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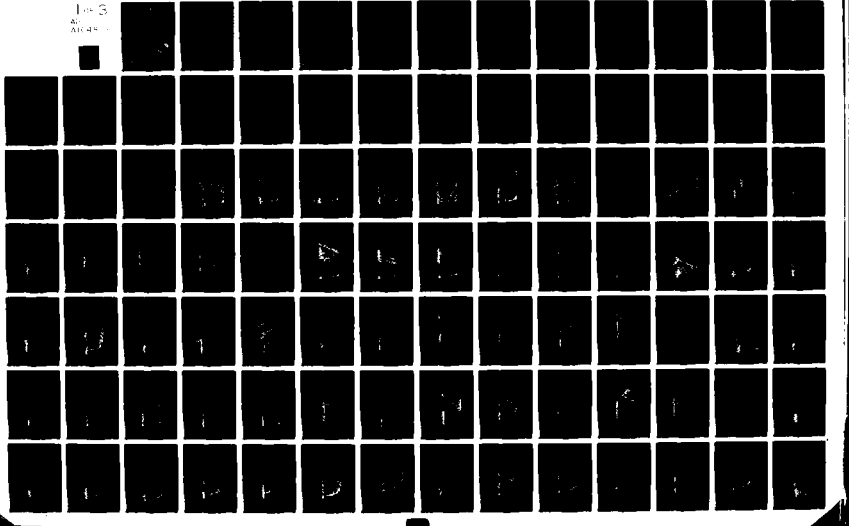

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MICROBIAL METHANE FERMENTATION KINETICS FOR TOXICANT EXPOSURE

by

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Methane fermentation of industrial wastes offers the dual potential of pollution abatement and energy conservation. This report presents results of experiments designed to investigate the effects of toxic substances on methane bacteria. Toxicants studied were calcium, cadmium, chromium, nickel, sulfide, chloroform, dichloroethylene, trichloroethylene, ethyl benzene, cationic surfactants, gasoline, jet fuel, and hydrazine. The effect of toxicant concentration, solids retention time, and temperature on methanogenic (contd.)

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response, acclimation, and reversibility was studied. Experiments included slug and continuous addition of toxicants.

The magnitude of the decrease in methane production, the length of decreased or zero gas production time, and the rate of return to full gas production is dependent on toxicant type and initial slug-dose concentration, exposure time, solids retention time, and temperature. Response patterns were remarkably similar for all toxicants. Extended periods of zero methane production were not indicative of ultimate process failure and recover rates were too high to be explained by bacterial regrowth. There exists a critical, initial slug-dose concentration beyond which the ability of the methanogens to recover quickly is severely impaired. Although a higher value of solids retention time may result in a less severe response to slug and continuous addition of toxicants, there was no clear pattern that applied to all toxicants. A sufficiently high SRT should, however, guard against washout of still-viable methanogens prior to recovery and/or acclimation. Higher temperature generally resulted in less severe responses. All toxicants exhibited some reversibility and most exhibited acclimation potential; the extent of reversibility and acclimation was dependent on experimental conditions.

ABSTRACT

Methane fermentation of industrial wastes offers the dual potential of pollution abatement and energy conservation when compared to more commonly used aerobic processes. In addition, only about ten percent as much excess biological sludge is produced. Utilization of the process has been limited due to several misconceptions, one of which is the inability of anaerobic processes to withstand exposure to toxicants. This report presents results of experiments designed to investigate the effects of toxic substances on methane bacteria. Toxicants studied were calcium, cadmium, chromium (III and VI), nickel, sulfide, chloroform, dichloroethylene, trichloroethylene, ethyl benzene, cationic surfactants (Hyamine 1622 and Hyamine 3500), regular gasoline, jet fuel (JP-4), and hydrazine. The effect of toxicant concentration, solids retention time (SRT: 15, 25, and 50 days), and temperature (25°, 35°, and 42.5°C.) on methanogenic response, acclimation, and reversibility was studied. Experiments included slug and continuous addition of toxicants.

Results from slug addition experiments showed that the magnitude of the decrease in methane production, the length of decreased or zero gas production time, and the rate of return to full gas production is dependent on toxicant type and initial slug-dose concentration, exposure time, SRT, and temperature. Response patterns were remarkably similar for all toxicants, and could generally be described by an empirical expression similar to the classical dissolved oxygen sag curve. Conceptual models were also developed to describe observed behavior. Extended periods of zero methane production (in excess of 40 days) were not indicative of ultimate process failure and recovery rates were too high to be explained by bacterial regrowth. There exists a critical, initial slug-dose concentration beyond which the ability of the methanogens to recover quickly is severely impaired. Once this concentration is exceeded, recovery times become quite protracted. Although higher values of SRT (25 and 50 days) may result in less severe responses to the toxicants, there is no clear pattern regarding recovery times that applies to all toxicants. The effect of SRT is a complex interaction of cell age, biomass concentration, and toxicant washout. Higher temperatures generally result in less severe responses, 35°C being the 'preferred' temperature. All toxicants exhibited some reversibility and most exhibited acclimation potential; the extent of reversibility and acclimation was dependent on experimental conditions.

Threshold concentrations (those causing the onset of decreased methane production) were very dependent on temperature and were between 5000 and 15,000 mg/l for calcium, less than 50 mg/l for cadmium, between 5 and 60 mg/l for chromium III, less than 10 mg/l for chromium VI, between 50 and 100 mg/l for nickel, less than 50 mg/l for sulfide, less than 5 mg/l for chloroform, less than 50 mg/l for both dichloroethylene and trichloroethylene, between 250 and 500 mg/l for ethyl benzene, between 5 and 50 mg/l for Hyamine 1622, between 1 and 10 mg/l for Hyamine 3500, less than 2500 mg/l for gasoline, between 1000 and 7500 for JP-4, and less than 10 mg/l for hydrazine.

Results from continuous addition experiments confirmed the significant acclimation potential of the methanogenic bacteria. Under optimal experimental conditions, 200 mg/l nickel, 20 mg/l chloroform, and 50 mg/l hydrazine could be tolerated with no decrease in system performance. The magnitude of the effect of SRT on response was once again shown to be dependent on toxicant type, concentration and temperature. Cell age and biomass concentration are undoubtedly contributing factors. In general, 25 and 50-day SRT will yield less severe responses. 35°C was the preferred temperature.

The importance of SRT is manifested in acclimation potential; lower SRT values result in washout of still-viable methanogens. A sufficiently high SRT will guard against such washout.

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INTRODUCTION

Methane fermentation processes have been used for many years to stabilize municipal wastewater sludges and have recently been applied to several types of industrial wastewaters. Pollutant removal by methane fermentation offers several significant advantages over more conventionally applied treatment:

1. No oxygen is required
2. Methane is produced and can be used as a supplemental energy source
3. Less biological sludge is produced
4. Less nutrients need to be added.

These advantages are dramatically demonstrated by the potential economic savings of about $100 per ton of organic removed and net energy savings of near 20×10^6 BTU per ton of organic removed when anaerobic treatment is compared with aerobic treatment.

Even when the above advantages are considered, applications of methane fermentation are limited and those systems in existence suffer from a somewhat unwarranted reputation for unreliability. Much of the hesitation in applying the process and many of the bad experiences occurring after installation result from a lack of understanding of process fundamentals. Effects of pH, alkalinity, temperature, mixing, and organic loading are fairly well understood, if not well applied. One of the major unknowns is how methane fermentation responds to toxicity. It is the general goal of this research to better elucidate the toxicity phenomena as it relates to methane bacteria. Fundamental information regarding toxicity phenomena is necessary if we are to fully realize the advantages listed above for treating industrial wastewaters using methane fermentation.

There are a number of compounds and classes of compounds that have been reported as toxic or inhibitory to methanogenic bacteria. A partial listing includes:

Heavy Metals	Antibiotics

Ammonium, cations in general	Disinfectants
Cyanide	Pesticides
Detergents	Organics (general)

The usual source of the above classes of compounds is industry, and the list of specific compounds is virtually limitless. For this study, fifteen relatively common toxicants were selected as representative of industrial toxicants:

Cation - Calcium (Ca^{2+})
Anion - Sulfide (S^{2-})
Heavy Metals - Nickel (Ni^{2+}), Cadmium (Cd^{2+}), Chromium (Cr^{3+} and Cr^{6+})
Surfactant - Cationic (Hyamine 1622 and Hyamine 3500)
Organic Solvents - Chloroform, Ethyl Benzine, Trichloroethylene, Dichloroethylene
Fuels - Gasoline, Jet Fuel (JP-4)
General - Hydrazine

Cr^{3+} and dichloroethylene were added to investigate differences in behavior from Cr^{6+} and trichloroethylene. In addition, two cationic surfactants were tested.

BACKGROUND AND LITERATURE REVIEW

The purpose of this section is to review the known literature pertaining to the inhibition of methane fermentation systems by the toxicants selected for this study. In addition, brief descriptions of the anaerobic process, toxicity phenomena, and the methods of toxicant application will be presented.

PROCESS DESCRIPTION

The anaerobic treatment process involves a complex series of digestive and fermentative reactions in which organic materials are converted into carbon dioxide (CO_2) and methane (CH_4) gases. Methane, being relatively insoluble in water, leaves the system, resulting in the stabilization of influent organic matter. The process occurs in four stages: 1) initial digestion of macromolecular materials by extracellular enzymes, such as proteases and lipases, to soluble materials; 2) conversion of the soluble materials to organic acids and alcohols by acid-producing fermentative organisms; 3) fermentation of the organic acids and alcohols to acetate, CO_2 and H_2; and 4) the conversion of H_2 and CO_2 and acetate to CH_4 by methanogenic bacteria (Brock, 1979).

The acid-producing fermentative organisms are facultative and obligate anaerobic bacteria. These bacteria include Clostridium spp., Peptococcus anaerobes, Bifidobacterium spp., Desulphovibrio spp., Corynebacterium spp., Lactobacillus, Actinomyces, Staphlococcus and Escherichia coli (Metcalf and Eddy, 1979).

The methanogenic bacteria are strict anaerobes. They are in general more sensitive than the acid formers and have very slow growth rates. As a result, their growth is usually considered to limit the rate of the overall conversion process. Waste stabilization occurs only when methane is formed. The principal genera of bacteria include the rods Methanobacterium and Methanobacillus and the spheres Methanococcus and Methanosarcina (Metcalf and Eddy, 1979).

The stability of anaerobic processes depends primarily upon the methanogenic bacteria, which are very sensitive to changes in the environment. Any adverse change such as a sudden change in pH or temperature, or the introduction of a toxicant into the digester may cause a decrease in gas

production, a lowering in the percentage of the methane gas produced, an increase in volatile acids concentration, and a subsequent drop of the pH as the buffer capacity is exceeded.

In order to operate an anaerobic treatment system efficiently, it is important to maintain several environmental factors (McCarty, 1964; Dague, 1968; Metcalf and Eddy, 1979). The reactor contents should be free of dissolved oxygen and other inhibitory materials. The pH of the liquid should range from 6.6 to 7.6. Sufficient alkalinity should be available to ensure that the pH does not fall below about 6.2, since the methanogens will not function efficiently below this value. The ensure proper growth of the bacteria, sufficient nutrients (especially nitrogen, phosphorous and iron) must be available. Finally temperature must be controlled. The optimum range for mesophilic bacteria is 30° to 38°C., and 49° to 57°C. for thermophilic bacteria.

TOXICITY

Toxicity is a relative term, its definition is dependent upon concentration. The concentration at which a material becomes toxic or inhibitory may vary from a fraction of a mg/l several thousand mg/l. The general effect which results from the addition of most substances to a biological system is illustrated in Figure 1. At very low concentrations, stimulation of activity usually occurs. This stimulatory concentration may range from only a fraction of a mg/l for some heavy metals to several hundred mg/l for sodium or calcium salts (McCarty, 1964). As the concentration increases beyond the stimulatory range, the rate of biological activity begins to decrease. A point is reached where the rate of activity is less than that achieved in the absence of the material. This point is known as the cross-over concentration, beyond which toxicity or inhibition occurs. A material may be judged toxic because it may cause an adverse shift in the microbial population of a biological waste treatment system. It might also cause the process to be more susceptible to a change in temperature or pH (Gaudy and Gaudy, 1980).

METHODS OF APPLICATION

Inhibitory substances may be introduced into a biological waste treatment process in several ways, as shown in Figure 2. The first method of

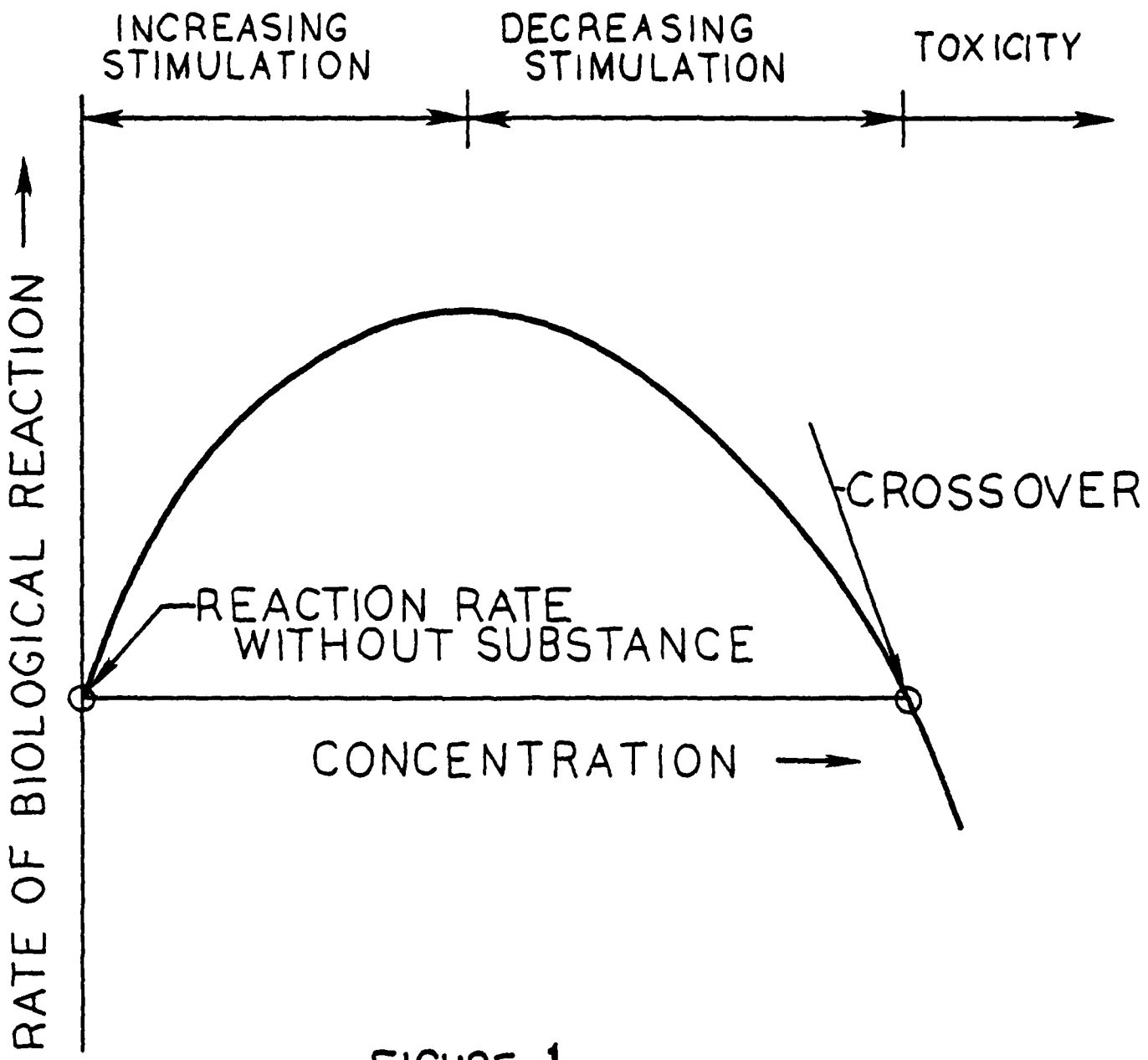

FIGURE 1

GENERAL EFFECT OF SUBSTANCES ON BIOLOGICAL REACTIONS

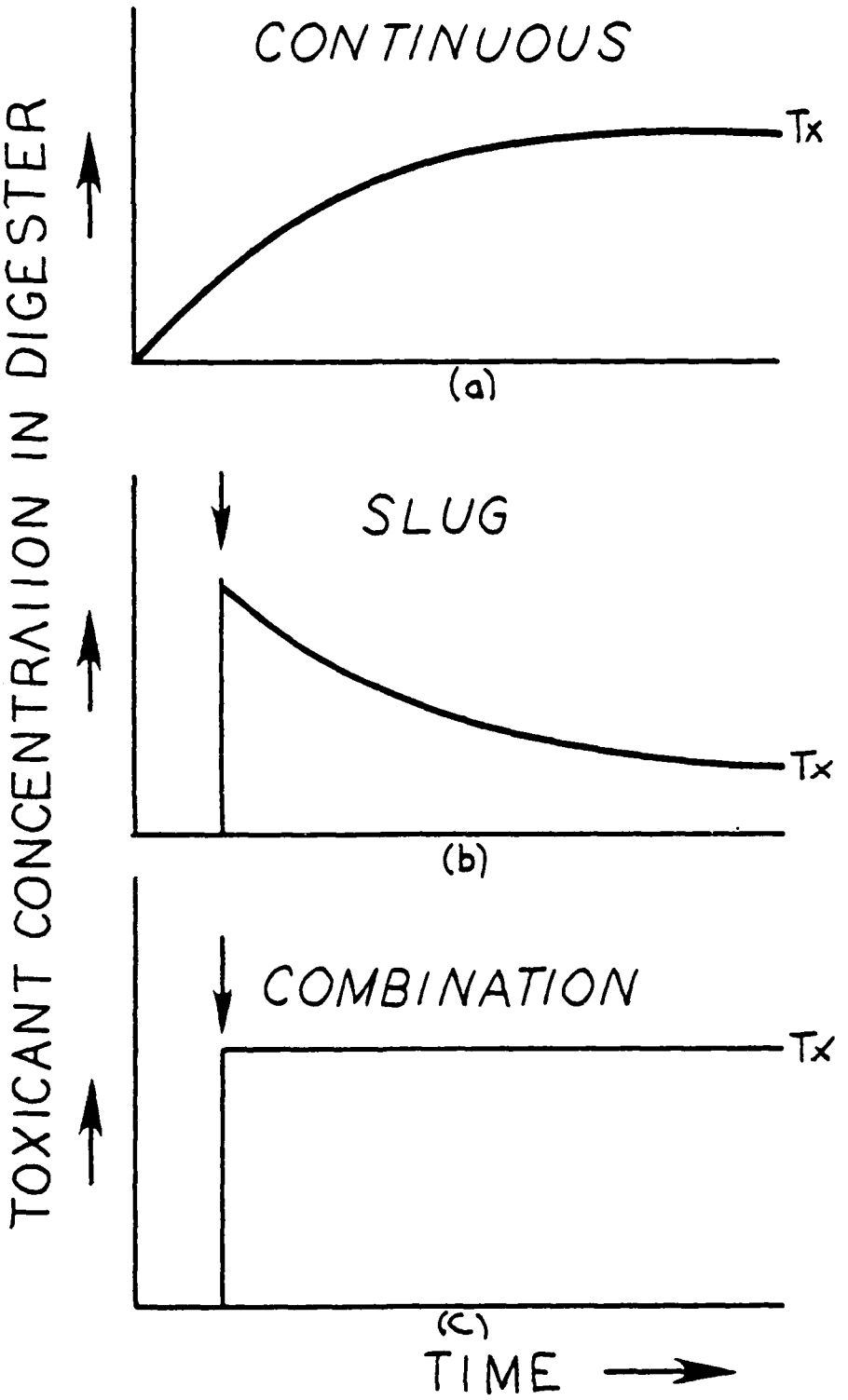

FIGURE 2

METHODS OF TOXICANT APPLICATION

toxicant application shown is termed continuous addition. The continuous addition of toxicant allows for the gradual increase in concentration of toxicant within the system. In this manner, methanogens are given the opportunity to acclimate to this gradual build-up. The second method shown is slug addition. This involves the addition of one large dose of toxicant, which is then removed gradually, the rate depending upon the biological solids retention time (SRT). Continuous and slug addition of toxicants simulate chronic and transient toxicity, respectively. These two methods were used in this study and will be explained further under EXPERIMENTAL METHODS. The last method shown is nominally termed combination application. This type of application combines the effects of the two described above. Initially, a slug concentration is applied and this concentration then continues to be applied in the influent in order to maintain a constant level regardless of SRT.

LITERATURE REVIEW

Inorganics

Data pertaining to calcium inhibition of methane fermentation is limited. McCarty and McKinney (1961) and Kugelman and Chin (1971) studied the effects of Ca^{2+} alone and in combination with other common cations. The latter authors found that Ca^{2+} concentrations 0.05M and above inhibited unacclimated methanogens, but that significant acclimation was possible.

Lawrence et al (1964) reported that soluble sulfides in excesss of 200 mg/l caused significant decreases in methane production. Lawrence and McCarty (1965) and Masselli et al (1967) recommended addition of sulfides to control heavy metal toxicity by sulfide precipitation, but cautioned against trading heavy metal toxicity for sulfide toxicity. Rudolfs and Amberg (1952) found a decreased gas production of near 30 percent following the addition of 200 mg/l sulfide. It is important to note that under anaerobic conditions, in a mixed culture of bacteria, sulfate is reduced to sulfide, meaning that high-sulfate carriage waters are potential sulfide toxicity problems.

It has long been realized that anaerobic systems are particularly vulnerable to high loadings of heavy metals. The most common single cause of stress in anaerobic digesters in England was reported to be heavy metal

toxicity (British Notes on Water Pollution, 1971). Mosey and Hughes (1975) report that many heavy metal ions react with the sulphydryl group of a wide range of enzynes, inactivating them. This action tends to hinder growth of the bacteria and in many cases kills them.

The toxicity of heavy metals depends upon the various chemical forms which the metal may assume under anaerobic conditions and at near-neutral pH levels. Mosey (1976) states that heavy metals only cause digestion failure when the concentration of their free ions (soluble) exceeds a certain threshold concentration, which is directly related to the concentration of divalent sulfide ions present in the digesting sludge. Work performed by Ghosh (1971) showed that although low concentrations of some heavy metals are extremely toxic, high concentrations could be tolerated if sufficient sulfide was available for precipitation. Lawrence and McCarty (1965) and Masselli et al (1967) also reported elimination of heavy metal toxicity with sulfide addition. Data in Table 1, compiled by Barth et al (1965), gives an indication of the difference between total and soluble concentrations that may exist in a digester.

Table 1

Total and Soluble Heavy Metal Content of Digesters
(after Barth et al, 1965)

Metal	Total Concentration (mg/l)	Soluble Concentration (mg/l)
Chromium (VI)	420	3.0
Copper	196	0.7
Nickel	70	1.6
Zinc	341	0.1

It may be readily seen that complexation and precipitation reactions may reduce the total metal concentration by a factor of over 100.

Soluble heavy metal concentrations reportedly associated with severe inhibition of anaerobic systems are 0.5 mg/l for copper, 3 mg/l for chromium VI, 2 mg/l for nickel and 1 mg/l for zinc (U.S. E.P.A., 1979; Mosey, 1976; DeWalle et al, 1979). Kugelman and Chin (1971) indicated that soluble metal concentrations of a few mg/l are all that is necessary to shut down

gas production.

Unlike most prior research, Hayes and Theis (1978) investigated the effects of heavy metals for both one-time, slug addition and semicontinuous addition. Table 2 is a summary of the results from Hayes and Theis (1978).

Table 2

Heavy Metal Toxicity Limits for Anaerobic Digestion

Heavy Metal	Semi-Continuous		Slug-Dose
	Inhibiting Concentration (mg/l)	Toxic Limit (mg/l)	Toxic Limit (mg/l)
Cr (III)	130	260	< 200
Cr (VI)	110	420	< 180
Cu	40	70	< 50
Ni	10	30	> 30
Cd	-	> 20	» 10
Pb	340	>340	» 250
Zn	400	600	<1700

It may be seen that with the exception of nickel and zinc, shock loading in the form of a slug dose produced a lower toxic limit than did semi-continuous addition. The authors related the order of decreasing toxicity on a weight-weight or molar basis as Ni>Cu>Pb>Cr>Zn. At the dosages used, cadmium produced no toxic effects.

Organics

Most of the research on surface active materials focused on anionic detergents. The ability of synthetic detergents, especially alkylbenzene sulfonates (ABS), to inhibit methane production is well known. Absorption of the detergents on sewage sludge solids prior to anaerobic digestion of those solids was the mechanism advanced in British Notes on Water Pollution (1971). Some acclimation to the detergents was reported. Pitter et al (1971) indicated that municipal sludge digestion was inhibited by linear

ABS concentrations greater than one percent of dry solids. Quaternary ammonium compounds, common additives to synthetic detergents, are known to cause inhibition to methane fermentation (Pearson, et al 1980; Speece, et al, 1979).

British Notes on Water Pollution (1971) reported that chlorinated hydrocarbons are widely used as solvents. They are used to degrease mechanical and electrical components and for dry-cleaning clothing. Some chlorinated hydrocarbons have been found to be extremely toxic to anaerobic digestion and have caused inhibition of a number of treatment plants in England. Chloroform was found to be the most toxic. Inhibition depended both on the solids content of the feed sludge and on the concentration of the chloroform in the wet sludge (mg/l) when other variables were excluded. Digesters became acclimated to different loadings of chloroform with replacement of between 0.5 and 6.0 percent of digester contents daily.

Bauchop (1967) used chloroform as a specific inhibitor for methane formation in suspensions of rumen fluid. Other investigators found that chloroform levels as low as 0.5 mg/l inhibited methanogenisis (Wolfe, 1971; Hovious et al, 1973; Mosey and Hughes, 1975; Lamb et al, 1977; Baresi et al, 1978). Continuous feeding of 10 mg/l of chloroform was found by Lamb et al (1977) to cause inhibition in sewage treatment plants in Britain.

Contrary to previous studies, research by Yang et al (1980) revealed that with acclimation, submerged anaerobic filters could tolerate 20 to 40 mg/l of chloroform without inhibition of methane production. Application of a slug dose of 200 mg/l to an 'acclimated filter' resulted in a severe reduction in methane production the following day, however, complete recovery was observed within four days.

Hovious et al (1973) tested ethyl benzene at concentrations of 150 to 1000 mg/l and found little or no inhibition. However, Chou et al (1977) found that an ethyl benzene level of 200 mg/l reduced activity by about 25 percent and a 60 percent reduction at 1000 mg/l. Ethylene dichloride, a solvent very similar in structure to the dichloroethylene investigated in the present study, has been reported to severely inhibit methane fermentation, inhibition starting at concentrations as low as 5 mg/l (Hovious et al, 1973; Stuckey et al, 1980).

Little data are available on anaerobic toxicity of fuels and we could find none on hydrazine toxicity. Rudolfs (1937) reported that gasoline

inhibited anaerobic digestion of municipal sludge, the percent gasoline to sludge volatile solids ratio being the parameter of importance. Speece et al (1979) found that injection of 500 ml of no-lead gasoline to an anaerobic filter temprarily reduced methane production by 70 percent. Hovious et al (1973) found that kerosene concentration of 500 mg/l only reduced anaerobic activity by nine percent.

BASIS FOR AND GOALS OF STUDY

BASIS FOR STUDY

From a review of the literature it appears that very little research has focused on patterns of recovery from toxicant exposure, acclimation and reversibility characteristics, or kinetics of anaerobic systems exposed to industrial toxicants. Little work has been done to evaluate the effect of temperature and SRT. The present study attempts to address these aspects.

GOALS OF THE STUDY

As stated in the proposal for this study, the goals are:

1. To evaluate the relationship between concentration of toxicant and the inhibition or toxicity caused.
2. To determine acclimation characteristics of methane bacteria.
3. To examine the reversibility of the toxicity.
4. To quantify the kinetics of toxicity and inhibition so as to formulate a dynamic model to describe the experimental results.

Both slug and continuous addition of toxicants were examined. Acclimation was studied using repeated slug additions and gradual exposure via continuous addition. Reversibility was studied by observing recovery patterns and by conducting specific reversibility experiments. Kinetics were investigated using data generated by experiments addressing goals one through three.

EXPERIMENTAL METHODS

INTRODUCTION

It is generally well accepted that the conversion of organic acids to methane by methanogenic bacteria is the 'rate limiting' step during high rate, anaerobic fermentation of most complex organics (McCarty, 1966; Lawrence and McCarty, 1969; Kugelman and Chin, 1971). Studie_ by Jeris and McCarty (1965), Smith and Mah (1966) and O'Rourke (1968) have shown that acetic acid is the most prevalent volatile acid intermediate formed in the methane fermentation of complex organics such as fats, carbohydrates and proteins. Research has shown that approximately 70 percent of the methane generated from anaerobic degradation of complex organics comes from acetate conversion to methane (Jeris and McCarty, 1965; Smith and Mah, 1966), thus defining acetate as the key intermediate in anaerobic biological treatment. Therefore, acetate enrichment cultures were selected to study the response of methane fermentation systems to addition of toxicants. Toxicity was evaluated using a serum bottle modification of the Hungate technique as described by Miller and Wolin (1974).

INOCULUM SOURCE

The original acetate enrichment culture was developed with sludge from an anaerobic digester. This system, a 400-liter, complete-mix (CSTR) reactor operated at a 50-day solids retention time (SRT or θ_c) and a temperature of 35°C, has been maintained in our laboratory for five years on an inorganic nutrient solution (Table 3) with acetate as the sole organic carbon source, except for 10 mg/l cysteine. Two additional seed cultures (20 liters each) have been maintained at θ_c values of 25 and 12.5 days for the past two years. Acetic acid is fed at the rate of 1050 mg/l per day to all three systems.

During toxicity experiments, aliquots of these cultures were anaerobically removed from the proper seed reactor and transferred to prepared serum bottles, 50 ml per bottle, using a syringe.

Table 3. Nutrient Salt Solution

Constituent	Conc. (mg/l)
NH_4Cl	400
KCl	400
$MgSO_4 .6H_2O$	400
Na_2S	100
$(NH_4)_2 HPO_4$	80
$FeCl_2.6H_2O$	40
$CoCl_2$	4
KI	10
Sodium Hexa Meta Phosphate	10
Cysteine	10
$MnCl_2$	0.5
$NH_4V_2O_3$	0.5
$Zn\ Cl_2$	0.5
$NiCl_2$	0.5
$Na_2Mo\ O_4.2H_2O$	0.5
H_3BO_3	0.5
$NaHCO_3$	6000

SERUM BOTTLE TECHNIQUE

A technique similar to that described by Owen et al (1979) was used to evaluate toxicity. The source of methanogenic bacteria was an acetate enrichment culture.

Serum Bottle Preparation

Serum bottles of 150 ml capacity were rinsed thoroughly with tap water, washed with a 1:1 HCl solution, and copiously rinsed with tap water. Bottles were then completely submerged in clean tap water and inverted, allowing the air to be replaced by water. The water was then displaced using an oxygen-free gas stream containing 67% N_2 and 33% CO_2. Serum bottle stoppers were inserted while the bottles were still submerged.

The oxygen-free bottles could then be inoculated with methane bacteria, nutrients, and potential toxicants. Once inoculated, the bottles could be operated in a batch or semi-continuous mode.

Semi-Continuous Operation

Semi-continuous operation involved a 24-hour cycle of feeding, wasting, and reading daily gas production. Total gas production was measured by displacement of a colored, acidic, salt-saturated solution. A syringe needle, connected to a specially designed graduated cylinder containing the colored solution, pierced the serum bottle stopper and the fluid was displaced via release of pressure from the bottle.

Following gas production measurement, glacial acetic acid (HAc) was added to compensate for that consumed during the previous 24-hour cycle and bring the HAc level back to 1050 mg/l for the start of a new 24-hour cycle. Consumption was estimated using the following:

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

From the above stoichiometry, feeding 1050 mg/l HAc to a 50-ml culture volume should result in production of about 22 ml of CH_4 biologically (assuming no synthesis) and about 22 ml CO_2 through chemical neutralization. The amount of CO_2 produced each day can be estimated from this stoichiometry and knowing how much HAc was added the previous day. Methane production was calculated by subtracting this estimated CO_2 production from the total measured gas production. The volume of HAc required to maintain a constant level of 1050 mg/l HAc in the serum bottle was determined using the

calculated methane production and the equation. This is better illustrated using the example in Table 4. Errors may occur due to the assumption that all the CO_2 produced was in the gas phase, however, this potential error has not significantly affected reported results.

Table 4

Daily Gas Production Calculation Example

$$CH_3COOH = CH_4 + CO_2$$

$$\left(\frac{1.05\text{mg HAc}}{\mu\text{l HAc}}\right)\left(\frac{1\text{m-mole HAc}}{60\text{ mg HAc}}\right)\left(\frac{22.4\text{ ml } CH_4}{\text{m-mole HAc}}\right)\left(\frac{(273+35)^\circ C}{273^\circ C}\right) = \frac{0.44\text{ ml } CH_4}{\mu\text{l HAc}}$$

Theoretically, 1.0 µl HAc = 0.44 ml CO_2 + 0.44 ml CH_4

Therefore,

Daily methane production =

Total gas production - $\frac{0.44\text{ ml } CO_2}{\mu\text{l HAc}}$ (µl HAc fed previous day)

The solids retention time was controlled by removing or wasting a specific quantity of the enrichment culture (1, 2 or 3.33 ml for SRT values of 50, 25 and 15 days, respectively) using a pre-set, automatic syringe. Initially, it was desired to operate the serum bottles at the same SRTs as the inoculum digesters. There was no problem adapting the serum bottles to the 25-day and 50-day SRTs, but the 12.5 SRT could not maintain stable gas production. This was probably due to being so close to the 'washout' SRT (bacterial generation time). Therefore, a 15-day SRT, which proved to be more stable, was developed from the 25-day SRT inoculum digester. Following removal of culture contents for SRT control, an appropriate volume of the nutrient salt solution listed in Table 3, containing 1050 mg/l HAc, was then added, completing the daily cycle. During continuous toxicant addition studies, toxicant was added at this time also.

Toxicant Introduction

Prior to addition of toxicants, serum bottles were operated as described above until methane production stabilized at about 22 ml/day. Toxicants were then added.

Slug-Dose Studies. Slug doses of the candidate toxicant were added from a stock solution using syringes. For some organics, direct injection of the liquid was practiced. Injection of the toxicants was done immediately after the start of a new 24-hour cycle to minimize substrate utilization prior to toxicant exposure.

Response to toxicant exposure was monitored using daily methane production. Acclimation potential of the methanogens was studied by injecting a second slug dose of toxicant at twice the original concentration once the methane production had returned to the control level of 22 ml/day. Third and fourth injections were made, each at double the previous concentration, in some bottles.

Continuous-Addition Studies. After the serum bottles had stabilized producing about 22 ml methane per day, toxicants were introduced as part of the nutrient salt solution. Individual nutrient salt solutions were prepared periodically for each of the three toxicant concentrations. The nutrient salt solution for the control serum bottles contained no toxicant. All of the serum bottles were maintained for a minimum of three SRTs in order to provide for the opportunity to expose the methanogenic bacteria to approximately 95 percent or more of the final desired concentration, assuming no other chemical or biological reactions take place.

Reversibility Studies. Studies on the reversibility of the toxicity phenomena were conducted by first injecting the toxicant to give the desired concentration in the serum bottle. Then, after the desired exposure time (one hour, one day, or four days), the serum bottles were placed directly into a centrifuge and centrifuged for 15 minutes at 3000g. The serum bottles were then carefully inverted and the supernatant was completely removed using a syringe. A 75/25 percent mixture of N_2/CO_2 was introduced as the supernatant was withdrawn. Then, unadulterated supernatant from the acetate enrichment culture was injected into the serum bottle to replace the adulterated supernatant withdrawn. Daily feeding and gas measurement were then continued as described above.

ANALYTICAL TECHNIQUES

Volatile suspended solids (VSS) were measured using glass fiber filters as described in Standard Methods (1975). COD was analyzed using the technique of Jirka and Carter (1975), pH was measured using a Fisher Accumet Model 250A or a Corning Model 12 pH meter. Heavy metals were analyzed with a Perkin Elmer Model 903 atomic absorption spectrophotometer.

Acetate was measured using a Carle Model AGC-311 (10% SP 1200/1% H_3PO_4 on Chromasorb W-AW 80.100 packing)or a Shimadzu GC-6AM Series (FAL-MoH_3PO_4 (Supelco) on Chromasorb W-AW 80/100 packing) gas chromatograph. Samples for analysis were filtered or centrifuged and the liquid adjusted to pH 2-3 using solid meta-phosphoric acid.

INFINITE DILUTION TECHNIQUE

An "infinite dilution" technique has been developed to rapidly determine K_s values for soluble substrates. Williamson and McCarty (1974) developed this method to calculate K_s, the Monod half-velocity coefficient, for autotrophic oxidation of nitrite and ammonium. The experimental time periods can be less than a few hours so that significant shifts in bacterial populations can be avoided. This technique is especially useful for very small (in the mg/l range) half-velocity coefficients.

Theory

A concentrated feed solution is continuously fed to a completely-mixed reactor without effluent recycle or wasting. The use of a concentrated feed minimizes the flowrate into the reactor, hence, the increase in reaction volume over the few hours of the experiment is neglible.

