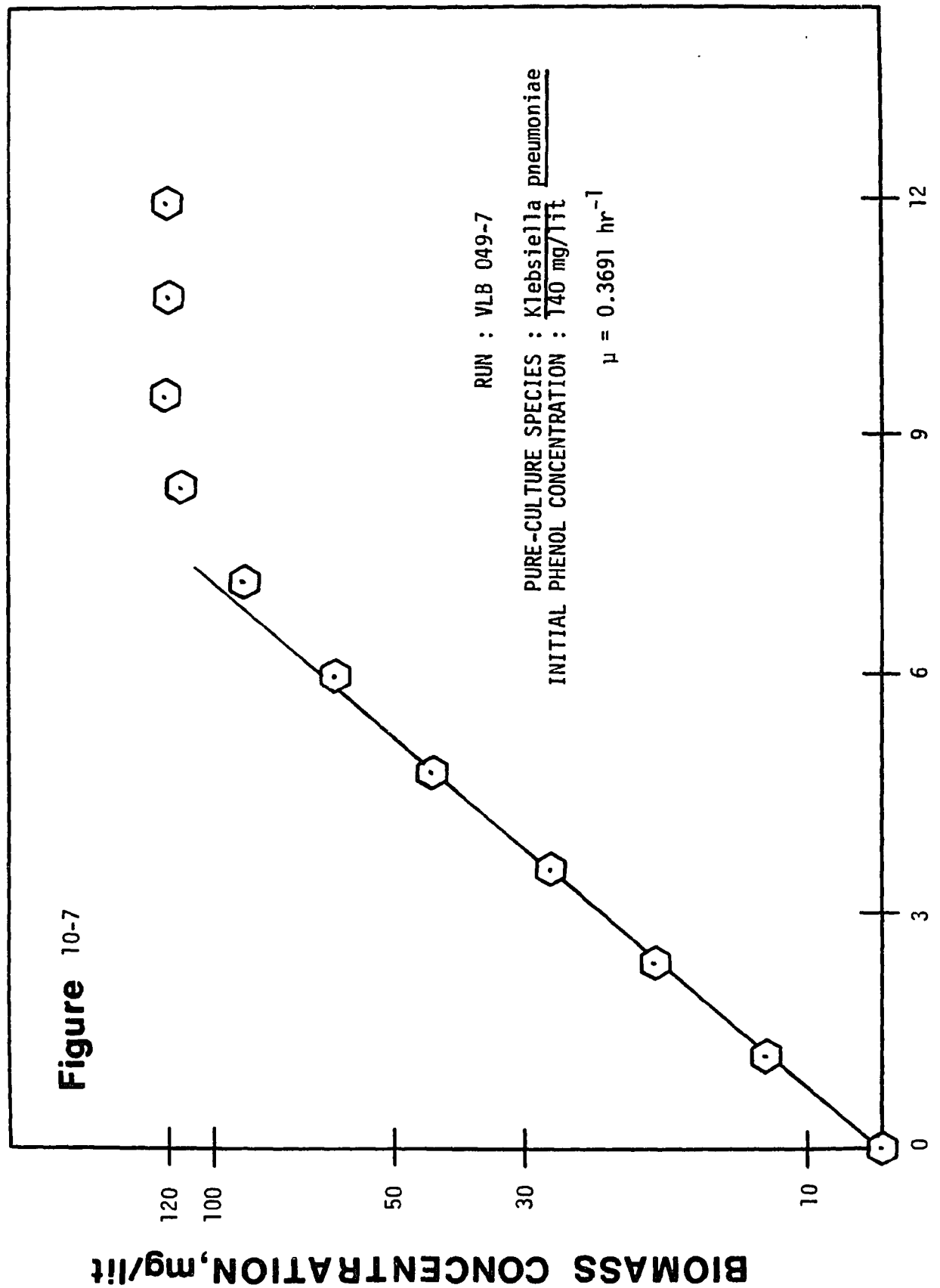

BIOMASS CONCENTRATION, mg/lit
120
100
50
30
10
0
3
6
9
12
TIME, hr.
RUN : VLB 049-7
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 140 mg/lit
μ = 0.3691 hr⁻¹

Figure 10-7

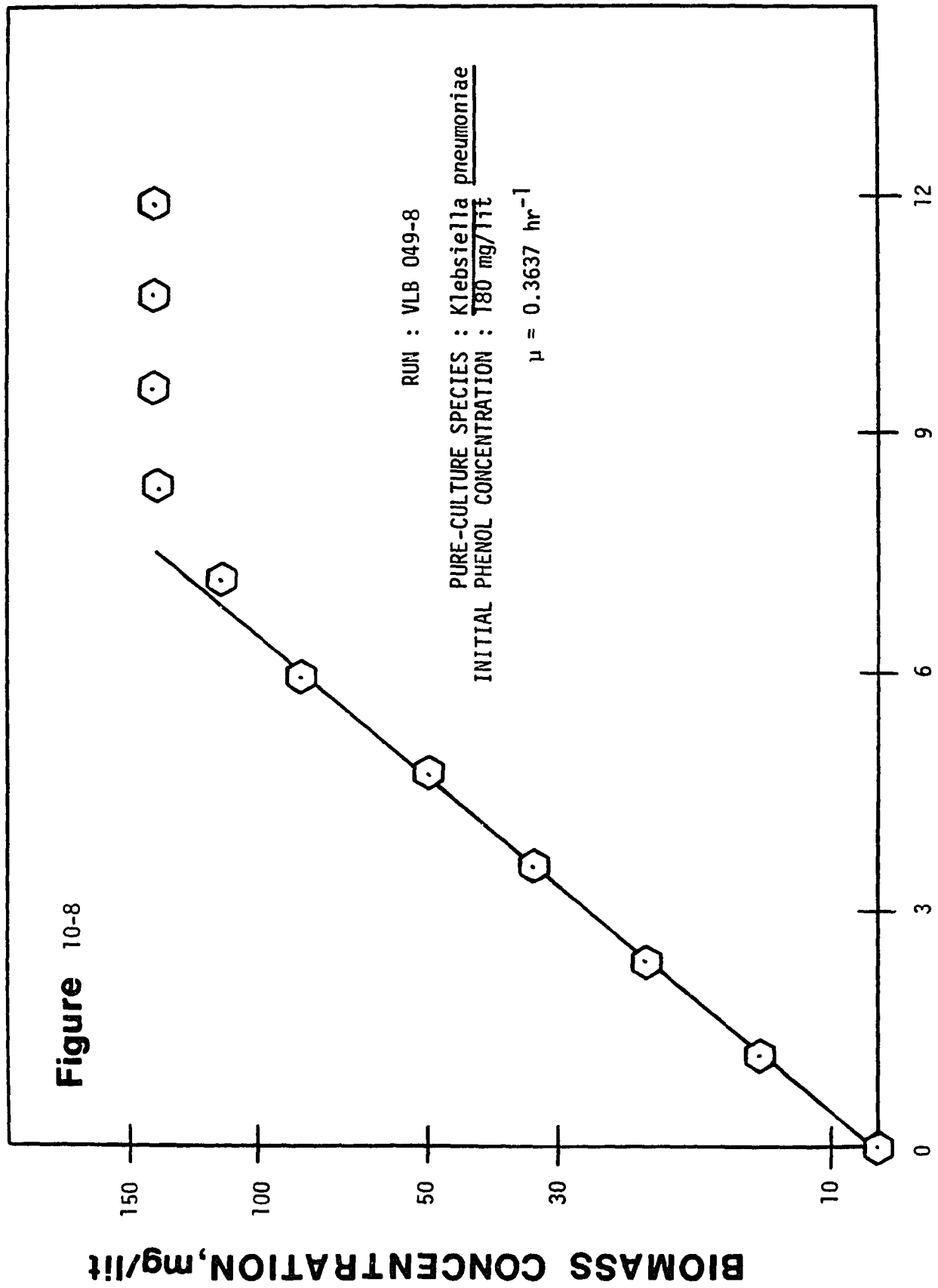

RUN : VLB 049-8
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 180 mg/lit
μ = 0.3637 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
150
100
50
30
10
0
3
6
9
12
TIME, hr.

Figure 10-8

Figure 11

Growth Curves of *Pseudomonas putida* at the following Initial Phenol Concentrations (Semi-log plots) (Run VLB 051-1 to VLB 051-8)

11-1	20 mg/lit
11-2	40 mg/lit
11-3	50 mg/lit
11-4	80 mg/lit
11-5	100 mg/lit
11-6	120 mg/lit
11-7	140 mg/lit
11-8	180 mg/lit

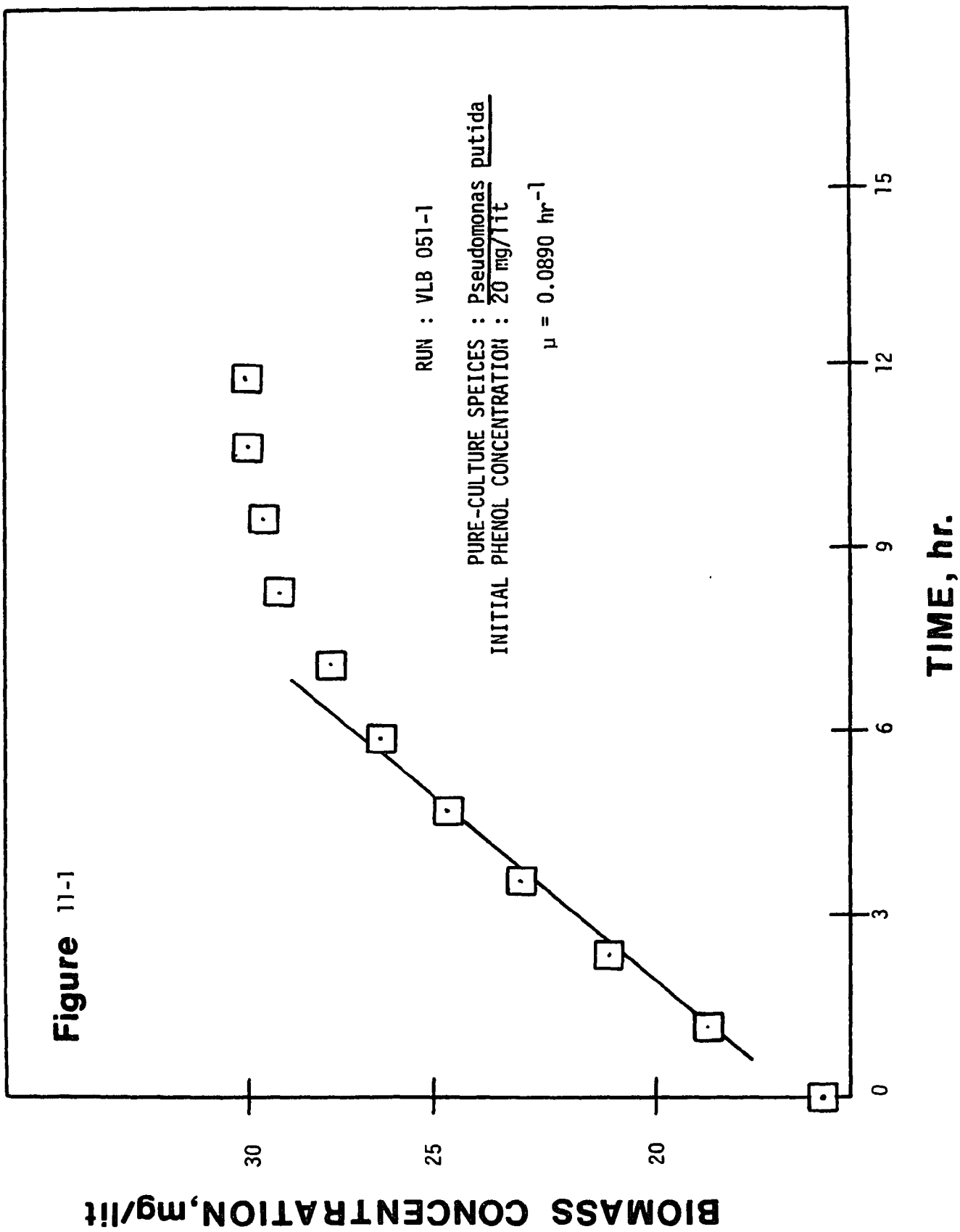

RUN : VLB 051-1
PURE-CULTURE SPEICES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20 mg/lit
μ = 0.0890 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
TIME, hr.
0
3
6
9
12
15
20
25
30

Figure 11-1

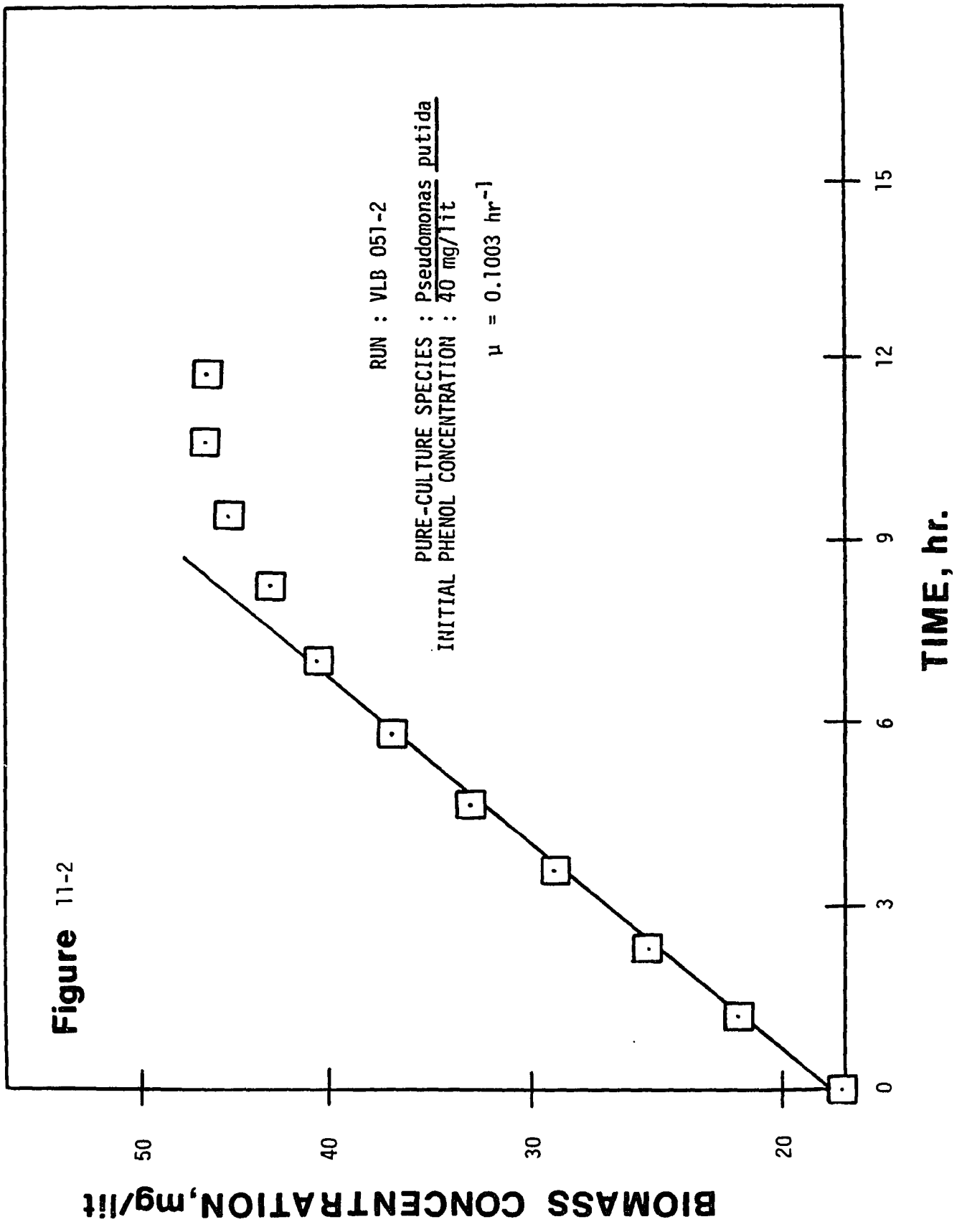

RUN : VLB 051-2
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 40 mg/lit
μ = 0.1003 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
TIME, hr.
20
30
40
50
0
3
6
9
12
15

Figure 11-2

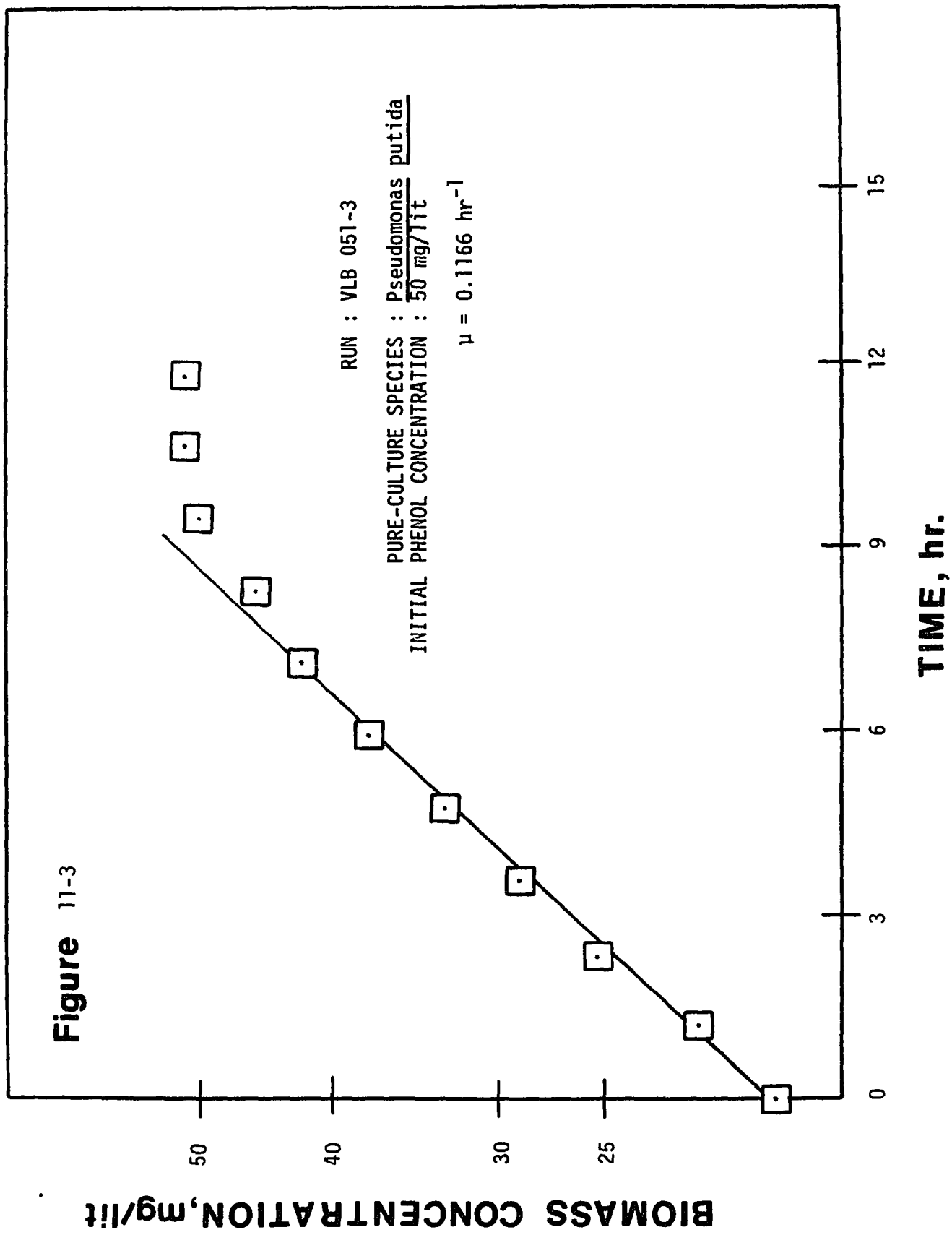

RUN : VLB 051-3
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 50 mg/lit
μ = 0.1166 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
50
40
30
25
0
3
6
9
12
15
TIME, hr.

Figure 11-3

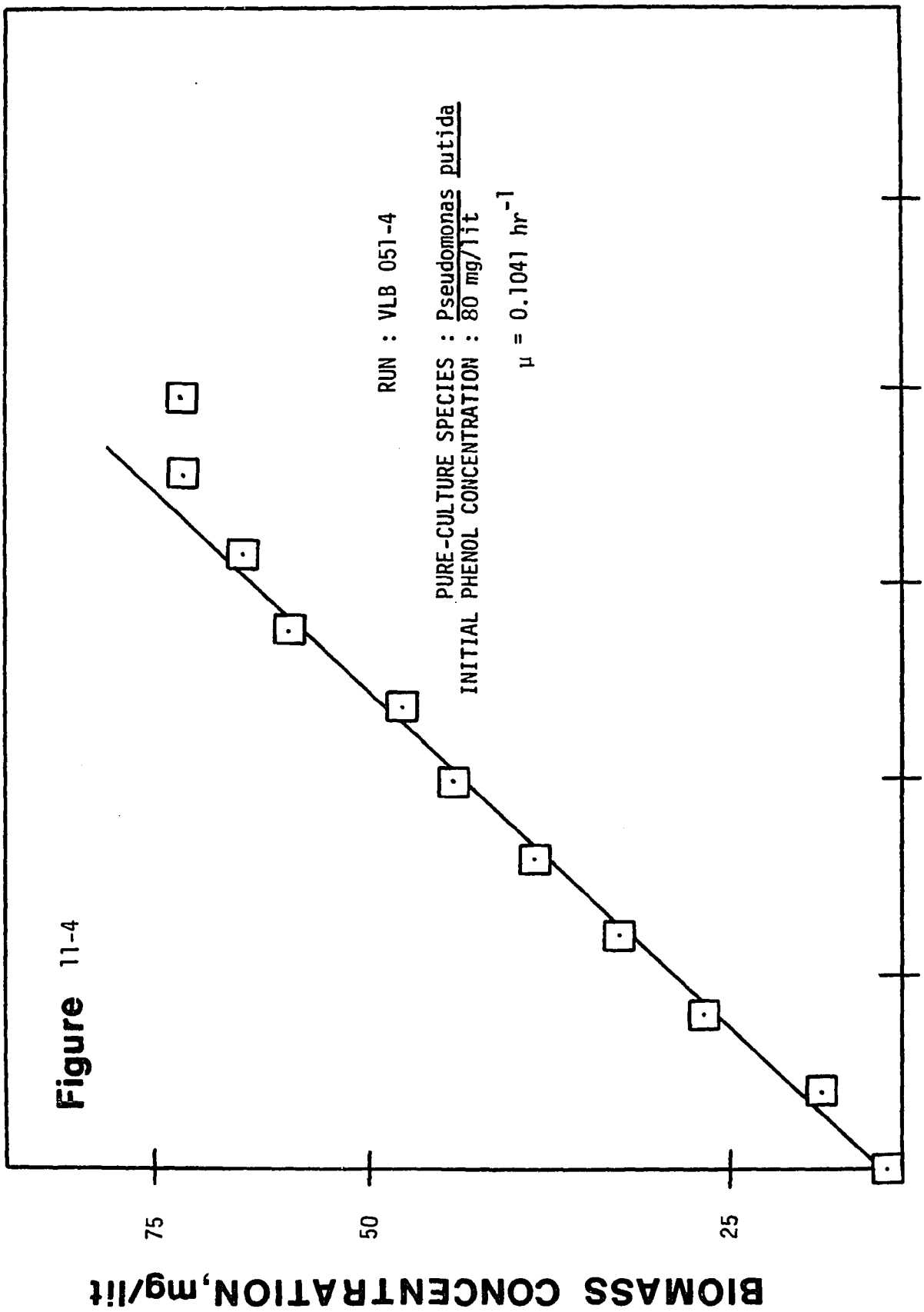

RUN : VLB 051-4
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 80 mg/lit
μ = 0.1041 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
TIME, hr.
0
3
6
9
12
15
25
50
75

Figure 11-4

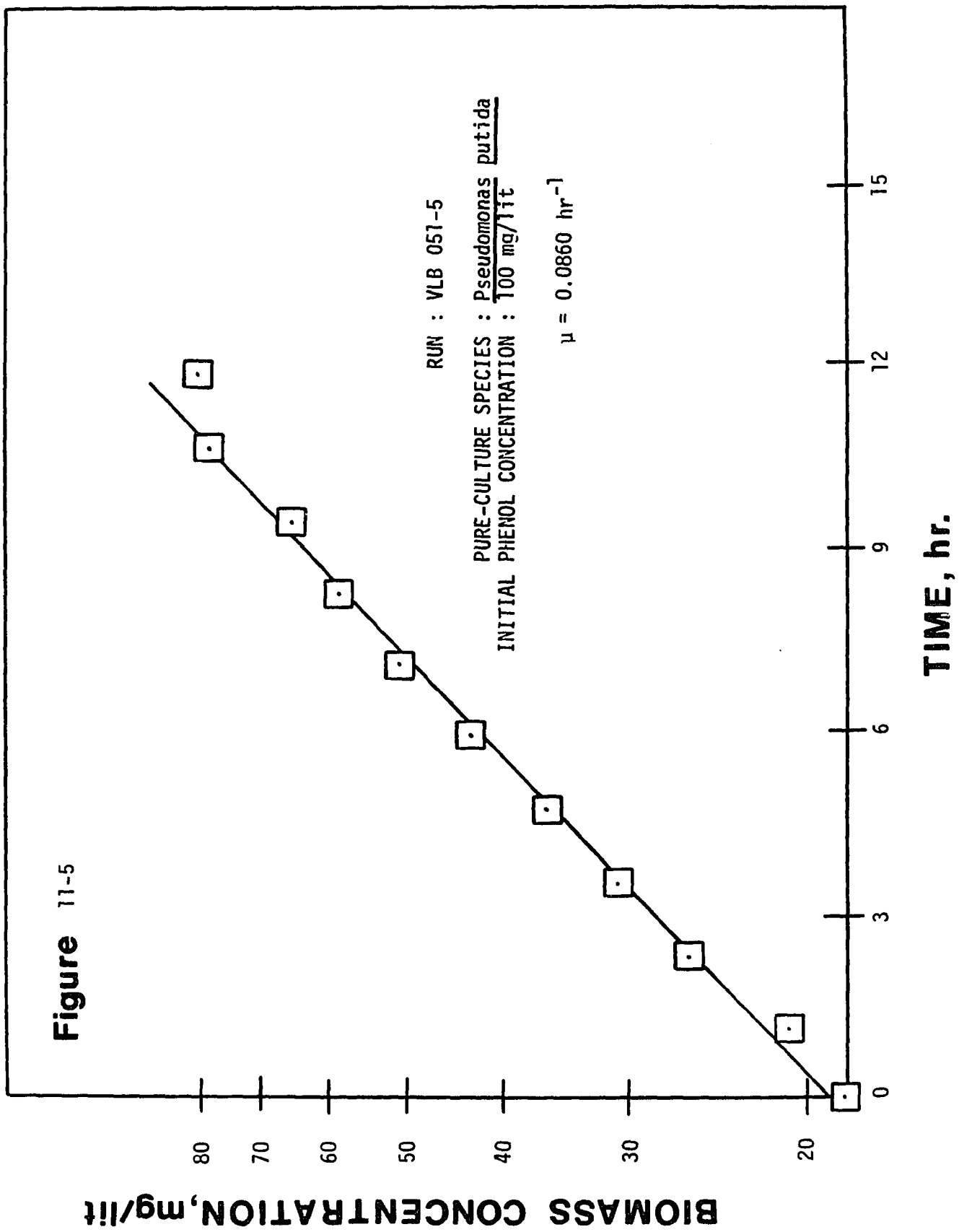

BIOMASS CONCENTRATION, mg/lit
20
30
40
50
60
70
80
0
3
6
9
12
15
TIME, hr.
RUN : VLB 051-5
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 100 mg/lit
μ = 0.0860 hr⁻¹

Figure 11-5

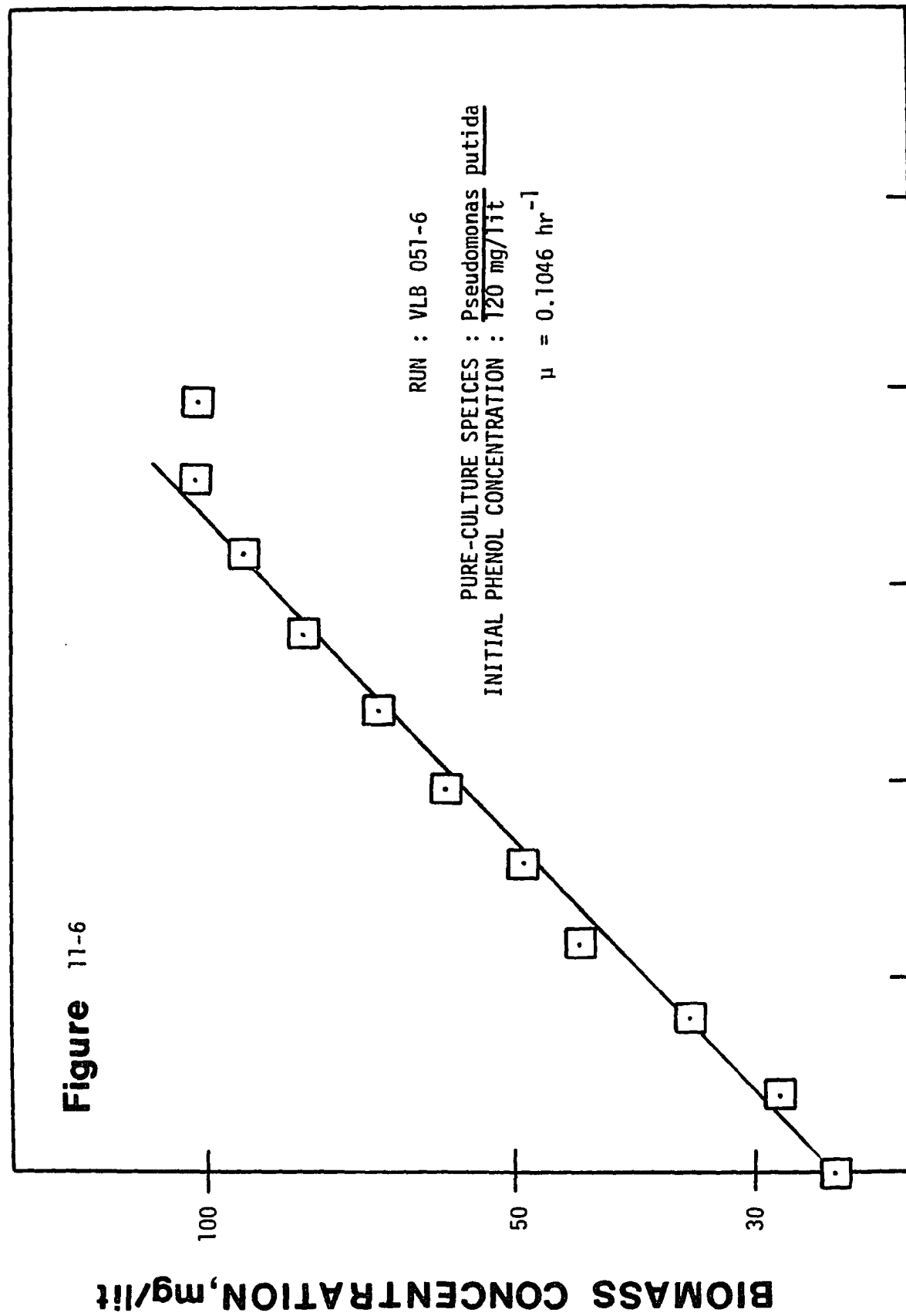

BIOMASS CONCENTRATION, mg/lit
100
50
30
0
3
6
9
12
15
TIME, hr.
RUN : VLB 051-6
PURE-CULTURE SPEICES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 120 mg/lit
μ = 0.1046 hr⁻¹

Figure 11-6

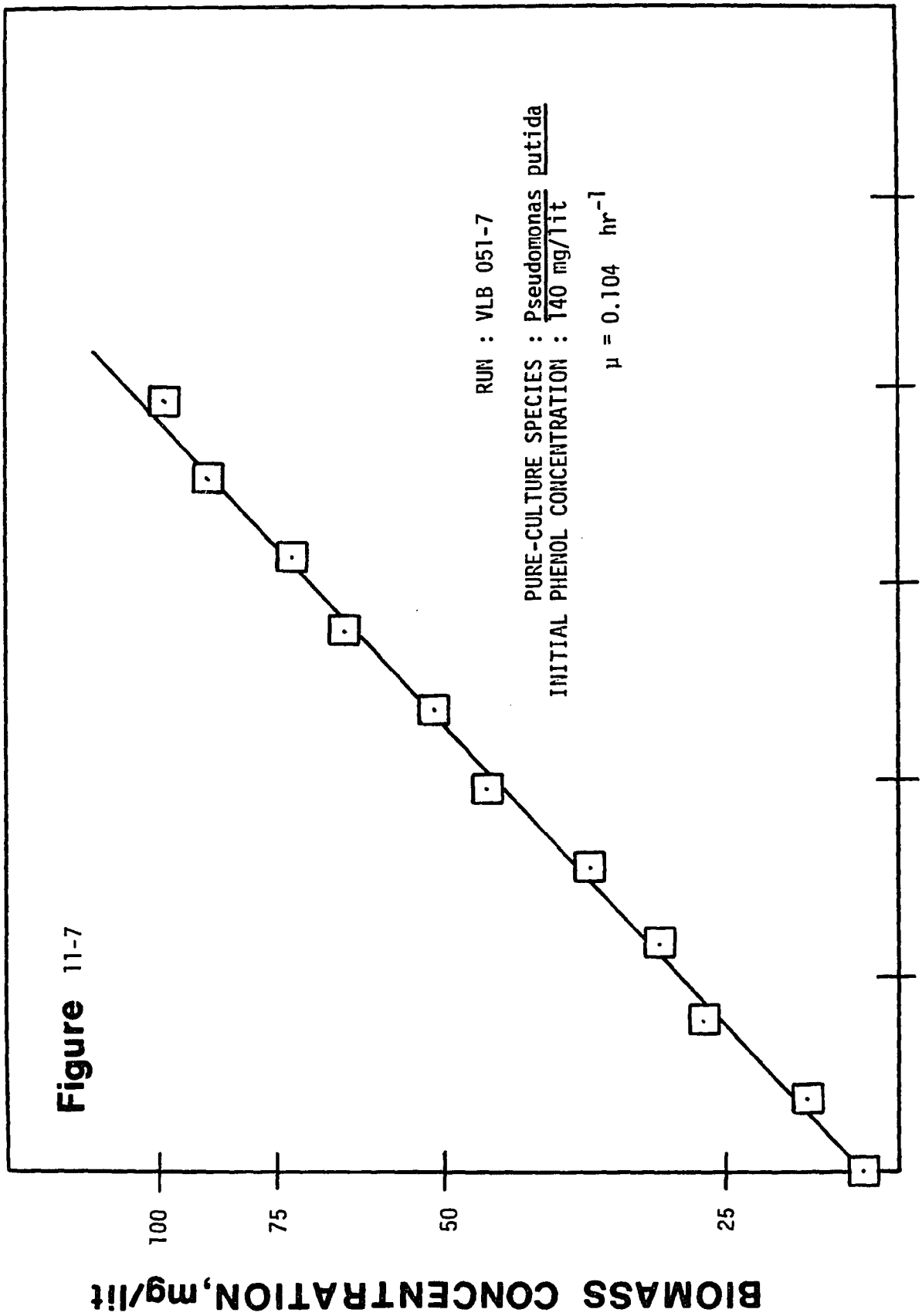

RUN : VLB 051-7
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 140 mg/lit
μ = 0.104 hr⁻¹
BIOMASS CONCENTRATION, mg/lit
100
75
50
25
0
3
6
9
12
15
TIME, hr.

Figure 11-7

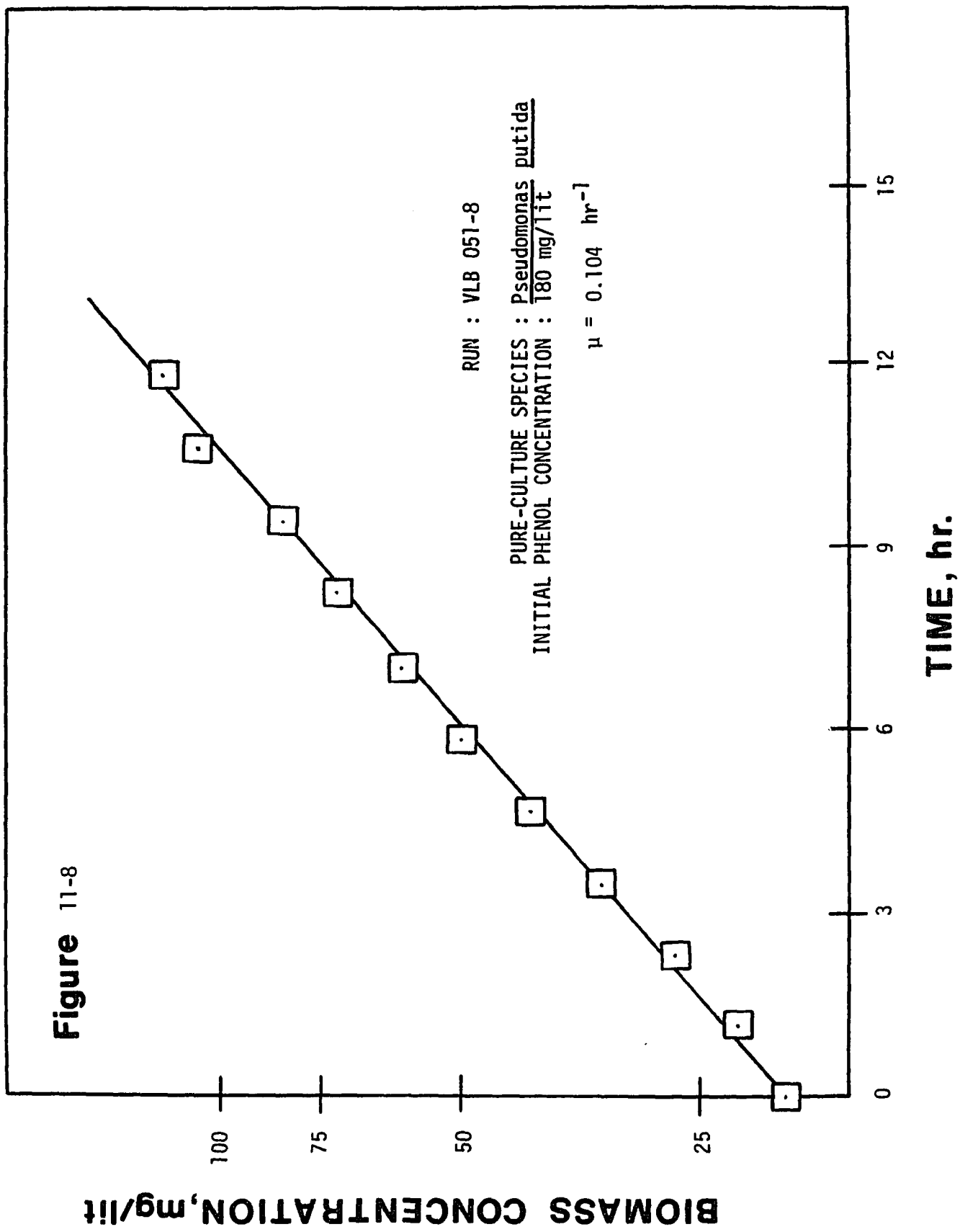

BIOMASS CONCENTRATION, mg/lit
100
75
50
25
0
3
6
9
12
15
TIME, hr.
RUN : VLB 051-8
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 180 mg/lit
μ = 0.104 hr⁻¹

Figure 11-8

Figure 12

Phenol Degradation of Serratia liquefaciens at the following Initial Phenol Concentrations (Run VLB 045-1 to VLB 045-8)

12-1	20 mg/lit
12-2	40 mg/lit
12-3	50 mg/lit
12-4	80 mg/lit
12-5	100 mg/lit
12-6	120 mg/lit
12-7	140 mg/lit
12-8	180 mg/lit.

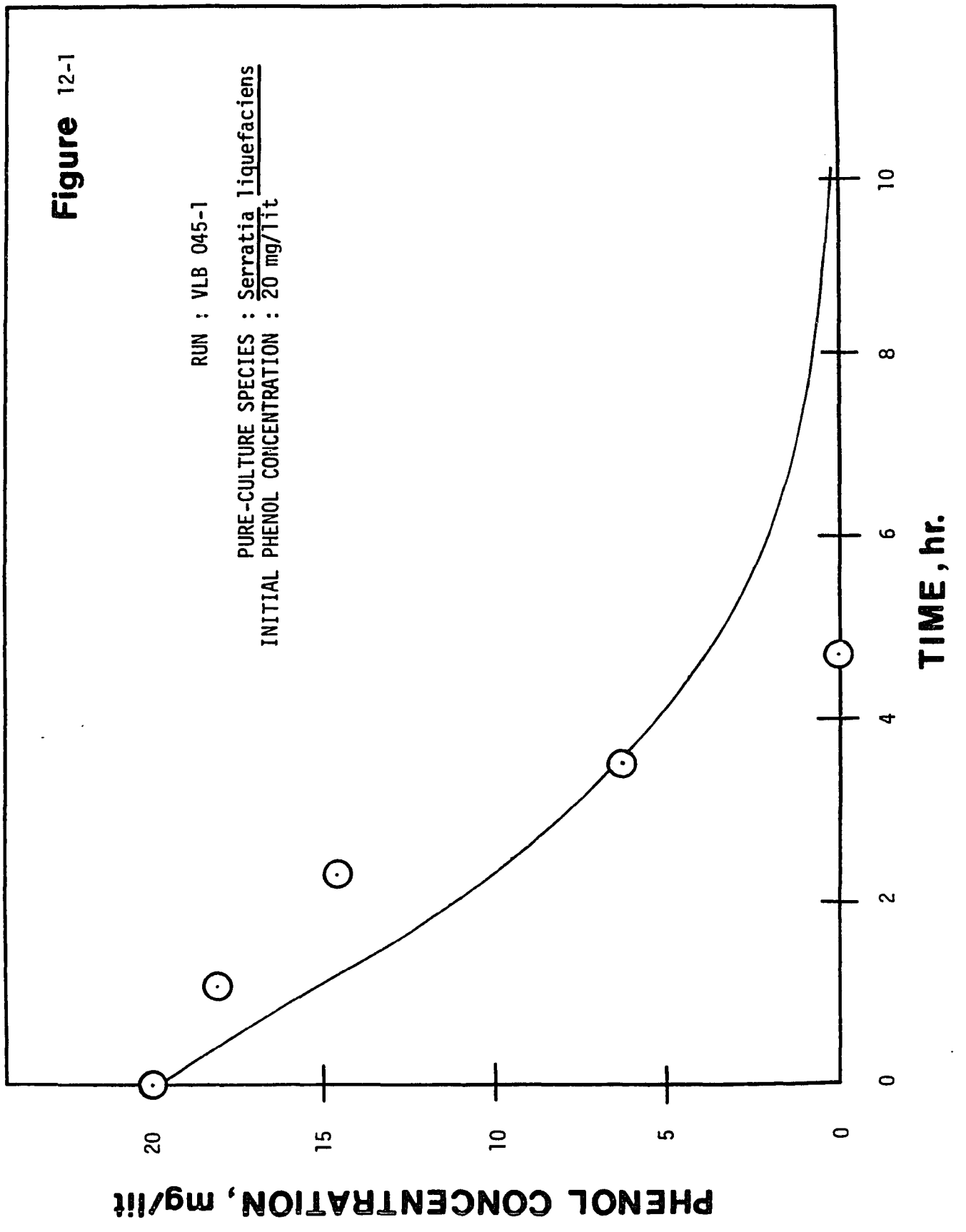
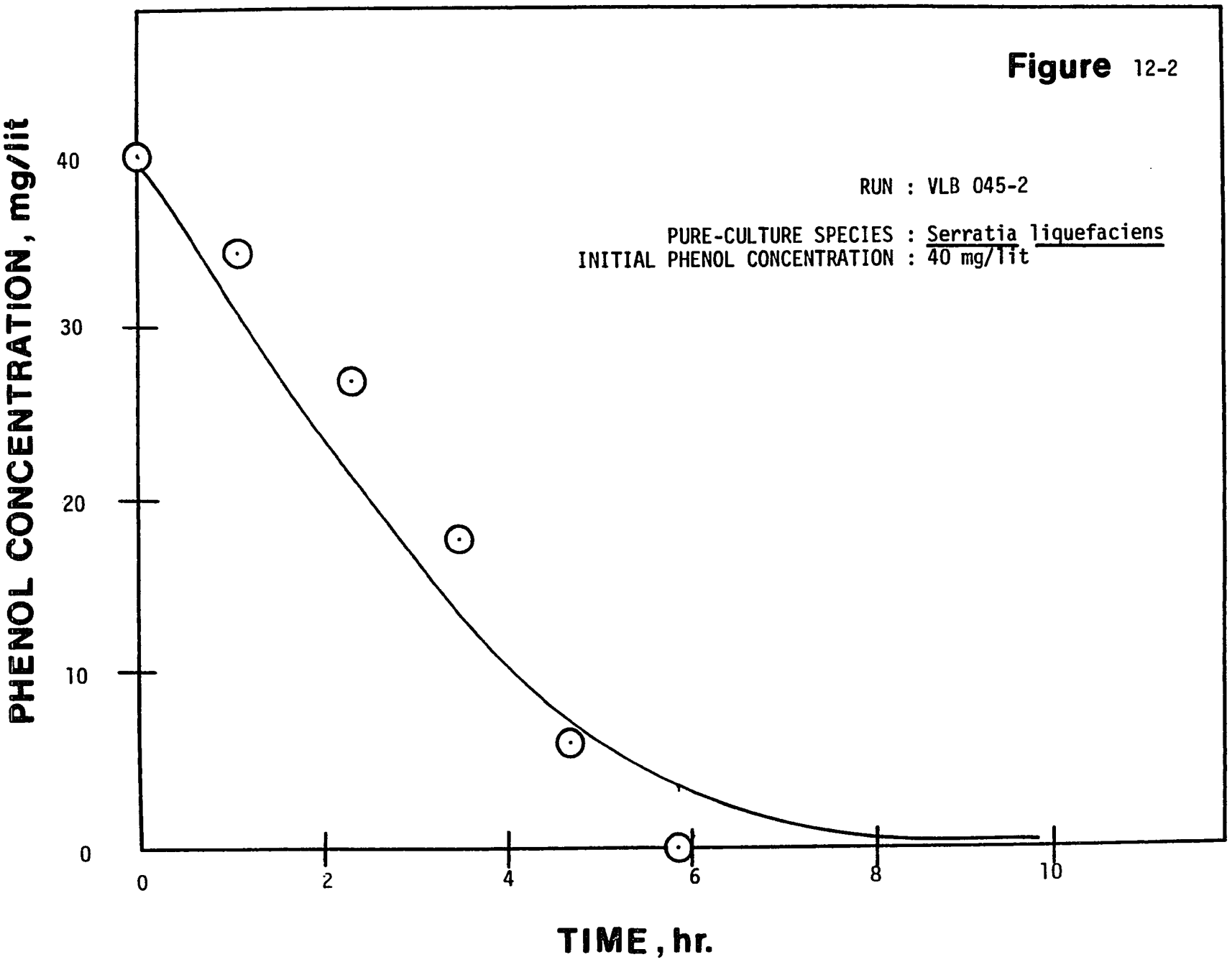


Figure 12-1
RUN : VLB 045-1
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 20 mg/lit
PHENOL CONCENTRATION, mg/lit
0
5
10
15
20
0
2
4
6
8
10
TIME, hr.

Figure 12-2


RUN : VLB 045-2
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 40 mg/lit
PHENOL CONCENTRATION, mg/lit
40
30
20
10
0
0
2
4
6
8
10
TIME, hr.

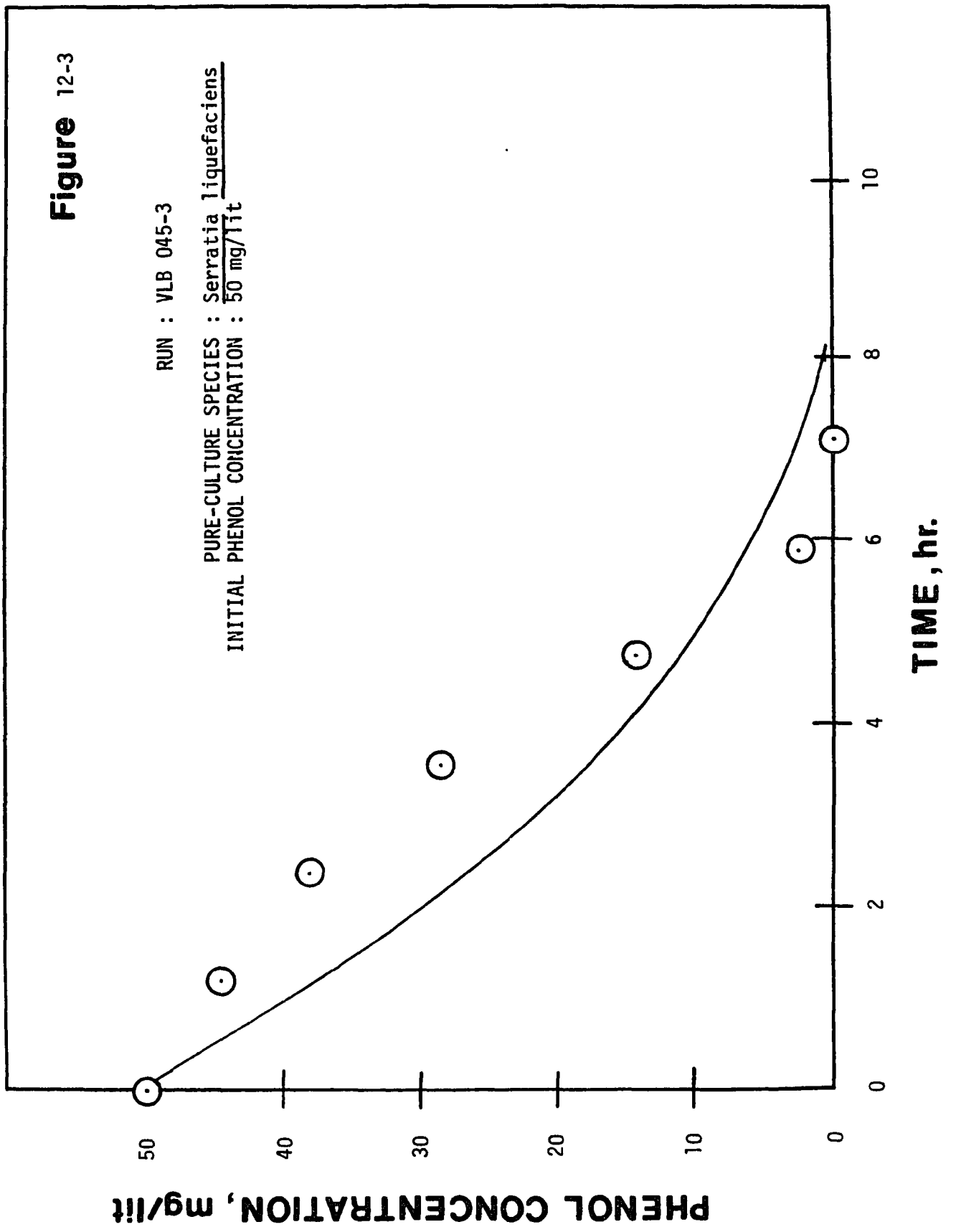


Figure 12-3
RUN : VLB 045-3
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 50 mg/lit
PHENOL CONCENTRATION, mg/lit
0
10
20
30
40
50
0
2
4
6
8
10
TIME, hr.

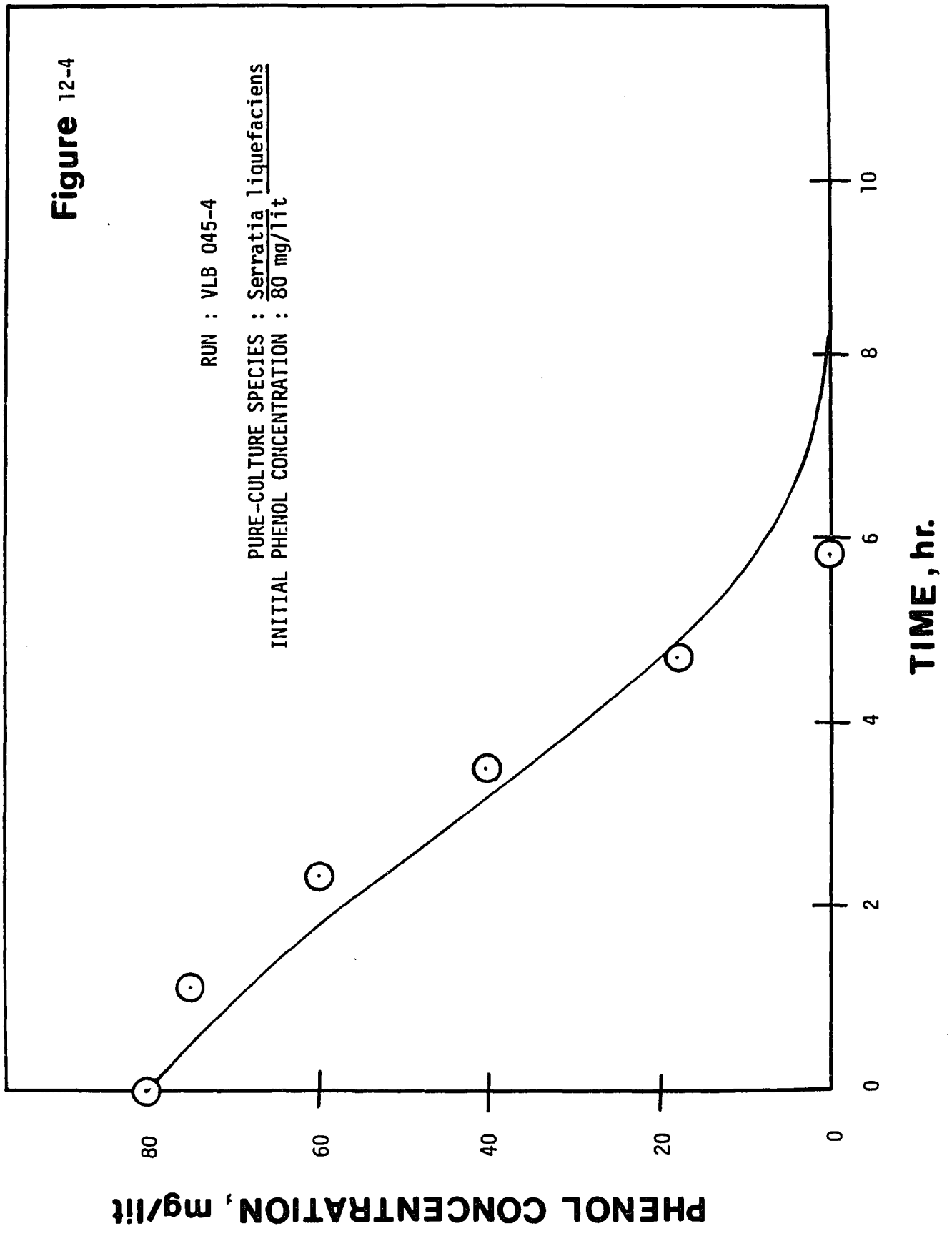


Figure 12-4
RUN : VLB 045-4
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 80 mg/lit
PHENOL CONCENTRATION, mg/lit
0
20
40
60
80
0
2
4
6
8
10
TIME, hr.

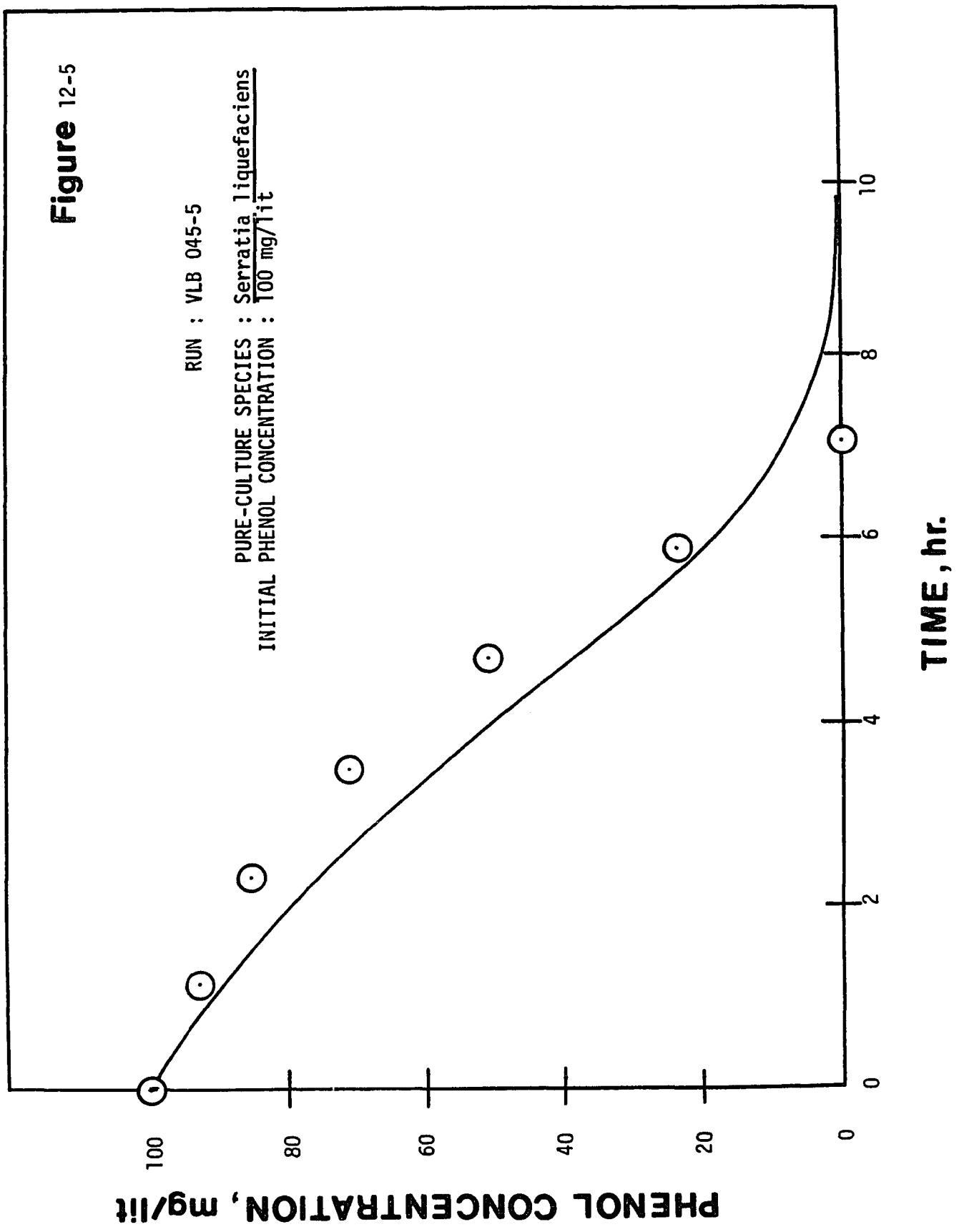


Figure 12-5
RUN : VLB 045-5
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 100 mg/lit
PHENOL CONCENTRATION, mg/lit
0
20
40
60
80
100
0
2
4
6
8
10
TIME, hr.

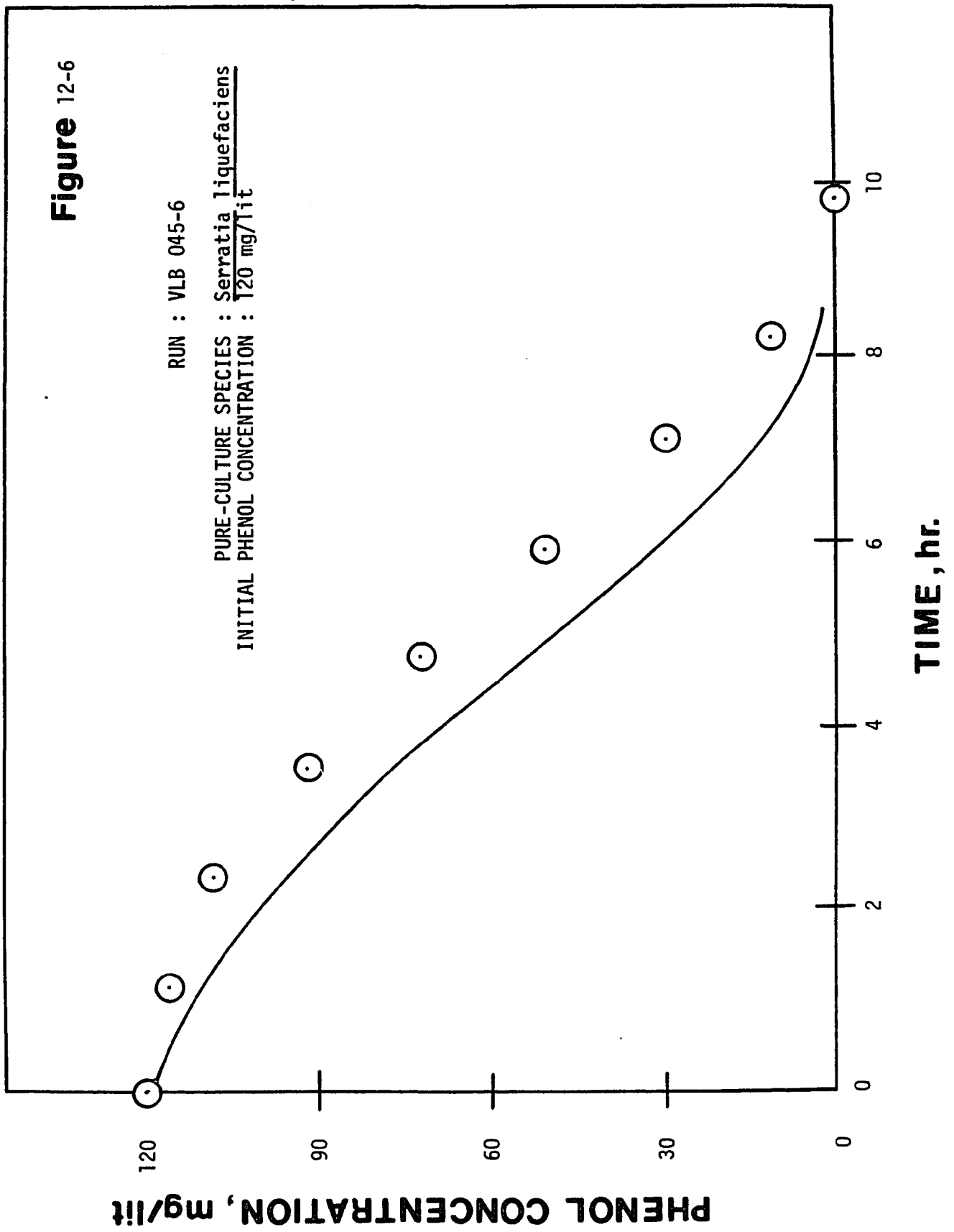


Figure 12-6
RUN : VLB 045-6
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 120 mg/lit
PHENOL CONCENTRATION, mg/lit
120
90
60
30
0
0
2
4
6
8
10
TIME, hr.

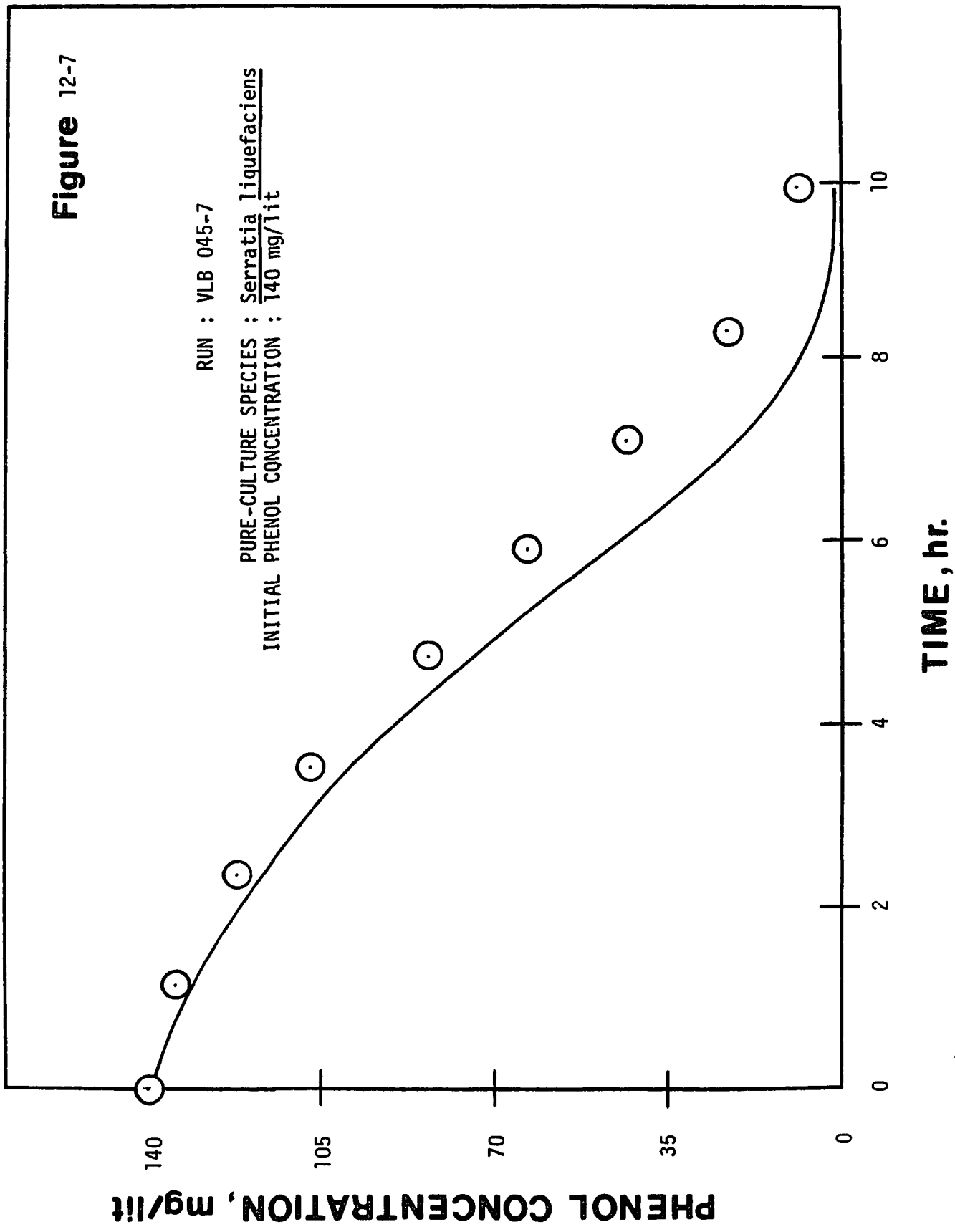


Figure 12-7
RUN : VLB 045-7
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 140 mg/lit
PHENOL CONCENTRATION, mg/lit
140
105
70
35
0
0
2
4
6
8
10
TIME, hr.