The Michaelis-Menten expression for substrate utilization is:

$$-\frac{dS}{dt} = \frac{kSX}{S + K_s}$$

where S = rate-limiting substrate concentration (mass/volume)
t = time
k = maximum substrate utilization rate ($time^{-1}$)
X = organism concentration (mass/volume)
K_s = half velocity coefficient (mass/volume)

A mass balance for substrate in the reactor gives:

$$\frac{dS}{dt} = \frac{S_f Q}{V} - \frac{kSX}{S_f K_s}$$

where S_f = substrate concentration in the feed solution (mass/volume)
Q = feed flowrate (volume/time)
V = reactor volume

Assuming that a steady-state substrate concentration will be reached when the mass flowrate, $S_f Q$, is maintained at less than the maximum utilization rate, kXV, then;

$$\frac{dS}{dt} = 0 = \frac{S_f Q}{V} - \frac{kSX}{S_f K_s}$$

or $$\frac{kS}{S_f K_s} = \frac{S_f Q}{XV}$$

Using this technique, the steady-state substrate concentrations are measured for a number of bacterial suspensions fed at a series of constant mass flow rates varying from 0 to kXV. From the data obtained the entire substrate utilization rate versus S curve can be drawn and values of k and K_s can be obtained. Using the Lineweaver-Burke procedure, the above equation may be rewritten as follows;

$$\frac{XV}{S_f Q} = \frac{K_s}{k}\frac{1}{S} + \frac{1}{k}$$

The slope of a plot of $\frac{1}{S}$ versus $\frac{XV}{S_f Q}$ equals $\frac{K_s}{k}$ and the ordinate intercept equals $\frac{1}{k}$.

Procedures

The reactors were half-gallon plastic aspirator bottles. The top opening of each bottle was fixed with a feed inlet and a gas outlet and was sealed. Prior to system start-up the reactor volumes were displaced by an oxygen-free gas stream containing 67% N_2 and 33% CO_2. The reactors were

filled with 1.5-liter aliquots drawn from the inoculum source. Two-ml samples were withdrawn at appropriate intervals from a sample port at the reactor base. Temperature was maintained at 35°C. Mixing was provided by magnetic stir bars. Gas evacuated from the reactors was bubbled through an acid/salt solution and was released to the atmosphere.

The microorganism concentration, as volatile suspended solids (VSS), of the inoculum source was measured for each experiment. At the conclusion of each experiment, reactor VSS was measured.

Feed solution was displaced from feed bottles and into the reactors by a gas mixture produced by electrolysis pumps. The feed solution contained approximately 10,000 mg/l as acetate and was prepared using distilled water and either glacial acetic acid or potassium acetate. The potassium concentrations were determined to be well below inhibitory levels. Reactor pH was checked before and after each experiment.

The substrate concentrations within the reactors and the feed solutions were measured as acetic acid by gas chromotography (GC). All samples for GC analysis were acidified to a pH less than 2 and then refrigerated.

A batch serum bottle technique was used to evaluate values of k that could be compared to the k obtained by the infinite dilution experiments. Approximately ten serum bottles were inoculated with 50-ml of culture and greater than 2000 mg/l of potassium acetate solution each time inoculum was withdrawn from the inoculum source for an infinite dilution experiment. The acetate concentration in the serum bottles was assumed to be much greater than K_s for the bacterial suspension. Hence, utilization was assumed to be substrate unlimited. The maximum utilization rate could be obtained from the slope of a plot of gas produced in the serum bottles versus time. Gas production was measured by a displacement of an acidified salt solution. The serum bottles were maintained at 35°C.

RESULTS

SLUG ADDITION OF TOXICANTS

Daily methane production by serum bottles operated in the semi-continuous mode was recorded. Slug doses of cadmium, calcium, chloroform, chromium III, chromium VI, dichloroethylene, ethyl benzene, Hyamine 1622, Hyamine 3500, Hydrazine, gasoline, jet fuel (JP-4), nickel, and trichloroethylene were introduced into separate bottles after stabilizing at quasi steady-state methane generation levels. Each toxicant was tested at six concentrations, one solids retention time (50 days) and three temperatures (25°C, 35°C, and 42.5°C). Exposure to calcium, chloroform, chromium III, chromium VI, nickel, and sulfide was also investigated at a 25-day SRT and the three temperatures. These six toxicants were also studied at a 15-day SRT at 35°C.

Statistical analysis of the control serum bottles for each set of environmental conditions revealed that the mean daily methane production was 21.5 ml/day with a 95% confidence interval of ± 1.5 ml for systems at 35°C and 42.5°C. The average methane production at 25°C was closer to 21 ml/day with a 95% confidence interval of approximately ± 3 ml. For the 15-day, 35°C controls, the average methane production was 19.9 ml with a 95% confidence interval of ± 4 ml. Temperature fluctuations were similar in all three incubators, normally ± 0.5°C.

Calcium (Ca^{++})

Stock solutions of calcium were prepared with $CaCl_2$. Serum bottle concentrations of 5000, 10,000, 15,000, 20,000, 25,000 and 30,000 mg/l as Ca^{++} were introduced as slug doses.

Responses to calcium exposure were very dependent upon concentration and environmental conditions (Figures 3 to 9). With increasing temperature and decreasing SRT, there was an increasing tendency for an initial increase in gas production followed by the resumption of normal gas production or by the expected decrease in methane generation. The increase in calculated "methane" production is probably due to CO_2 generation from bicarbonate alkalinity upon $CaCO_3$ precipitation. Those serum bottles not showing a

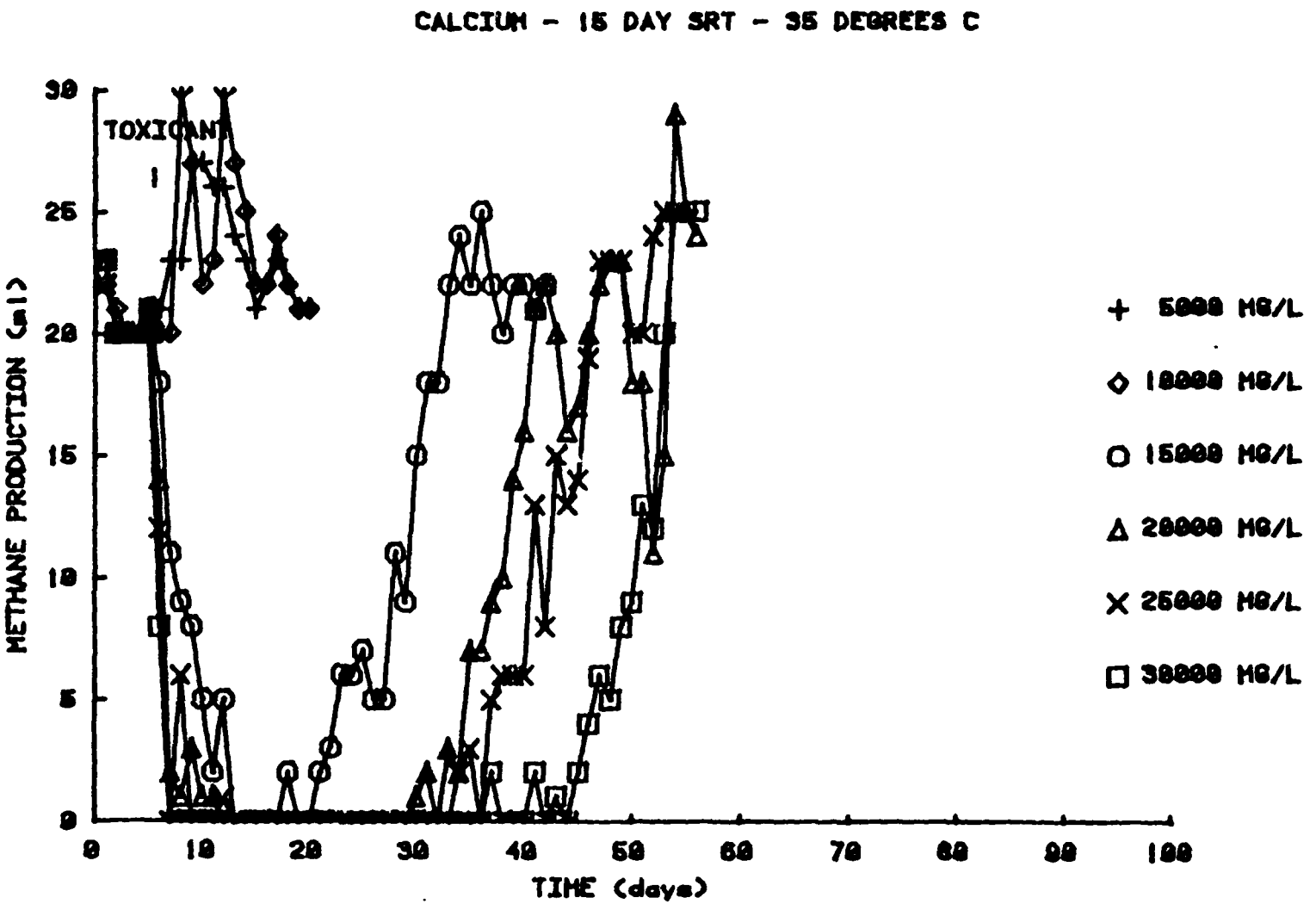

Figure 3. Response of Methanogens to Slug Doses of Calcium

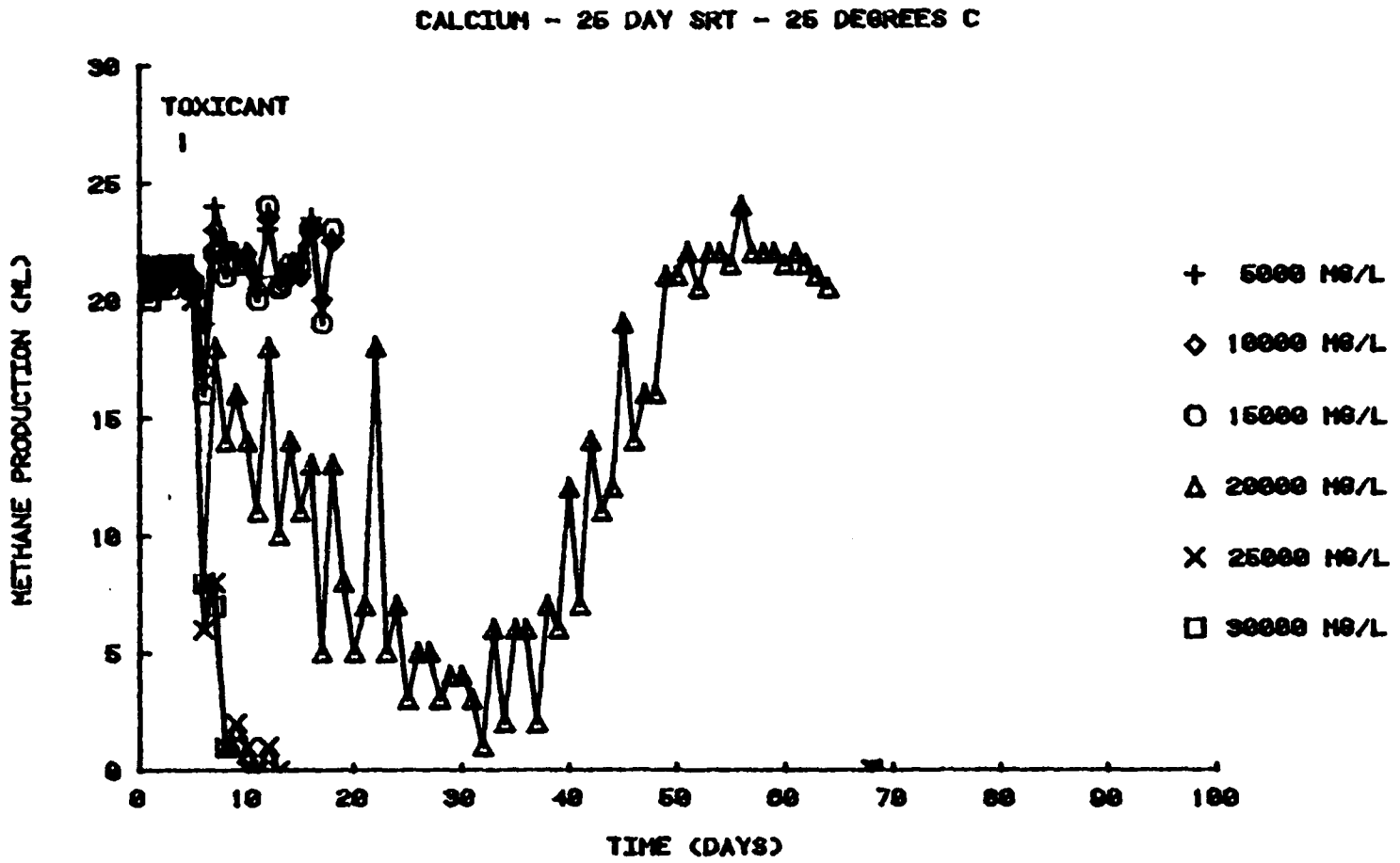

Figure 4. Response of Methanogens to Slug Doses of Calcium

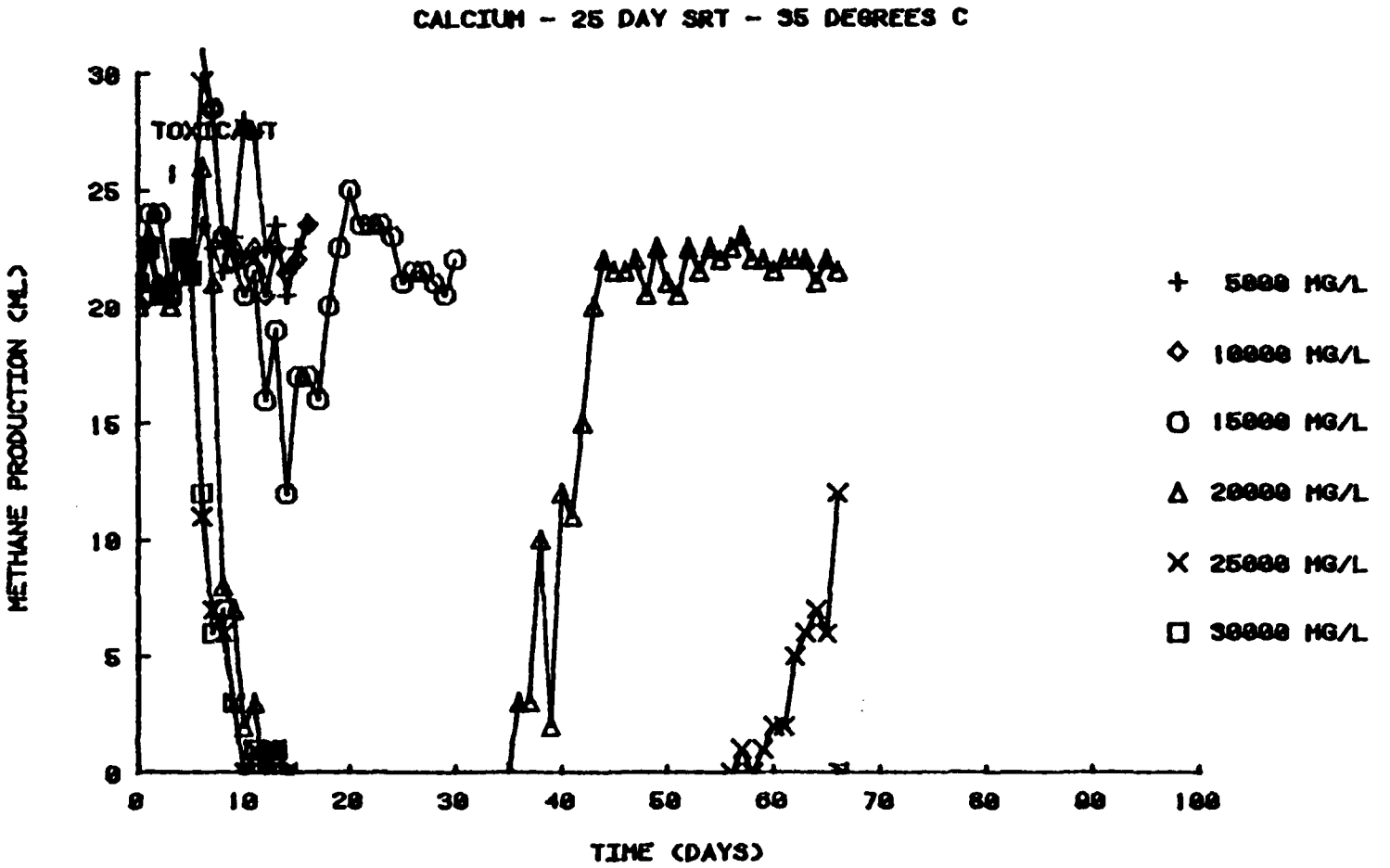

Figure 5. Response of Methanogens to Slug Doses of Calcium

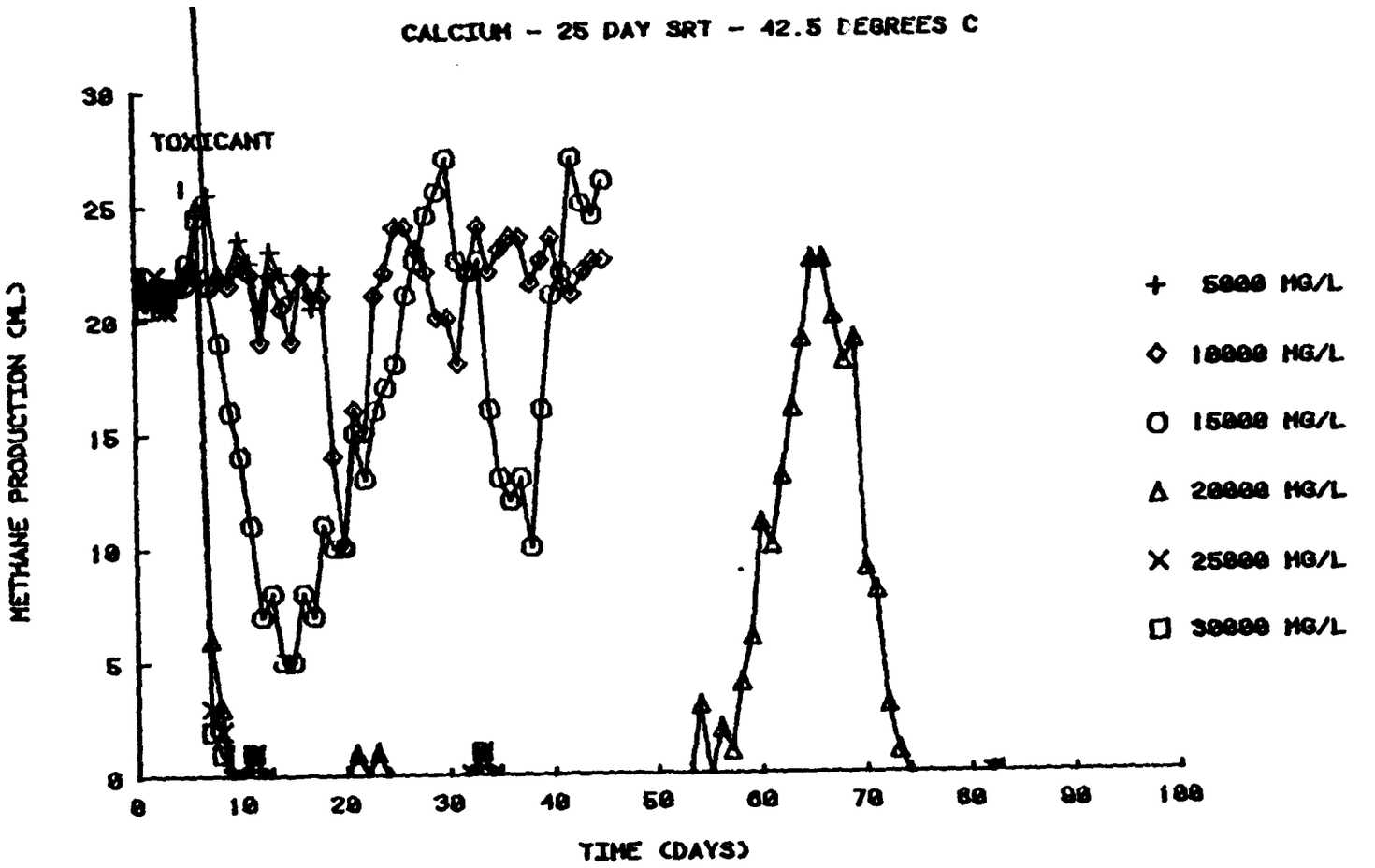

Figure 6. Response of Methanogens to Slug Doses of Calcium

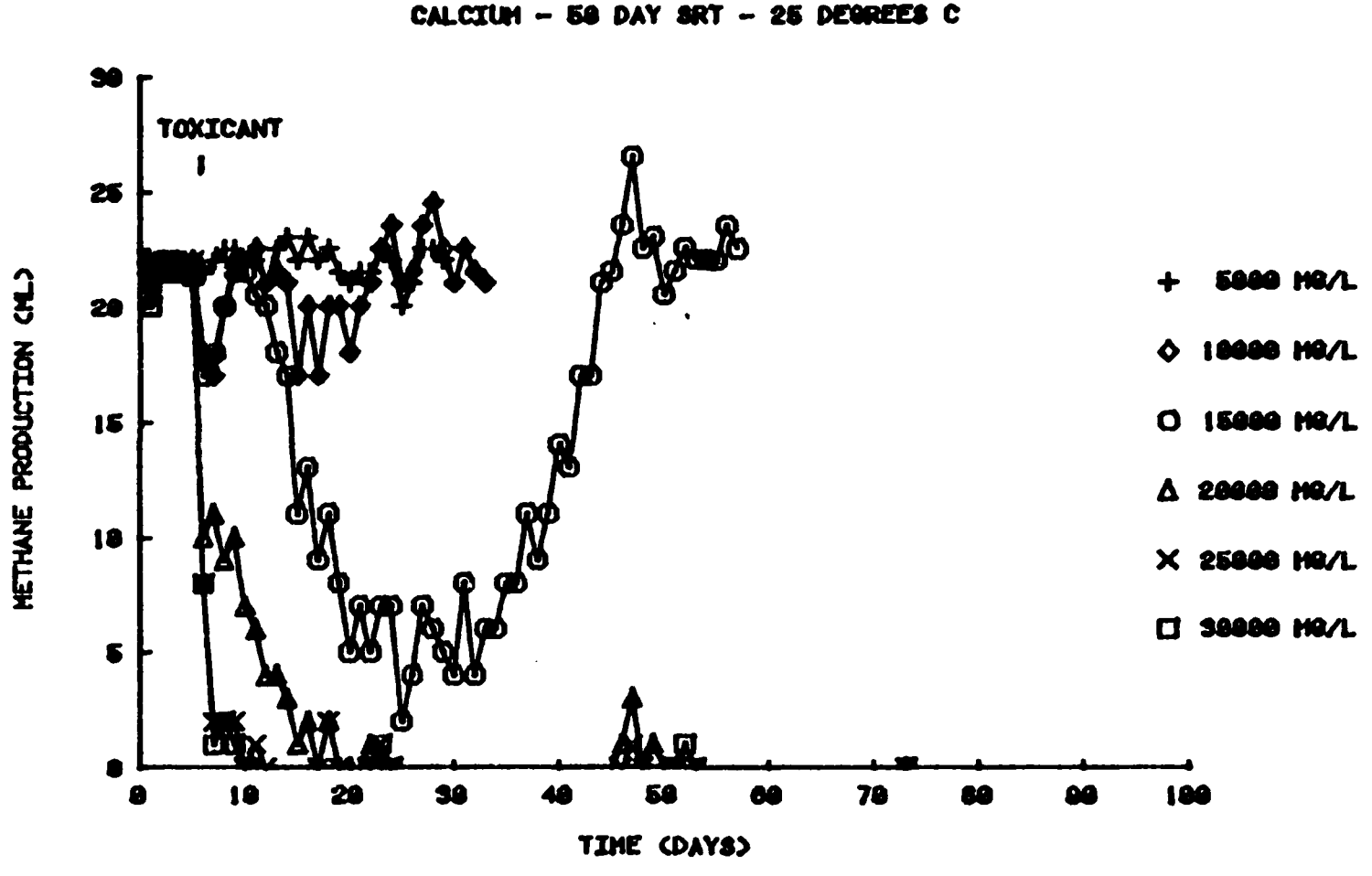

Figure 7. Response of Methanogens to Slug Doses of Calcium

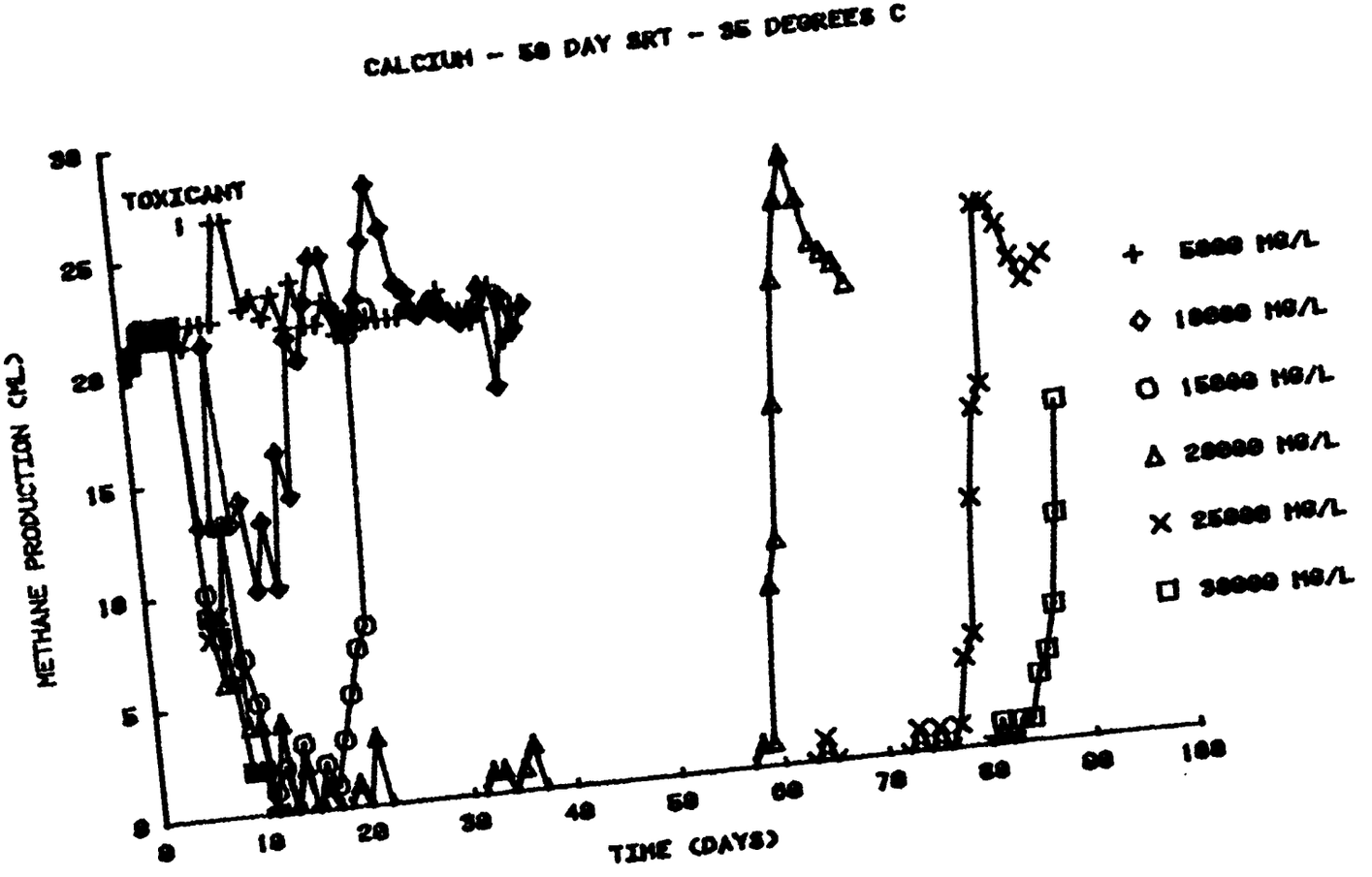

Figure 8. Response of Methanogens to Slug Doses of Calcium

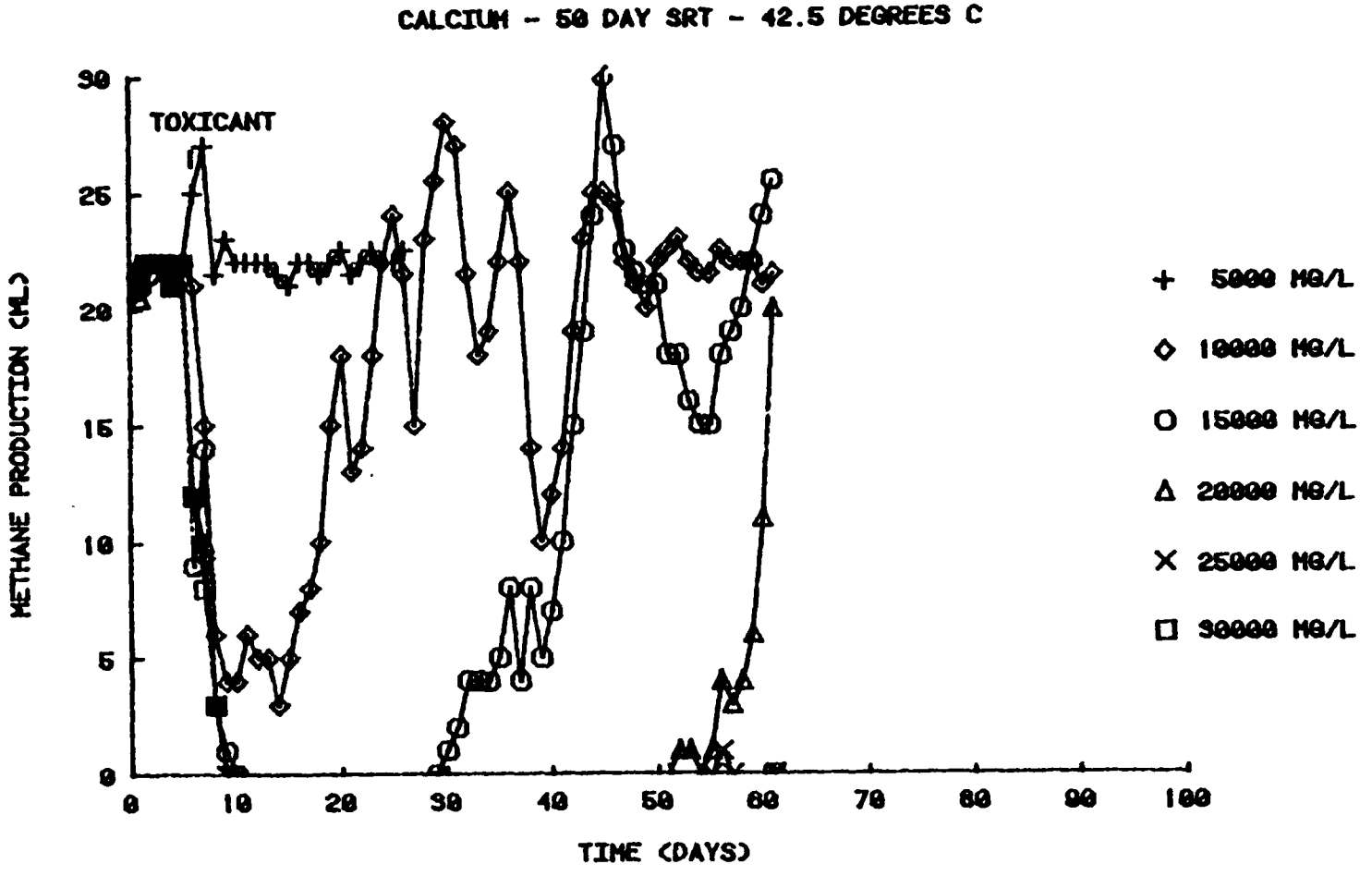

Figure 9. Response of Methanogens to Slug Doses of Calcium

sharp increase in gas production initially demonstrated the typical response curve. This curve is characterized by a rapid drop in gas production followed by a period of low or zero methane generation, the length dependent upon initial toxicant concentration and, finally, recovery to normal gas production. Recovery rates were generally slower than initial toxicant response rates and were slower for higher initial slug dose concentrations. One other unusual response to calcium was a residual toxicity effect exhibited at higher temperatures, noted by decreases in methane production after an initial return to control methane generation levels.

The severity of response increased significantly with increasing temperature. Bottles maintained at a 25-day SRT were much more able to cope with calcium toxicity than those at a 50-day SRT. For conditions tested, a temperature of 35°C with a 15-day SRT appears to be optimum conditions in terms of rate of recovery. These results were not expected, and may be due to the rapid washout of toxicant at this low SRT.

Although there are some signs of acclimation to 10,000 mg/l Ca^{++} at a 50-day SRT, significant acclimation was not clearly demonstrated (Figures 10 to 16). As a matter of fact, repeated injection of calcium appears to be detrimental.

Cadmium (Cd^{++})

Stock solutions of cadmium chloride were used to introduce serum bottle concentrations of 50, 75, 100, 125, 150 and 200 mg/l Cd^{++}. Cadmium can of course precipitate with the sulfide contained in the culture media. The maximum available sulfide present would be 97 mg/l as S. If all this sulfide reacted with the cadmium, a maximum of 340 mg/l of cadmium would precipitate. It is unlikely that all the sulfur would be available for precipitation.

Cadmium was the only toxicant to show an uncharacteristic response pattern in relation to initial toxicant concentrations. Normally, the severity of response to toxicant exposure increased with increasing concentrations. The least severe response to cadmium did occur after introduction of 50 mg/l Cd^{++}. However, an initial concentration of 200 mg/l Cd^{++}, the largest slug dose, resulted in the second fastest recovery, while the last bottles to recover were exposed to only 75 or 100 mg/l Cd^{++} (Figures 17 to

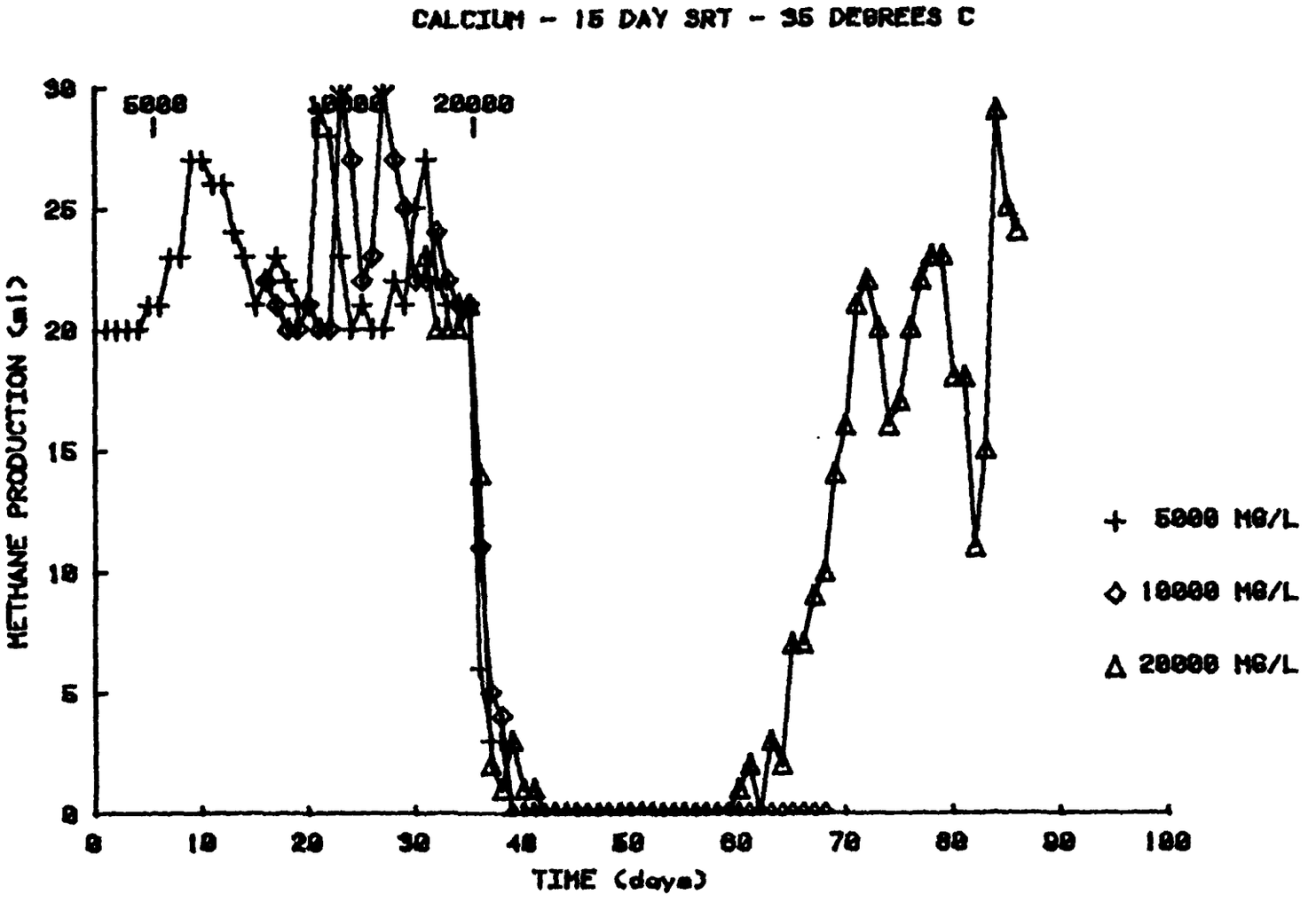

Figure 10. Acclimation of Methanogens to Slug Doses of Calcium

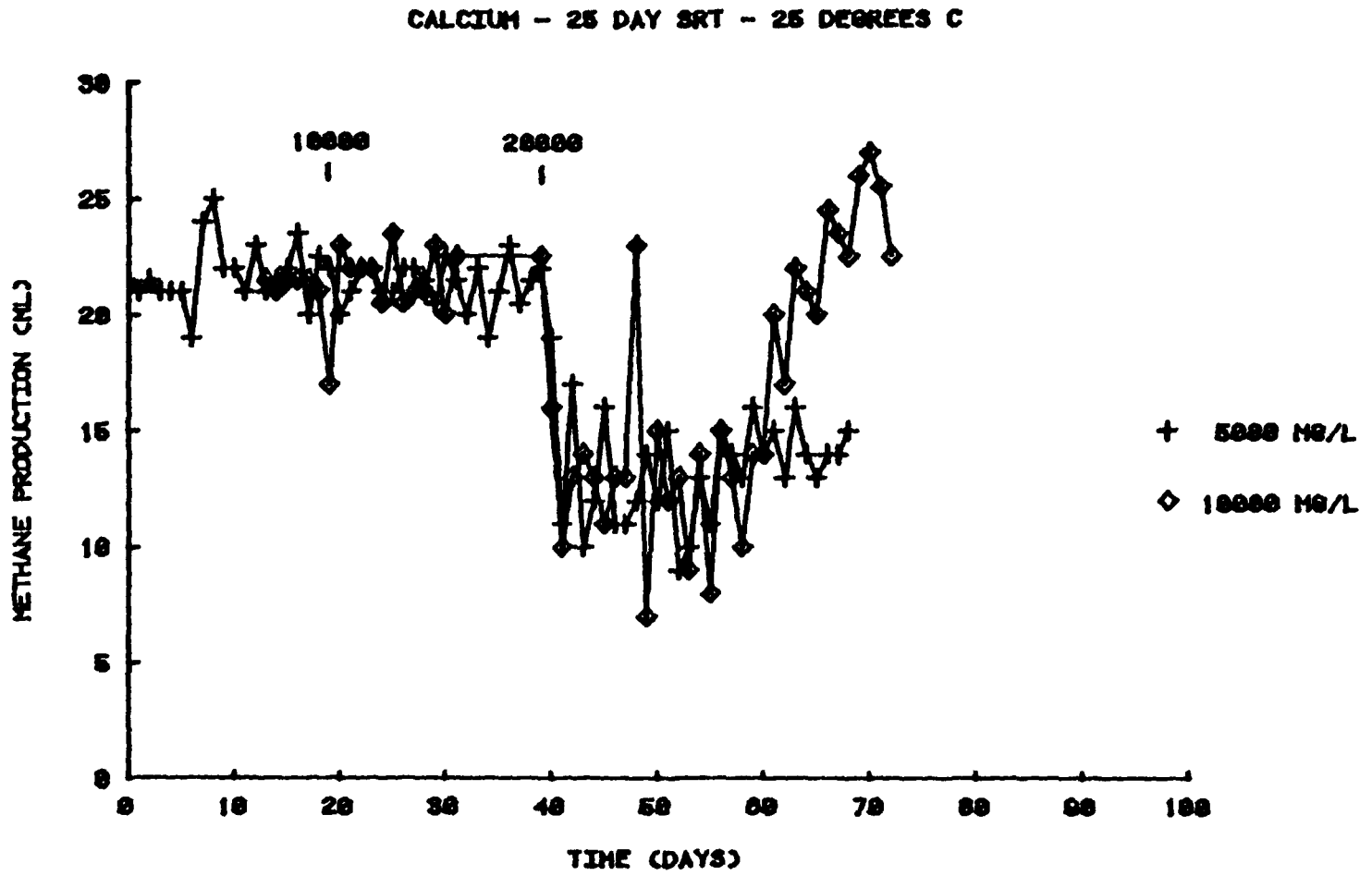

Figure 11. Acclimation of Methanogens to Slug Doses of Calcium

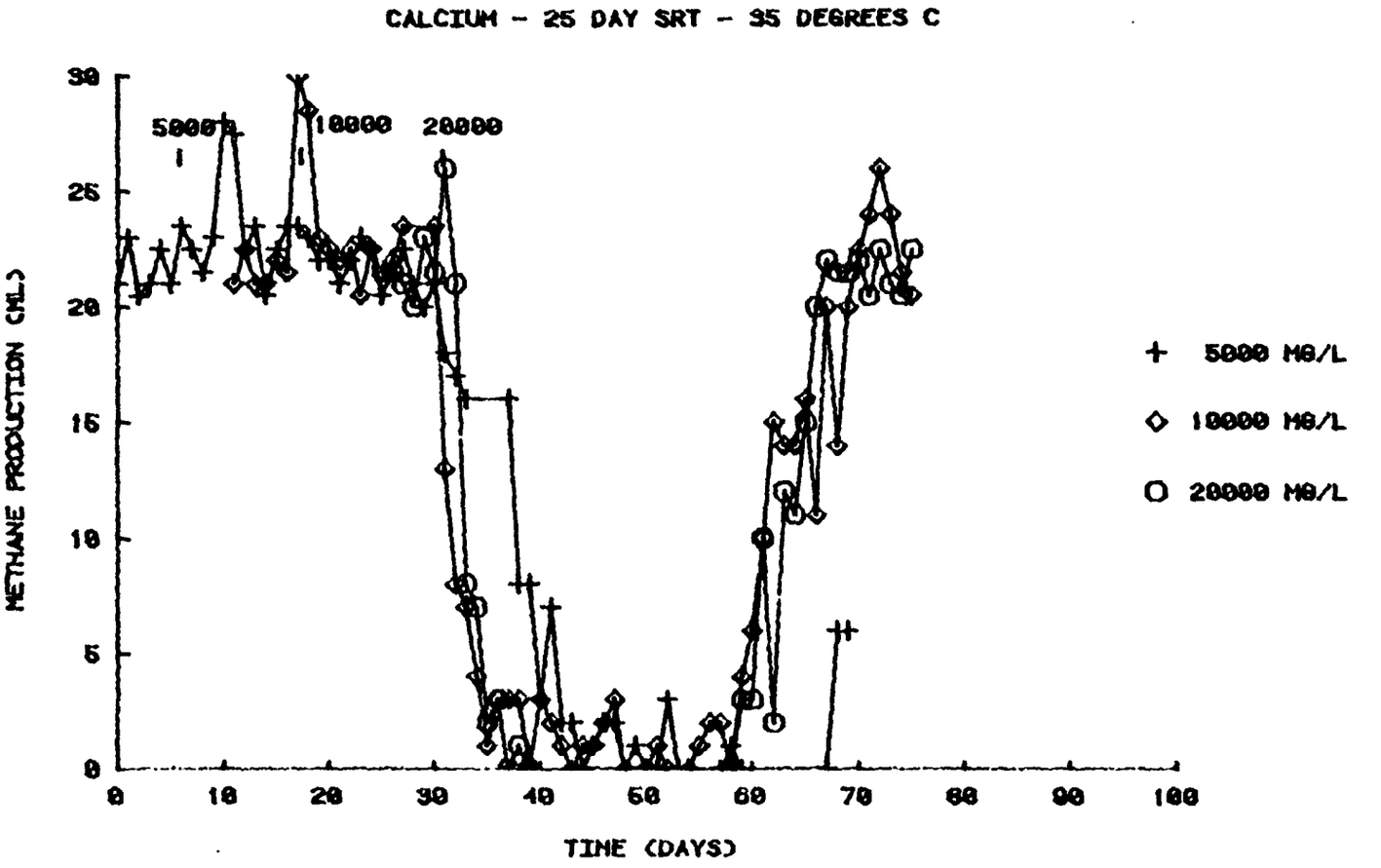

Figure 12. Acclimation of Methanogens to Slug Doses of Calcium

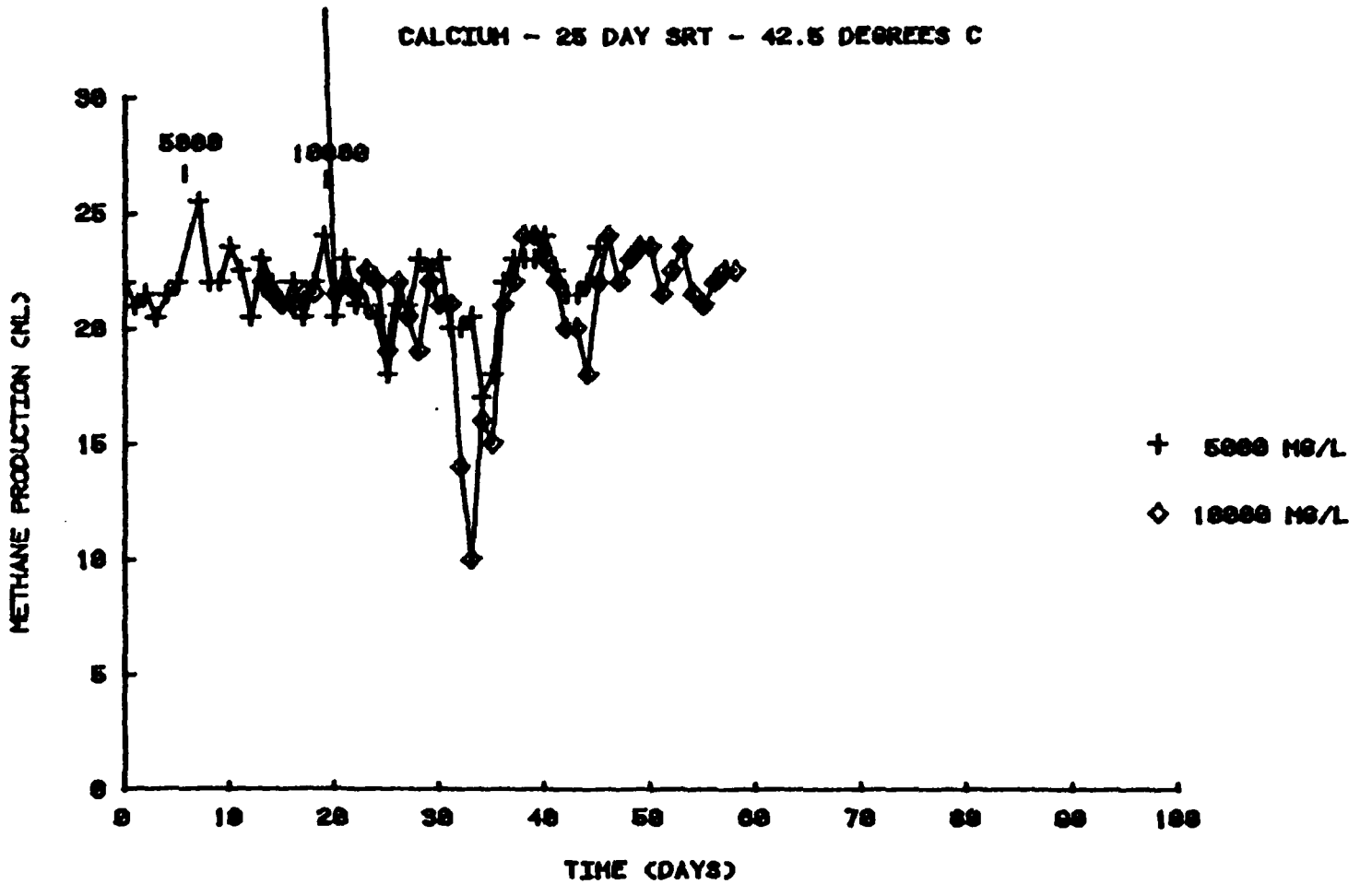

Figure 13. Acclimation of Methanogens to Slug Doses of Calcium

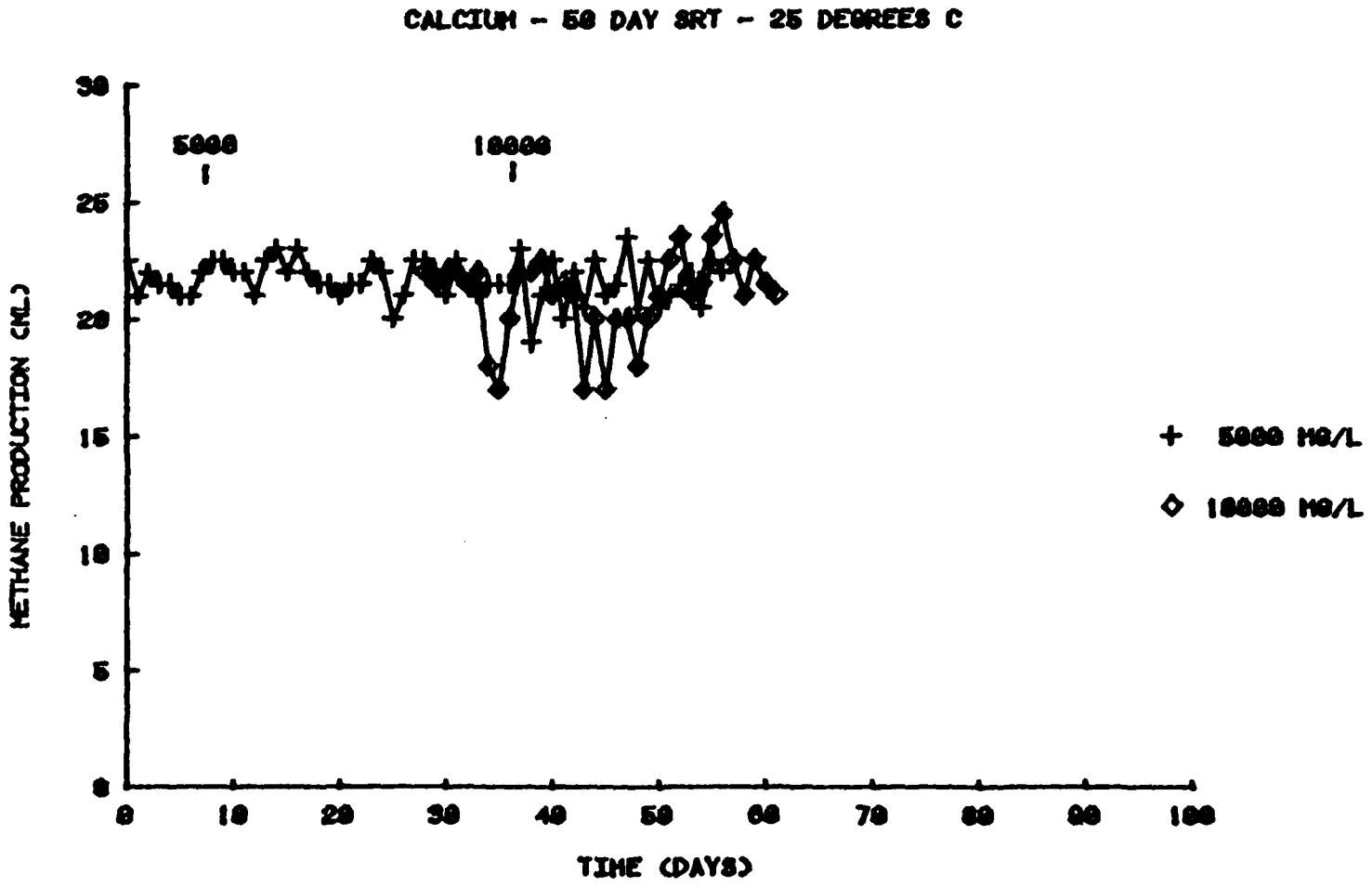

Figure 14. Acclimation of Methanogens to Slug Doses of Calcium

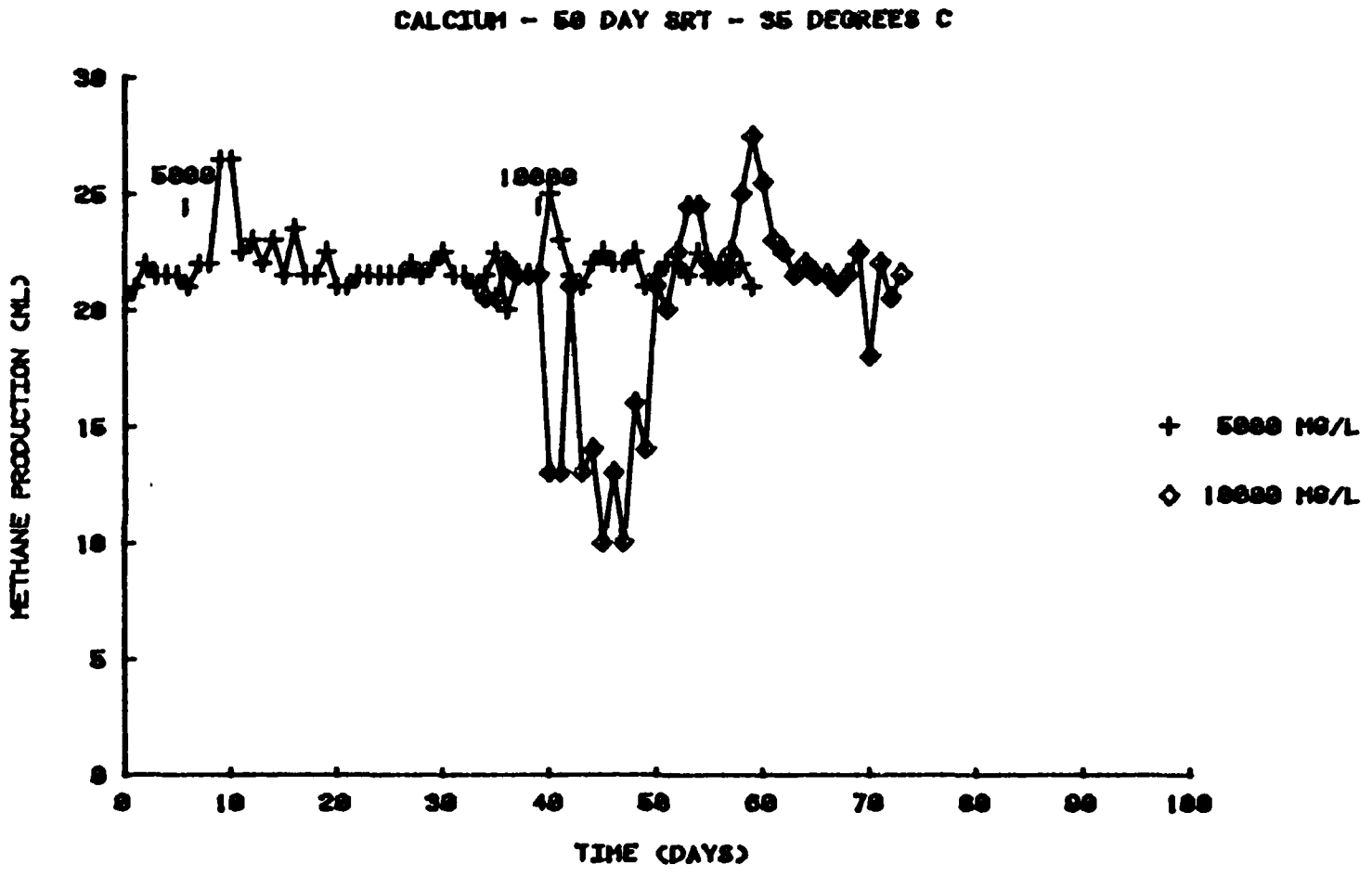

Figure 15. Acclimation of Methanogens to Slug Doses of Calcium

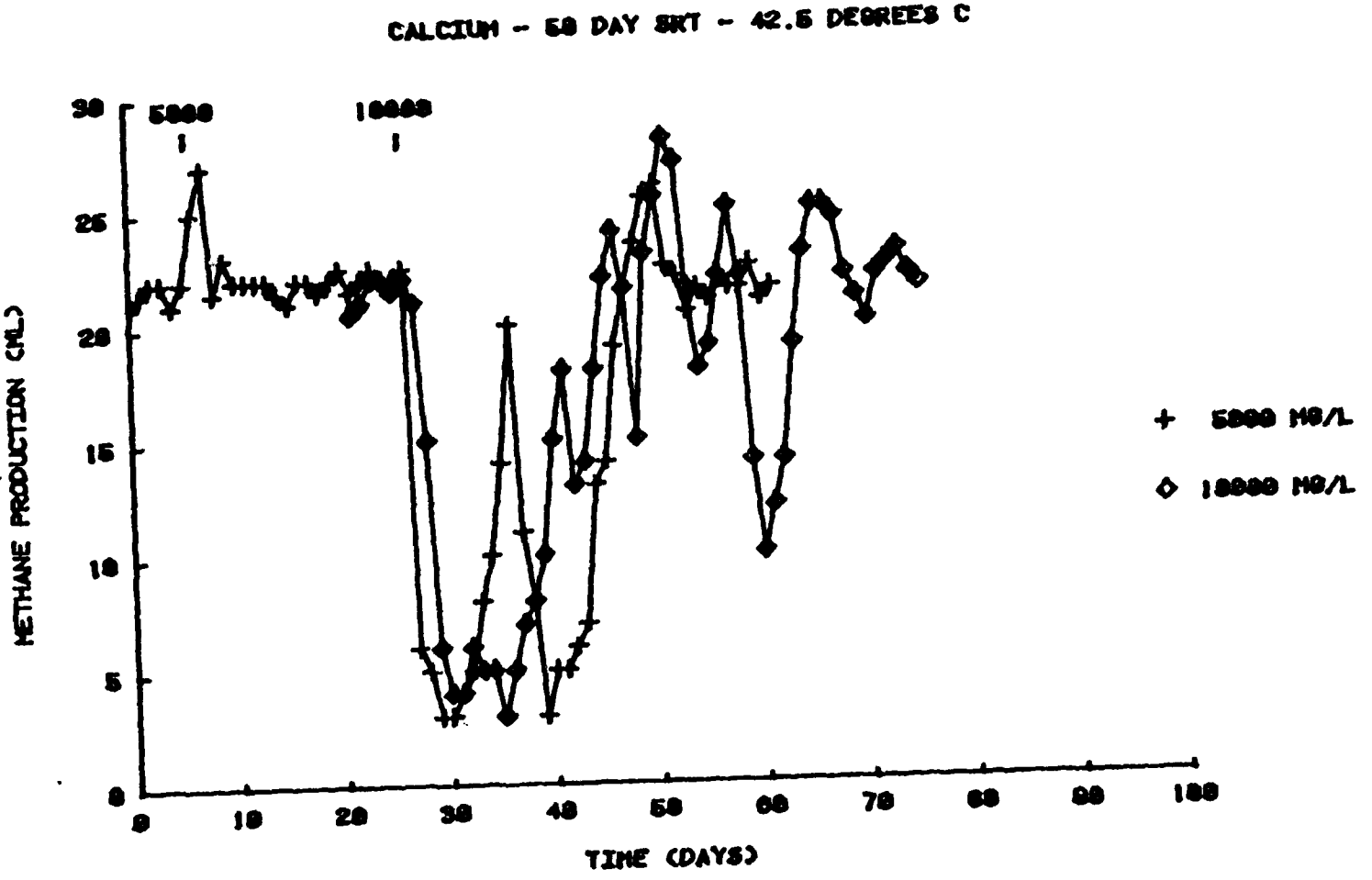

Figure 16. Acclimation of Methanogens to Slug Doses of Calcium

19). An unusually large experimental error could explain this anomaly. Recovery from toxicant exposure generally occurred quickest in serum bottles maintained at 35°C. At 42.5°C, the recoveries were only slightly slower, however, the recovery times and recovery rates at 25°C were considerably slower.

Data shown in Figures 20 to 22 indicate that methanogens can, to a certain degree, acclimate to cadmium exposure.

Chromium (III) (Cr^{+3})

Stock solution of $[Cr(H_2O)_4Cl_3]\cdot 2H_2O$ were used to introduce serum bottle concentrations of 5, 10, 20, 40, 60 and 100 mg/l Cr^{+3}. In the pH range observed during this study (6.5-7.4), chromium III can precipitate as a hydroxide thereby reducing the soluble concentration the methanogens "see." Data presented in Table 1 (BACKGROUND AND LITERATURE REVIEW) demonstrate this penomenon. Soluble Cr^{+3} was not measured in these studies.

Responses to chromium (III) were unusual in that the initial decrease in methane production did not necessarily occur after only one day, as was the case with most other toxicants studied. Responses, expressed as decreased methane production, were delayed longer as initial toxicant concentrations were decreased (Figures 23 to 29).

The effects of SRT and temperature were interdependent. Cultures maintained at 25°C seemed to be better able to cope with chromium (III) exposure when maintaining a 50-day SRT. Responses by 35°C cultures did not vary significantly with changing SRT; the 15-day system exhibited a somewhat more severe response. However, serum bottles kept at 42.5°C were less severely affected with a 25-day SRT.

At 35°C, the response to chromium (III) was least severe. Cultures reacted a little more severely at 42.5°C, and those bottles kept at 25°C showed the most severe response pattern.

Although there was some indication of acclimation potential for some systems (Figures 30, 34, 35), the data do not permit a firm conclusion (Figures 30 to 36). Upon exposure to a second or third slug dose, the responsive delays generally became much shorter, but in some cases were more severe. Thus, using time after toxicant exposure required to resume normal gas production as a criteria, acclimation was demonstrated at 42.5°C and