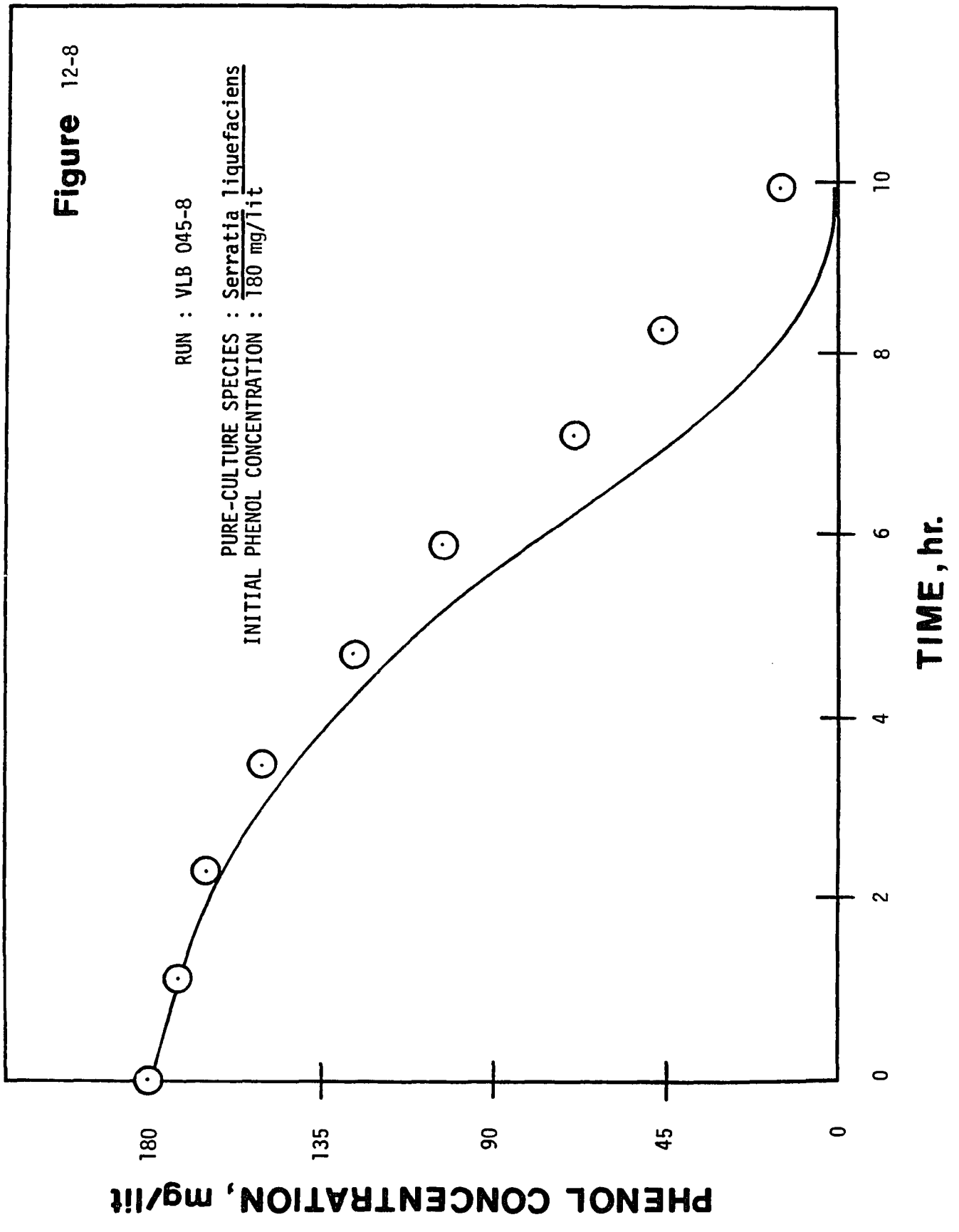


Figure 12-8
RUN : VLB 045-8
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 180 mg/lit
PHENOL CONCENTRATION, mg/lit
180
135
90
45
0
0
2
4
6
8
10
TIME, hr.

Figure 13

Phenol Degradation of Klebsiella pneumoniae at the following Initial Phenol Concentrations (Run VLB 049-1 to VLB 049-8)

13-1	20 mg/lit
13-2	40 mg/lit
13-3	50 mg/lit
13-4	80 mg/lit
13-5	100 mg/lit
13-6	120 mg/lit
13-7	140 mg/lit
13-8	180 mg/lit

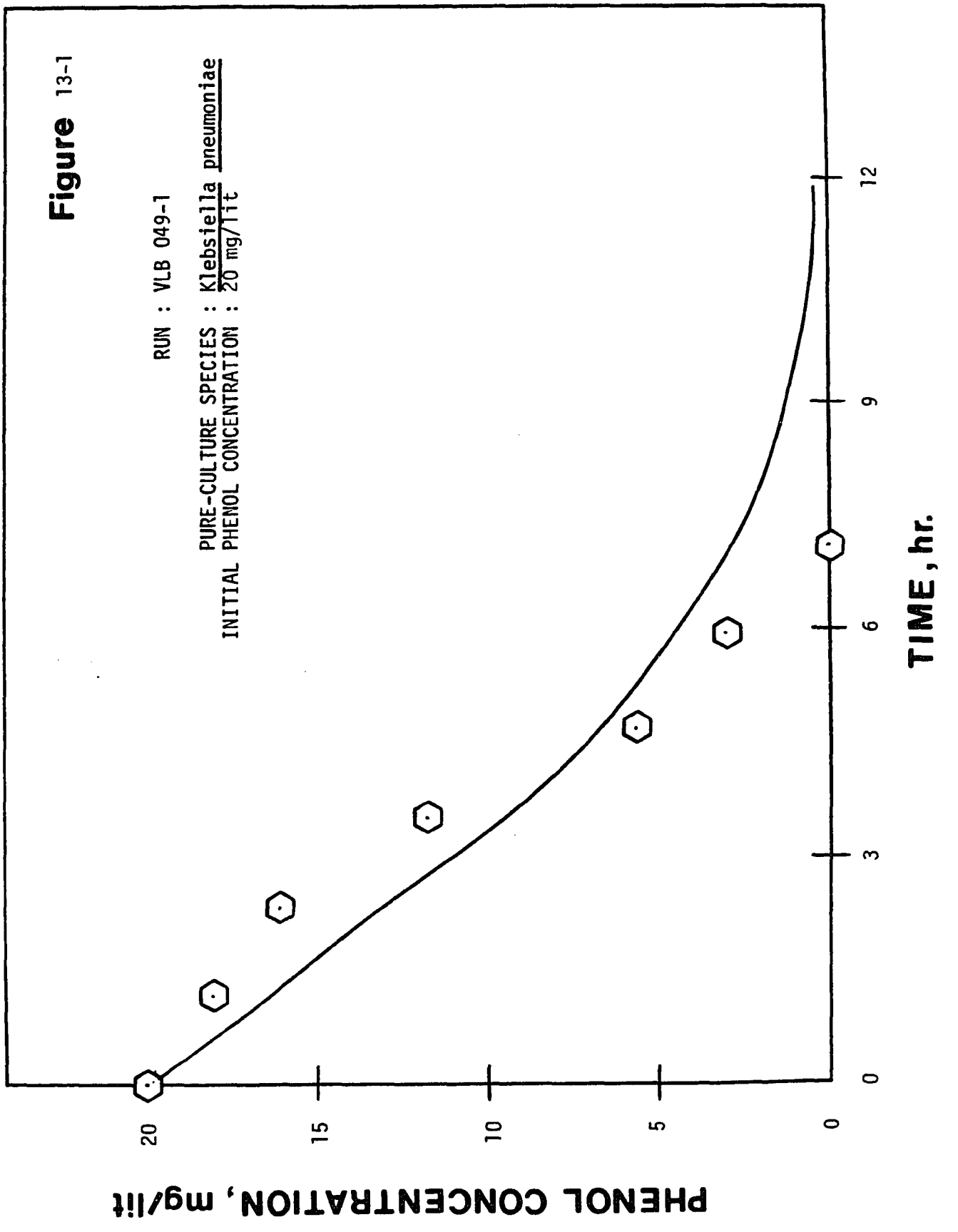


Figure 13-1
RUN : VLB 049-1
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20 mg/lit
PHENOL CONCENTRATION, mg/lit
20
15
10
5
0
0
3
6
9
12
TIME, hr.

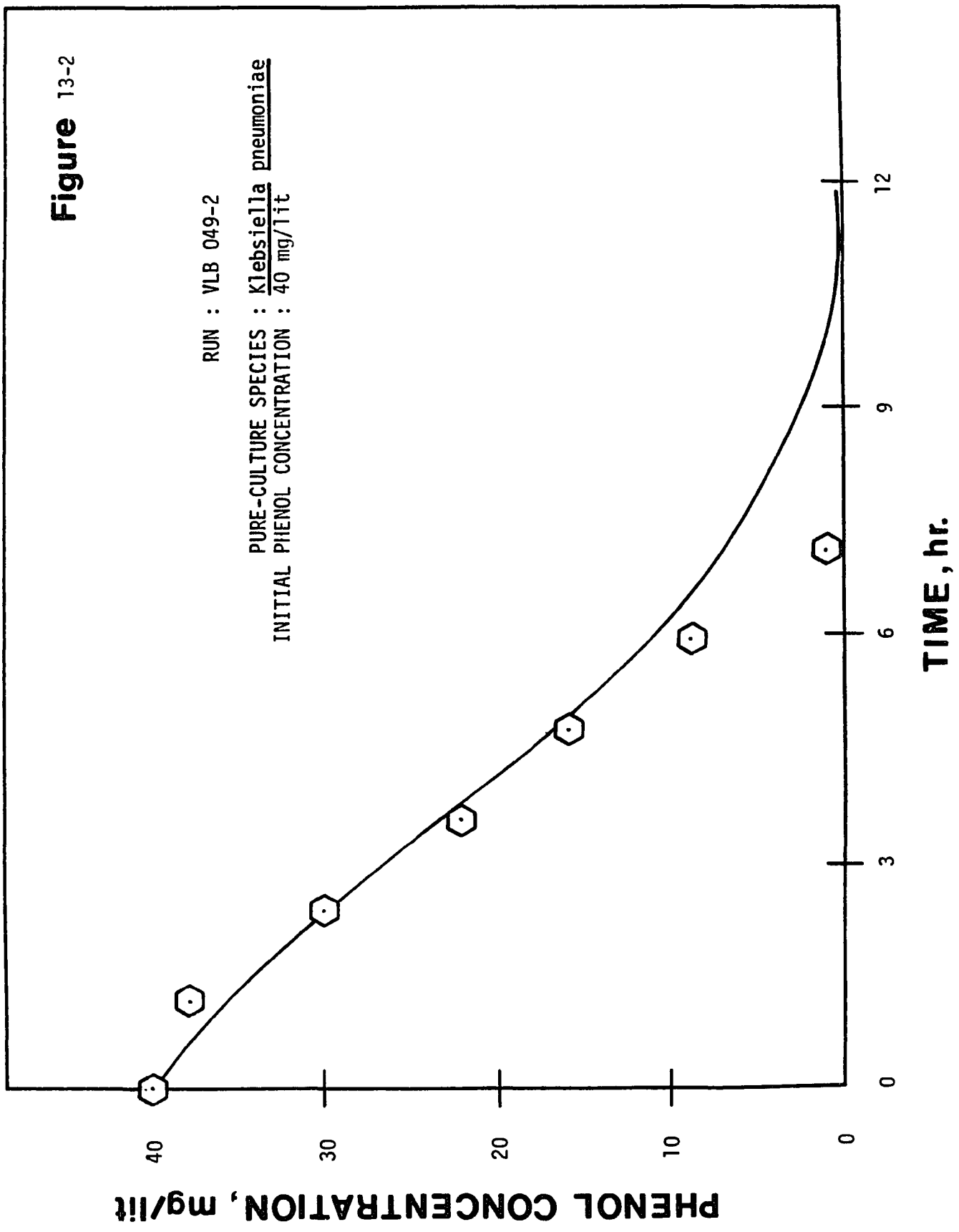


Figure 13-2
RUN : VLB 049-2
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 40 mg/lit
PHENOL CONCENTRATION, mg/lit
40
30
20
10
0
0
3
6
9
12
TIME, hr.

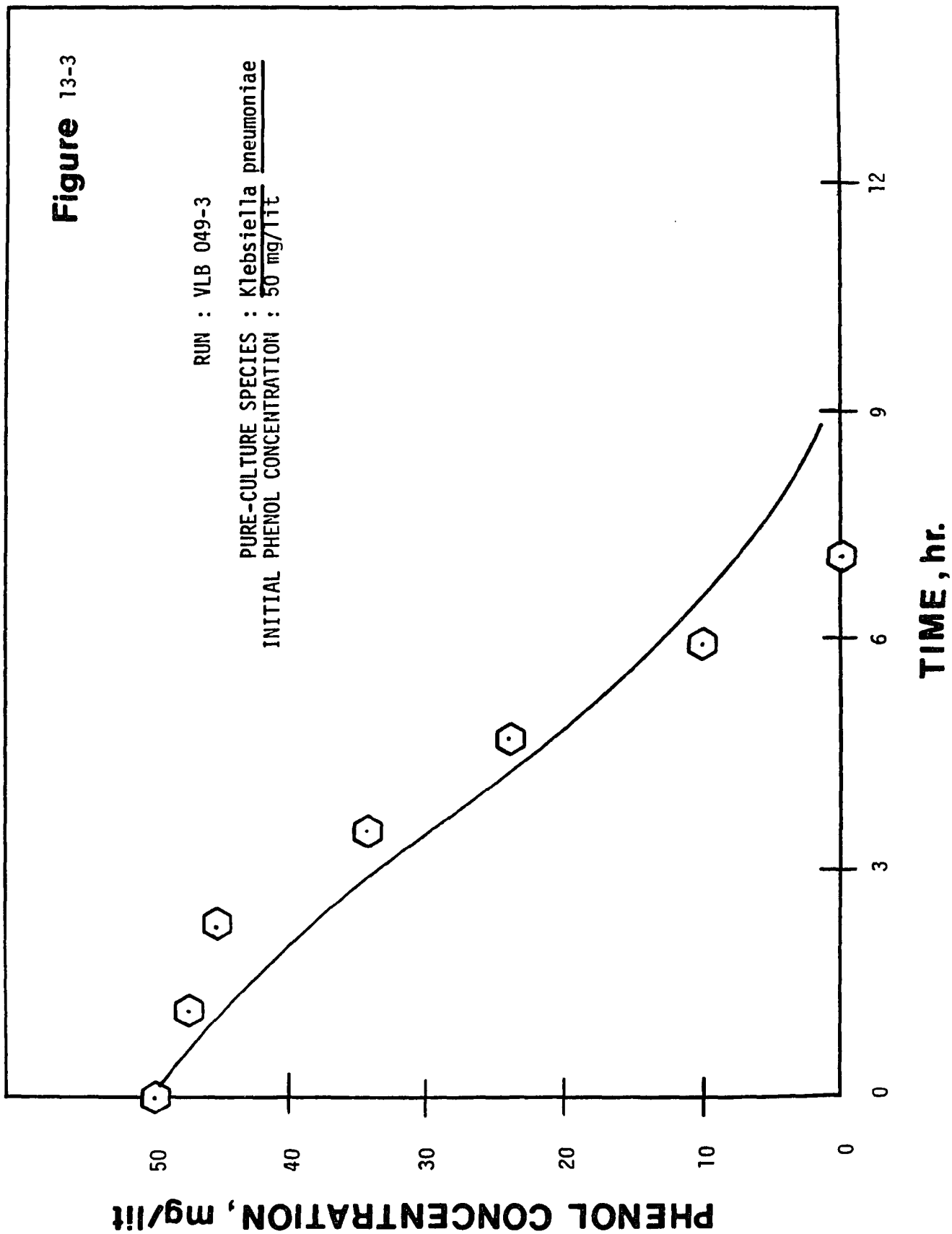


Figure 13-3
RUN : VLB 049-3
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 50 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
10
20
30
40
50
0
3
6
9
12

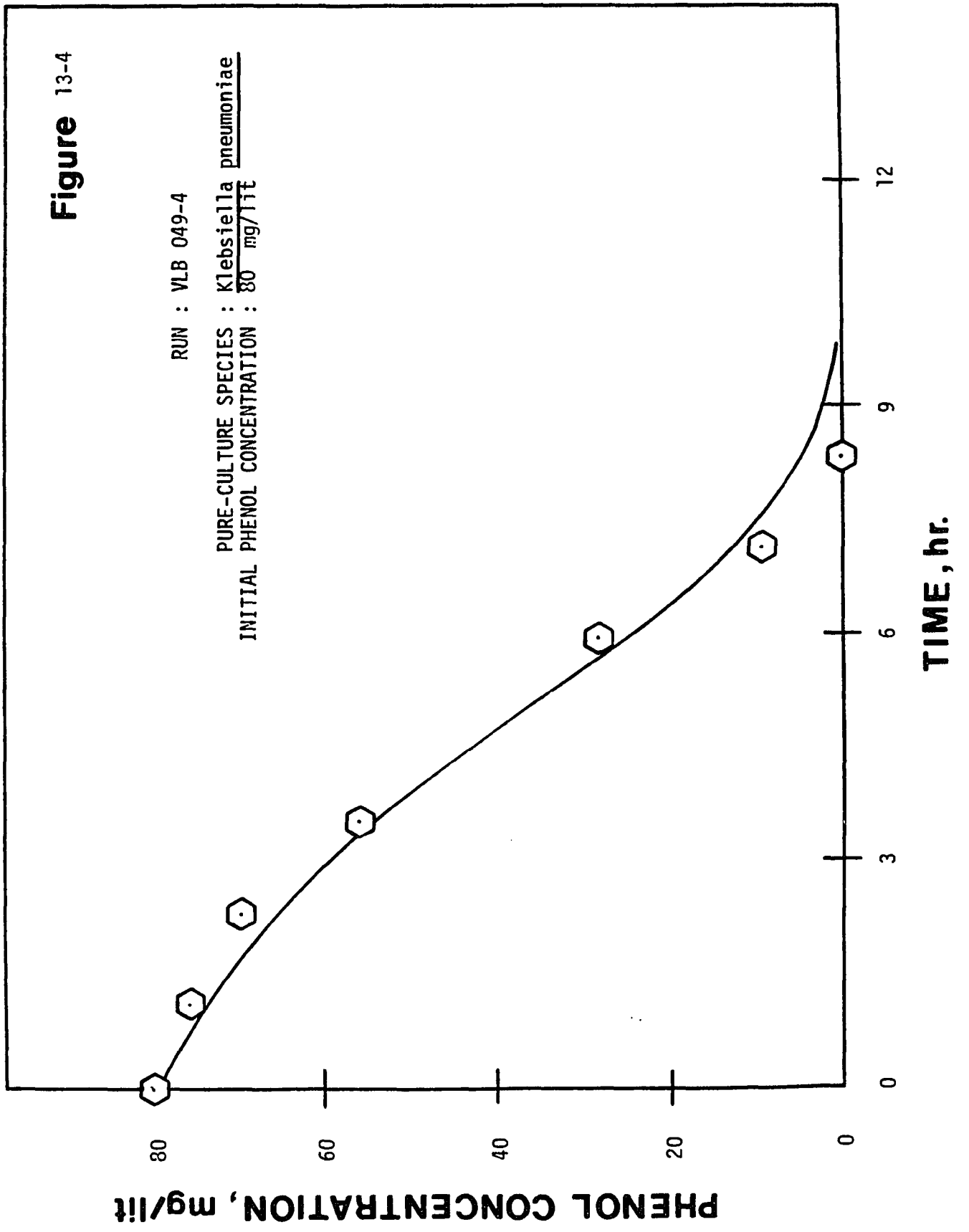


Figure 13-4
RUN : VLB 049-4
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 80 mg/lit
PHENOL CONCENTRATION, mg/lit
0
20
40
60
80
0
3
6
9
12
TIME, hr.

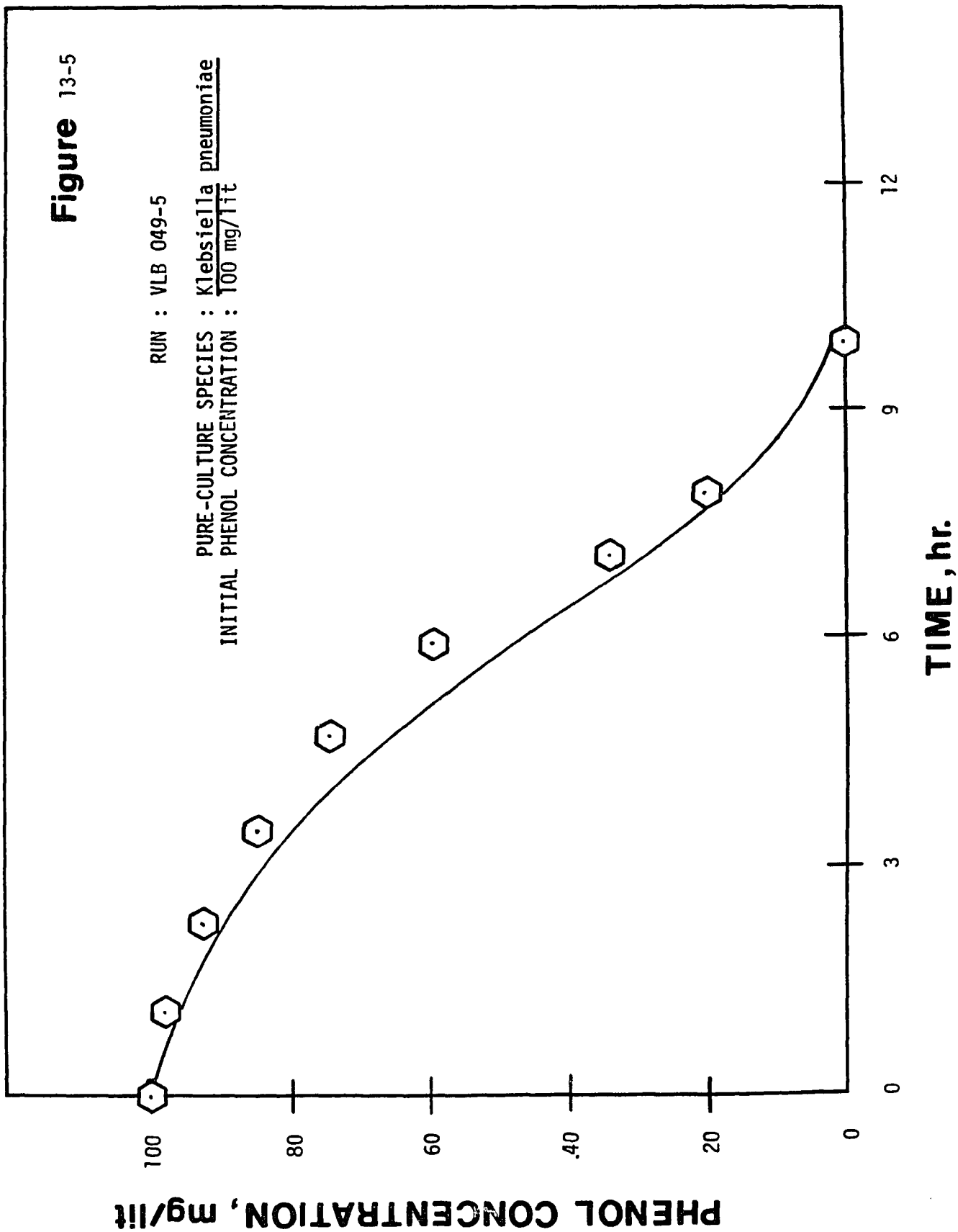


Figure 13-5
RUN : VLB 049-5
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 100 mg/lit
PHENOL CONCENTRATION, mg/lit
100
80
60
40
20
0
0
3
6
9
12
TIME, hr.

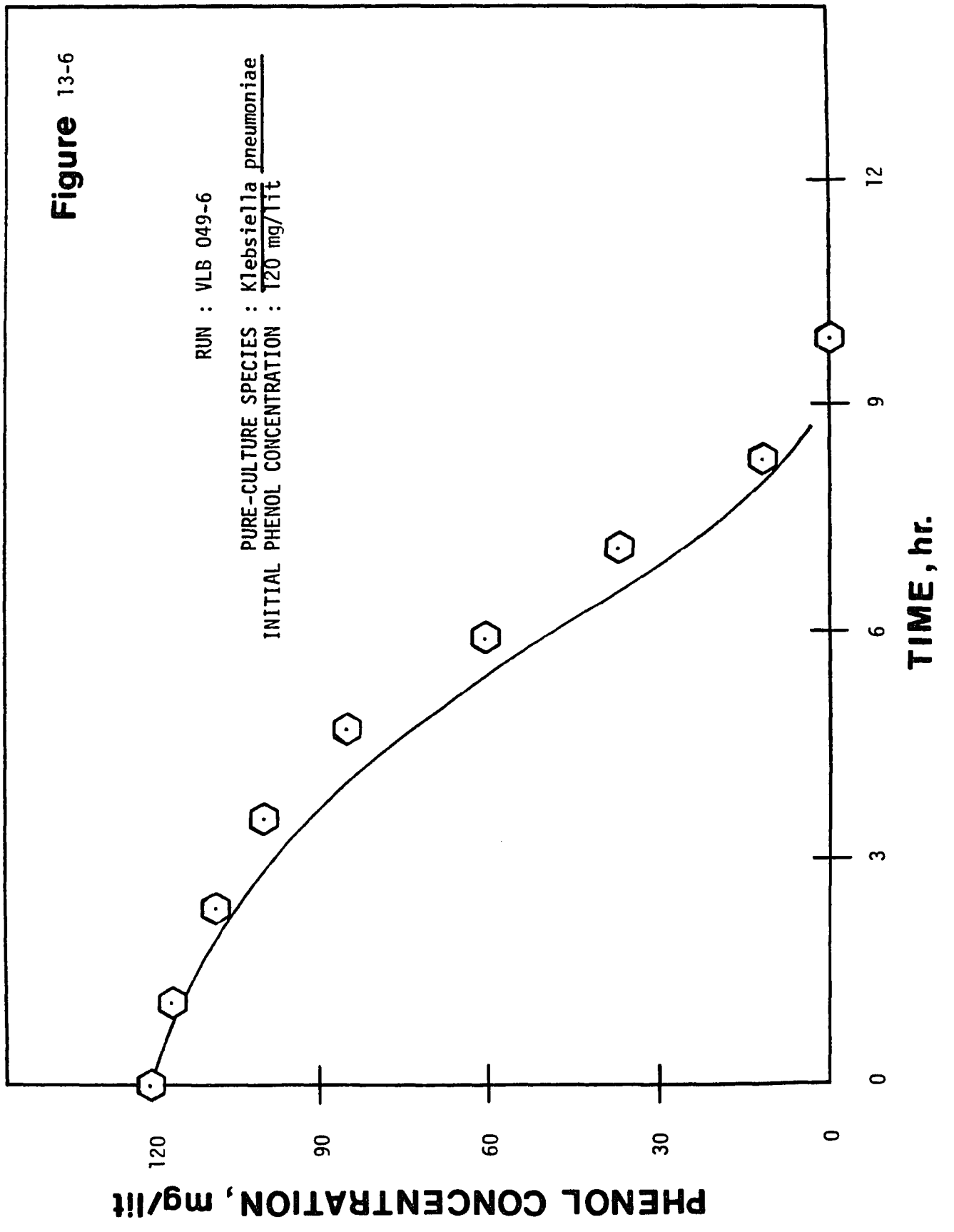


Figure 13-6
RUN : VLB 049-6
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 120 mg/lit
PHENOL CONCENTRATION, mg/lit
120
90
60
30
0
0
3
6
9
12
TIME, hr.

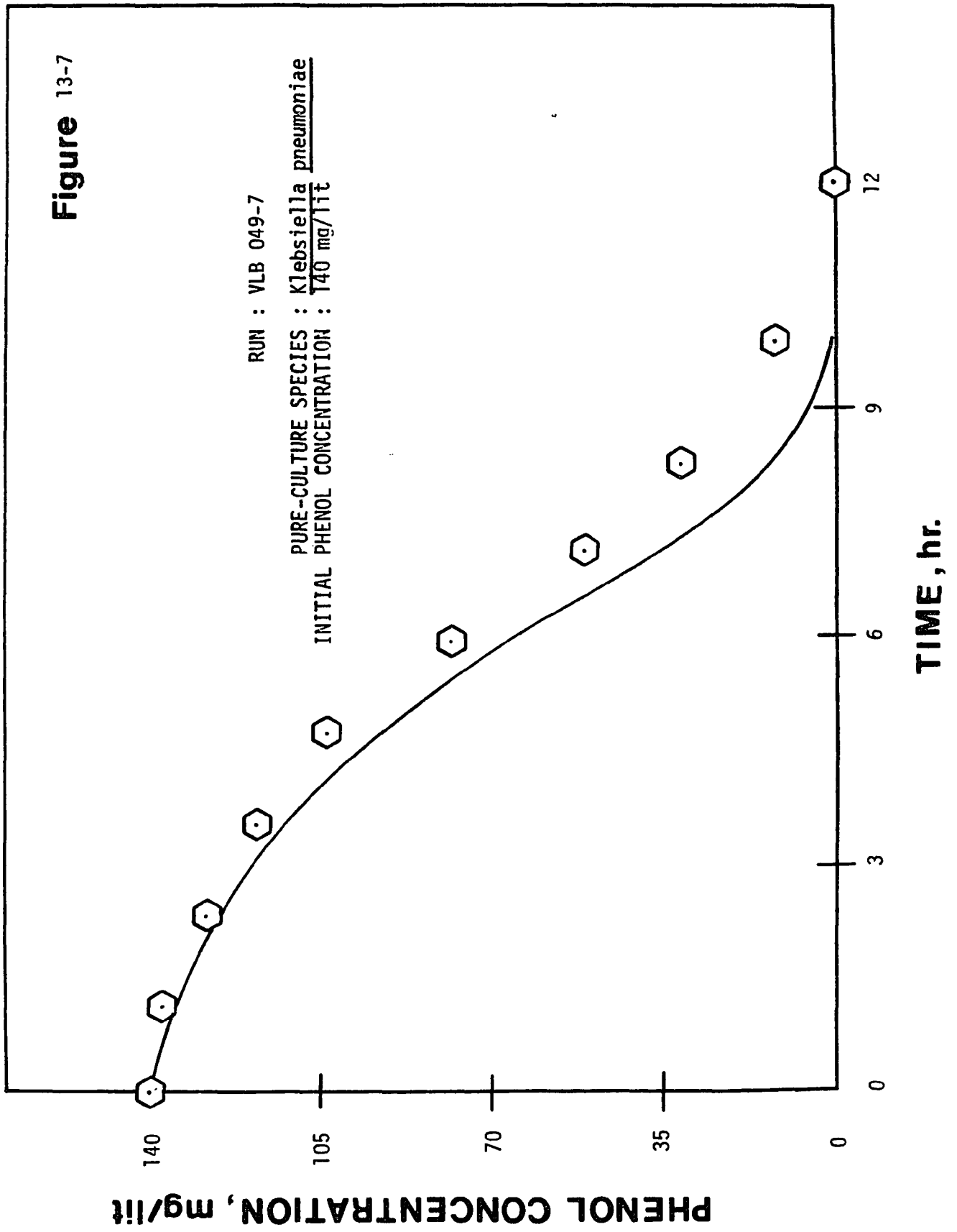


Figure 13-7
RUN : VLB 049-7
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 140 mg/lit
PHENOL CONCENTRATION, mg/lit
140
105
70
35
0
0
3
6
9
12
TIME, hr.

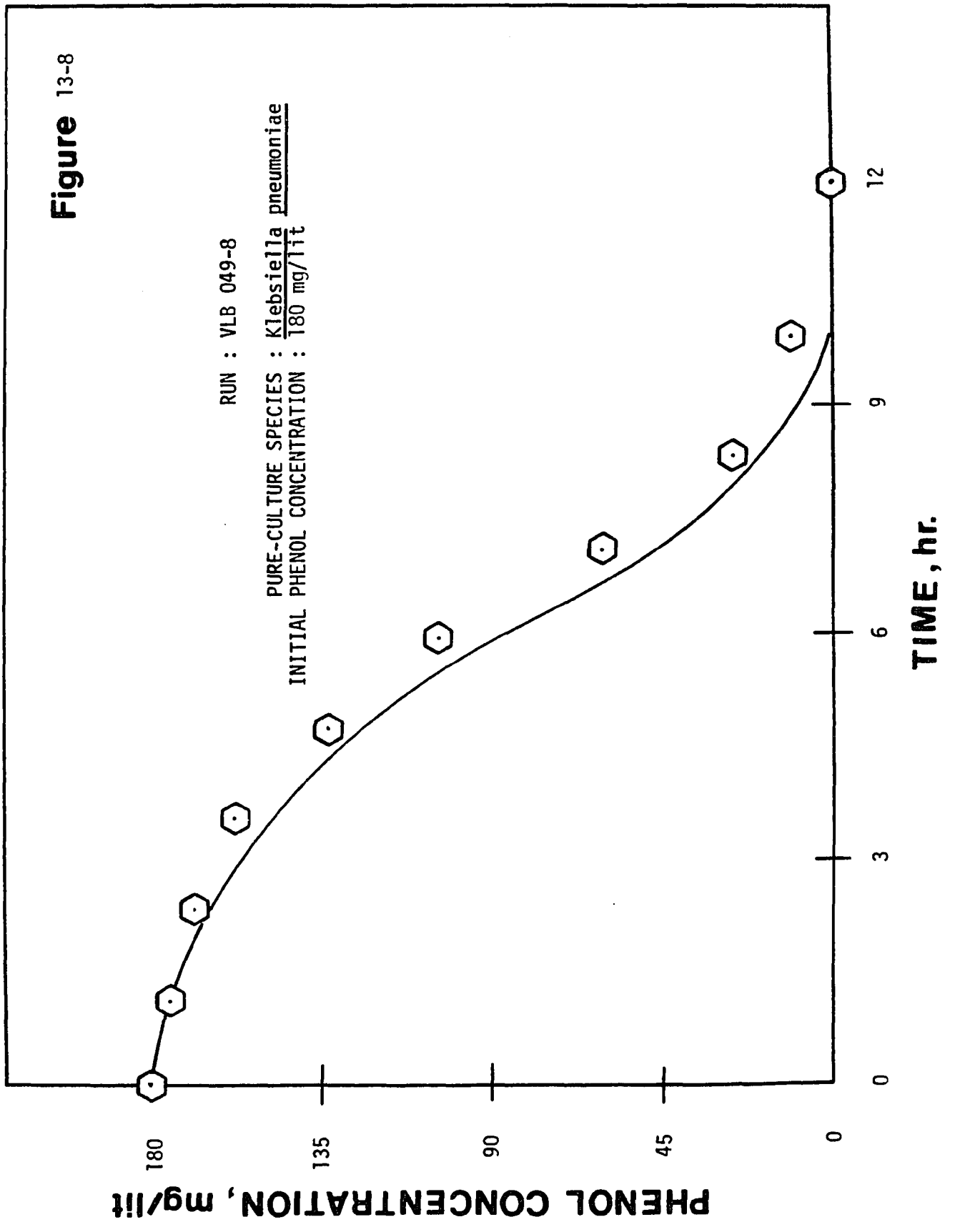


Figure 13-8
RUN : VLB 049-8
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 180 mg/lit
PHENOL CONCENTRATION, mg/lit
180
135
90
45
0
0
3
6
9
12
TIME, hr.

Figure 14

Phenol Degradation of *Pseudomonas putida* at the following Initial Phenol Concentrations (Run VLB 051-1 to VLB 051-8)

14-1	20 mg/lit
14-2	40 mg/lit
14-3	50 mg/lit
14-4	80 mg/lit
14-5	100 mg/lit
14-6	120 mg/lit
14-7	140 mg/lit
14-8	180 mg/lit

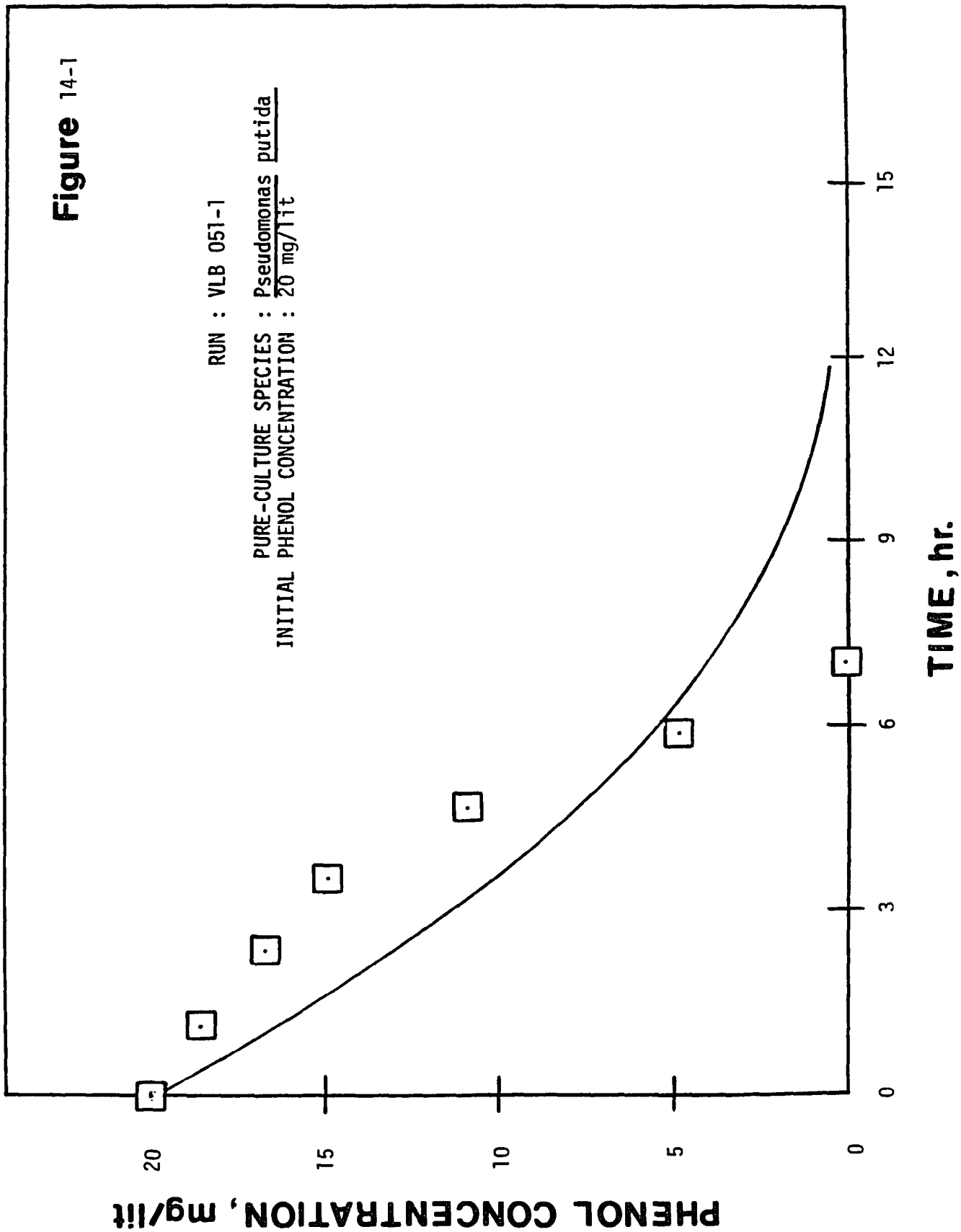


Figure 14-1
RUN : VLB 051-1
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20 mg/lit
PHENOL CONCENTRATION, mg/lit
0
5
10
15
20
0
3
6
9
12
15
TIME, hr.

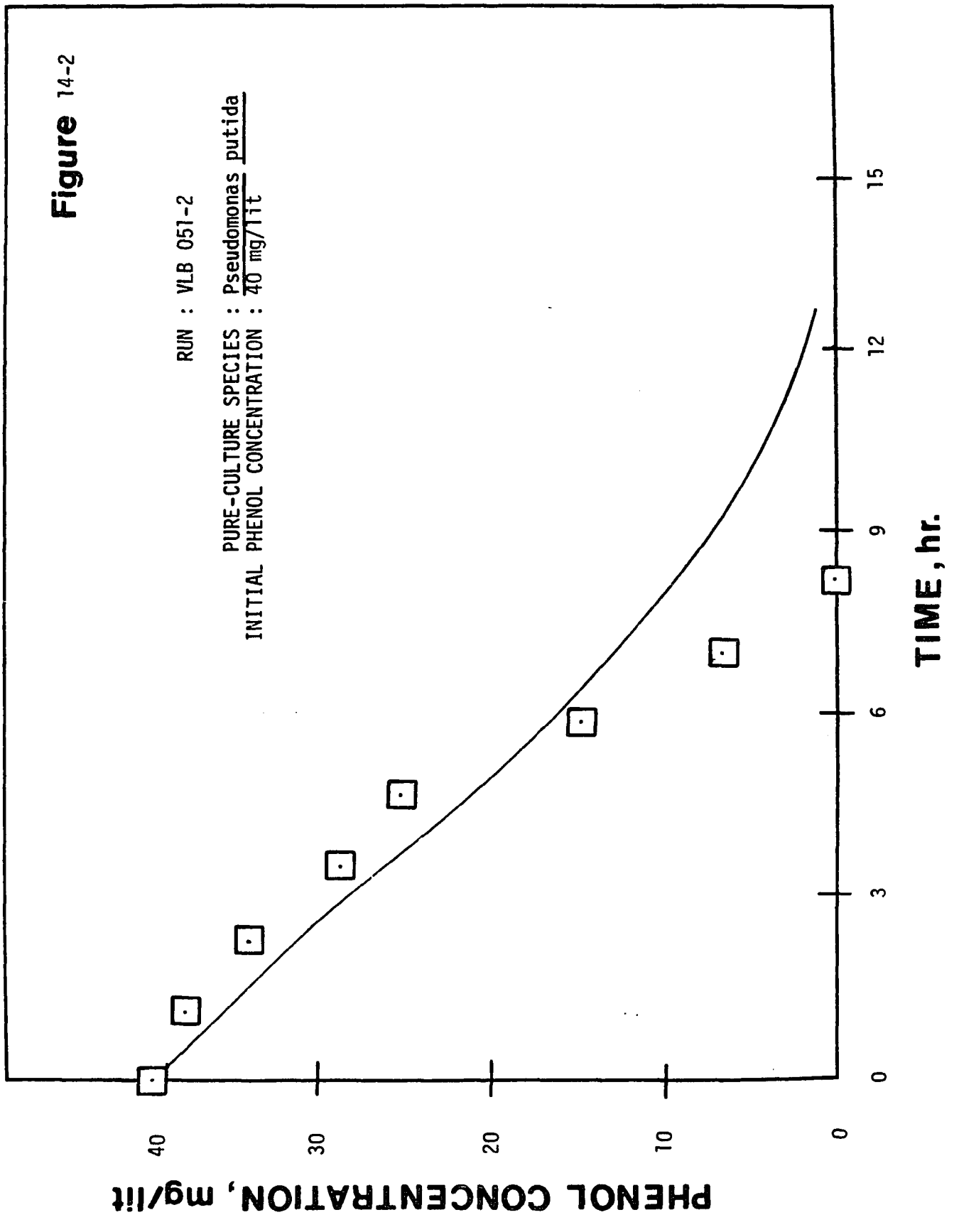


Figure 14-2
RUN : VLB 051-2
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 40 mg/lit
PHENOL CONCENTRATION, mg/lit
0
10
20
30
40
0
3
6
9
12
15
TIME, hr.

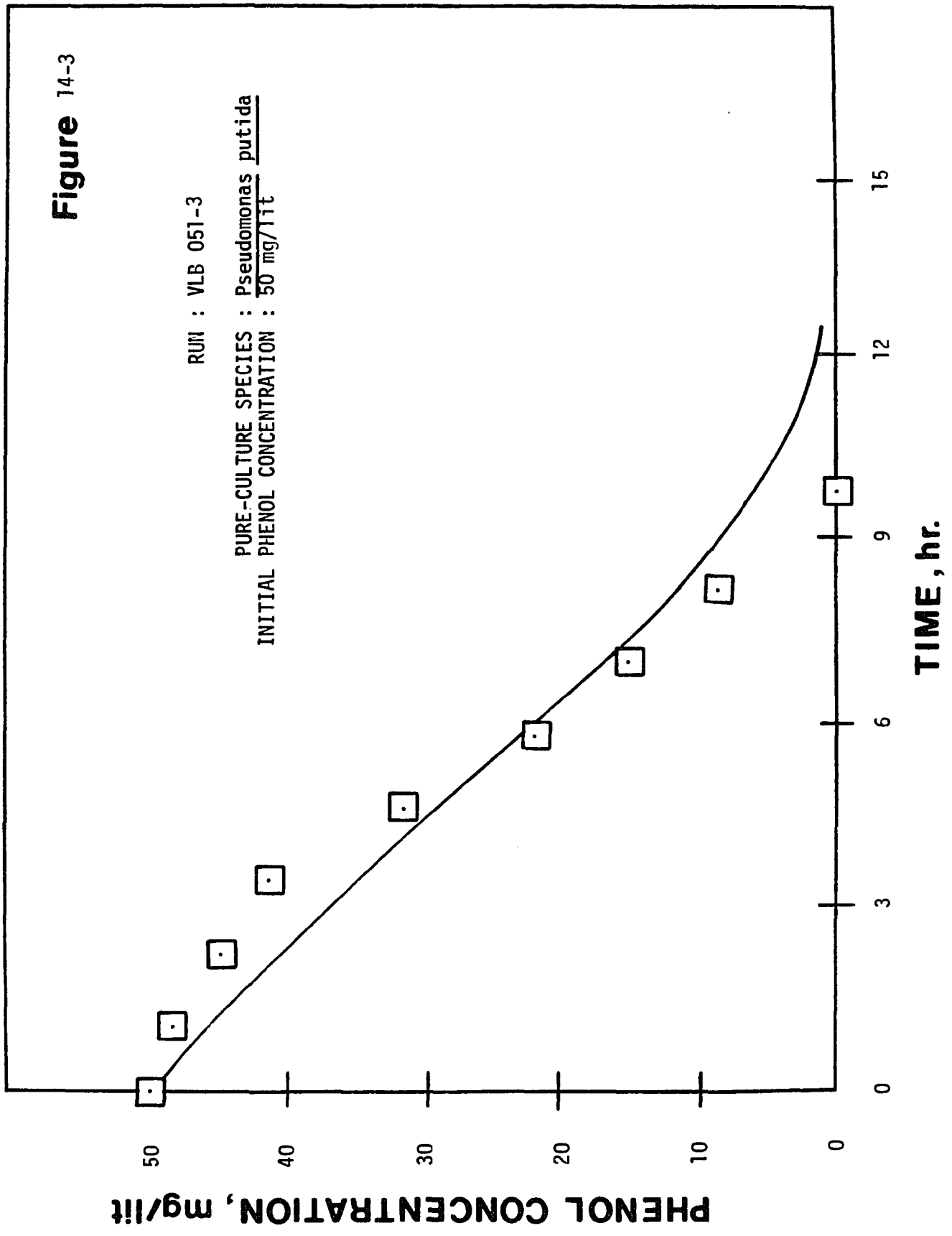


Figure 14-3
RUN : VLB 051-3
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 50 mg/lit
PHENOL CONCENTRATION, mg/lit
0
10
20
30
40
50
0
3
6
9
12
15
TIME, hr.

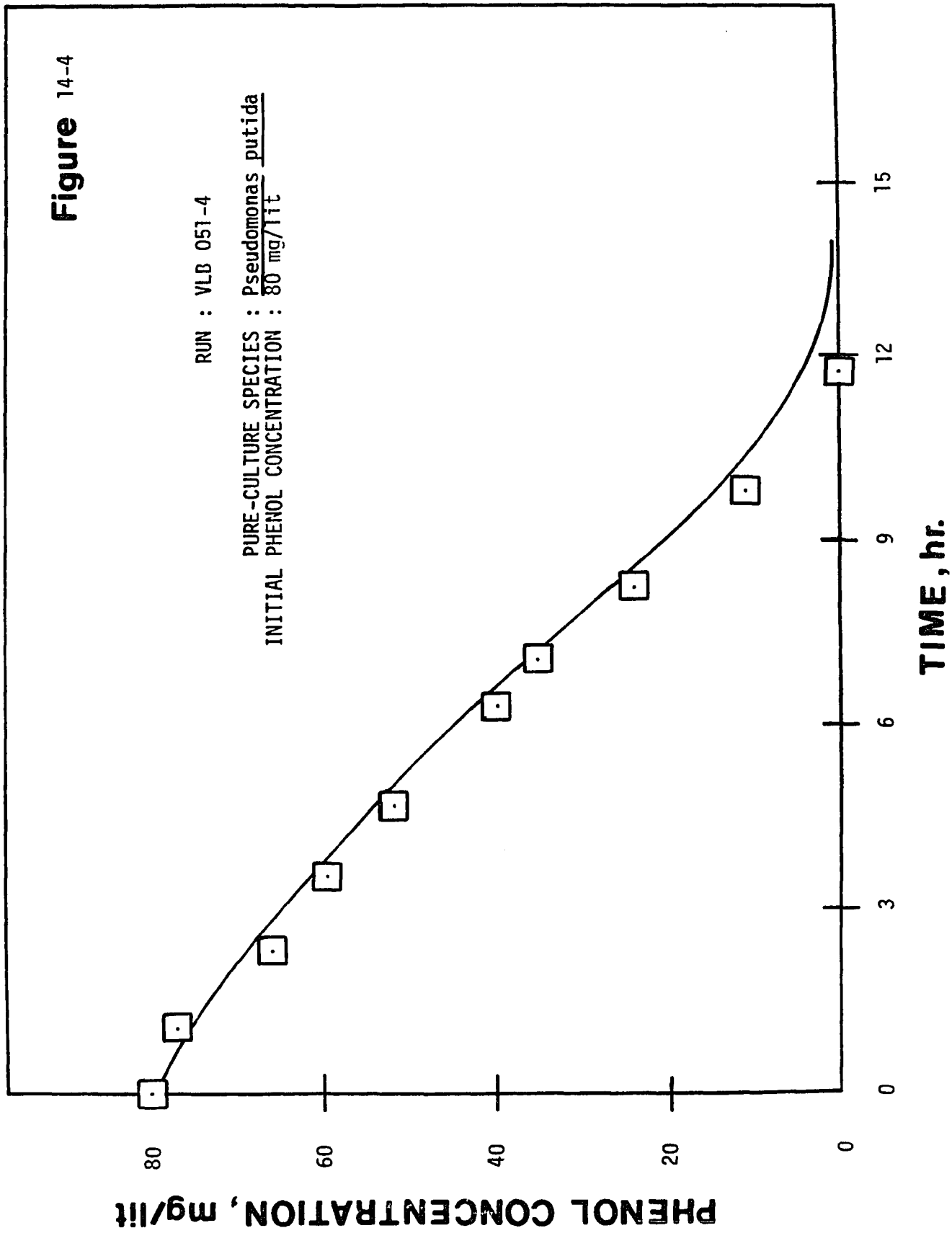


Figure 14-4
RUN : VLB 051-4
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 80 mg/lit
PHENOL CONCENTRATION, mg/lit
0
20
40
60
80
0
3
6
9
12
15
TIME, hr.

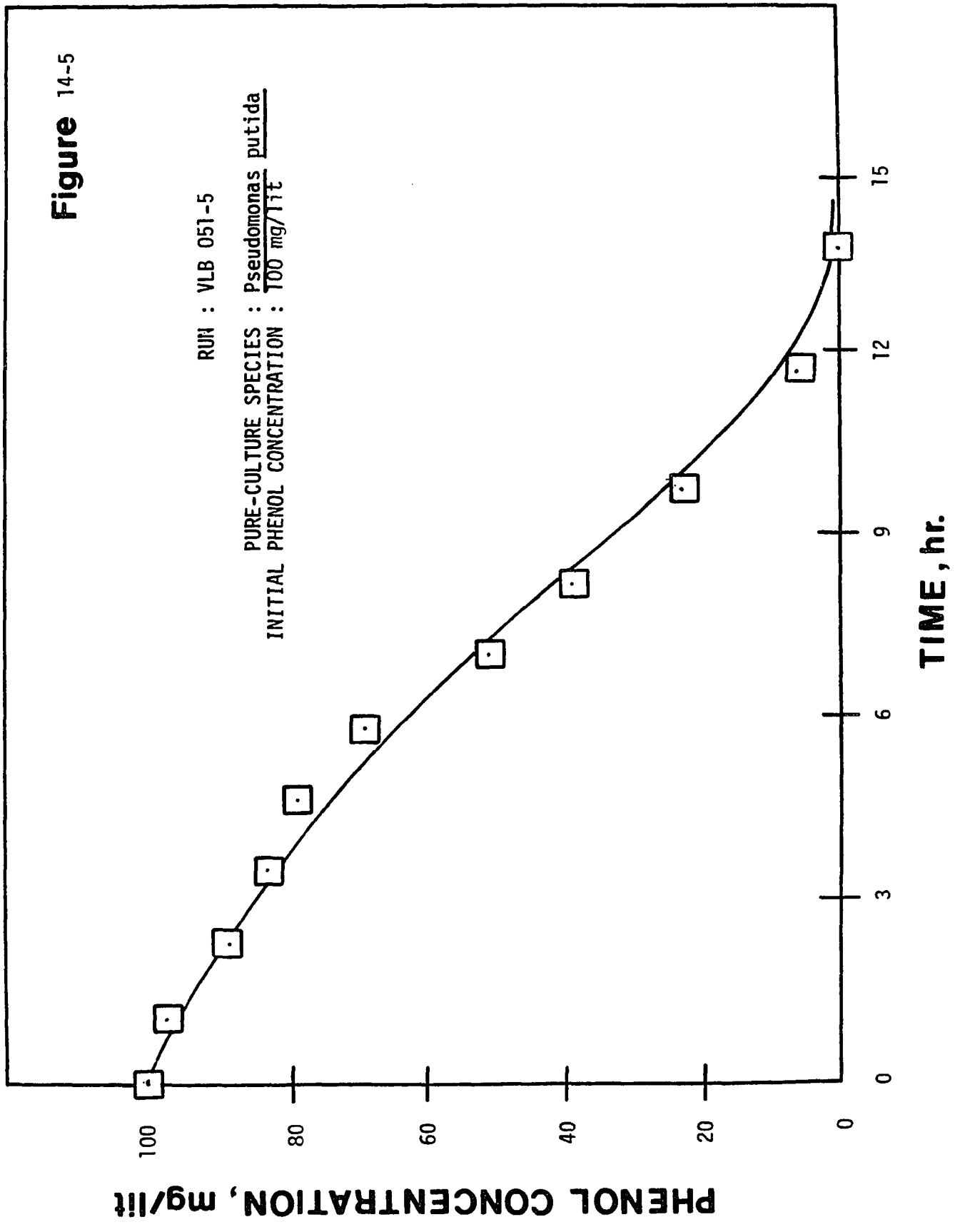


Figure 14-5
RUN : VLB 051-5
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 100 mg/lit
PHENOL CONCENTRATION, mg/lit
100
80
60
40
20
0
0
3
6
9
12
15
TIME, hr.

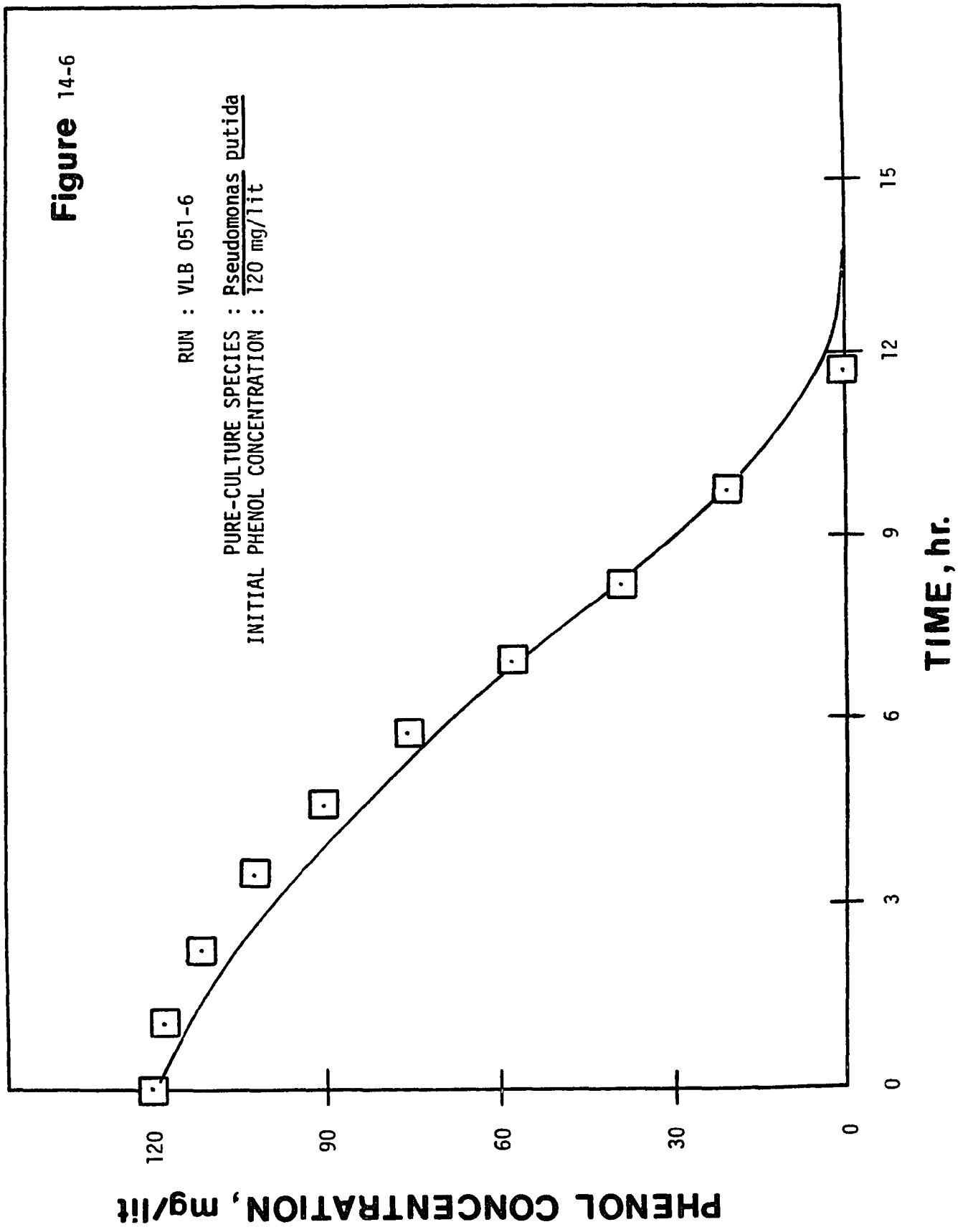


Figure 14-6
RUN : VLB 051-6
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 120 mg/lit
PHENOL CONCENTRATION, mg/lit
120
90
60
30
0
0
3
6
9
12
15
TIME, hr.

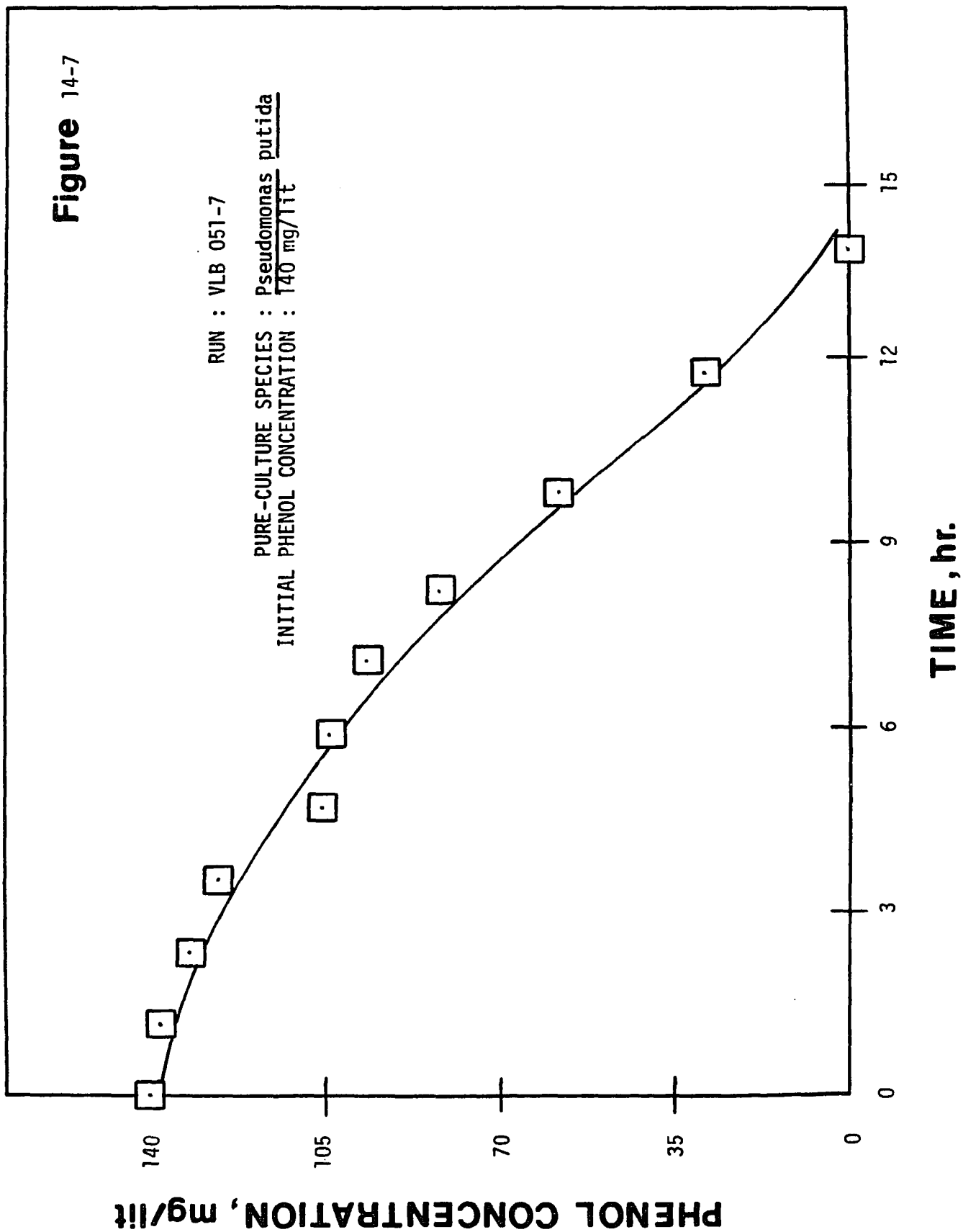


Figure 14-7
RUN : VLB 051-7
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 140 mg/lit
PHENOL CONCENTRATION, mg/lit
140
105
70
35
0
0
3
6
9
12
15
TIME, hr.

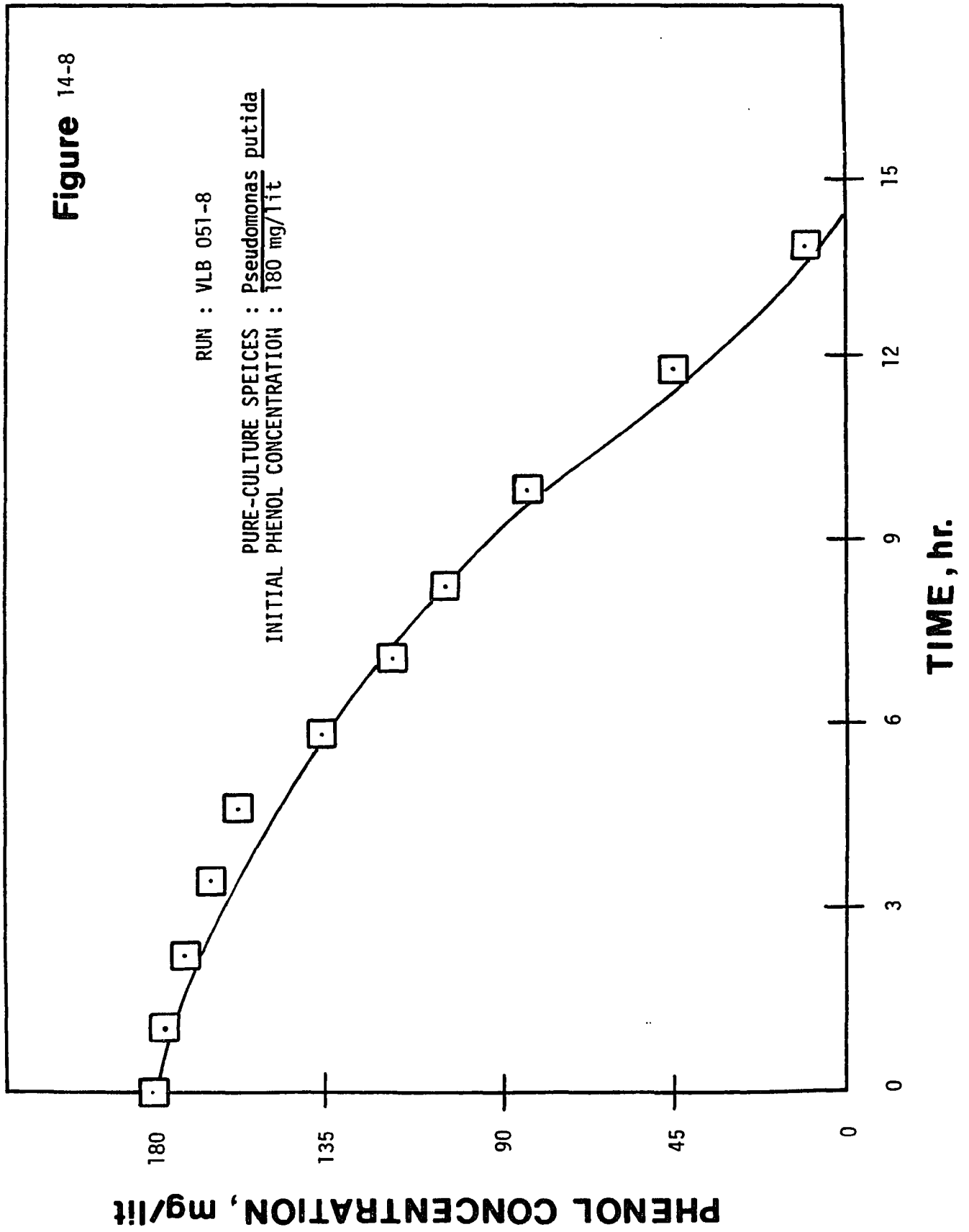


Figure 14-8
RUN : VLB 051-8
PURE-CULTURE SPEICES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 180 mg/lit
PHENOL CONCENTRATION, mg/lit
180
135
90
45
0
0
3
6
9
12
15
TIME, hr.

Figure 15

Specific Growth Rate vs. Initial Phenol Concentration
for Serratia liquefaciens

15-1 Run VLB 045
15-2 Run VLB 045-A
15-3 Run VLB 045-B

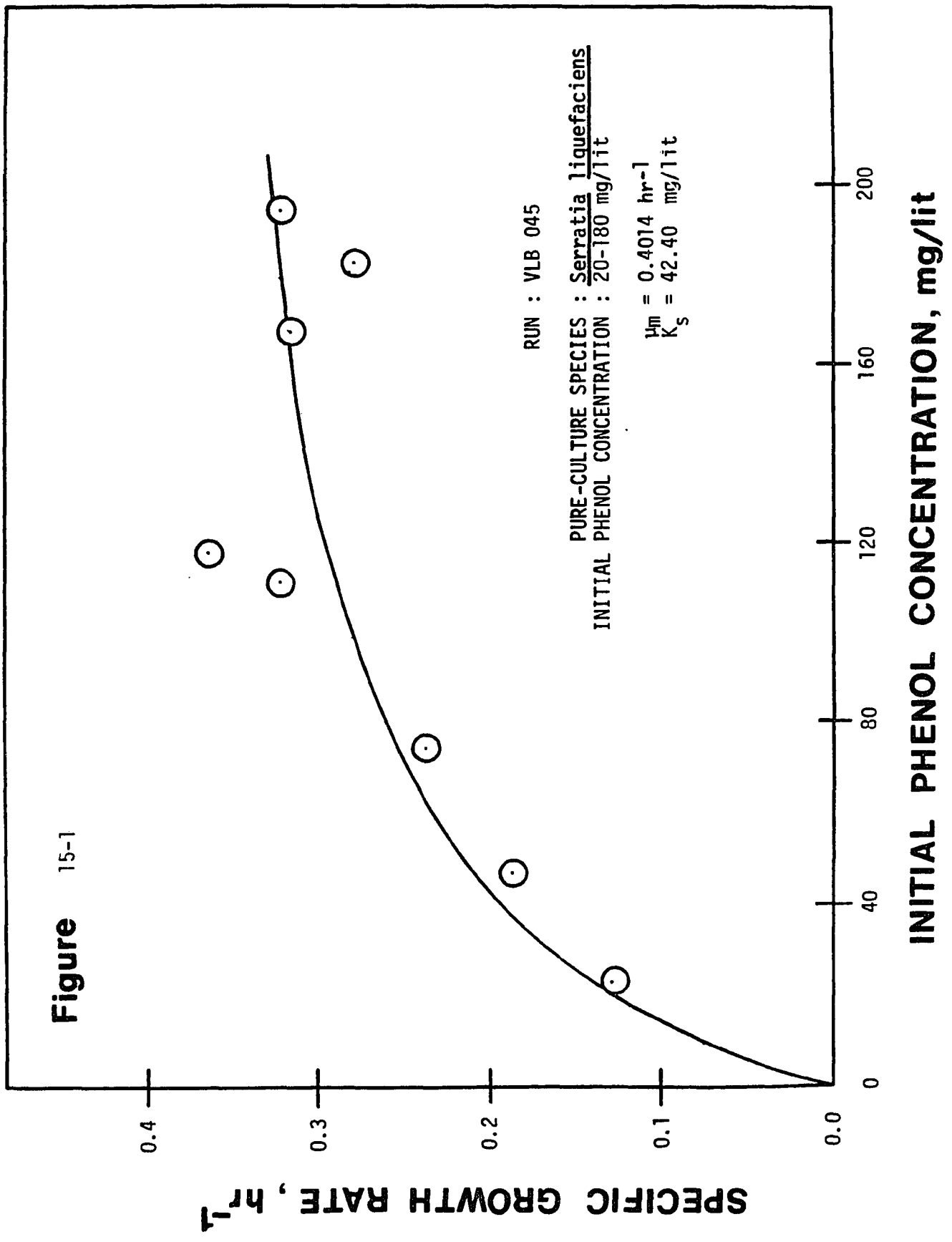


Figure 15-1
RUN : VLB 045
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.4014 hr^{-1}
K_s = 42.40 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.0
0.1
0.2
0.3
0.4
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

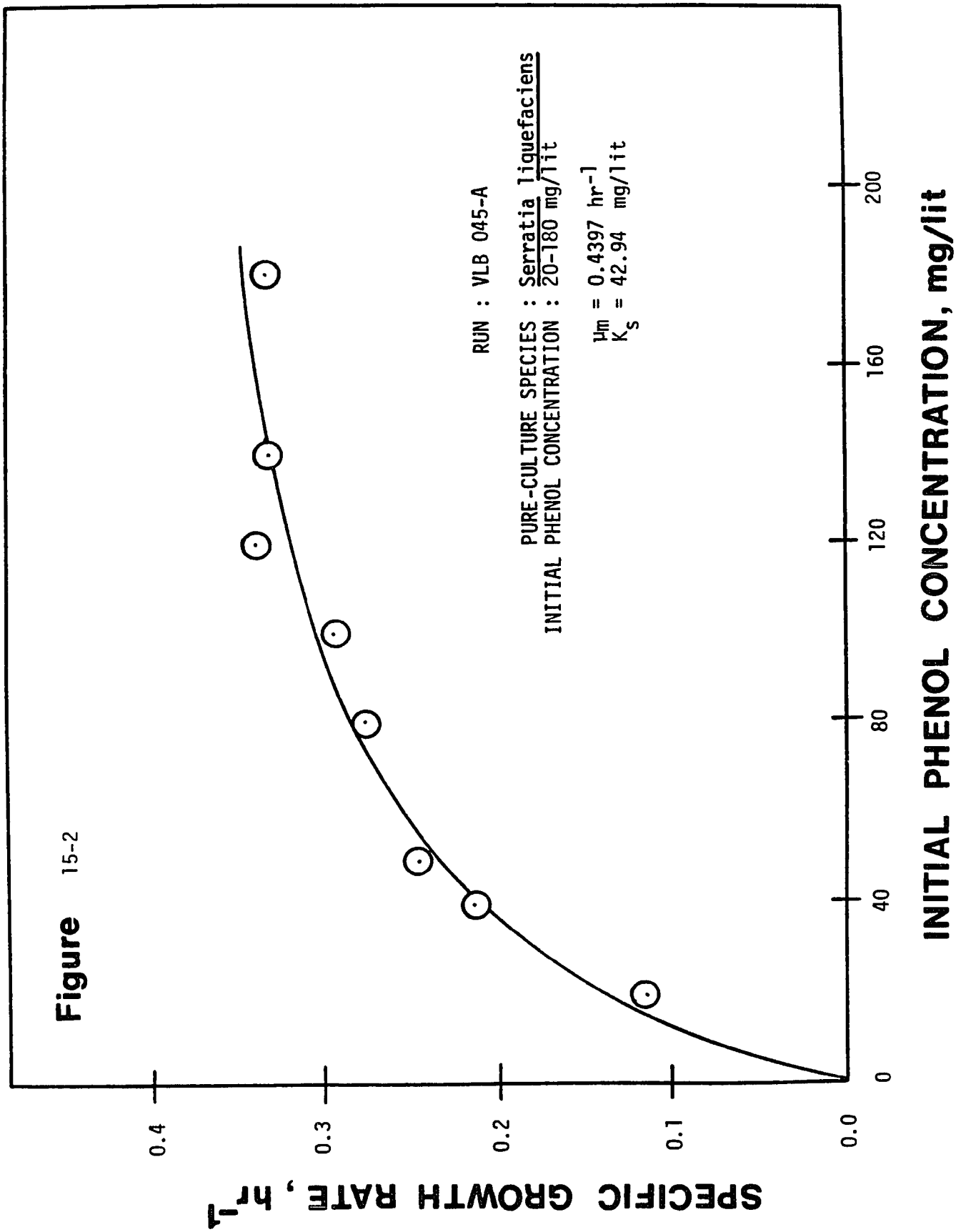

RUN : VLB 045-A
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.4397 hr^{-1}
K_s = 42.94 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.0
0.1
0.2
0.3
0.4
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

Figure 15-2

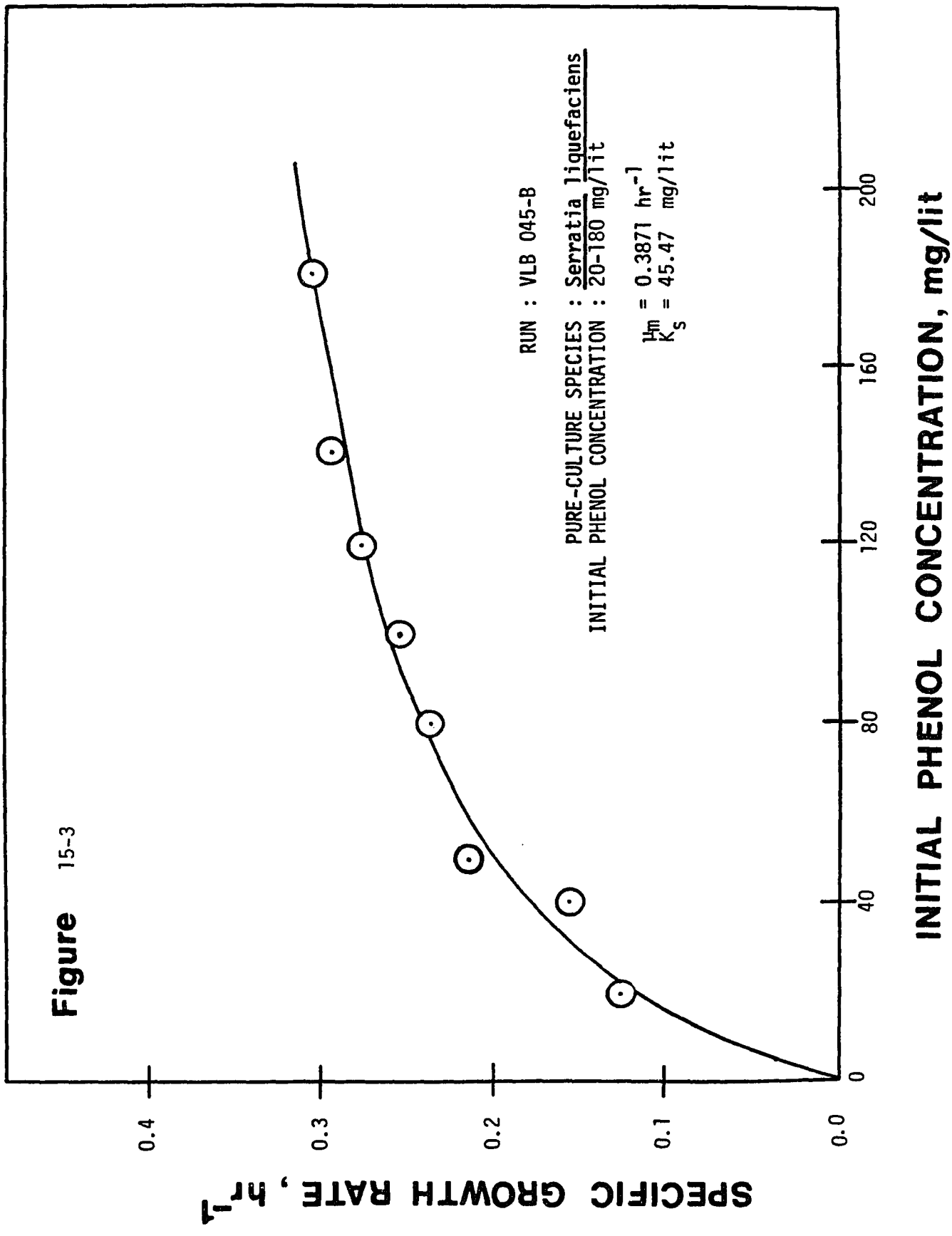


Figure 15-3
RUN : VLB 045-B
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.3871 hr^{-1}
K_s = 45.47 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.0
0.1
0.2
0.3
0.4
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

Figure 16

Specific Growth Rate vs. Initial Phenol Concentration
for Klebsiella pneumoniae

16-1	Run VLB 049
16-2	Run VLB 049-A
16-3	Run VLB 049-B

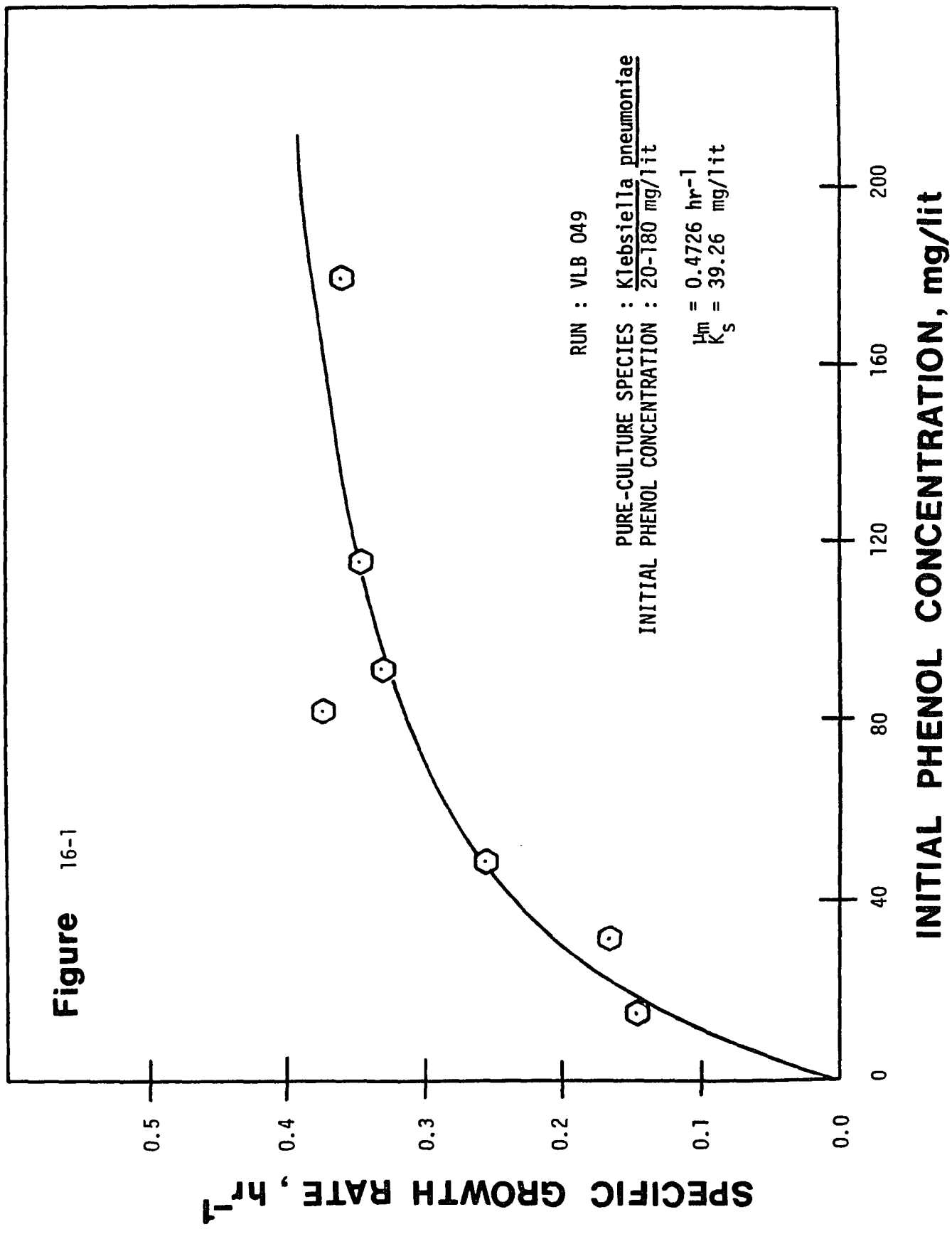


Figure 16-1
RUN : VLB 049
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.4726 hr^{-1}
K_s = 39.26 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.0
0.1
0.2
0.3
0.4
0.5
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

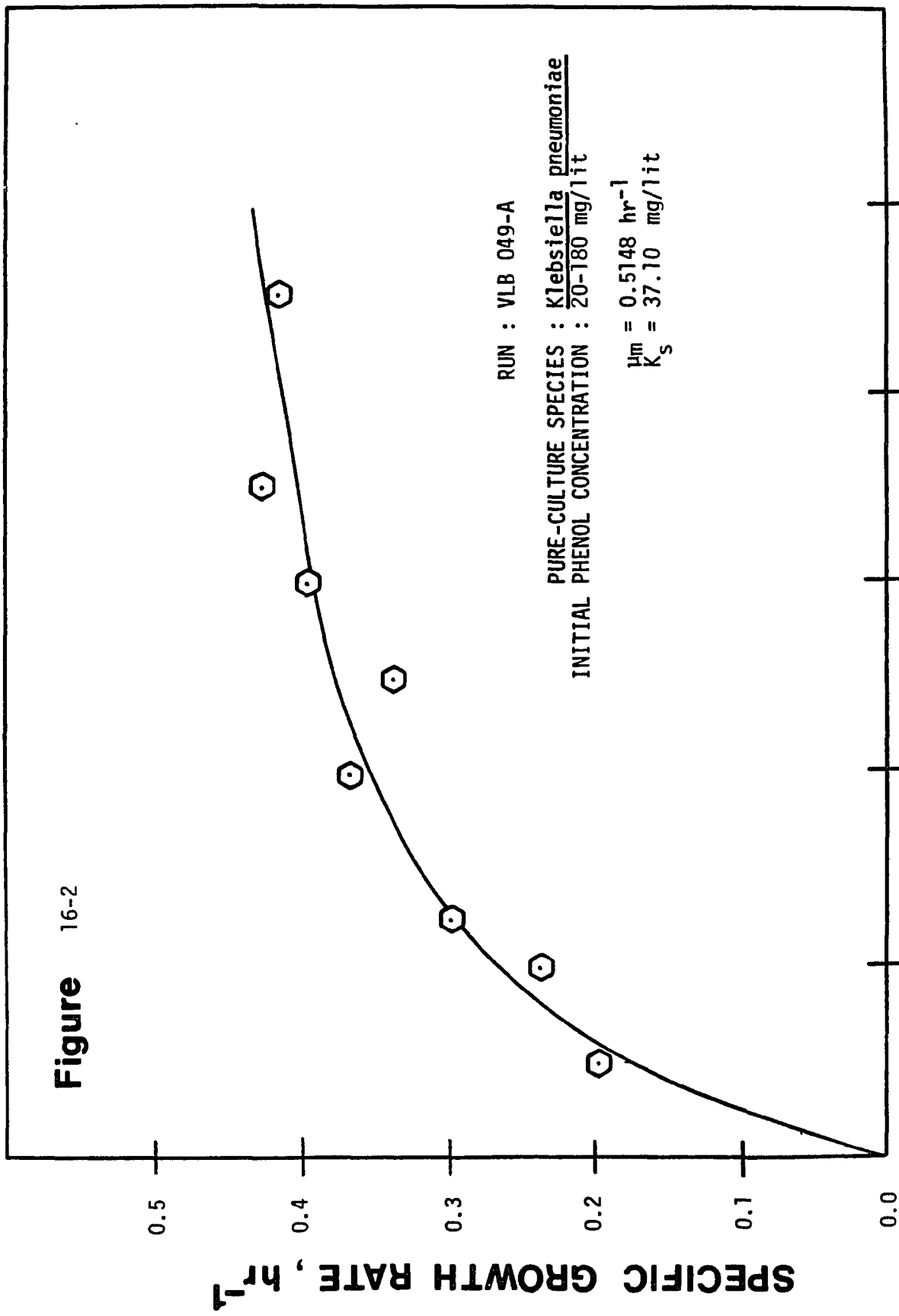


Figure 16-2
RUN : VLB 049-A
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.5148 hr^{-1}
K_s = 37.10 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.0
0.1
0.2
0.3
0.4
0.5
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

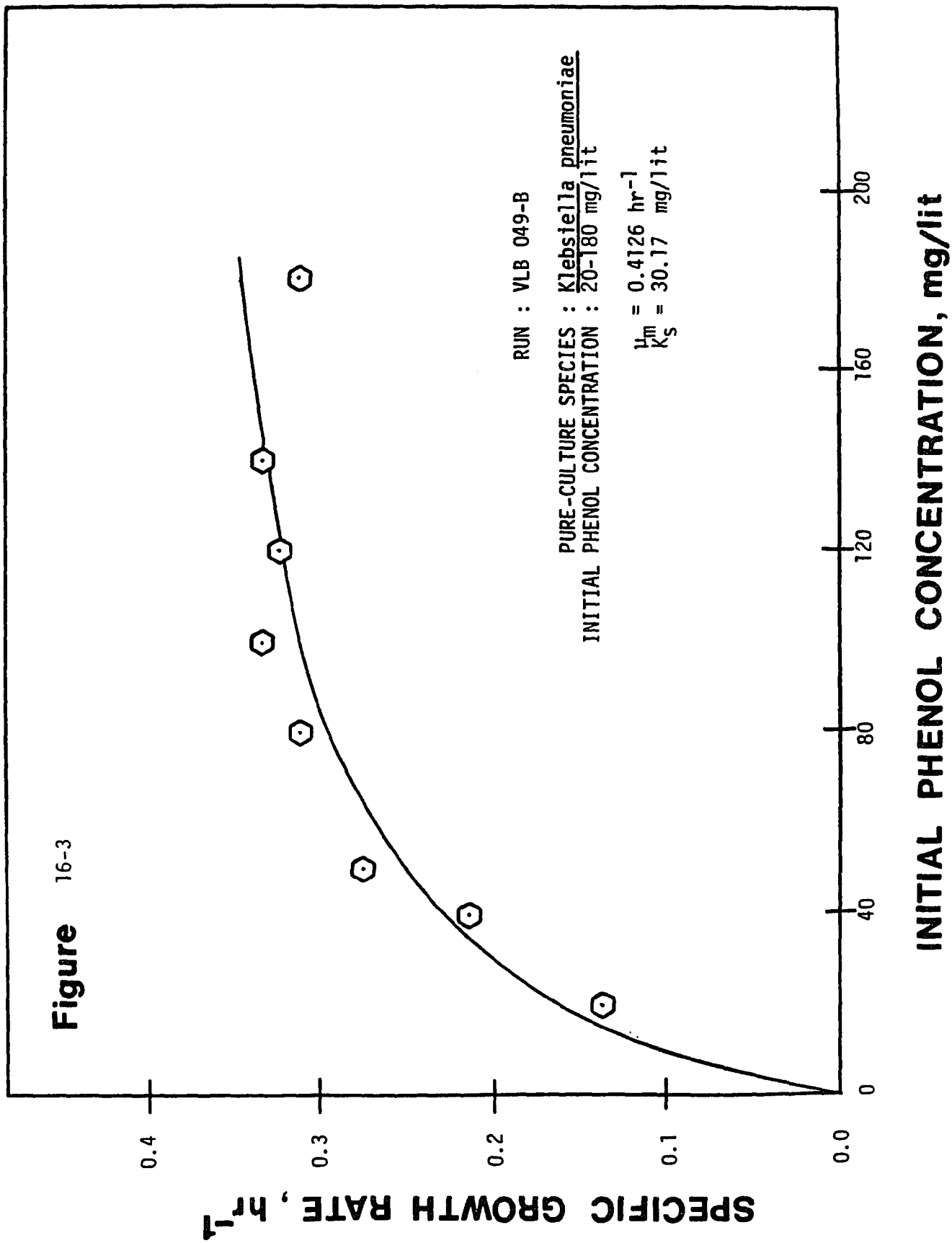


Figure 16-3
RUN : VLB 049-B
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.4126 hr^{-1}
K_s = 30.17 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.0
0.1
0.2
0.3
0.4
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

Figure 17

Specific Growth Rate vs. Initial Phenol Concentration
for *Pseudomonas putida*

17-1	Run VLB 051
17-2	Run VLB 051-A
17-3	Run VLB 051-B

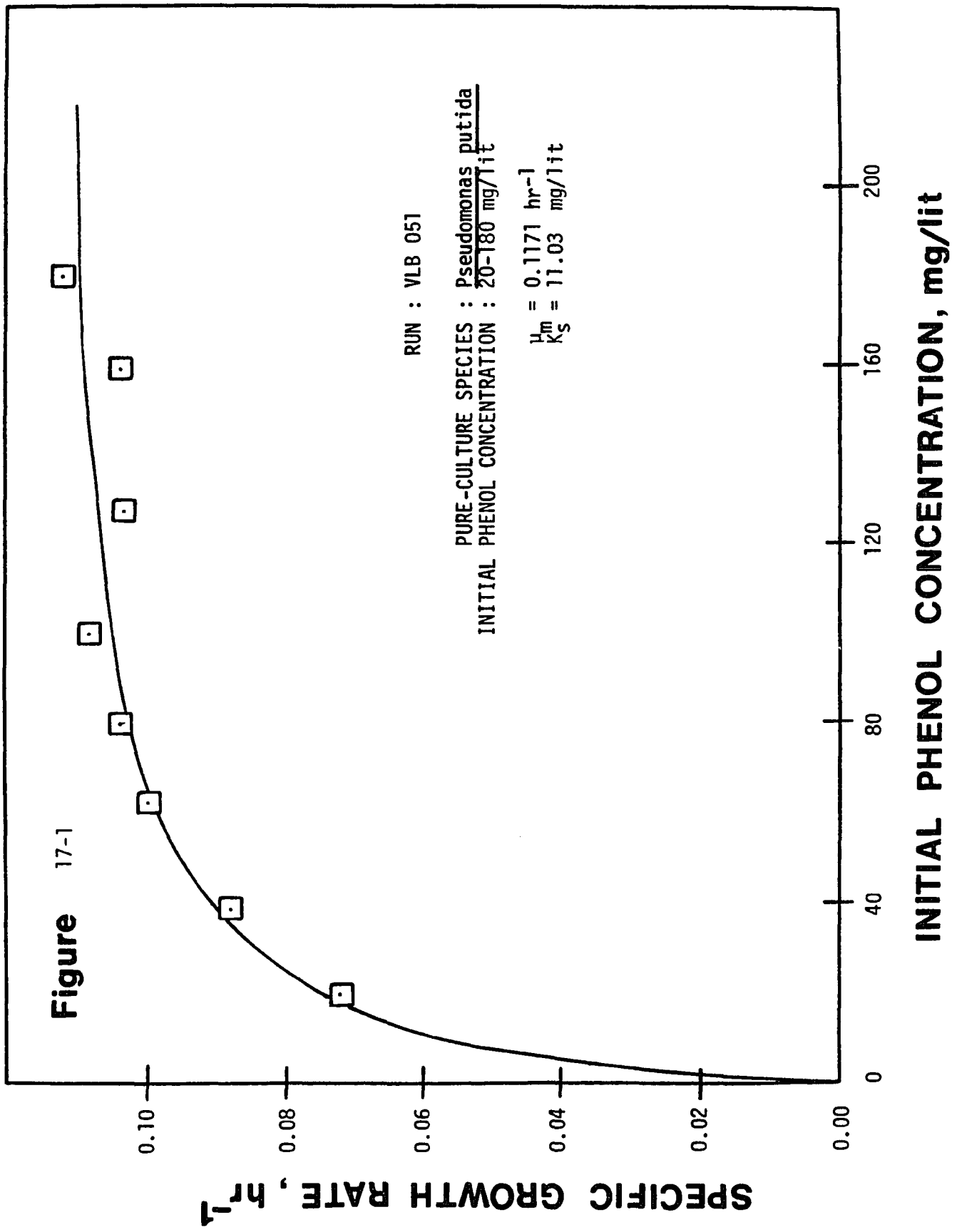


Figure 17-1
RUN : VLB 051
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.1171 hr^{-1}
K_s = 11.03 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.00
0.02
0.04
0.06
0.08
0.10
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

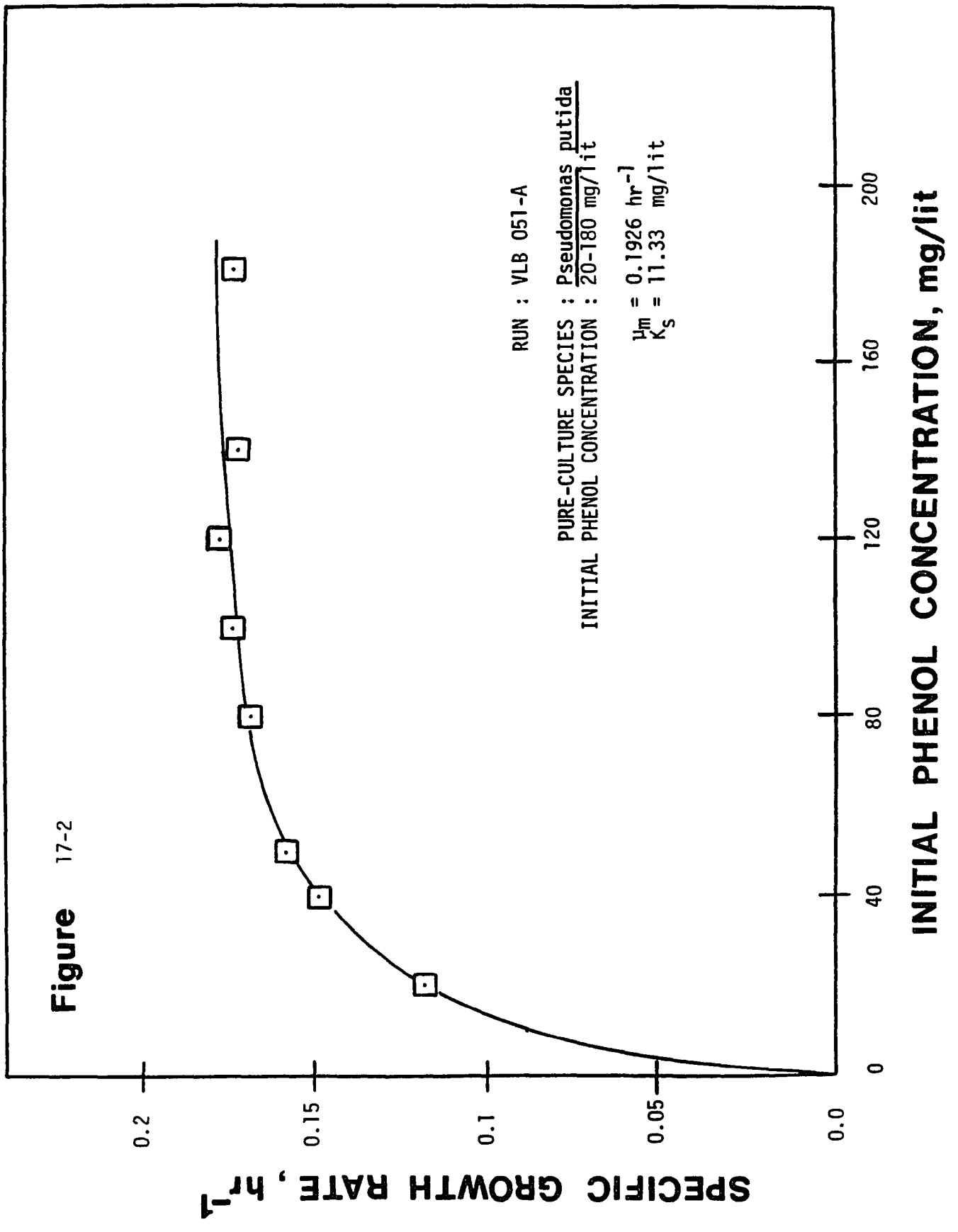


Figure 17-2
RUN : VLB 051-A
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.1926 hr^{-1}
K_s = 11.33 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.0
0.05
0.1
0.15
0.2
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

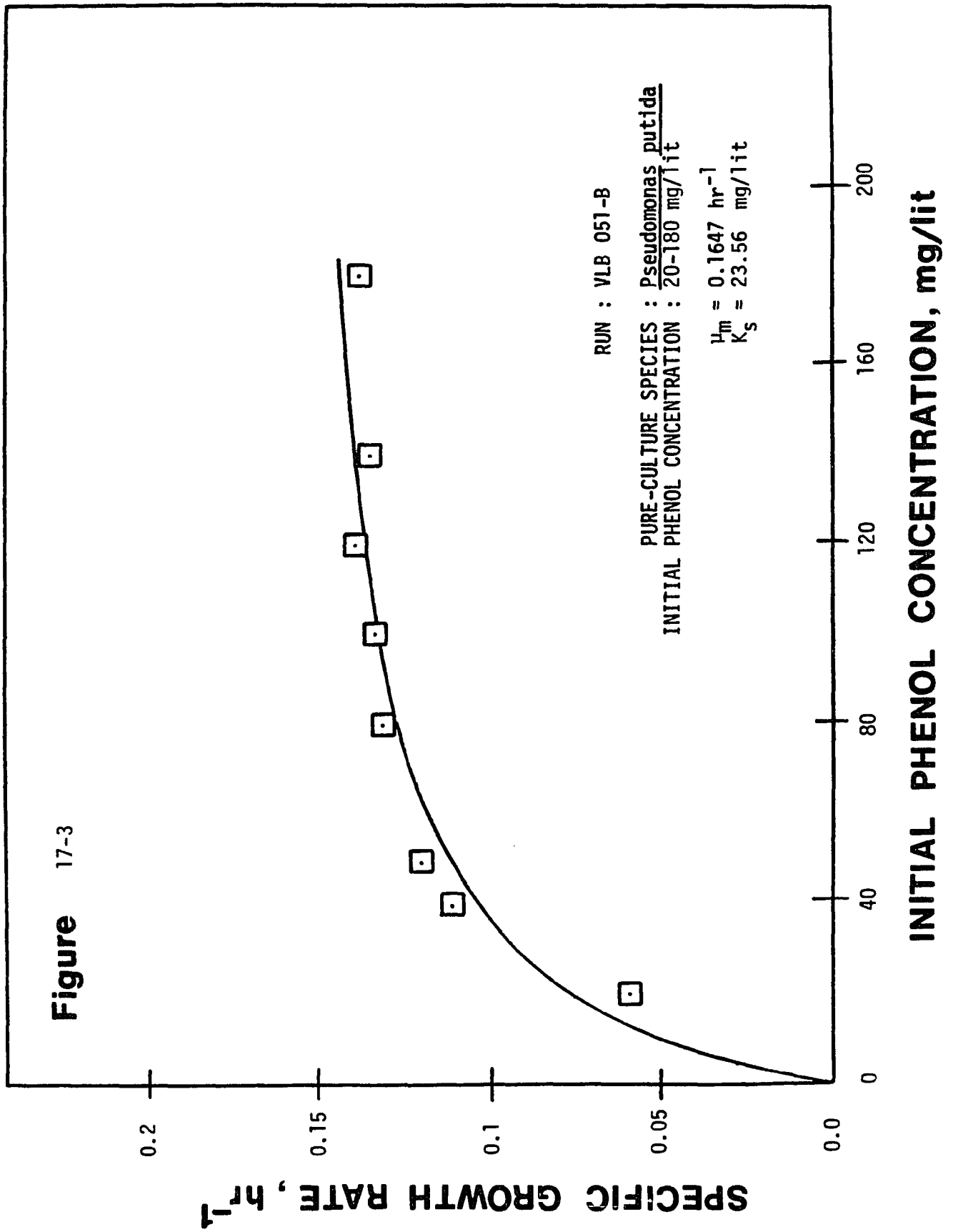


Figure 17-3
RUN : VLB 051-B
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20-180 mg/lit
μ_m = 0.1647 hr^{-1}
K_s = 23.56 mg/lit
SPECIFIC GROWTH RATE, hr^{-1}
0.0
0.05
0.1
0.15
0.2
0
40
80
120
160
200
INITIAL PHENOL CONCENTRATION, mg/lit

Figure 18

Yield Coefficients of *Serratia liquefaciens* at the following Initial Phenol Concentration (Run VLB 045-1 to VLB 045-8)

18-1	20 mg/lit
18-2	40 mg/lit
18-3	50 mg/lit
18-4	80 mg/lit
18-5	100 mg/lit
18-6	120 mg/lit
18-7	140 mg/lit
18-8	180 mg/lit

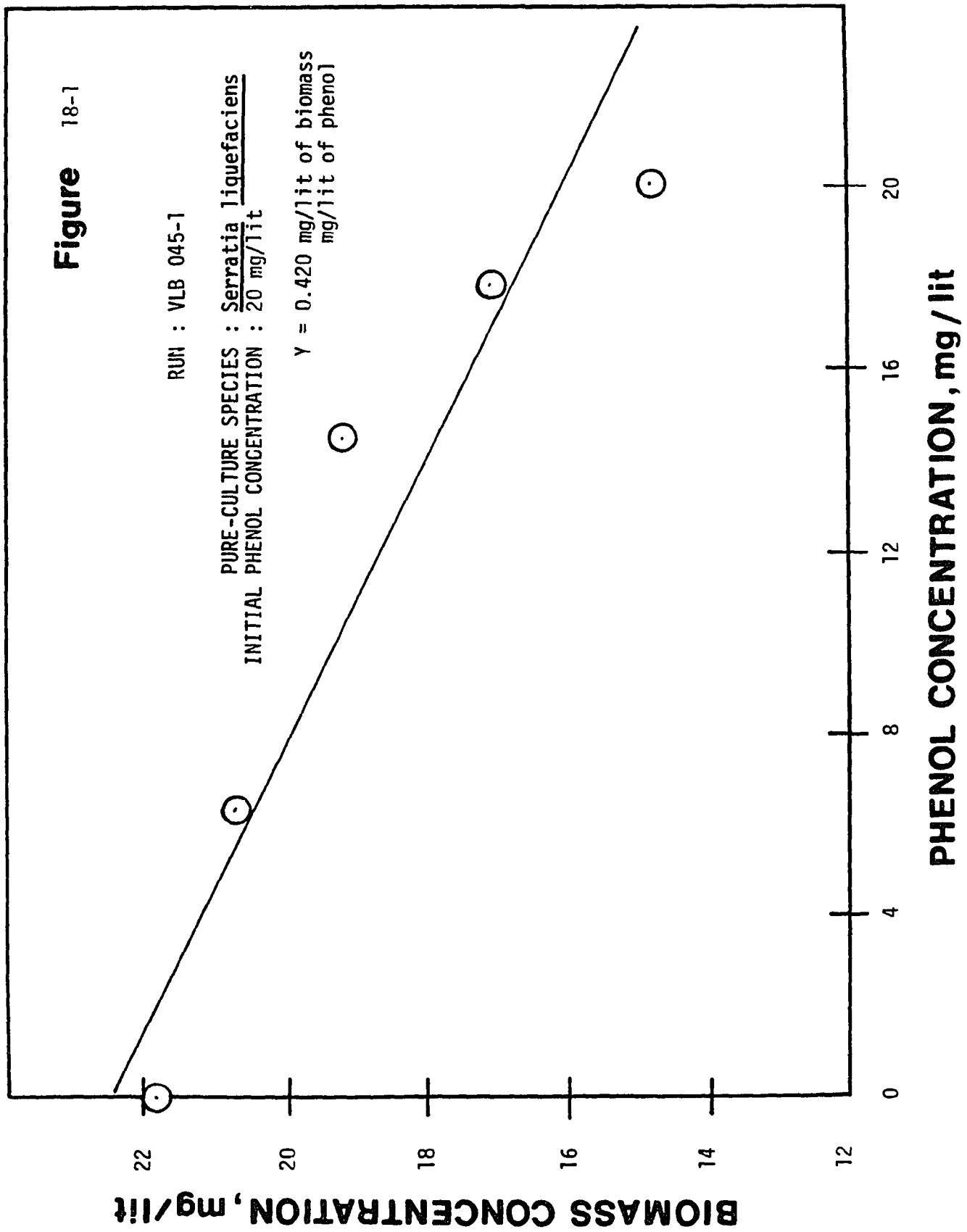


Figure 18-1
RUN : VLB 045-1
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 20 mg/lit
Y = 0.420 mg/lit of biomass
mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
22
20
18
16
14
12
0
4
8
12
16
20

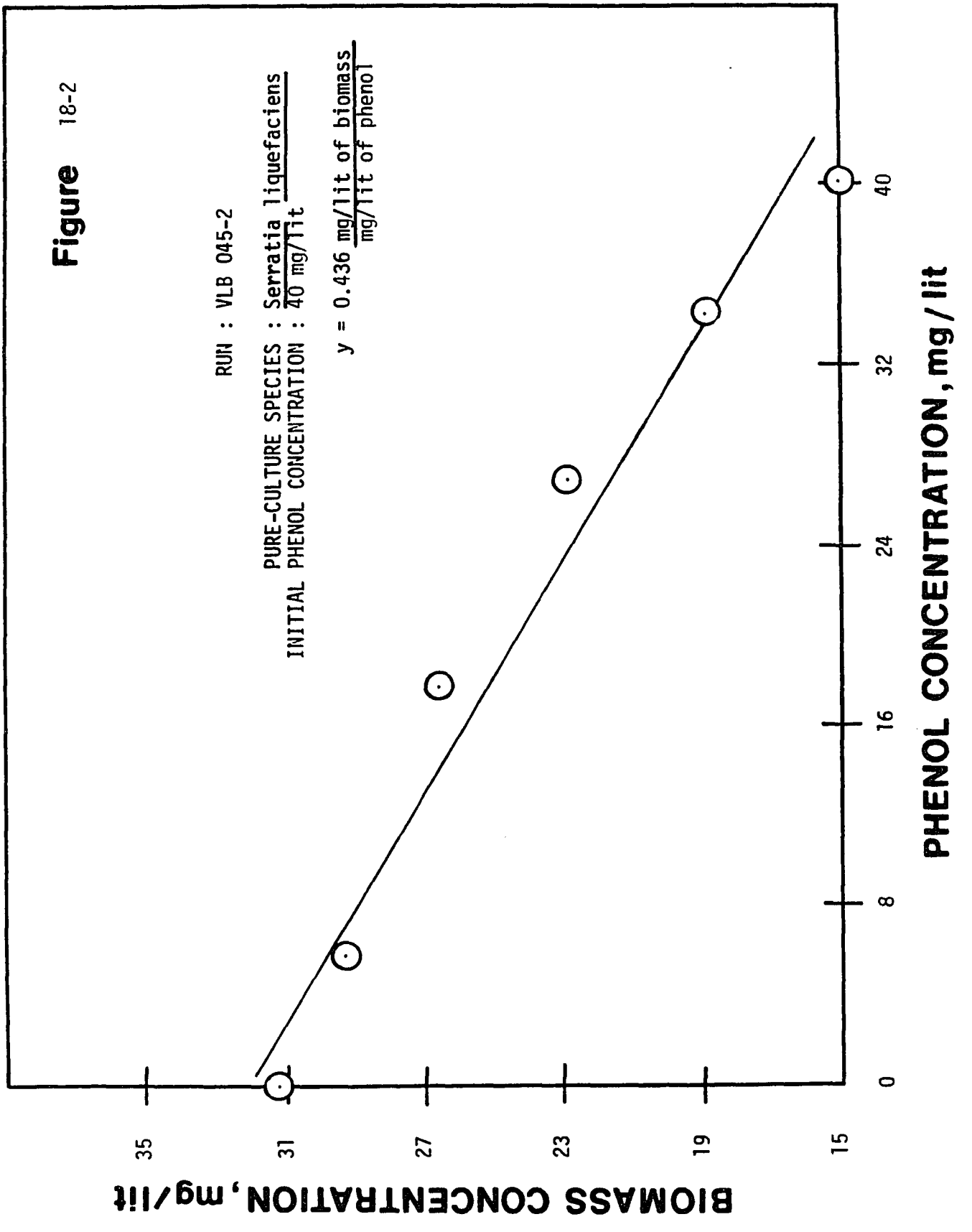


Figure 18-2
RUN : VLB 045-2
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 40 mg/lit
y = 0.436 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
35
31
27
23
19
15
0
8
16
24
32
40

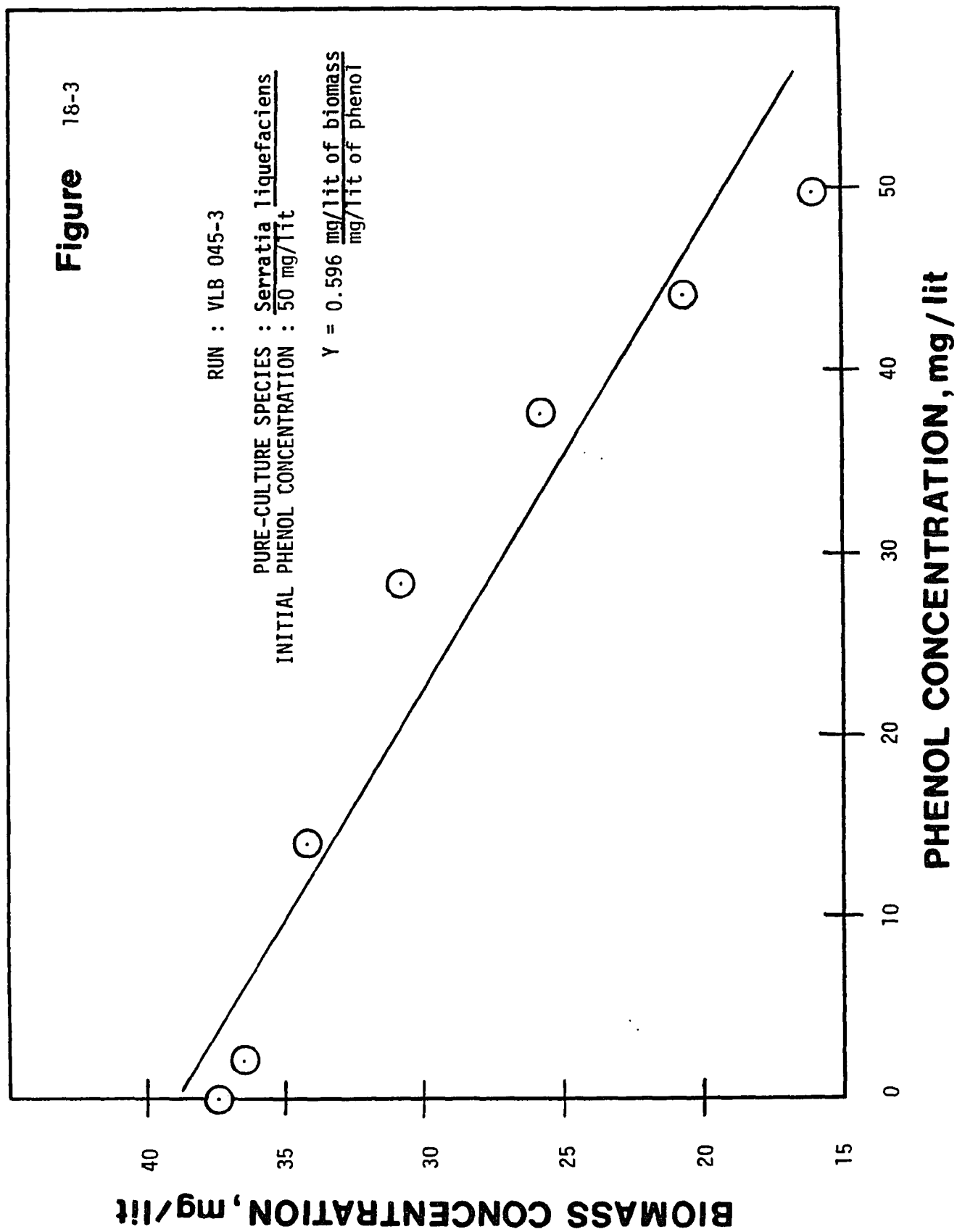


Figure 18-3
RUN : VLB 045-3
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 50 mg/lit
Y = 0.596 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
40
35
30
25
20
15
0
10
20
30
40
50

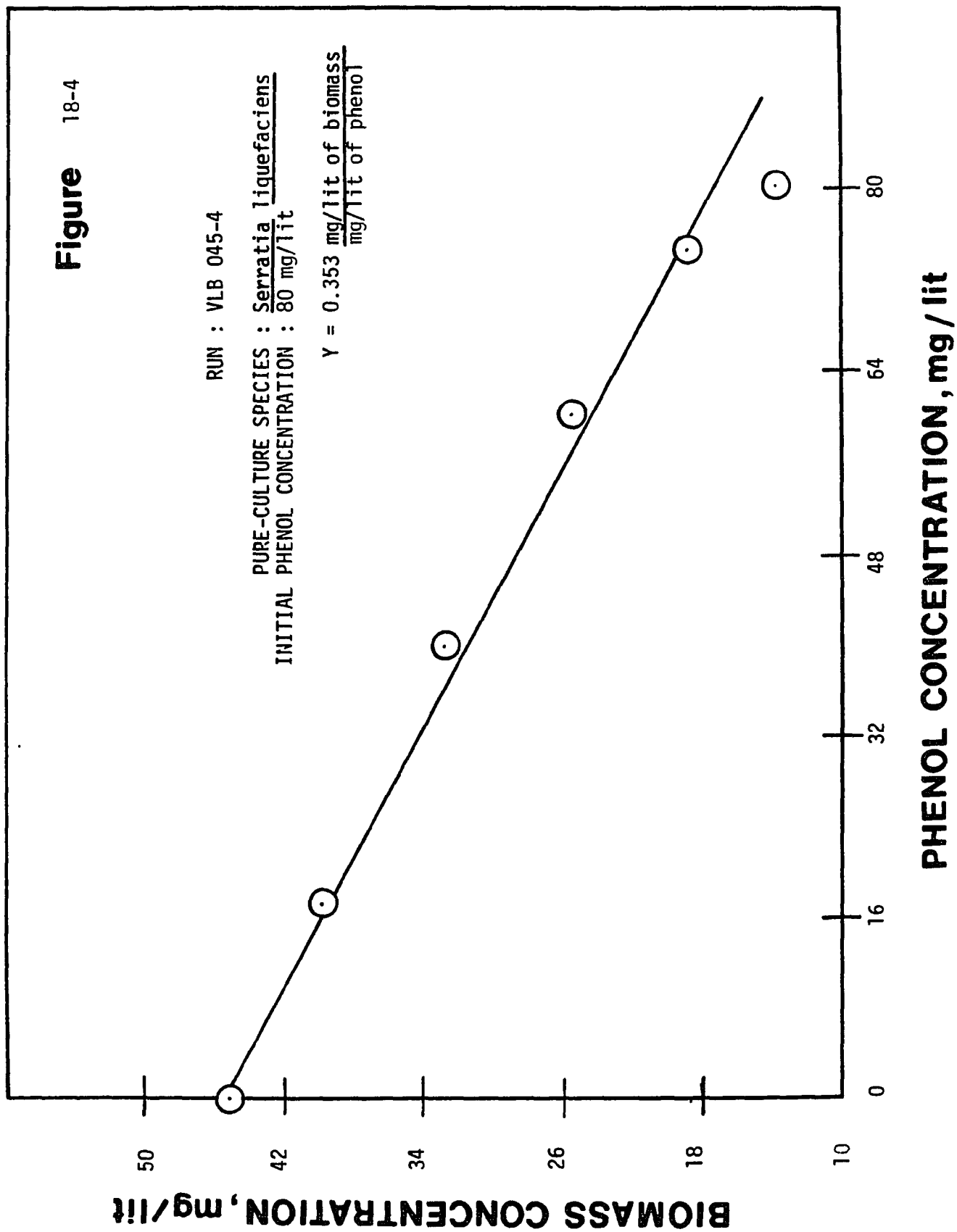


Figure 18-4
RUN : VLB 045-4
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 80 mg/lit
Y = 0.353 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
50
42
34
26
18
10
0
16
32
48
64
80
PHENOL CONCENTRATION, mg/lit

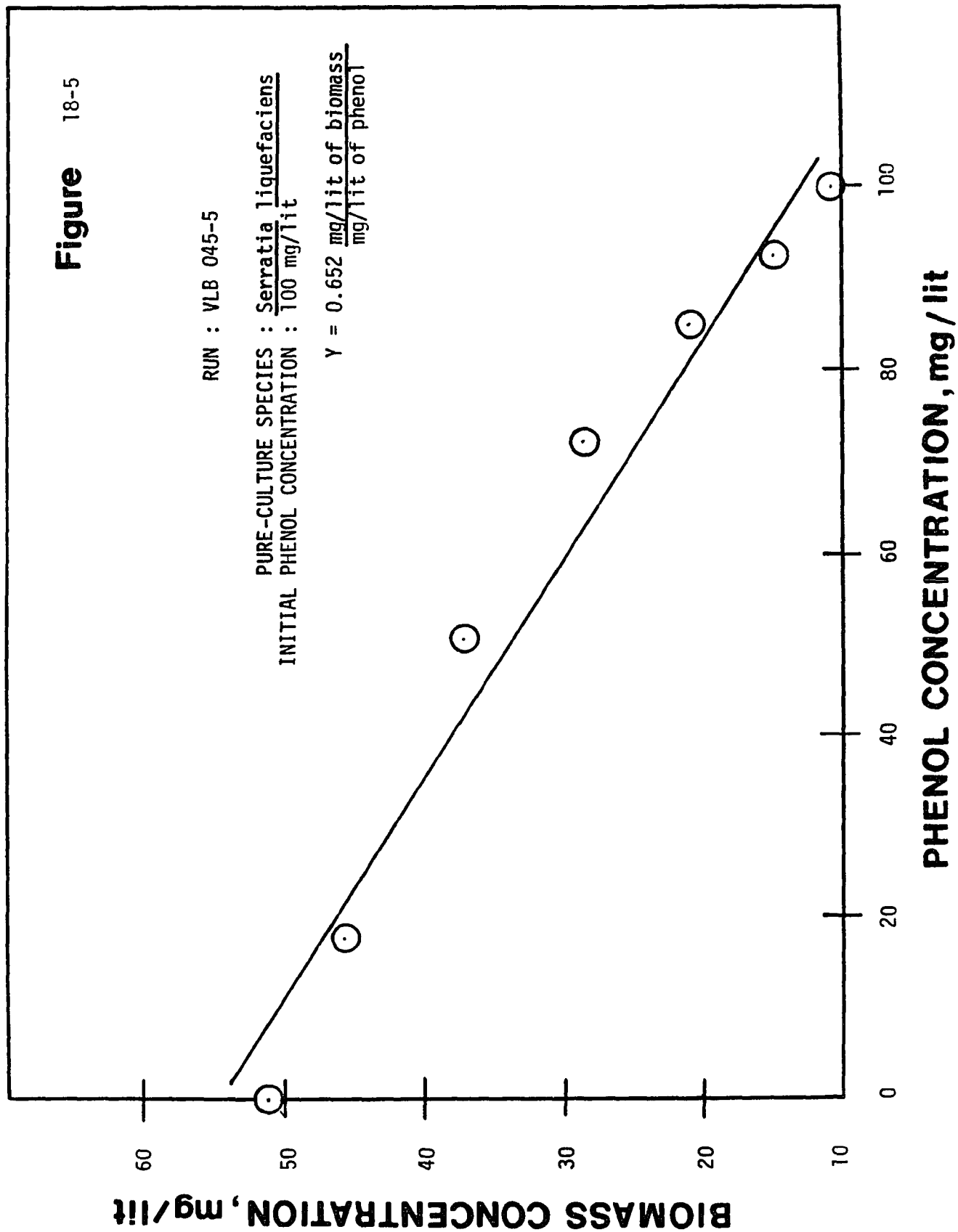


Figure 18-5
RUN : VLB 045-5
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 100 mg/lit
Y = 0.652 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
10
20
30
40
50
60
0
20
40
60
80
100

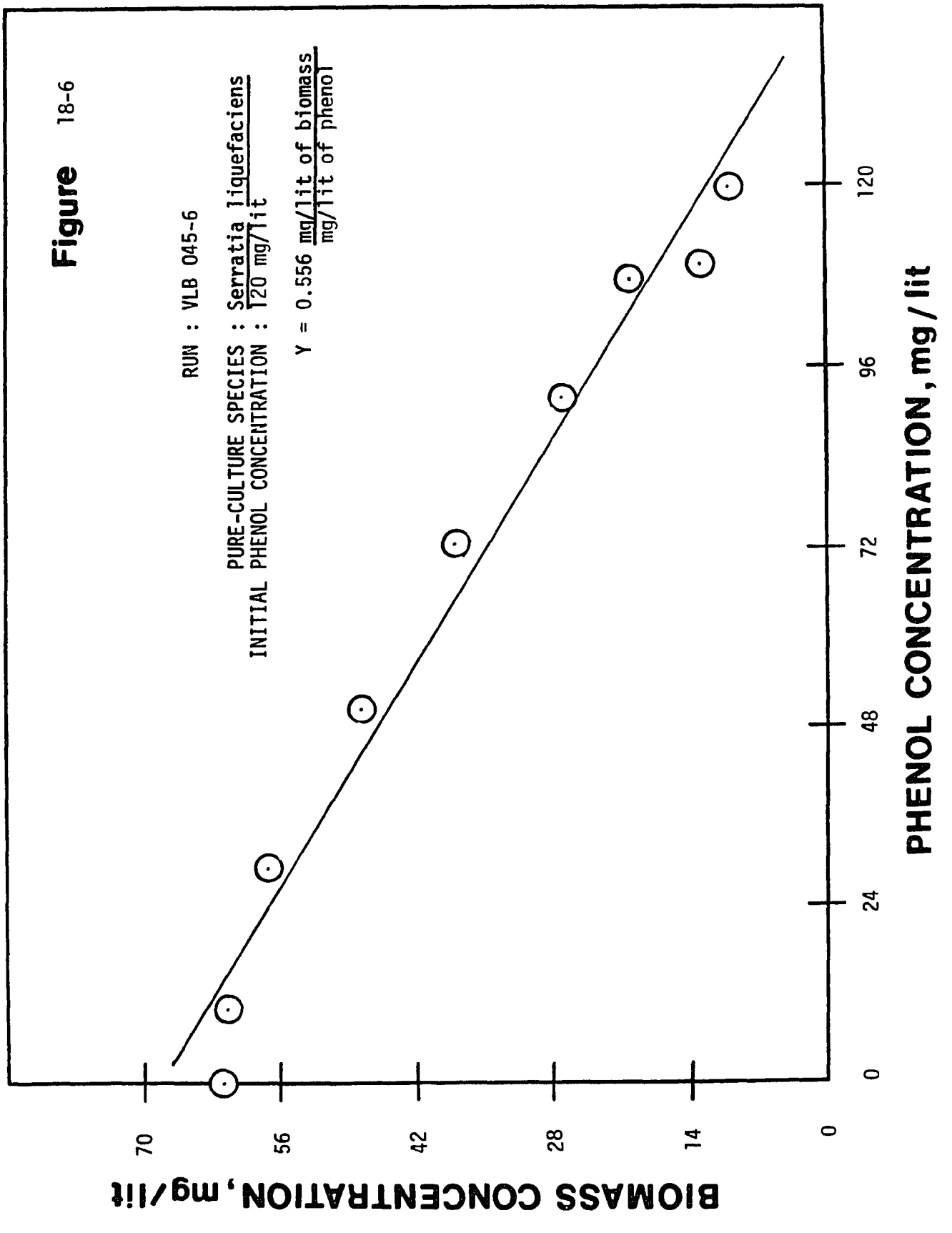


Figure 18-6
RUN : VLB 045-6
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 120 mg/lit
Y = 0.556 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
70
56
42
28
14
0
0
24
48
72
96
120
PHENOL CONCENTRATION, mg/lit

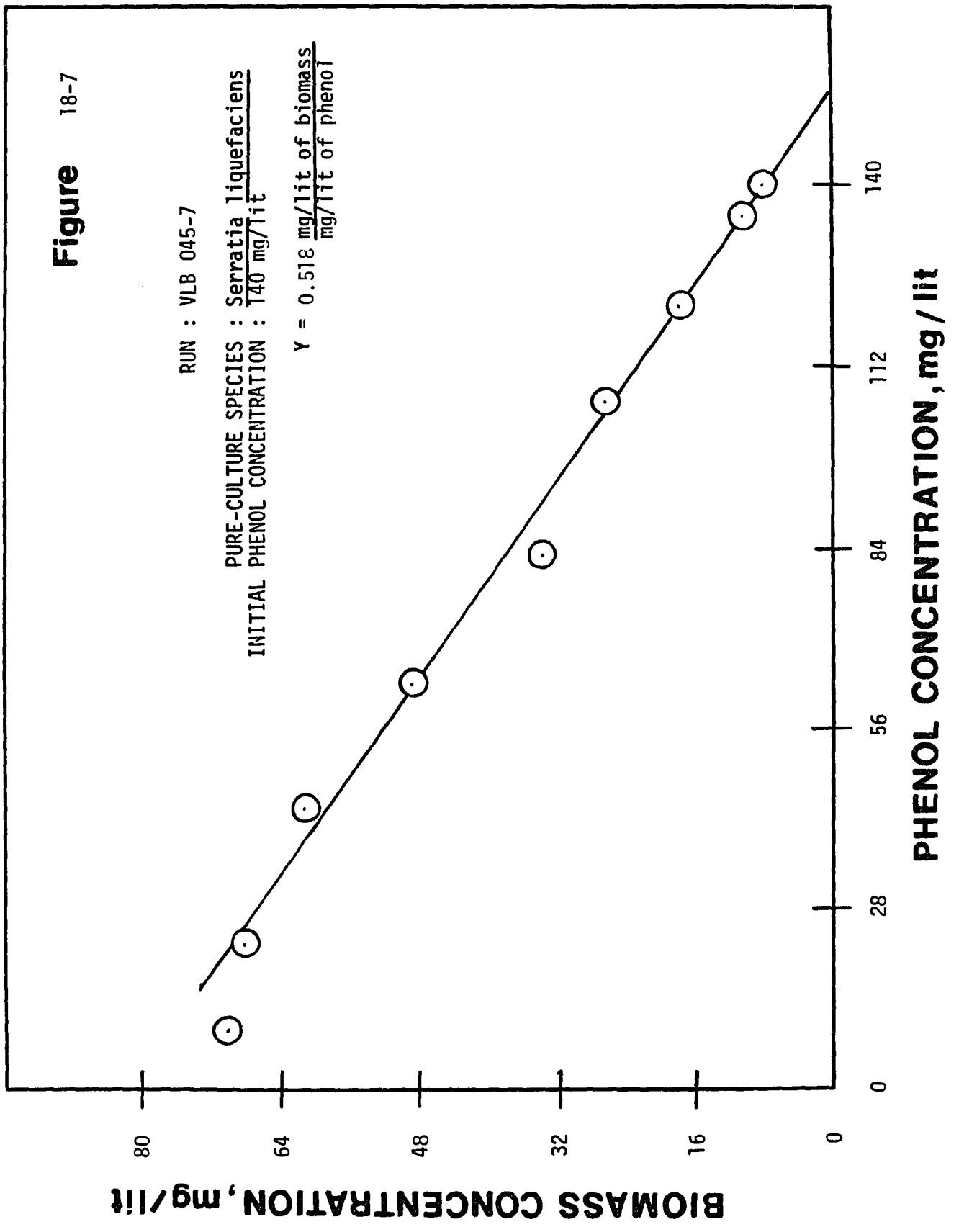


Figure 18-7
RUN : VLB 045-7
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 140 mg/lit
Y = 0.518 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
0
16
32
48
64
80
0
28
56
84
112
140
PHENOL CONCENTRATION, mg/lit

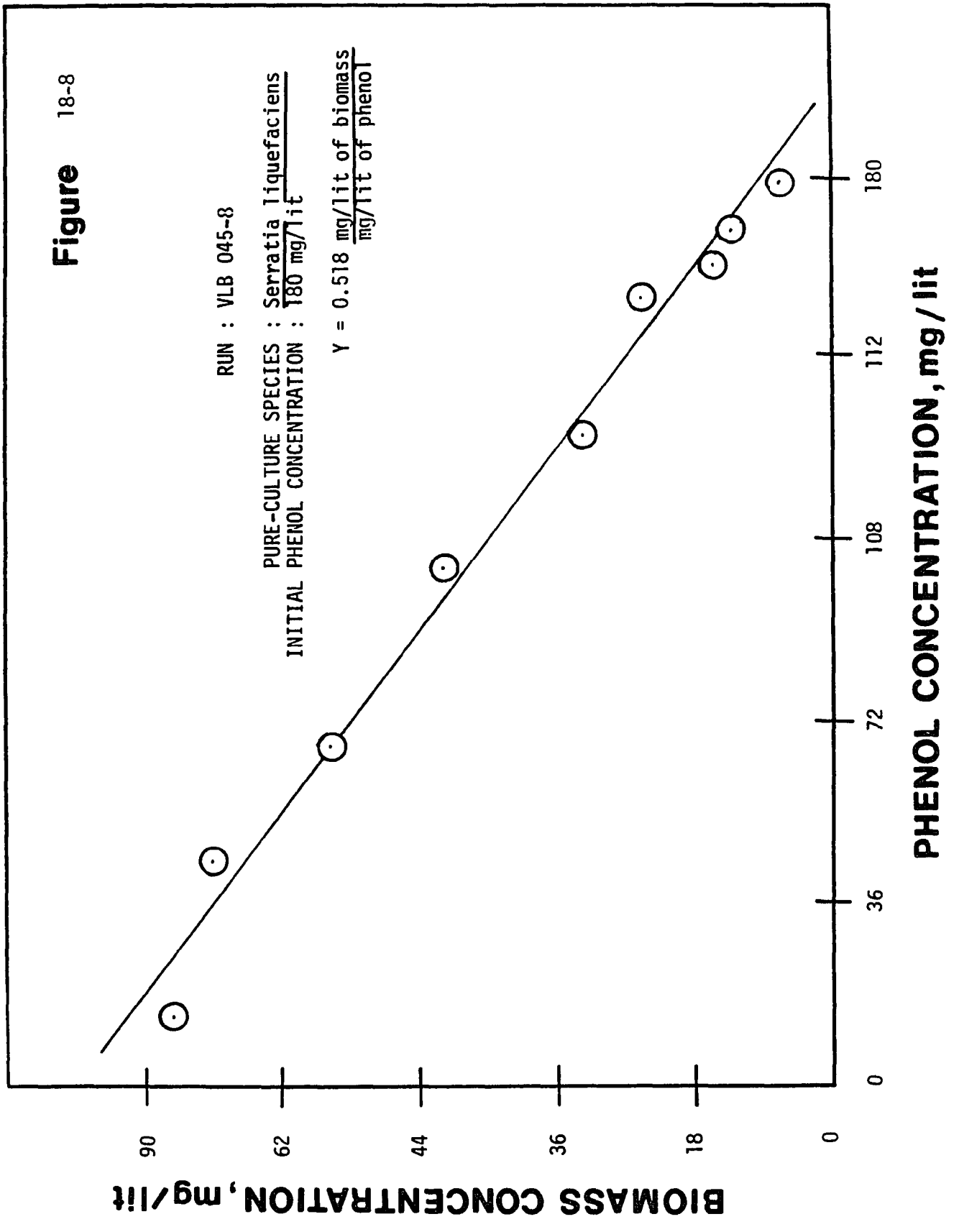


Figure 18-8
RUN : VLB 045-8
PURE-CULTURE SPECIES : Serratia liquefaciens
INITIAL PHENOL CONCENTRATION : 180 mg/lit
Y = 0.518 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
0
18
36
44
62
90
0
36
72
108
112
180
PHENOL CONCENTRATION, mg/lit

Figure 19

Yield Coefficients of Klebsiella pneumoniae at the following Initial Phenol Concentrations (Run VLB 049-1 to VLB 049-8)

19-1	20 mg/lit
19-2	40 mg/lit
19-3	50 mg/lit
19-4	80 mg/lit
19-5	100 mg/lit
19-6	120 mg/lit
19-7	140 mg/lit
19-8	180 mg/lit