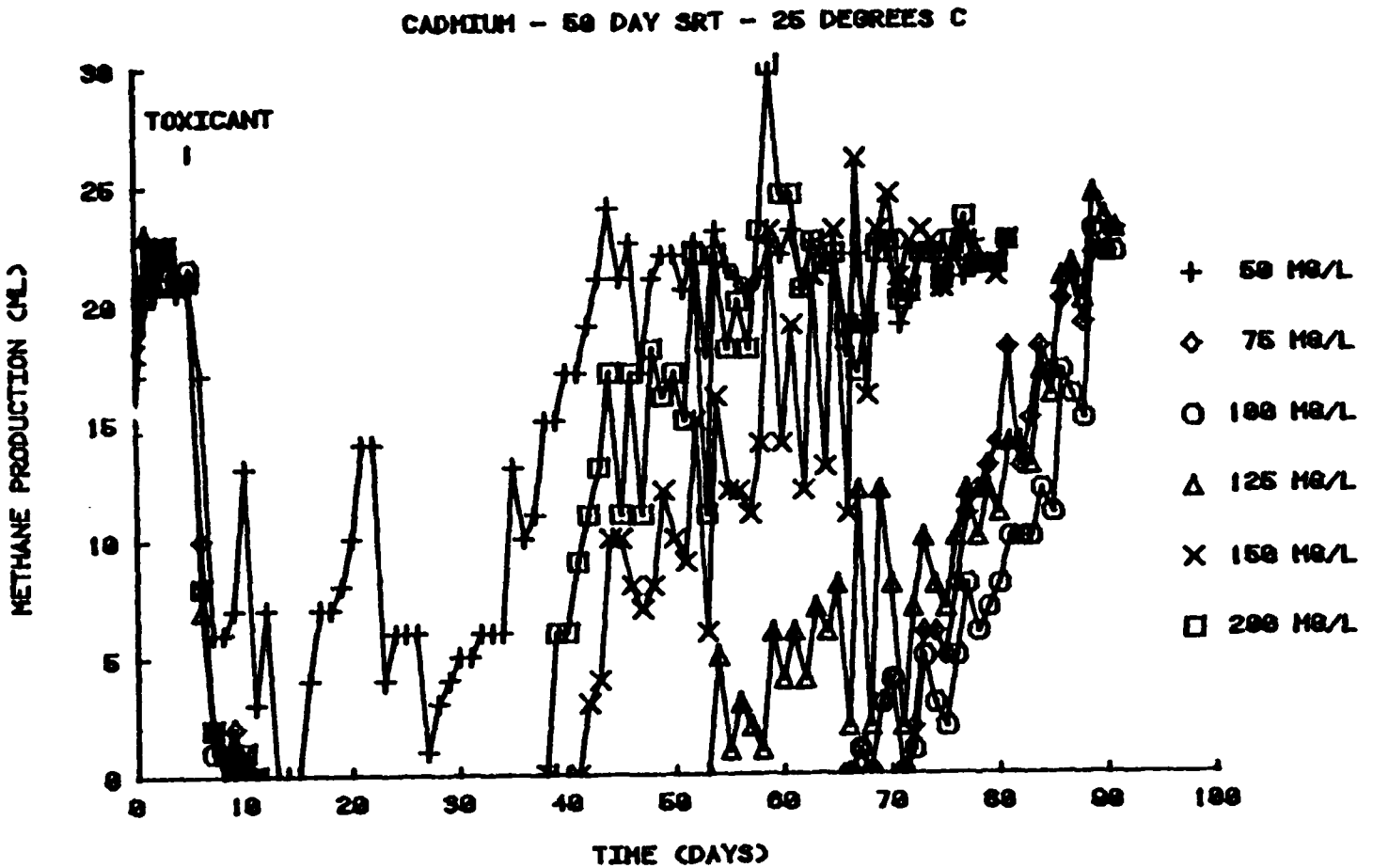

Figure 17. Response of Methanogens to Slug Doses of Cadmium

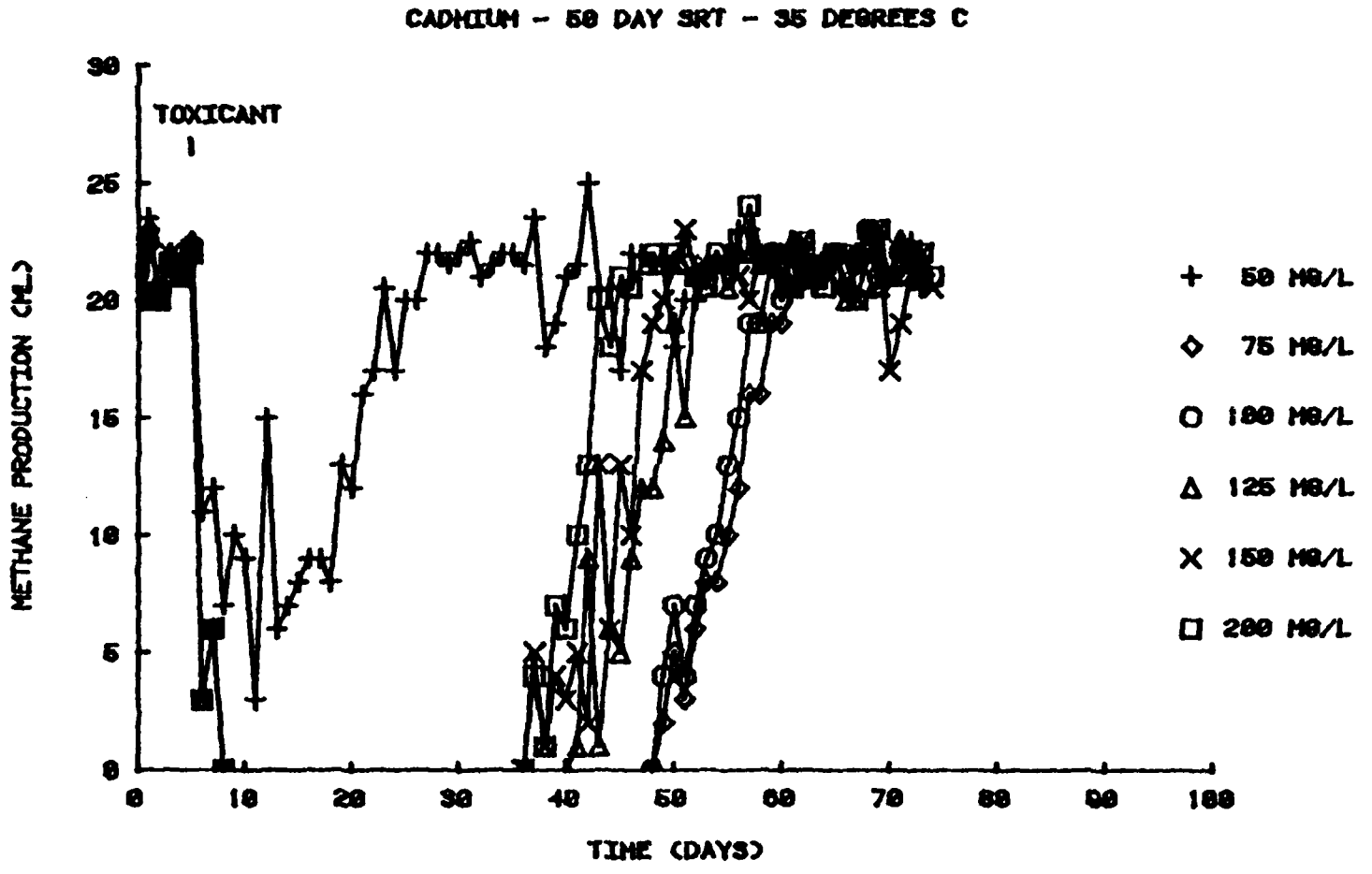

Figure 18. Response of Methanogens to Slug Doses of Cadmium

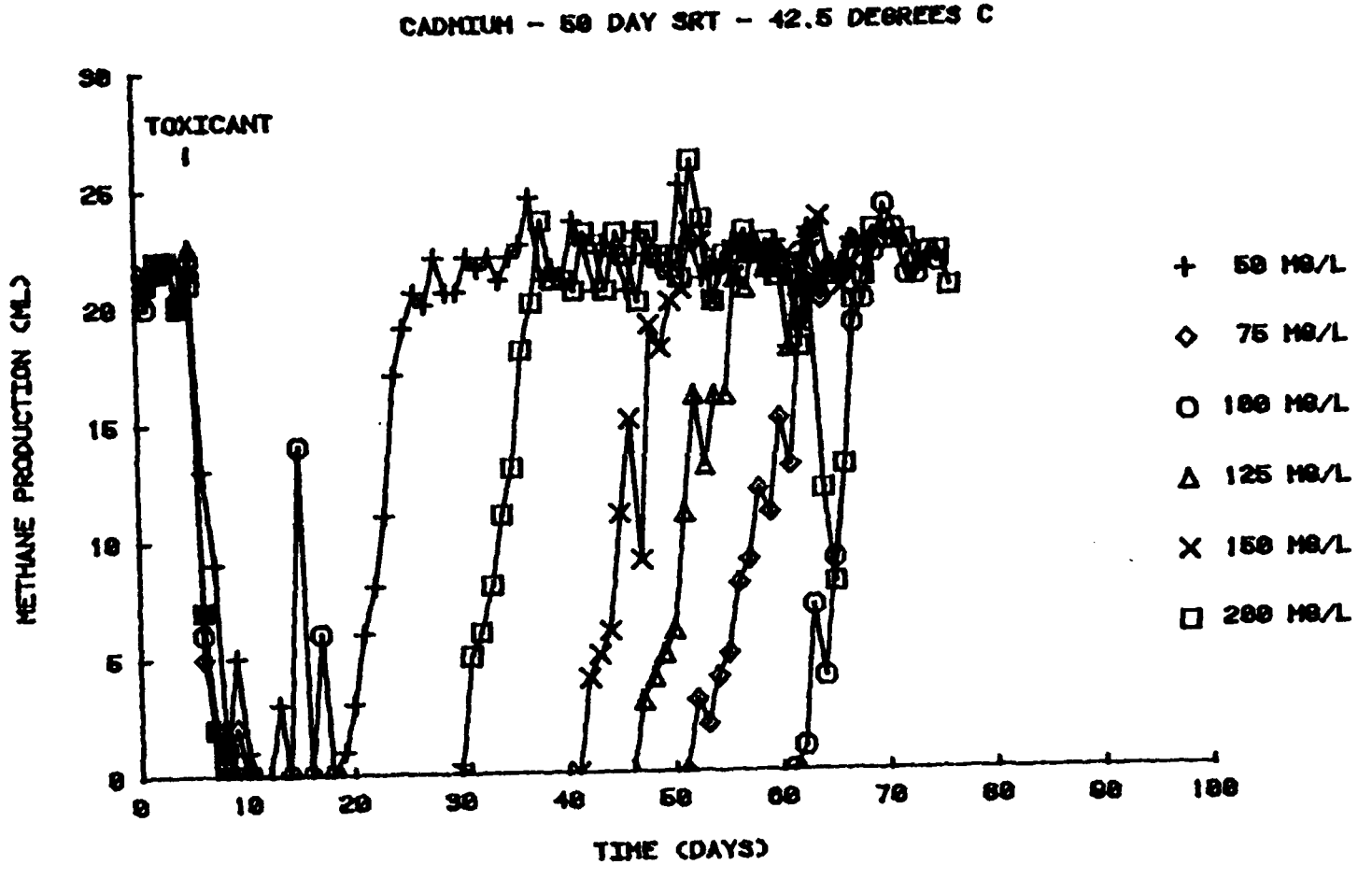

Figure 19. Response of Methanogens to Slug Doses of Cadmium

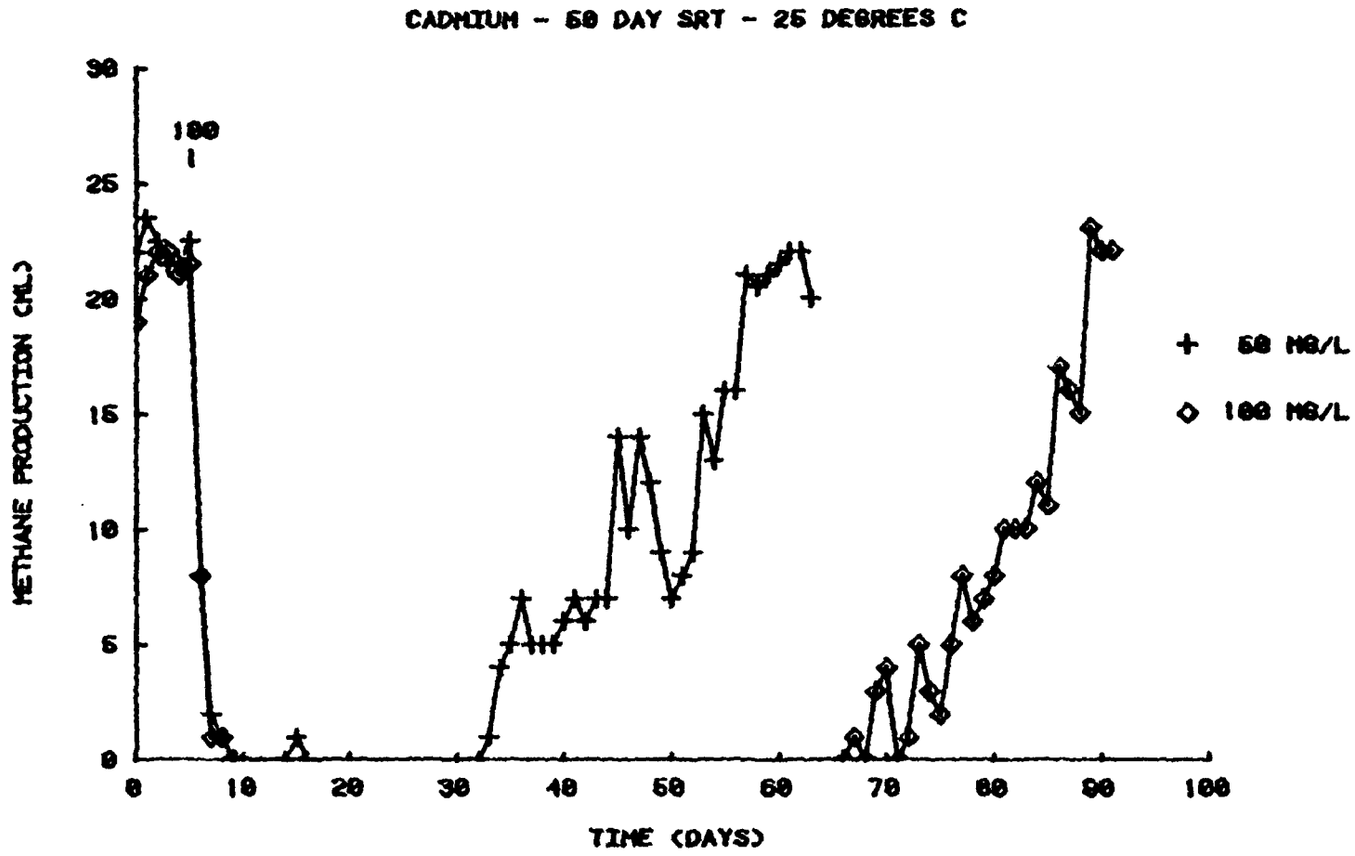

Figure 20. Acclimation of Methanogens to Slug Doses of Cadmium

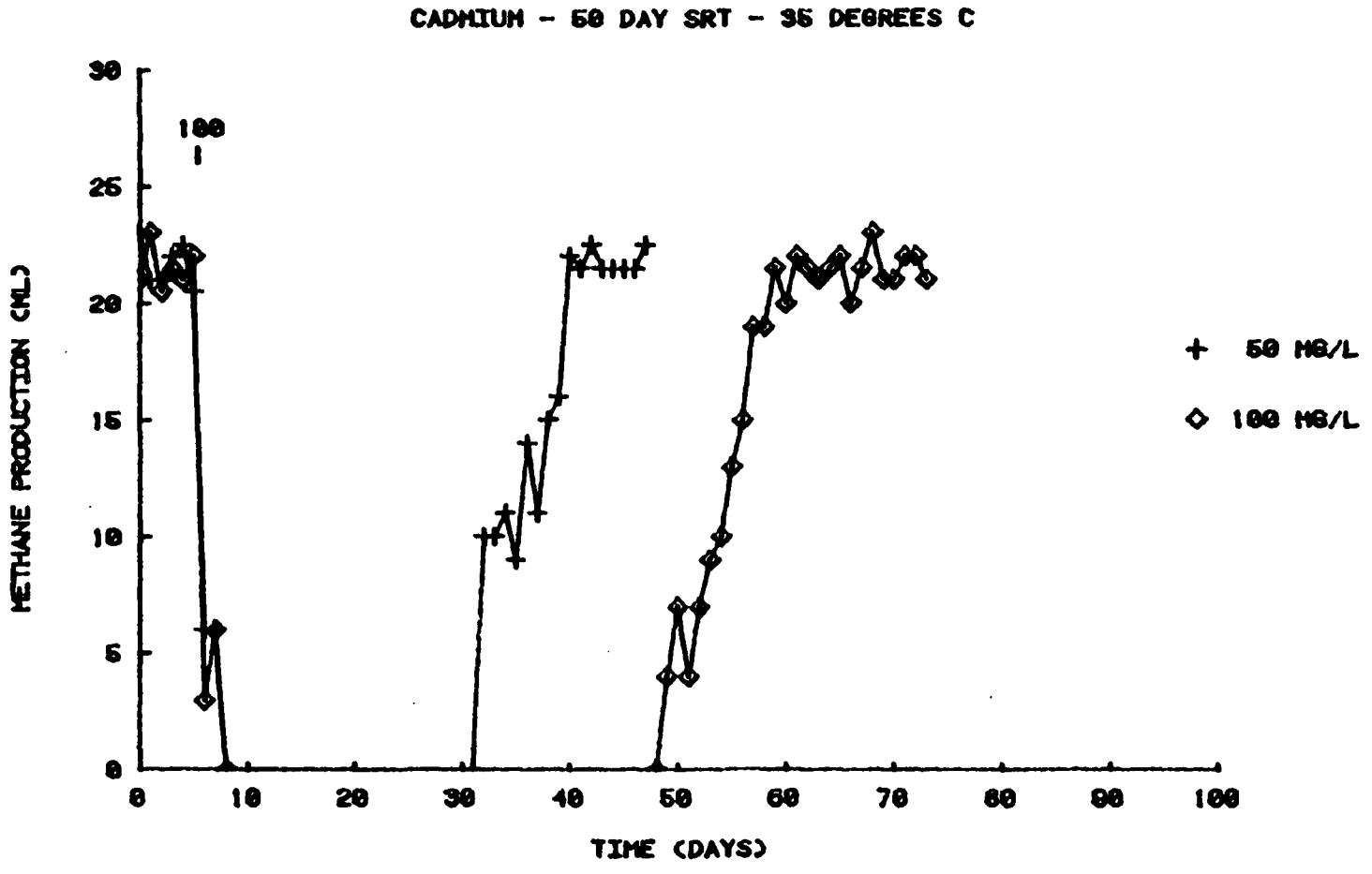

Figure 21. Acclimation of Methanogens to Slug Doses of Cadmium

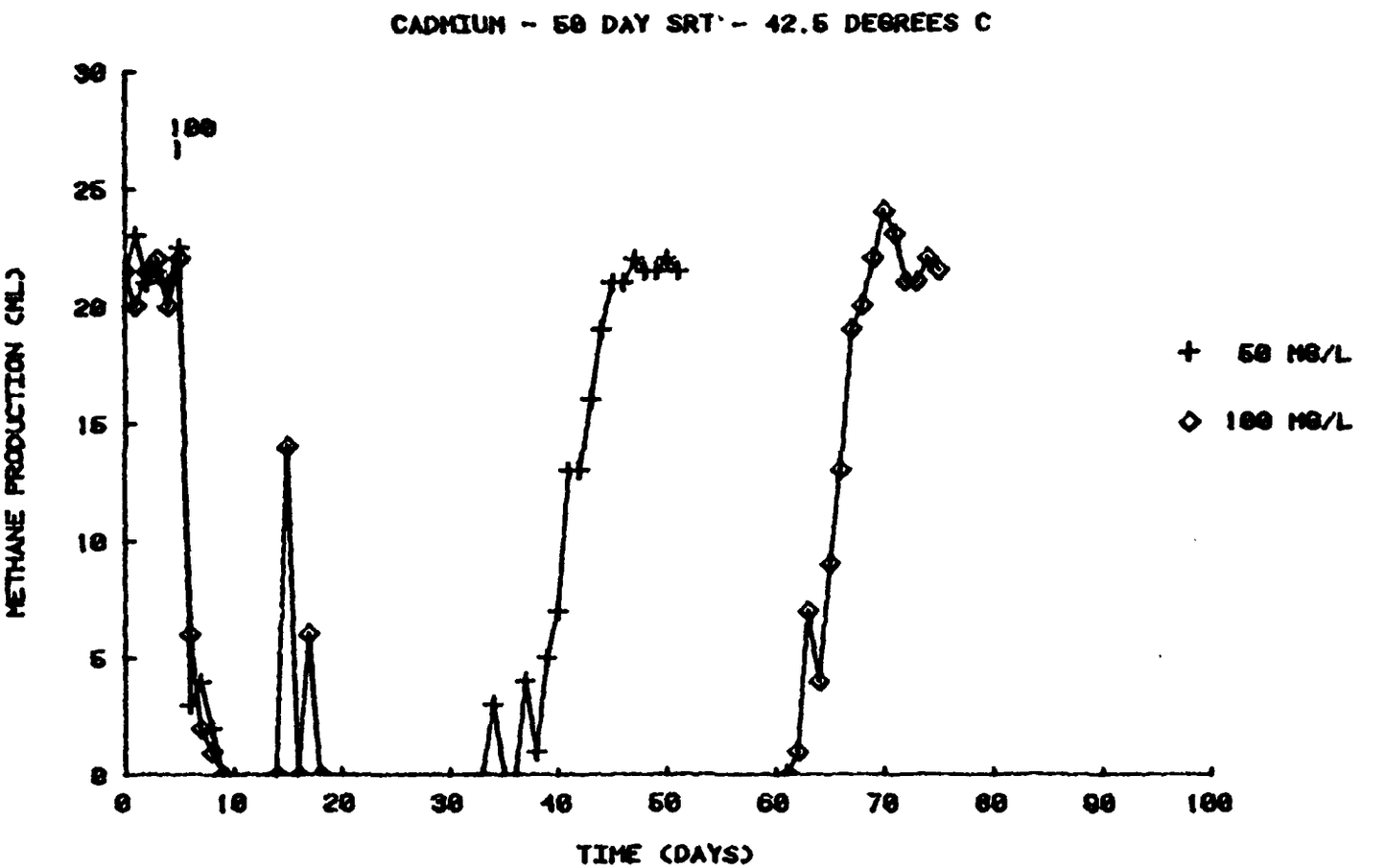

Figure 22. Acclimation of Methanogens to Slug Doses of Cadmium

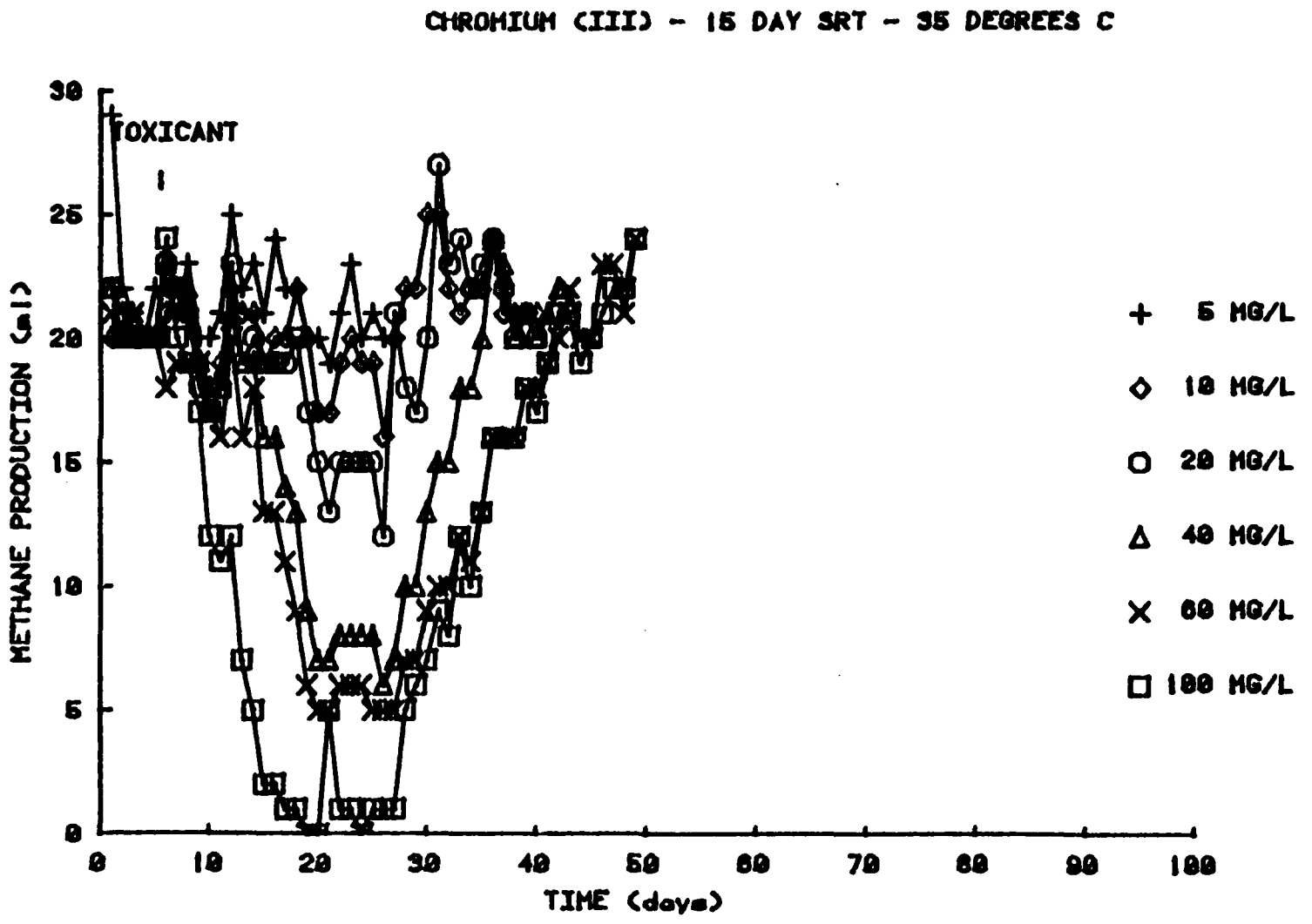

FIGURE 23. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

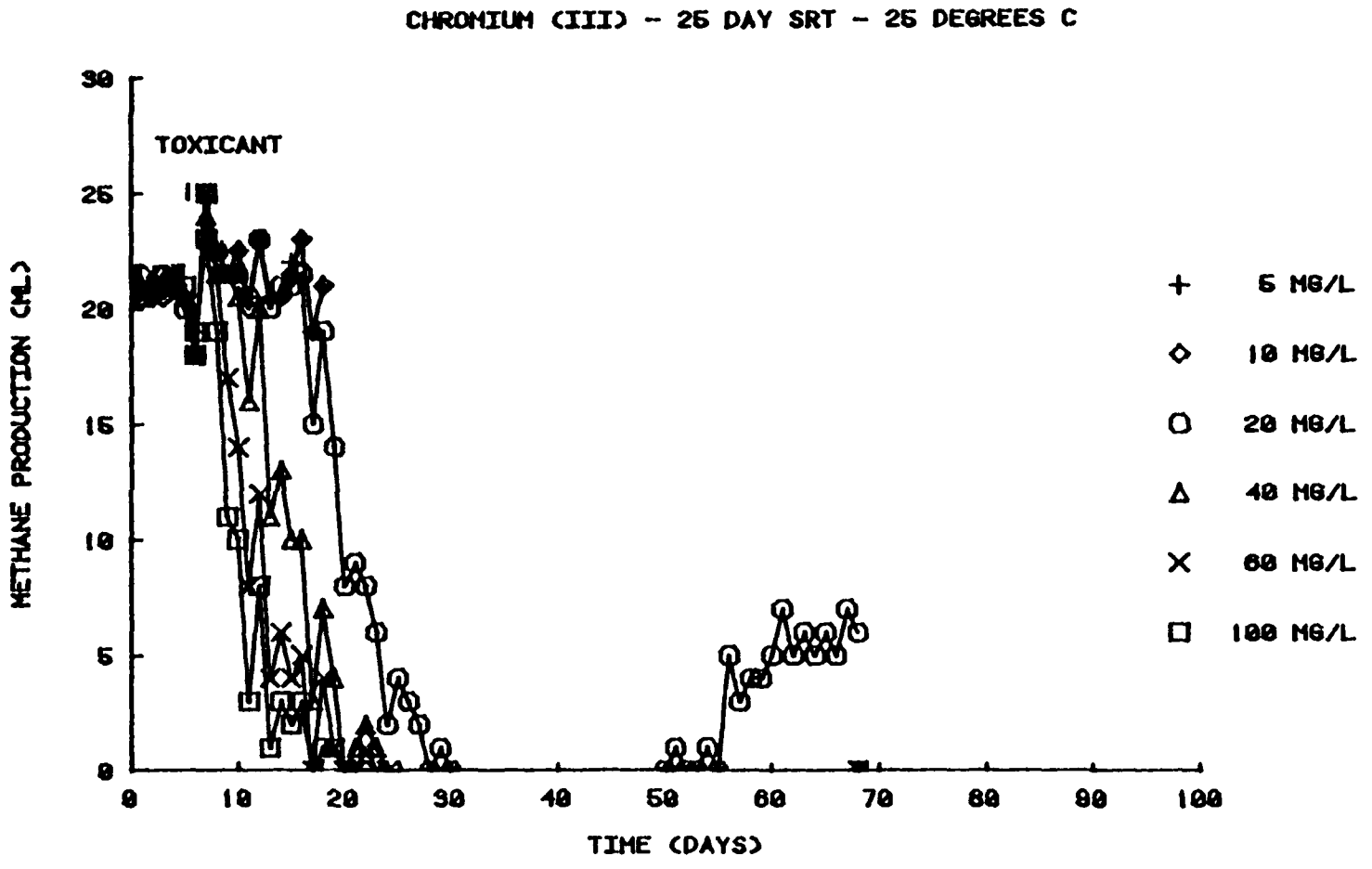

FIGURE 24. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

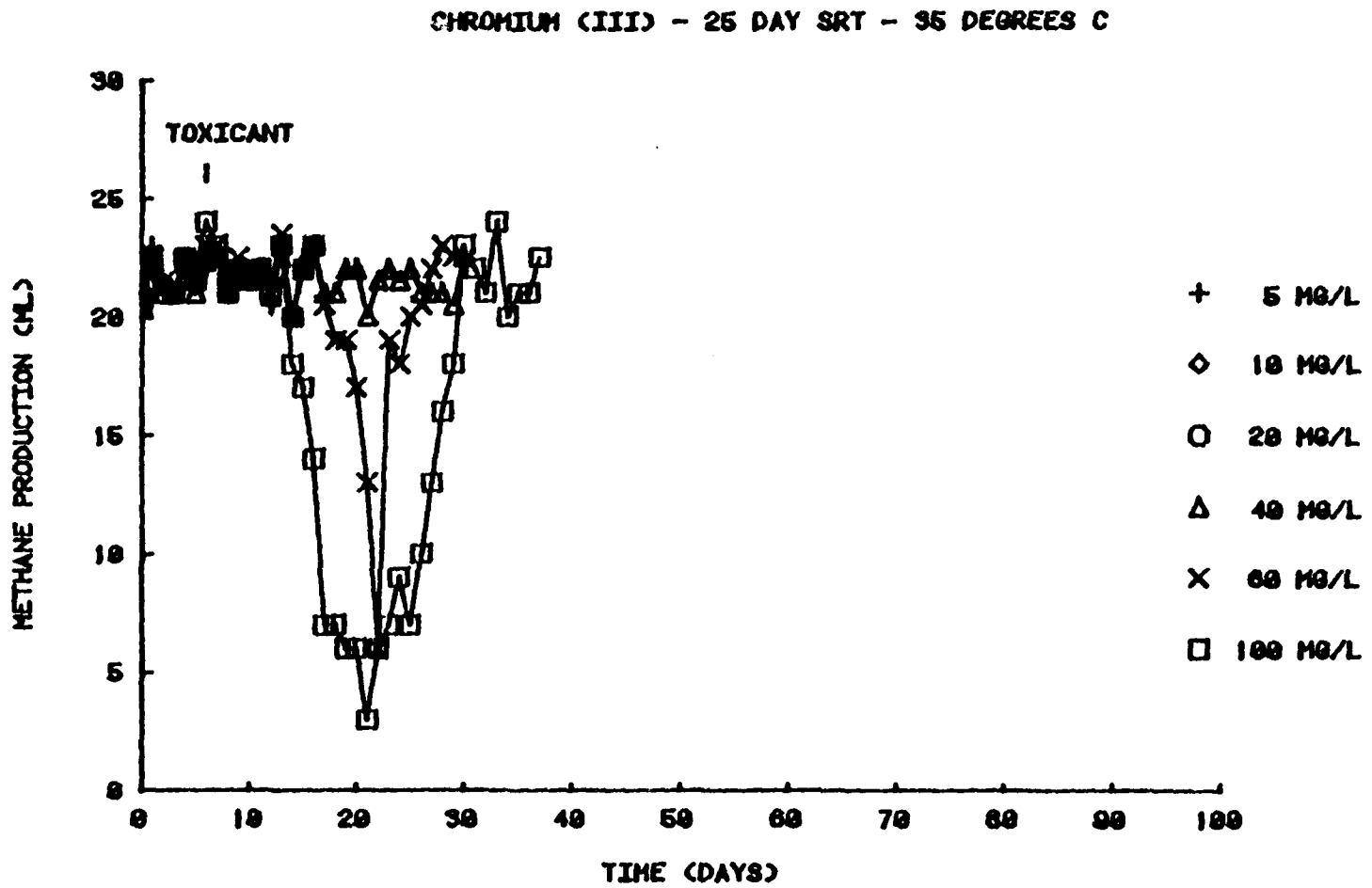

FIGURE 25. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

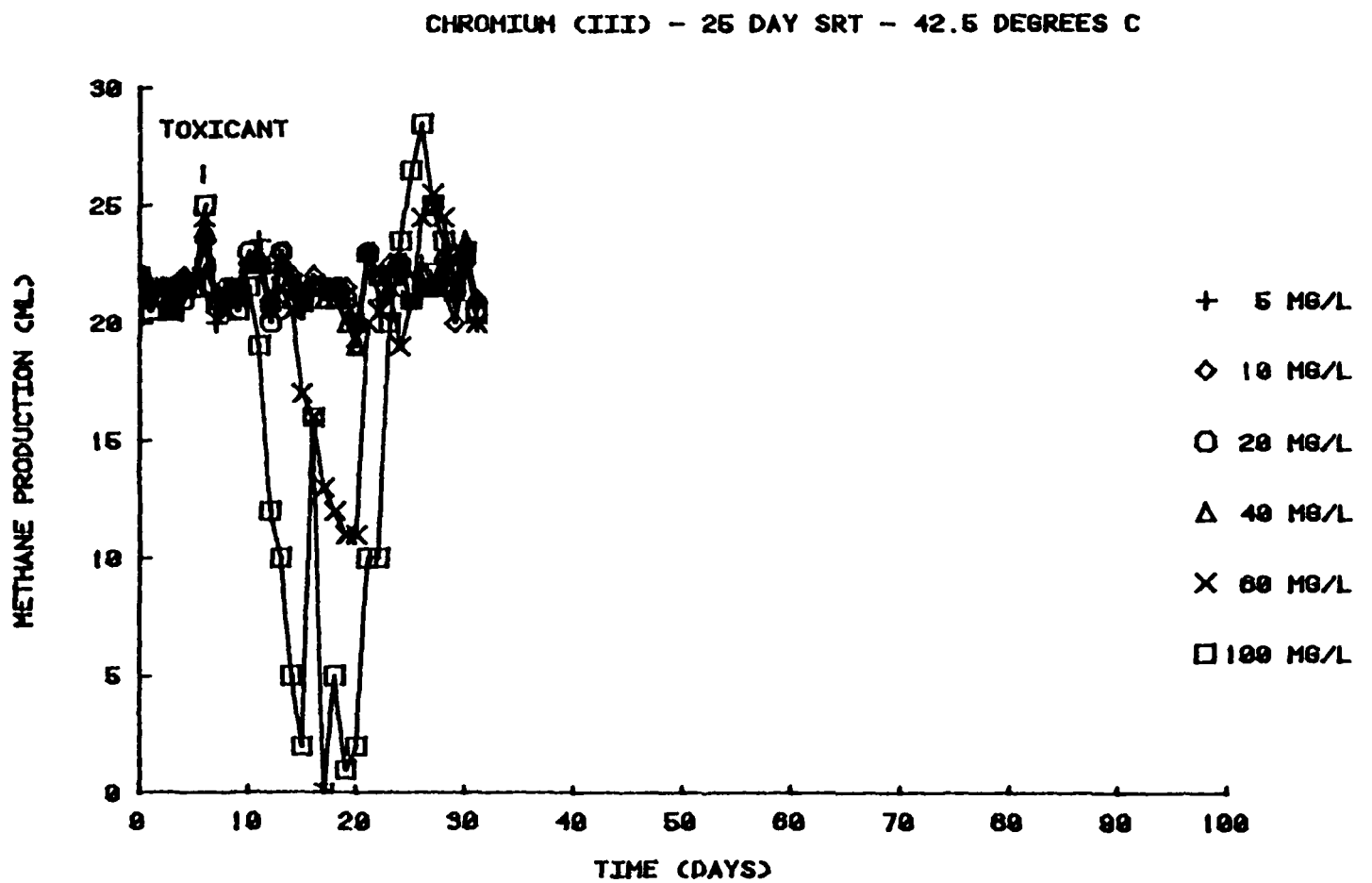

FIGURE 26. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

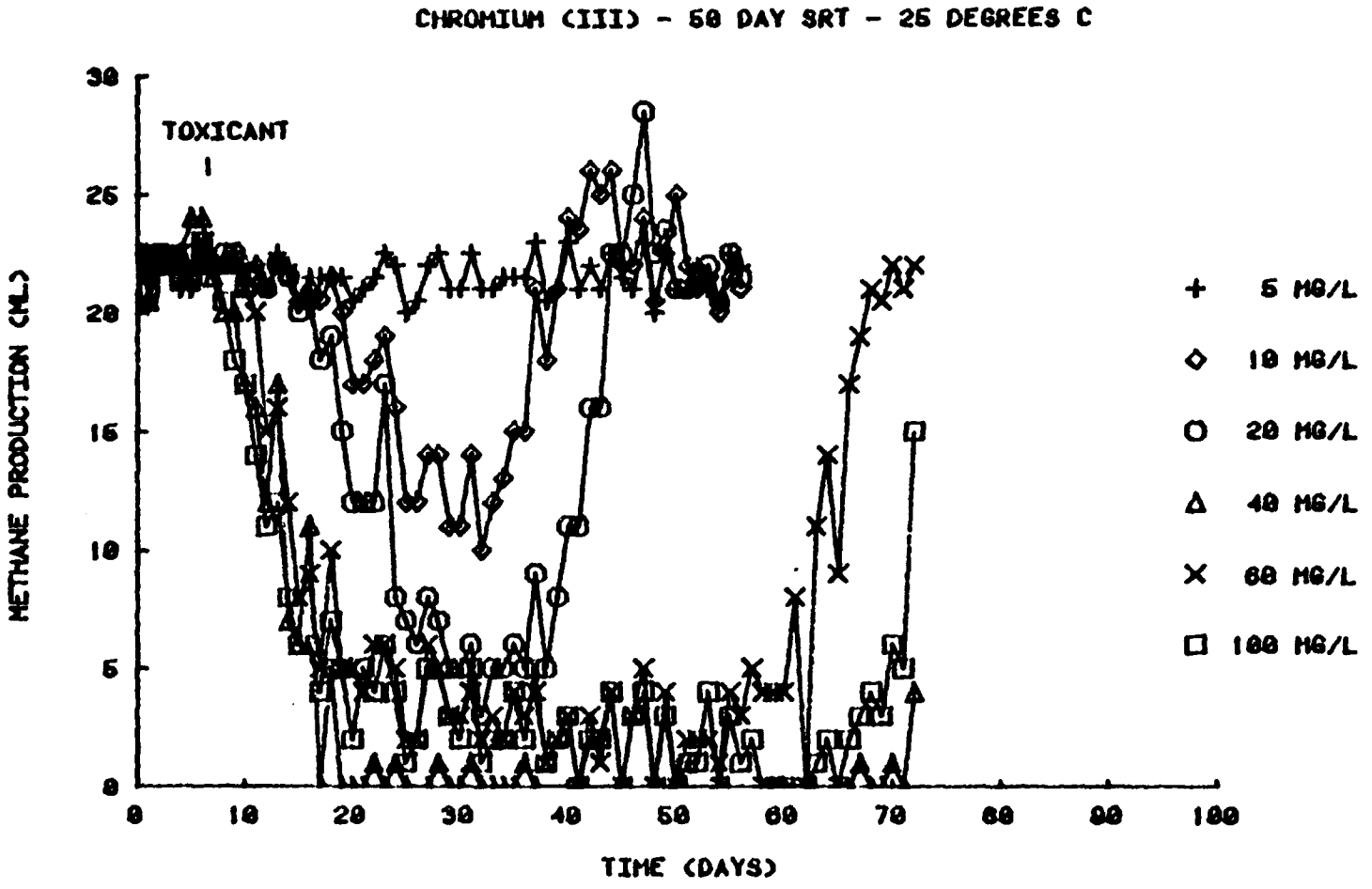

FIGURE 27. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

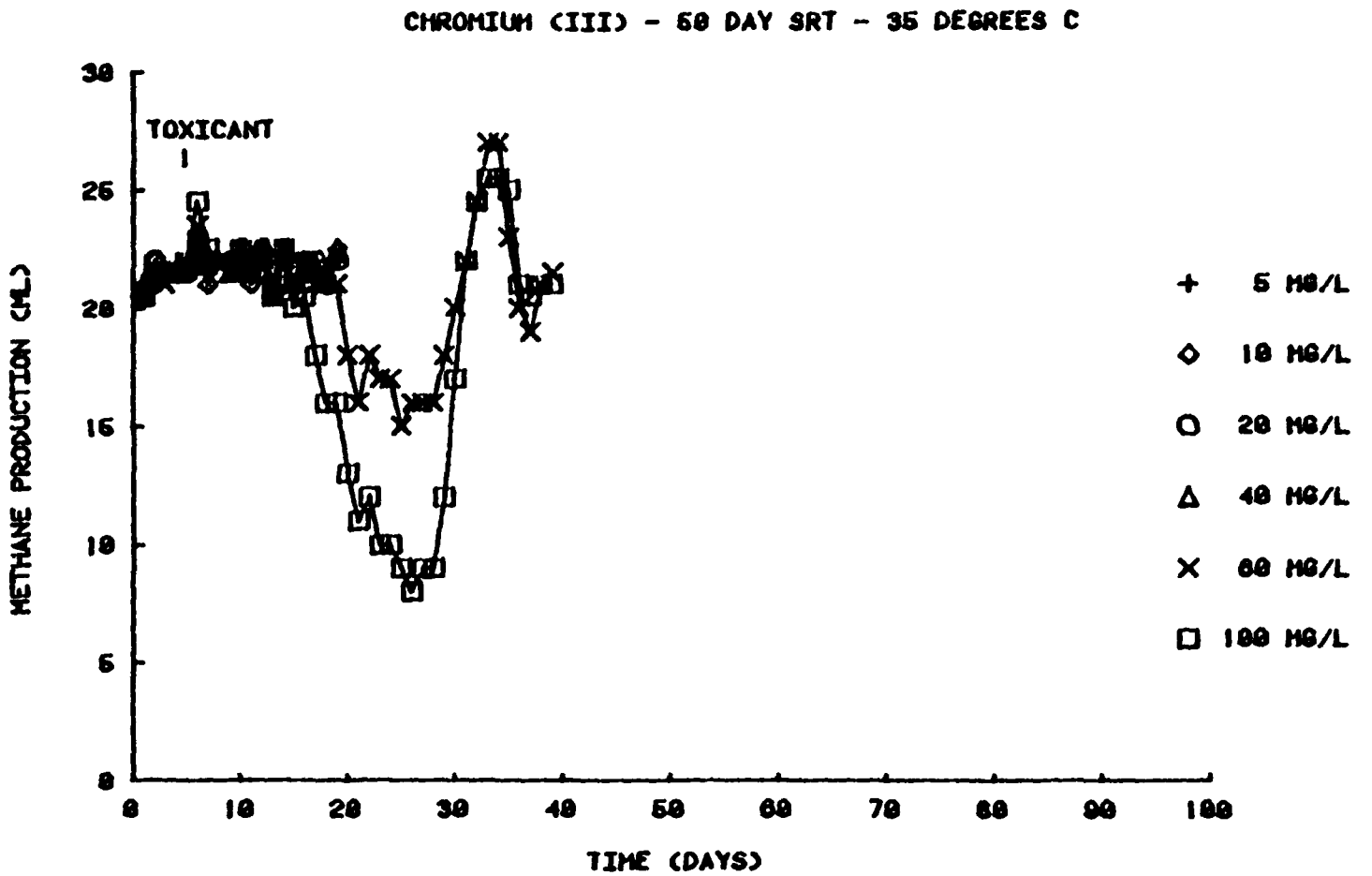

FIGURE 28. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

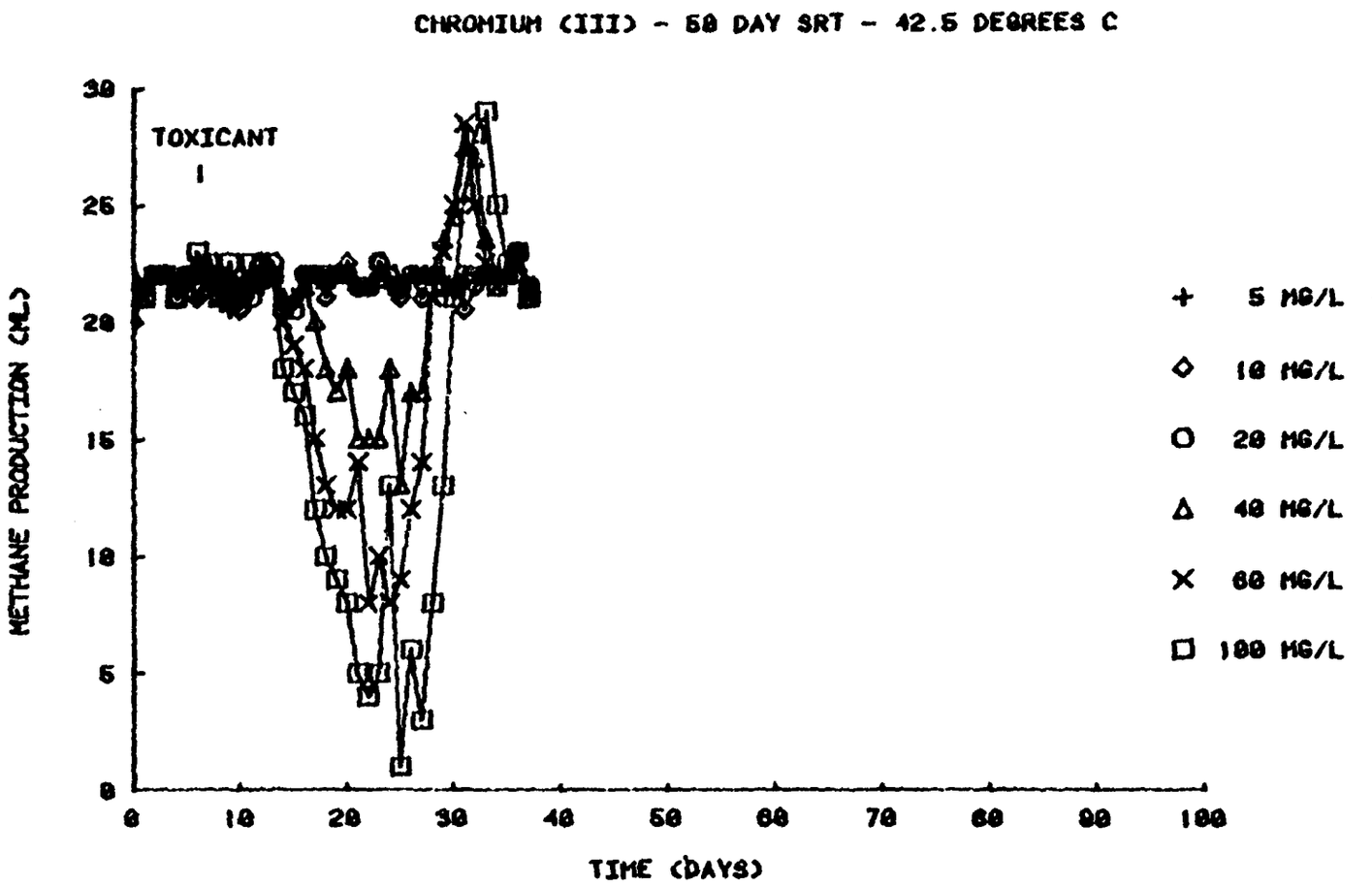

FIGURE 29. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

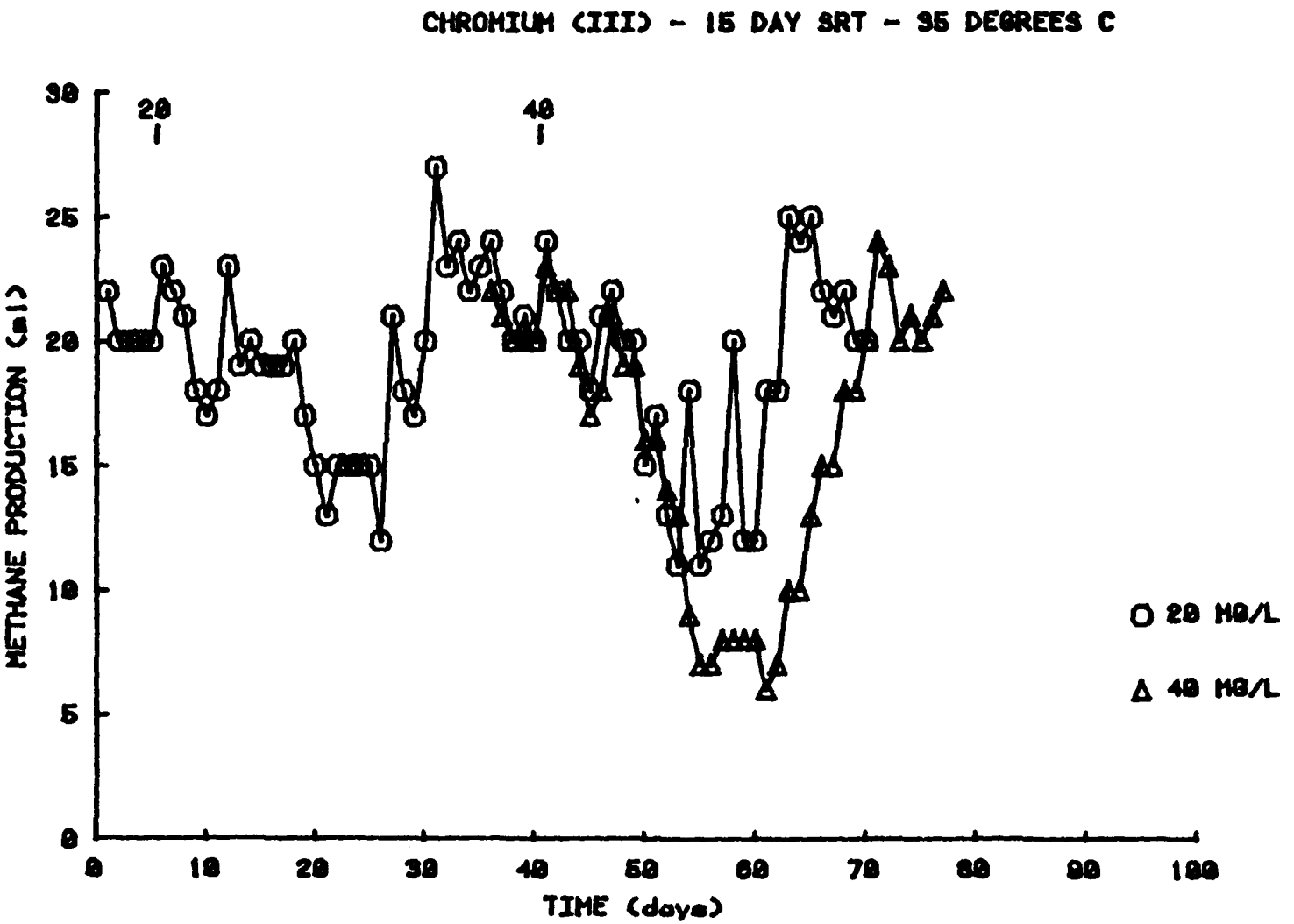

FIGURE 30. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

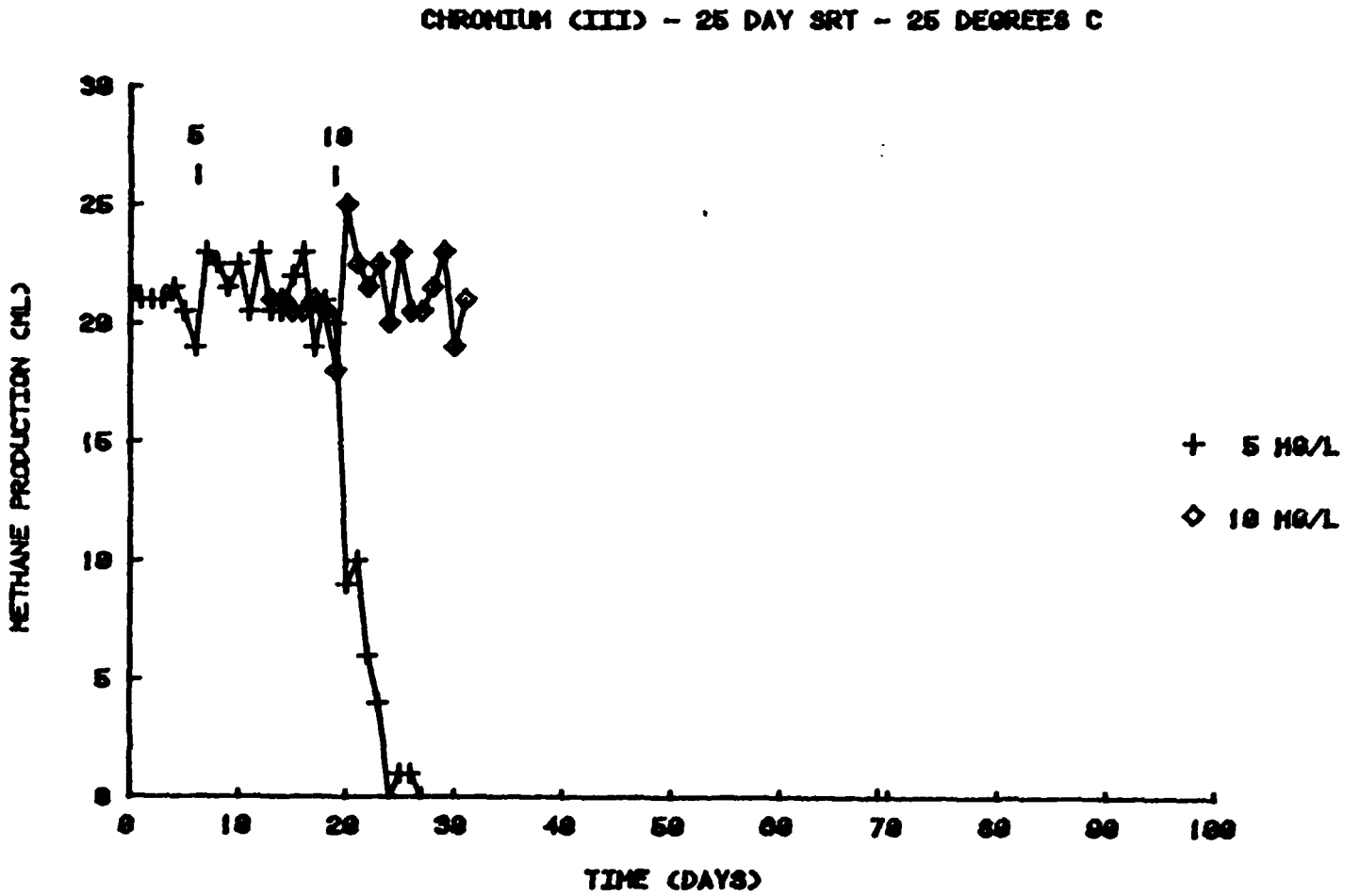

FIGURE 31. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

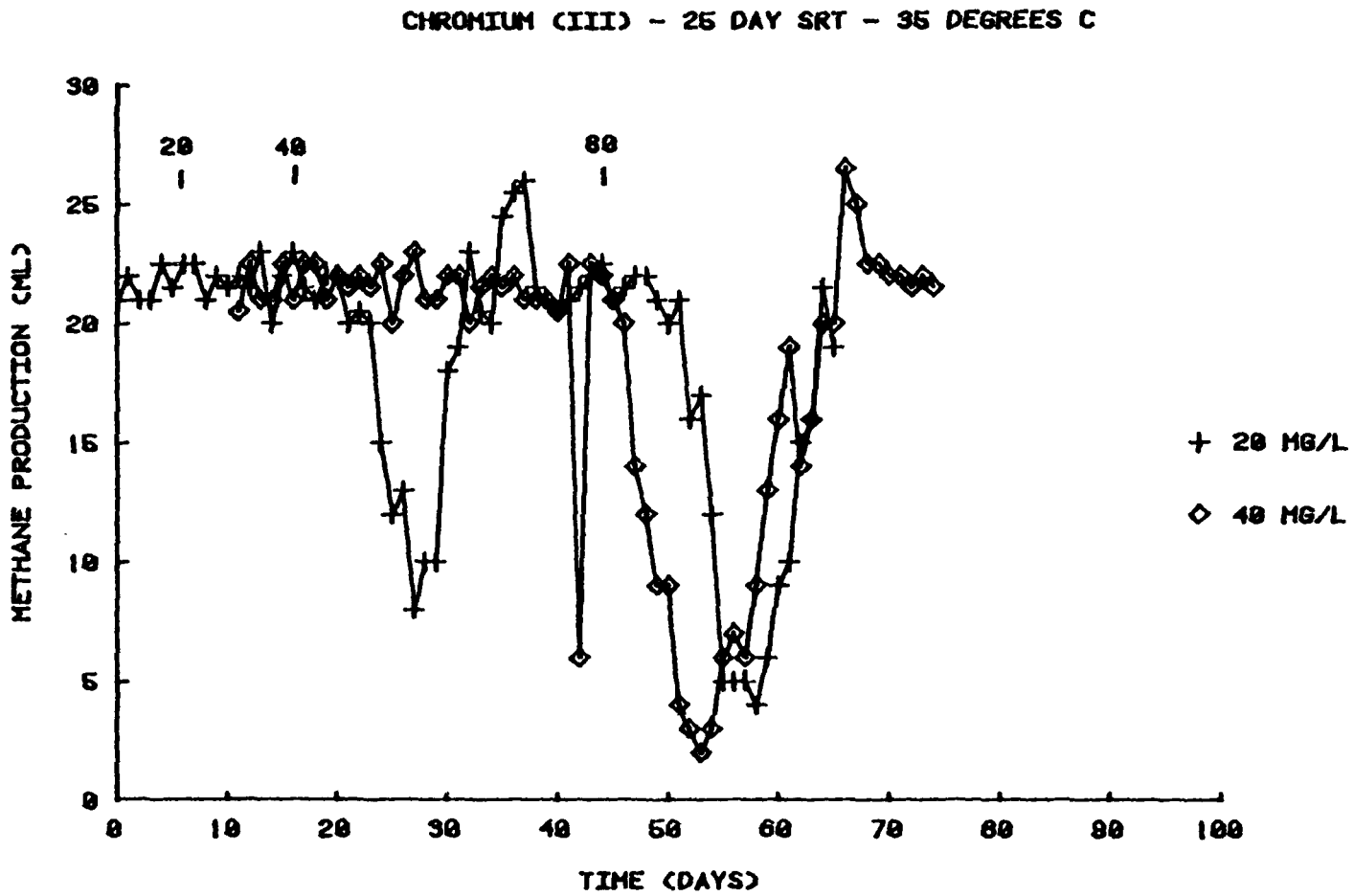

FIGURE 32. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

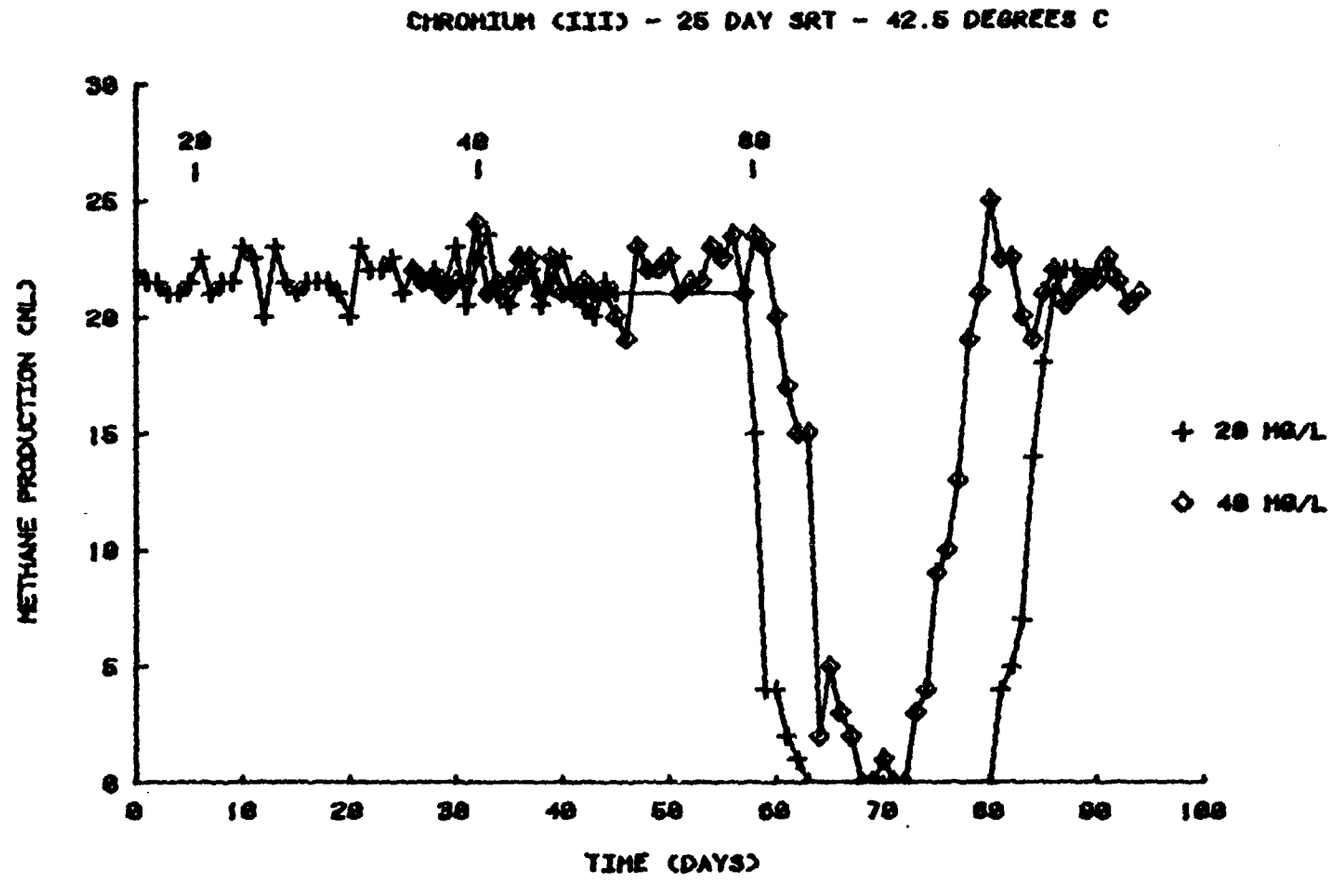

FIGURE 33. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

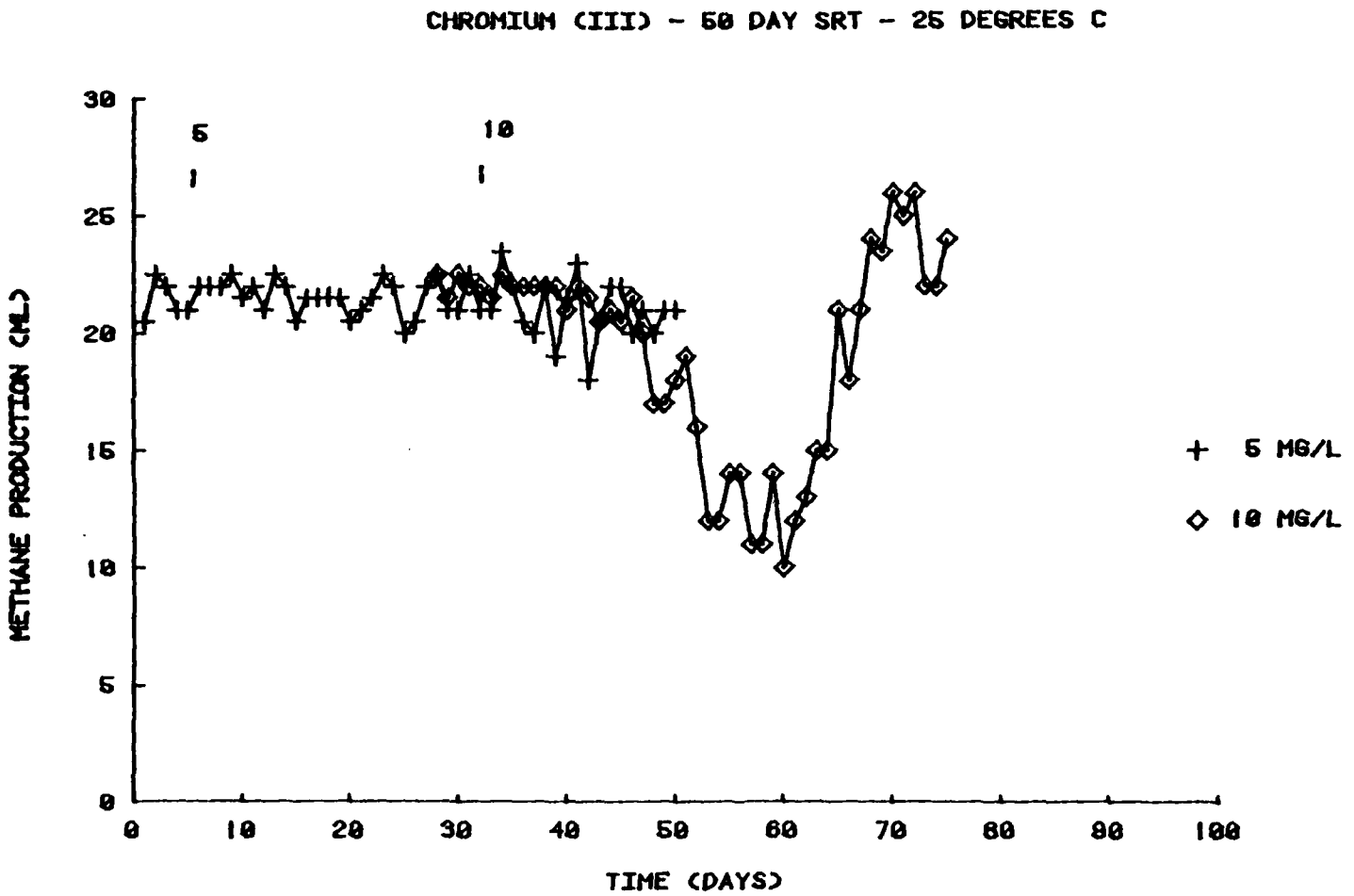

FIGURE 34. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

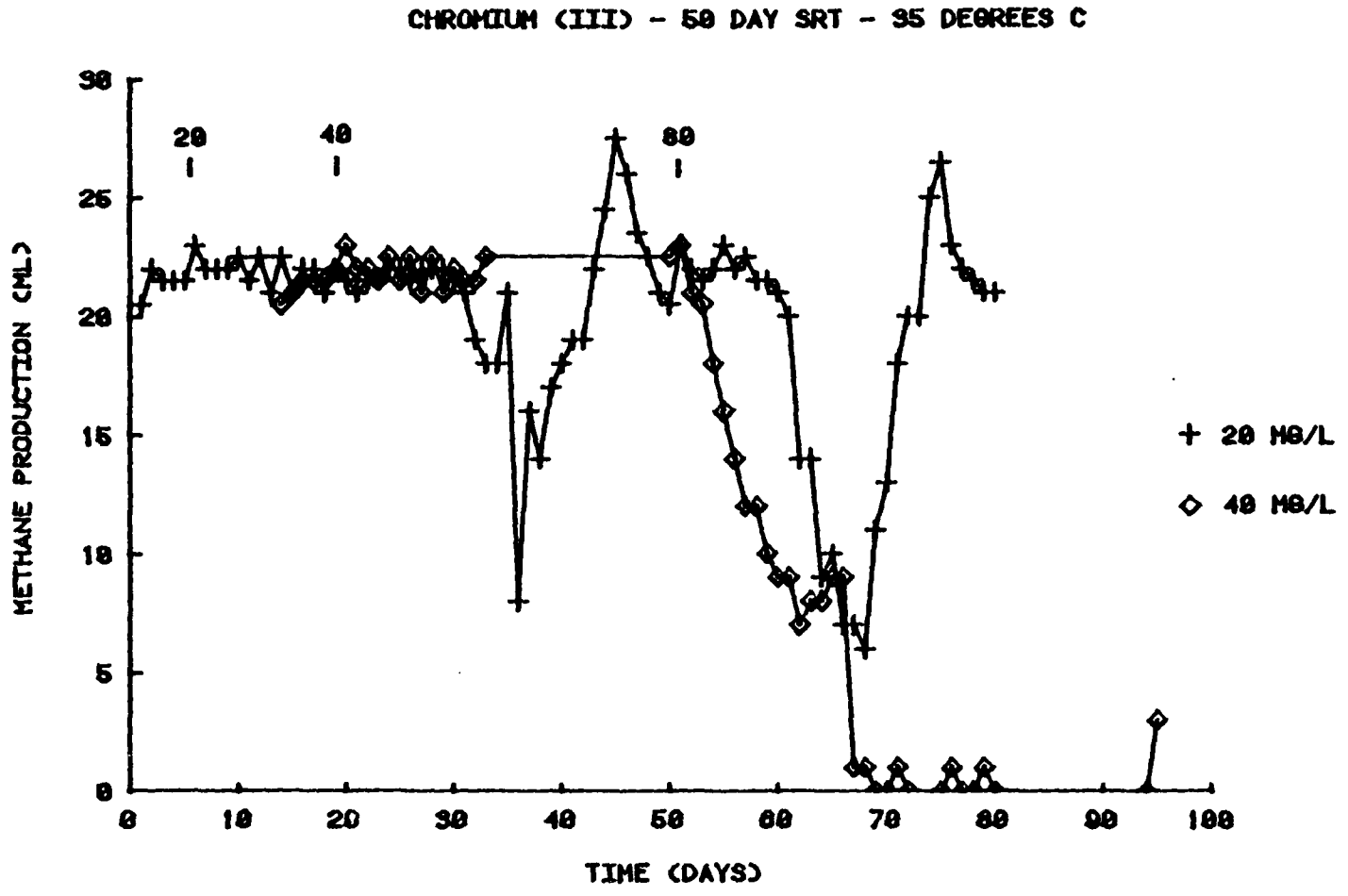

FIGURE 35. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

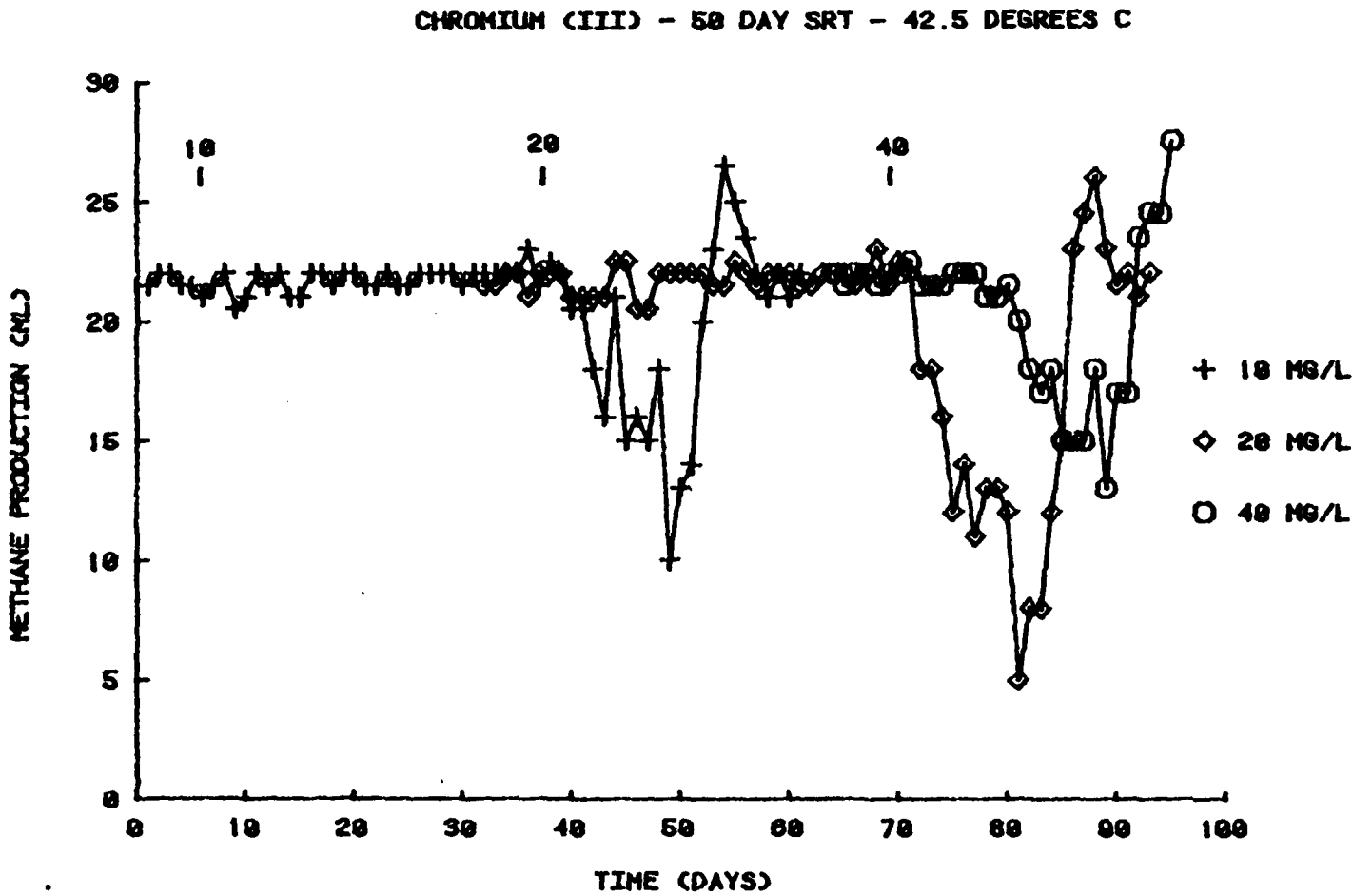

FIGURE 36. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM III