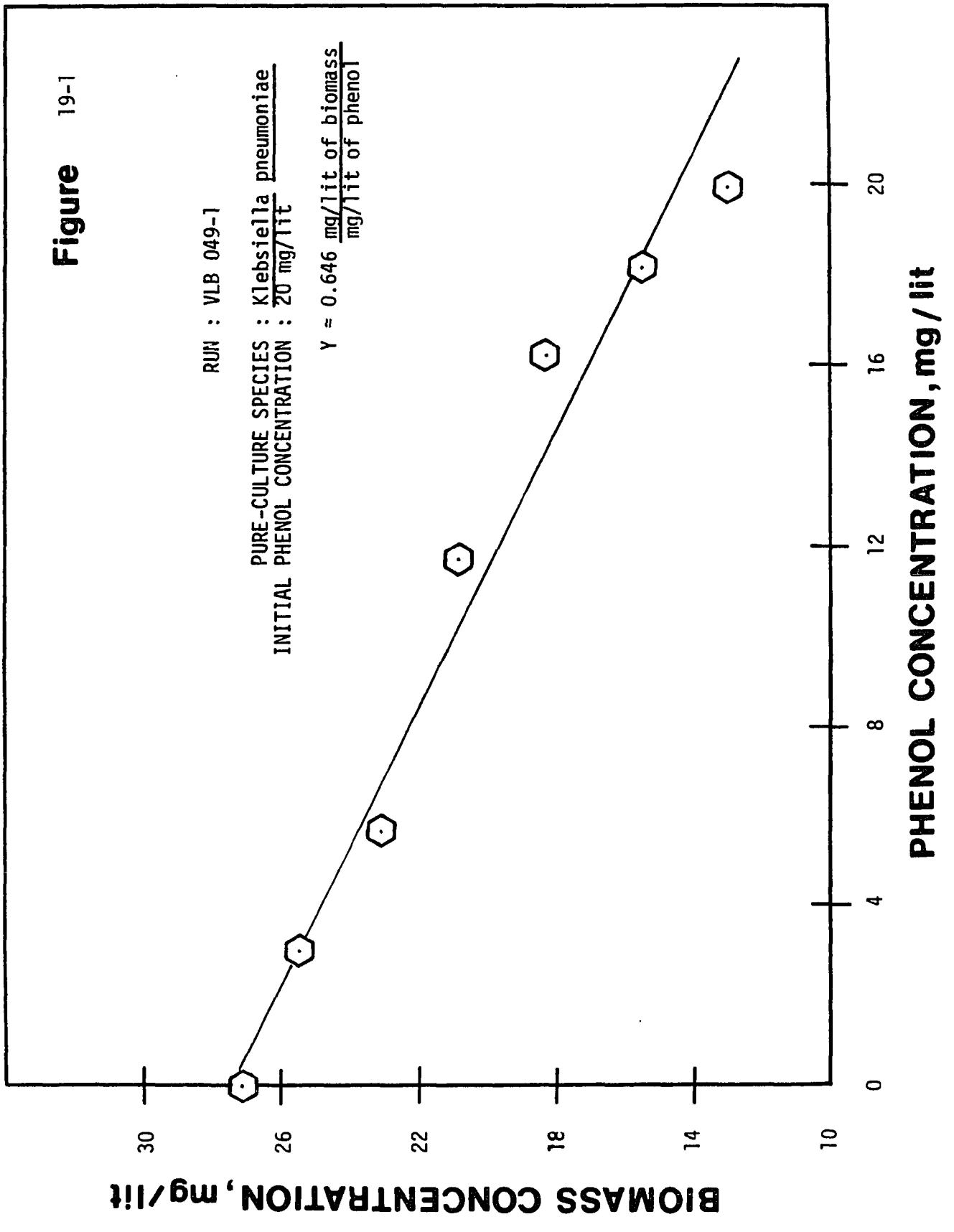


Figure 19-1
RUN : VLB 049-1
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20 mg/lit
Y = 0.646 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
10
14
18
22
26
30
0
4
8
12
16
20

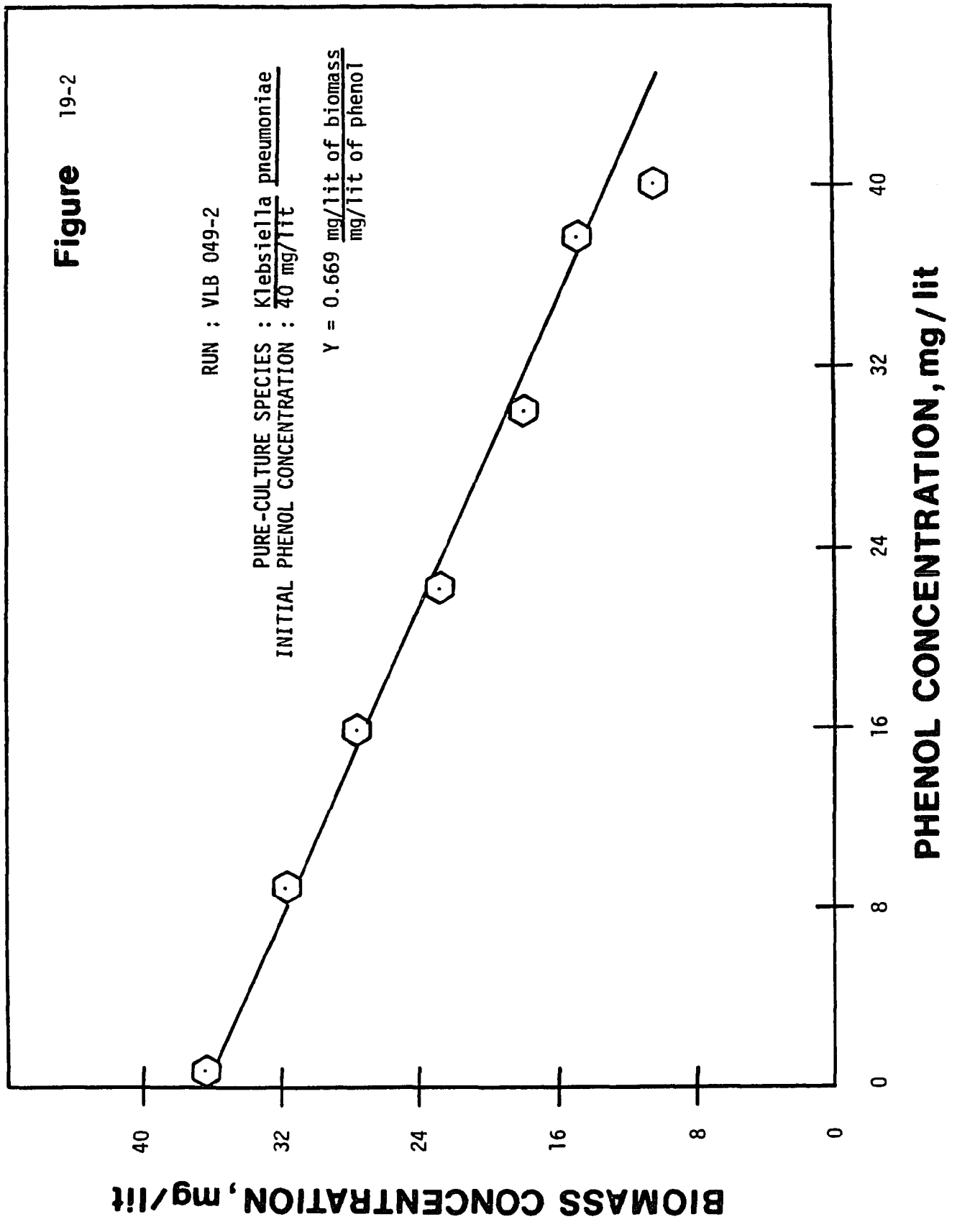


Figure 19-2
RUN : VLB 049-2
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 40 mg/lit
Y = 0.669 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
0
8
16
24
32
40
PHENOL CONCENTRATION, mg/lit
0
8
16
24
32
40

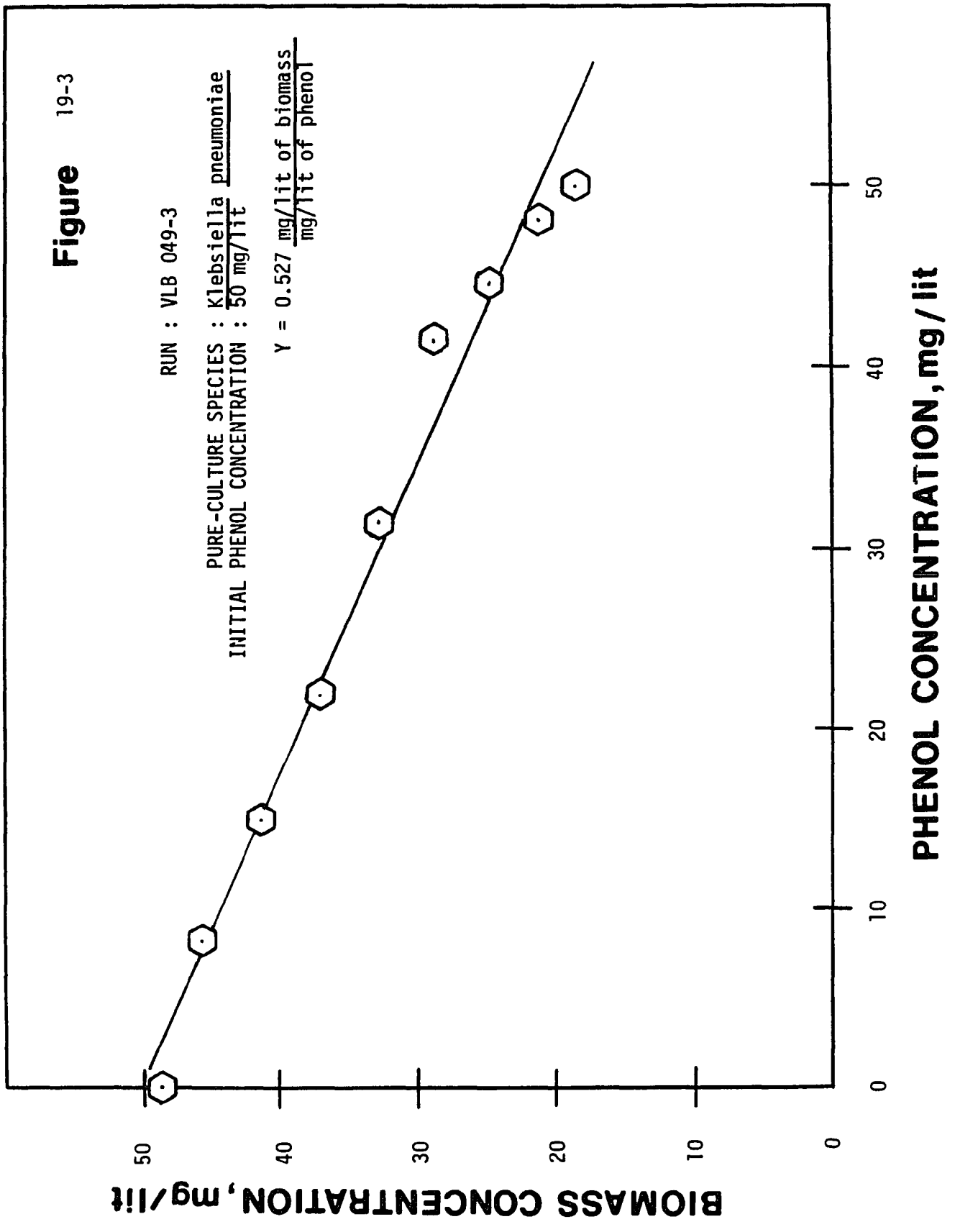


Figure 19-3
RUN : VLB 049-3
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 50 mg/lit
Y = 0.527 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
0
10
20
30
40
50

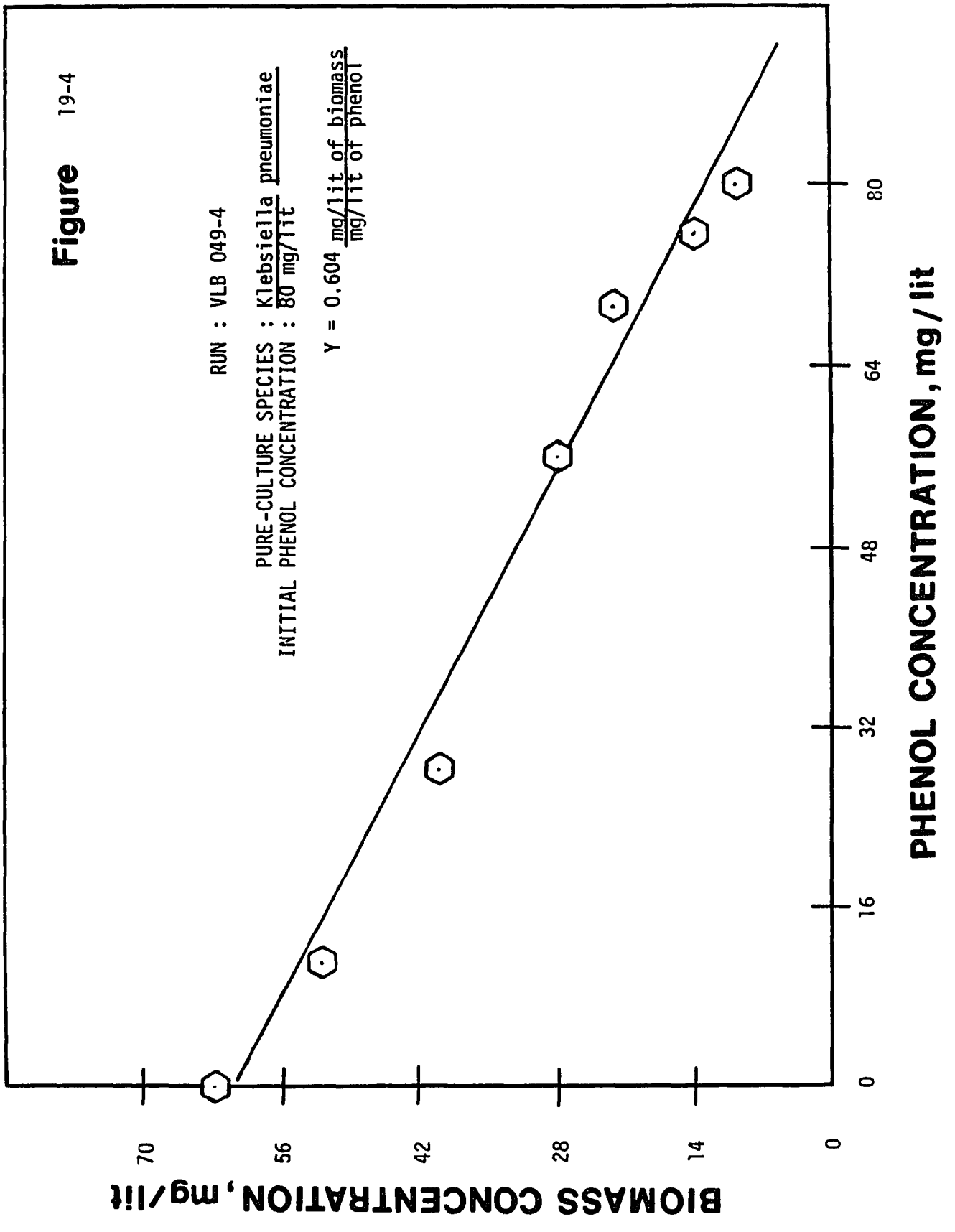


Figure 19-4
RUN : VLB 049-4
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 80 mg/lit
Y = 0.604 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
0
14
28
42
56
70
0
16
32
48
64
80
PHENOL CONCENTRATION, mg/lit

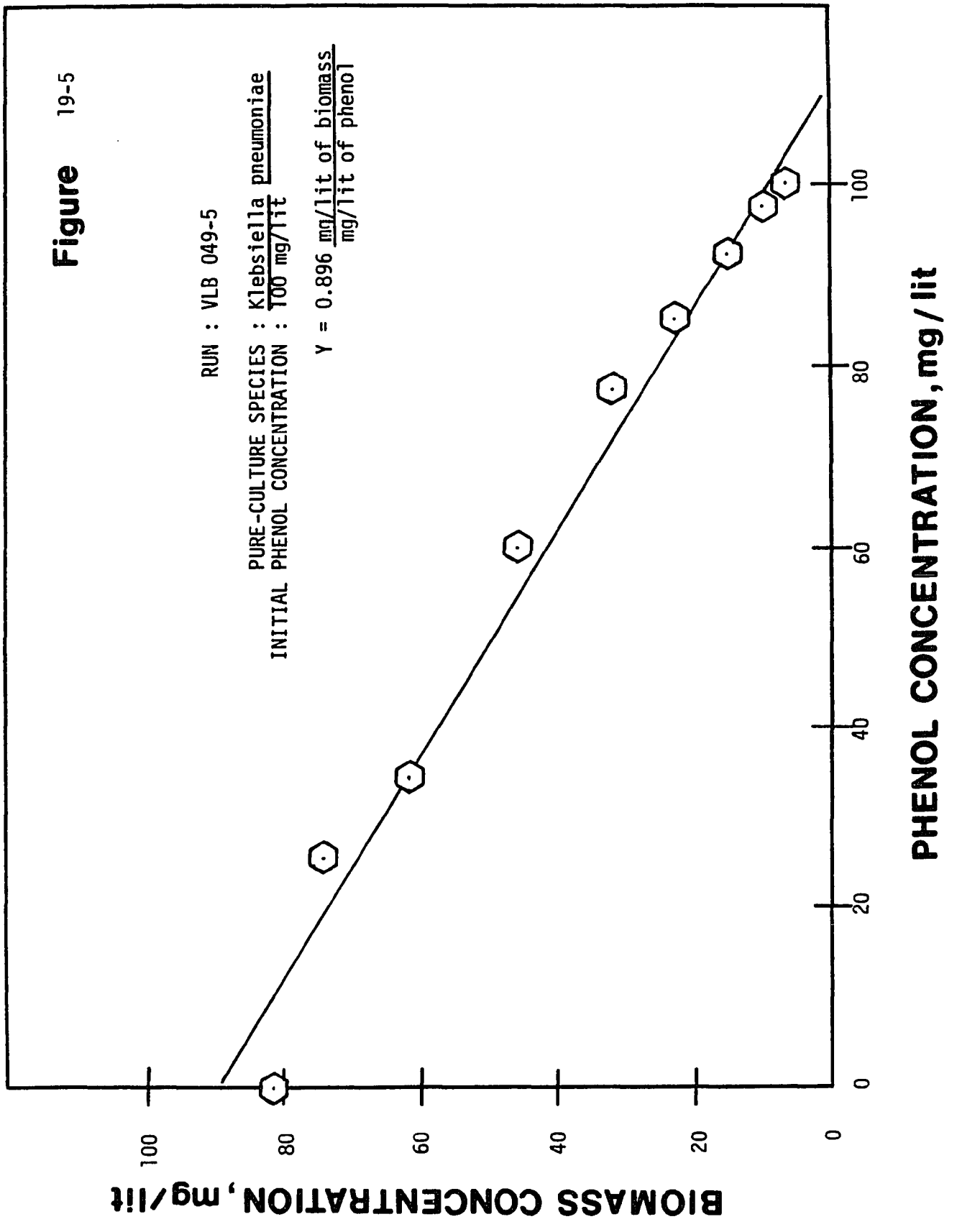


Figure 19-5
RUN : VLB 049-5
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 100 mg/lit
Y = 0.896 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
0
20
40
60
80
100

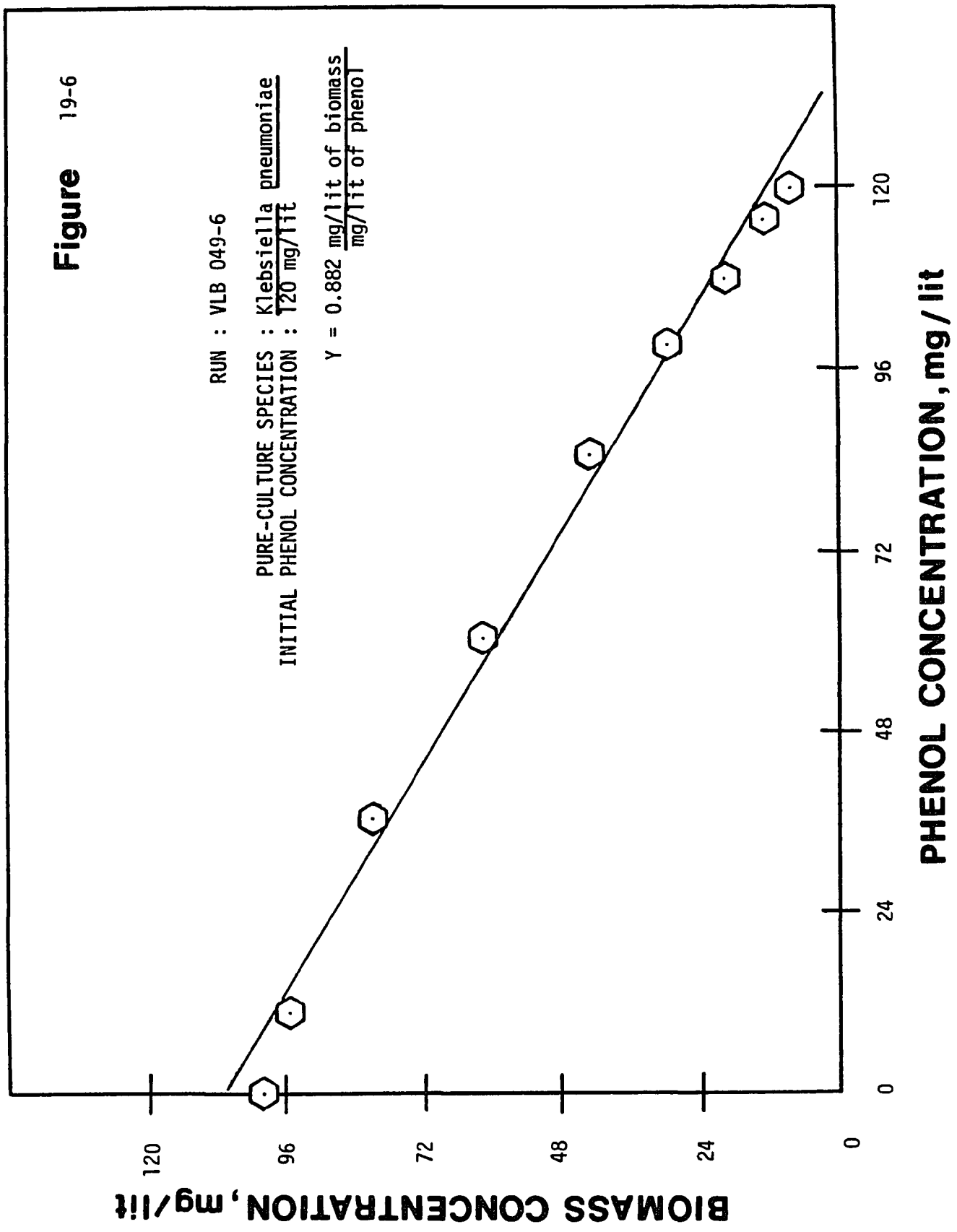


Figure 19-6
RUN : VLB 049-6
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 120 mg/lit
Y = 0.882 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
0
24
48
72
96
120

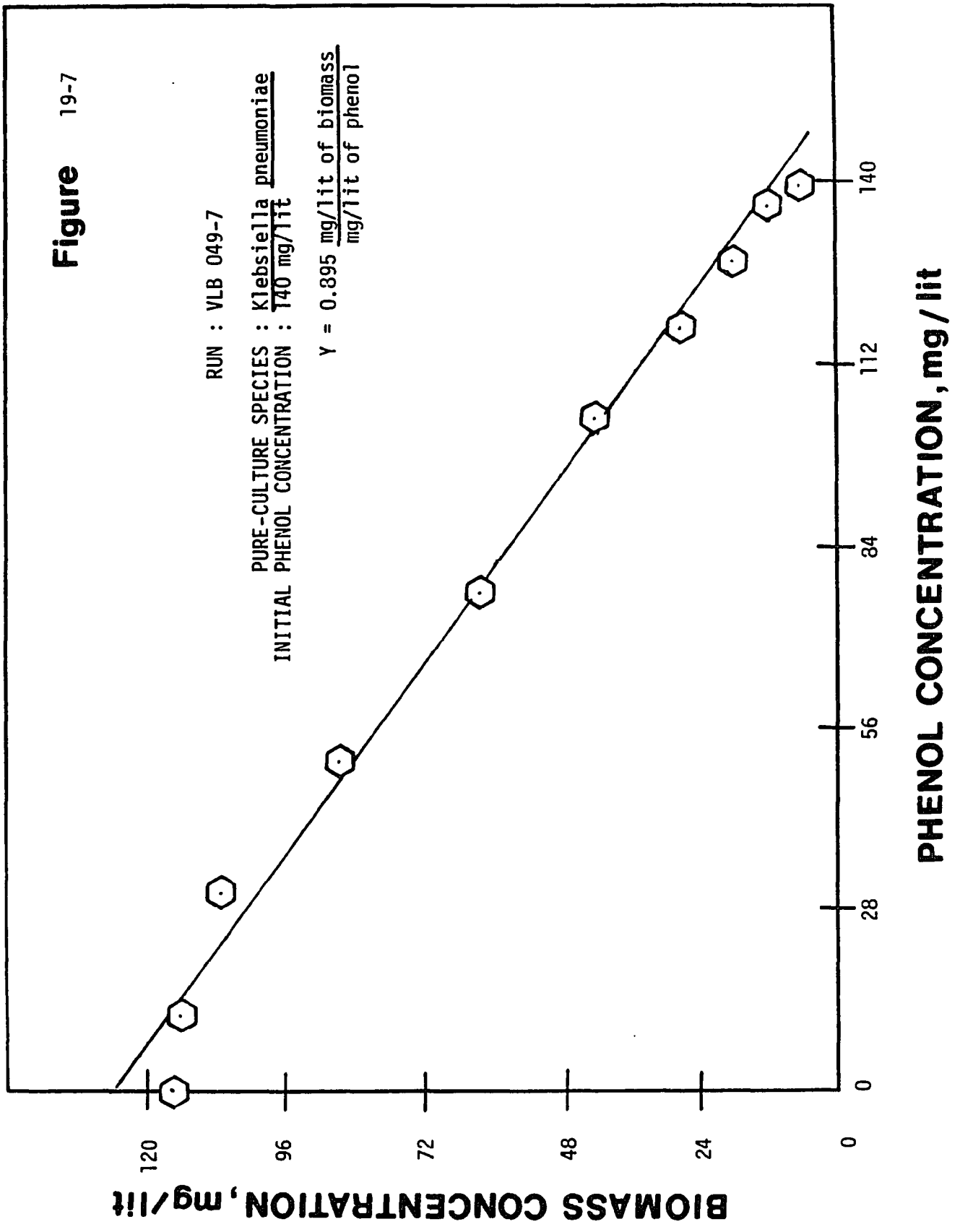


Figure 19-7
RUN : VLB 049-7
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 140 mg/lit
Y = 0.395 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
0
24
48
72
96
120
0
28
56
84
112
140
PHENOL CONCENTRATION, mg/lit

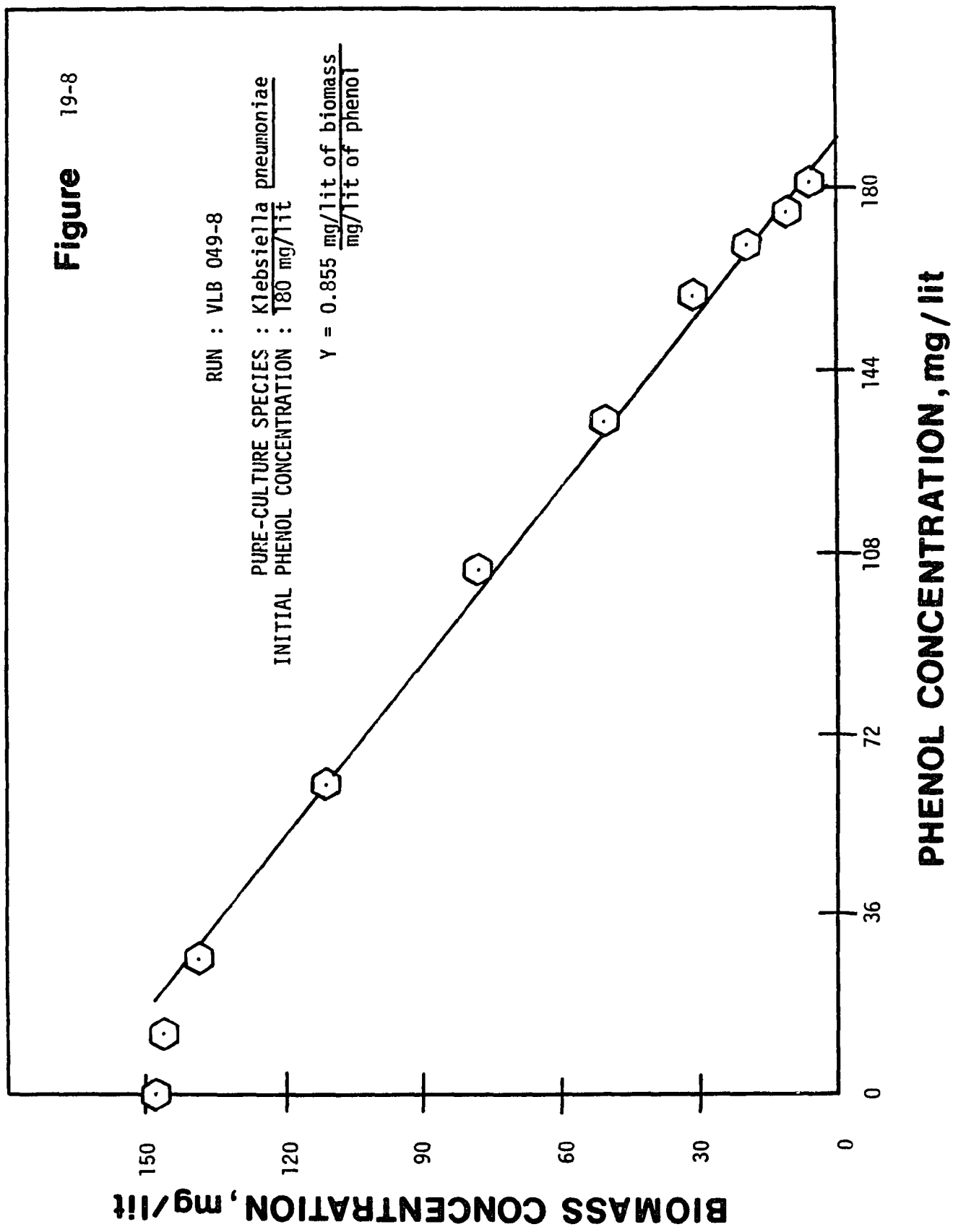


Figure 19-8
RUN : VLB 049-8
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 180 mg/lit
Y = 0.855 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
150
120
90
60
30
0
0
36
72
108
144
180
PHENOL CONCENTRATION, mg/lit

Figure 20

Yield Coefficients of Pseudomonas putida at the following Initial Phenol Concentrations (Run VLB 051-1 to VLB 051-8)

20-1	20 mg/lit
20-2	40 mg/lit
20-3	50 mg/lit
20-4	80 mg/lit
20-5	100 mg/lit
20-6	120 mg/lit
20-7	140 mg/lit
20-8	180 mg/lit

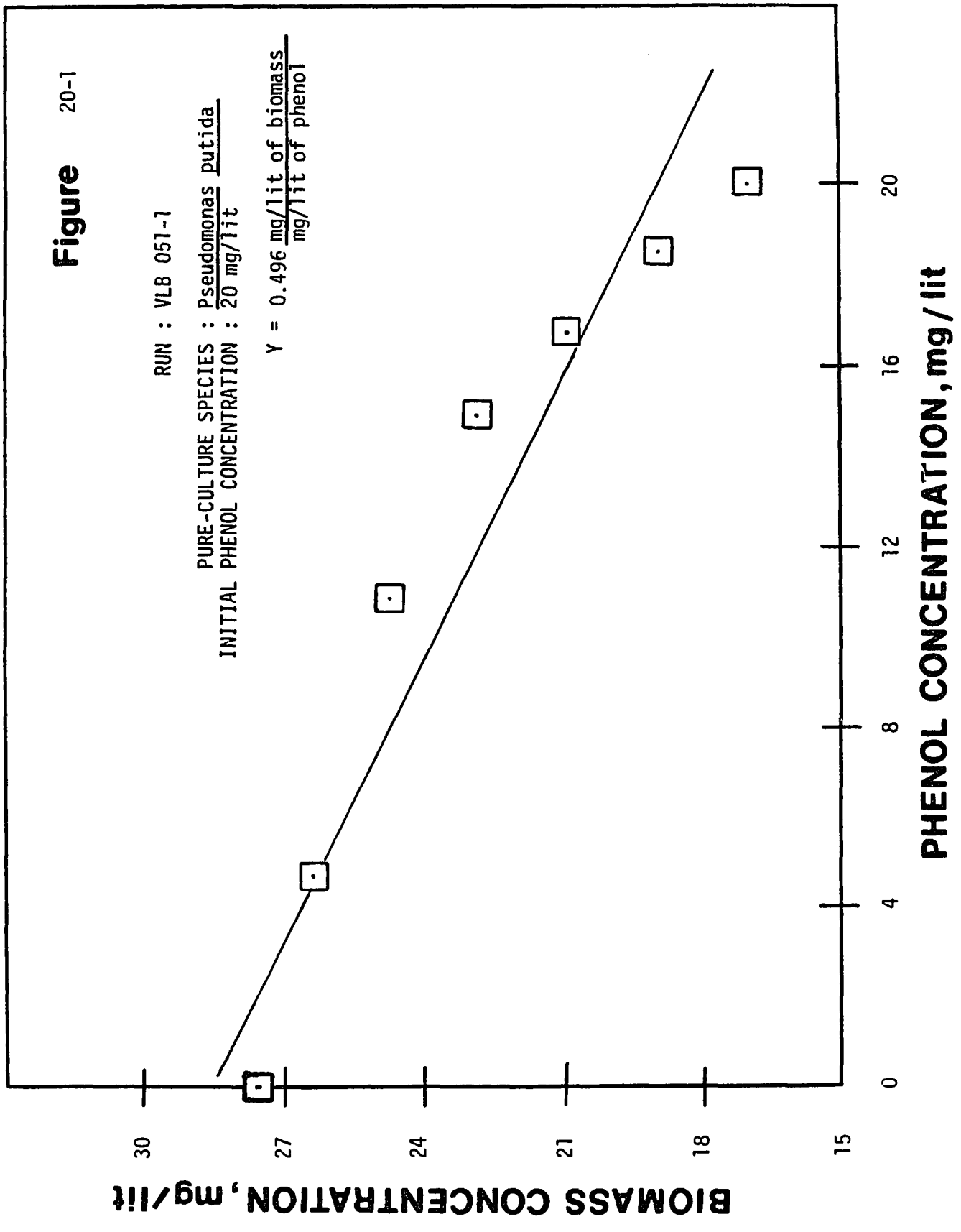


Figure 20-1
RUN : VLB 051-1
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20 mg/lit
Y = 0.496 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
30
27
24
21
18
15
0
4
8
12
16
20

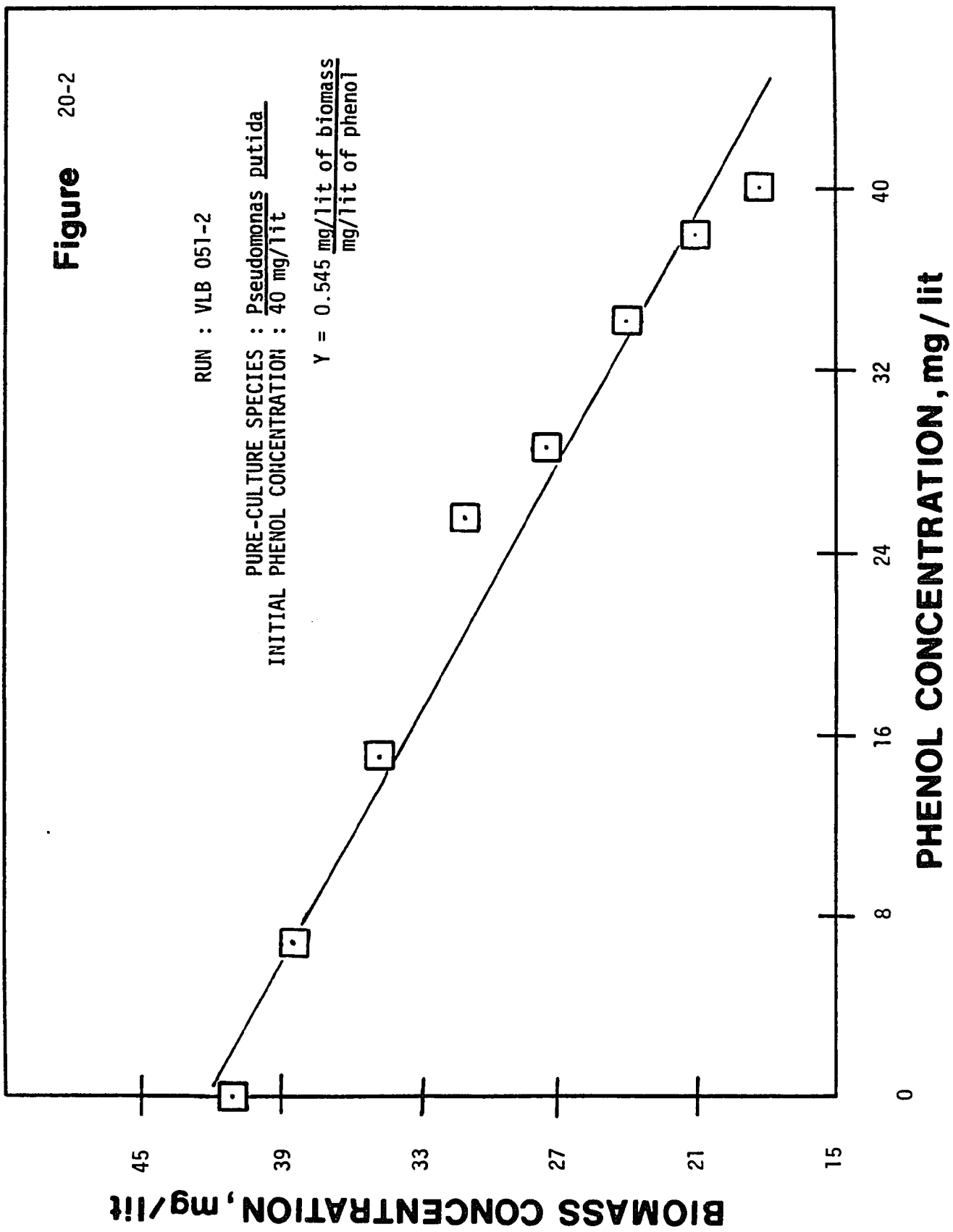


Figure 20-2
RUN : VLB 051-2
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 40 mg/lit
Y = 0.545 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
PHENOL CONCENTRATION, mg/lit
45
39
33
27
21
15
0
8
16
24
32
40

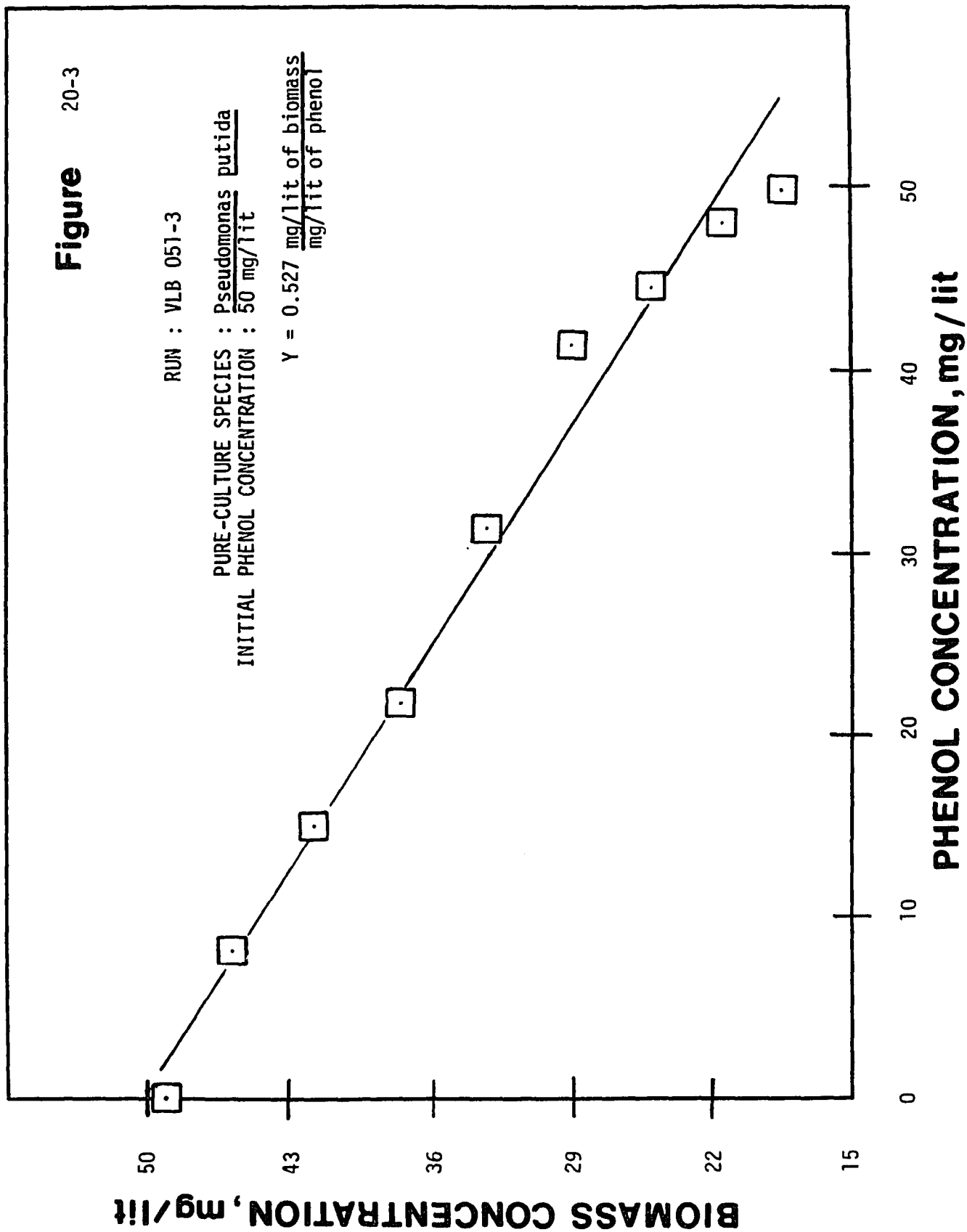


Figure 20-3
RUN : VLB 051-3
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 50 mg/lit
Y = 0.527 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
50
43
36
29
22
15
0
10
20
30
40
50
PHENOL CONCENTRATION, mg/lit

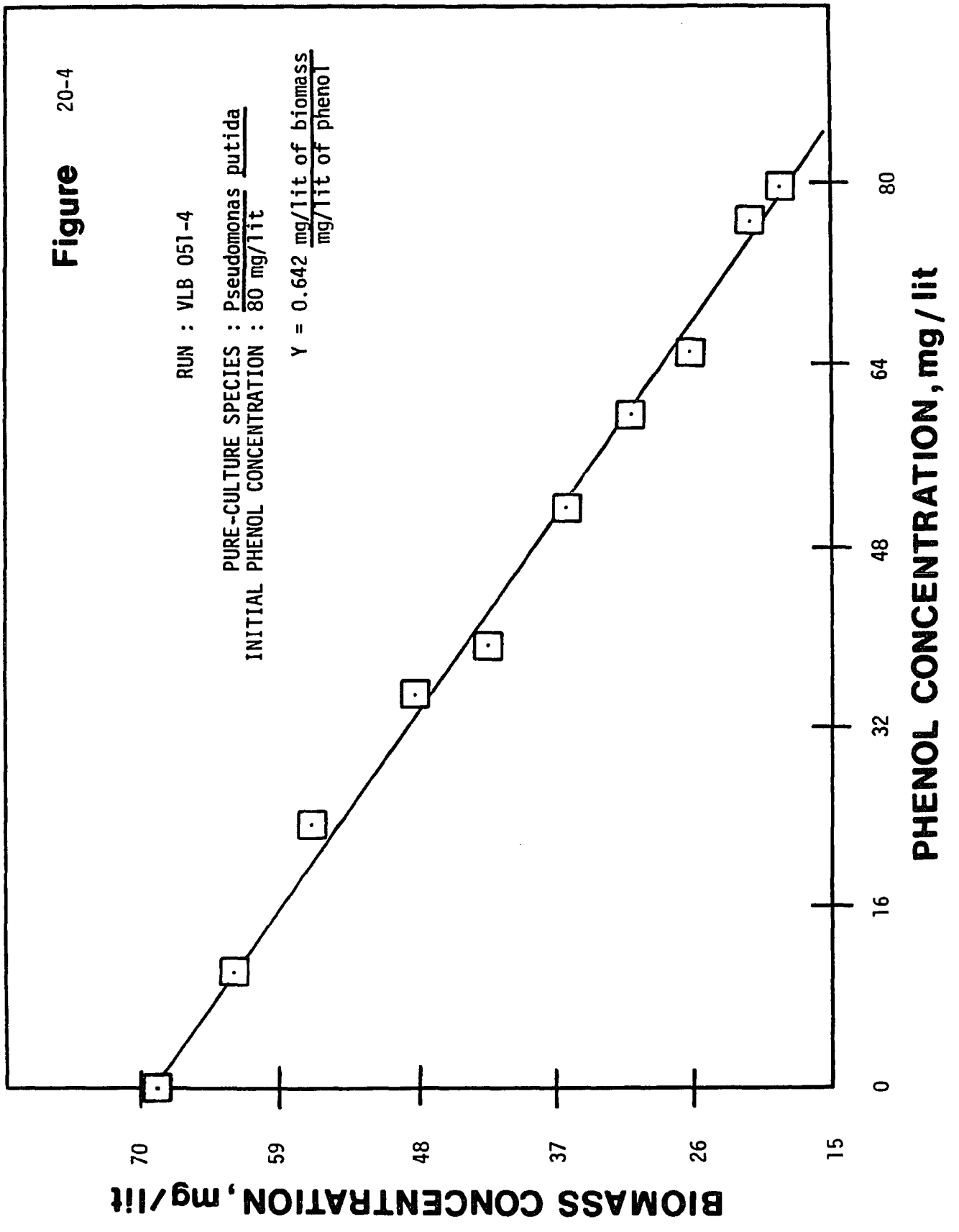


Figure 20-4
RUN : VLB 051-4
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 80 mg/lit
Y = 0.642 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
70
59
48
37
26
15
0
16
32
48
64
80
PHENOL CONCENTRATION, mg/lit

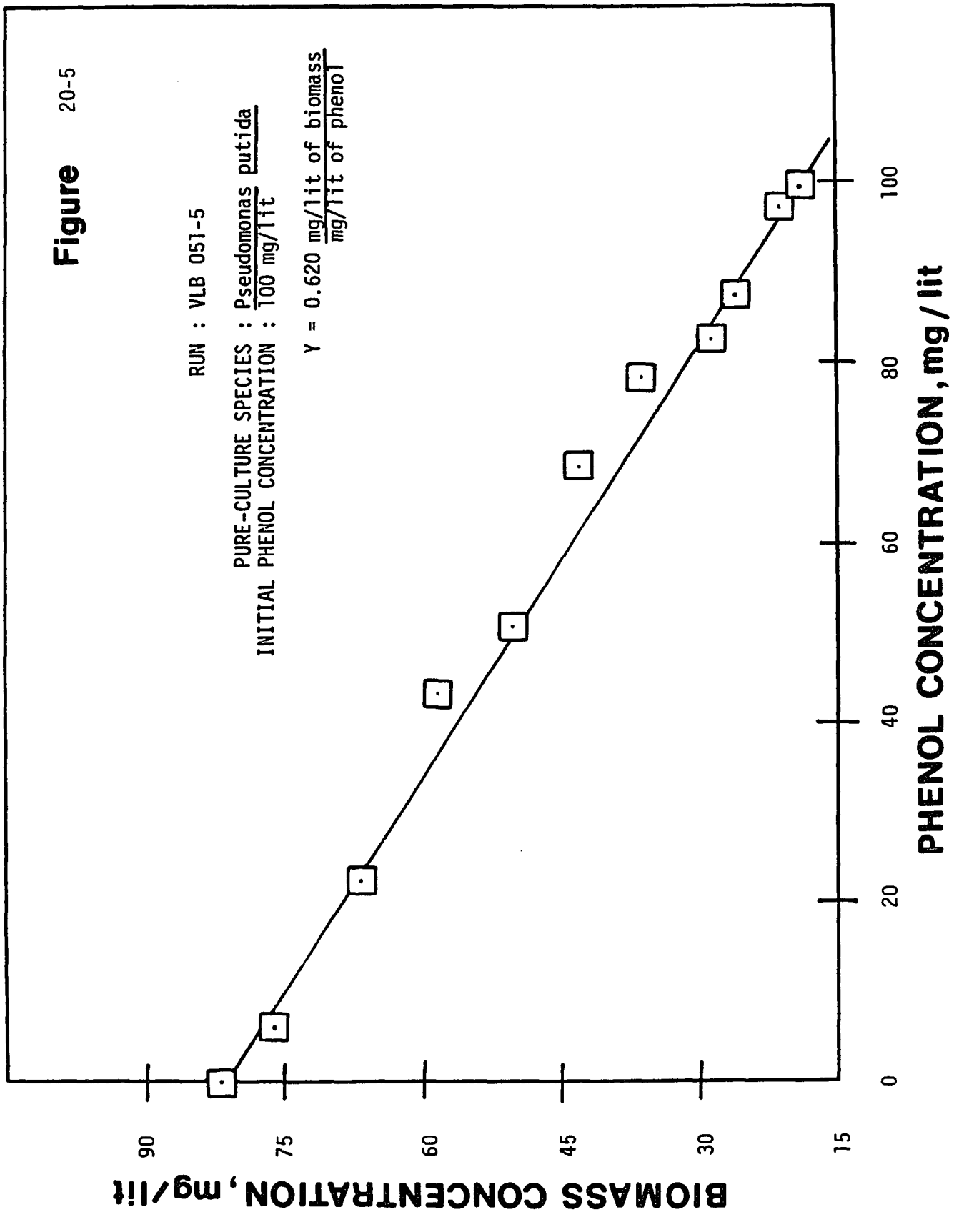


Figure 20-5
RUN : VLB 051-5
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 100 mg/lit
Y = 0.620 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
90
75
60
45
30
15
0
20
40
60
80
100
PHENOL CONCENTRATION, mg/lit

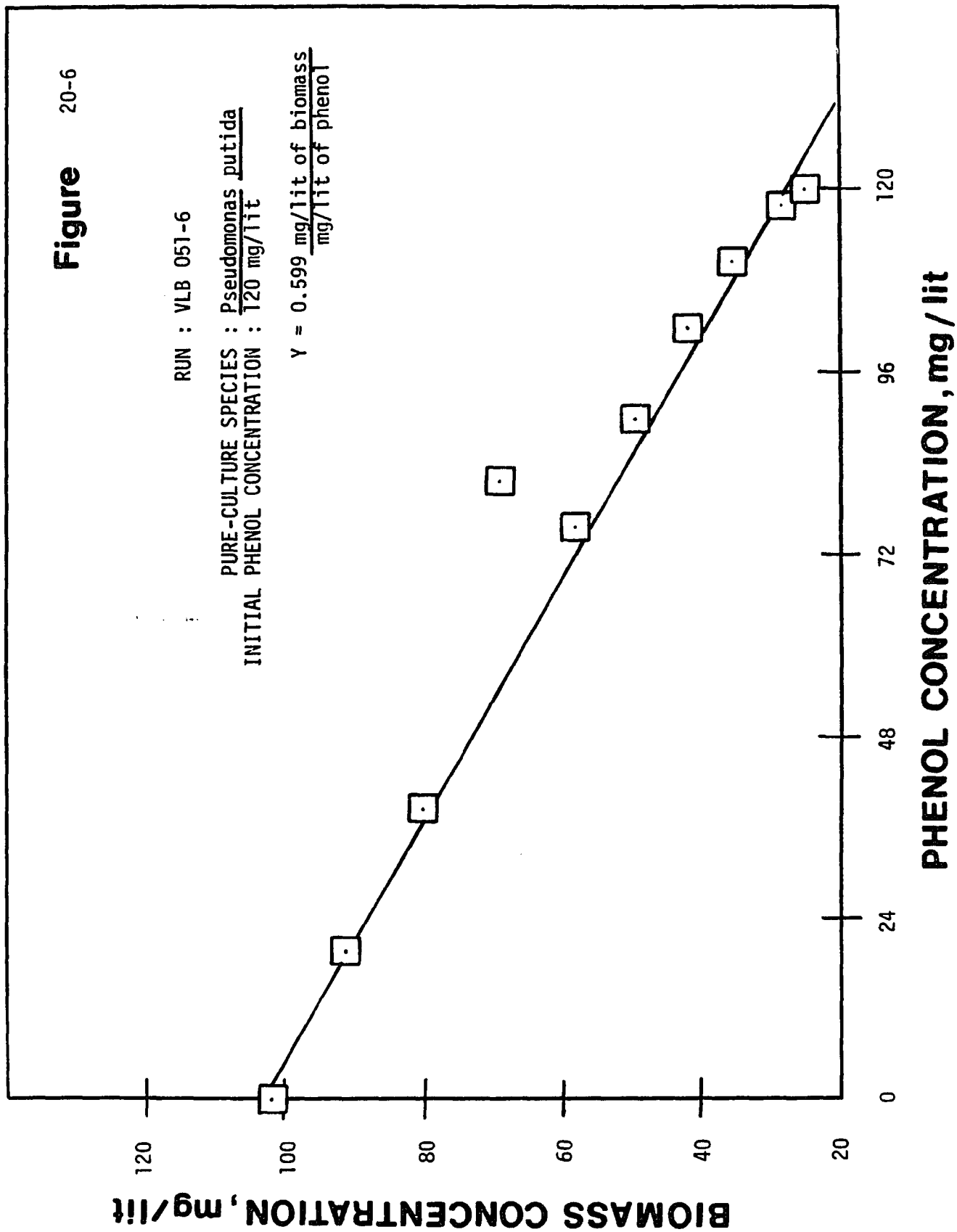


Figure 20-6
RUN : VLB 051-6
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 120 mg/lit
Y = 0.599 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
120
100
80
60
40
20
0
24
48
72
96
120
PHENOL CONCENTRATION, mg/lit

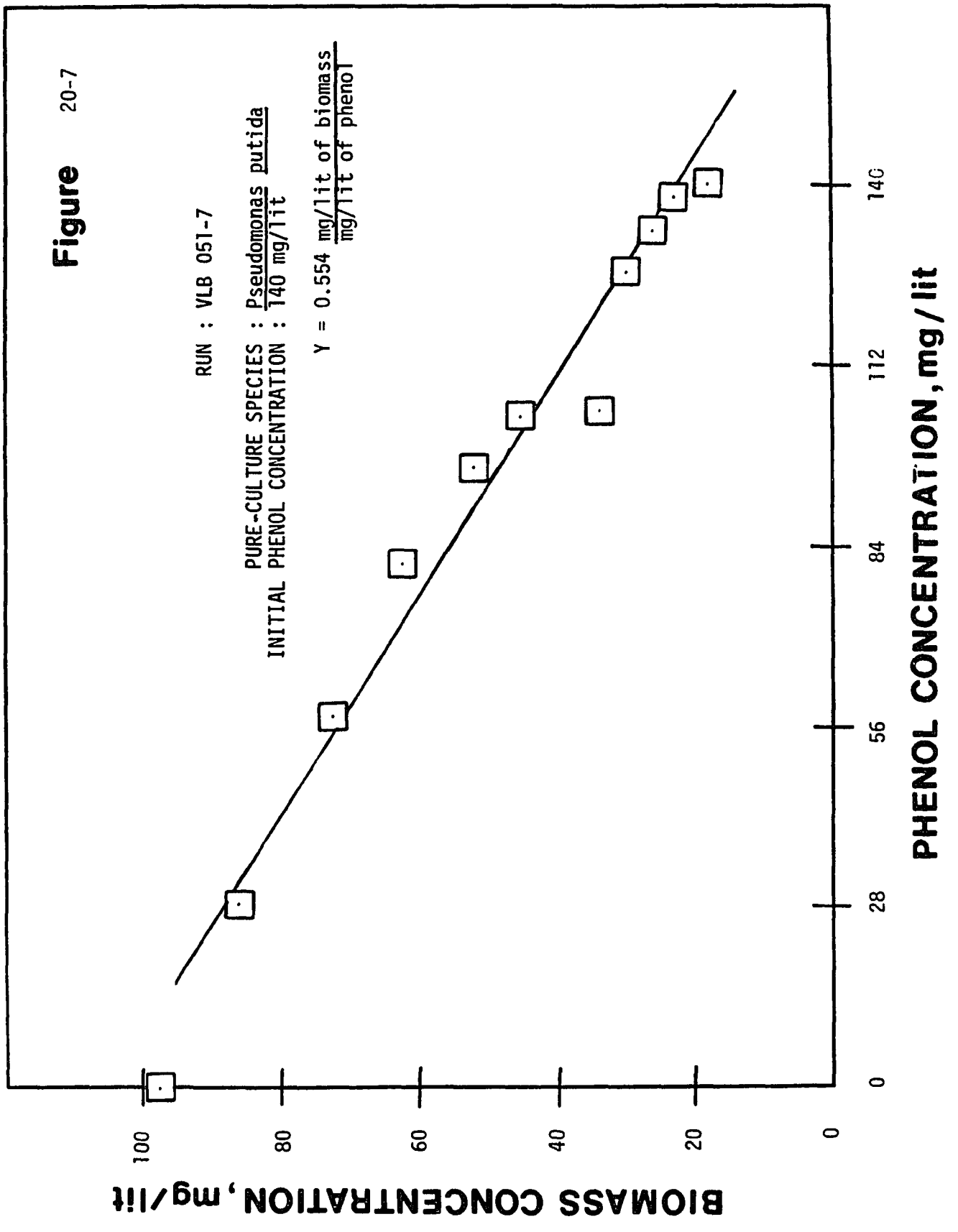


Figure 20-7
RUN : VLB 051-7
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 140 mg/lit
Y = 0.554 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
0
20
40
60
80
100
PHENOL CONCENTRATION, mg/lit
0
28
56
84
112
140

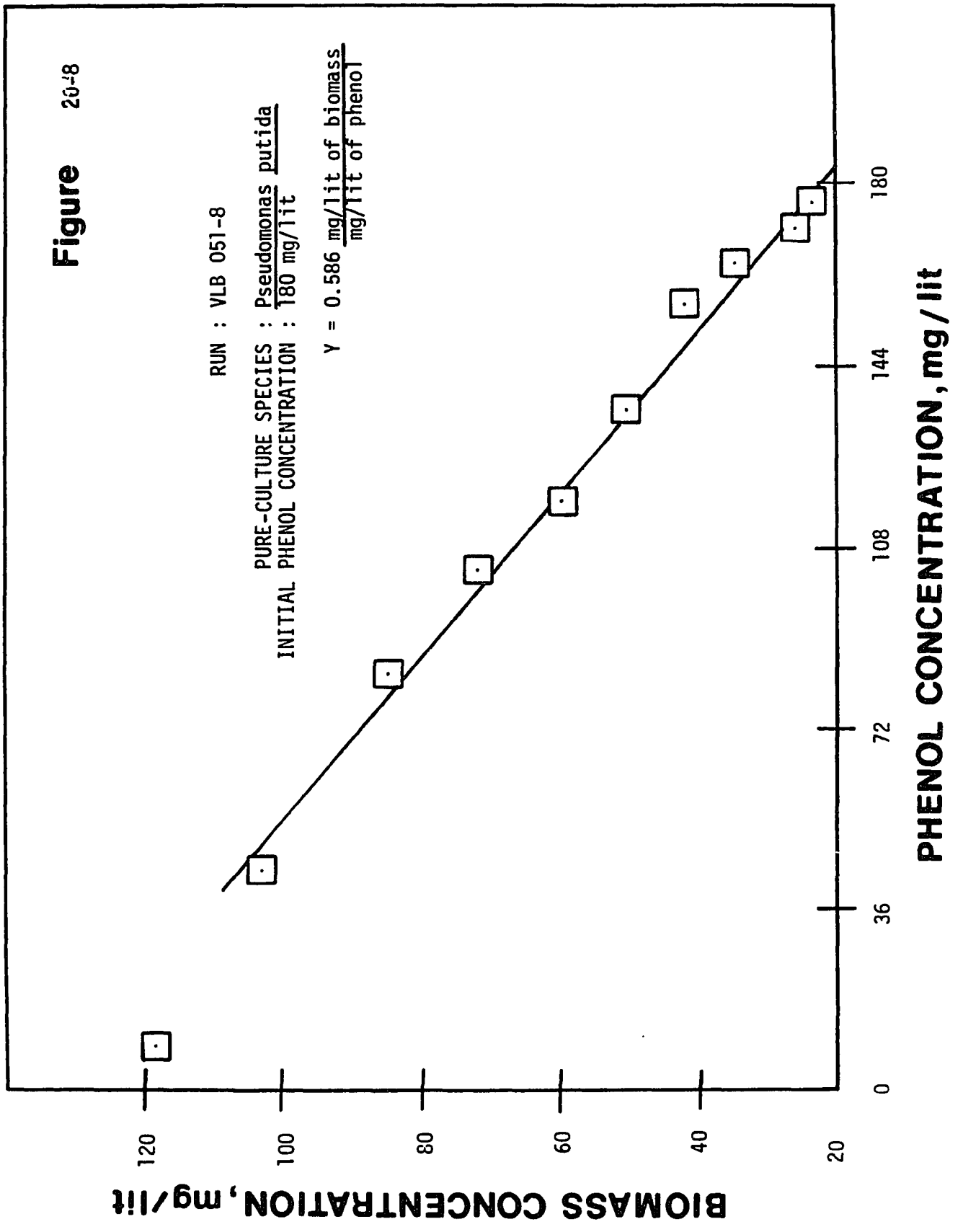


Figure 20-8
RUN : VLB 051-8
PURE-CULTURE SPECIES : Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 180 mg/lit
Y = 0.586 mg/lit of biomass / mg/lit of phenol
BIOMASS CONCENTRATION, mg/lit
120
100
80
60
40
20
0
36
72
108
144
180
PHENOL CONCENTRATION, mg/lit

Figure 21

Growth Curves of K. pneumoniae in the FSSAS (Filtered Supernatant Solution of Activated Sludge) at the following Initial Phenol Concentration (Run VLB 047-1 to VLB 047-8)

21-1	20 mg/lit
21-2	40 mg/lit
21-3	50 mg/lit
21-4	80 mg/lit
21-5	100 mg/lit
21-6	120 mg/lit
21-7	140 mg/lit
21-8	180 mg/lit

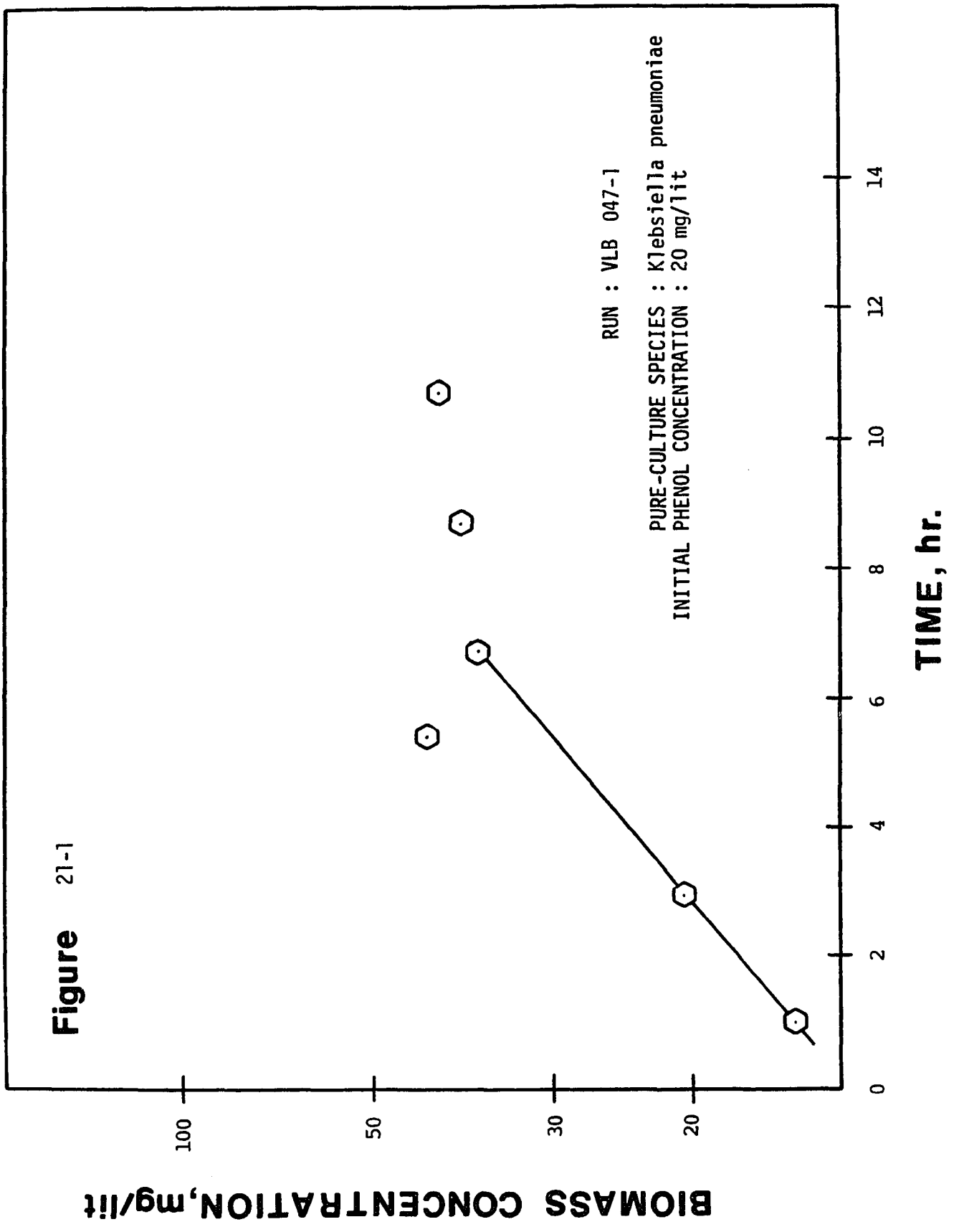


Figure 21-1
RUN : VLB 047-1
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20 mg/lit
BIOMASS CONCENTRATION, mg/lit
100
50
30
20
0
2
4
6
8
10
12
14
TIME, hr.

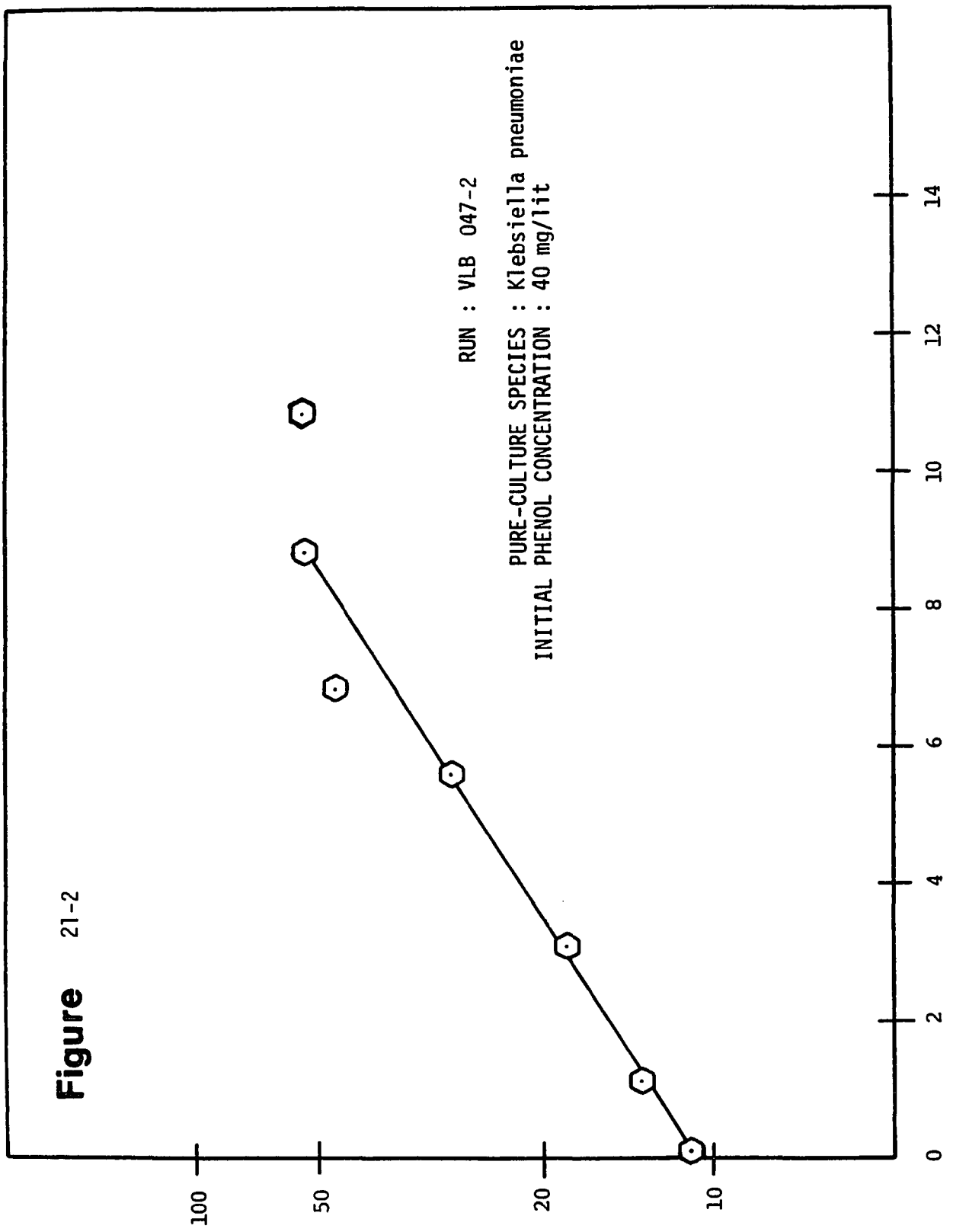


Figure 21-2
RUN : VLB 047-2
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 40 mg/lit
BIOMASS CONCENTRATION, mg/lit
100
50
20
10
0
2
4
6
8
10
12
14
TIME, hr.