50-day SRT. However, if the period of reduced gas production is used as the criterion, acclimation did not occur (Figure 36).

Chromium (VI) (Cr^{+6})

Slug dose concentrations of 5, 10, 20, 40, 60 and 100 mg/l Cr^{+6} were introduced to serum bottle cultures. The stock chromium (VI) solutions were prepared from Na_2CrO_4. Cr^{6+} can be reduced to Cr^{3+} in the anaerobic environment of the serum bottle. The fact that chemical reactions take place under such conditions is demonstrated by data in Table 1, which shows that of the 420 mg/l of Cr^{6+} present in a digester, only 3 mg/l was soluble. No attempt was made to ascertain the chemical fate of added Cr^{6+}.

Sharp and immediate reductions in methane generation resulted from the toxicant exposure. Recovery from the lower concentrations began after only a few days of exposure and proceeded at a rapid rate. Larger slug doses caused longer periods of low gas production and significantly slower recovery rates (Figures 37 to 43).

The dependence of toxicant response patterns on SRT again was shown to vary with temperature. The differences at 25°C between the 25-day and 50-day SRT cultures is not clear (Figures 38 and 41), but the 15-day SRT appears to be preferred by 35°C cultures (Figures 37, 39 and 42). At 42.5°C, responses to lower chromium (VI) concentrations are less severe with a 50-day SRT, but when long periods of zero gas production result from exposure to higher concentrations, the recovery appears faster in the 25-day SRT bottles (Figures 40 and 43).

As with chromium (III) exposure, the order of temperatures resulting in increasingly severe responses was 35°C, 42.5°C, and 25°C. Again the 25°C cultures seem to have been more severely affected by the toxicant than bottles maintained at the higher temperatures.

There was no evidence of acclimation to chromium (VI) at any combination of SRT and temperature (Figures 44 to 50). Responses to the toxicant appear to be independent of previous toxicant exposure, except that a build-up of chromium (VI) may have caused a more severe response as repetitive slug-dose concentrations were increased.

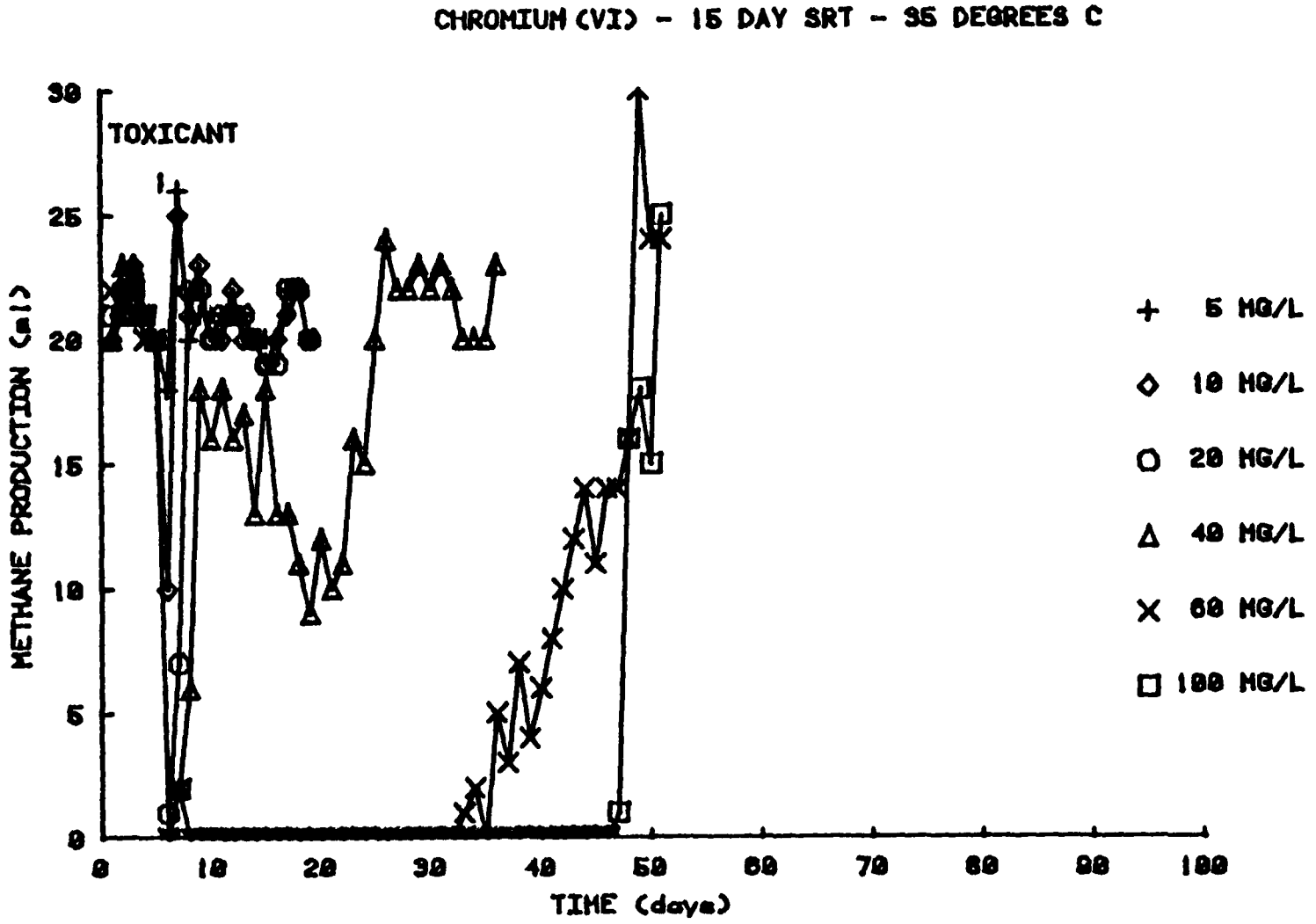

FIGURE 37. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

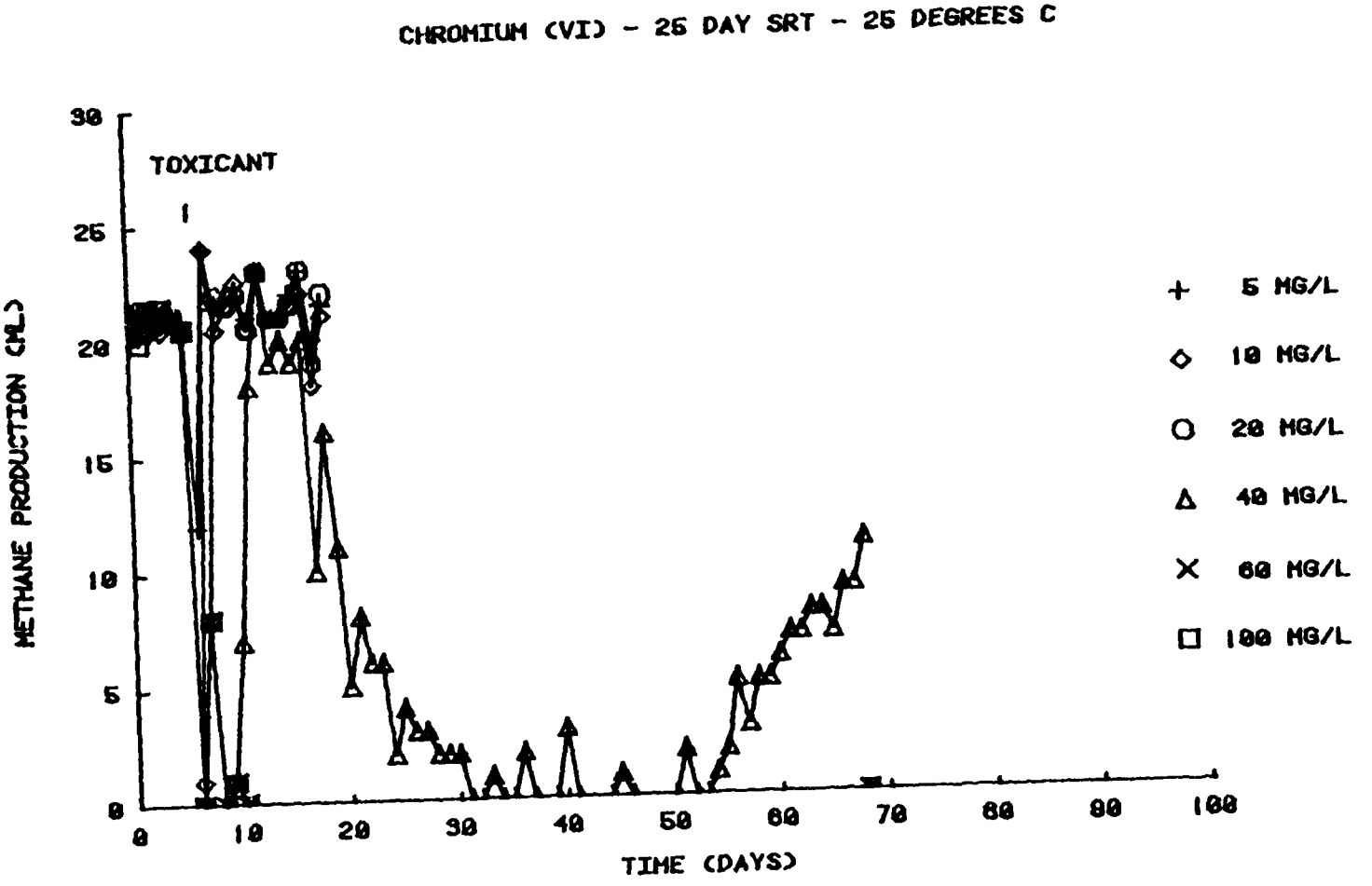

FIGURE 38. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

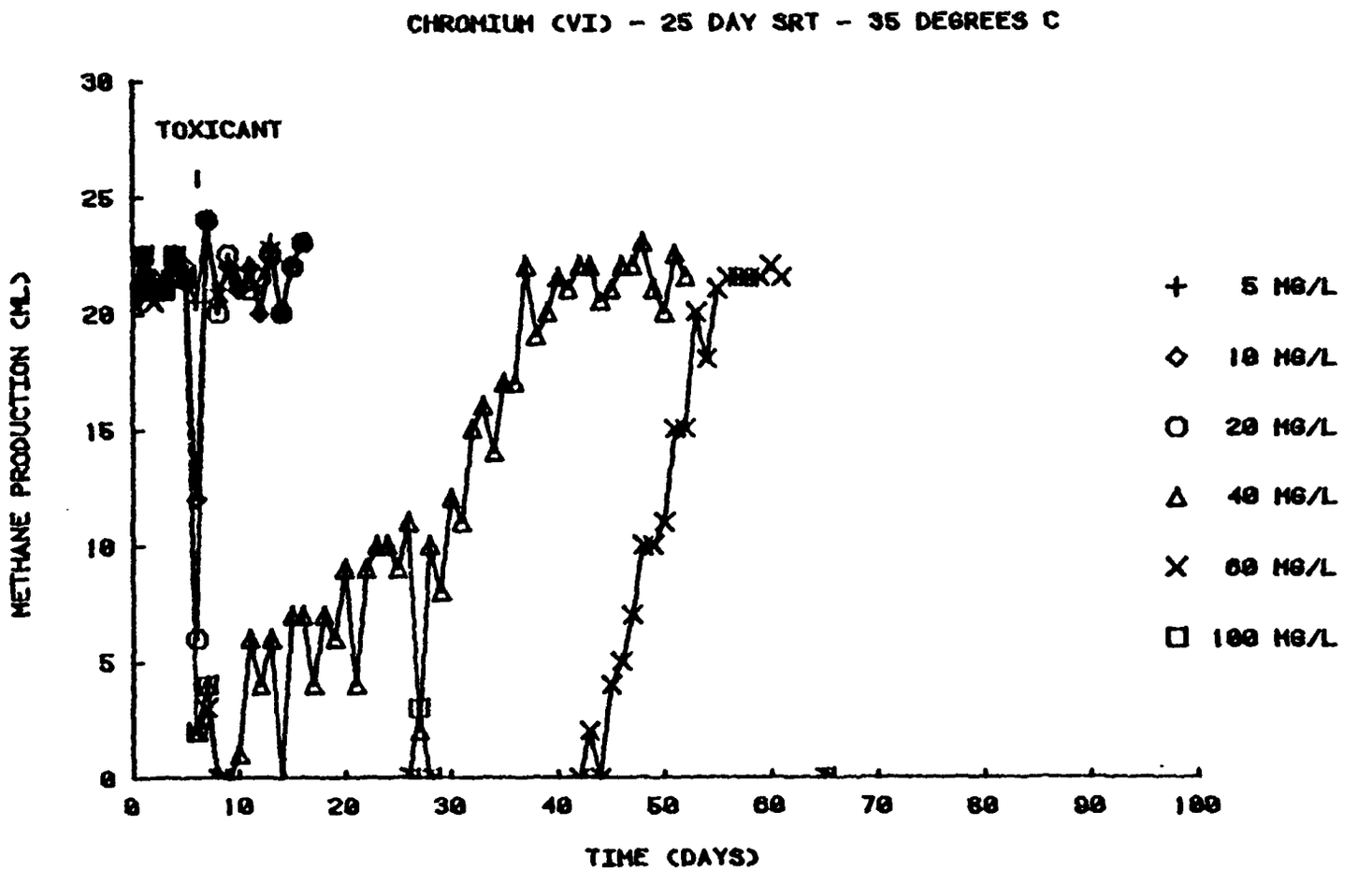

FIGURE 39. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

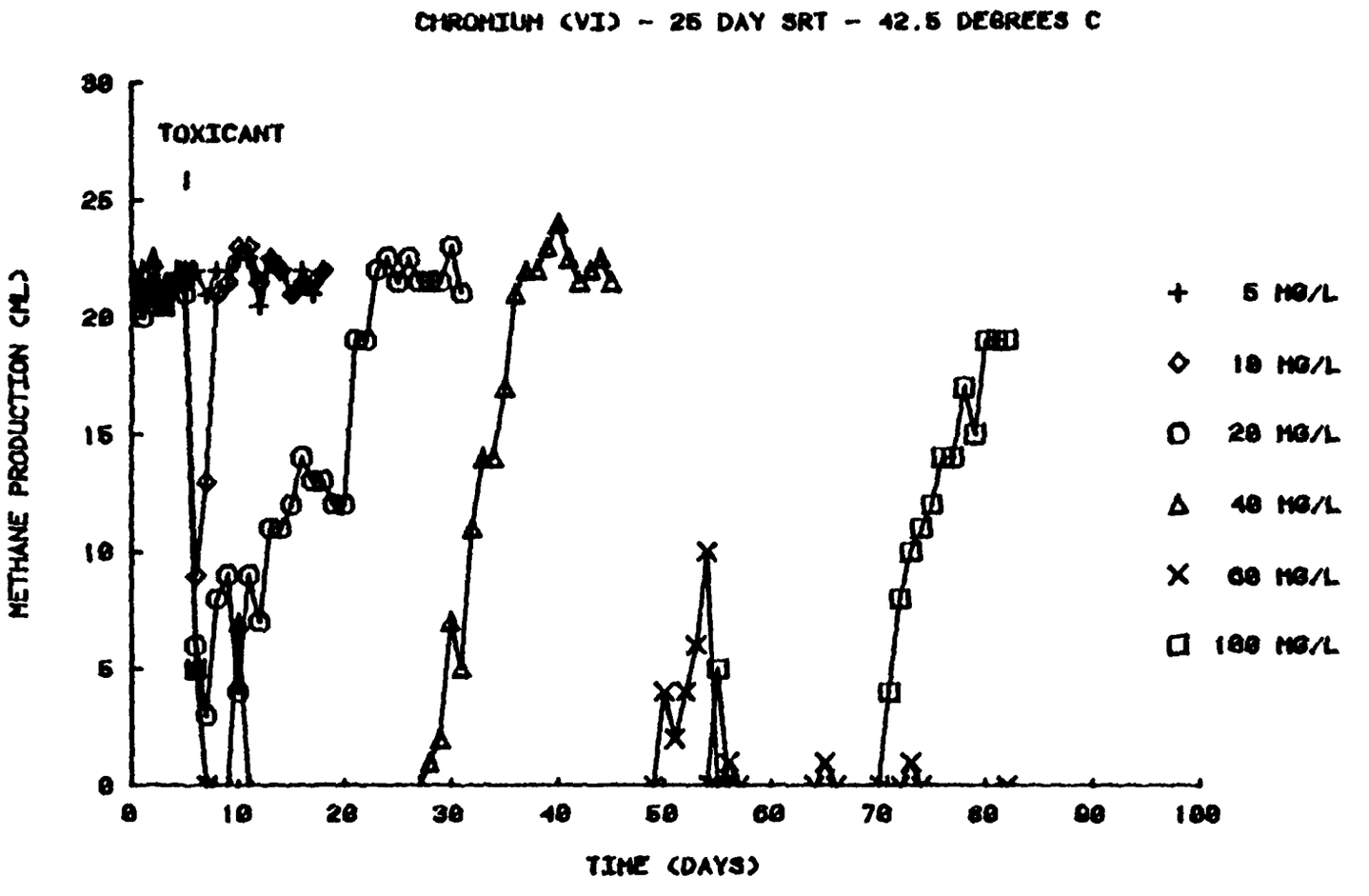

FIGURE 40. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

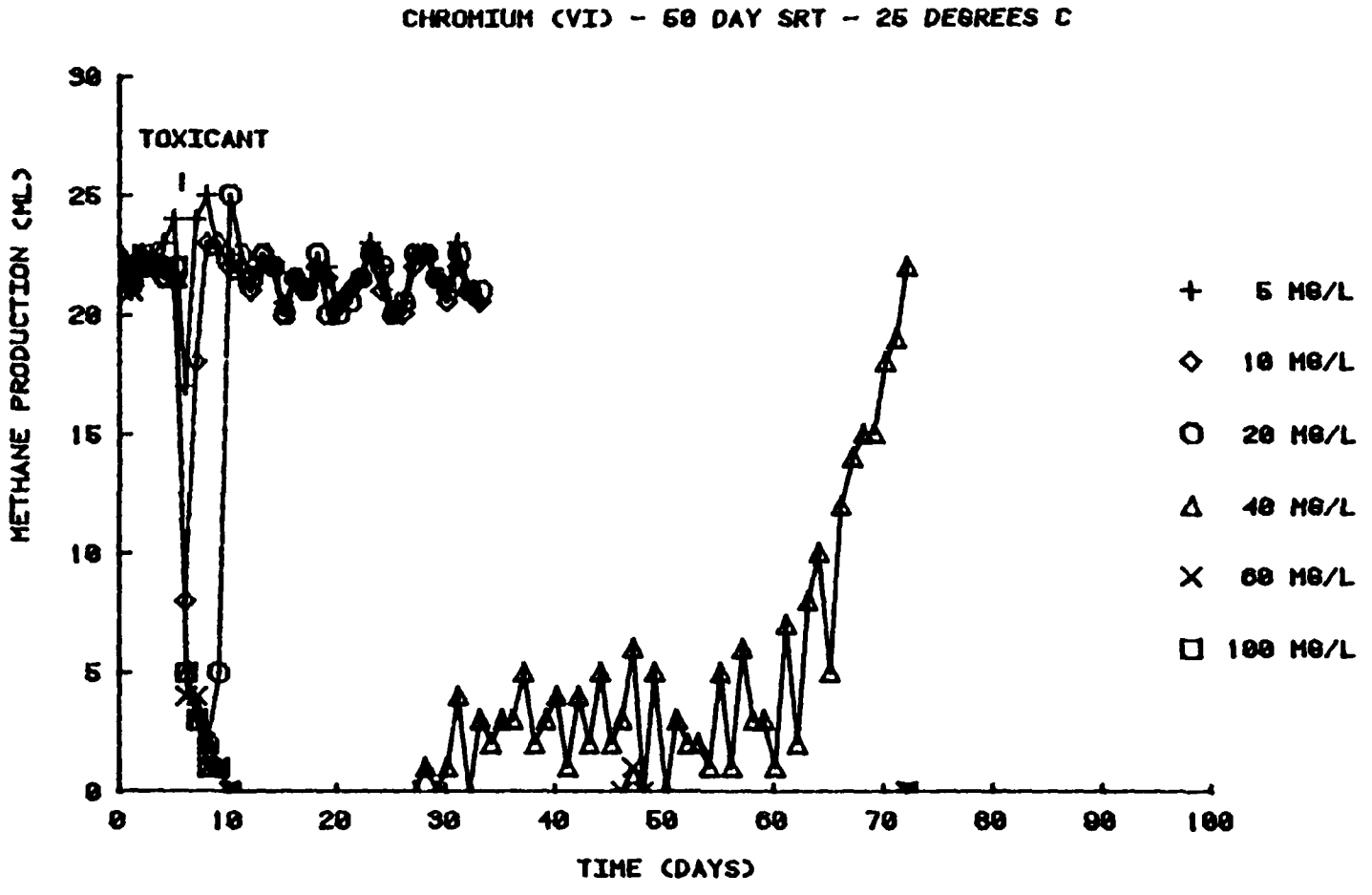

FIGURE 41. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

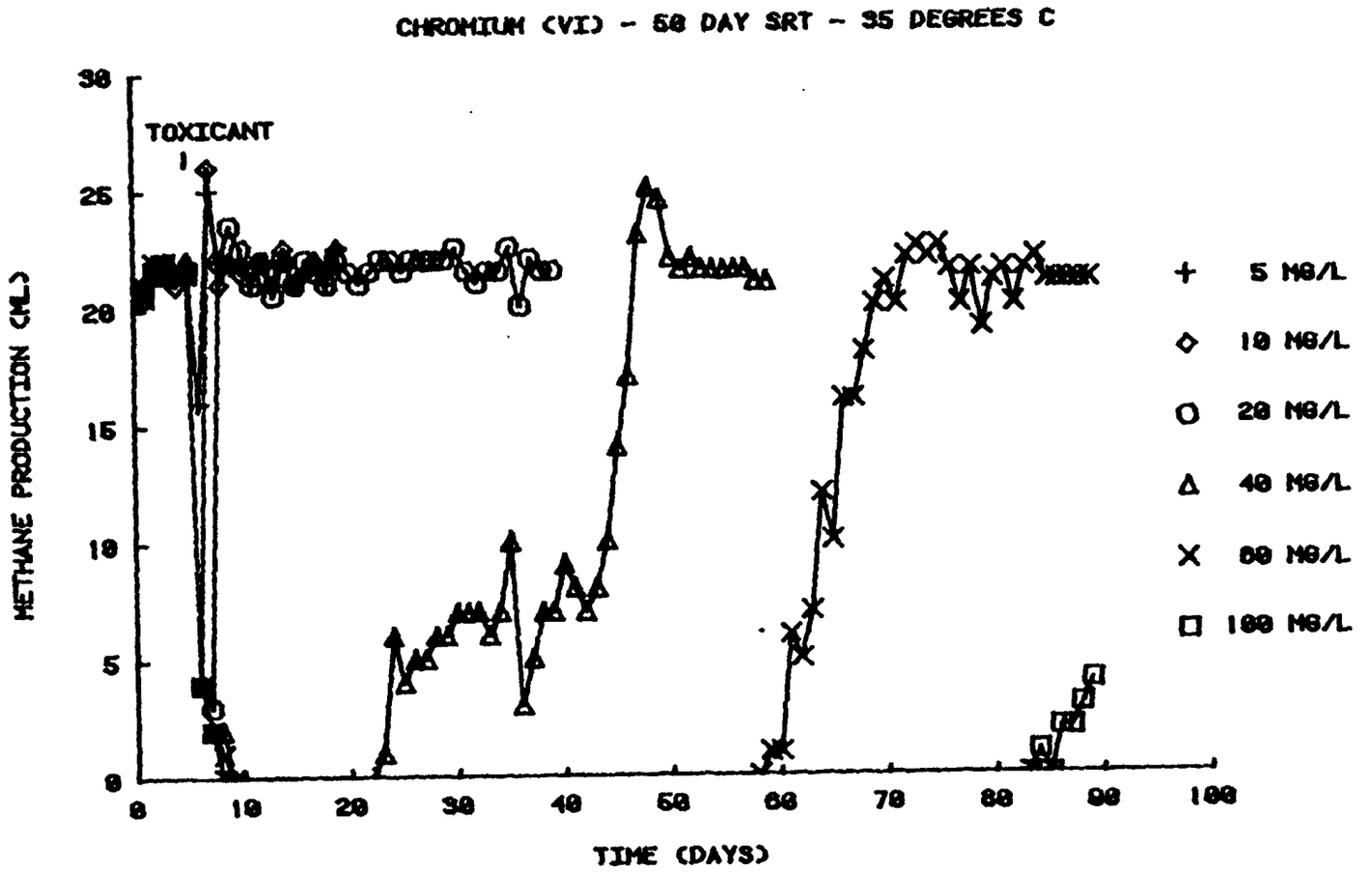

FIGURE 42. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

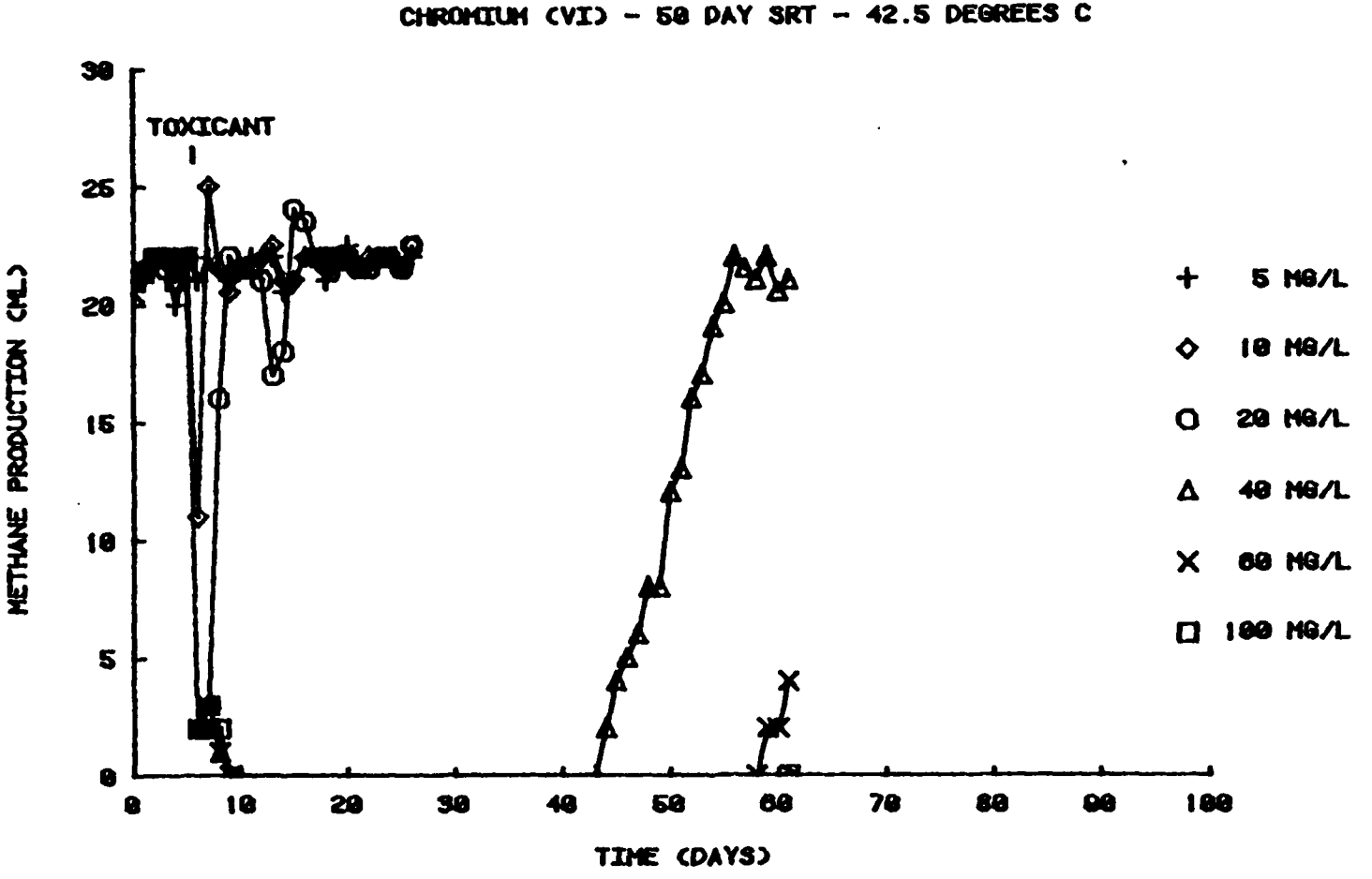

FIGURE 43. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

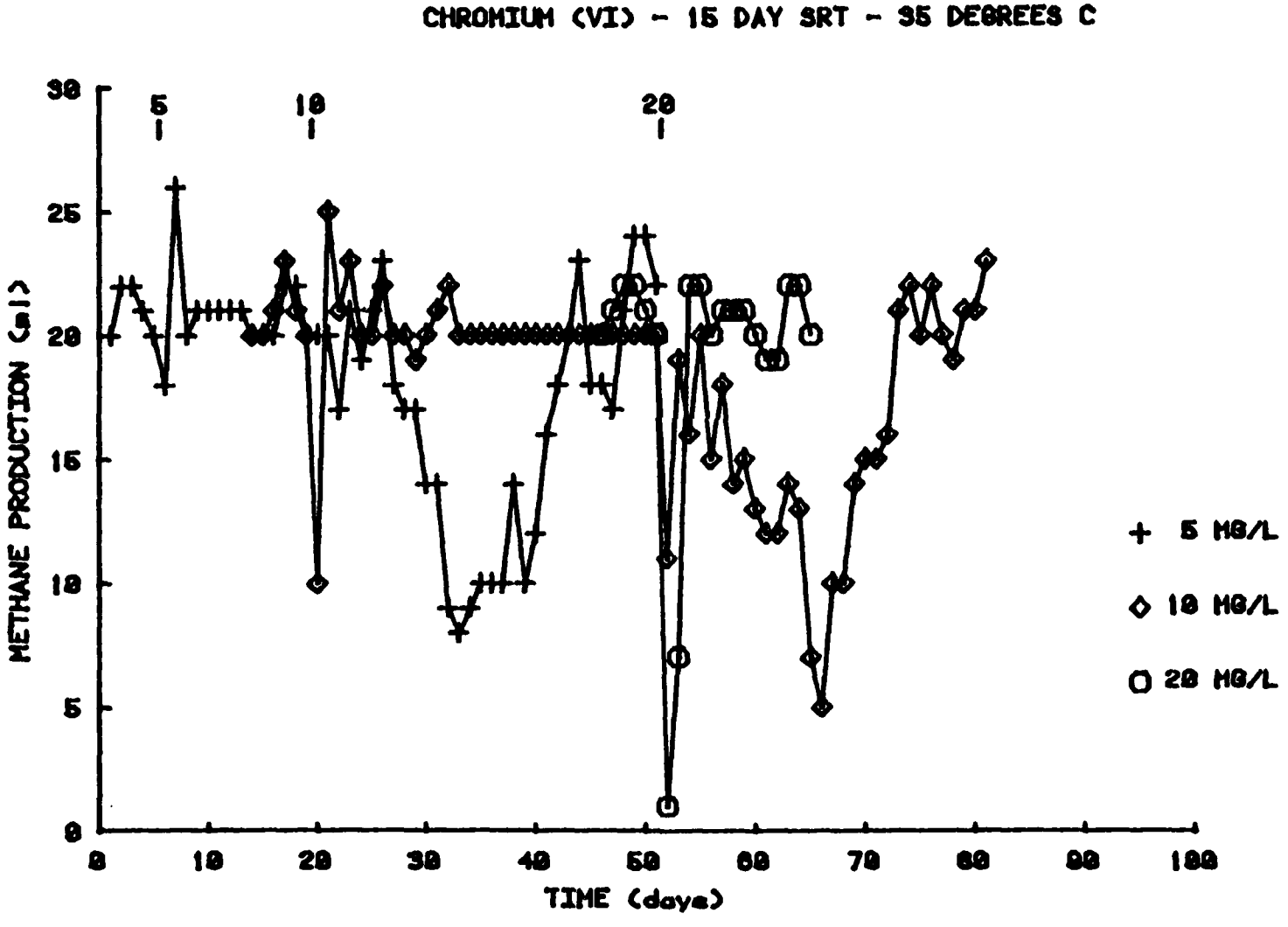

FIGURE 44. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

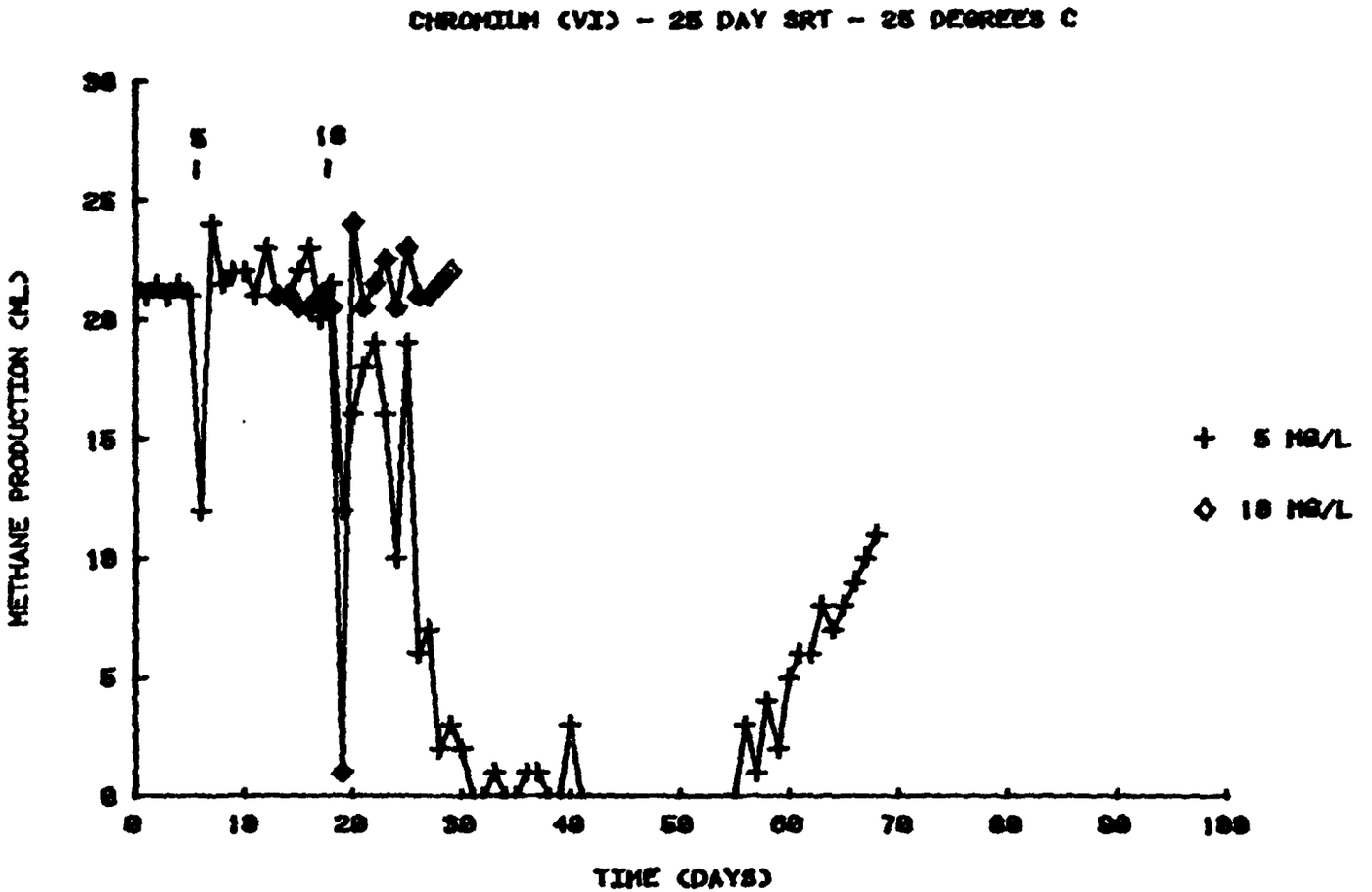

FIGURE 45. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

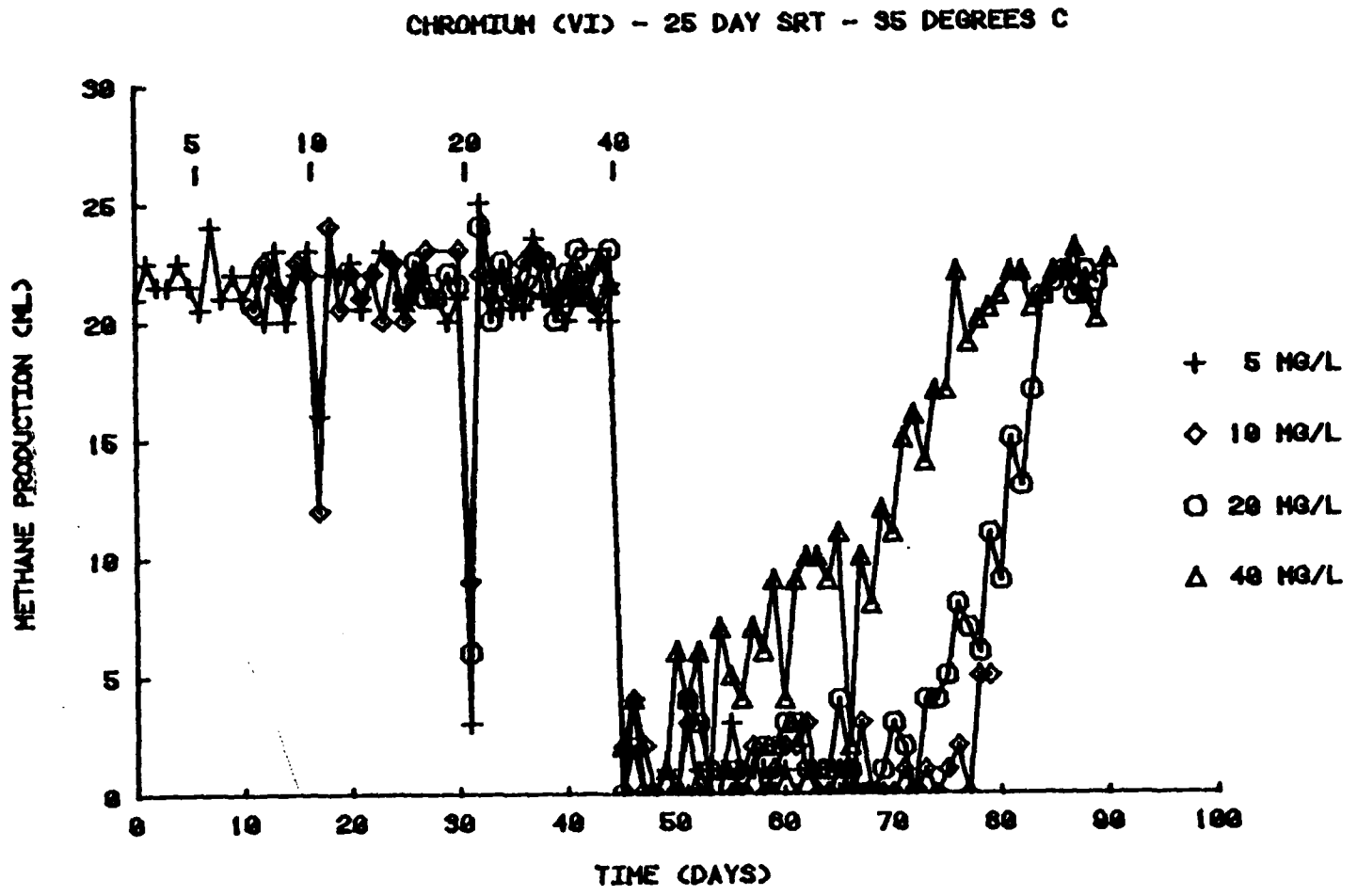

FIGURE 46. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

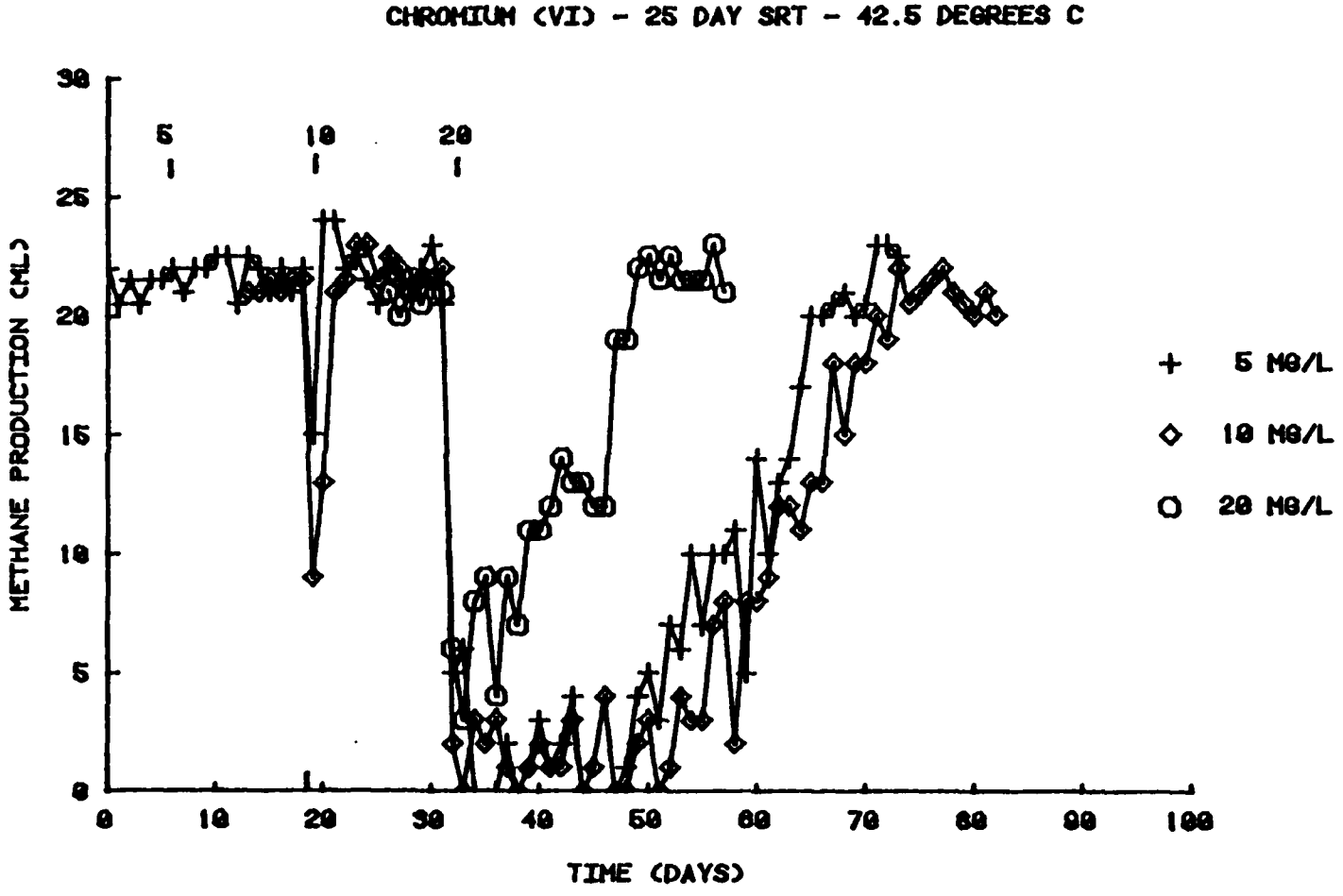

FIGURE 47. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

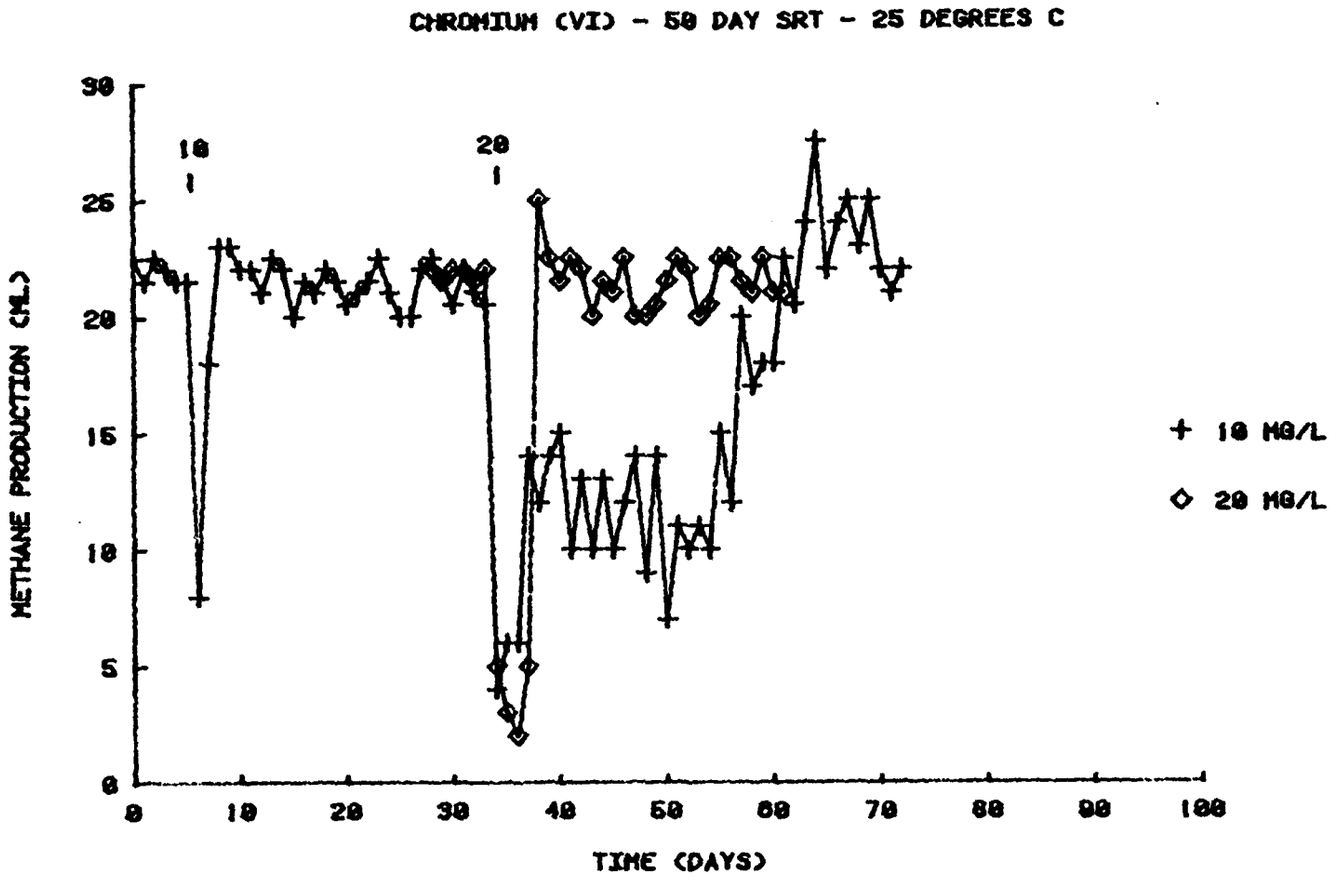

FIGURE 48. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

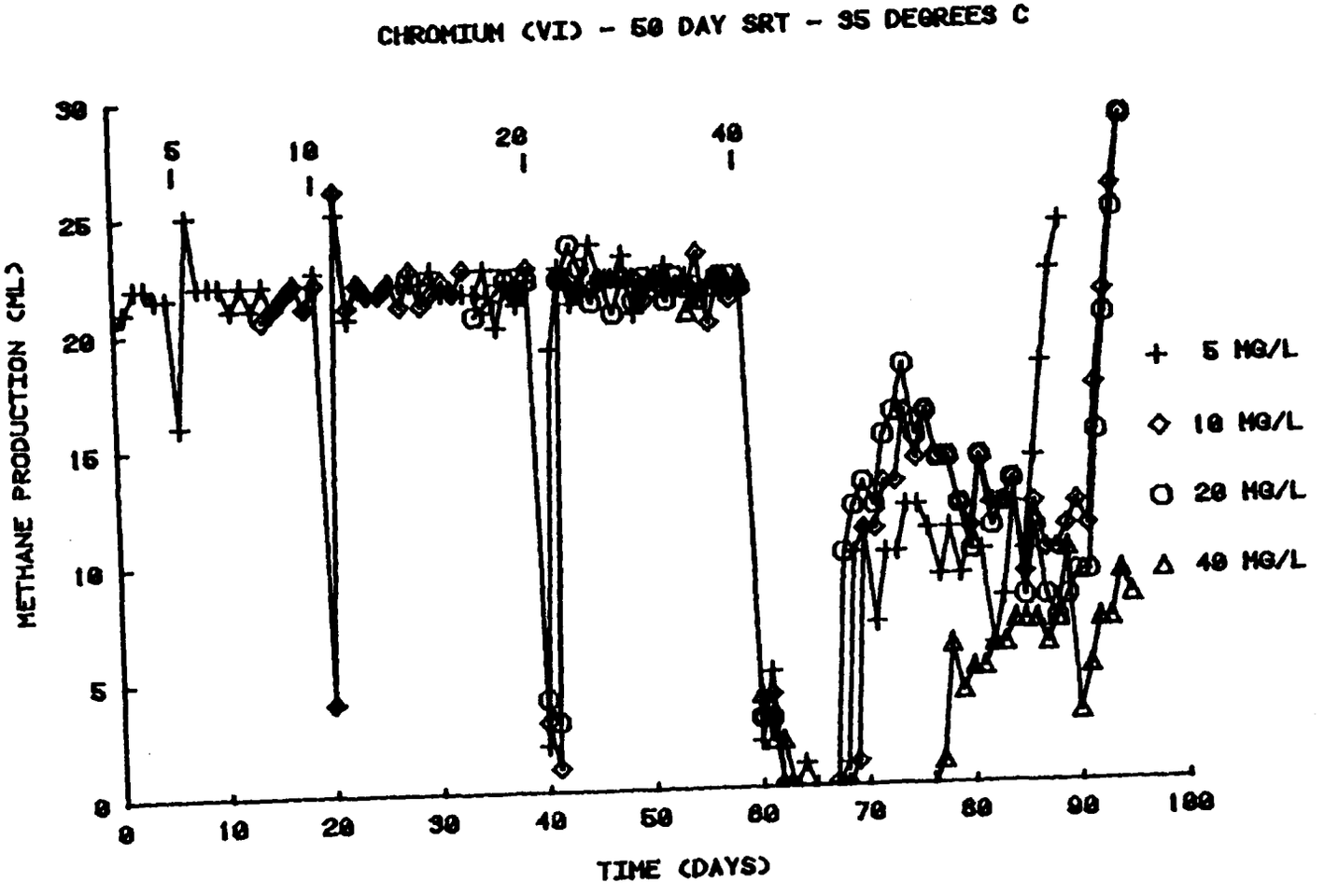

FIGURE 49. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

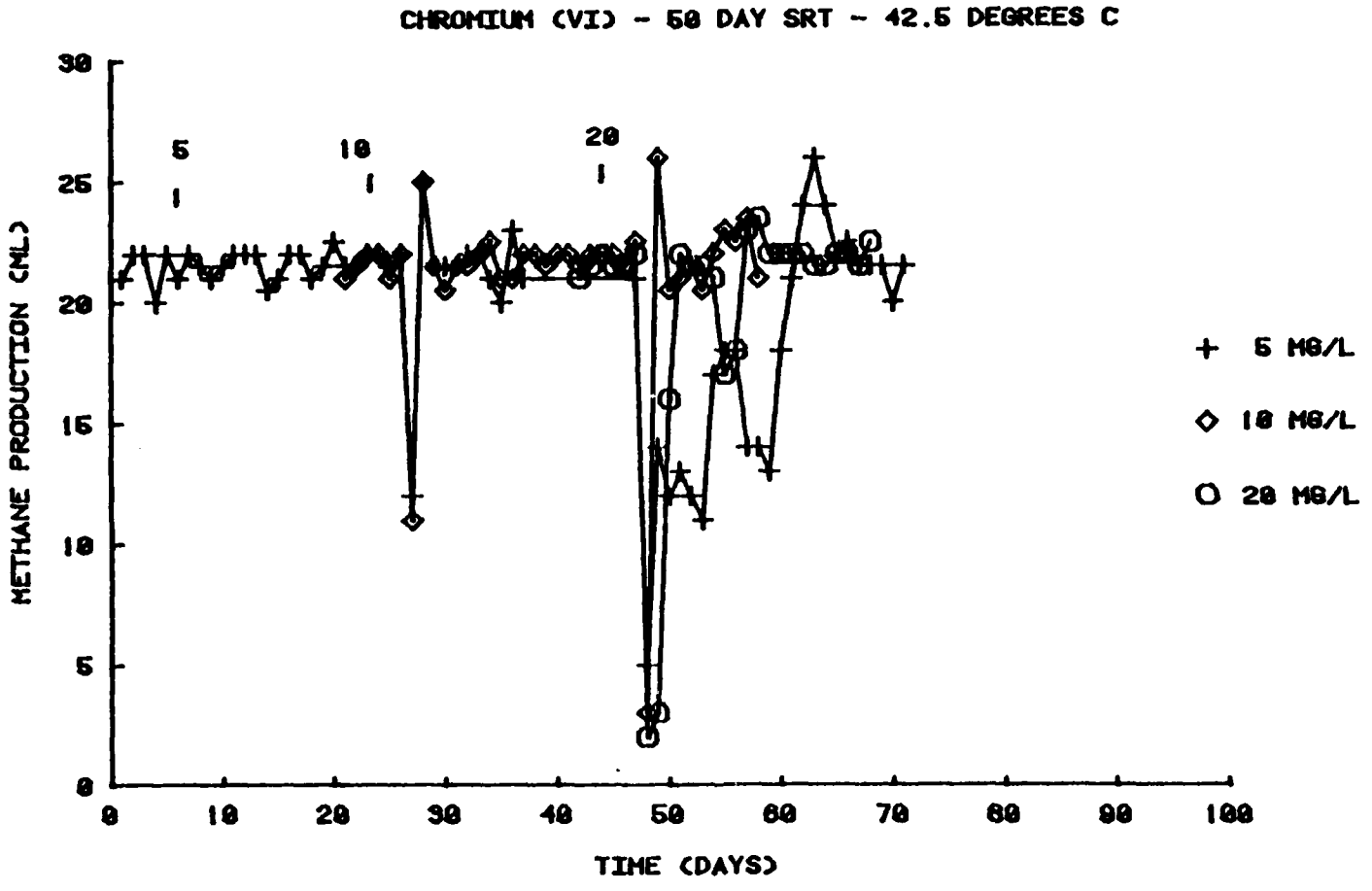

FIGURE 50. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHROMIUM VI

Nickel (Ni^{++})

Stock solutions of nickel chloride were used to introduce serum bottle concentrations of 50, 60, 70, 80, 90 and 100 mg/l Ni^{++}. Added nickel can precipitate with sulfide present in the serum bottles. Using the technique offered to estimate the maximum cadmium that could be precipitated, the maximum nickel that could precipitate is 178 mg/l. Again, it is unlikely that all the sulfur would be available for nickel precipitation.