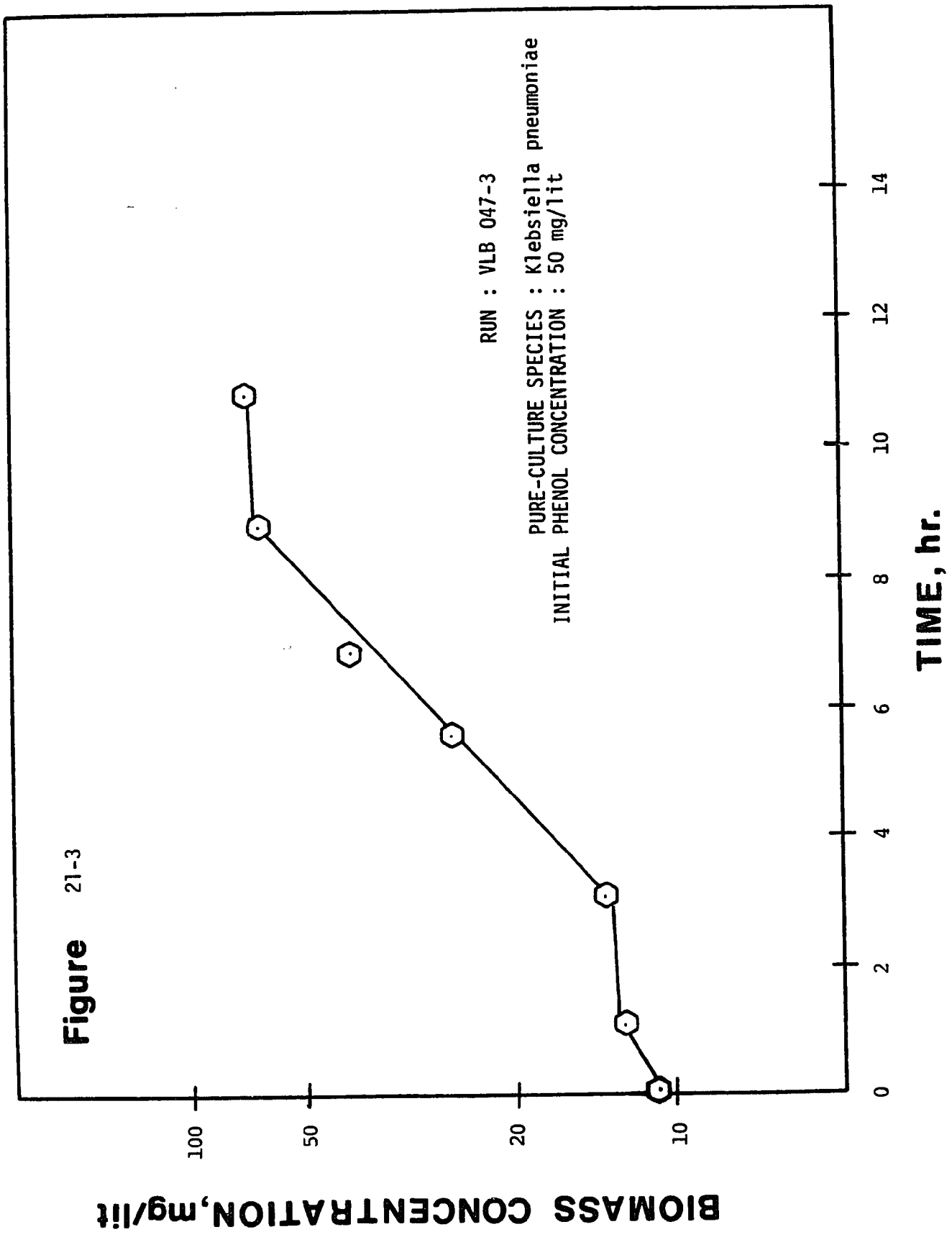

RUN : VLB 047-3
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 50 mg/lit
BIOMASS CONCENTRATION, mg/lit
100
50
20
10
0
2
4
6
8
10
12
14
TIME, hr.

Figure 21-3

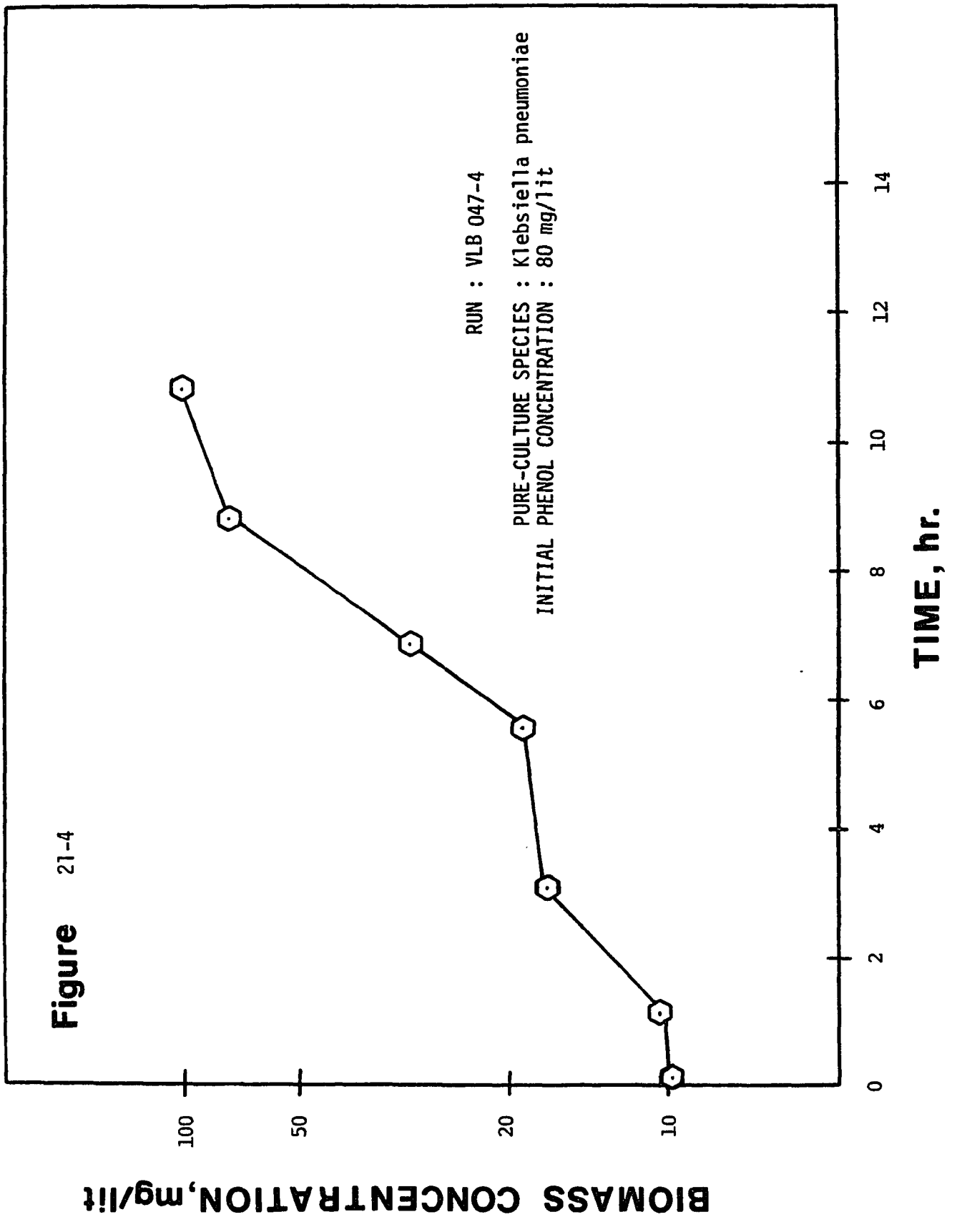


Figure 21-4
RUN : VLB 047-4
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 80 mg/lit
BIOMASS CONCENTRATION, mg/lit
100
50
20
10
0
2
4
6
8
10
12
14
TIME, hr.

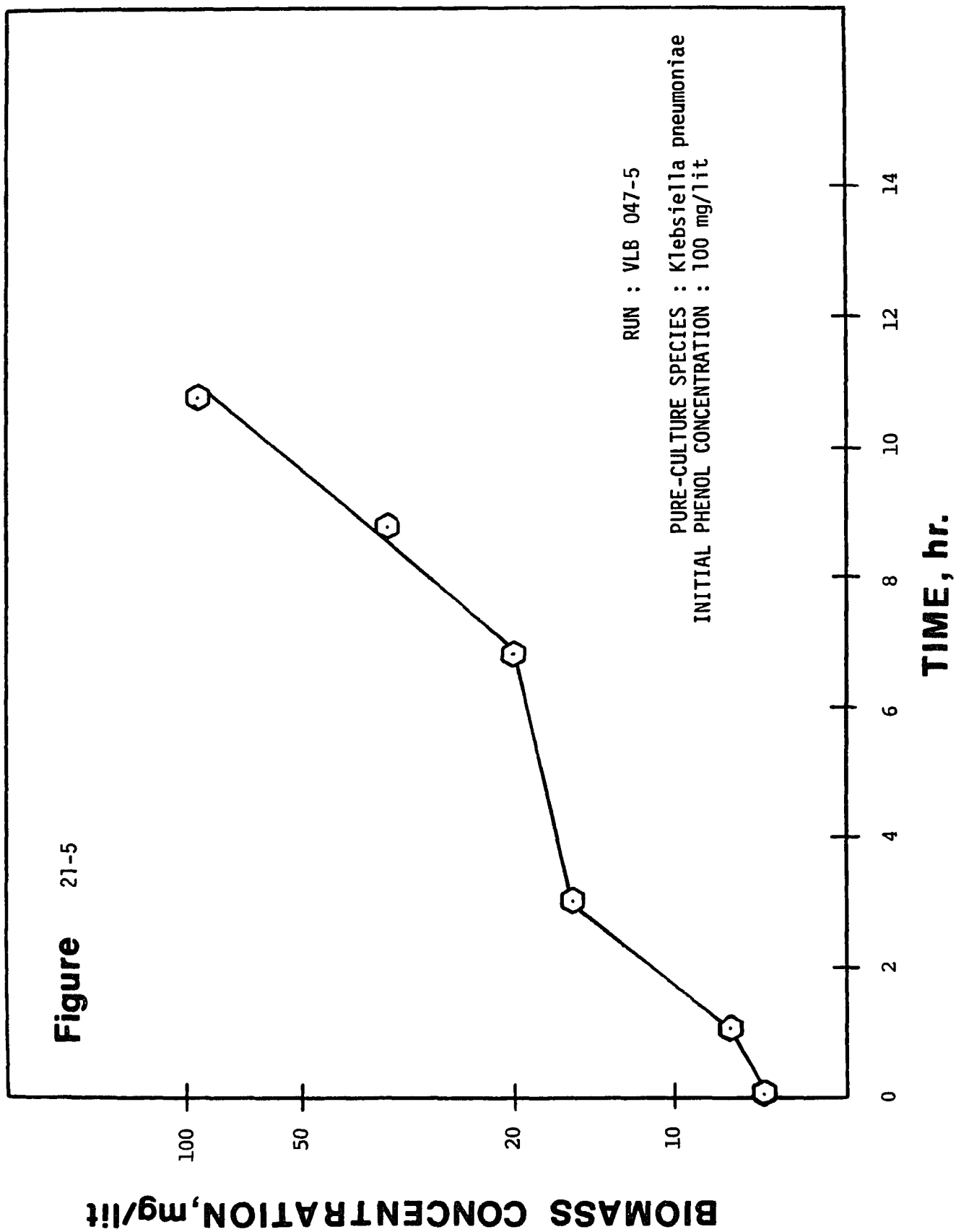


Figure 21-5
RUN : VLB 047-5
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 100 mg/lit
BIOMASS CONCENTRATION, mg/lit
100
50
20
10
0
2
4
6
8
10
12
14
TIME, hr.

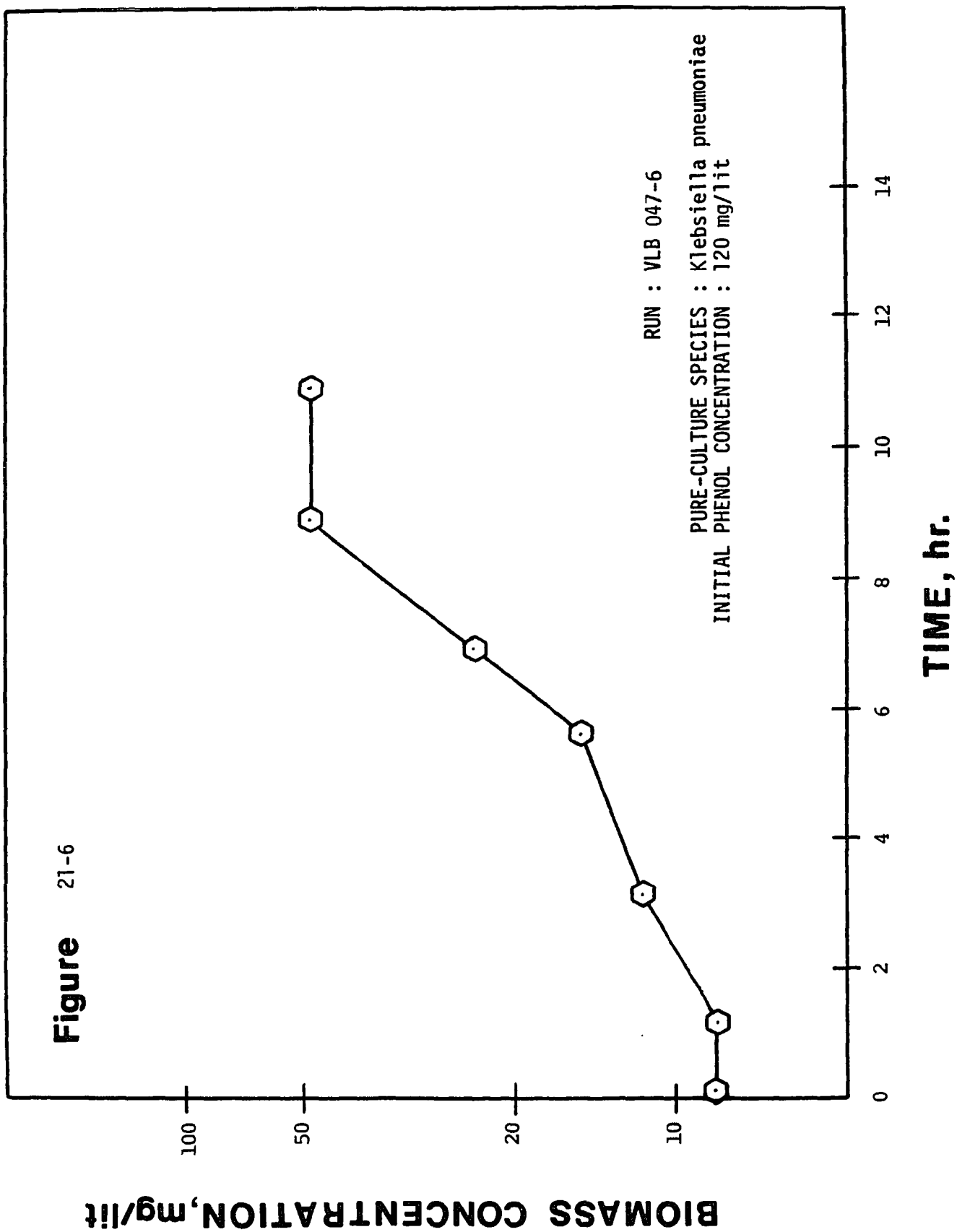


Figure 21-6
RUN : VLB 047-6
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 120 mg/lit
BIOMASS CONCENTRATION, mg/lit
100
50
20
10
0
2
4
6
8
10
12
14
TIME, hr.

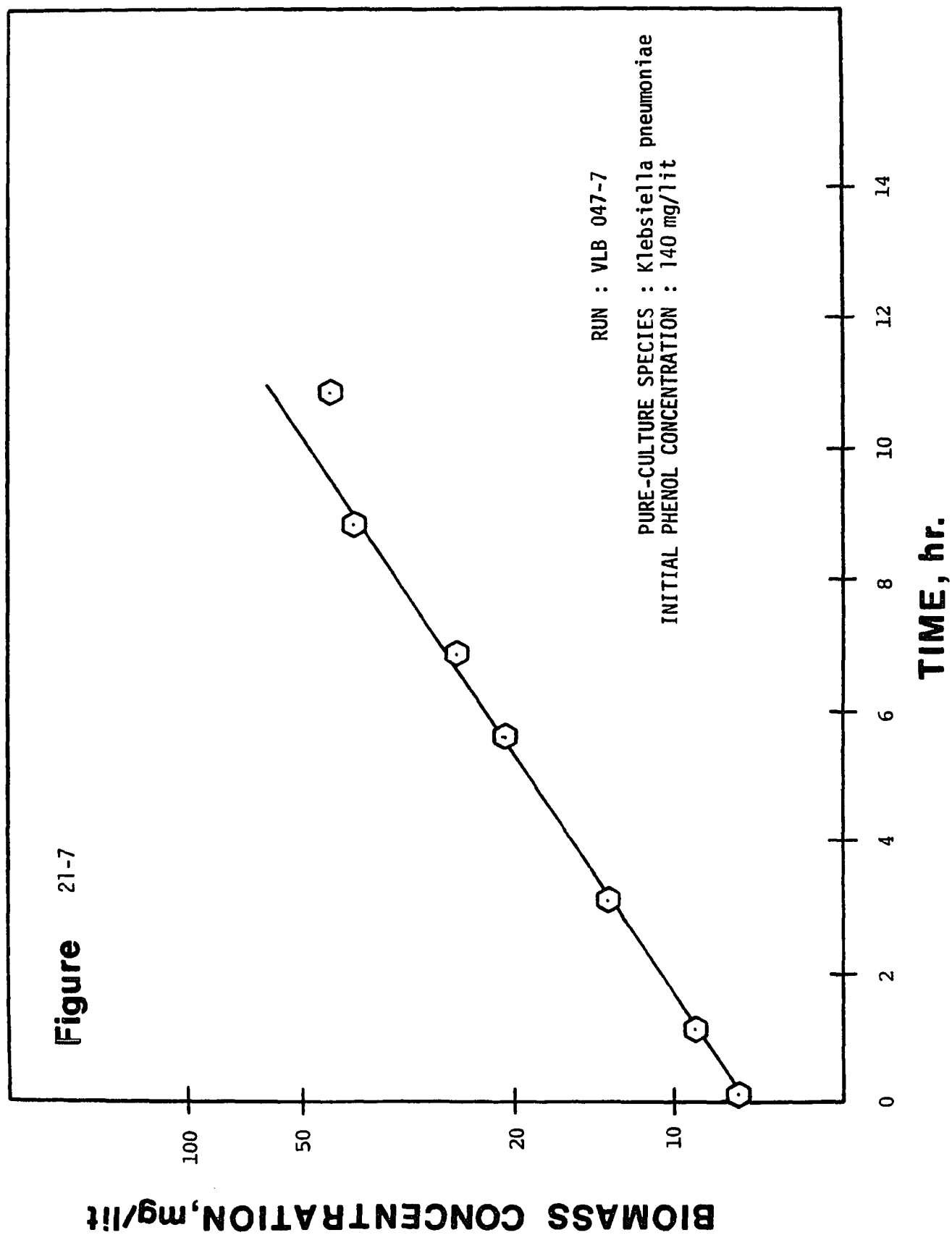


Figure 21-7
RUN : VLB 047-7
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 140 mg/lit
BIOMASS CONCENTRATION, mg/lit
100
50
20
10
0
2
4
6
8
10
12
14
TIME, hr.

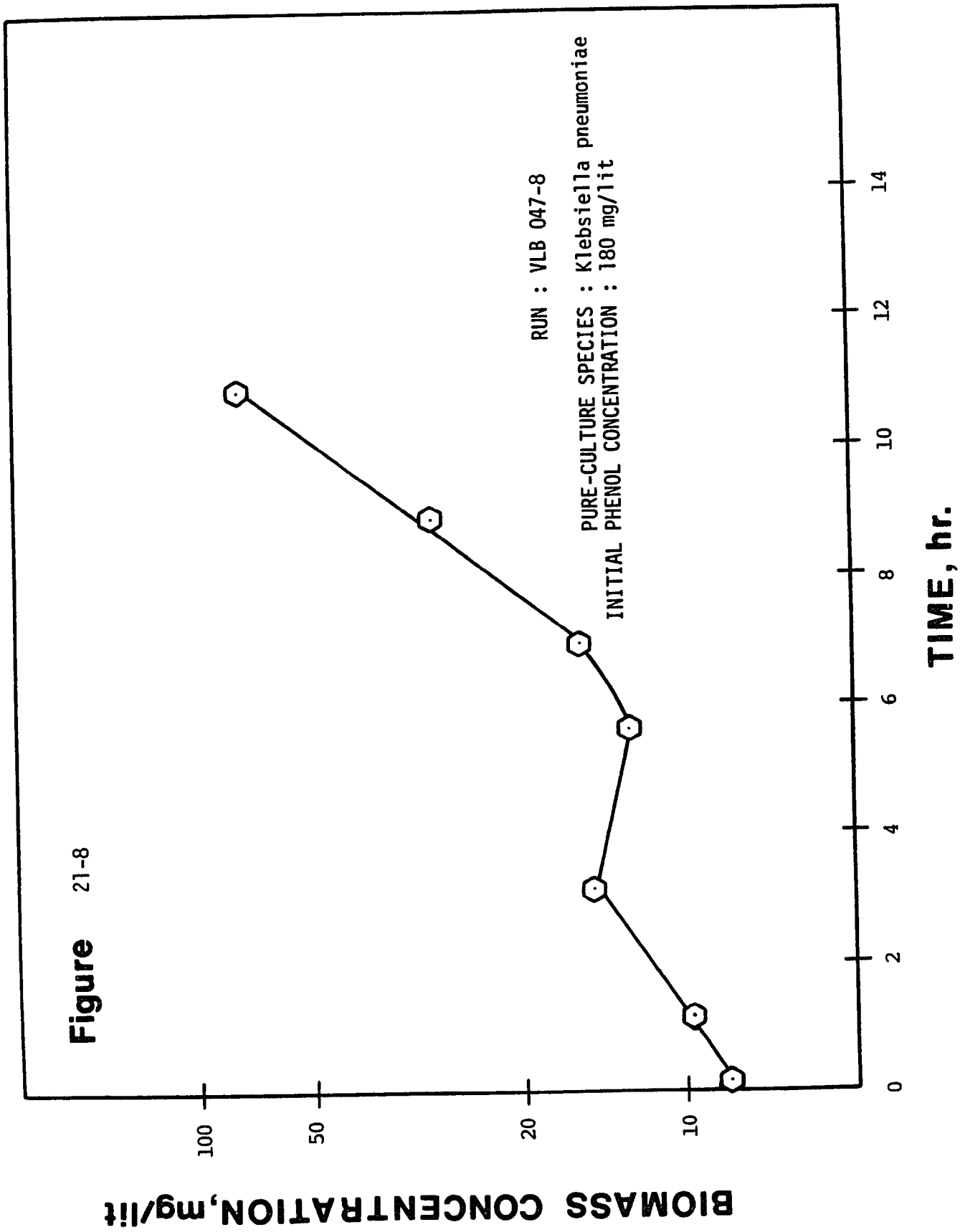


Figure 21-8
RUN : VLB 047-8
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 180 mg/lit
BIOMASS CONCENTRATION, mg/lit
100
50
20
10
0
2
4
6
8
10
12
14
TIME, hr.

Figure 22

Phenol Degradation of K. pneumoniae in the FSSAS (Filtered Supernatant Solution of Activated Sludge) at the following Initial Phenol Concentration (Run VLB 047-1 to VLB 047-8)

22-1	20 mg/lit
22-2	40 mg/lit
22-3	50 mg/lit
22-4	80 mg/lit
22-5	100 mg/lit
22-6	120 mg/lit
22-7	140 mg/lit
22-8	180 mg/lit

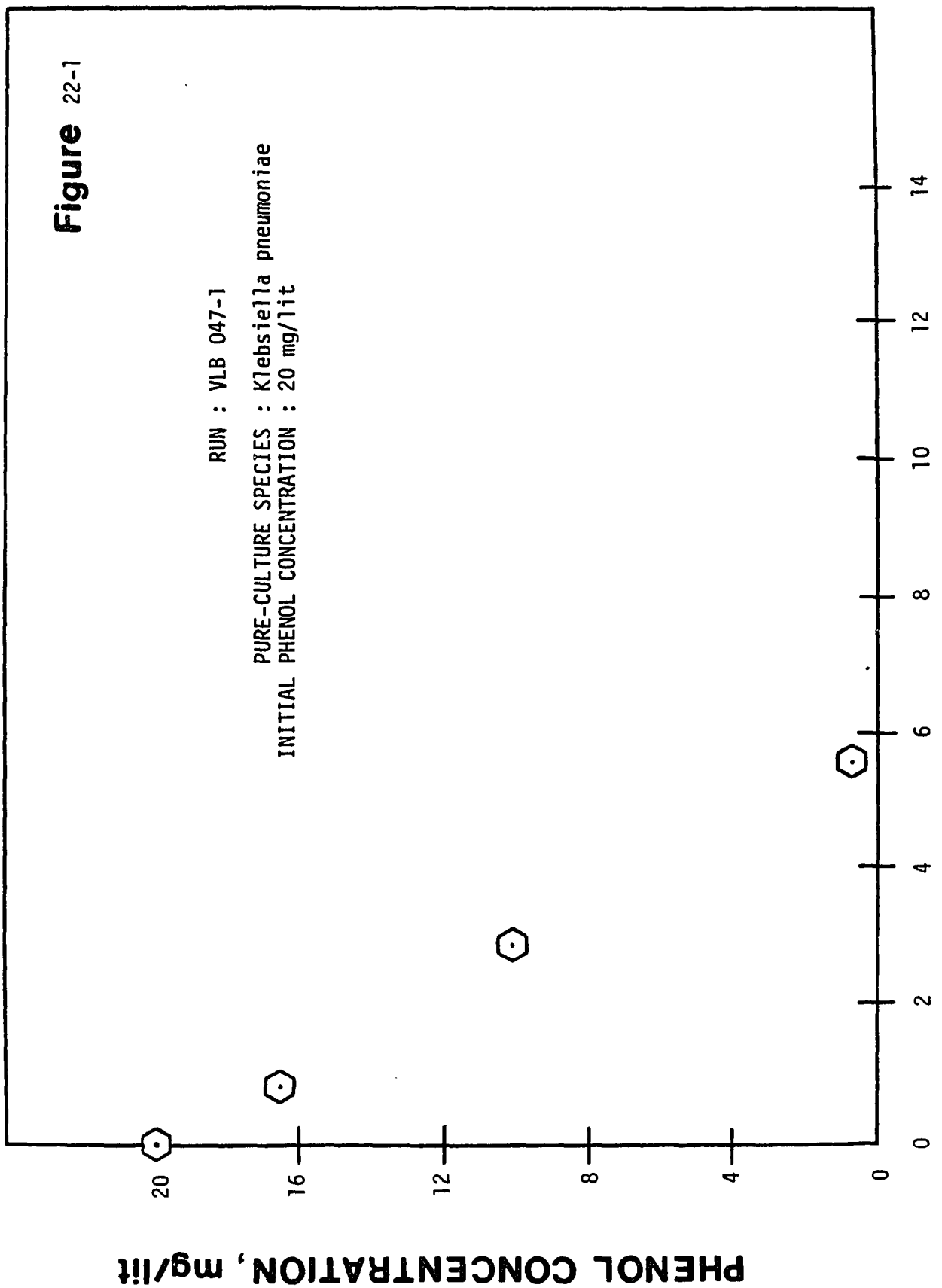


Figure 22-1
RUN : VLB 047-1
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
4
8
12
16
20
0
2
4
6
8
10
12
14

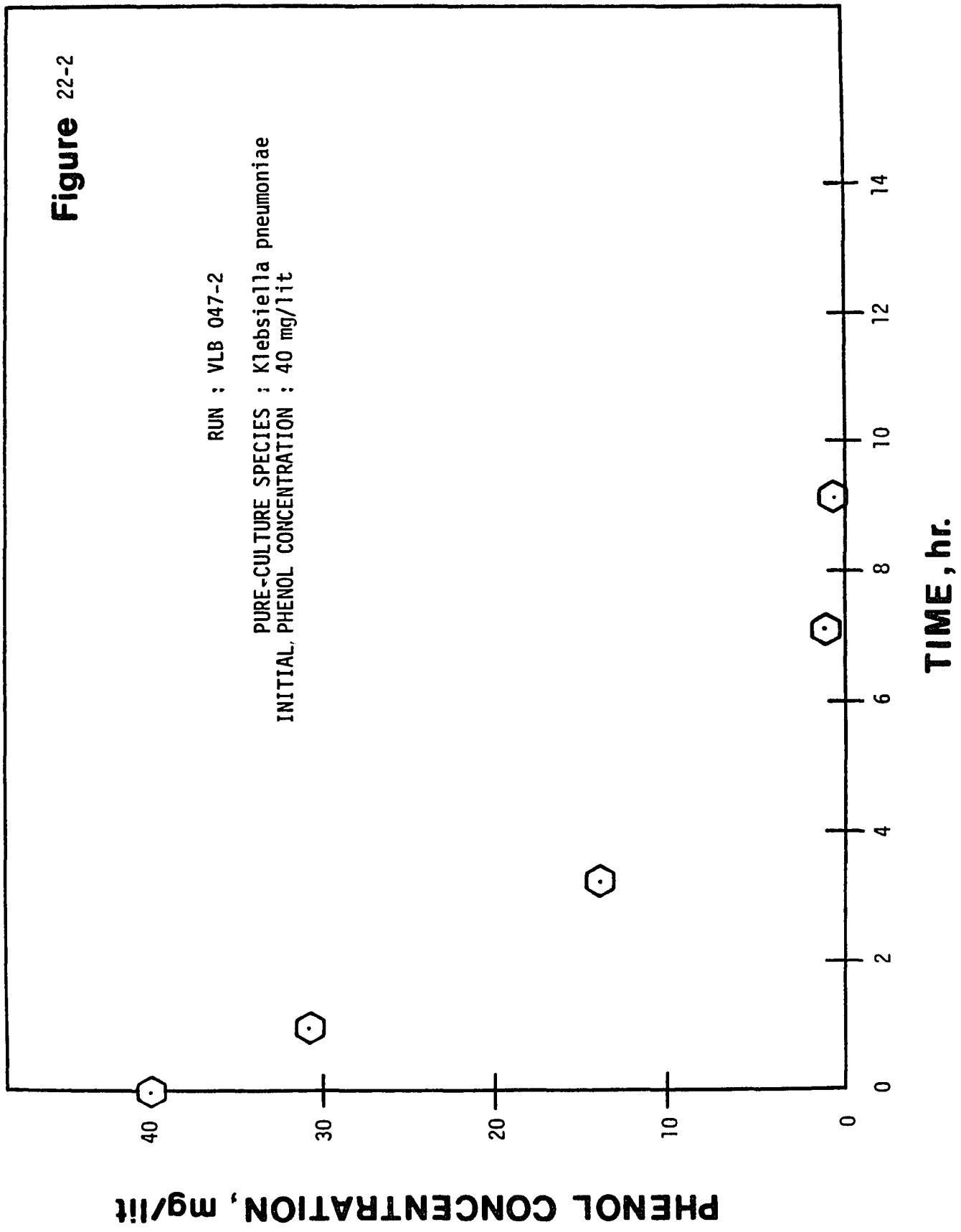


Figure 22-2
RUN : VLB 047-2
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 40 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
10
20
30
40
0
2
4
6
8
10
12
14

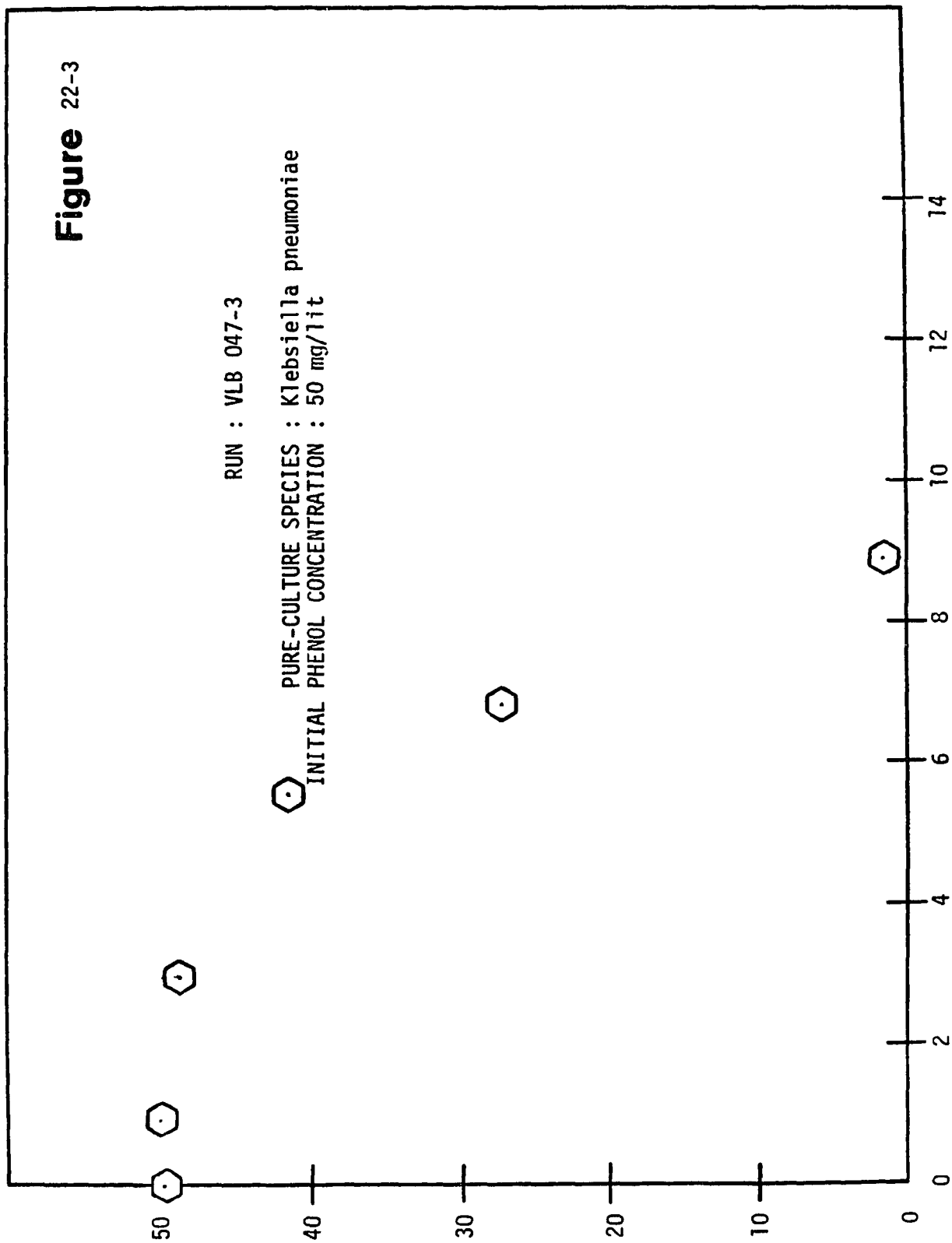


Figure 22-3
RUN : VLB 047-3
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 50 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
10
20
30
40
50
0
2
4
6
8
10
12
14

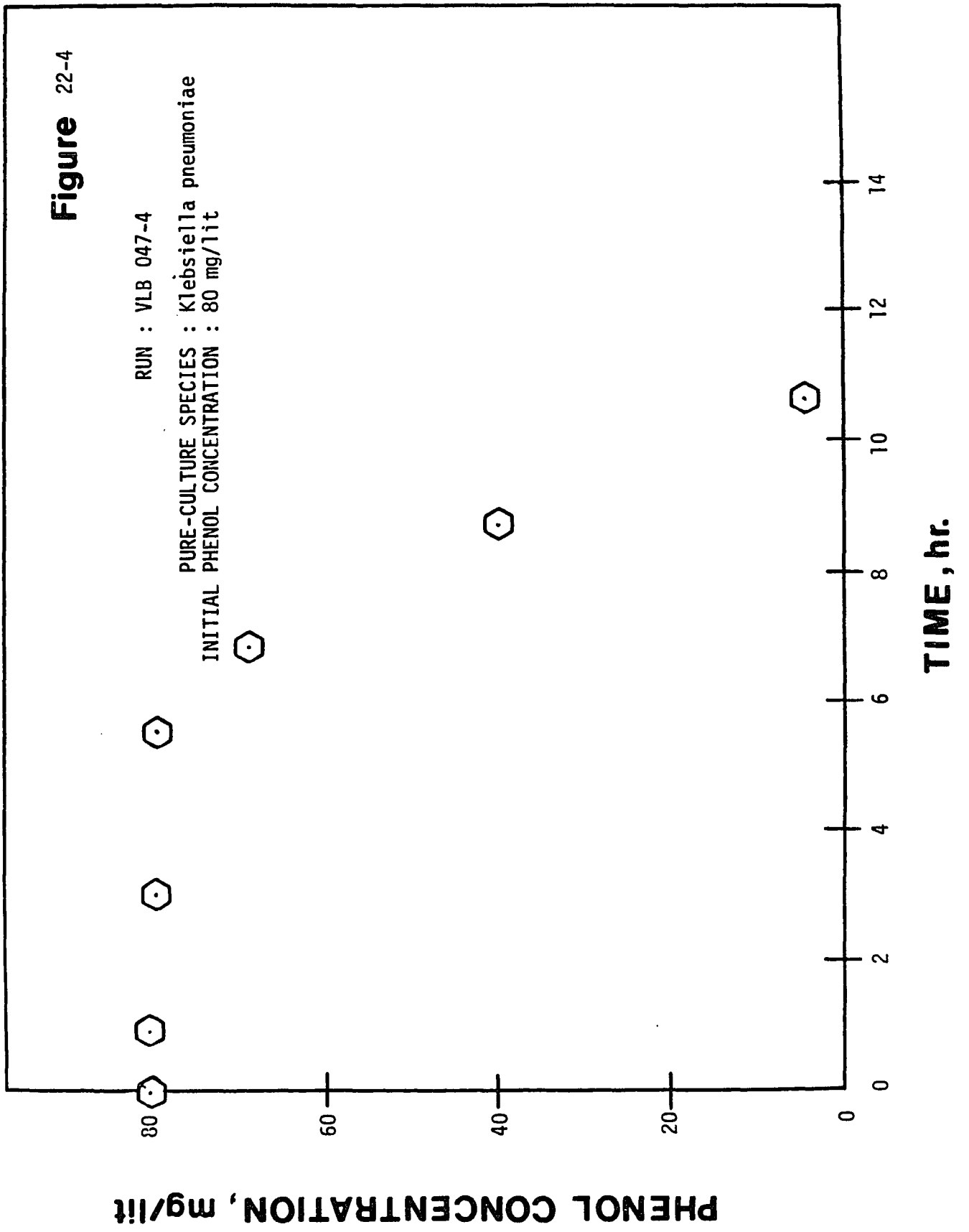


Figure 22-4
RUN : VLB 047-4
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 80 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
20
40
60
80
0
2
4
6
8
10
12
14

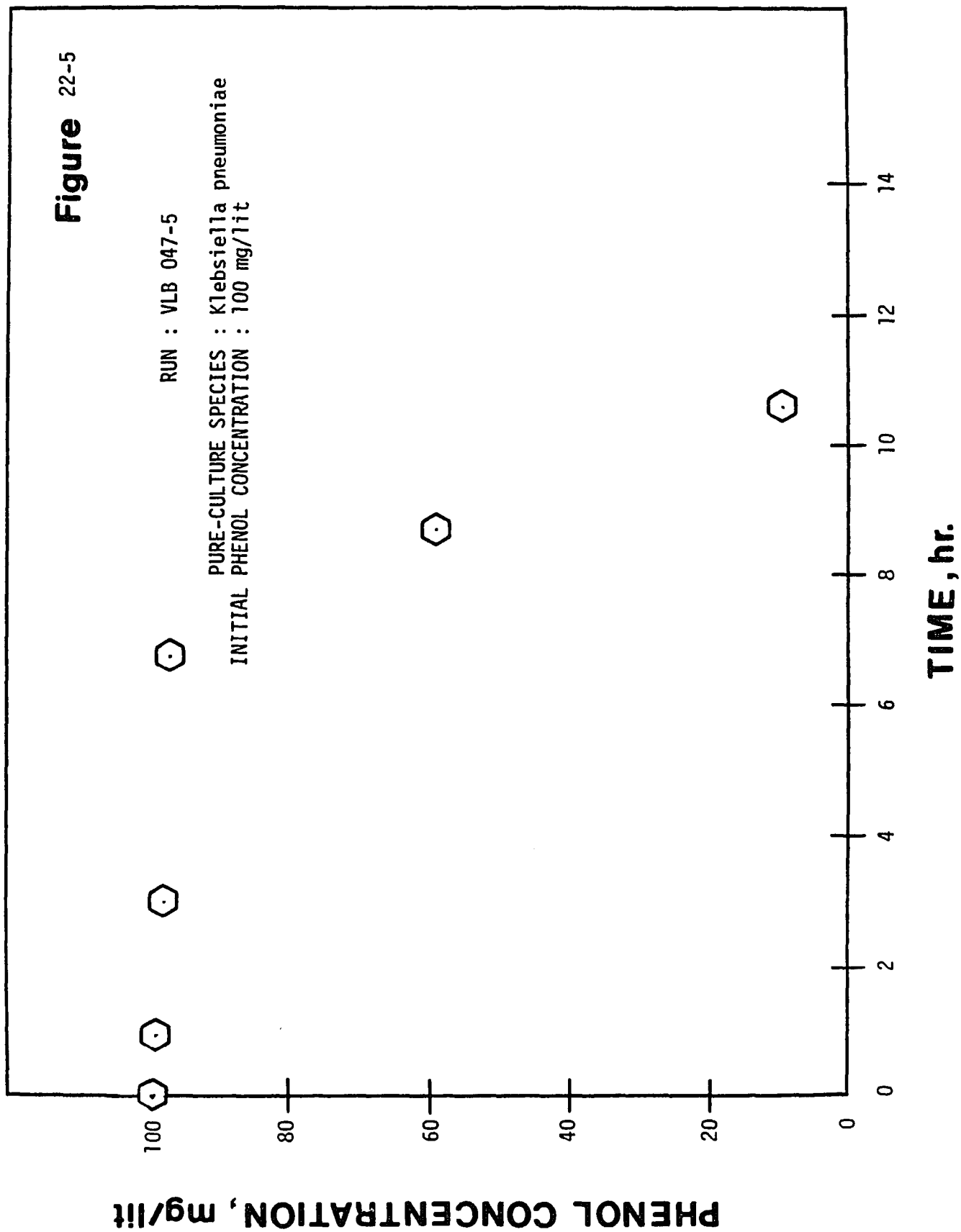


Figure 22-5
RUN : VLB 047-5
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 100 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
20
40
60
80
100
0
2
4
6
8
10
12
14

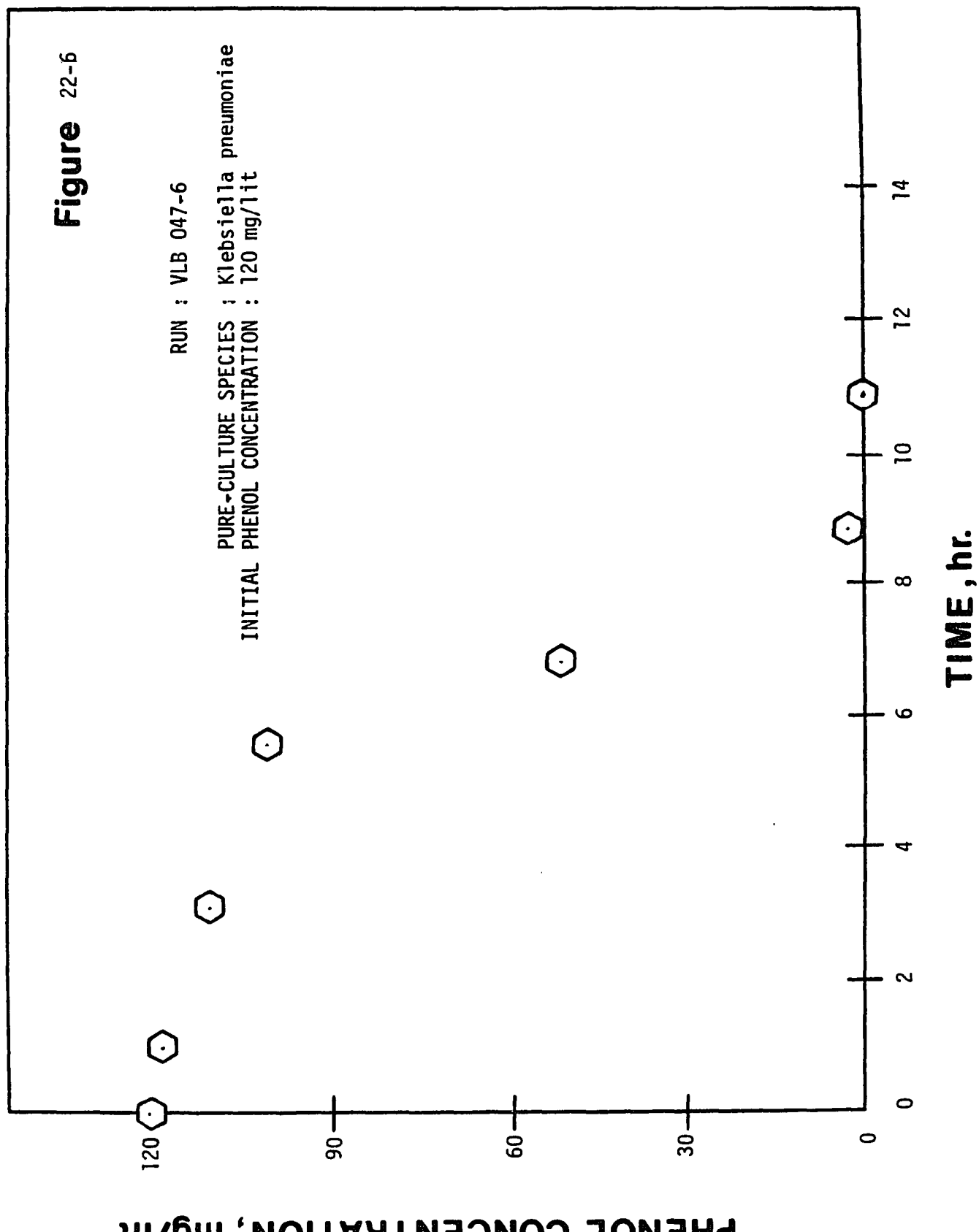


Figure 22-6
RUN : VLB 047-6
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 120 mg/lit
PHENOL CONCENTRATION, mg/lit
120
90
60
30
0
0
2
4
6
8
10
12
14
TIME, hr.

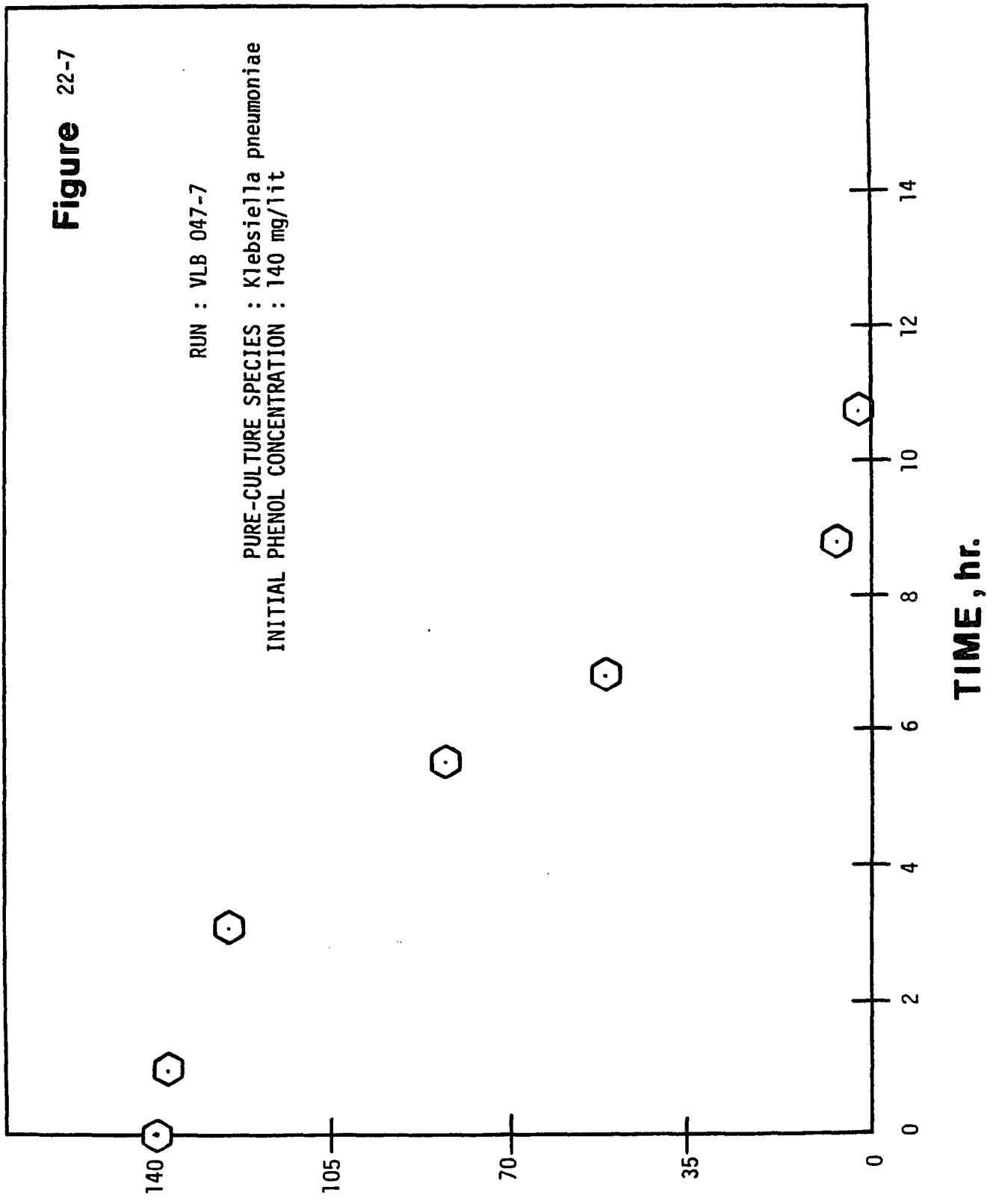


Figure 22-7
RUN : VLB 047-7
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 140 mg/lit
PHENOL CONCENTRATION, mg/lit
140
105
70
35
0
0
2
4
6
8
10
12
14
TIME, hr.

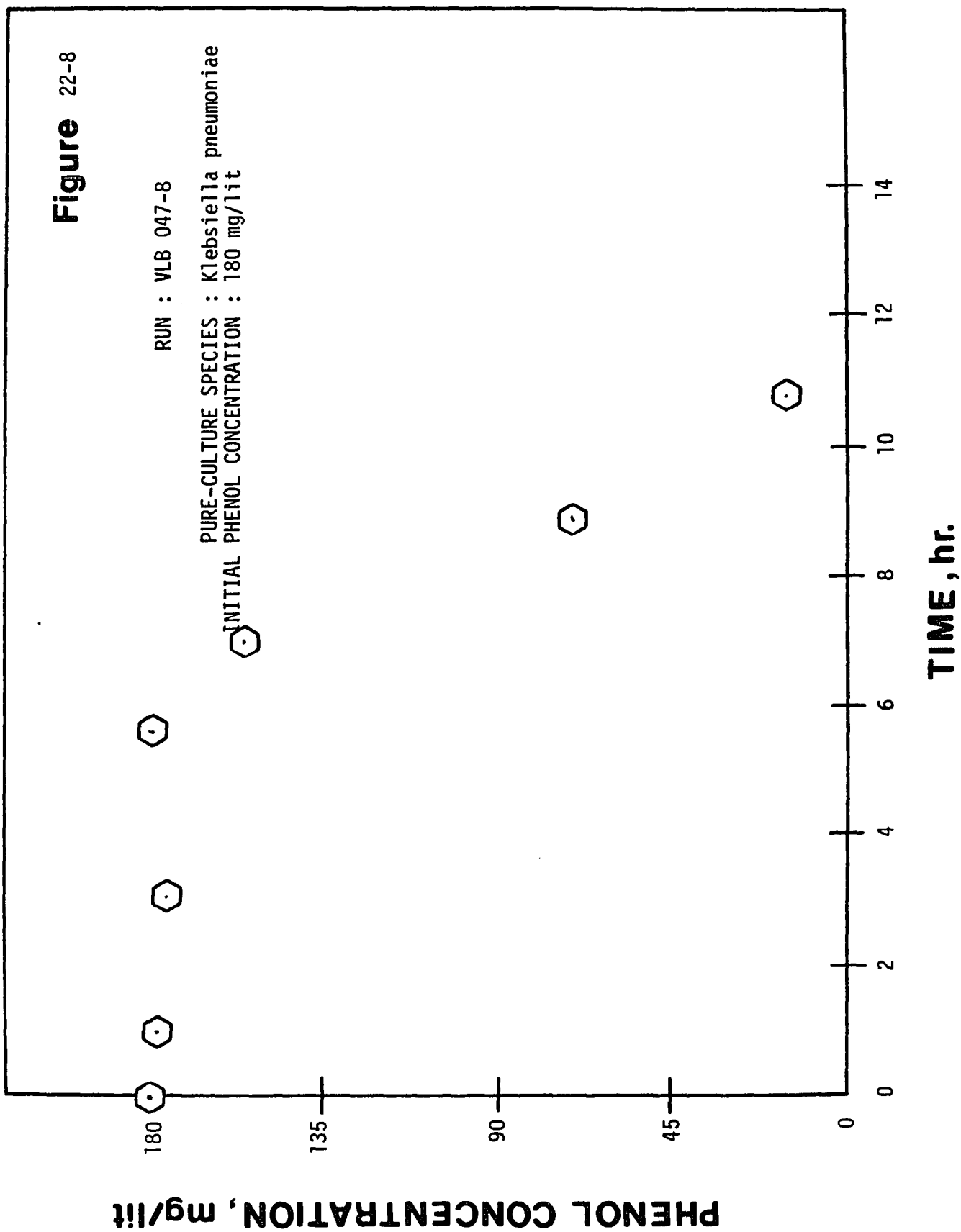


Figure 22-8
RUN : VLB 047-8
PURE-CULTURE SPECIES : Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 180 mg/lit
PHENOL CONCENTRATION, mg/lit
180
135
90
45
0
0
2
4
6
8
10
12
14
TIME, hr.

Figure 23

Growth Curves of the Mixed Culture
(*K. pneumoniae* and *P. putida*) at
the following Initial Phenol Concentrations
(Run VLB 070-1 to VLB 070-8)

23-1	20 mg/lit
23-2	40 mg/lit
23-3	50 mg/lit
23-4	80 mg/lit
23-5	100 mg/lit
23-6	120 mg/lit
23-7	140 mg/lit
23-8	180 mg/lit

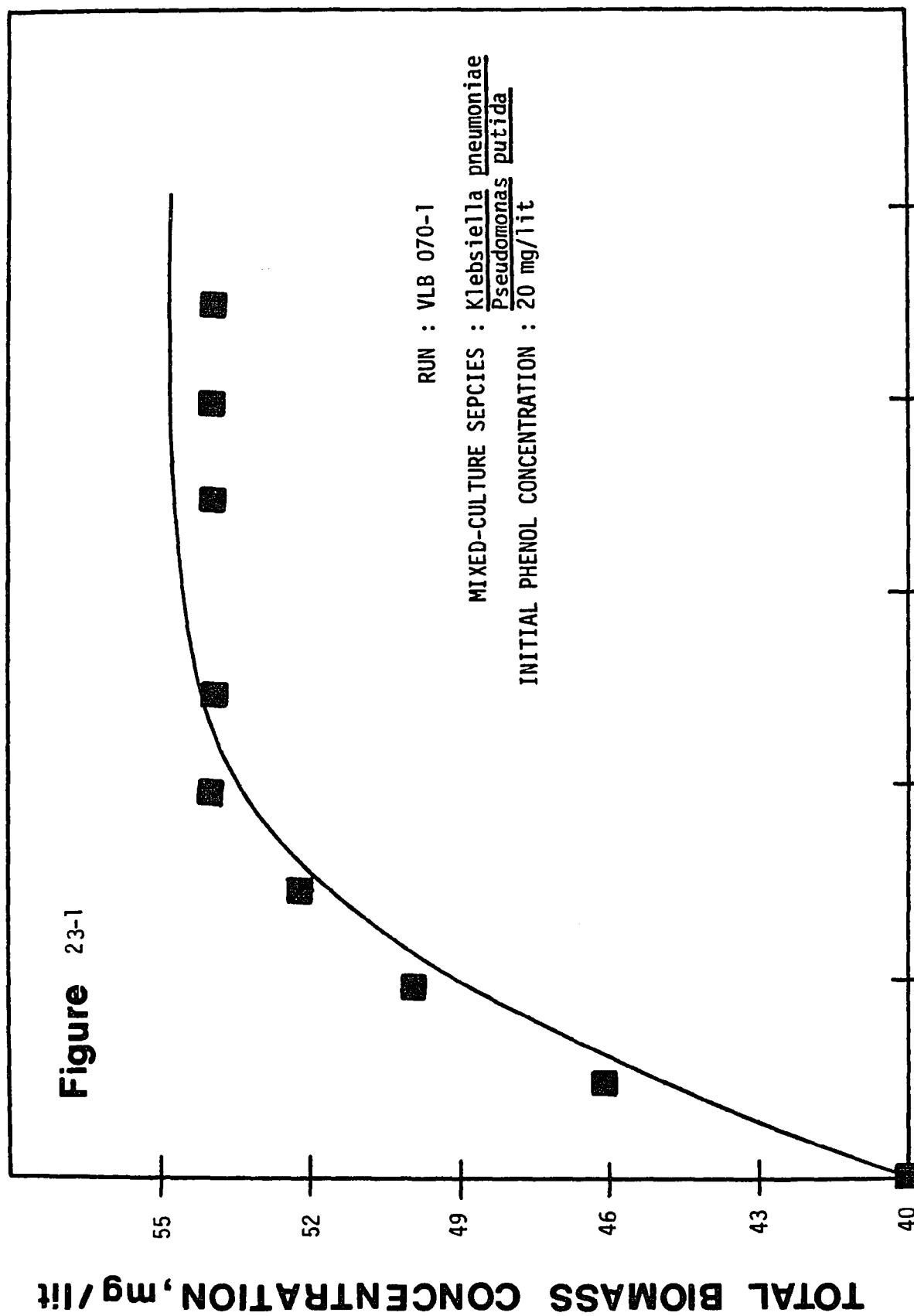

RUN : VLB 070-1
MIXED-CULTURE SEPCIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
TIME, hr.
40
43
46
49
52
55
0
2
4
6
8
10

Figure 23-1

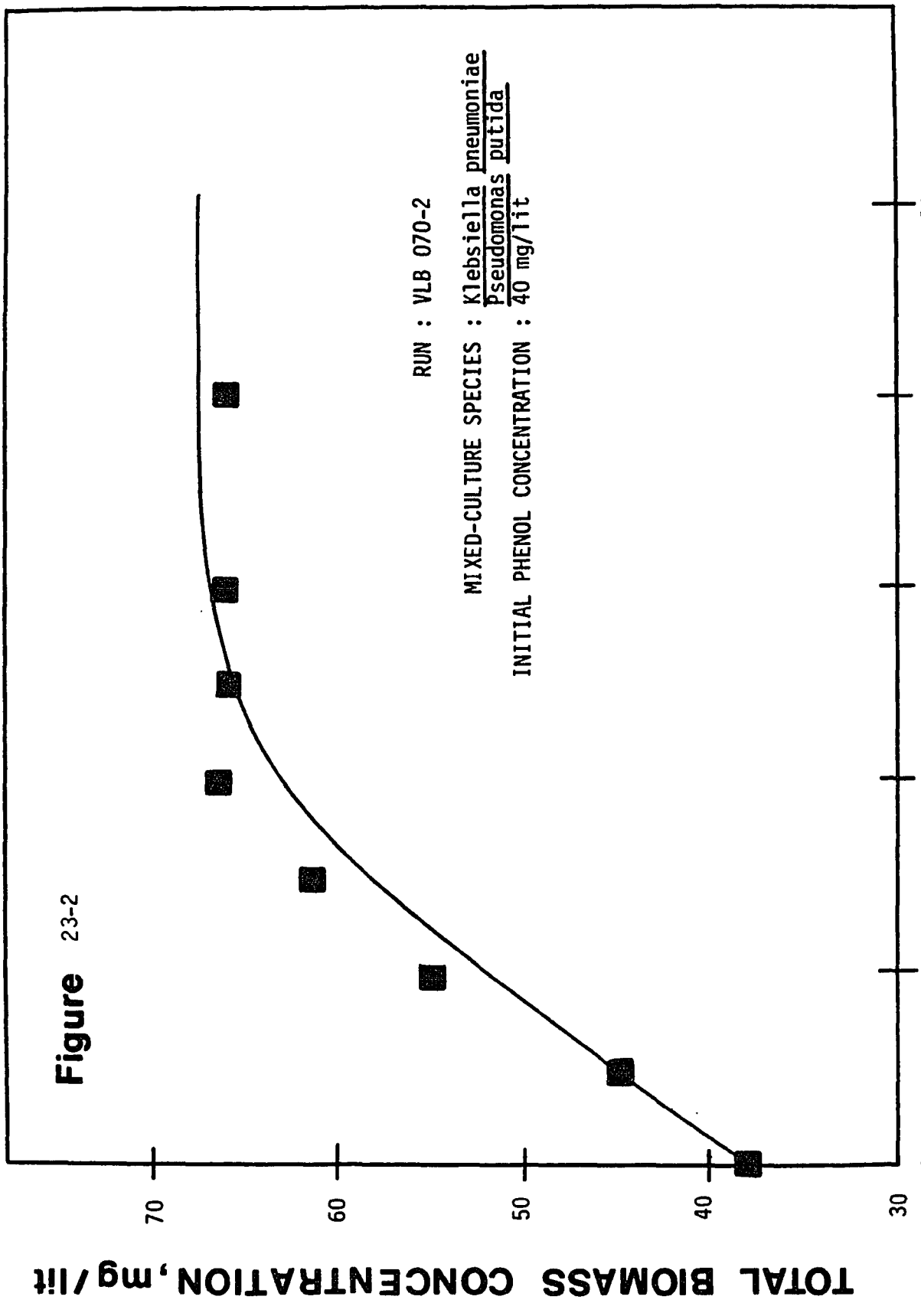

RUN : VLB 070-2
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 40 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
TIME, hr.
30
40
50
60
70
0
2
4
6
8
10

Figure 23-2

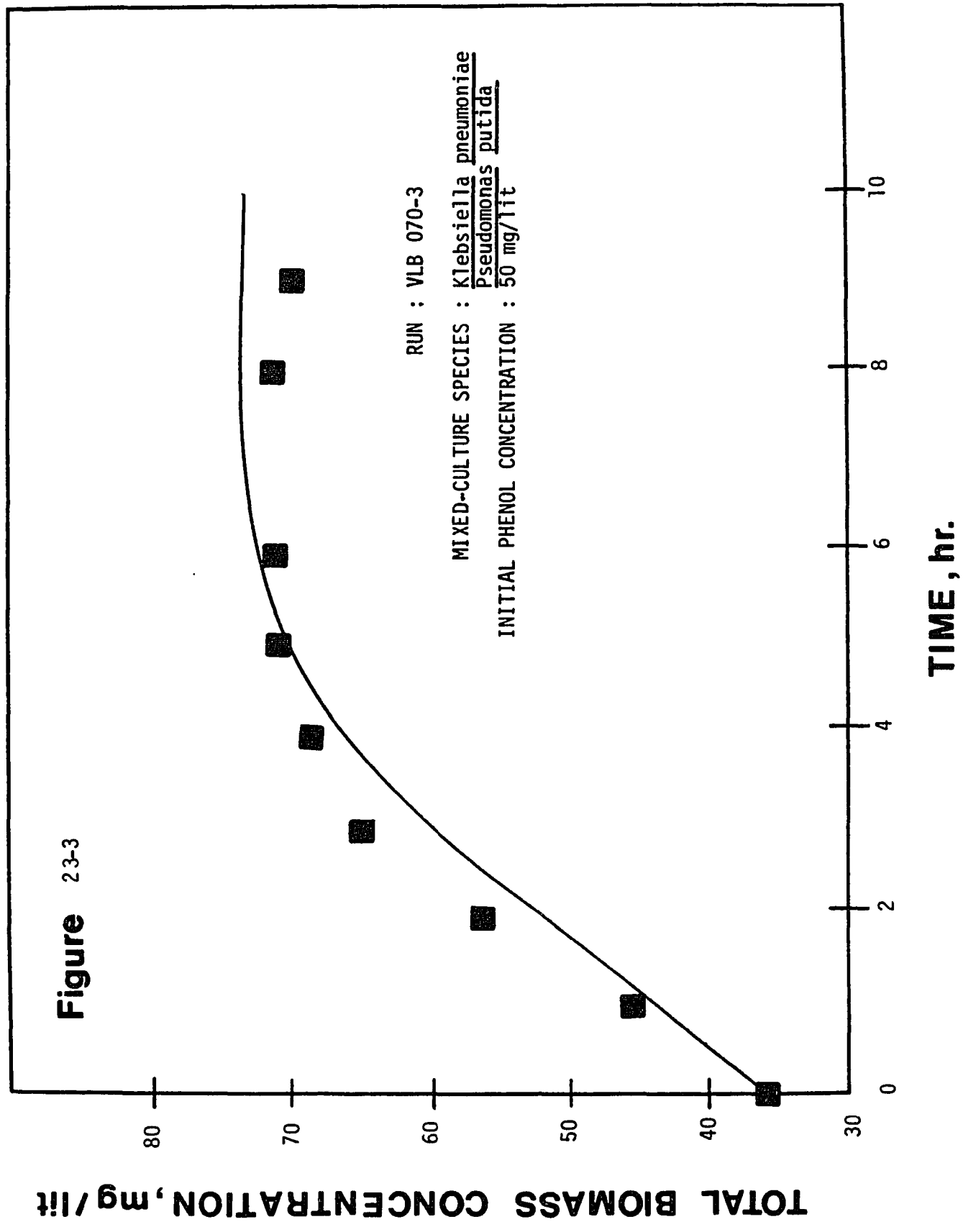

TOTAL BIOMASS CONCENTRATION, mg/lit
80
70
60
50
40
30
0
2
4
6
8
10
TIME, hr.
RUN : VLB 070-3
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 50 mg/lit

Figure 23-3

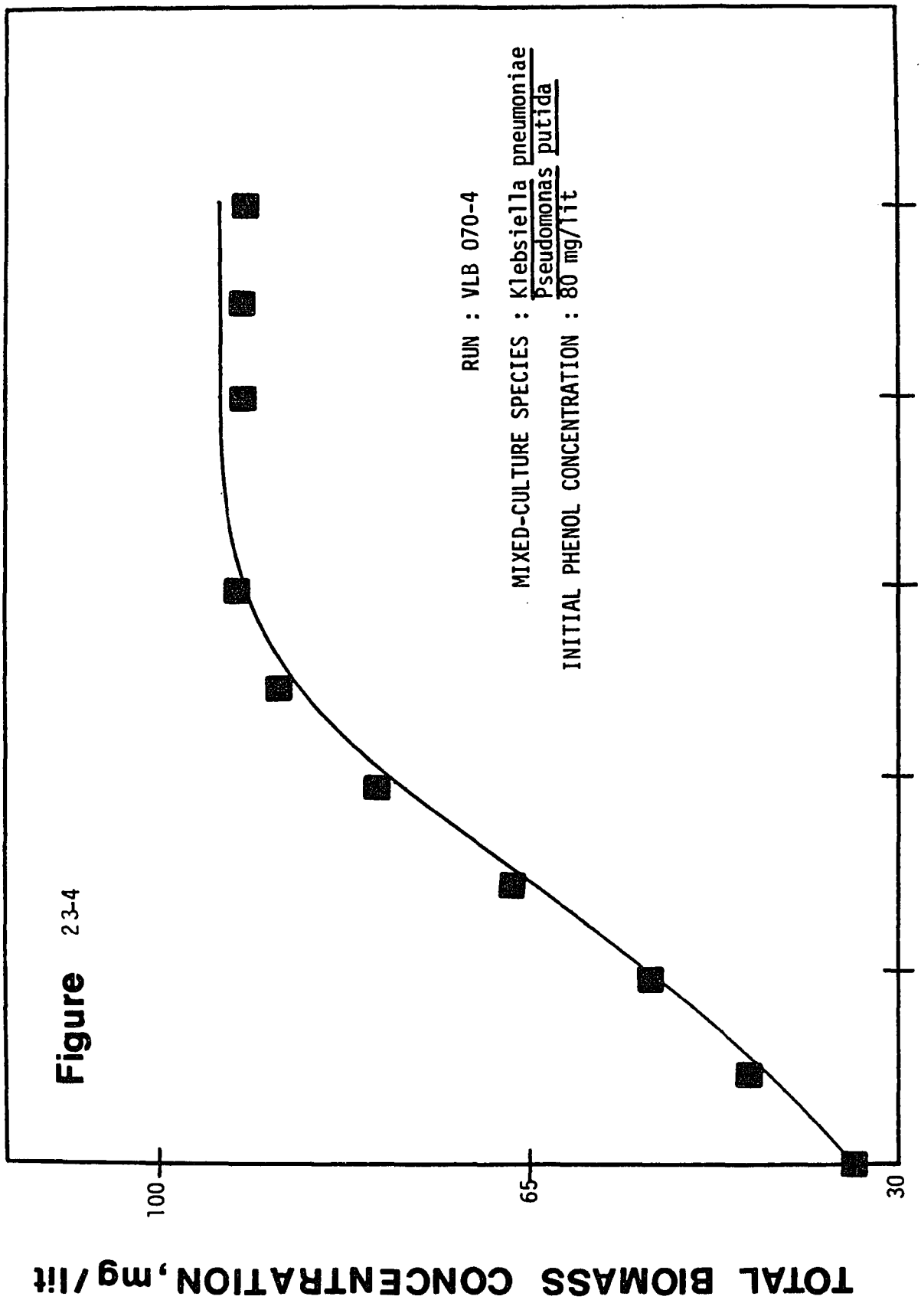

TOTAL BIOMASS CONCENTRATION, mg/lit
100
65
30
0
2
4
6
8
10
TIME, hr.
RUN : VLB 070-4
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 80 mg/lit

Figure 23-4

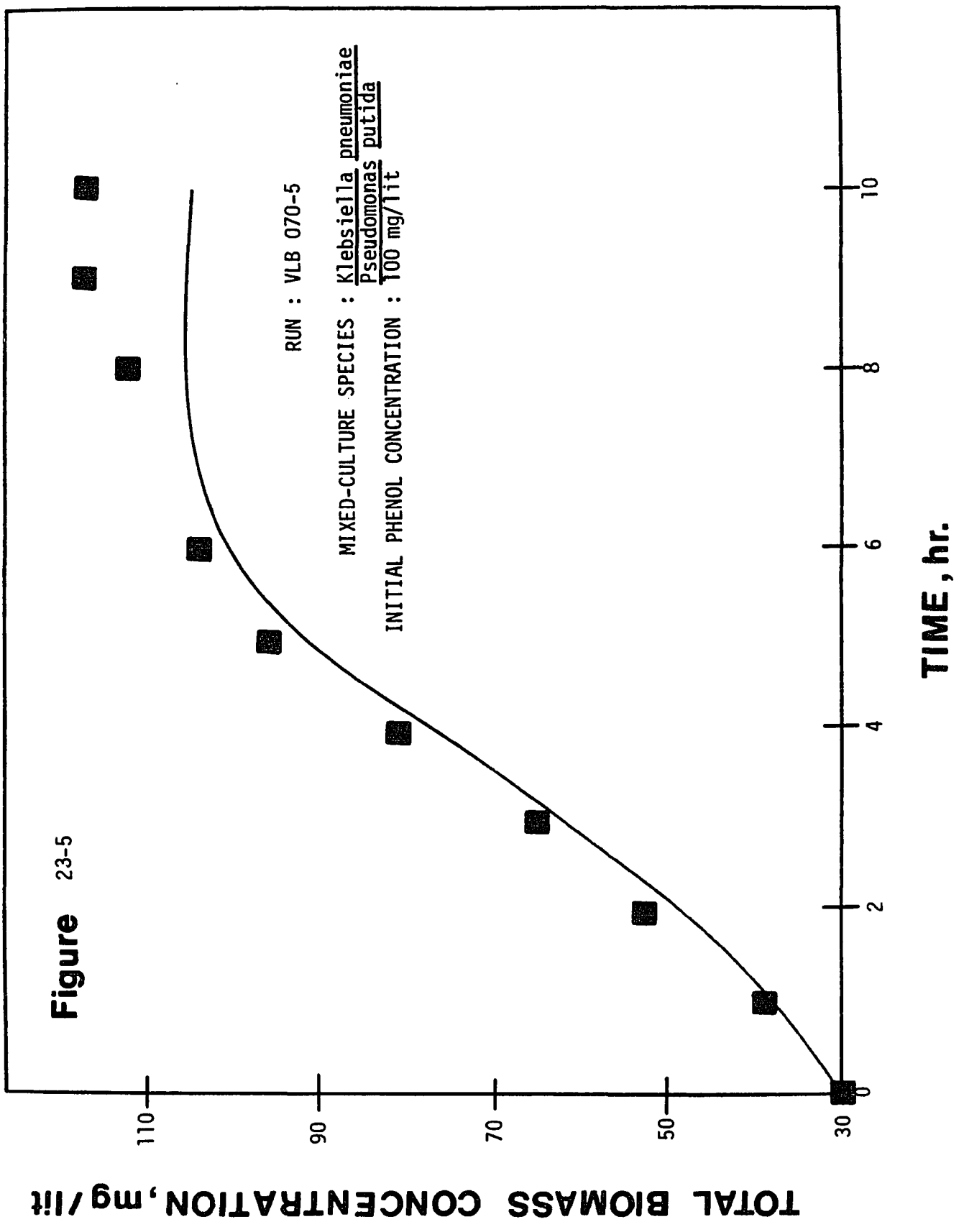


Figure 23-5
RUN : VLB 070-5
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 100 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
110
90
70
50
30
0
2
4
6
8
10
TIME, hr.

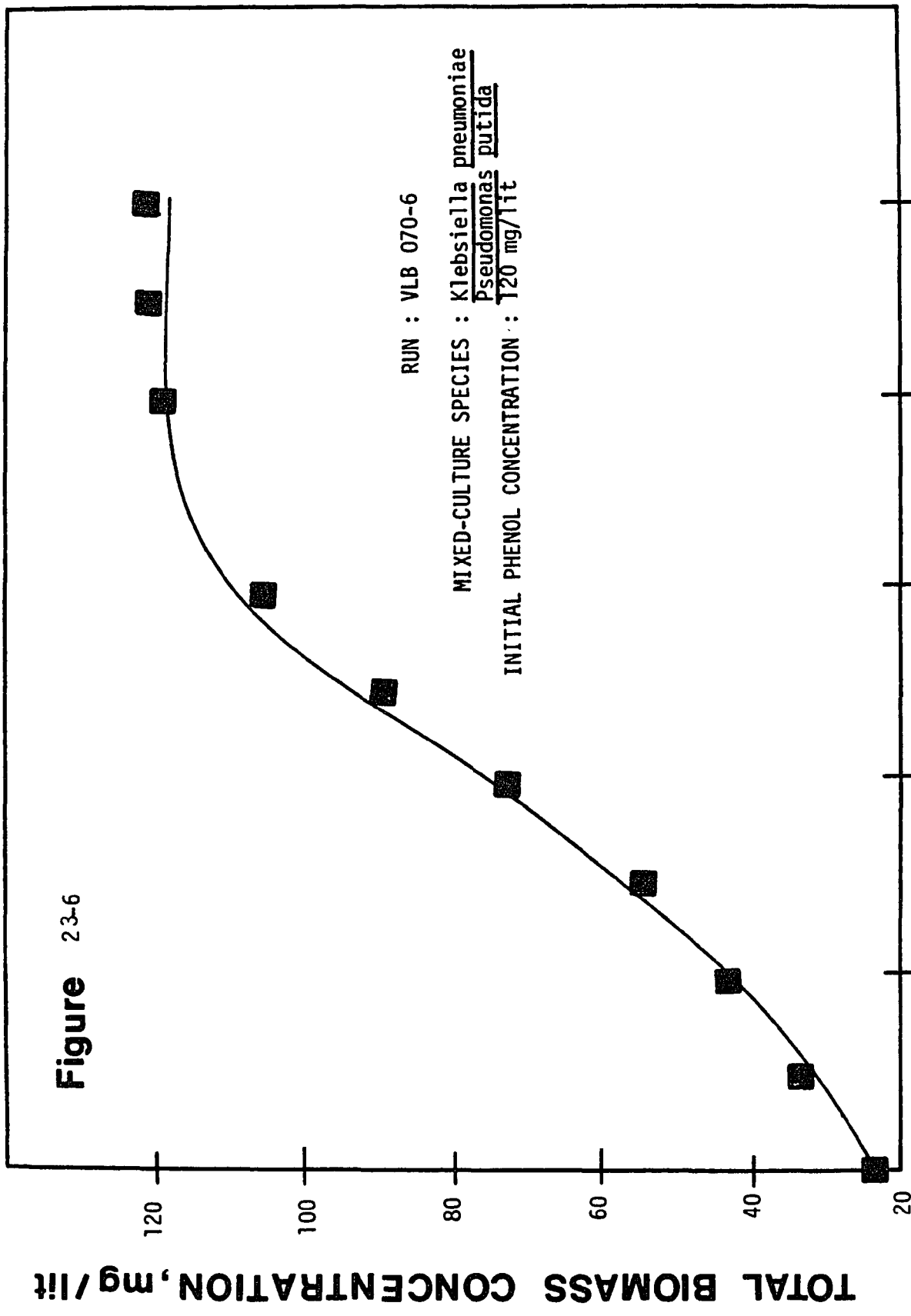

RUN : VLB 070-6
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 120 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
TIME, hr.
20
40
60
80
100
120
0
2
4
6
8
10

Figure 23-6

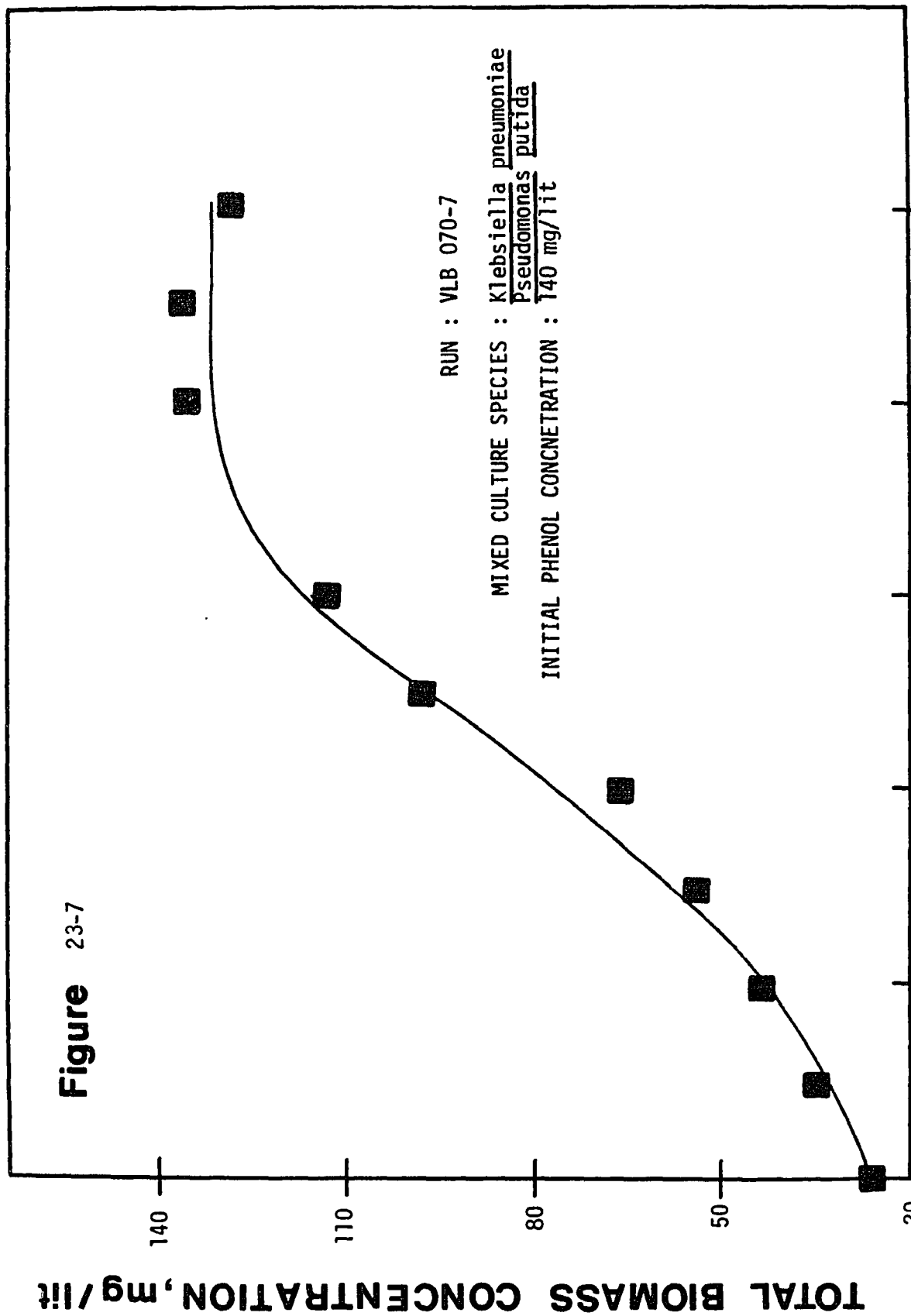

TOTAL BIOMASS CONCENTRATION, mg/lit
140
110
80
50
20
0
2
4
6
8
10
TIME, hr.
RUN : VLB 070-7
MIXED CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCNETRATION : 140 mg/lit

Figure 23-7

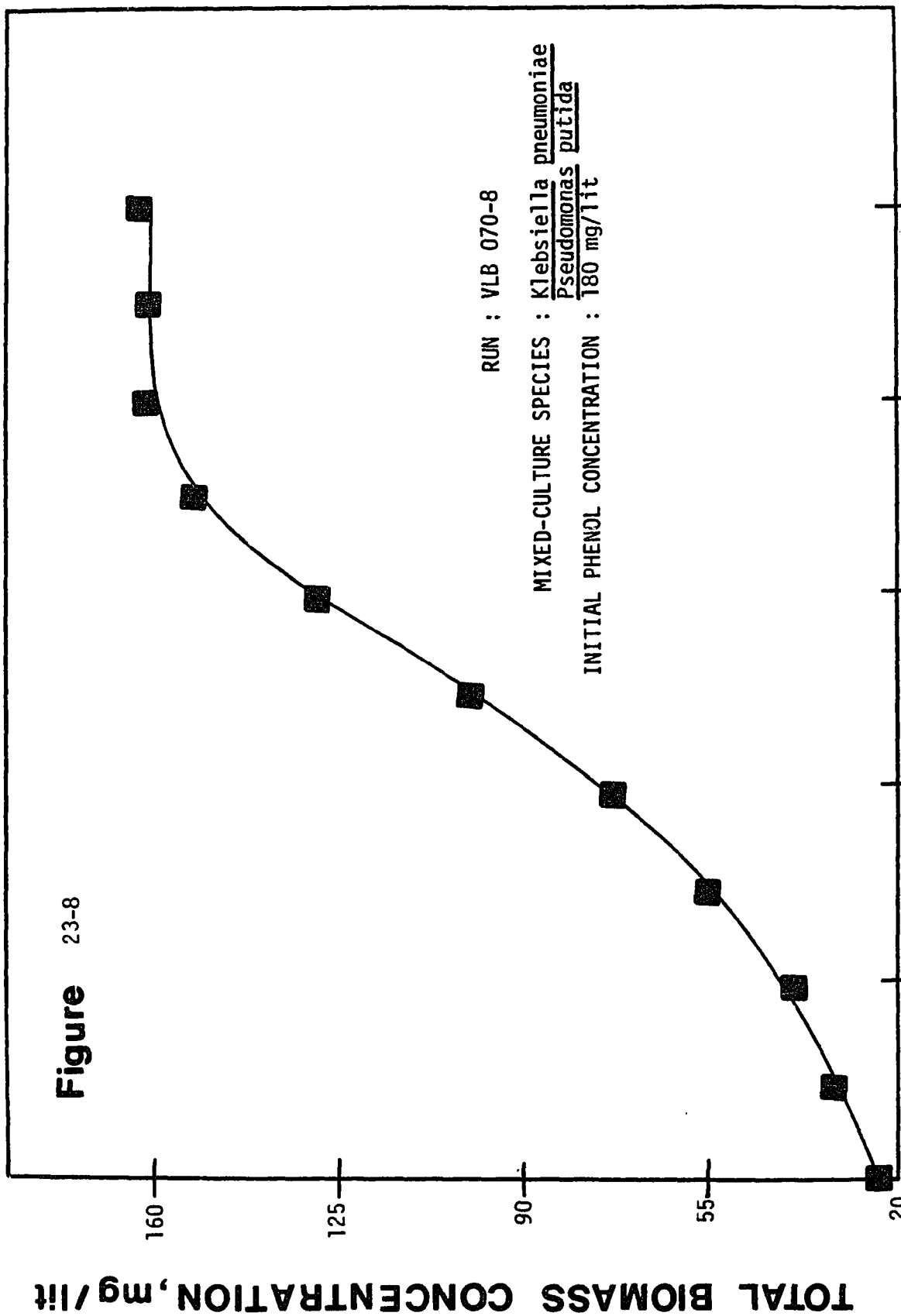


Figure 23-8
RUN : VLB 070-8
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 180 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
TIME, hr.
20
55
90
125
160
0
2
4
6
8
10

Figure 24

Growth Curves of the Mixed Culture
(*K. pneumoniae* and *S. liquefaciens*) at
the following Initial Phenol Concentrations
(Run VLB 071-1 to VLB 071-8)

24-1	20 mg/lit
24-2	40 mg/lit
24-3	50 mg/lit
24-4	80 mg/lit
24-5	100 mg/lit
24-6	120 mg/lit
24-7	140 mg/lit
24-8	180 mg/lit

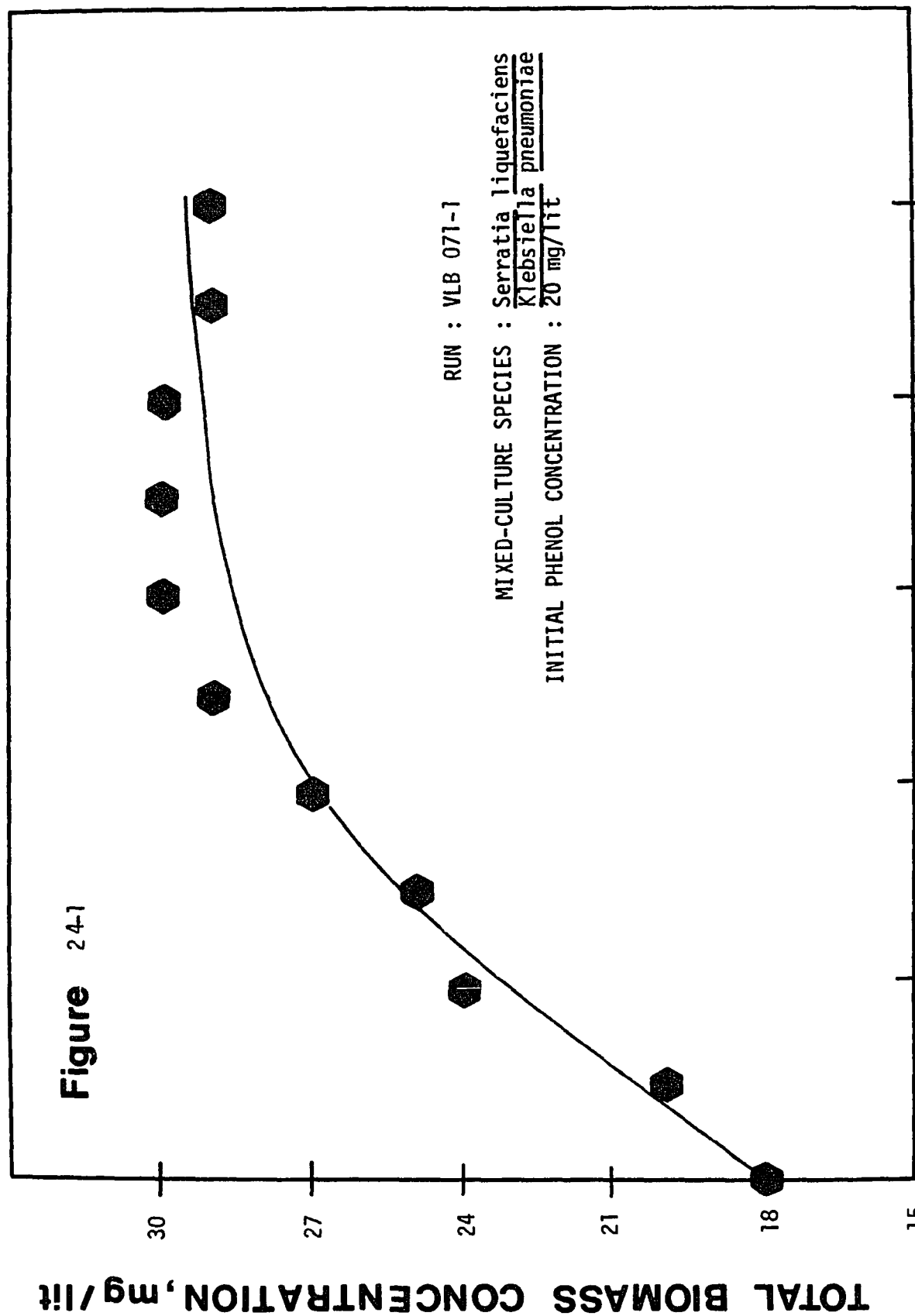

TOTAL BIOMASS CONCENTRATION, mg/lit
30
27
24
21
18
15
0
2
4
6
8
10
TIME, hr.
RUN : VLB 071-1
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20 mg/lit

Figure 24-1

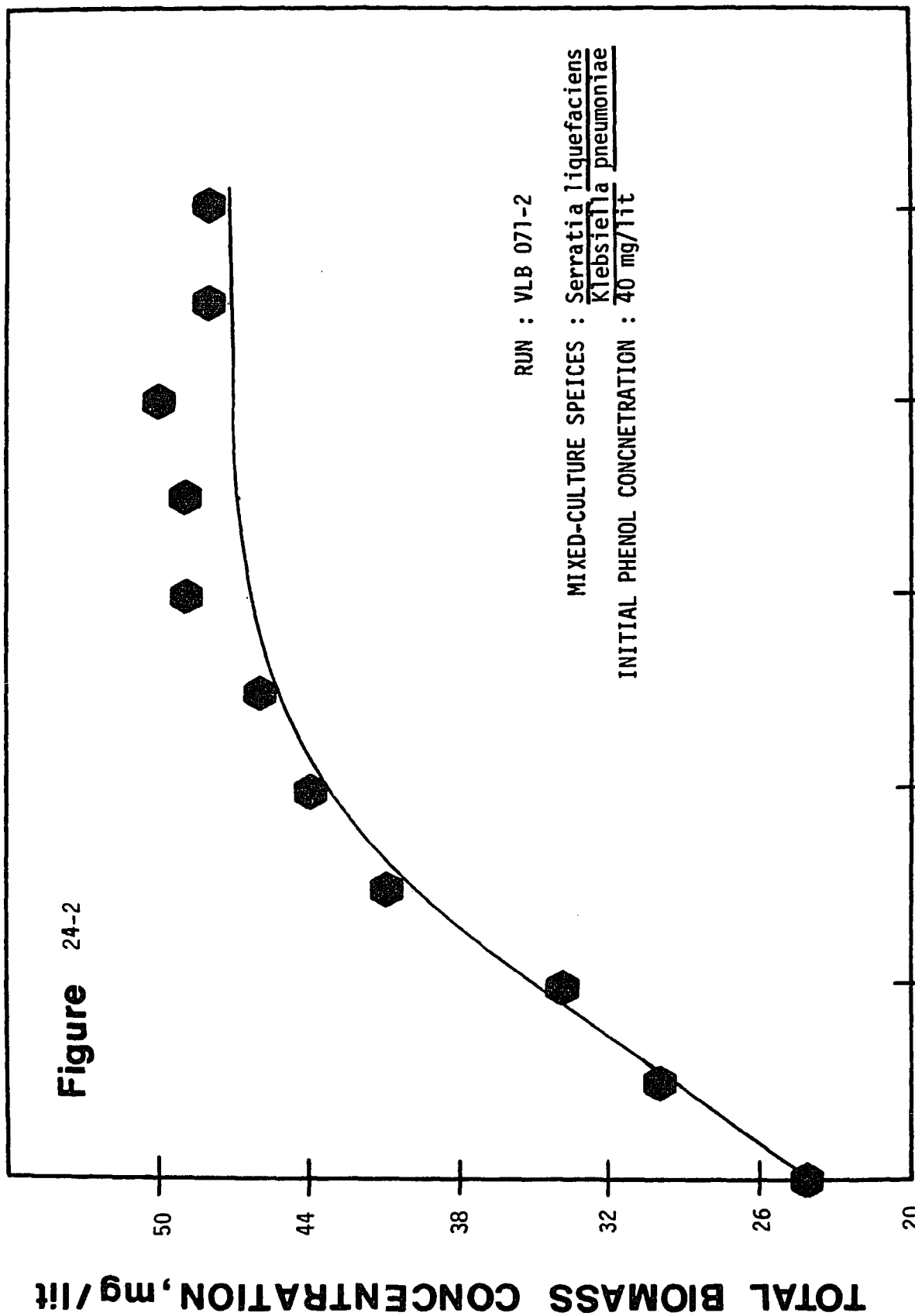

TOTAL BIOMASS CONCENTRATION, mg/lit
50
44
38
32
26
20
0
2
4
6
8
10
TIME, hr.
RUN : VLB 071-2
MIXED-CULTURE SPEICES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCNETRATION : 40 mg/lit

Figure 24-2

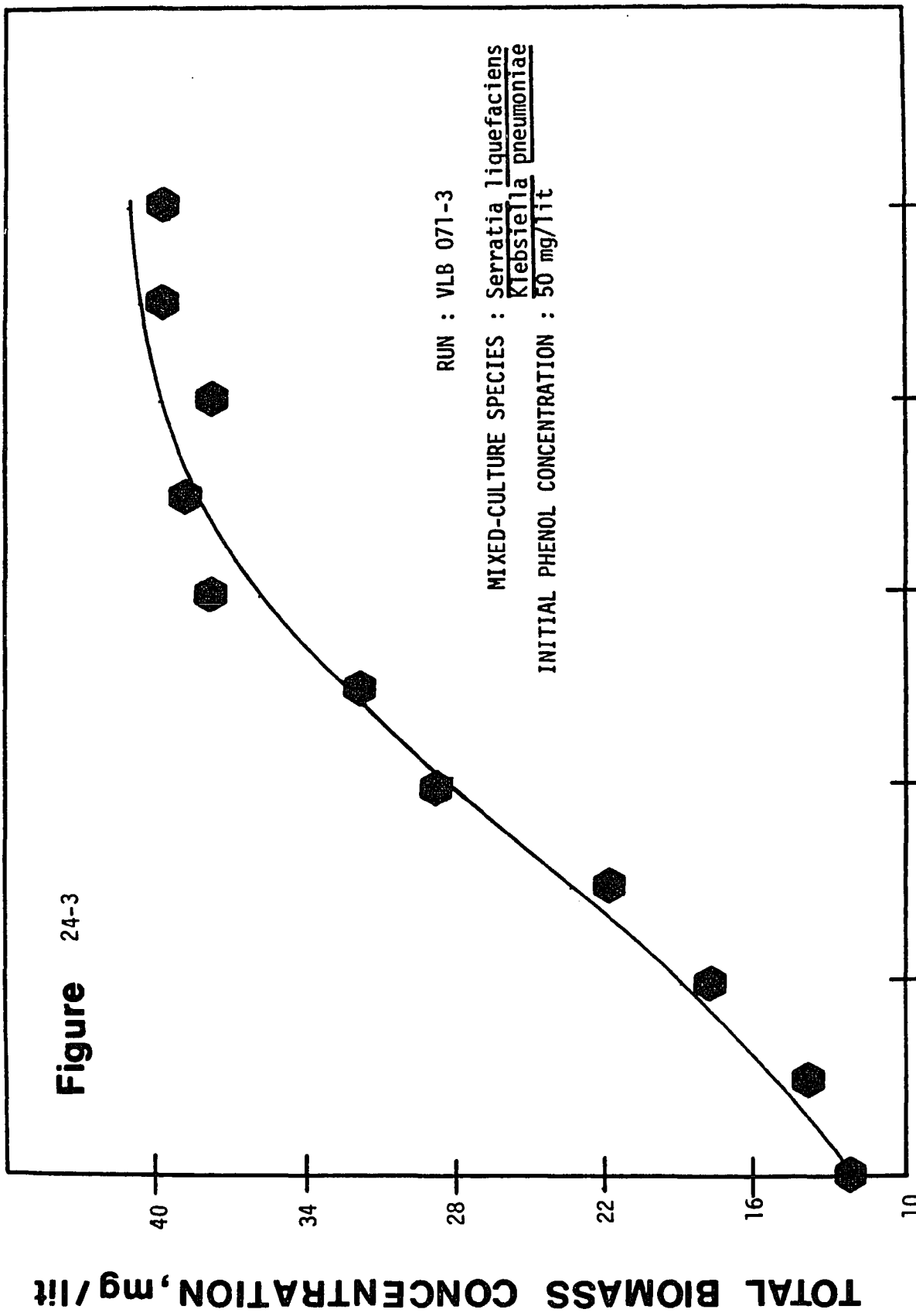

RUN : VLB 071-3
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 50 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
TIME, hr.
10
16
22
28
34
40
0
2
4
6
8
10

Figure 24-3

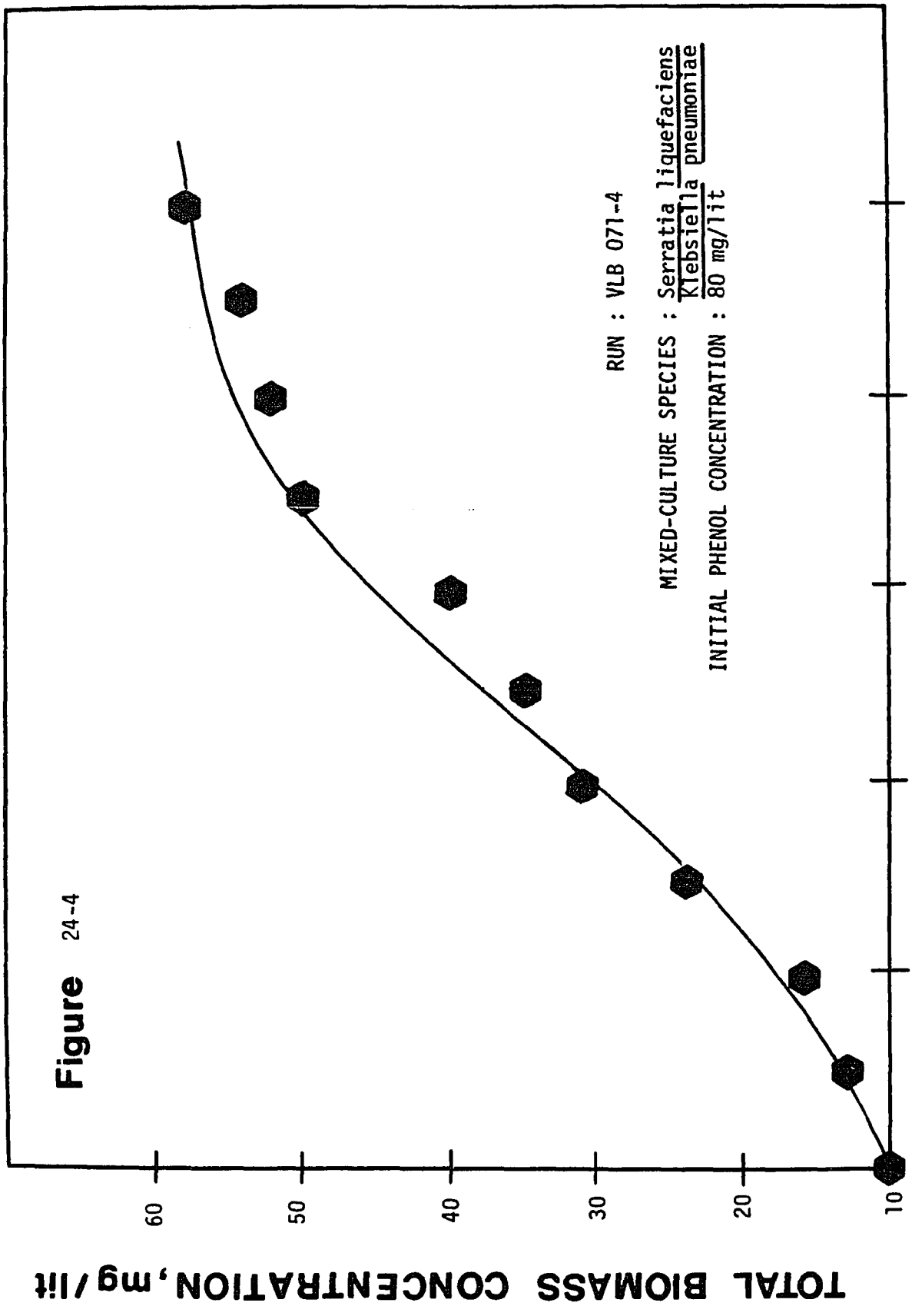

TOTAL BIOMASS CONCENTRATION, mg/lit
60
50
40
30
20
10
0
2
4
6
8
10
TIME, hr.
RUN : VLB 071-4
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 80 mg/lit

Figure 24-4

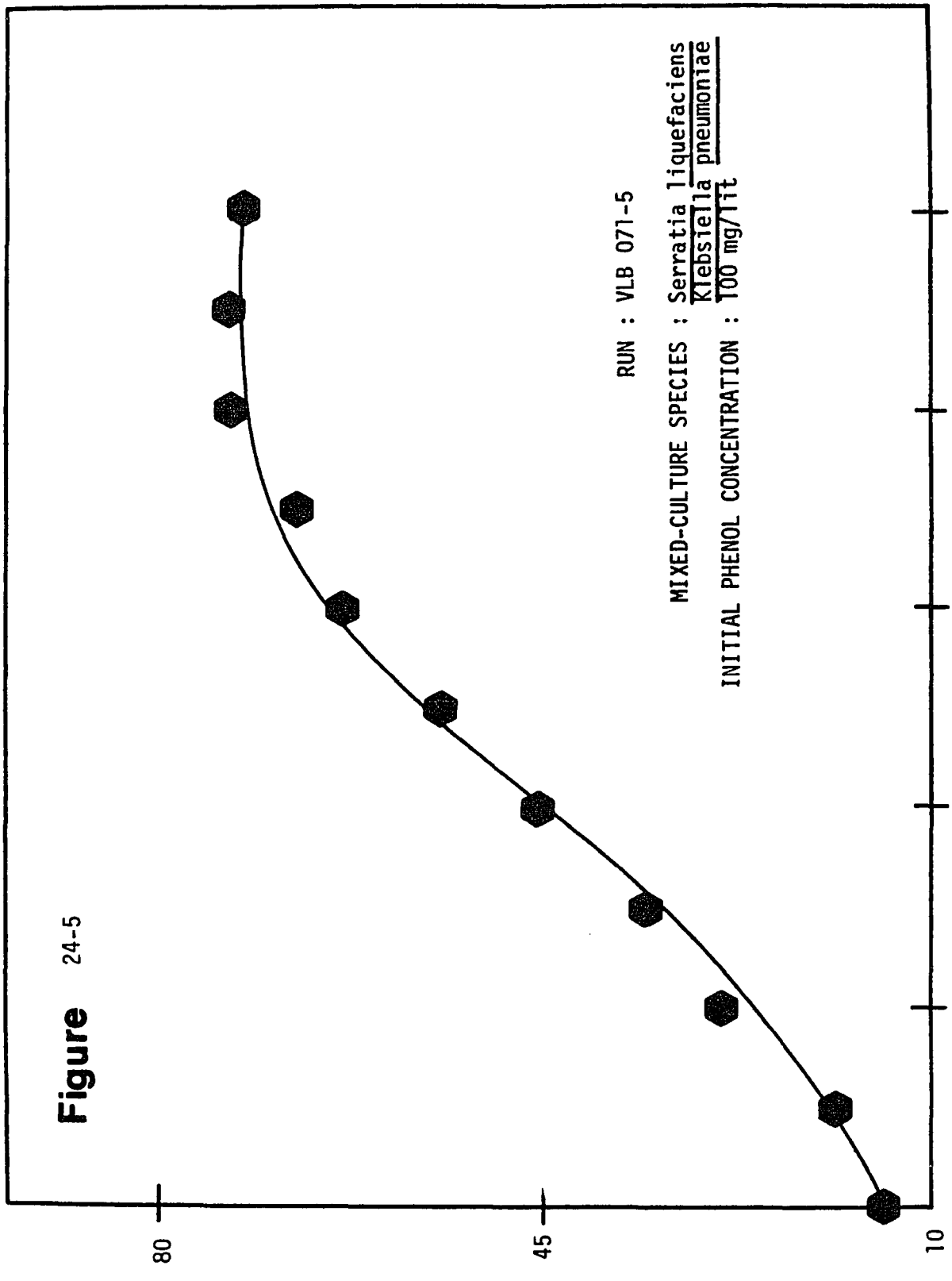


Figure 24-5
RUN : VLB 071-5
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 100 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
80
45
10
0
2
4
6
8
10
TIME, hr.

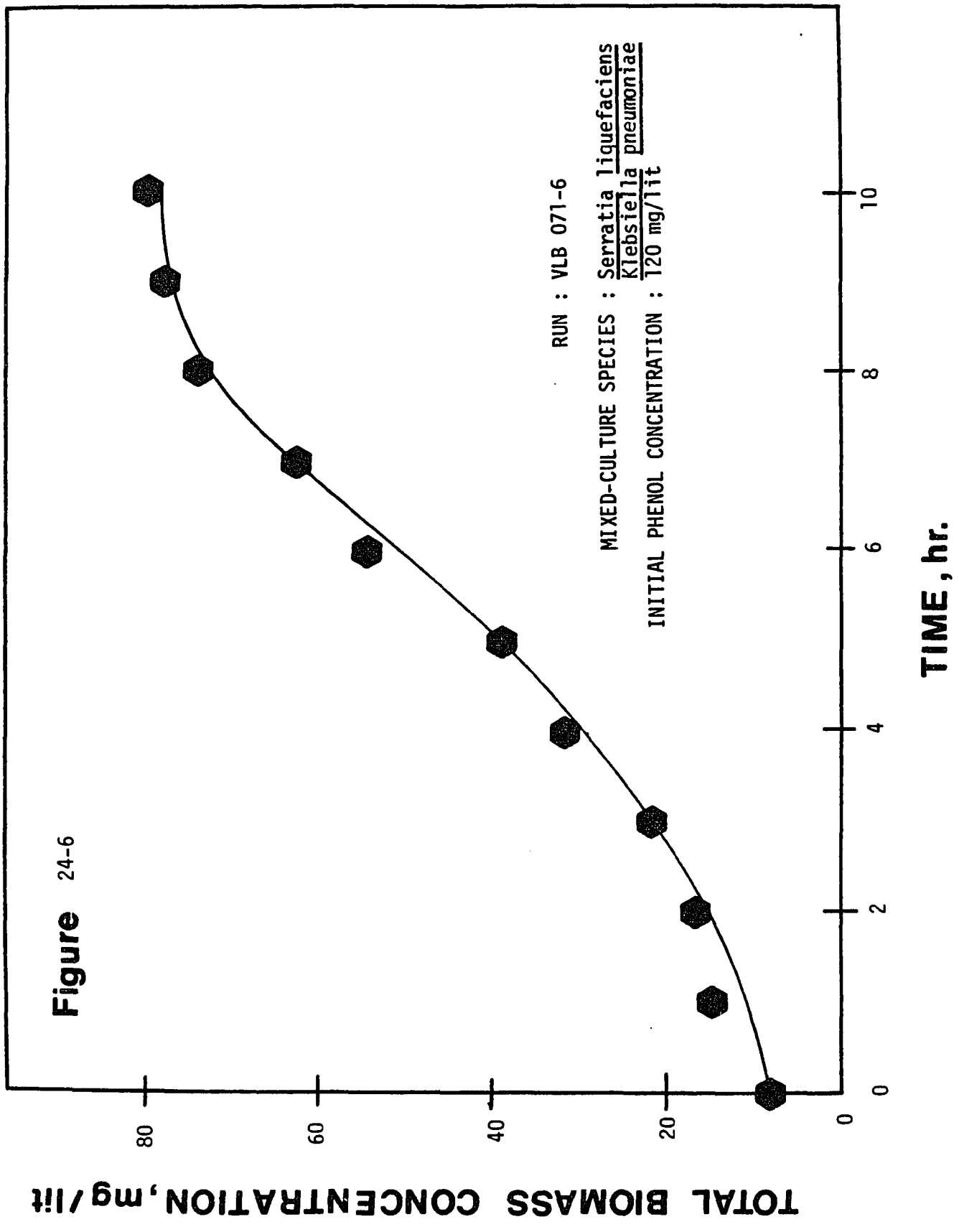

TOTAL BIOMASS CONCENTRATION, mg/lit
0
20
40
60
80
0
2
4
6
8
10
TIME, hr.
RUN : VLB 071-6
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 120 mg/lit

Figure 24-6

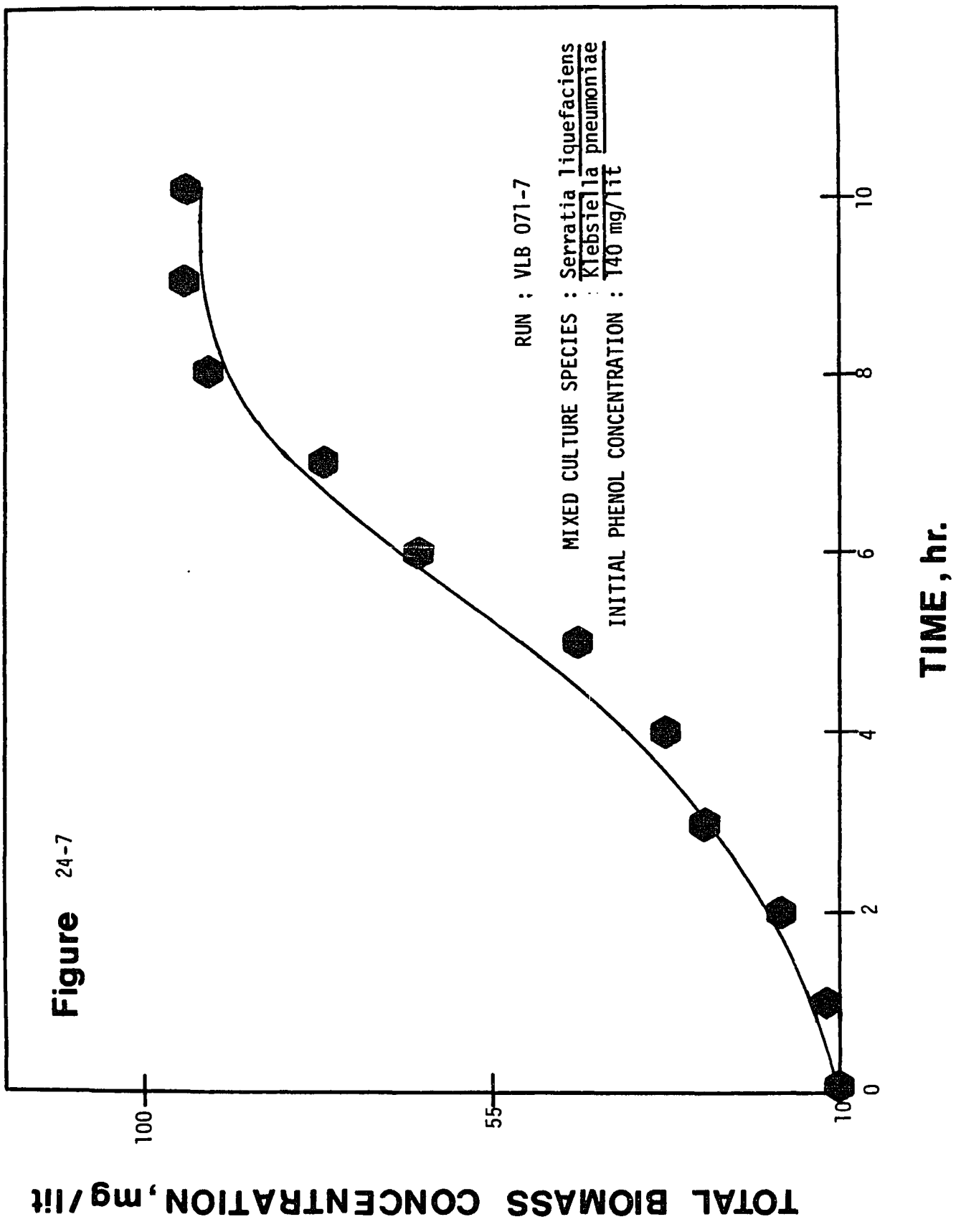

TOTAL BIOMASS CONCENTRATION, mg/lit
100
55
10
0
2
4
6
8
10
TIME, hr.
RUN : VLB 071-7
MIXED CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 140 mg/lit

Figure 24-7

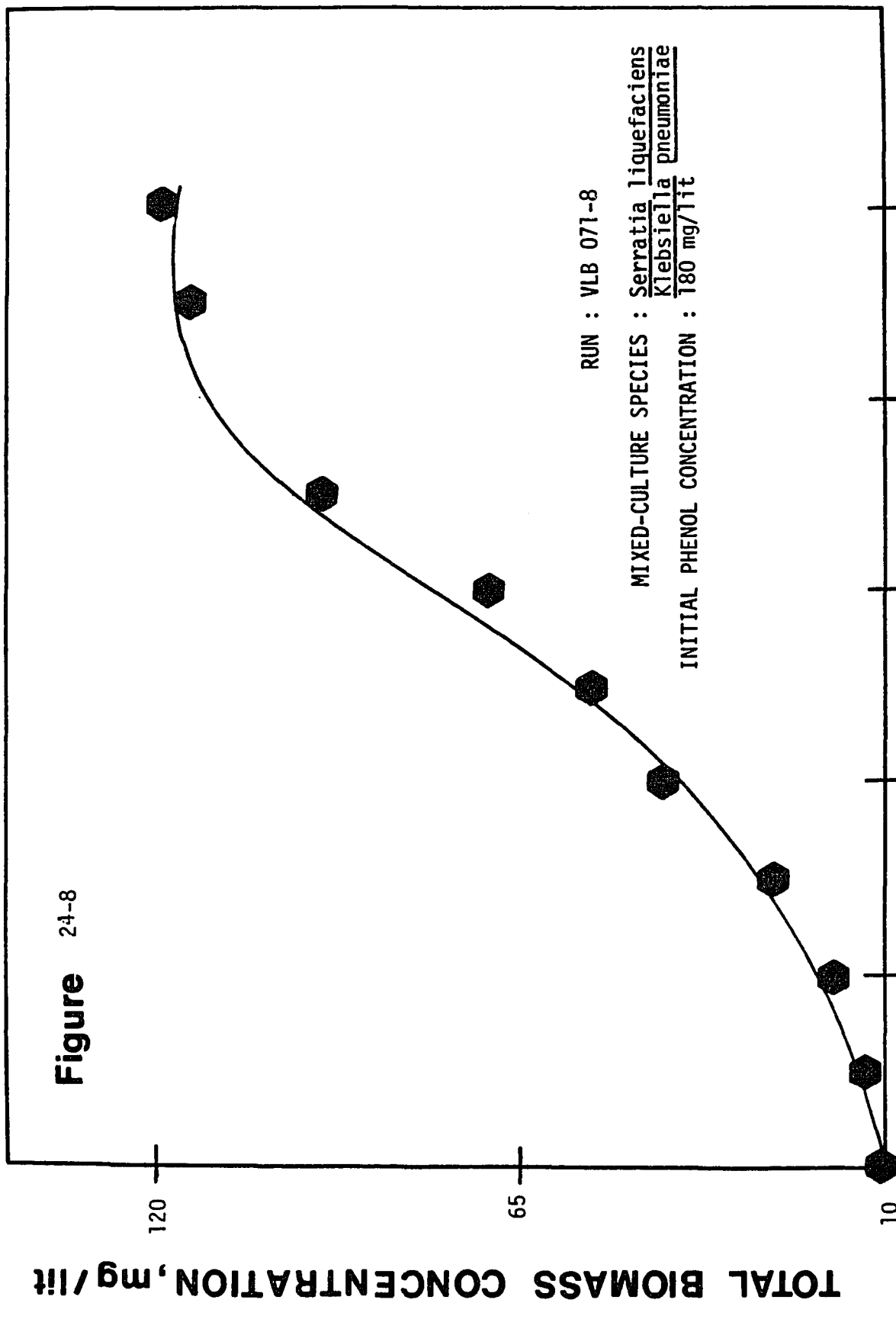


Figure 24-8
RUN : VLB 071-8
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 180 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
120
65
10
0
2
4
6
8
10
TIME, hr.

Figure 25

Growth Curves of the Mixed Culture
(*P. putida* and *S. liquefaciens*) at
the following Initial Phenol Concentrations
(Run VLB 072-1 to VLB 072-8)

25-1	20 mg/lit
25-2	40 mg/lit
25-3	50 mg/lit
25-4	80 mg/lit
25-5	100 mg/lit
25-6	120 mg/lit
25-7	140 mg/lit
25-8	180 mg/lit

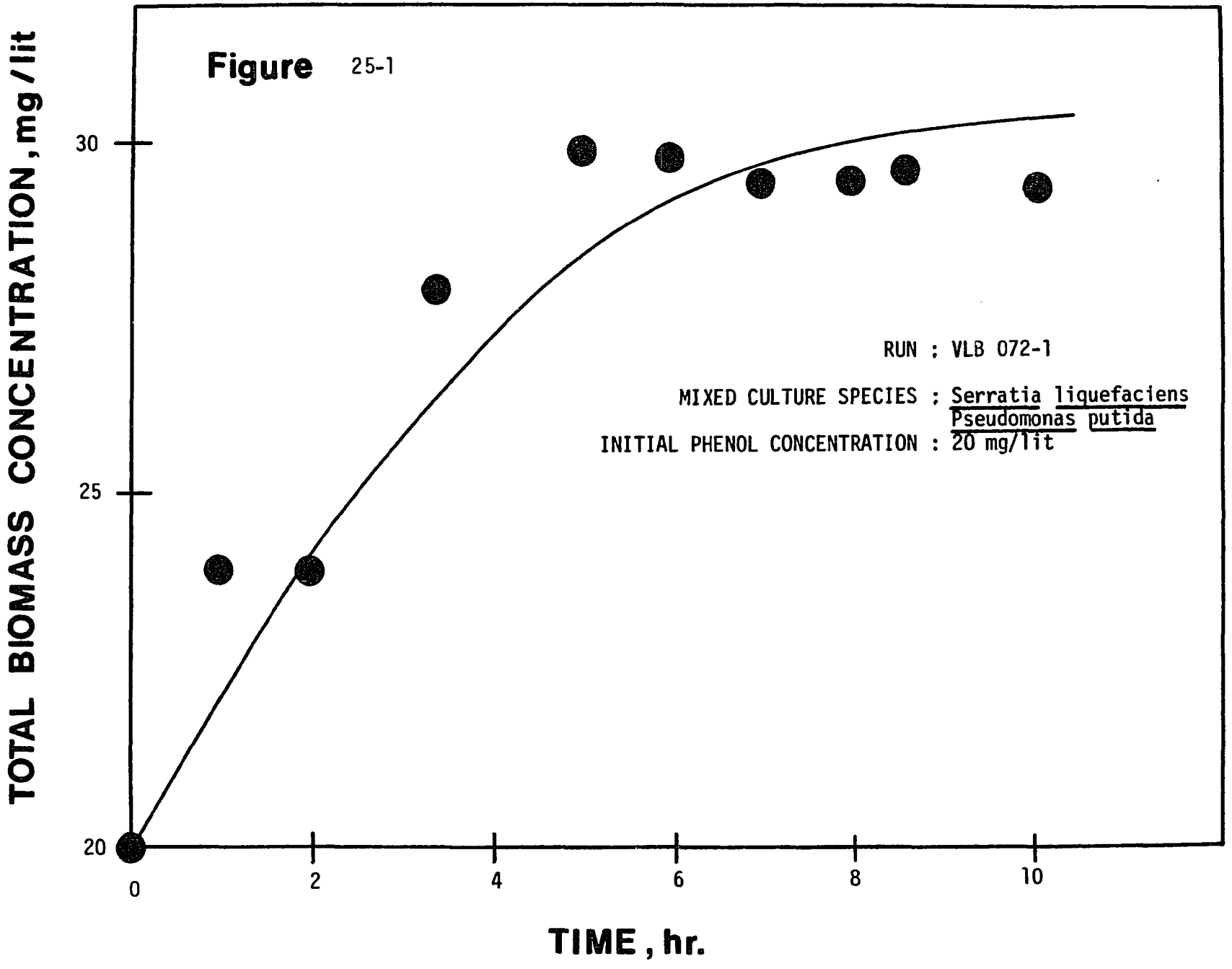


Figure 25-1
TOTAL BIOMASS CONCENTRATION, mg/lit
30
25
20
0
2
4
6
8
10
TIME, hr.
RUN : VLB 072-1
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20 mg/lit

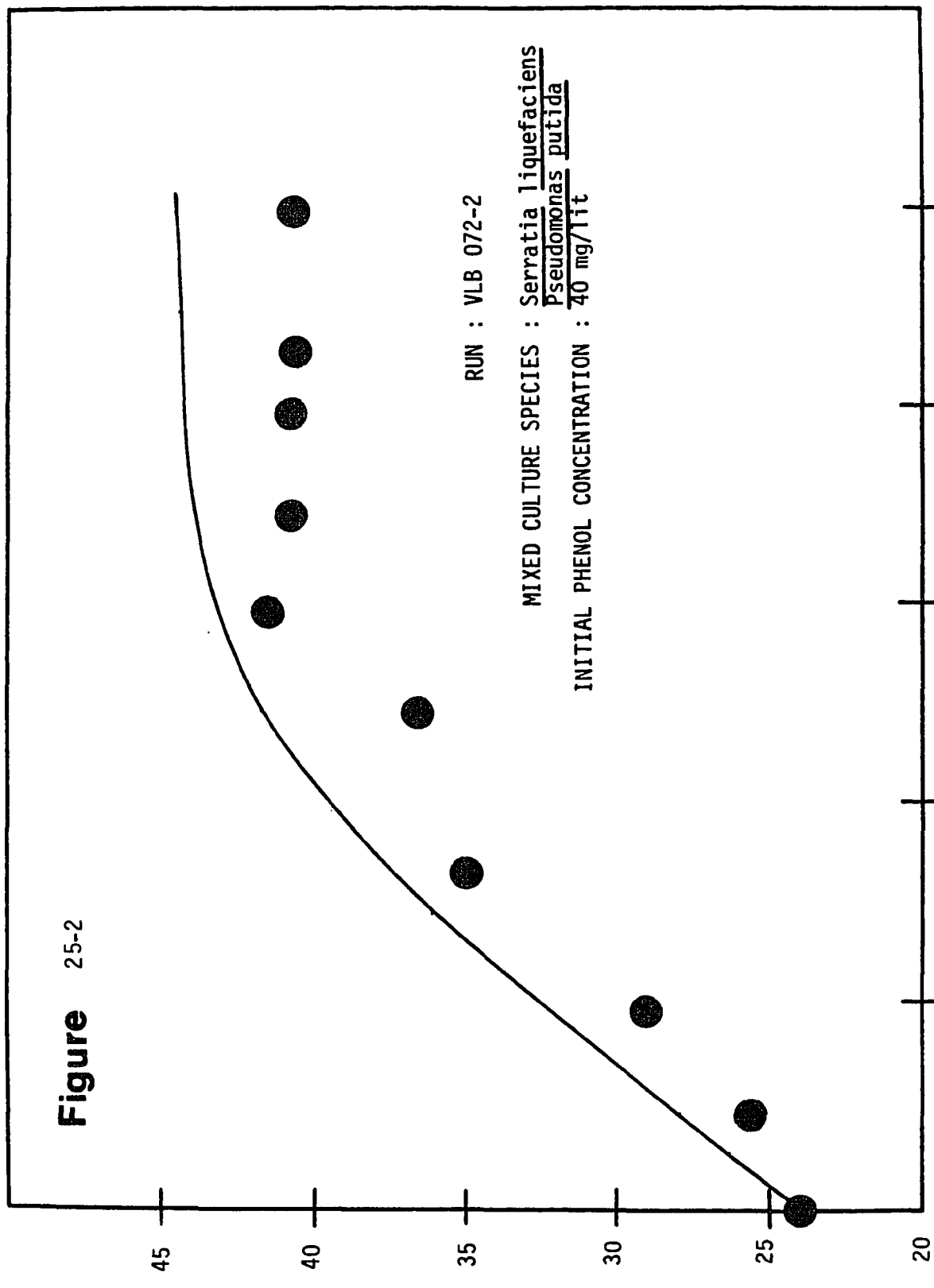

TOTAL BIOMASS CONCENTRATION, mg/lit
45
40
35
30
25
20
0
2
4
6
8
10
TIME, hr.
RUN : VLB 072-2
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 40 mg/lit

Figure 25-2

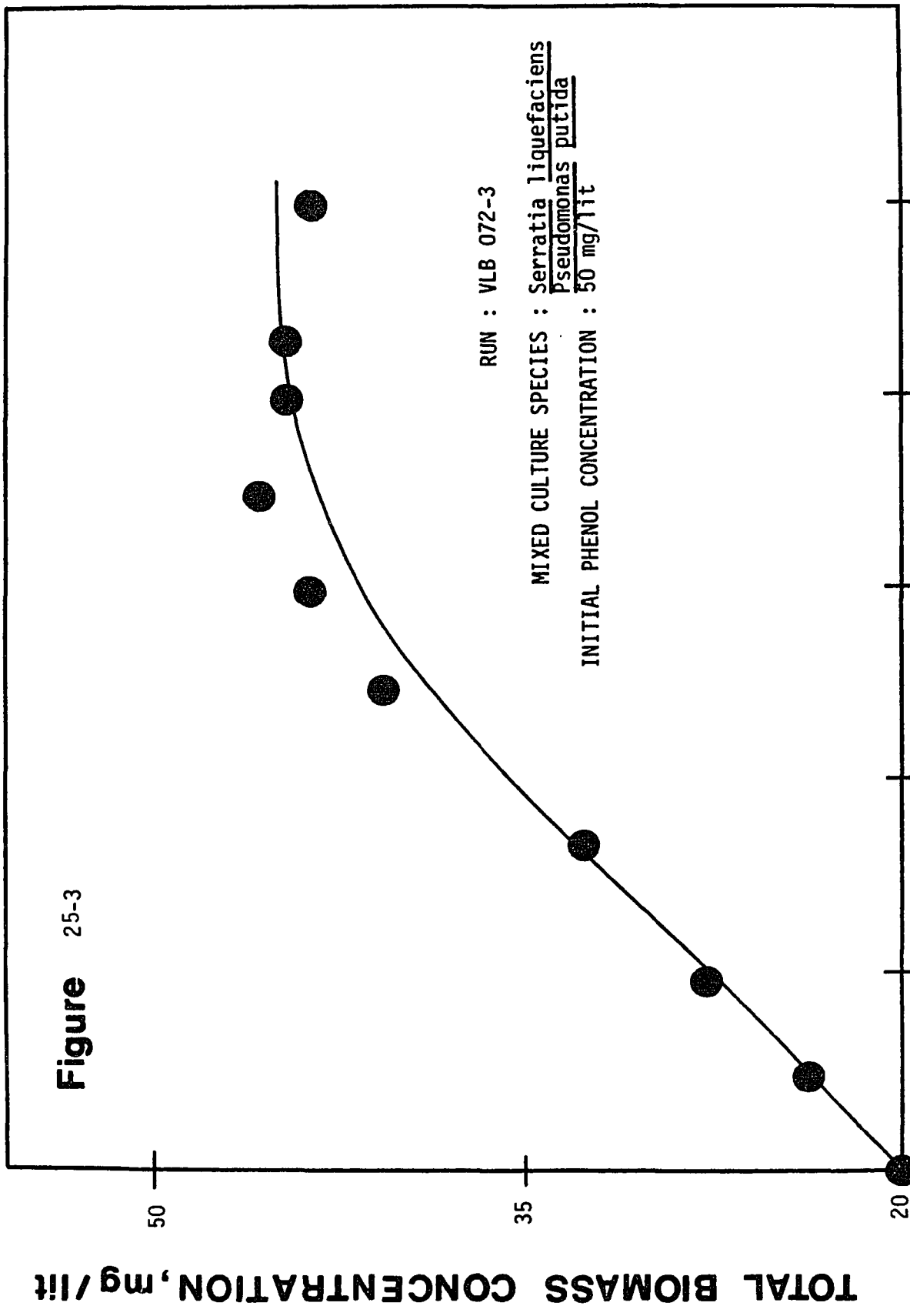

RUN : VLB 072-3
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 50 mg/lit
TOTAL BIOMASS CONCENTRATION, mg/lit
TIME, hr.
20
35
50
0
2
4
6
8
10

Figure 25-3

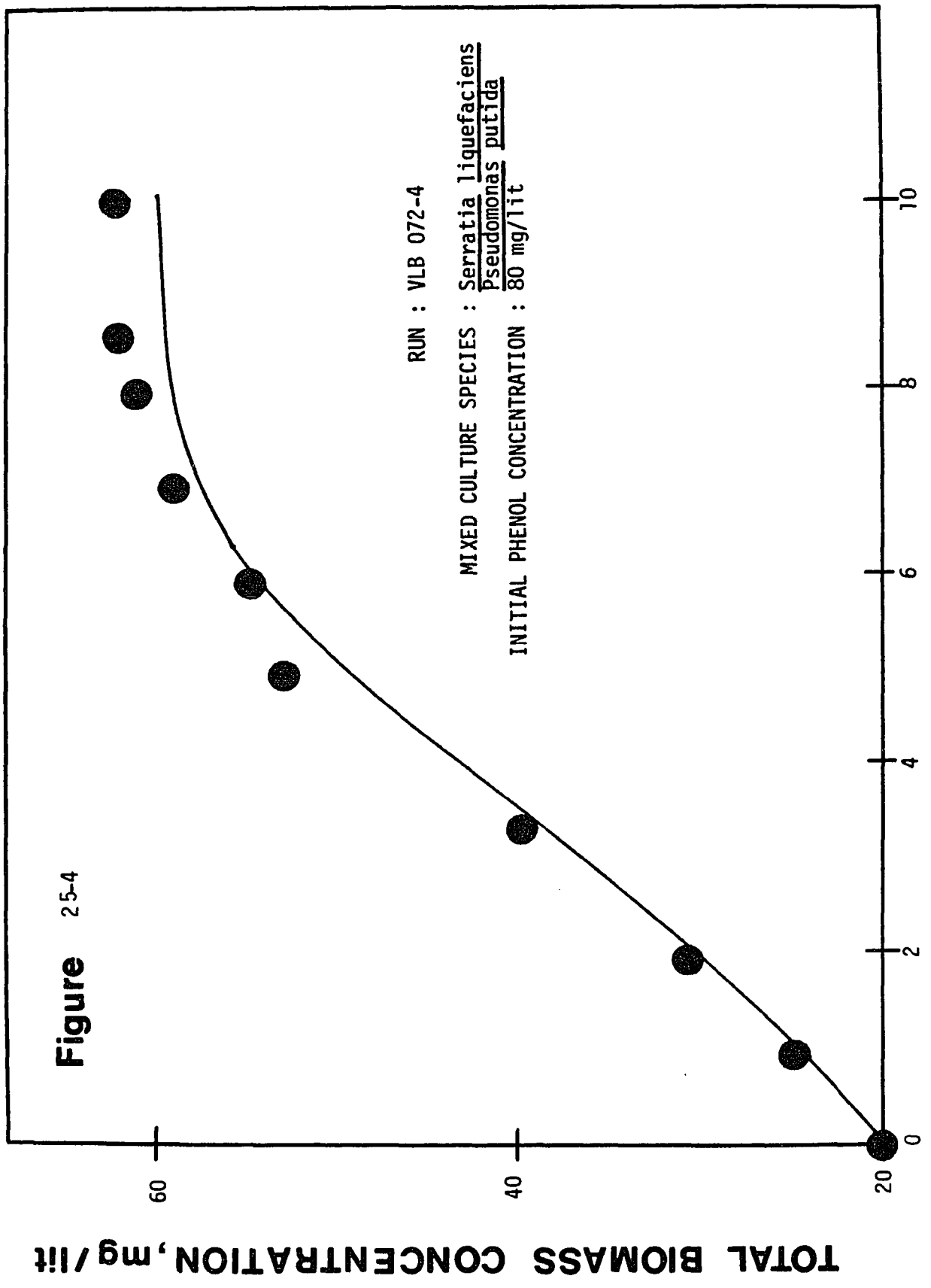

TOTAL BIOMASS CONCENTRATION, mg/lit
20
40
60
0
2
4
6
8
10
TIME, hr.
RUN : VLB 072-4
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 80 mg/lit