Delayed response to the toxicant occurred at all three temperatures and all SRTs (Figures 51 to 57). The longest delays generally occurred at 35°C and the shortest delays, 1 to 2 days, were prevalent in the 42.5°C bottles. The delays also decreased with increasing slug dose concentrations.

Those cultures maintained with a 25-day SRT (Figures 52 to 54) appeared much more capable of tolerating nickel exposure than those with a 50-day SRT (Figures 55 to 57). Comparison of SRT at 35°C indicates that the 15-day system (Figure 51) experienced a slightly less severe response.

For a 25-day SRT, the effects of nickel were minimal at 25°C, the toxicity effects increasing at 35°C and 42.5°C (Figures 52 to 54). With a 50-day SRT, a temperature of 35°C was preferable and 42.5°C again resulted in the most severe responses (Figures 55 to 57).

Significant acclimation characteristics were demonstrated at SRTs of 25 and 50 days for temperatures of 25°C and 42.5°C (Figures 59, 61, 62 and 64). However, cultures maintained at 35°C did not exhibit significant acclimation capabilities (Figures 58, 60, and 63) for the concentrations tested.

Sulfide ($S^{=}$)

Slug dose concentrations of 50, 100, 150, 200, 300 and 500 mg/l $S^{=}$ in serum bottles were provided from stock solutions of $Na_2S \cdot 9H_2O$.

The characteristic response to sulfide was a drop to the minimum methane production after one day of toxicant exposure followed by a sharp increase in gas generation on the second day. After this sharp, initial recovery, a slower recovery rate predominated until the full methane production level was reached (Figures 65 to 71).

Recovery rates were generally faster in 50-day SRT systems, with the difference in rates increasing with increasing toxicant concentration.