Figure 25-4

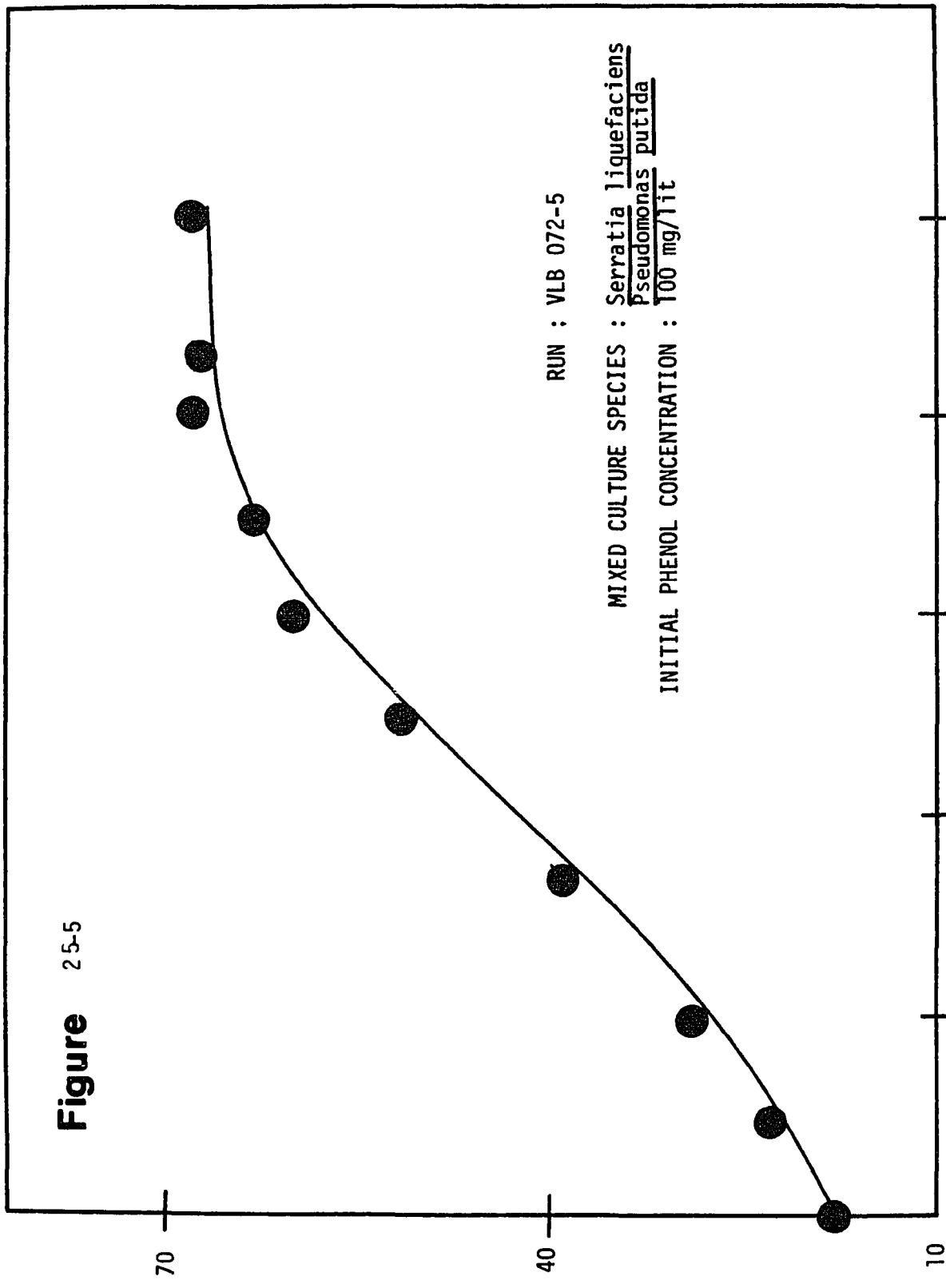

TOTAL BIOMASS CONCENTRATION, mg/lit
70
40
10
0
2
4
6
8
10
TIME, hr.
RUN : VLB 072-5
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 100 mg/lit

Figure 25-5

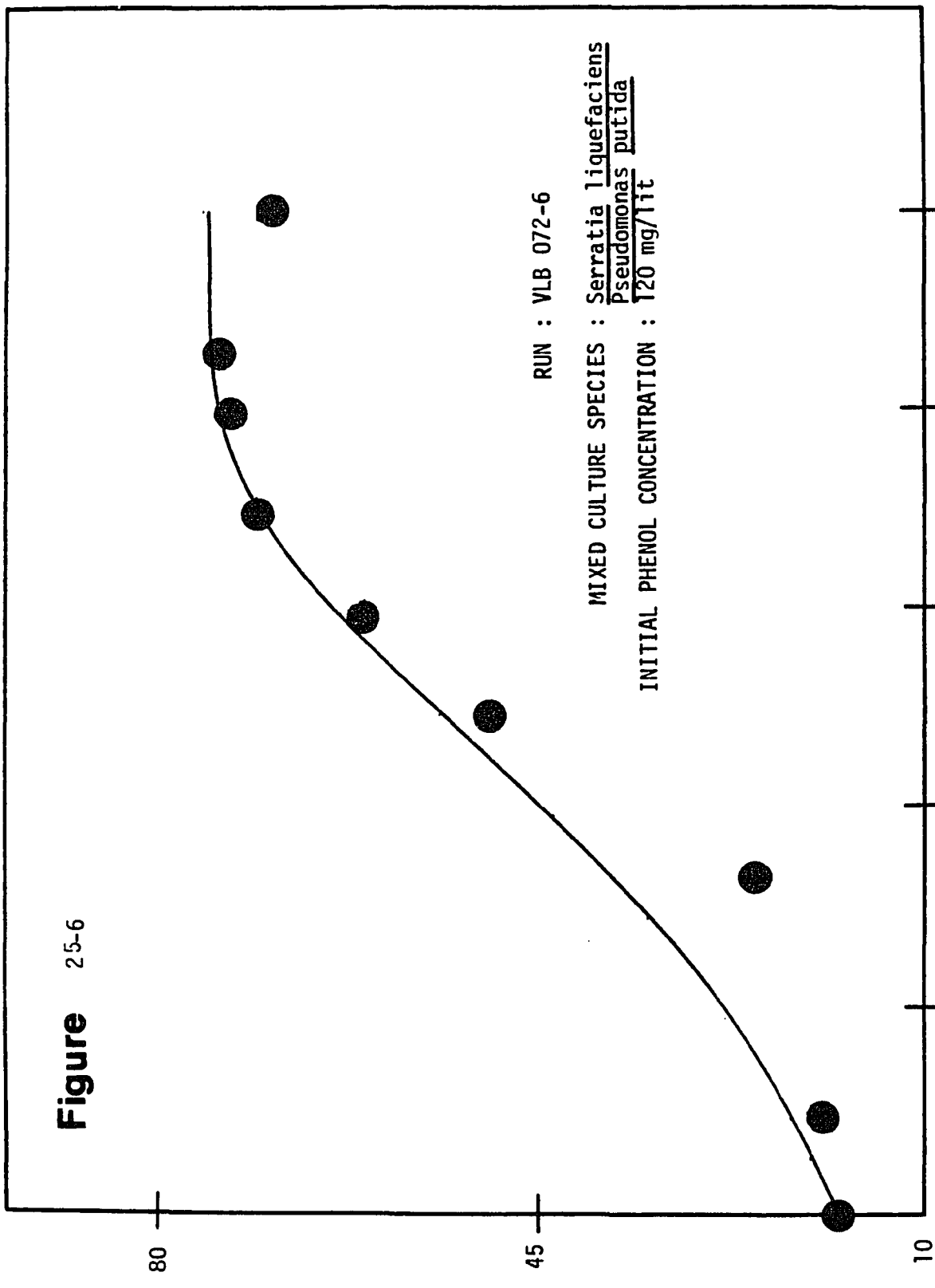

TOTAL BIOMASS CONCENTRATION, mg/lit
80
45
10
0
2
4
6
8
10
TIME, hr.
RUN : VLB 072-6
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 120 mg/lit

Figure 25-6

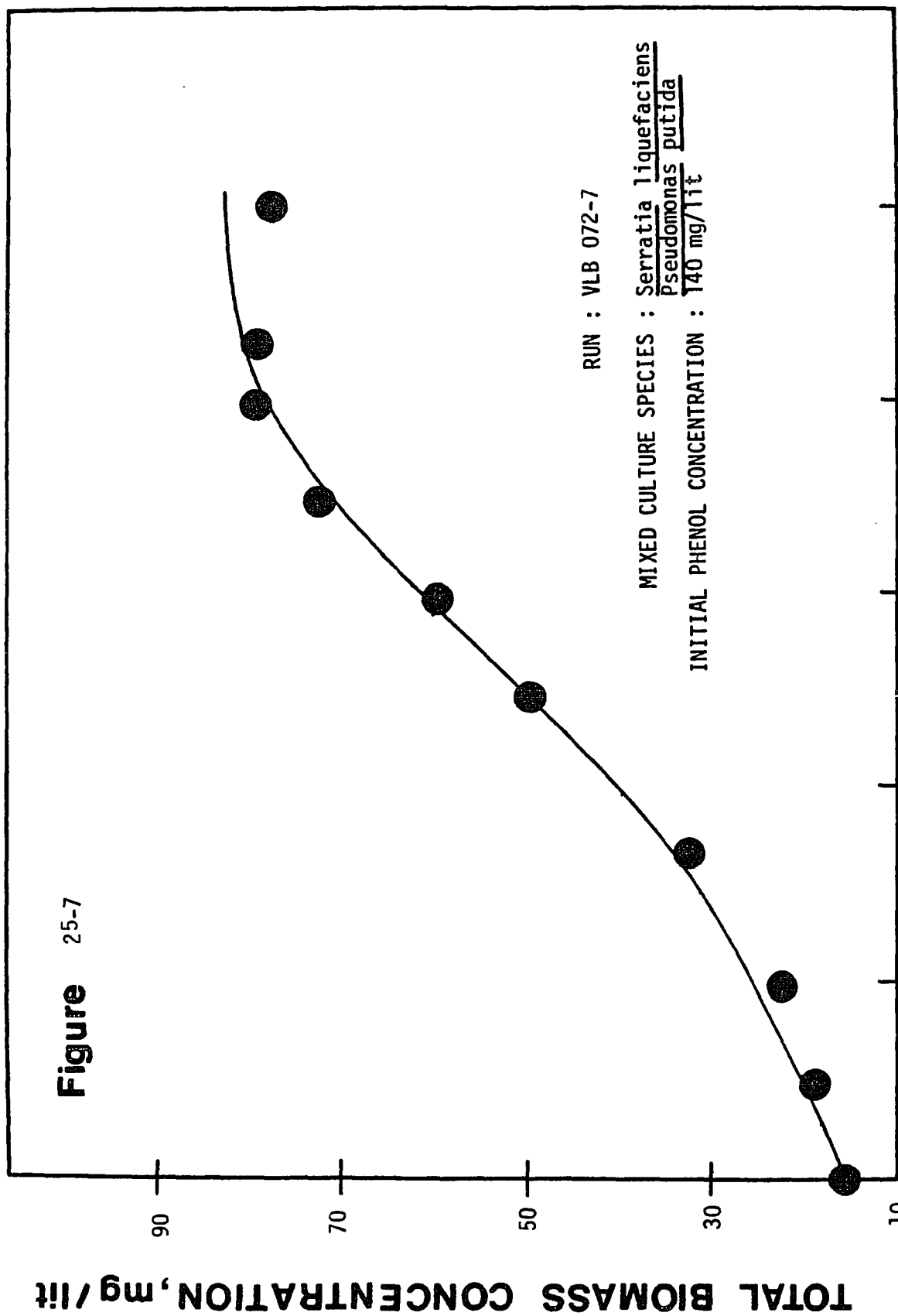

TOTAL BIOMASS CONCENTRATION, mg/lit
90
70
50
30
10
0
2
4
6
8
10
TIME, hr.
RUN : VLB 072-7
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 140 mg/lit

Figure 25-7

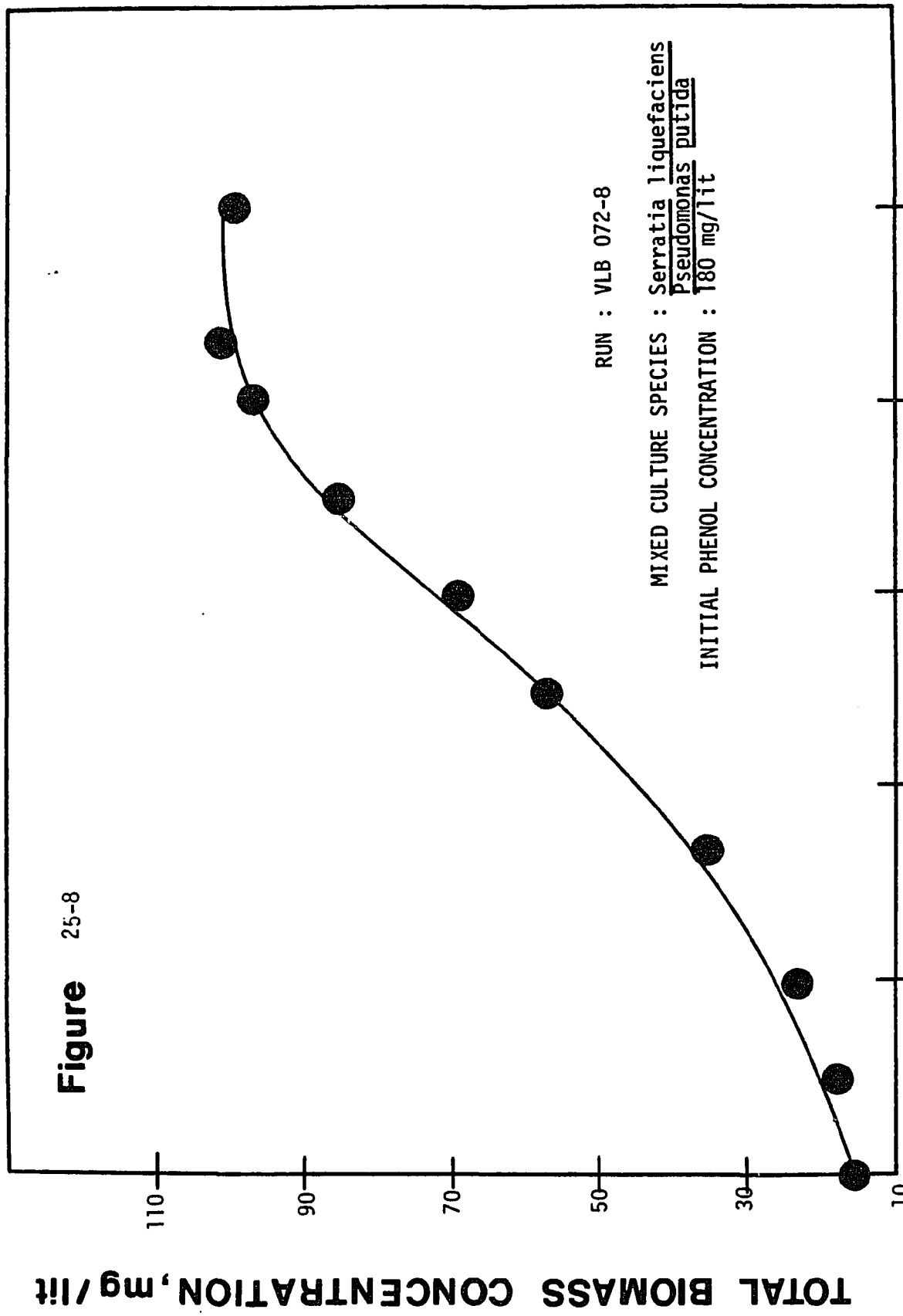

TOTAL BIOMASS CONCENTRATION, mg/lit
110
90
70
50
30
10
0
2
4
6
8
10
TIME, hr.
RUN : VLB 072-8
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 180 mg/lit

Figure 25-8

Figure 26

Phenol Degradation of the Mixed Culture
(*K. pneumoniae* and *P. putida*) at
the following Initial Phenol Concentrations
(Run VLB 070-1 to VLB 070-8)

26-1	20 mg/lit
26-2	40 mg/lit
26-3	50 mg/lit
26-4	80 mg/lit
26-5	100 mg/lit
26-6	120 mg/lit
26-7	140 mg/lit
26-8	180 mg/lit

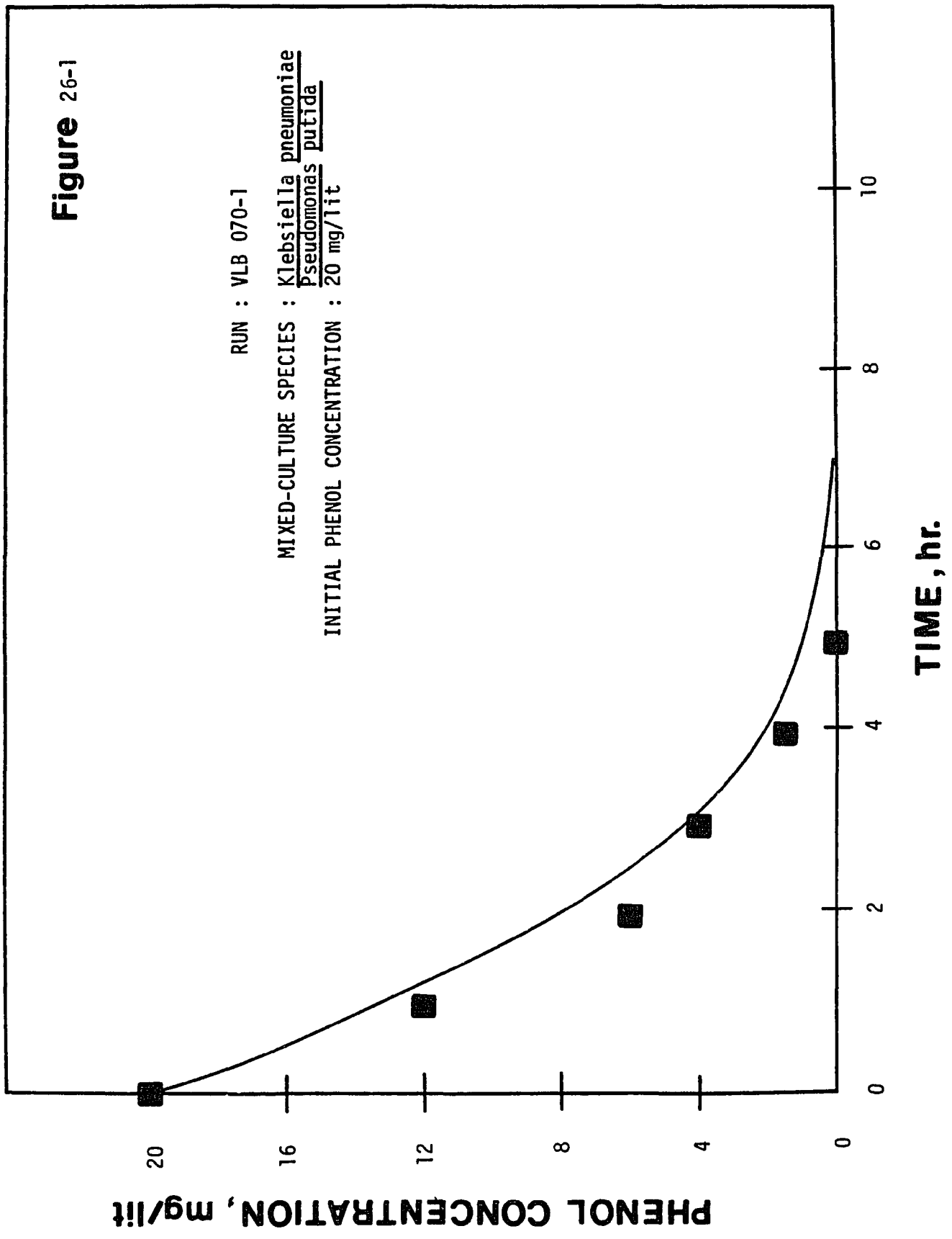


Figure 26-1
RUN : VLB 070-1
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
4
8
12
16
20
0
2
4
6
8
10

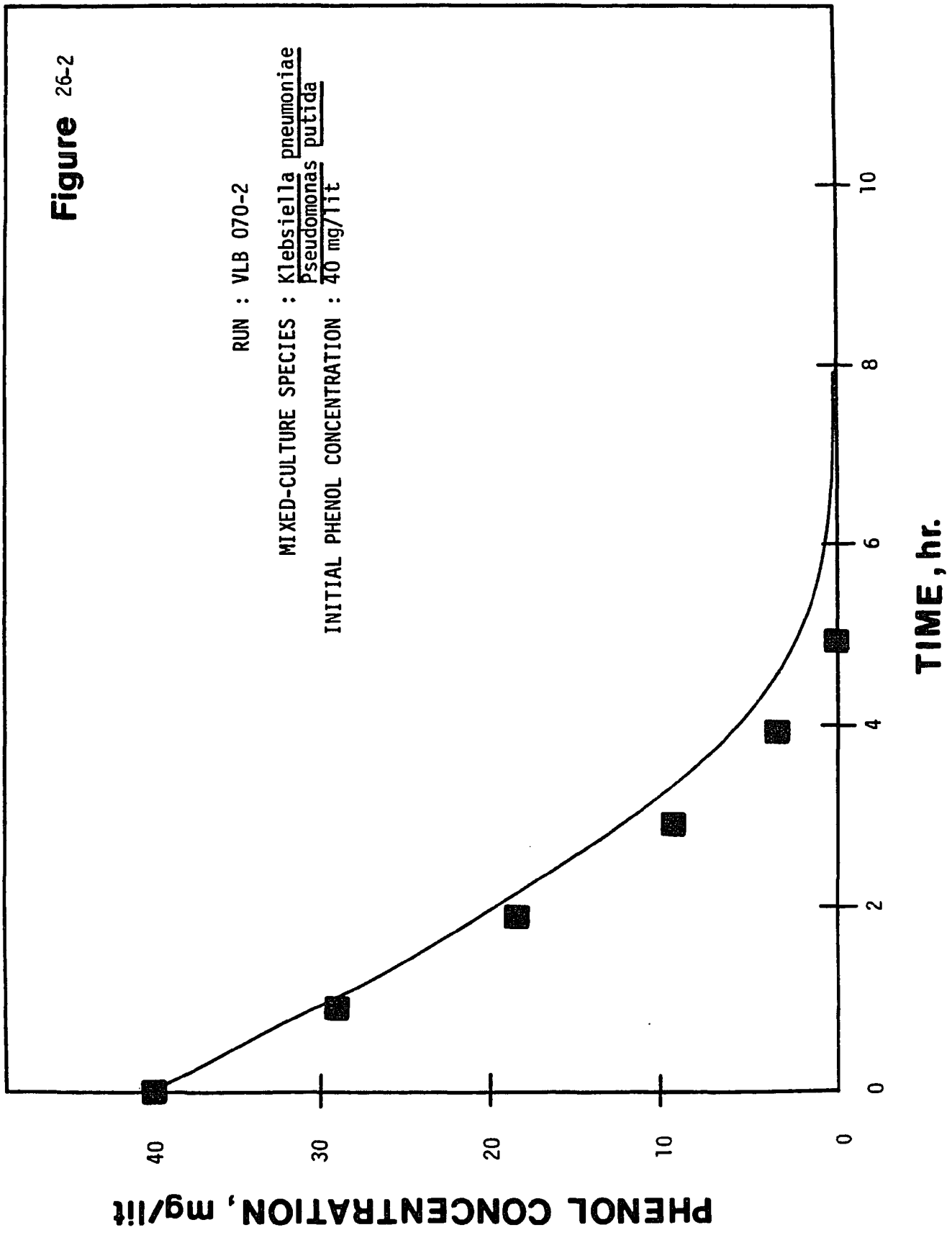


Figure 26-2
RUN : VLB 070-2
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 40 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
10
20
30
40
0
2
4
6
8
10

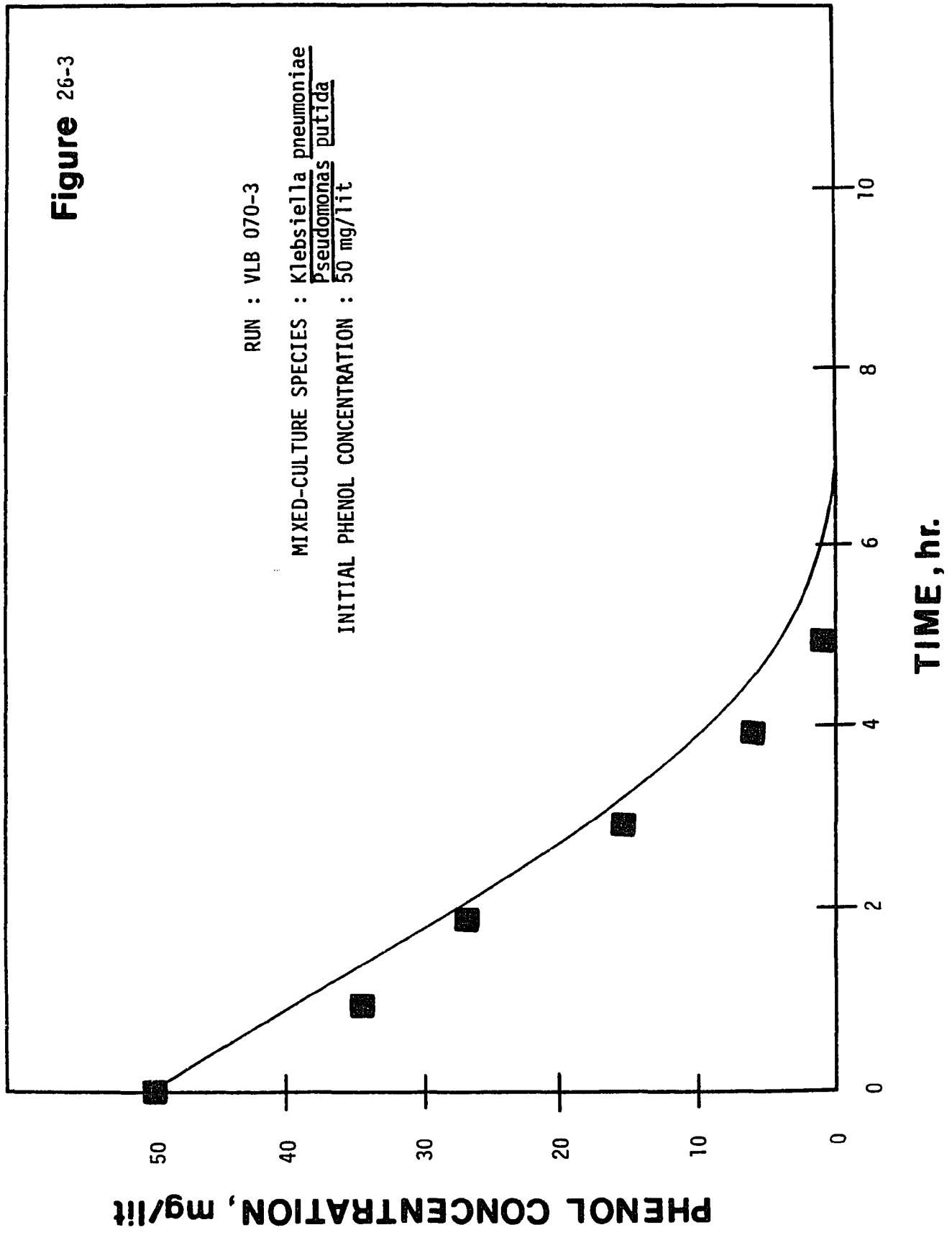


Figure 26-3
RUN : VLB 070-3
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 50 mg/lit
PHENOL CONCENTRATION, mg/lit
0
10
20
30
40
50
0
2
4
6
8
10
TIME, hr.

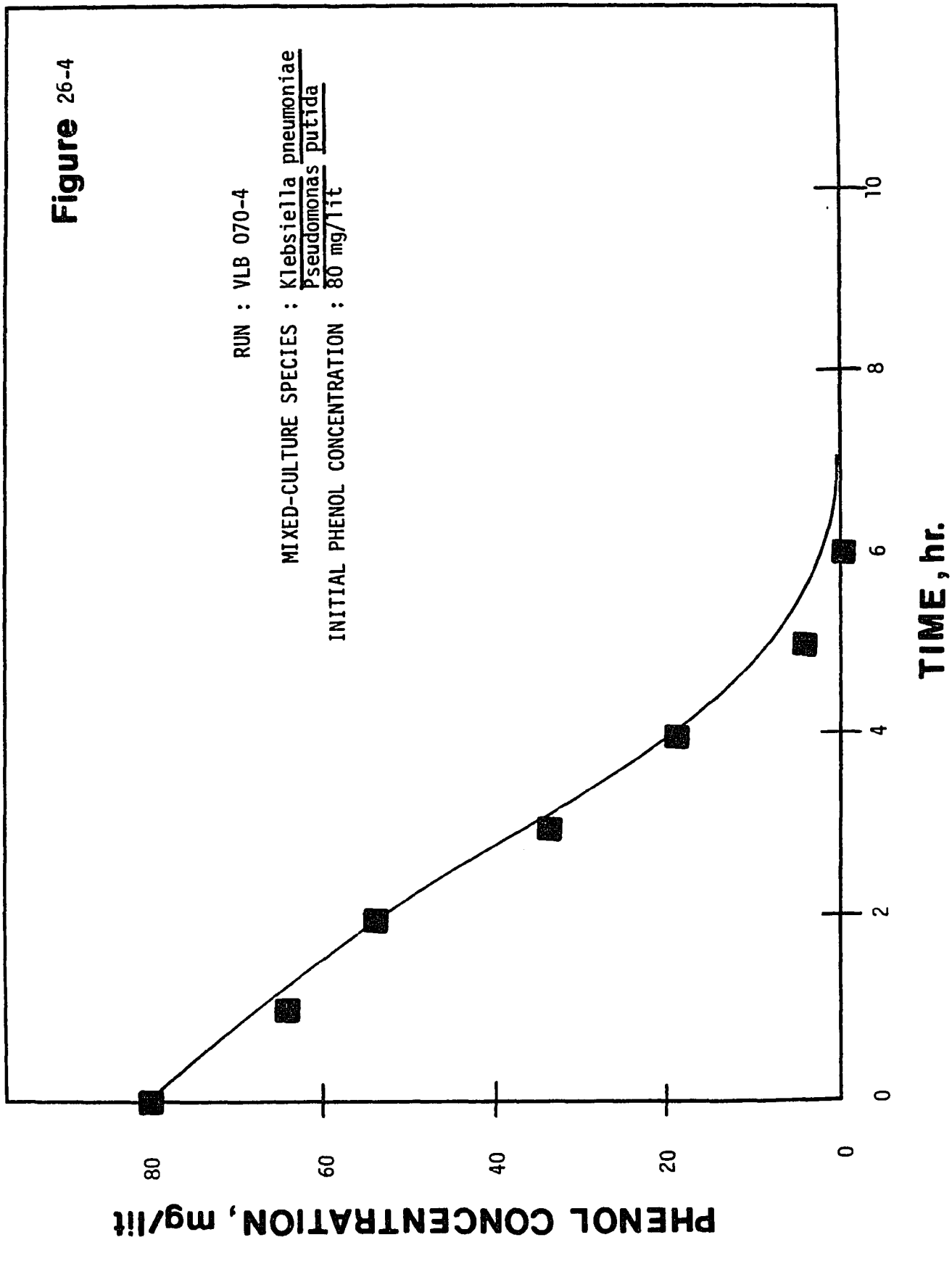


Figure 26-4
RUN : VLB 070-4
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 80 mg/lit
PHENOL CONCENTRATION, mg/lit
0
20
40
60
80
0
2
4
6
8
10
TIME, hr.

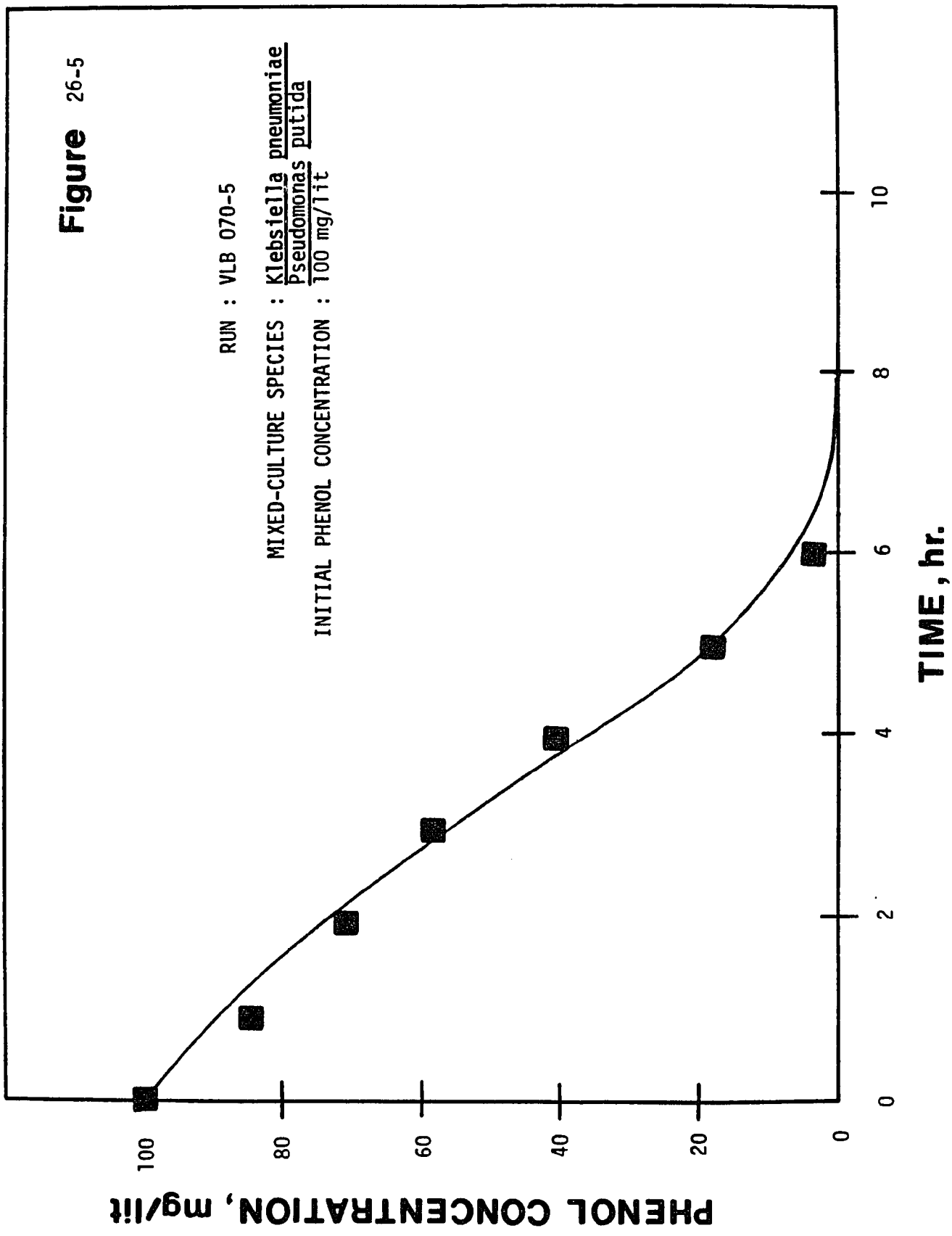


Figure 26-5
RUN : VLB 070-5
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 100 mg/lit
PHENOL CONCENTRATION, mg/lit
100
80
60
40
20
0
0
2
4
6
8
10
TIME, hr.

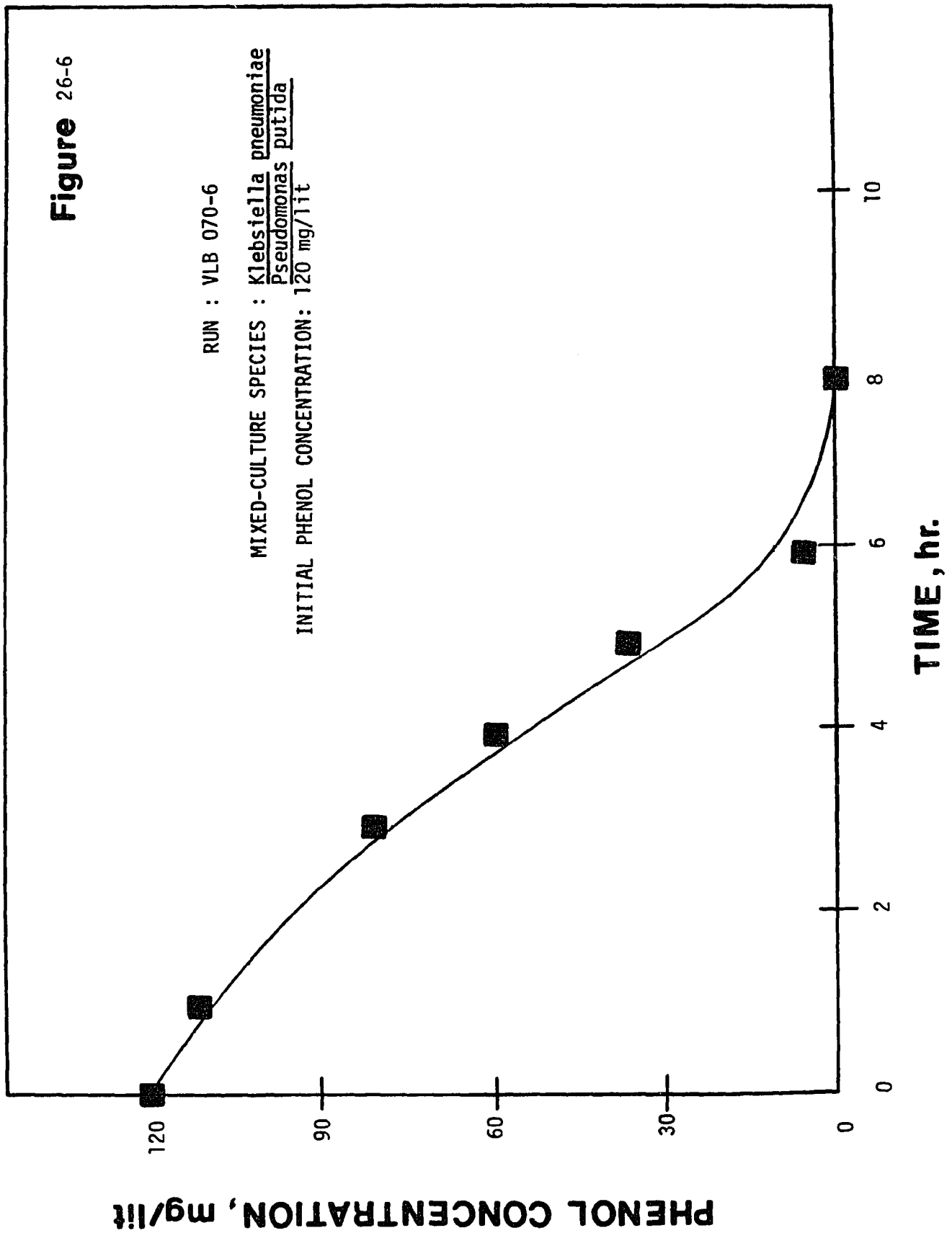


Figure 26-6
RUN : VLB 070-6
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION: 120 mg/lit
PHENOL CONCENTRATION, mg/lit
120
90
60
30
0
0
2
4
6
8
10
TIME, hr.

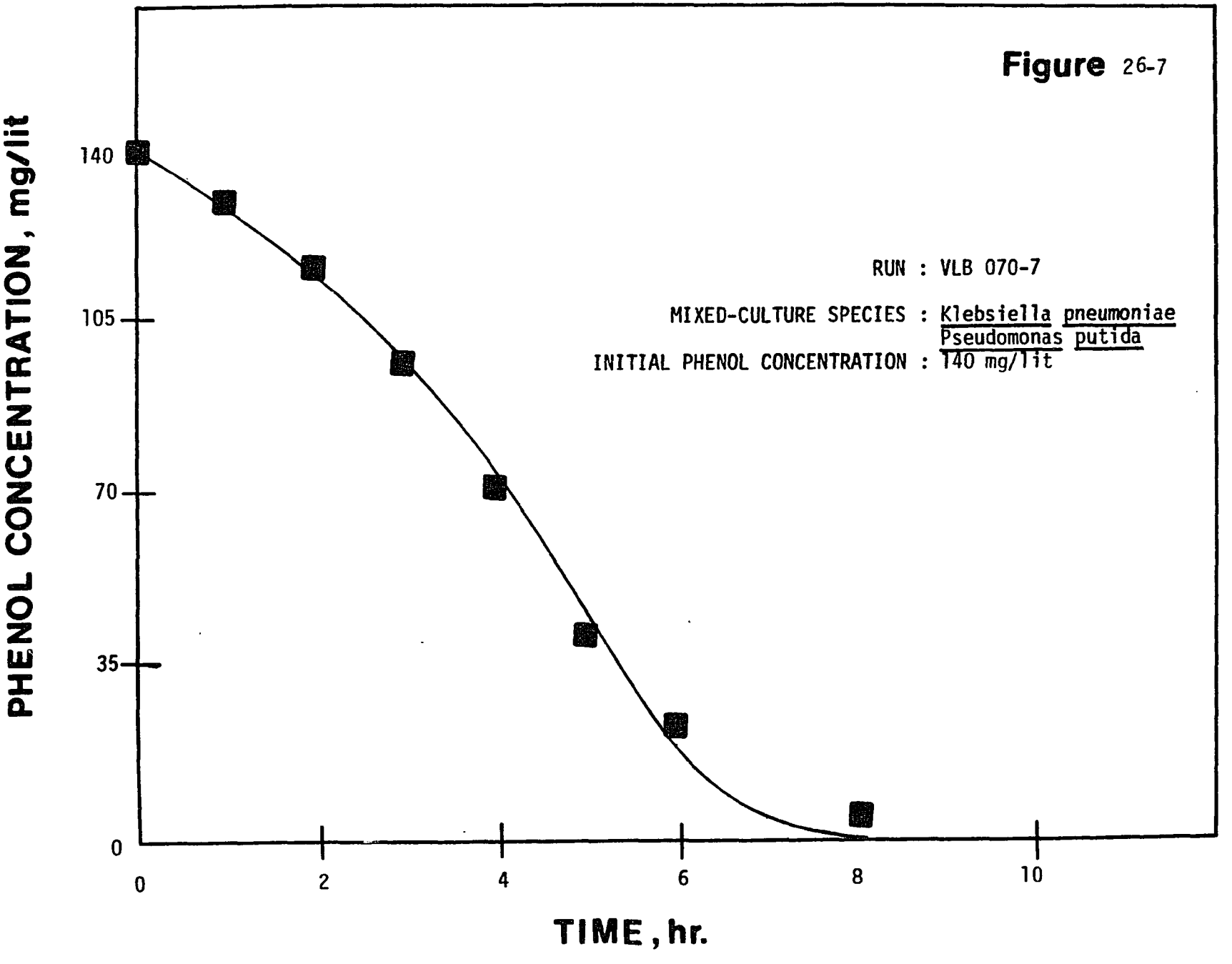


Figure 26-7
RUN : VLB 070-7
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 140 mg/lit
PHENOL CONCENTRATION, mg/lit
140
105
70
35
0
0
2
4
6
8
10
TIME, hr.

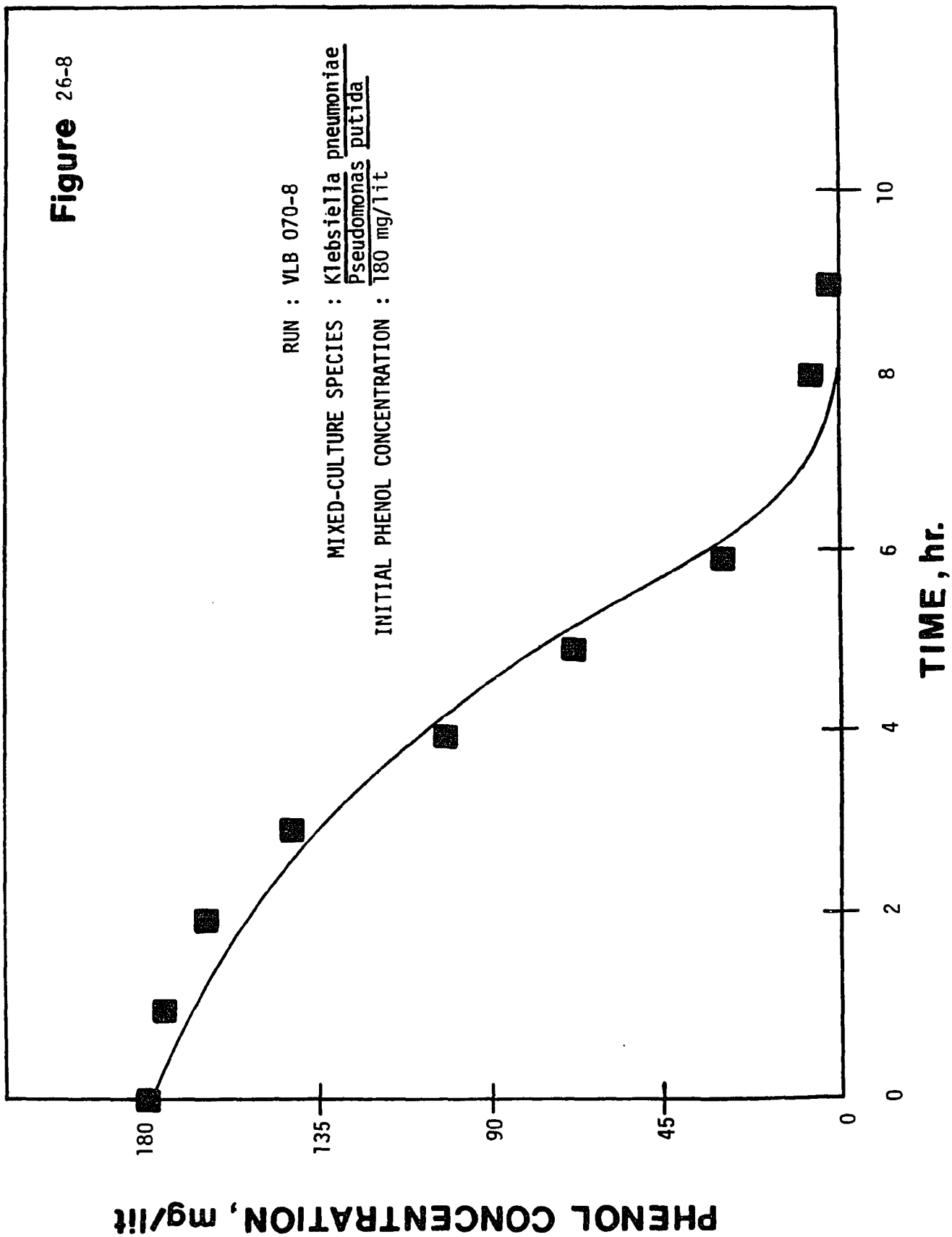


Figure 26-8
RUN : VLB 070-8
MIXED-CULTURE SPECIES : Klebsiella pneumoniae
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 180 mg/lit
PHENOL CONCENTRATION, mg/lit
180
135
90
45
0
0
2
4
6
8
10
TIME, hr.

Figure 27

Phenol Degradation of the Mixed Culture (*K. pneumoniae* and *S. liquefaciens*) at the following Initial Phenol Concentrations (Run VLB 071-1 to VLB 071-8)

27-1	20 mg/lit
27-2	40 mg/lit
27-3	50 mg/lit
27-4	80 mg/lit
27-5	100 mg/lit
27-6	120 mg/lit
27-7	140 mg/lit
27-8	180 mg/lit

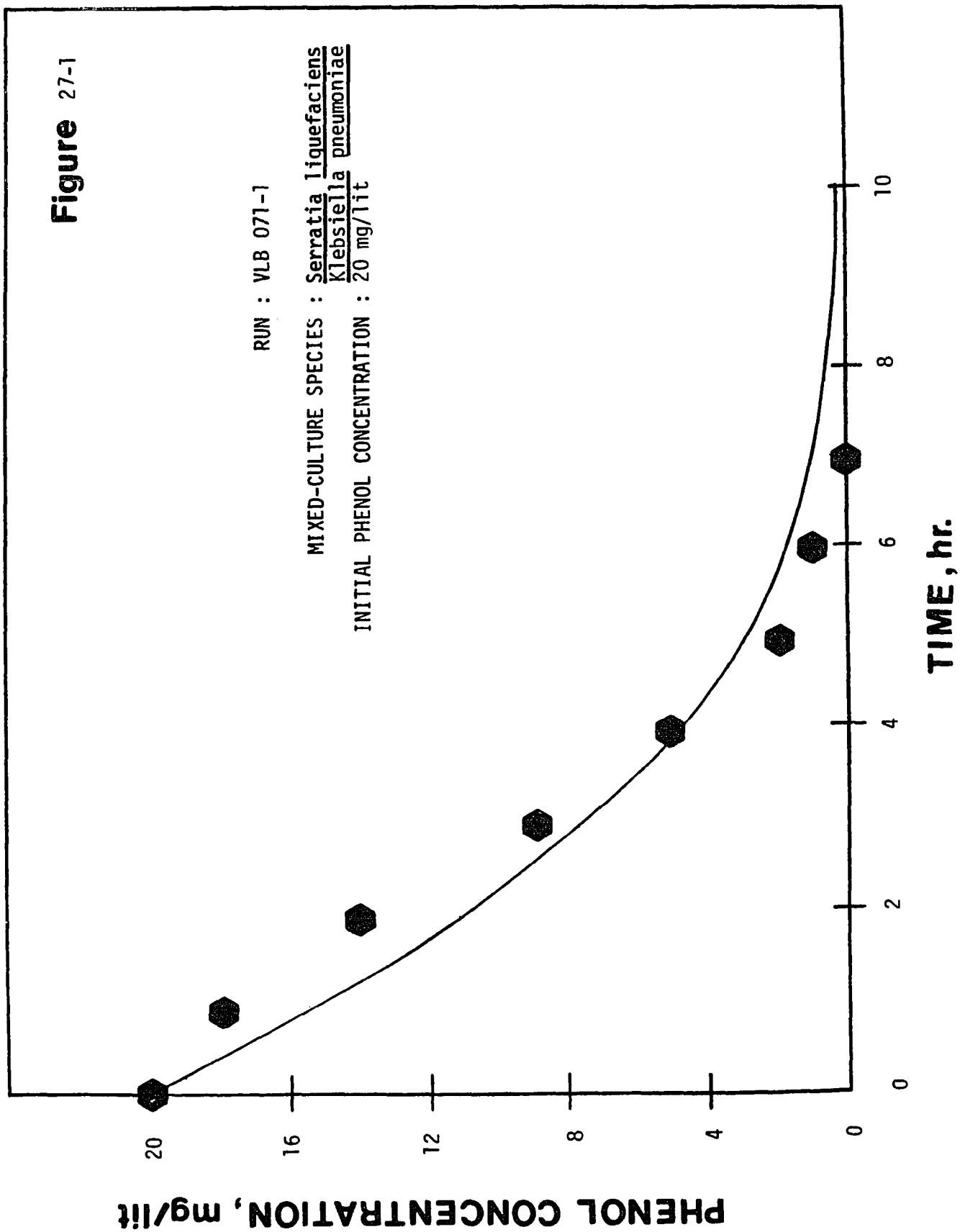


Figure 27-1
RUN : VLB 071-1
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 20 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
4
8
12
16
20
0
2
4
6
8
10

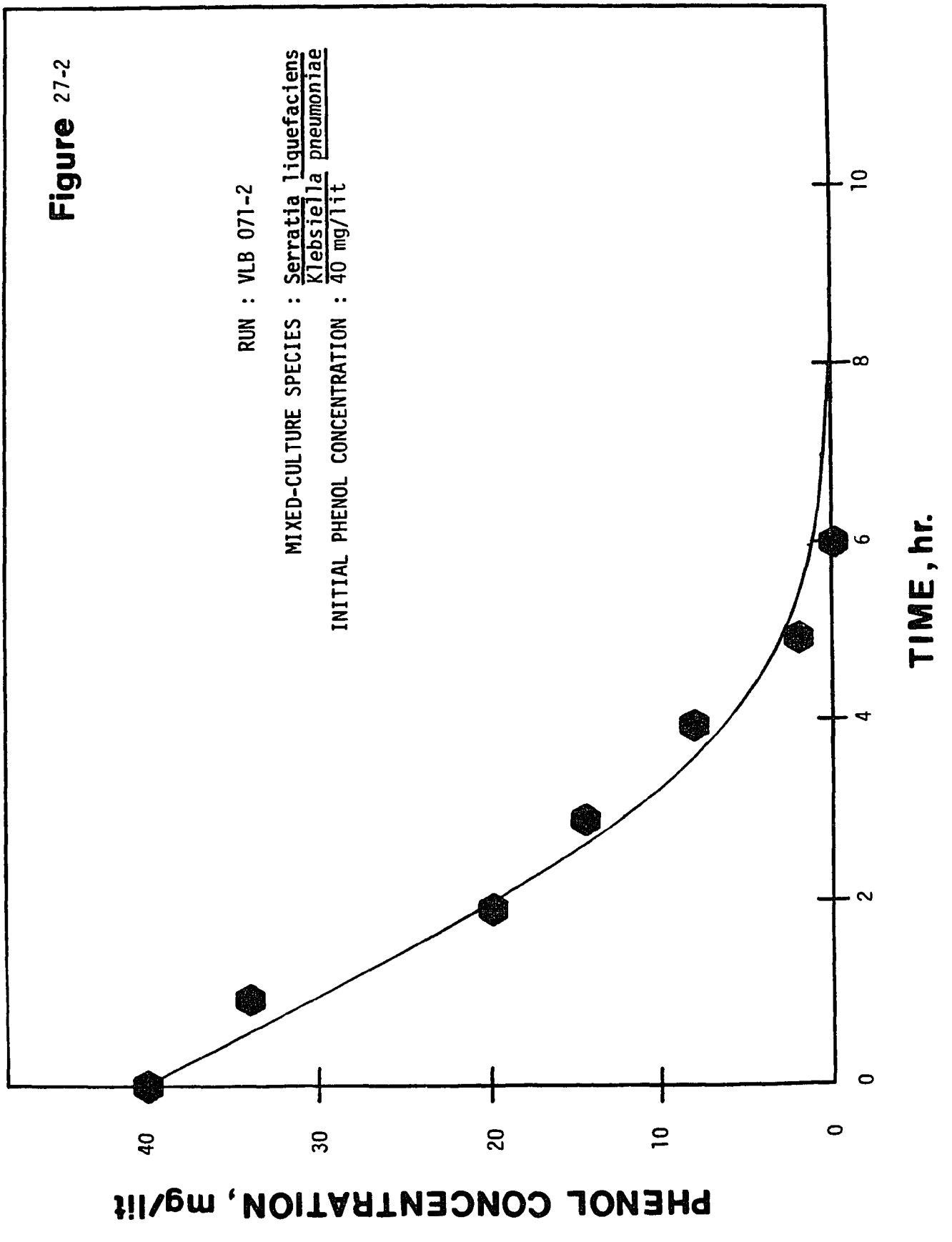


Figure 27-2
RUN : VLB 071-2
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 40 mg/lit
PHENOL CONCENTRATION, mg/lit
40
30
20
10
0
0
2
4
6
8
10
TIME, hr.

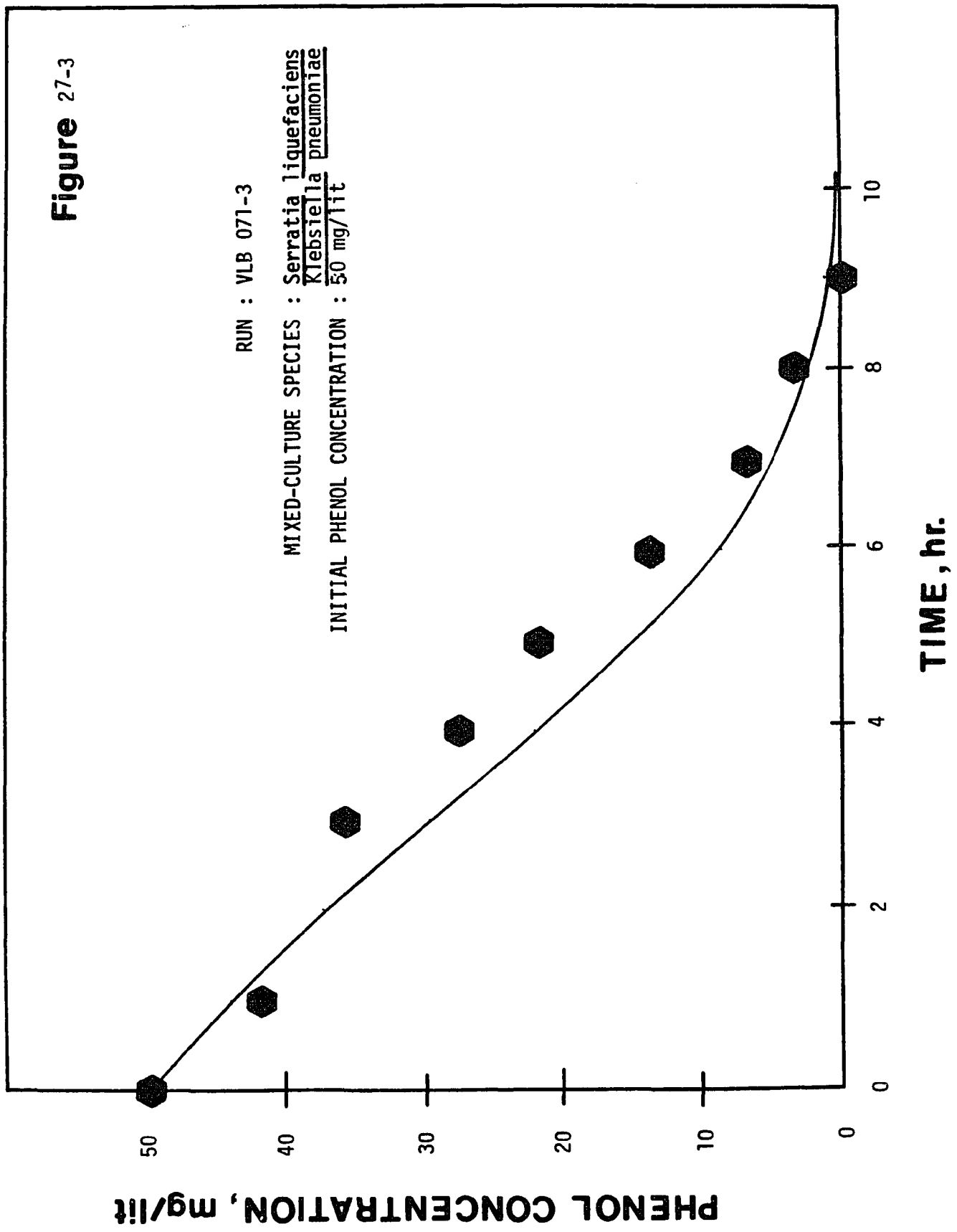


Figure 27-3
RUN : VLB 071-3
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 50 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
10
20
30
40
50
0
2
4
6
8
10

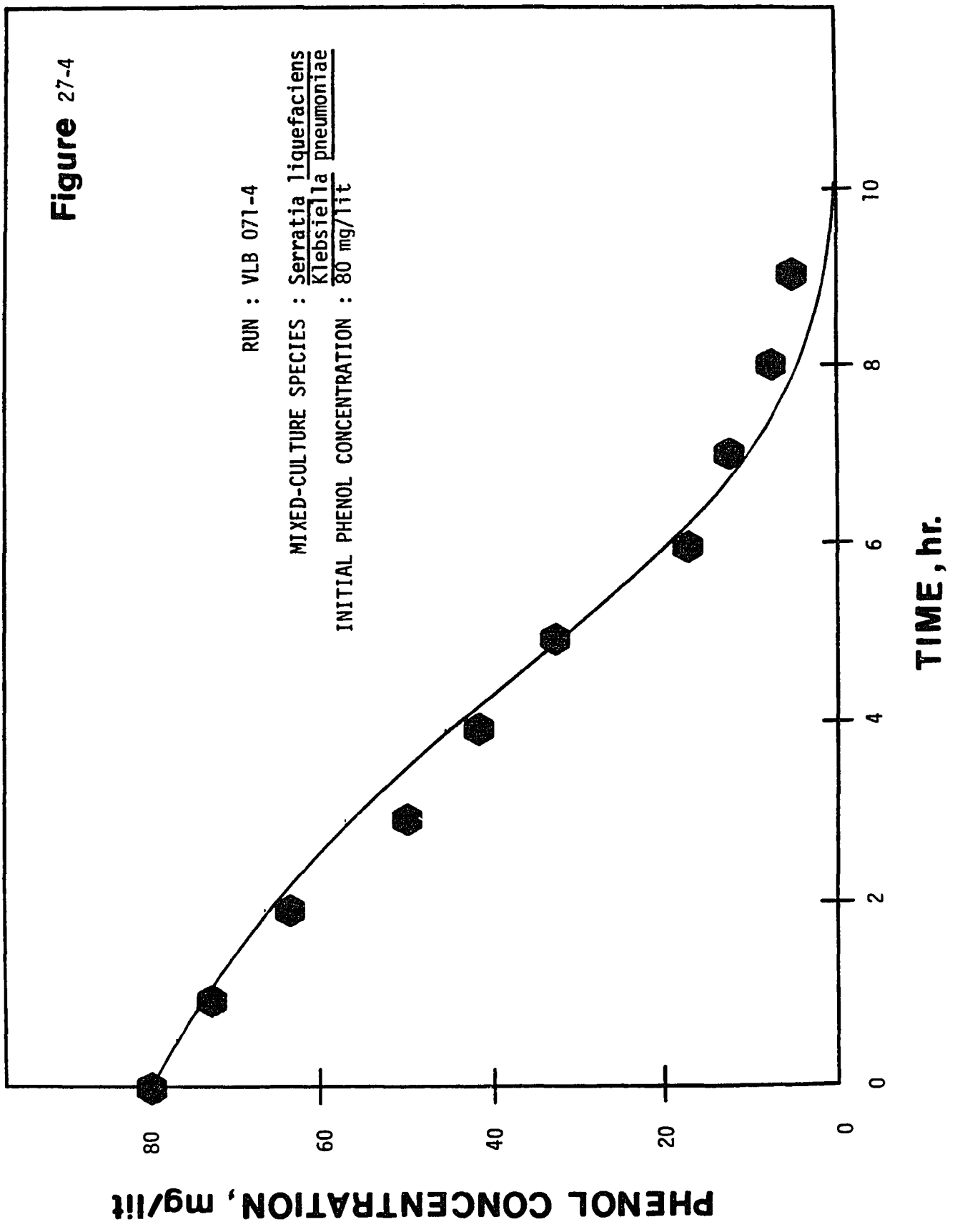


Figure 27-4
RUN : VLB 071-4
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 80 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
20
40
60
80
0
2
4
6
8
10

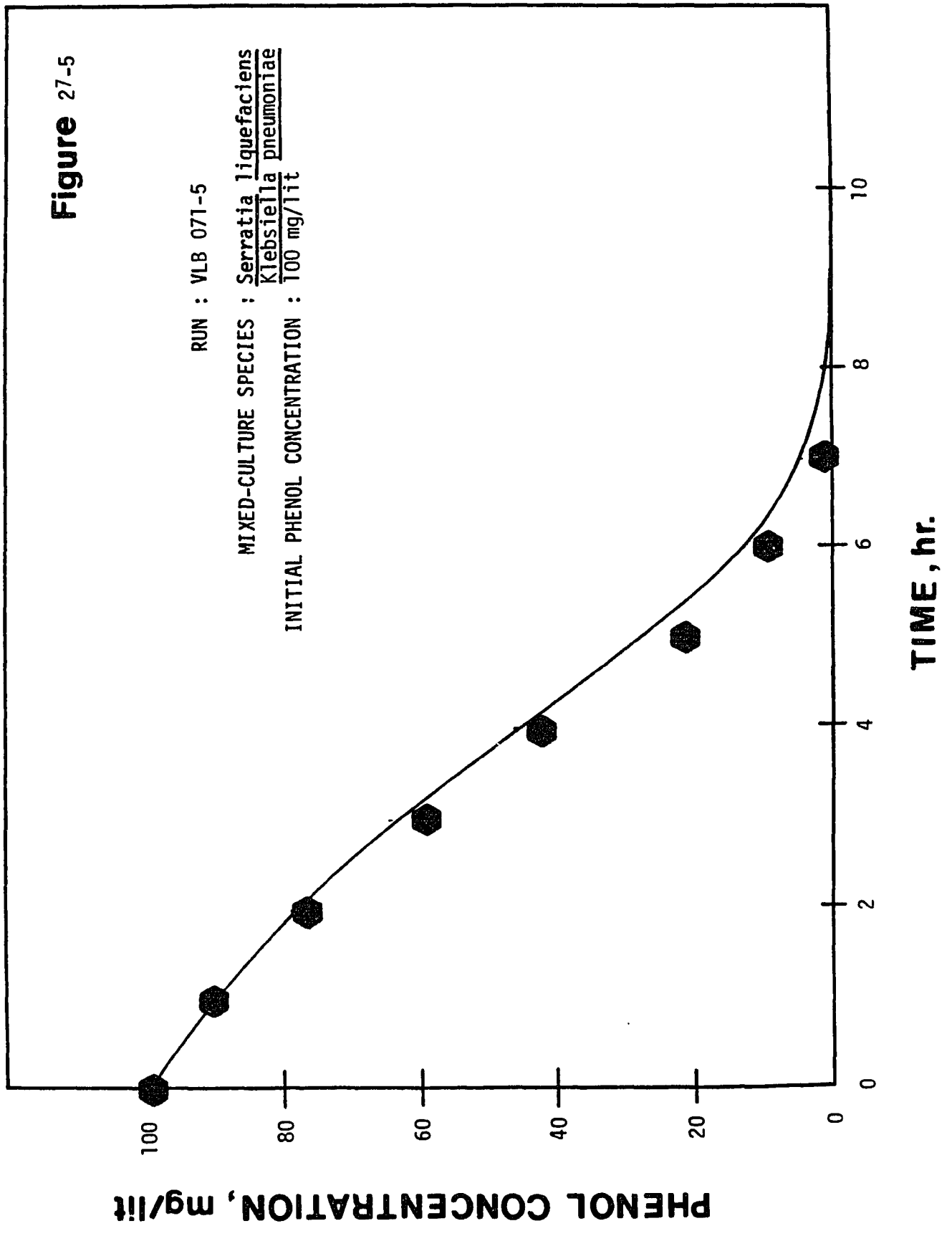


Figure 27-5
RUN : VLB 071-5
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 100 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
20
40
60
80
100
0
2
4
6
8
10

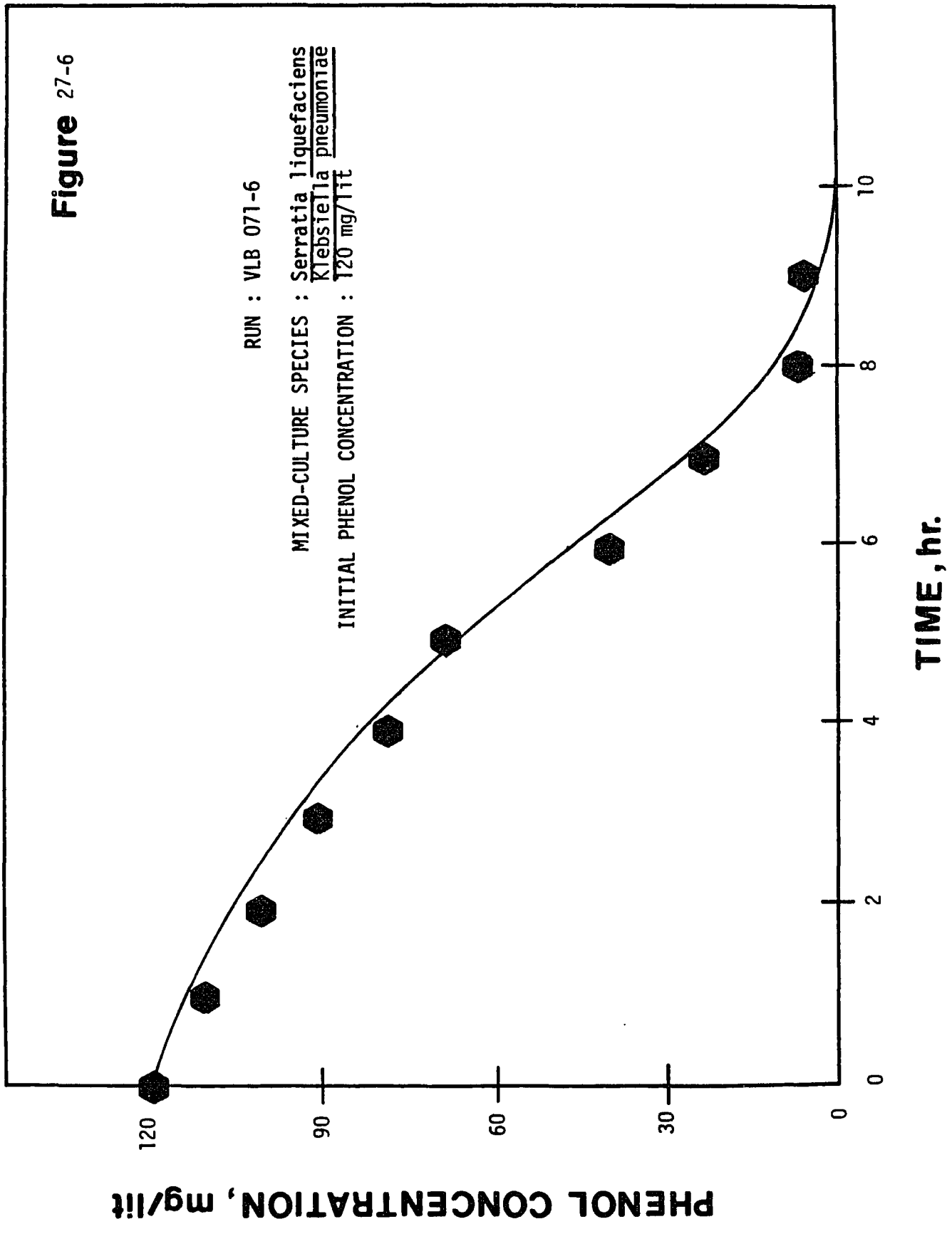


Figure 27-6
RUN : VLB 071-6
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 120 mg/lit
PHENOL CONCENTRATION, mg/lit
120
90
60
30
0
0
2
4
6
8
10
TIME, hr.

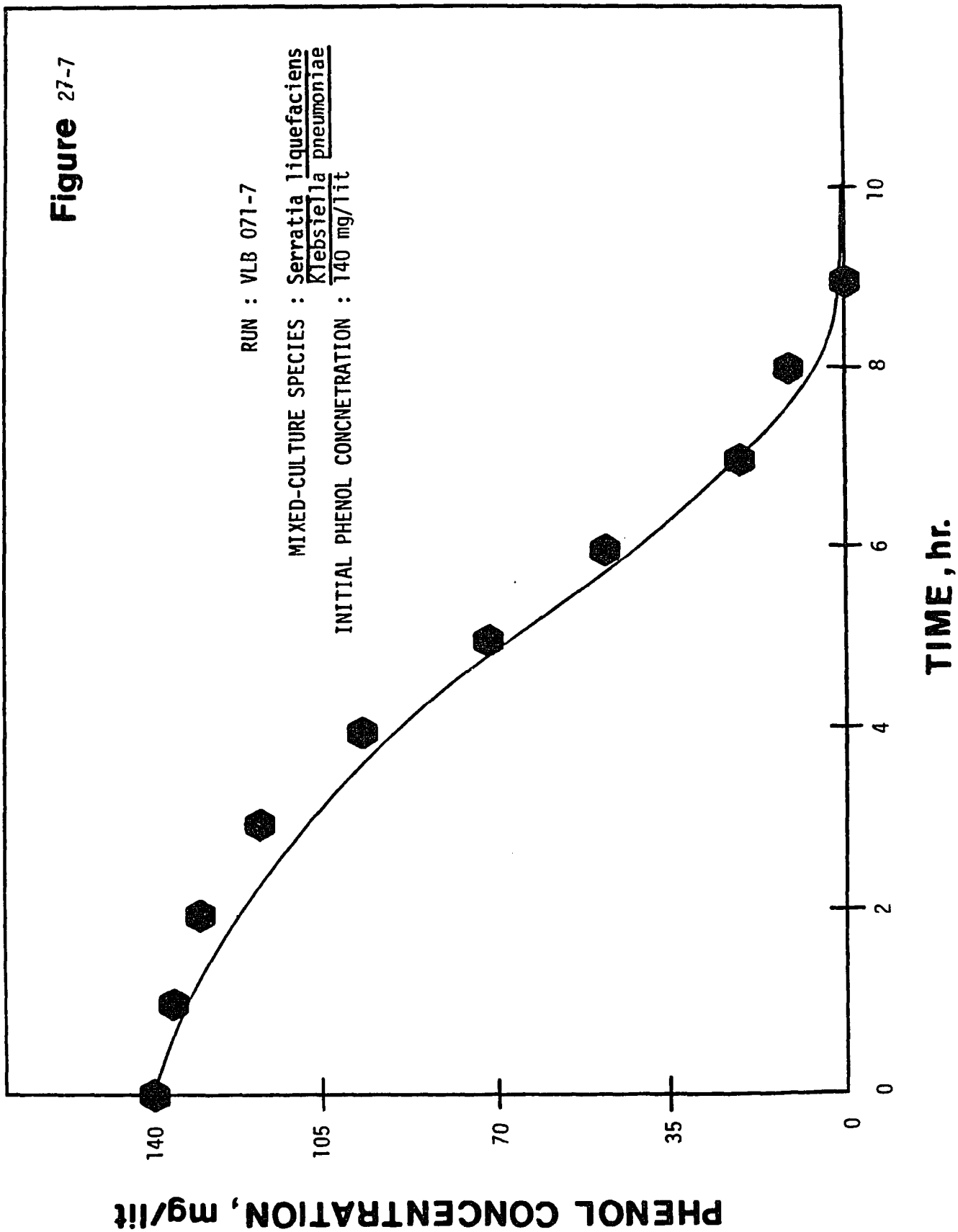


Figure 27-7
RUN : VLB 071-7
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCNETRATION : 140 mg/lit
PHENOL CONCENTRATION, mg/lit
140
105
70
35
0
0
2
4
6
8
10
TIME, hr.

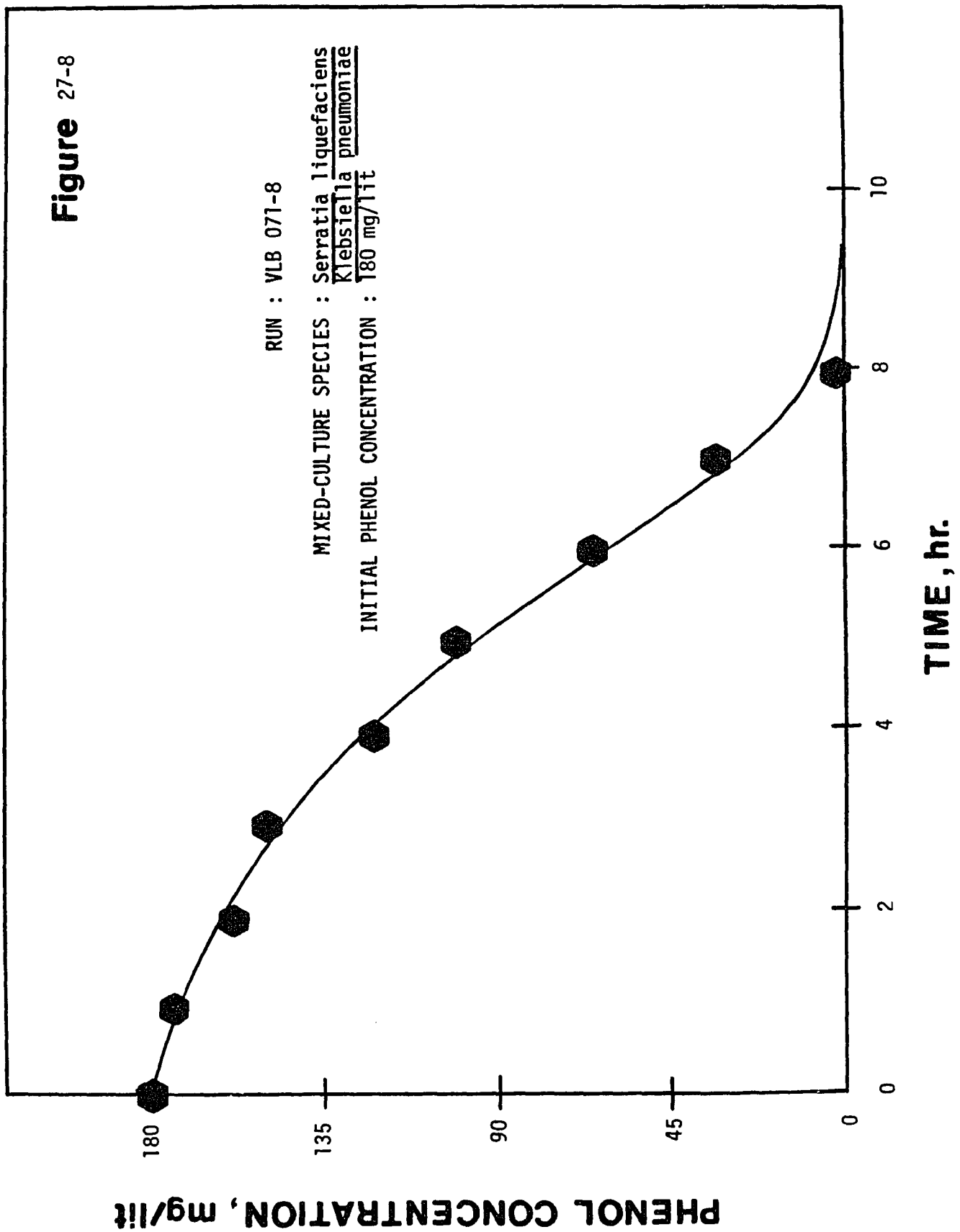


Figure 27-8
RUN : VLB 071-8
MIXED-CULTURE SPECIES : Serratia liquefaciens
Klebsiella pneumoniae
INITIAL PHENOL CONCENTRATION : 180 mg/lit
PHENOL CONCENTRATION, mg/lit
180
135
90
45
0
0
2
4
6
8
10
TIME, hr.

Figure 28

Phenol Degradation of the Mixed Culture
(*P. putida* and *S. liquefaciens*) at
the following Initial Phenol Concentrations
(Run VLB 072-1 to VLB 072-8)

28-1	20 mg/lit
28-2	40 mg/lit
28-3	50 mg/lit
28-4	80 mg/lit
28-5	100 mg/lit
28-6	120 mg/lit
28-7	140 mg/lit
28-8	180 mg/lit

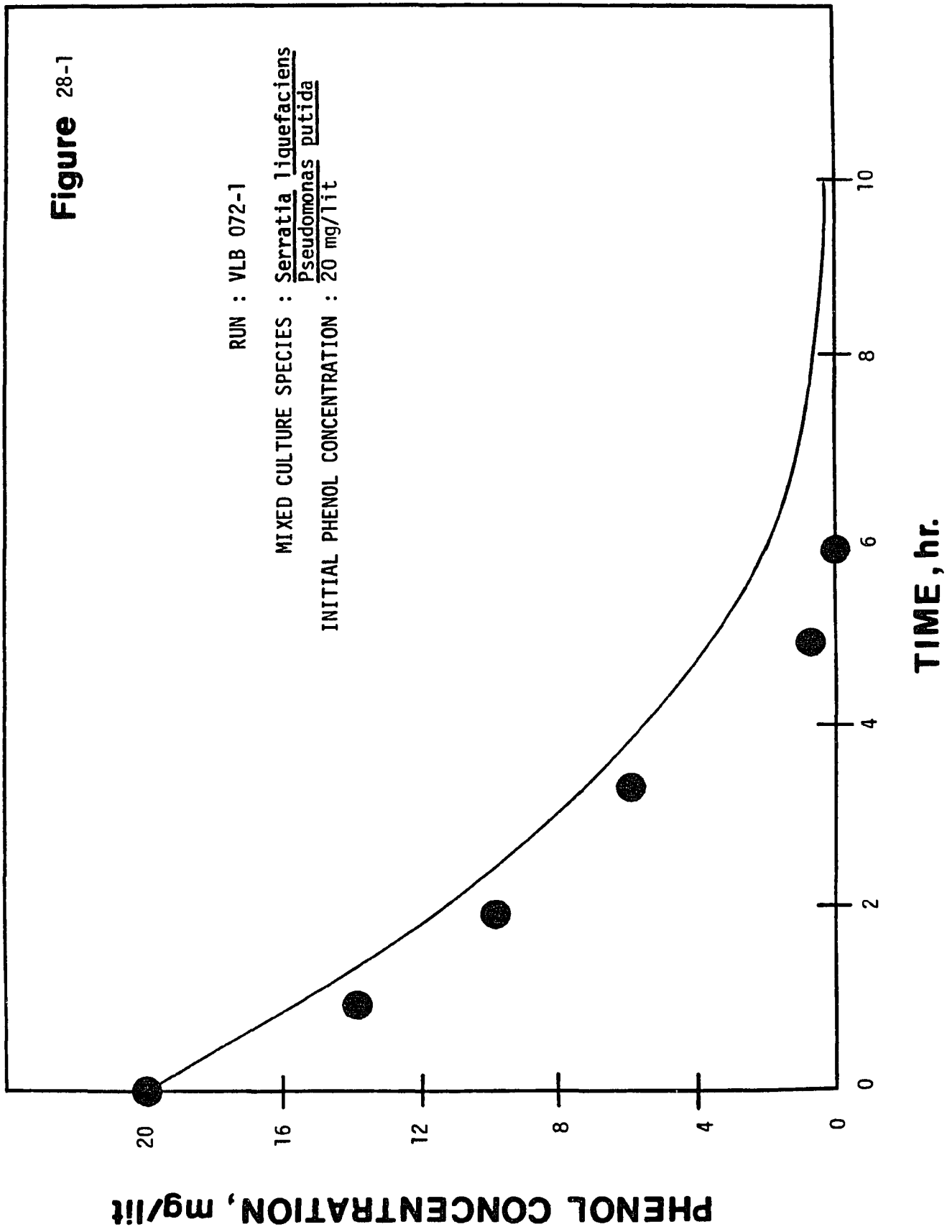


Figure 28-1
RUN : VLB 072-1
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 20 mg/lit
PHENOL CONCENTRATION, mg/lit
20
16
12
8
4
0
0
2
4
6
8
10
TIME, hr.

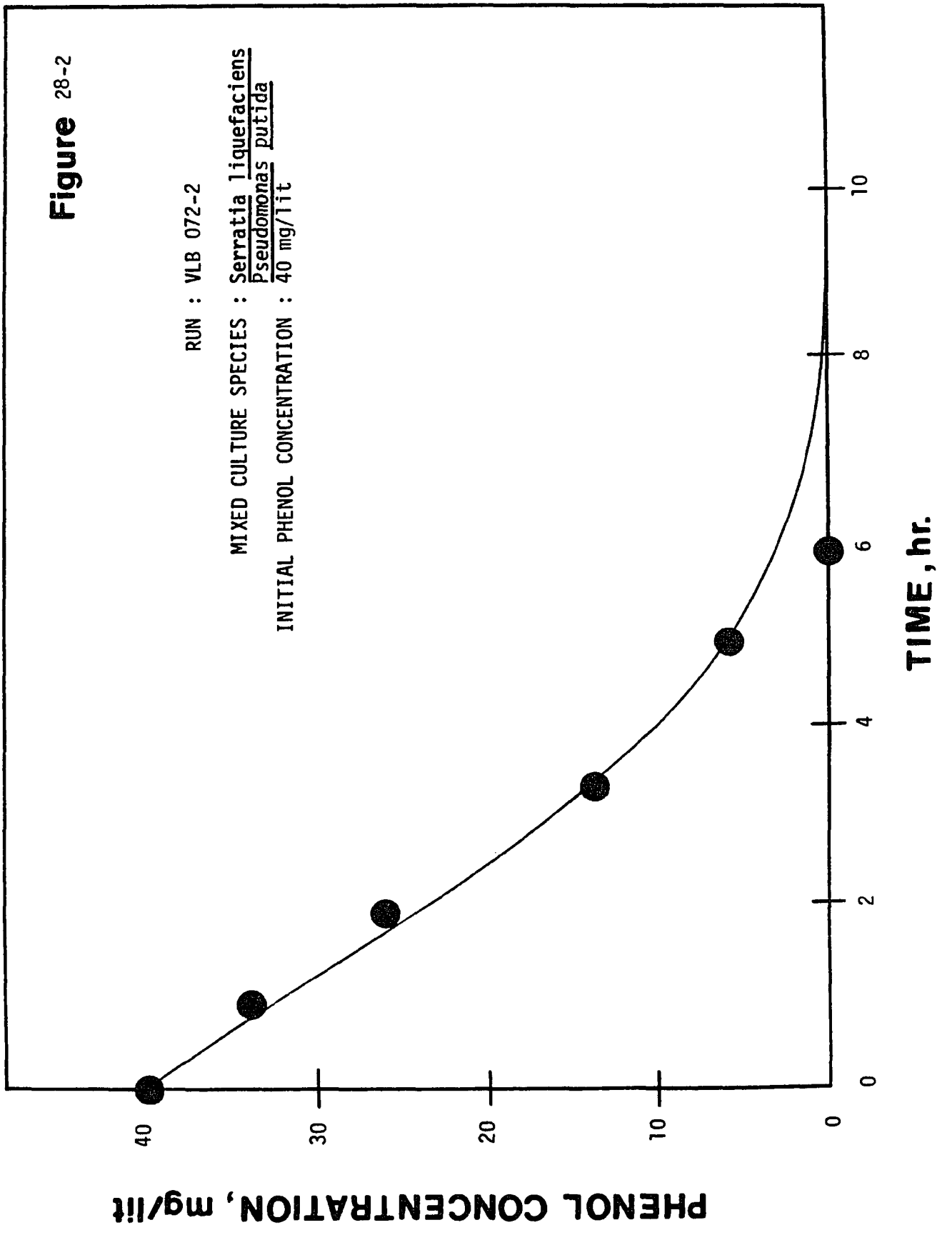


Figure 28-2
RUN : VLB 072-2
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 40 mg/lit
PHENOL CONCENTRATION, mg/lit
0
10
20
30
40
0
2
4
6
8
10
TIME, hr.

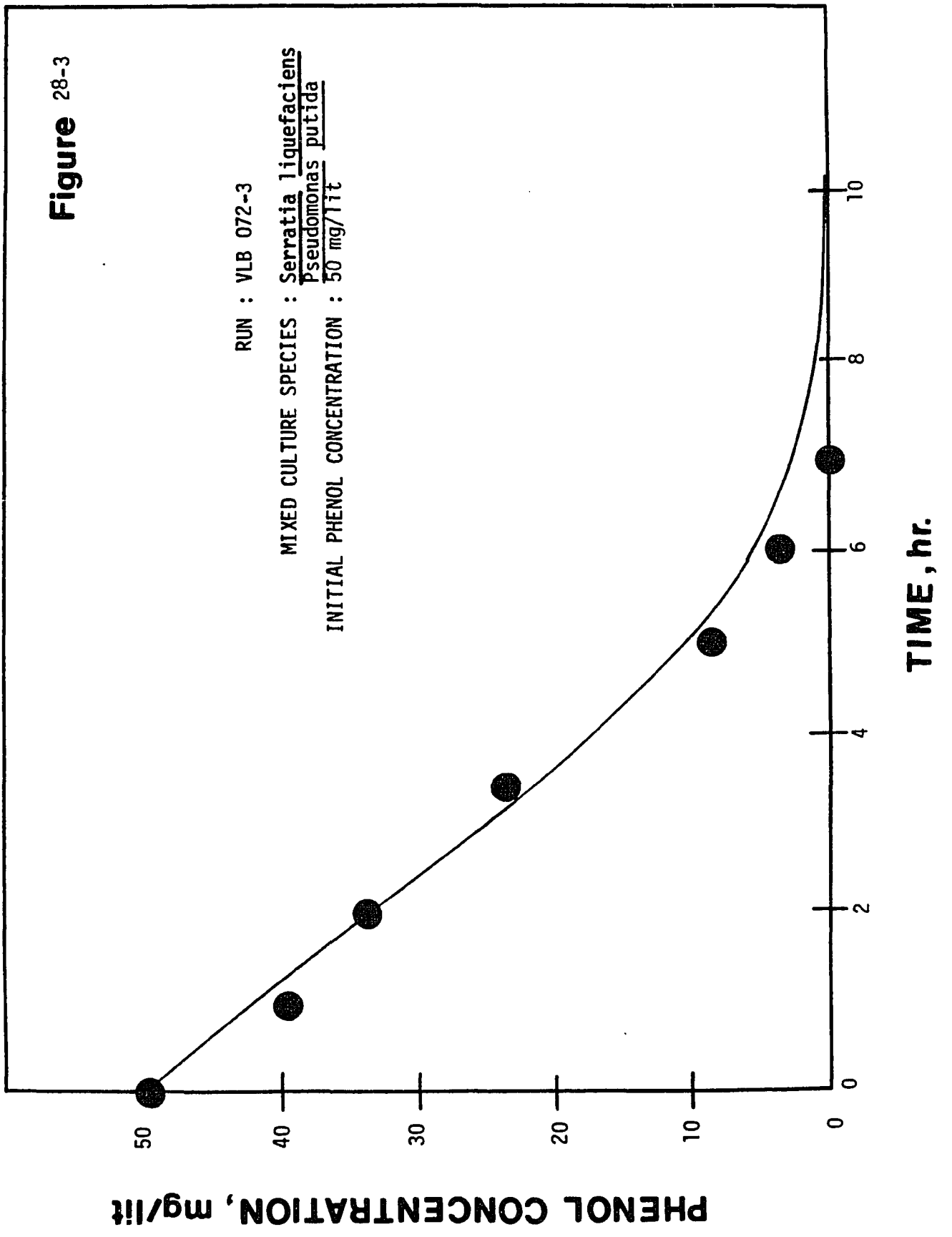


Figure 28-3
RUN : VLB 072-3
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 50 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
10
20
30
40
50
0
2
4
6
8
10

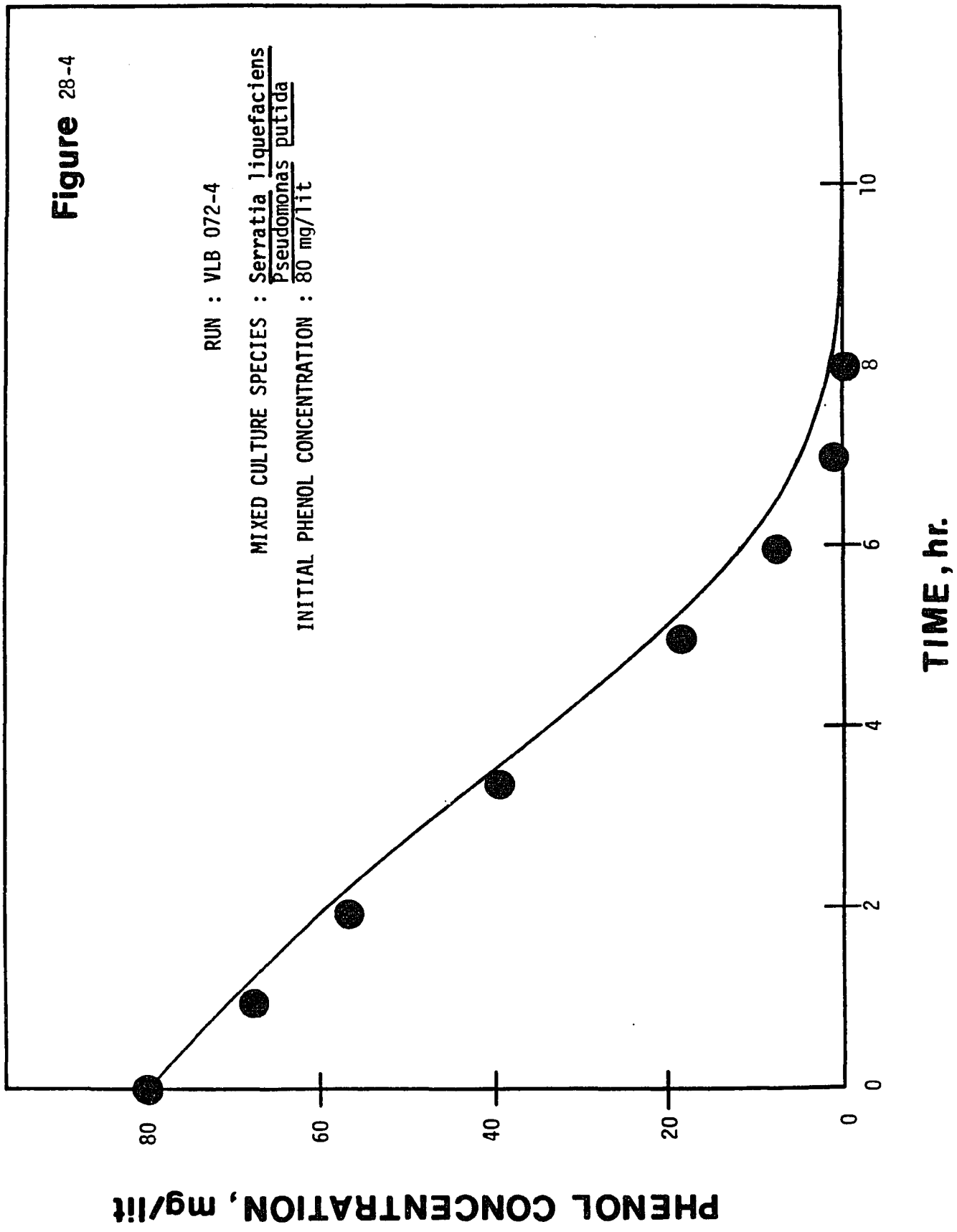


Figure 28-4
RUN : VLB 072-4
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 80 mg/lit
PHENOL CONCENTRATION, mg/lit
TIME, hr.
0
20
40
60
80
0
2
4
6
8
10

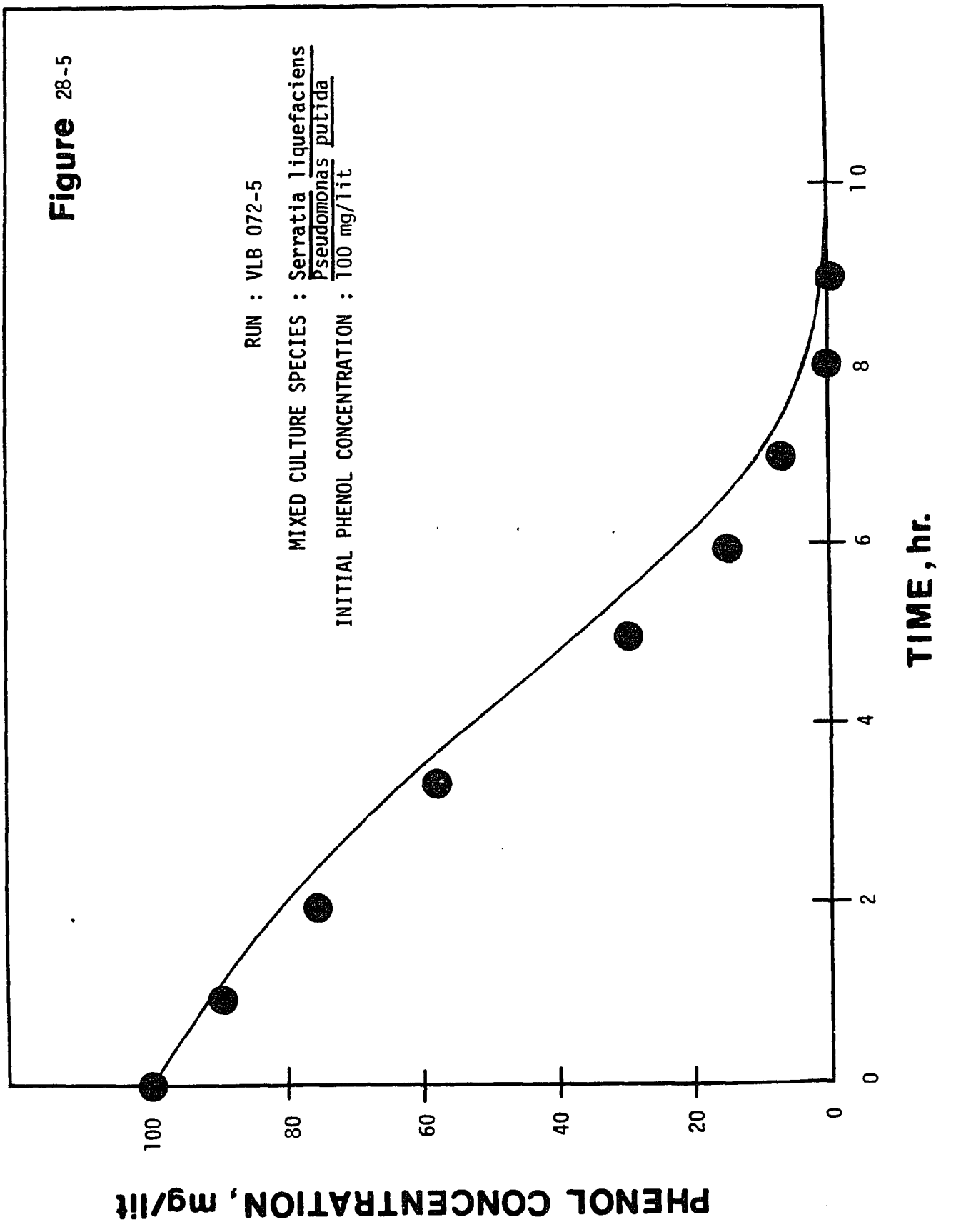


Figure 28-5
RUN : VLB 072-5
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 100 mg/lit
PHENOL CONCENTRATION, mg/lit
100
80
60
40
20
0
0
2
4
6
8
10
TIME, hr.

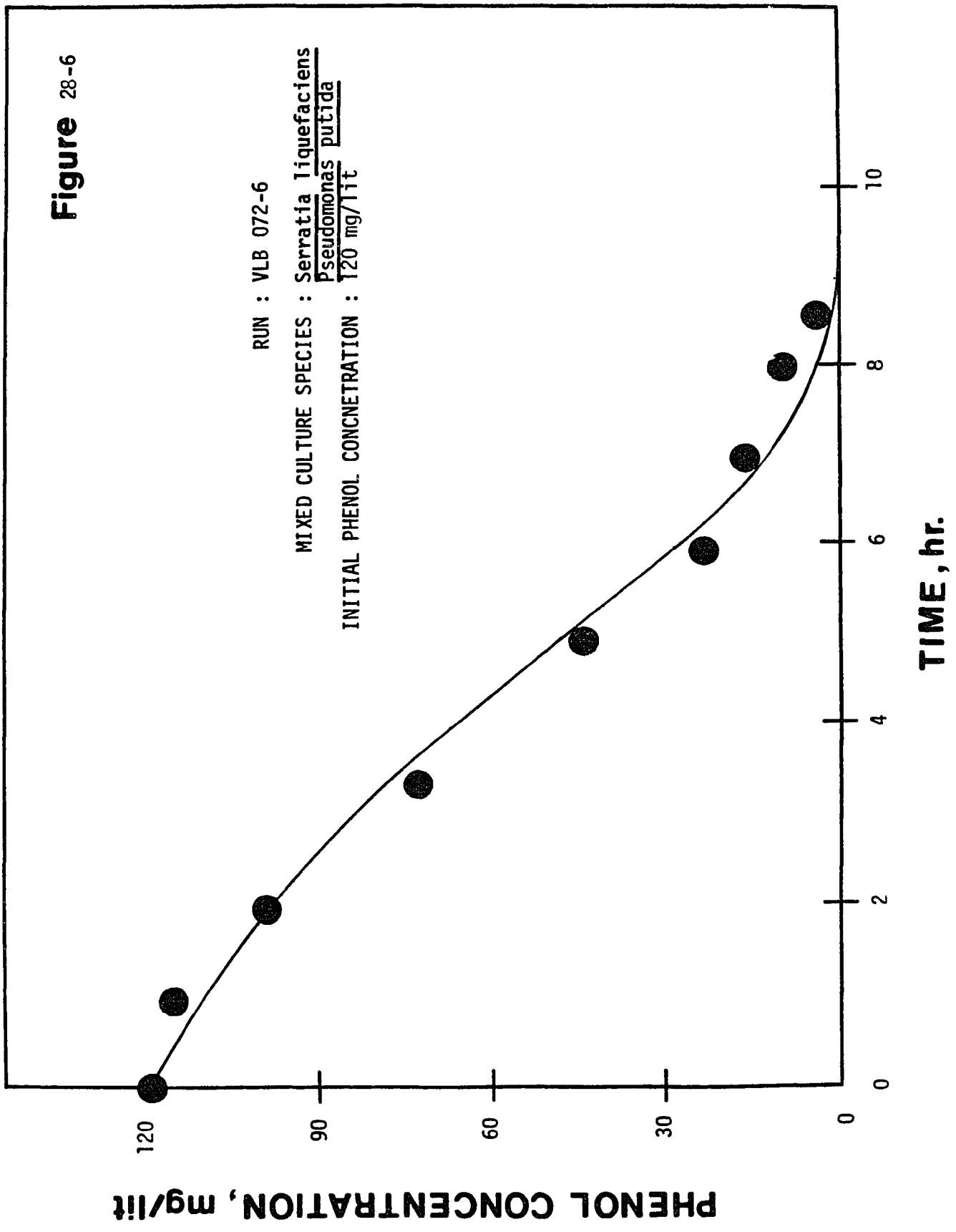


Figure 28-6
RUN : VLB 072-6
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCNETRATION : 120 mg/lit
PHENOL CONCENTRATION, mg/lit
120
90
60
30
0
0
2
4
6
8
10
TIME, hr.

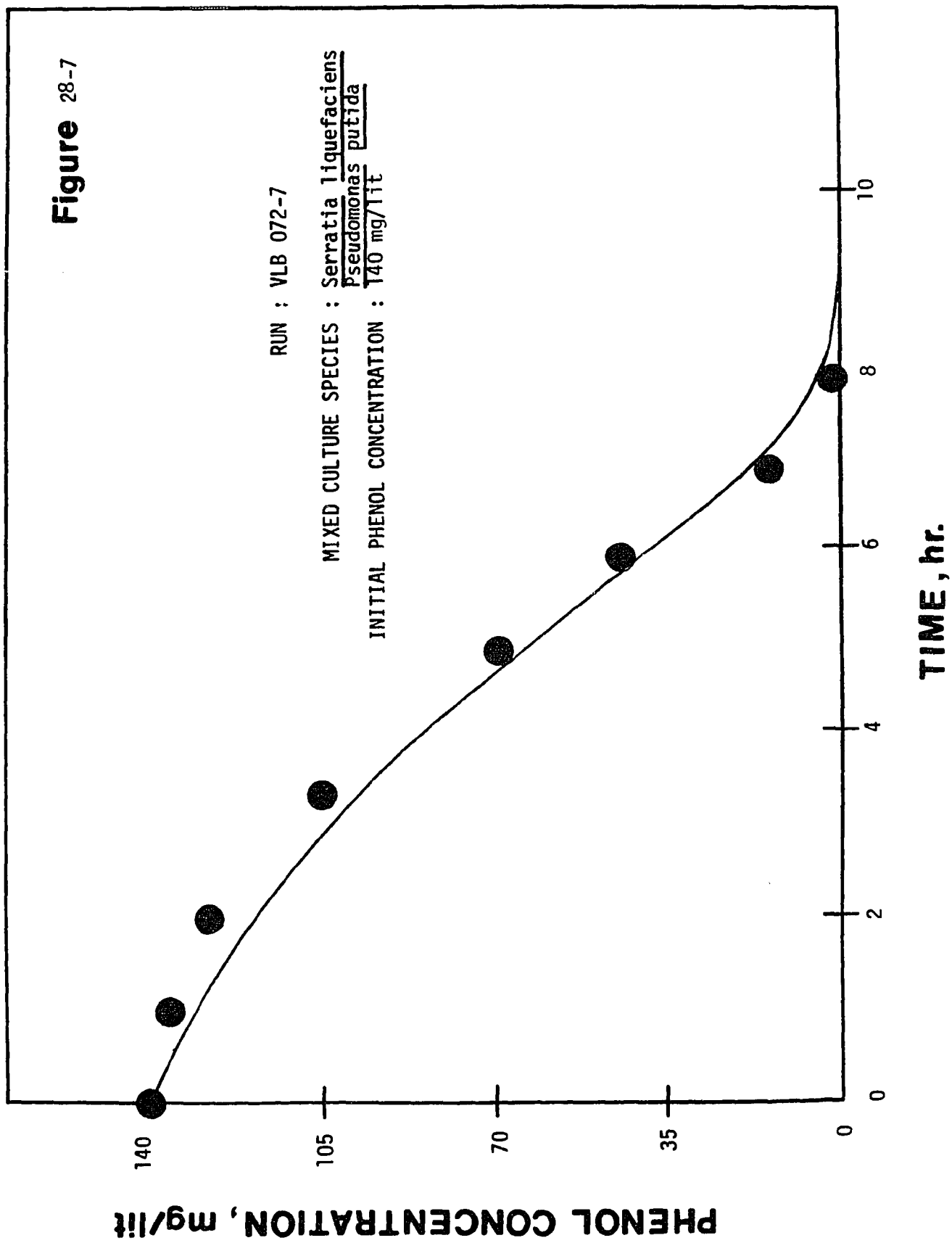


Figure 28-7
RUN : VLB 072-7
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 140 mg/lit
PHENOL CONCENTRATION, mg/lit
140
105
70
35
0
0
2
4
6
8
10
TIME, hr.

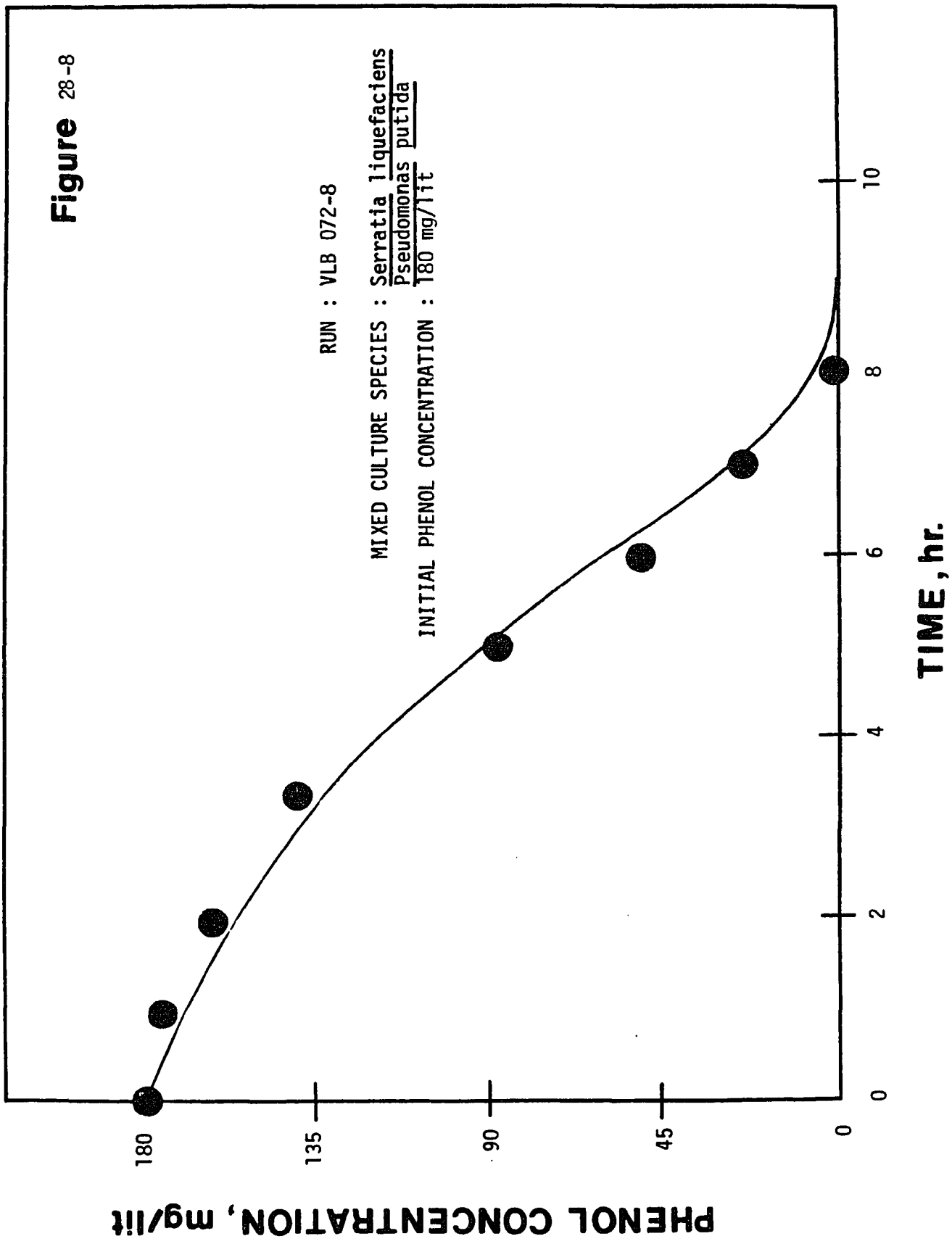


Figure 28-8
RUN : VLB 072-8
MIXED CULTURE SPECIES : Serratia liquefaciens
Pseudomonas putida
INITIAL PHENOL CONCENTRATION : 180 mg/lit
PHENOL CONCENTRATION, mg/lit
180
135
90
45
0
0
2
4
6
8
10
TIME, hr.

APPENDIX 1

NON-LINEAR LEAST SQUARES SUBROUTINE (20, 37)

Marquardt's method of obtaining a least squares fit to a set of data with a function in which the parameters are nonlinear has been implemented as a FORTRAN IV subroutine. The user specifies the function to be fitted to the data by writing a subroutine to evaluate the function. This subroutine may also evaluate the partial derivatives of the function with respect to the parameters, or the user may have the fitting program estimate the partials. Since NLLSQ is written as a subroutine having many options, the user is free to use it as a part of some larger calculation and to provide his own plotting section after the printed output, to compute the correlation matrix of the parameters, and perform a nonlinear confidence region calculation.

A. INTRODUCTION

Fitting a function containing adjustable parameters to a set of data so as to minimize the sum of the squares of the residuals is a simple problem if

the adjustable parameters enter the function linearly. If their occurance is nonlinear, however, the mathematical problem of finding the solution is considerably more difficult, and an iterative method must be used to converge on the correct parameter values. Marquardt has devised an iterative scheme for nonlinear least squares fitting which combines the advantages of the gradient and the Taylor series methods. At each step of the iteration a compromise is made between the parameter corrections predicted by the two methods. When the iterative process is in its early stage, the compromise criterion favors the gradient method, since the Taylor series method is likely to be unstable far from convergence. As the parameters get closet to their correct values, more use is made of the Taylor series method, and this avoids the very slow convergence of the gradient method in the neighborhood of the correct solution. The method also contains safeguards to maintain stability during the intermediate stages.

B. USE OF NLLSQ

NLLSQ is called by the user's program in a call statement:

CALL NLLSQ (Y, X, B, RRR, NARRAY, ARRAY, IB, FMT)

All of the arguments are arrays whose dimensions are specified in the user's program. When NLLSQ is called, these arrays must contain the specific information.

C. USE OF MODEL SUBROUTINE

The user specifies the function to be fitted to the data by supplying a subroutine named MODEL. THis subroutine is called sequentially for each of the N data points. and for each point it must evaluate the function and the residual using values of the parameters provided in a COMMON block. The MODEL subroutine should also compute values for the partial parametersm if it is possible to do this. If no partial derivative values are computed by MODEL, the program will estimate the value of the partials by evaluating the function at two values of the parameter separated by some incremental value.

The MODEL subroutine must have the following calling sequence:

```
CALL MODEL (F,Y,X,RRR,I,JP)
```

and must contain the common statement:

```
COMMON/BLK1/B(45),P(20),RE,N,M,K
```

```
C      XXXXXXXXXX NON-LINEAR REGRESSION PROGRAM XXXXXXXXX
C
C             THE PROGRAM HAS BEEN REVISED FOR THE
C                   SIMULATION OF THE DATA OF
C                    'SPECIFIC GROWTH RATE'
C                              AND
C               'INITIAL SUBSTRATE CONCENTRATION'
C
C      XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
C
      PROGRAM REGRES
      REAL PARAM(10),Y(250),X(250),A(8)
      INTEGER N,NA(8),IB(10)
      DIMENSION NAME(80),TITLE(80)
      NCRD=1
      NPRT=2
      READ(NCRD,*)NSETS
      DO 50 J=1,NSETS
      WRITE(NPRT,9000)
9000   FORMAT('1')
      READ(NCRD,901) (NAME(I),I=1,80)
      WRITE(NPRT,801) (NAME(I),I=1,80)
C      READ(NCRD,*)  PARAM(1)
      READ(NCRD,*)NP
      WRITE(NPRT,9010)NP
9010   FORMAT('0',5X,'The number of data points = ',G13.4)
      DO 110 I =1,NP
C           READ(NCRD,*) X(I),Y(I)
              READ(NCRD,*)Y(I),X(I)
C
C
C      Y(I)=SPECIFIC GROWTH RATE
C      X(I)=SUBSTRATE CONCENTRATION
110    CONTINUE
      PARAM(1)=2.
      PARAM(2)=30.
      NA(1)=NP
      NA(2)=1
      NA(3)=2
      NA(4)=0
      NA(5)=2
      NA(6)=1
      NA(7)=NPRT
      NA(8)=50
```

```
      DO 120 I=1,8
           A(I)=0.0
120   CONTINUE
      CALL NLLSQ(Y,X,PARAM,RRR,NA,A,IB)
50    CONTINUE
      WRITE(NPRT,1999)PARAM(1),PARAM(2)
 1999 FORMAT('0',7X,'PAR(1)=',F15.6,/,8X,'PAR(2)=',
     #F15.6)
      STOP
901   FORMAT(80A1)
902   FORMAT(5F10.5)
903   FORMAT(I2)
904   FORMAT(2F20.10)
909   FORMAT(F10.5)
801   FORMAT(' '80A1)
      END
```

APPENDIX 2

OUTPUT OF THE NON-LINEAR REGRESSION PROGRAM
(Data from Run VLB 051-B of Pseudomonas putida)

```
NO OF DATA POINTS IS   8   NO OF PARAMETERS IS  2   NO OF INDEPENDENT V
ARIABLES IS  1

DELTA= 0.99999990E-05   E= 0.50000000E-04   FF= 0.40000000E 01   GAMCR=
 0.60000000E 02
  T= 0.20000000E 01   TAU= 0.99999990E-03   ZETA= 0.10000000E-30   AL=
0.10000000E 00

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXX



NO OF ITERATIONS =  1

PHI = 0.69498150E-02    LAMBDA = 0.10000000E-01

PARAMETERS
   0.13770860E 00   0.32541060E 02

GAMMA = 0.40297710E 02    LENGTH OF DB = 0.35564710E 01

DB CORRECTION VECTOR
  -0.18622910E 01   0.25410620E 01


CORRELATION COEFFICIENTS


COLUMNS   1  THROUGH   2
   1.0000   0.9152
   0.9152   1.0000

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXX



NO OF ITERATIONS =  2

PHI = 0.85601970E-03    LAMBDA = 0.10000000E-02

PARAMETERS
   0.16117060E 00   0.17221600E 02

GAMMA = 0.67228620E 01    LENGTH OF DB = 0.58221510E-01

DB CORRECTION VECTOR
   0.23461950E-01  -0.15319440E 02


CORRELATION COEFFICIENTS


COLUMNS   1  THROUGH   2
   1.0000   0.8530
   0.8530   1.0000
```

```
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXXXX


NO OF ITERATIONS =  3

PHI = 0.51036240E-03    LAMBDA = 0.10000000E-03

PARAMETERS
   0.16519790E 00   0.23620370E 02

GAMMA = 0.54445290E 02    LENGTH OF DB = 0.28599060E-01

DB CORRECTION VECTOR
   0.40273850E-02   0.63987780E 01


CORRELATION COEFFICIENTS


COLUMNS    1  THROUGH   2
   1.0000   0.8866
   0.8866   1.0000

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXXXX


NO OF ITERATIONS =  4

PHI = 0.51029600E-03    LAMBDA = 0.99999990E-05

PARAMETERS
   0.16518360E 00   0.23928220E 02

GAMMA = 0.40157050E 02    LENGTH OF DB = 0.10684240E-02

DB CORRECTION VECTOR
  -0.14298490E-04   0.30785980E 00


CORRELATION COEFFICIENTS


COLUMNS    1  THROUGH   2
   1.0000   0.9025
   0.9025   1.0000
```

```
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX


NO OF ITERATIONS =  5

PHI = 0.50979240E-03    LAMBDA = 0.99999940E-06

PARAMETERS
   0.16453920E 00   0.23455640E 02

GAMMA = 0.48286590E 02    LENGTH OF DB = 0.80925630E-02

DB CORRECTION VECTOR
  -0.64441880E-03  -0.47257420E 00


CORRELATION COEFFICIENTS


COLUMNS   1  THROUGH   2
   1.0000   0.8985
   0.8985   1.0000

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX


NO OF ITERATIONS =  6

PHI = 0.50977450E-03    LAMBDA = 0.99999900E-07

PARAMETERS
   0.16469190E 00   0.23544280E 02

GAMMA = 0.22170130E 01    LENGTH OF DB = 0.35604360E-02

DB CORRECTION VECTOR
   0.15271160E-03   0.88651470E-01


CORRELATION COEFFICIENTS


COLUMNS   1  THROUGH   2
   1.0000   0.9044
   0.9044   1.0000
```

```
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXXXX


NO OF ITERATIONS =   7

PHI = 0.50977290E-03    LAMBDA = 0.99999890E-08

PARAMETERS
   0.16471900E 00   0.23559180E 02

GAMMA = 0.14168150E 02    LENGTH OF DB = 0.48477050E-02

DB CORRECTION VECTOR
   0.27162290E-04   0.14908110E-01


CORRELATION COEFFICIENTS


COLUMNS    1  THROUGH    2
   1.0000   0.8991
   0.8991   1.0000

CONVERGENCE BY GAMMA EPSILON TEST

CORRECTION VECTOR FOR LA[illegible] ITERATION WAS NOT USED




NO OF ITERATIONS =   8

PHI = 0.50977290E-03    LAMBDA = 0.99999890E-08

PARAMETERS
   0.16471900E 00   0.23559180E 02

GAMMA = 0.42023510E 02    LENGTH OF DB = 0.67293830E-03

DB CORRECTION VECTOR
   0.18886730E-05   0.94520390E-03


PTP MATRIX


COLUMNS    1  THROUGH    2
   0.42856660E 01  -0.69211340E-02
  -0.69211340E-02   0.13825890E-04


PTP CORRELATION COEFFICIENTS


COLUMNS    1  THROUGH    2
   1.0000  -0.8991
  -0.8991   1.0000
```

PTP INVERSE

COLUMNS 1 THROUGH 2
0.12180250E 01 0.60973430E 03
0.60973430E 03 0.37755640E 06

PARAMETER CORRELATION COEFFICIENTS

COLUMNS 1 THROUGH 2
1.0000 0.8991
0.8991 1.0000

	OBSERVED	PREDICTED	RESIDUAL
1	0.59999990E-01	0.75629940E-01	-0.15629950E-01
2	0.11199990E 00	0.10366330E 00	0.83366030E-02
3	0.12099990E 00	0.11196350E 00	0.90364210E-02
4	0.13200000E 00	0.12724620E 00	0.47537680E-02
5	0.13499990E 00	0.13331170E 00	0.16882410E-02
6	0.13999990E 00	0.13768730E 00	0.23126600E-02
7	0.13599990E 00	0.14099270E 00	-0.49927230E-02
8	0.13800000E 00	0.14565490E 00	-0.76549640E-02

AAD= 0.6801E-02BIAS= -0.2687E-03RMS= 0.6372E-04

The absolute maximum error = 0.1563E-01

The error sum of the square = 0.5098E-03

The variance = 0.8496E-04

The standard deviation = 0.8534E-02

SUPPORT PLANE PARA	STD ERROR	ONE - PARAMETER LOWER	UPPER	LOWER UPPER
1	0.10172800E-01	0.14437340E 00	0.18506460E 00	0.1359459
OE 00	0.19349200E 00			
2	0.56637420E 01	0.12231700E 02	0.34886670E 02	0.7539718
OE 01	0.39578650E 02			

APPENDIX 3

COMPUTER PROGRAM OF INTEGRATING ROUTINE (77)

The program is used for integrating a system of ordinary differential equations. It integrates by switching between a 4th order explicit Runge-Kutta and a 3rd order semi-implicit Michelsen integrating routine. This program is ideal for locally stiff and/or numerically unstable systems. The relative merits of the explicit routine over the semi-implicit one are speed and accuracy. However, the latter routine is unconditionally stable. Computation time is minimized by integrating implicitly in the locality of numerical instability.

The routine is capable of integrating an ODE system with a maximum of nine equations if the system is non-autonomous and of ten equations if autonomous.

The program has been configured in such a way that it contains the part of the operating code that remains unchanged. The file [SYSEQ.FOR] which contains all the necessary code that is characteristic to the ODE system that is to be integrated.

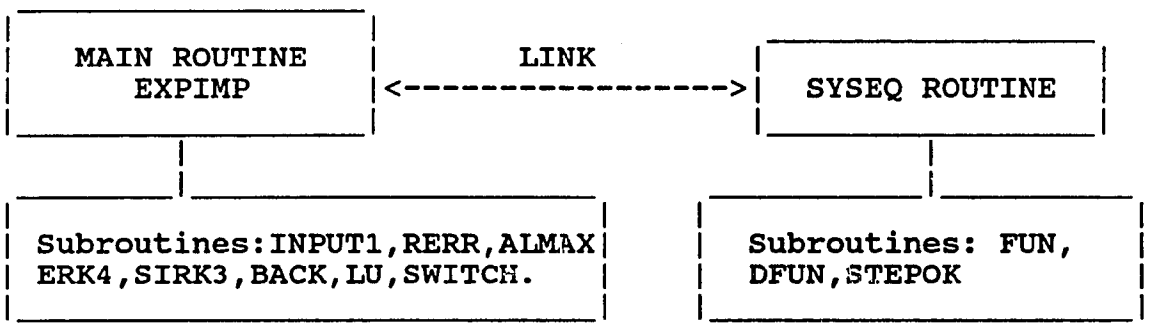
A datafile with filename [INPUT] should be created if data will not be enter interactively (from a terminal). The data should be entered in **FREE FORMAT.**

A. DESCRIPTION OF SUBROUTINES

-SUBROUTINE-	-UTILITY-
FUNCTION RERR	: Calculates the maximum error per iterating step and determines whether it is within specified tolerance.
FUNCTION ALMAX	: Estimates the maximum eigenvalue of the system for stability purposes.
ERK4	: Explicit Fourth Order Runge-Kutta.
SIRK3	: Third Order Semi-Implicit Runge-Kutta (Michelsen)
BACK	: Used for back substitution after LU decomposition
LU	: Used for L-U decomposition of Jacobian
*** Subroutines	BACK and LU are used by SIRK3 ***
SWITCH	: Switching Algorithm that controls decision making as to which integrating method should be used.
FUN	: Contains system equations F(i)
DFUN	: Contains Jacobian of system DF(i,j)
INPUT1	: Used to input initial conditions SY(i)
STEPOK	: Output routine used after successful iteration to print integration information at specified NTABs

Subroutines FUN,DFUN,STEPOK are created in separate file. [SYSEQ.FOR] by the user since they are system specific.

B. BLOCK DIAGRAM OF CODE:



```
*************************************************************
*                      ### EXPIMP ###                       *
*                                                           *
*     PROGRAM TO SOLVE A SYSTEM OF O.D.E.s BY SWITCHING     *
*     BETWEEN MICHELSEN'S METHOD AND 4TH ORDER EXPLICIT     *
*     RUNGE KUTTA METHOD ACCORDING TO THE STIFFNESS OF      *
*                       THE SYSTEM.                         *
*                                                           *
*************************************************************
      IMPLICIT REAL*8(A-H,O-Z)
      DIMENSION SY(10)
      COMMON ID/BLOCK1/A(10,10)/BLOCK2/B(10,10)/
     *BLOCK3/C(10,10)
      COMMON /MISES/ ITER
      COMMON /BLOCK5/HMIN,NOK,XOK,XOK1,MOVE,IAM/
     *BLOCK7/PAR(20)
C IM=2 USE ERK4
C IM=3 USE SIRKS
C SY(I) INITIAL VALUE OF Y(I)
C TOL TOLERANCE
C HO INITIAL STEP SIZE
C NFAIL NO. OF FAILURE
C TIME=TIME INTERVAL
C NEQ=NO. EQUATIONS
      OPEN(2,FILE='INPUT')
      OPEN(3,FILE='OUTPUT',STATUS='NEW')
C  ........
C          DATA INPUT
C                      ............
      WRITE(*,743)
 743  FORMAT(/2X,'HELLO!!!  Welcome to Expimp. I hope you
     *have a most'/12X,'fascinating integrated trip on
     *thevergeof numerical stability.'/12X,
     *'FASTEN SWITCHBELTS -- STANDBY FOR TAKE-OFF')
      WRITE(*,601)
 601  FORMAT(/2X,'*** SUPPLY IREAD: 1-INPUT FROM TERMINAL'
     */21X,'2-INPUT FILE [INPUT]')
 320  READ(*,*)IREAD
      IF(IREAD.NE.1.AND.IREAD.NE.2)THEN
      WRITE(*,501)
 501  FORMAT(/2X,'*** IMPROPER IREAD SUPPLIED -
     *TRY AGAIN :')
      GOTO 320
      ENDIF
      GOTO (101,102),IREAD
 101  WRITE(*,602)
 602  FORMAT(/2X,'* NUMBER OF EQUATIONS OF NON-AUTONOMOUS:')
  READ(*,*)NEQ1
  WRITE(*,603)
 603  FORMAT(/2X,'*** SUPPLY ID AND IM ....')
```

```
      WRITE(*,604)
604   FORMAT(/2X,'* ID: 1-NON-AUTONOMOUS, 0-AUTONOMOUS
     *SYSTEM'/2X,'* IM: 2-START WITH EXPLICIT R-K,
     *3-START WITH IMPLICIT R-K')
  READ(*,*)ID,IM
321   CONTINUE
  IF(ID.NE.0.AND.ID.NE.1)THEN
  WRITE(*,502)
502   FORMAT(/2X,'*** IMPROPER ID SUPPLIED - TRY AGAIN...')
  READ(*,*)ID
  GOTO 321
  ENDIF
322   CONTINUE
  IF(IM.NE.2.AND.IM.NE.3)THEN
  WRITE(*,503)
503   FORMAT(/2X,'*** IMPROPER IM SUPPLIED - TRY AGAIN...')
  READ(*,*)IM
  GOTO 322
  ENDIF
  WRITE(*,*)'*** ENTER NUMBER OF SYSTEM PARAMETERS, NPN:'
  READ(*,*)NPN
  WRITE(*,605)
605   FORMAT(/2X,'*** RELATIVE TOLERANCE AND INITIAL
     *STEP-SIZE:')
  READ(*,*)TOL,HO
  WRITE(*,606)
606   FORMAT(/2X,'*** INITIAL, TERMINAL TIMES, AND NTAB:')
       WRITE(*,616)
616   FORMAT(/2X,'*** [NTAB IS THE NUBER OF TIME
     *SUB-INTERVALS]')
  READ(*,*)XST,TIME,NTAB
  WRITE(*,607)
607   FORMAT(/2X,'*** NUMBER OF ITERATIONS IN POWER METHOD:')
  READ(*,*)ITER
  WRITE(*,609)
609   FORMAT(/2X,'*** SUPPLY MINIMUM INTEGRATING STEP-SIZE:')
  READ(*,*)HMIN
  WRITE(*,610)
610   FORMAT(/2X,'*** NUMBER OF ALLOWABLE SUCCESSFUL SIRK3
     *ITERATIONS')
  READ(*,*)NOK
  WRITE(*,611)
611   FORMAT(/2X,'*** RATIO OF STABLE STEP-SIZE TO IMPLICIT
     *INTEGRATING STEP [XOK]'/2X,'AND DESIRED FRACTION OF
     *STABLE STEP TO BE USED IN EXPLICIT'/2X,'INTEGRATION
     *[XOK1], UPON RETURN FROM SIRK3.')
  READ(*,*)XOK,XOK1
  WRITE(*,612)
612   FORMAT(/2X,'*** MAXIMUM UNSUCCESFUL SIRK3 ITERATIONS
     8[MOVE],'/7X,'PRIOR TO RETURN TO EXPLICIT INTEGRATION ?')
```

```
   READ(*,*)MOVE
   WRITE(*,613)
 613  FORMAT(/2X,'IN CASE HMIN HAS TO BE ADJUSTED, WILL RUN
     *BE UNATTENDED ?'/8X,' 1-YES, 0-NO')
   READ(*,*)IAM
   WRITE(*,*)'*** ENTER SYSTEM PARAMETERS :'
   DO 879 I=1,NPN
 879  READ(2,*)PAR(I)
   GOTO 111
C  .........
C       Reading data from file INPUT if non-interactive mode
C       is used.
C
            ..........
 102  READ(2,*)NEQ1
   READ(2,*)ID,IM
   READ(2,*)NPN
   READ(2,*)TOL,HO
   READ(2,*)XST,TIME,NTAB
   READ(2,*)ITER
   READ(2,*)HMIN
   READ(2,*)NOK
   READ(2,*)XOK,XOK1
   READ(2,*)MOVE
   READ(2,*)IAM
   DO 798 I=1,NPN
 798  READ(2,*)PAR(I)
C
C ****** WARNING: If program will run UNATTENDED and input
C         is from data-file then DO NOT FORGET to add IDEC
C         at the end of the datafile. ******
C
 111  CONTINUE
C
C *** This routine sets the program execution after
C     receiving initial values of variables from the
C     input subroutine...
C
   CALL INPUT1(NEQ1,SY,IREAD)
C
C       If system is non-autonomous convert it to autonomous
C
   IF(ID.EQ.1)THEN
   NEQ=NEQ1+1
   SY(NEQ)=XST
   ELSE
   NEQ=NEQ1
   ENDIF
```

```
C  .........
C              Printing Output heading and initial values
C .........
   WRITE(3,151)
 151   FORMAT(/15X,'*** SWITCHING ALGORITHM ***')
   WRITE(3,6001)NEQ1,ID,IM
 6001 FORMAT(/5X,'NUMBER OF EQUATIONS:',I3,2X,'ID=',
     *I2,2X,'IM=',I2)
   WRITE(3,6002)ITER
 6002 FORMAT(/5X,'NUMBER OF ITERATIONS IN POWER METHOD:',I3)
   WRITE(3,6003)TOL,HO
 6003 FORMAT(/5X,'RELATIVE TOLERANCE:',D12.4/2X,
     *'INITIAL STEP SIZE:',&D12.4)
   WRITE(3,100)
   WRITE(3,200)XST,IM,HO,SY(1)
   DO 15 I=2,NEQ1
  15   WRITE(3,300)SY(I)
 100   FORMAT(/6X,'TIME',2X,'IM',2X,'NF',4X,' H ',5X,
     *'VARIABLES....')
 200   FORMAT(/1X,F9.4,2X,I2,6X,F8.4,2X,D12.5)
 300   FORMAT(/30X,D12.5)
   WRITE(3,876)
 876   FORMAT(//20X,'##### INTEGRATION BEGINS #####'/)
C
C       Integrating begins with output generated at
C       specified tabs.
C
   XTAB=TIME/FLOAT(NTAB)
   X1=XST
   DO 10 I=1,NTAB
   IF(HO.GT.XTAB)HO=XTAB
   X2=X1+XTAB
   CALL SWITCH(NEQ,NEQ1,X1,X2,HO,SY,IM,TOL,IWHAT)
   IF(IWHAT.EQ.1)GOTO 30
  10   X1=X2
  30   CONTINUE
   STOP
   END
```

APPENDIX 4

RESULTS OF THE INTEGRATION PROGRAM

*** SWITCHING ALGORITHM ***

NUMBER OF EQUATIONS: 3 ID= 0 IM= 3

NUMBER OF ITERATIONS IN POWER METHOD: 50

RELATIVE TOLERANCE: .1000D-04
INITIAL STEP SIZE: .1000D+00

TIME	IM	NF	H	VARIABLES....
.0000	3		.1000	.80000D+01
				.80000D+01
				.12000D+03

INTEGRATION BEGINS

.0000	3	0	.100000	.16000D+02	.12000D+03
*** Switch to EXP4. Unsuccessful SIRK3. Accuracy problem. ***					
.4000	2	0	.300000	.18223D+02	.11602D+03
.8000	2	0	.400000	.20731D+02	.11154D+03
1.2000	2	0	.400000	.23554D+02	.10651D+03
1.6000	2	0	.400000	.26718D+02	.10089D+03
2.0000	2	0	.400000	.30247D+02	.94642D+02
2.4000	2	0	.400000	.34159D+02	.87734D+02
2.8000	2	0	.400000	.38460D+02	.80160D+02
3.2000	2	0	.400000	.43139D+02	.71942D+02
3.6000	2	0	.400000	.48160D+02	.63147D+02
4.0000	2	0	.400000	.53448D+02	.53911D+02
4.4000	2	0	.400000	.58875D+02	.44456D+02
4.8000	2	0	.400000	.64253D+02	.35112D+02
5.2000	2	0	.400000	.69328D+02	.26314D+02
5.6000	2	2	.200000	.73817D+02	.18551D+02
6.0000	2	0	.108736	.77473D+02	.12242D+02
6.4000	2	2	.752708E-01	.80180D+02	.75776D+01
6.8000	2	2	.132059	.82003D+02	.44426D+01
7.2000	2	0	.486594E-01	.83131D+02	.25021D+01
7.6000	2	0	.820117E-01	.83789D+02	.13725D+01
8.0000	2	0	.973370E-01	.84157D+02	.74094D+00
8.4000	2	0	.106106	.84357D+02	.39637D+00
8.8000	2	0	.110925	.84465D+02	.21098D+00
9.2000	2	0	.113531	.84523D+02	.11199D+00
9.6000	2	0	.114926	.84554D+02	.59364D-01
10.0000	2	0	.115669	.84570D+02	.31442D-01