Cultures incubated at 25°C were most severely affected by sulfide, the

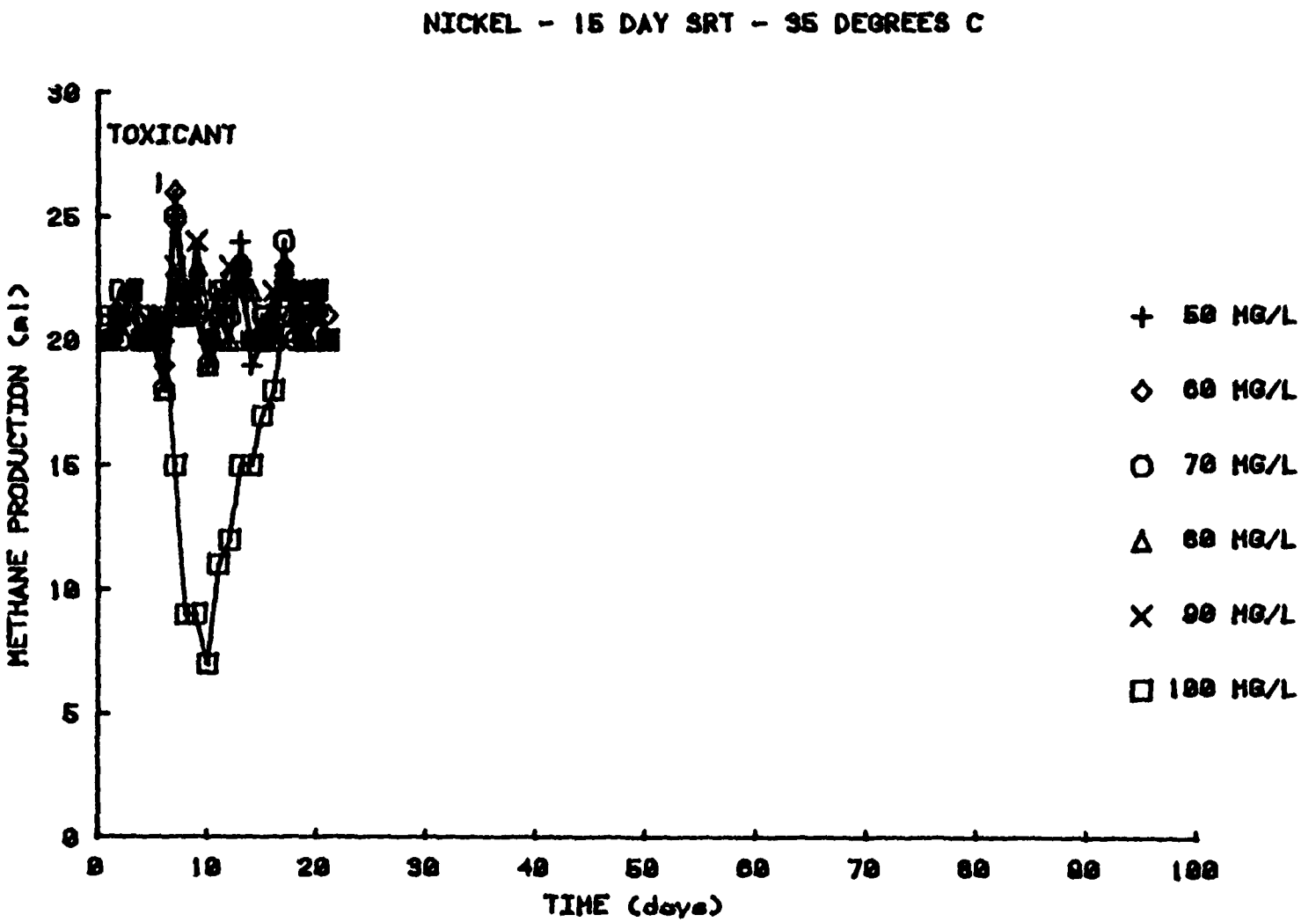

FIGURE 51. RESPONSE OF METHANOGENS TO SLUG DOSES OF NICKEL

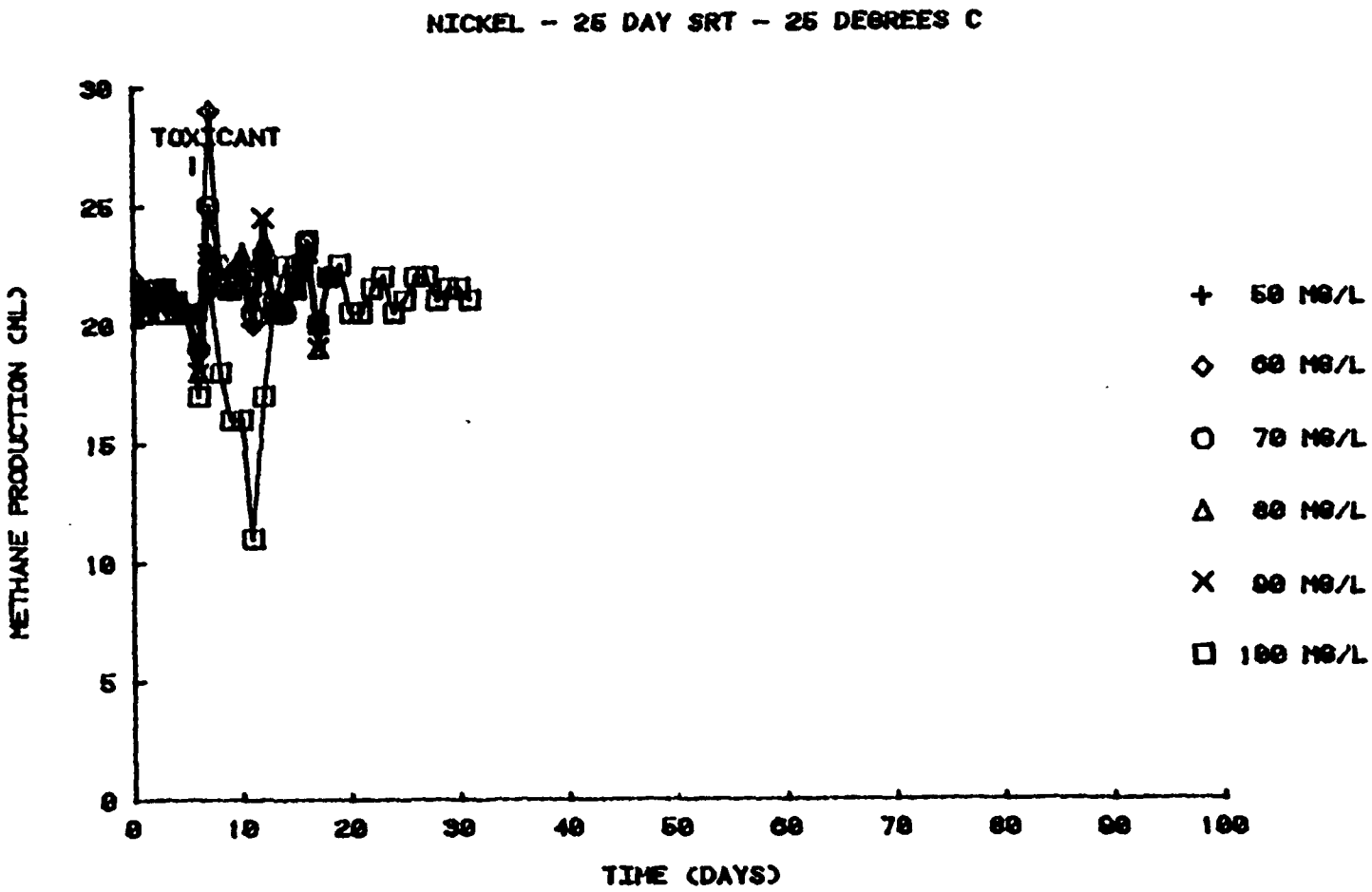

FIGURE 52. RESPONSE OF METHANOGENS TO SLUG DOSES OF NICKEL

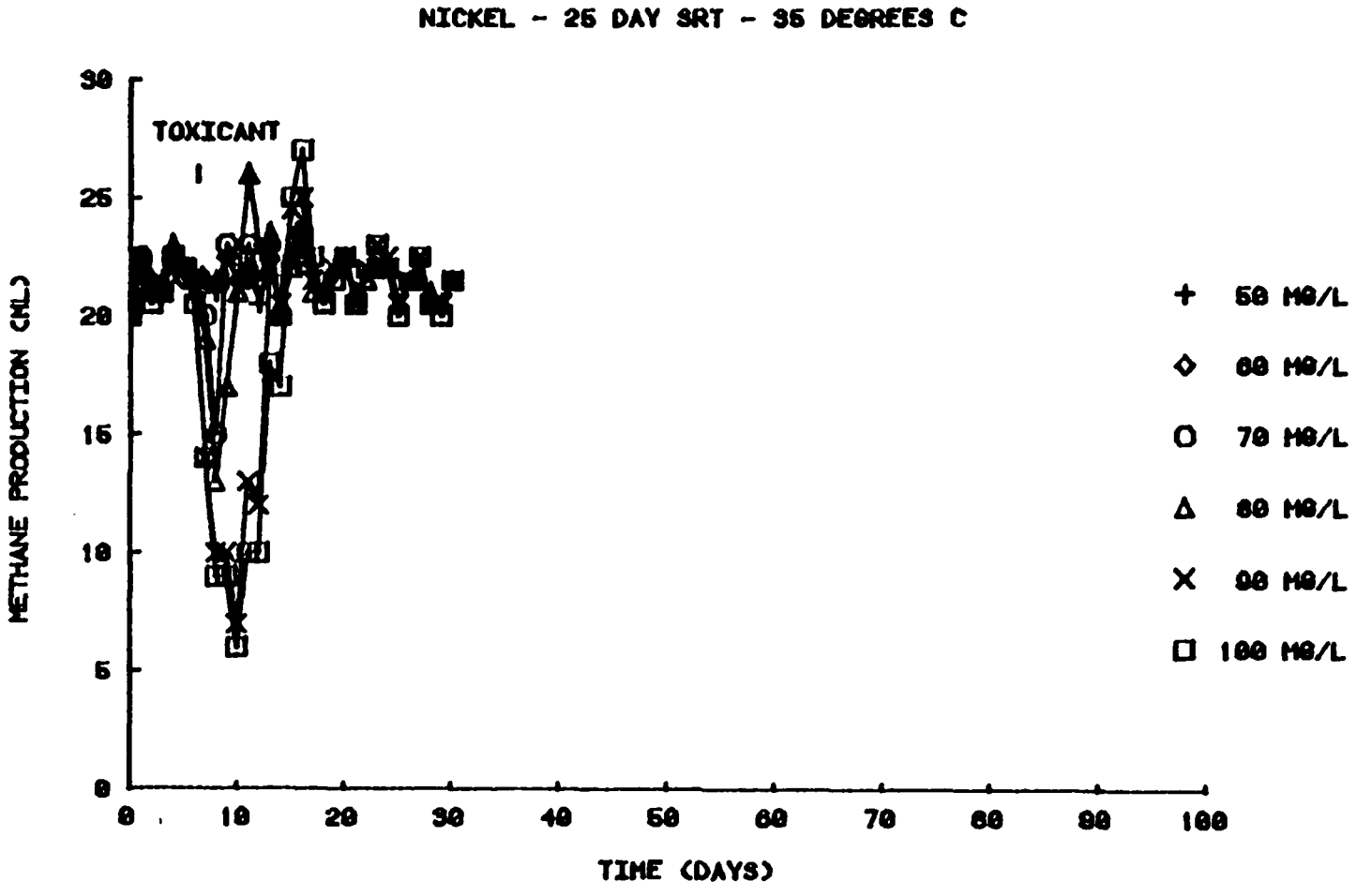

FIGURE 53. RESPONSE OF METHANOGENS TO SLUG DOSES OF NICKEL

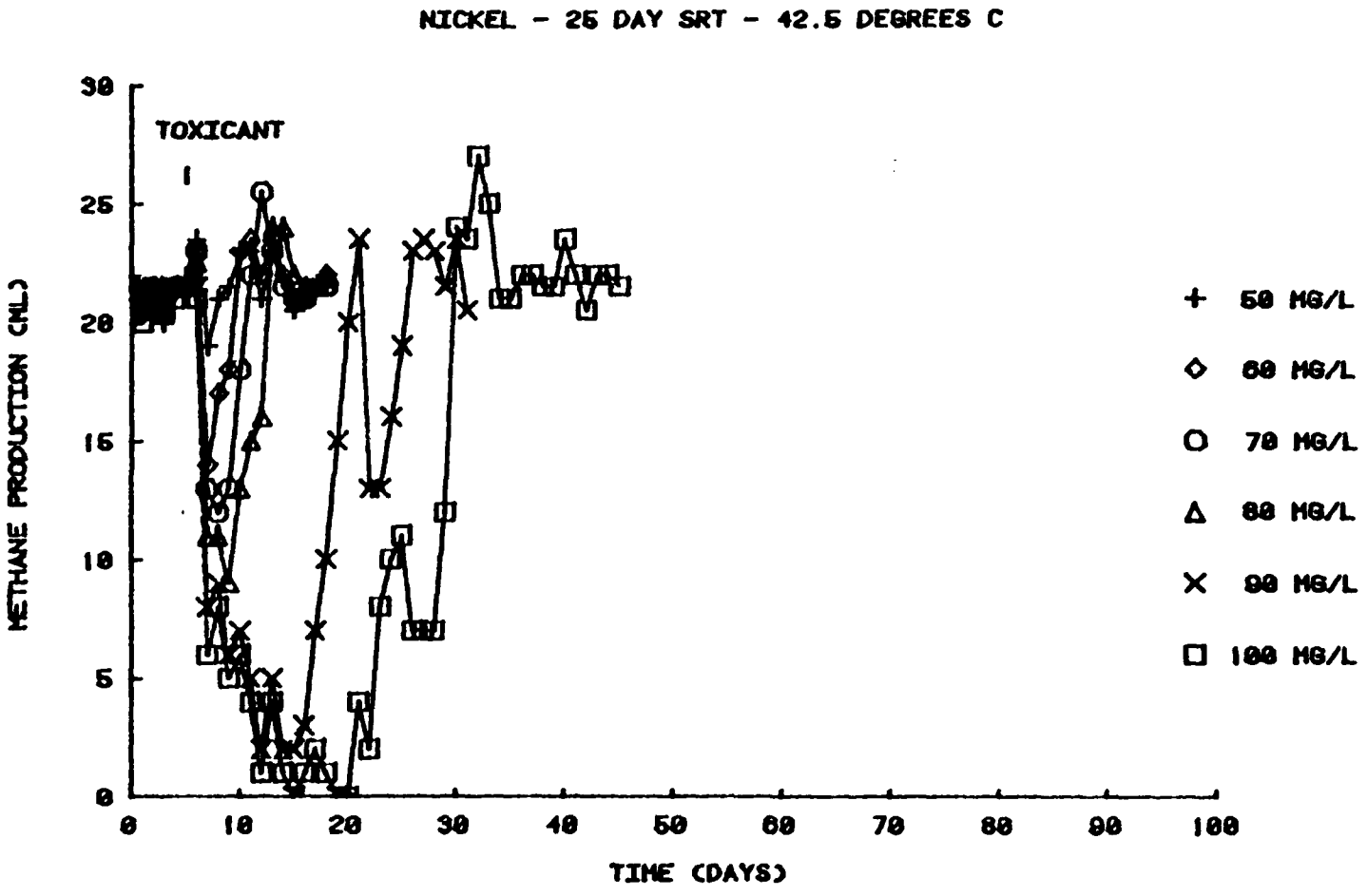

FIGURE 54. RESPONSE OF METHANOGENS TO SLUG DOSES OF NICKEL

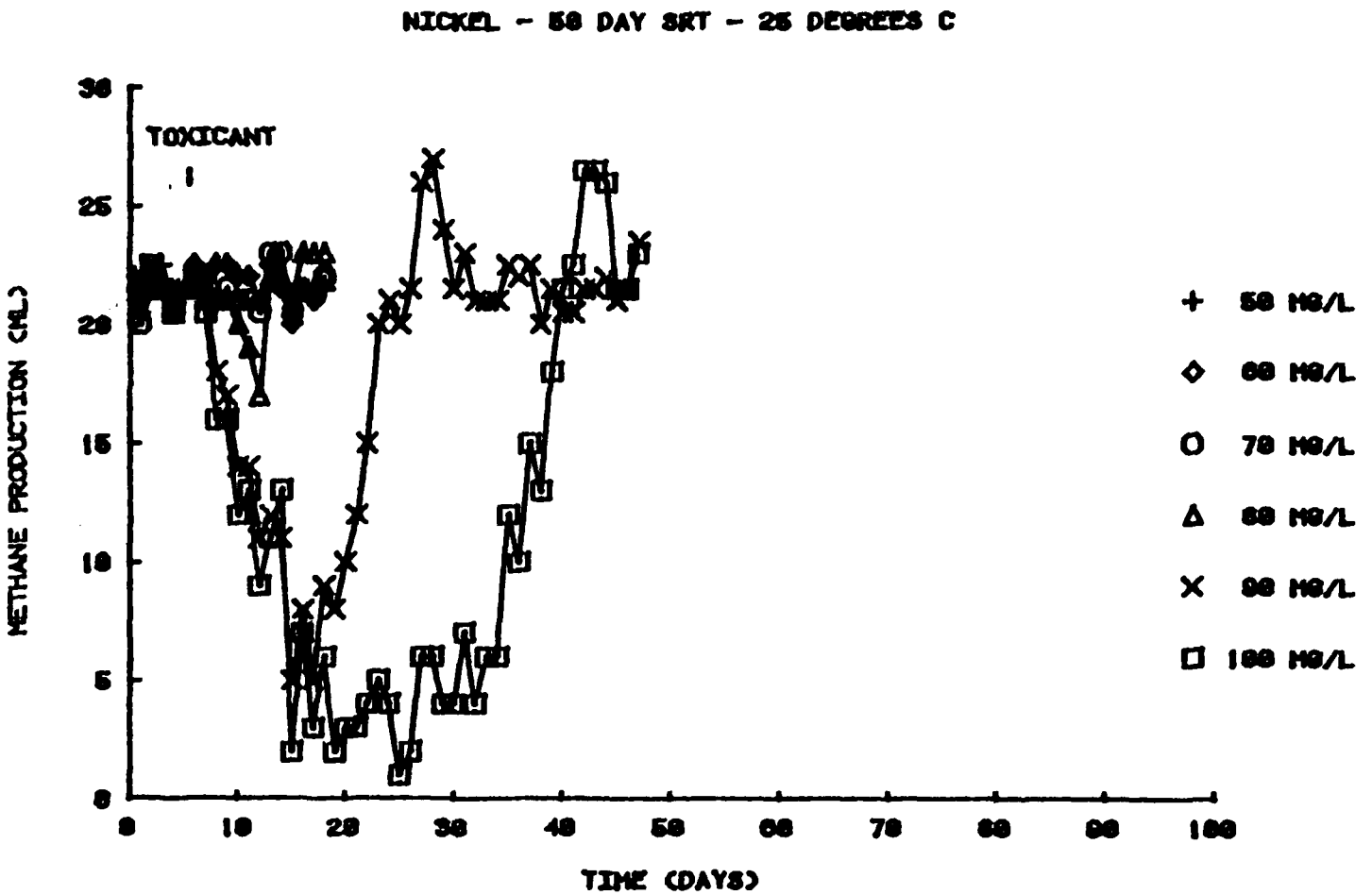

FIGURE 55. RESPONSE OF METHANOGENS TO SLUG DOSES OF NICKEL

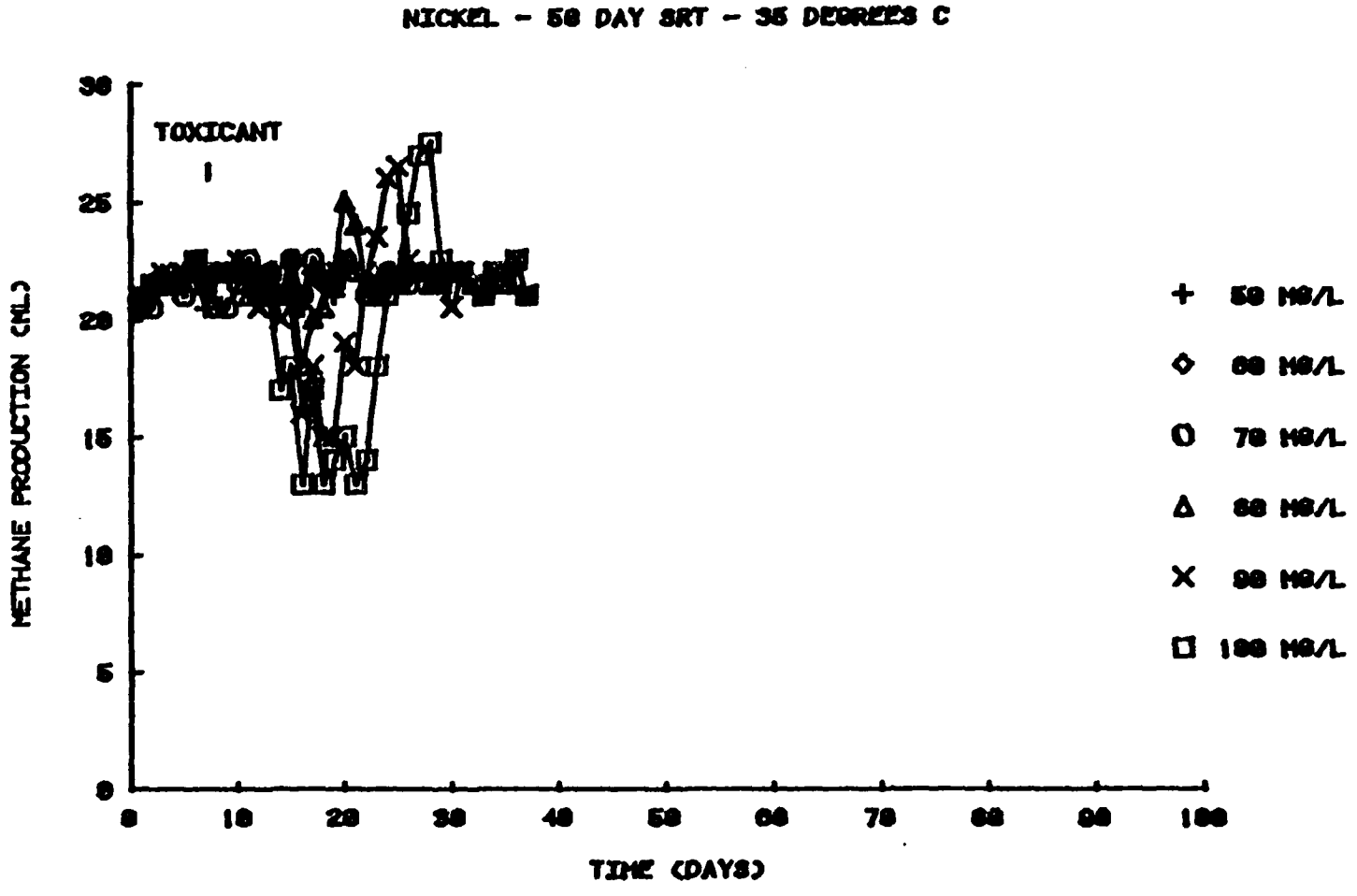

FIGURE 56. RESPONSE OF METHANOGENS TO SLUG DOSES OF NICKEL

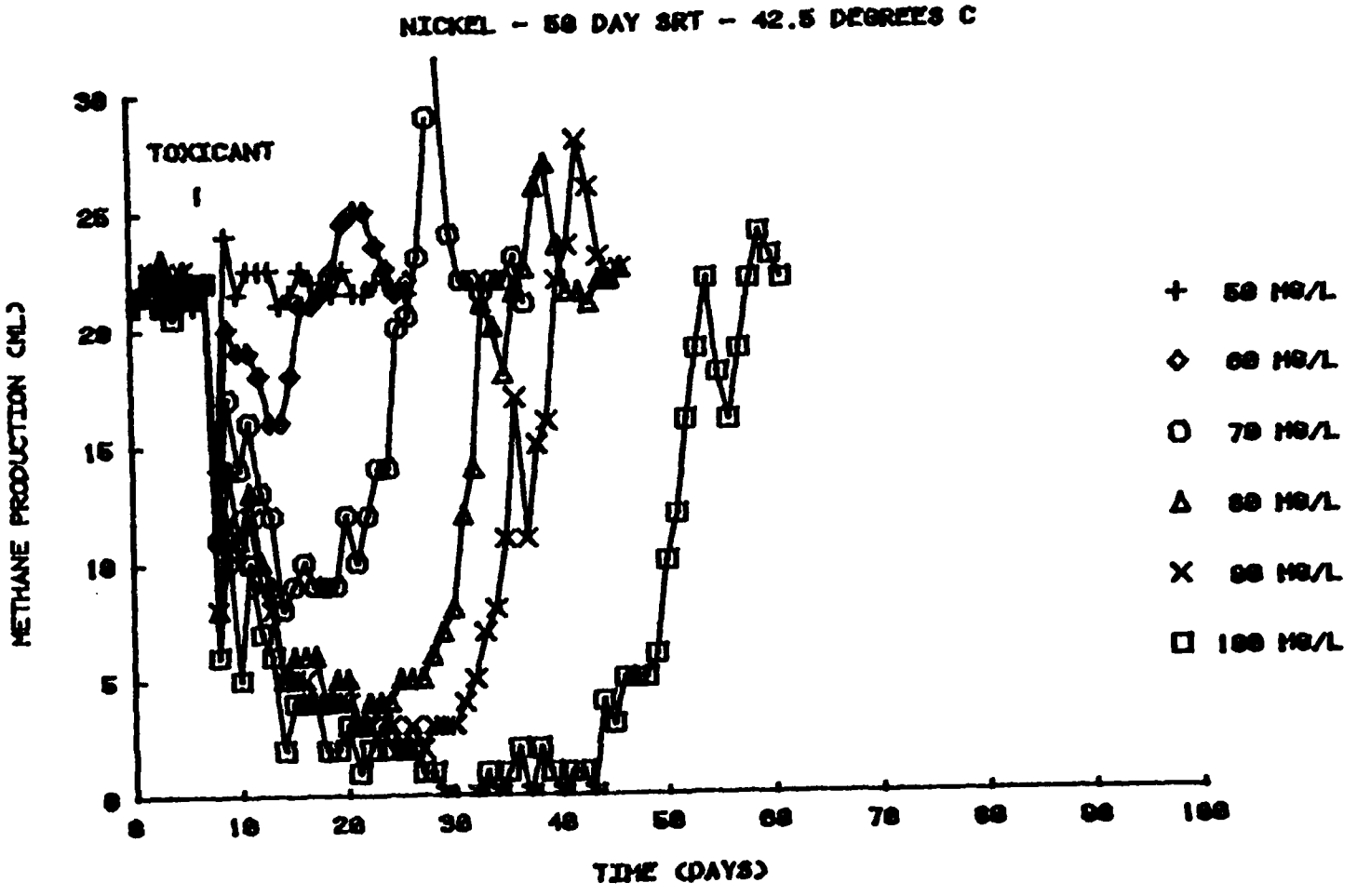

FIGURE 57. RESPONSE OF METHANOGENS TO SLUG DOSES OF NICKEL

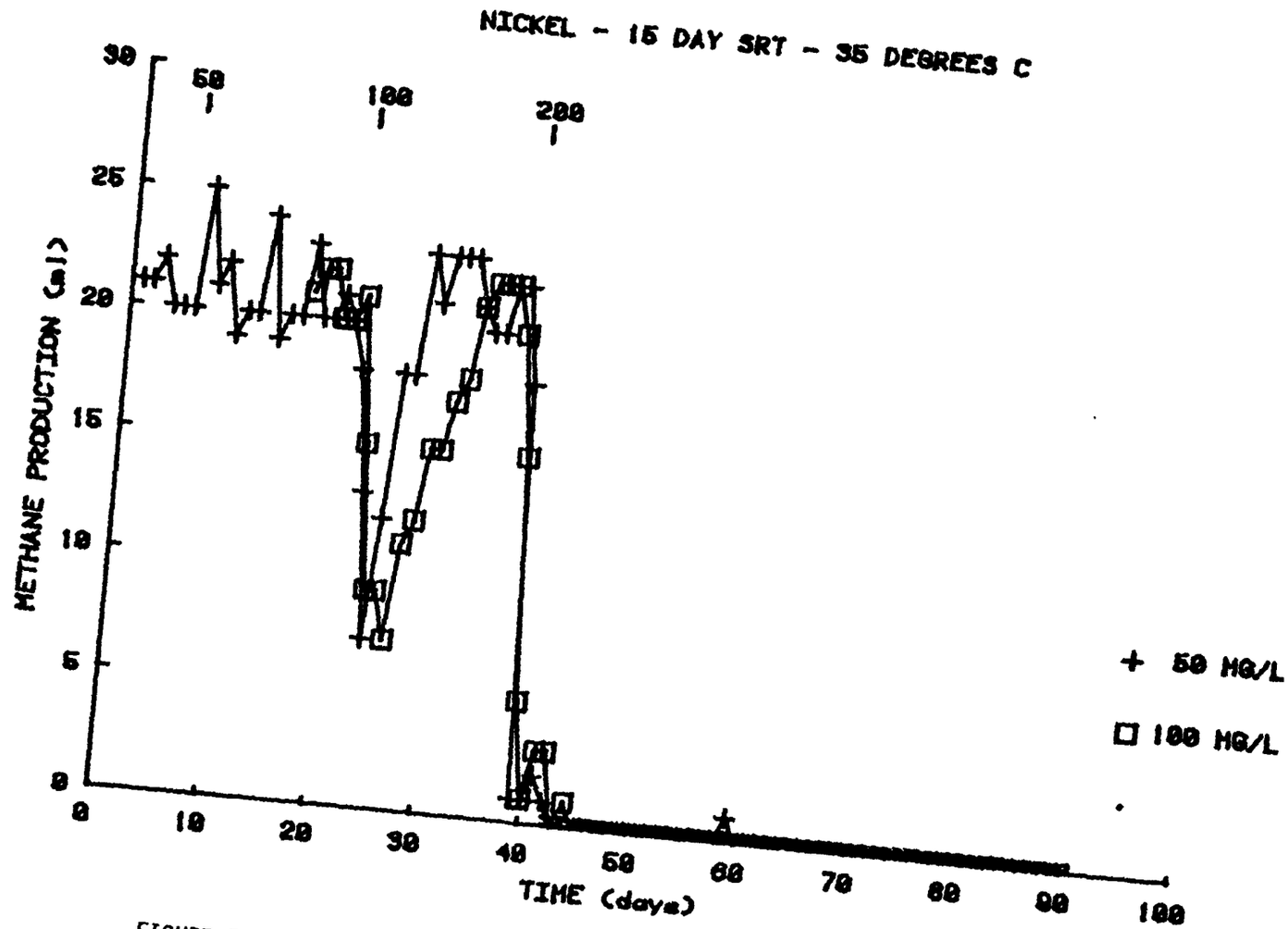

FIGURE 58. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF NICKEL

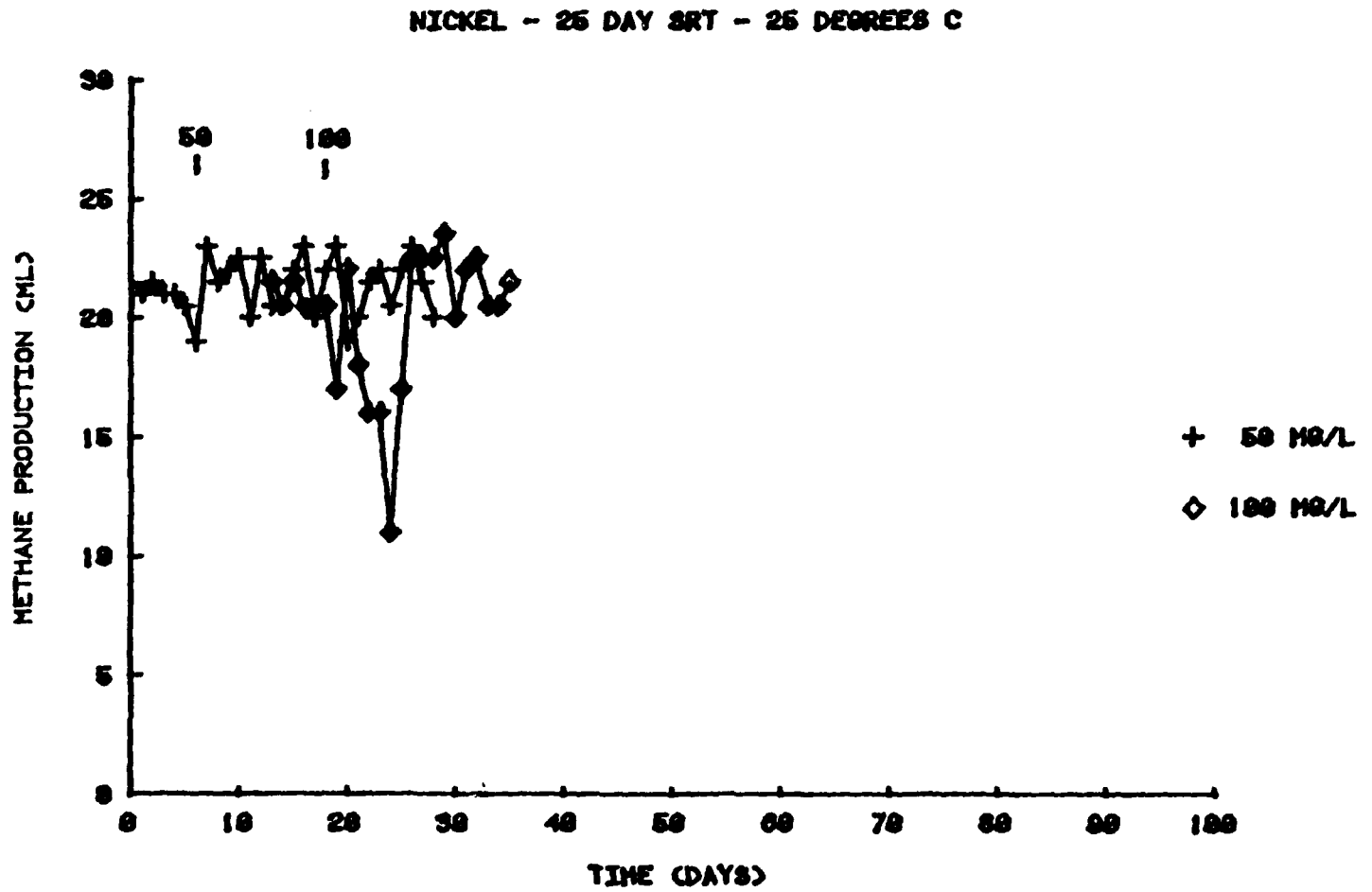

FIGURE 59. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF NICKEL

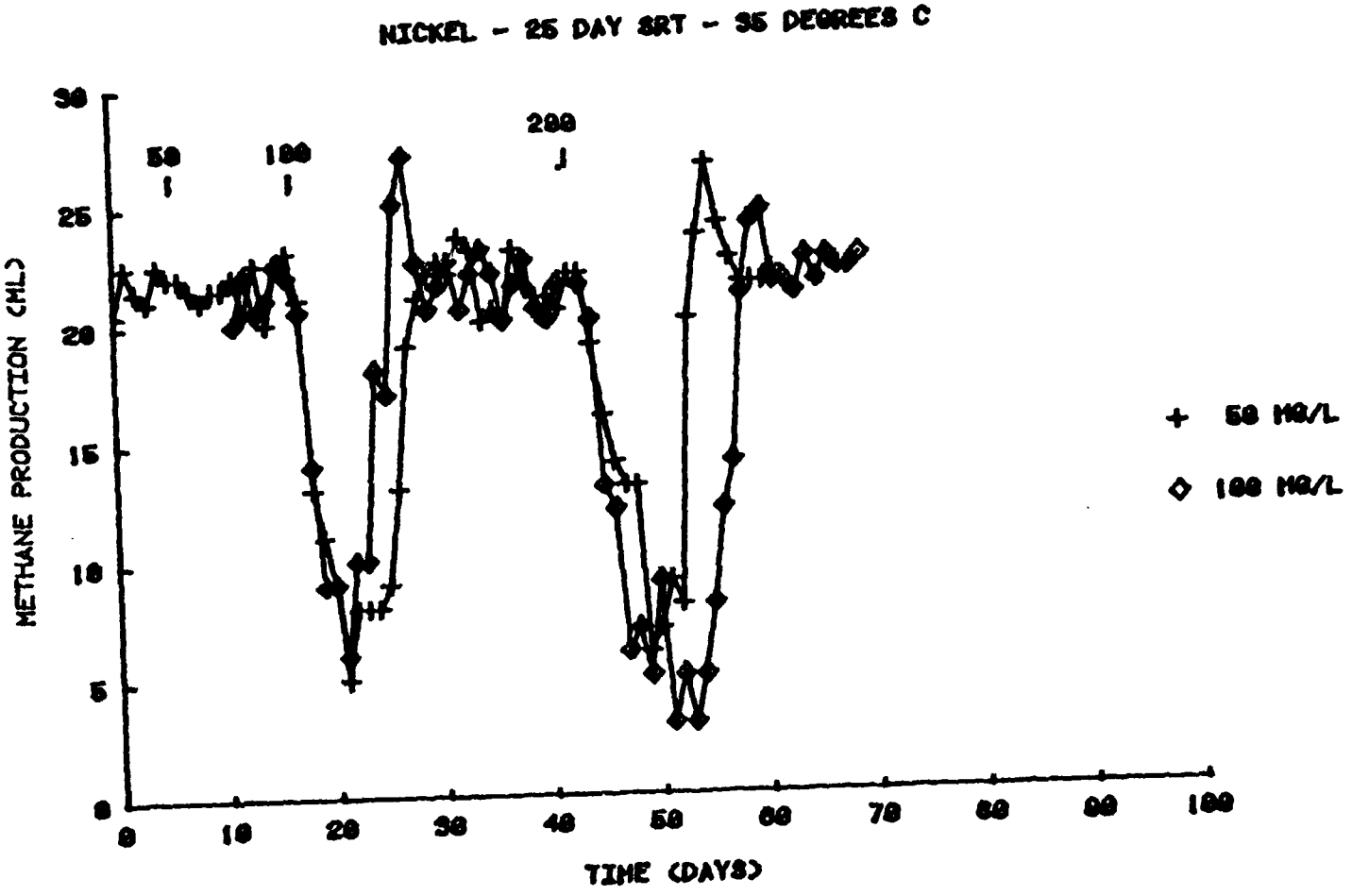

FIGURE 60. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF NICKEL

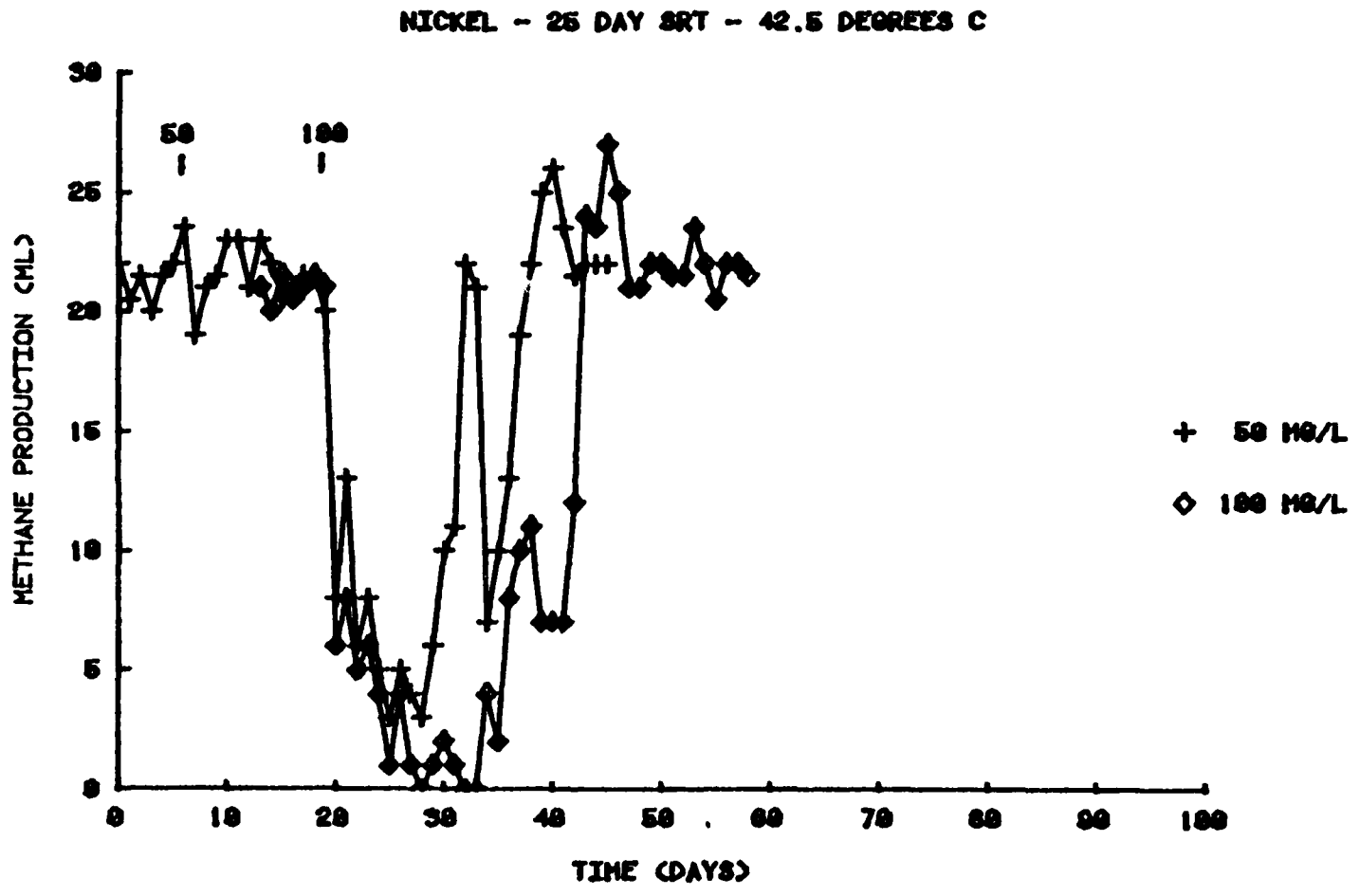

FIGURE 61. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF NICKEL

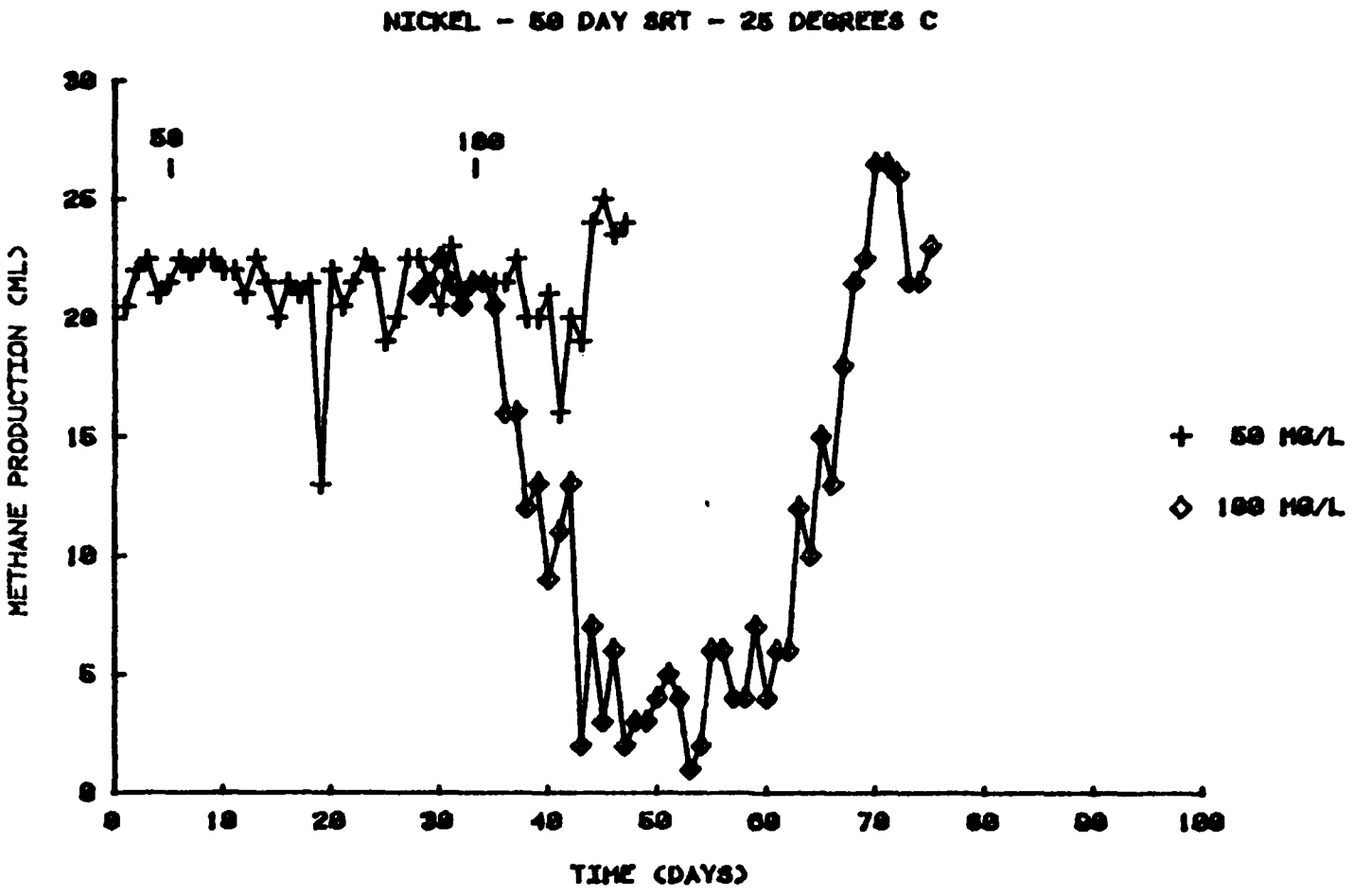

FIGURE 62. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF NICKEL

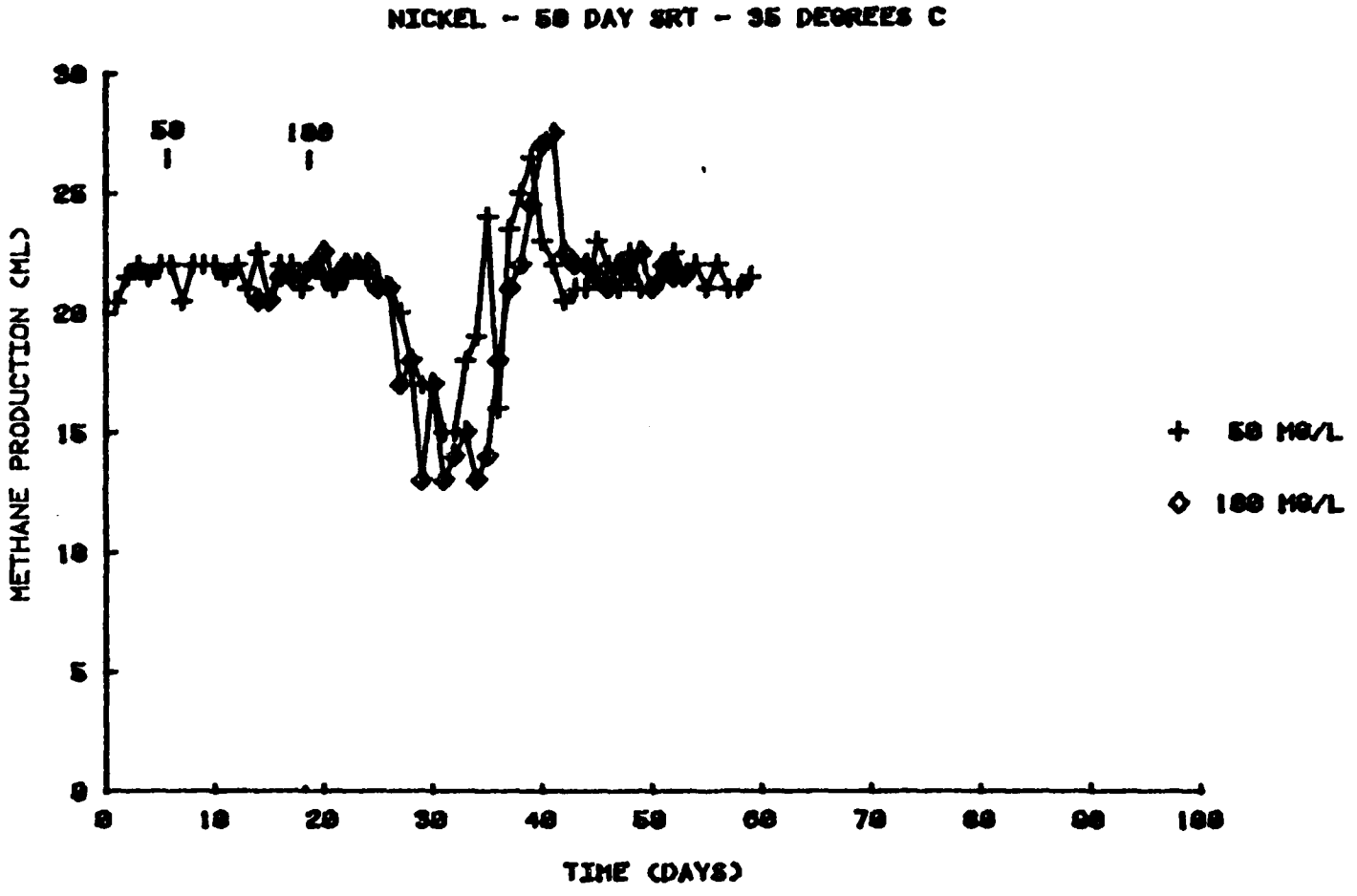

FIGURE 63. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF NICKEL

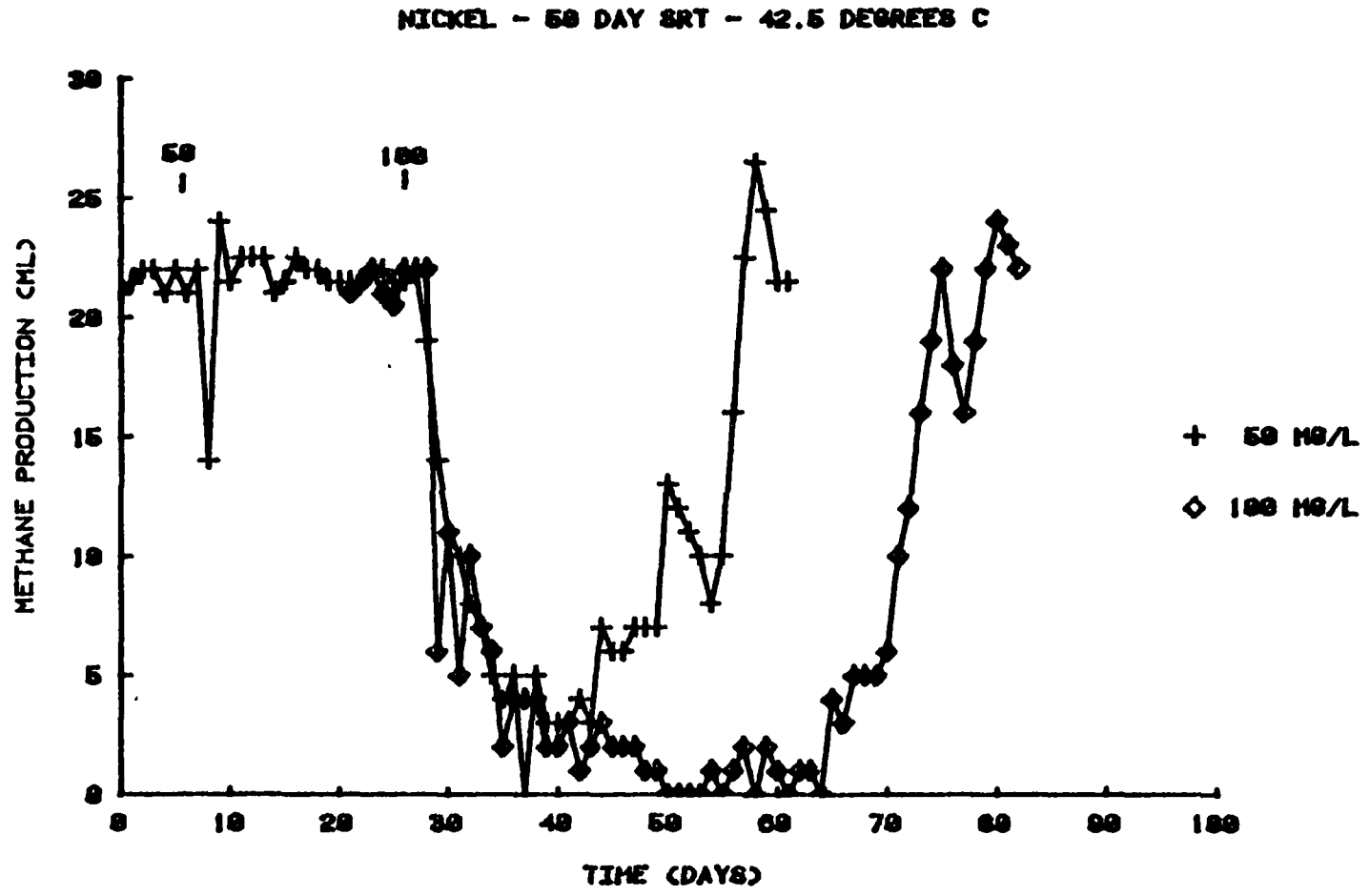

FIGURE 64. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF NICKEL

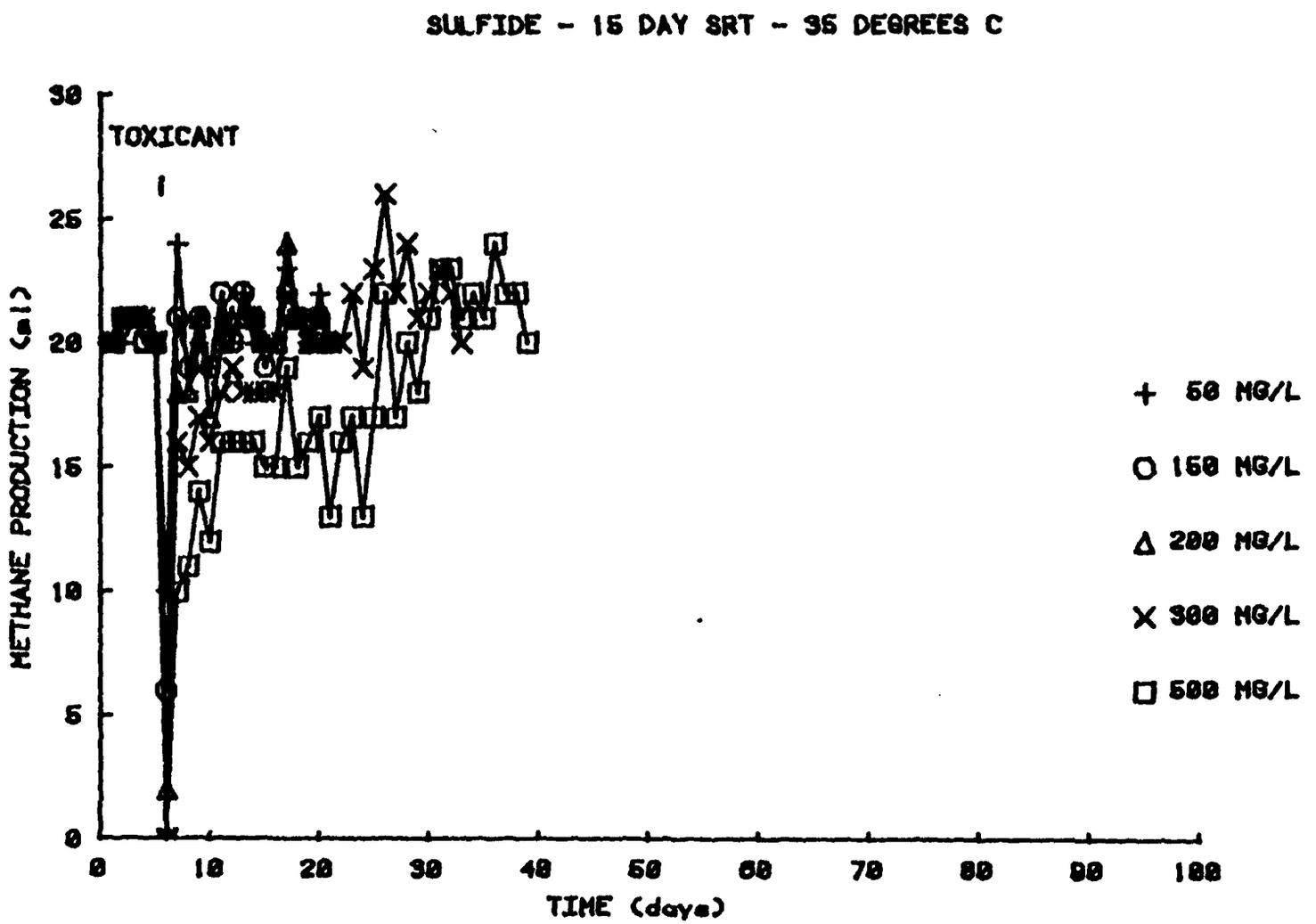

FIGURE 65. RESPONSE OF METHANOGENS TO SLUG DOSES OF SULFIDE

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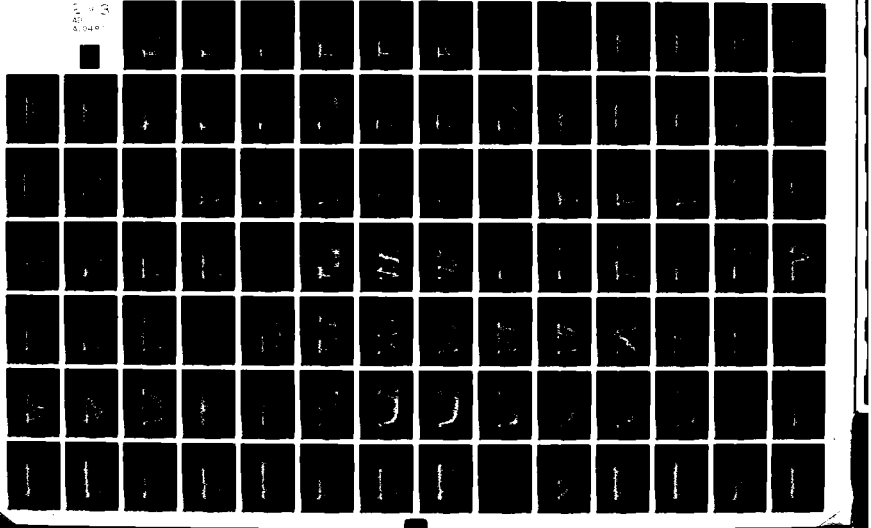
MICROBIAL METHANE FERMENTATION KINETICS FOR TOXICANT EXPOSURE.(U)
AUG 81 G F PARKIN, W M KOCHER, S W MILLER F49620-79-C-0190
UNCLASSIFIED AFOSR-TR-81-0680 NL



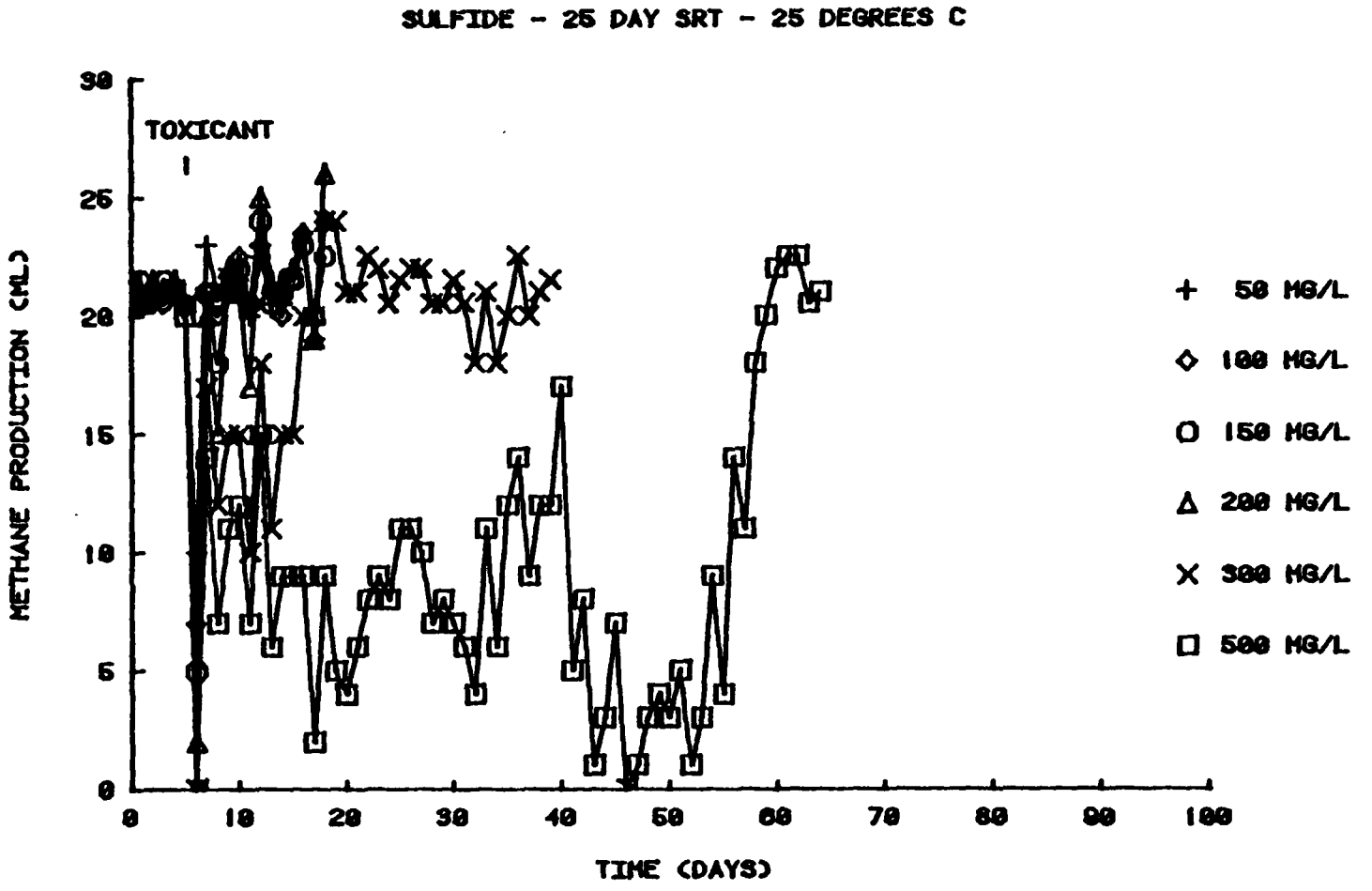


FIGURE 66. RESPONSE OF METHANOGENS TO SLUG DOSES OF SULFIDE

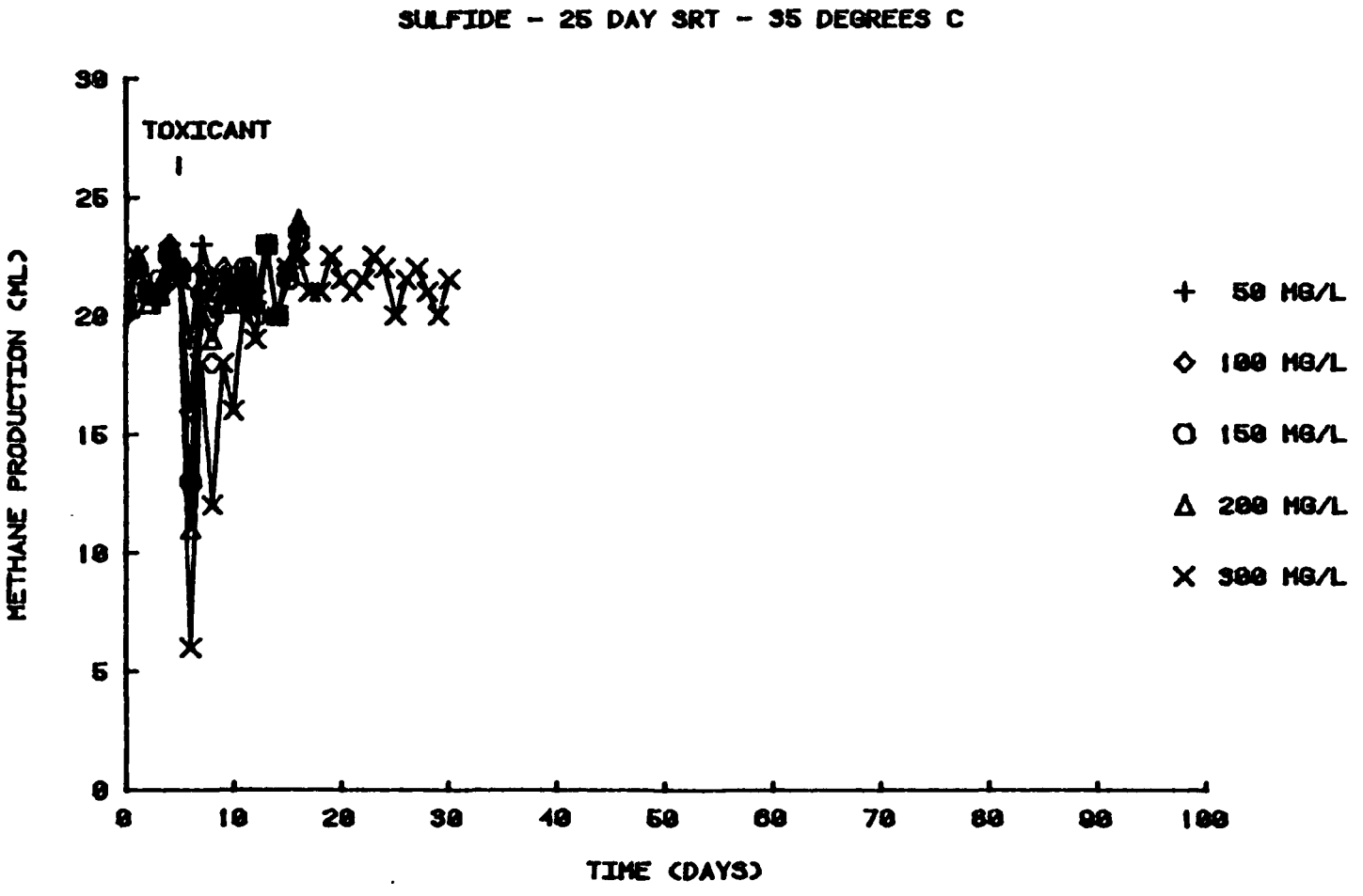


FIGURE 67. RESPONSE OF METHANOGENS TO SLUG DOSES OF SULFIDE

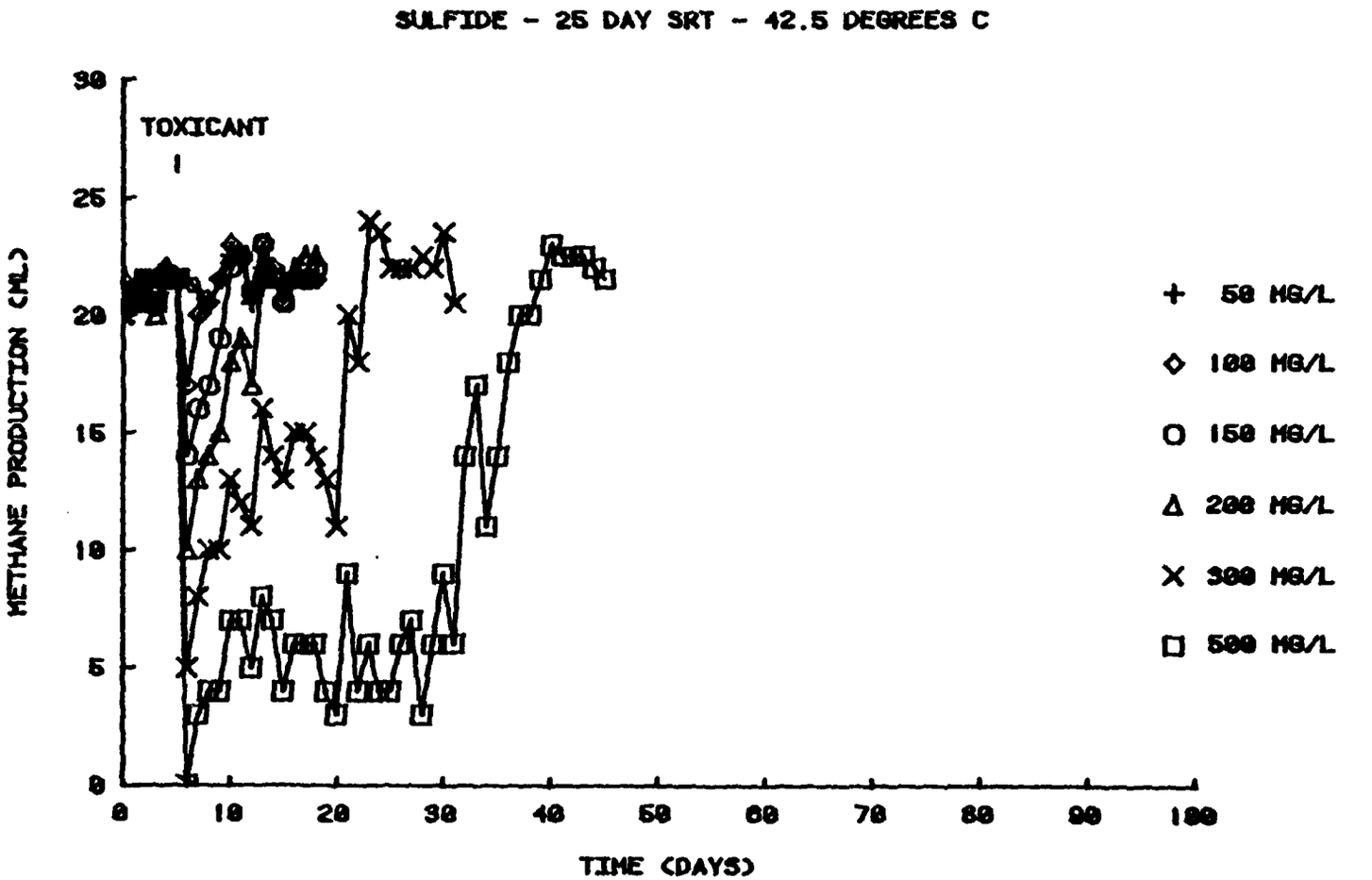


FIGURE 68. RESPONSE OF METHANOGENS TO SLUG DOSES OF SULFIDE

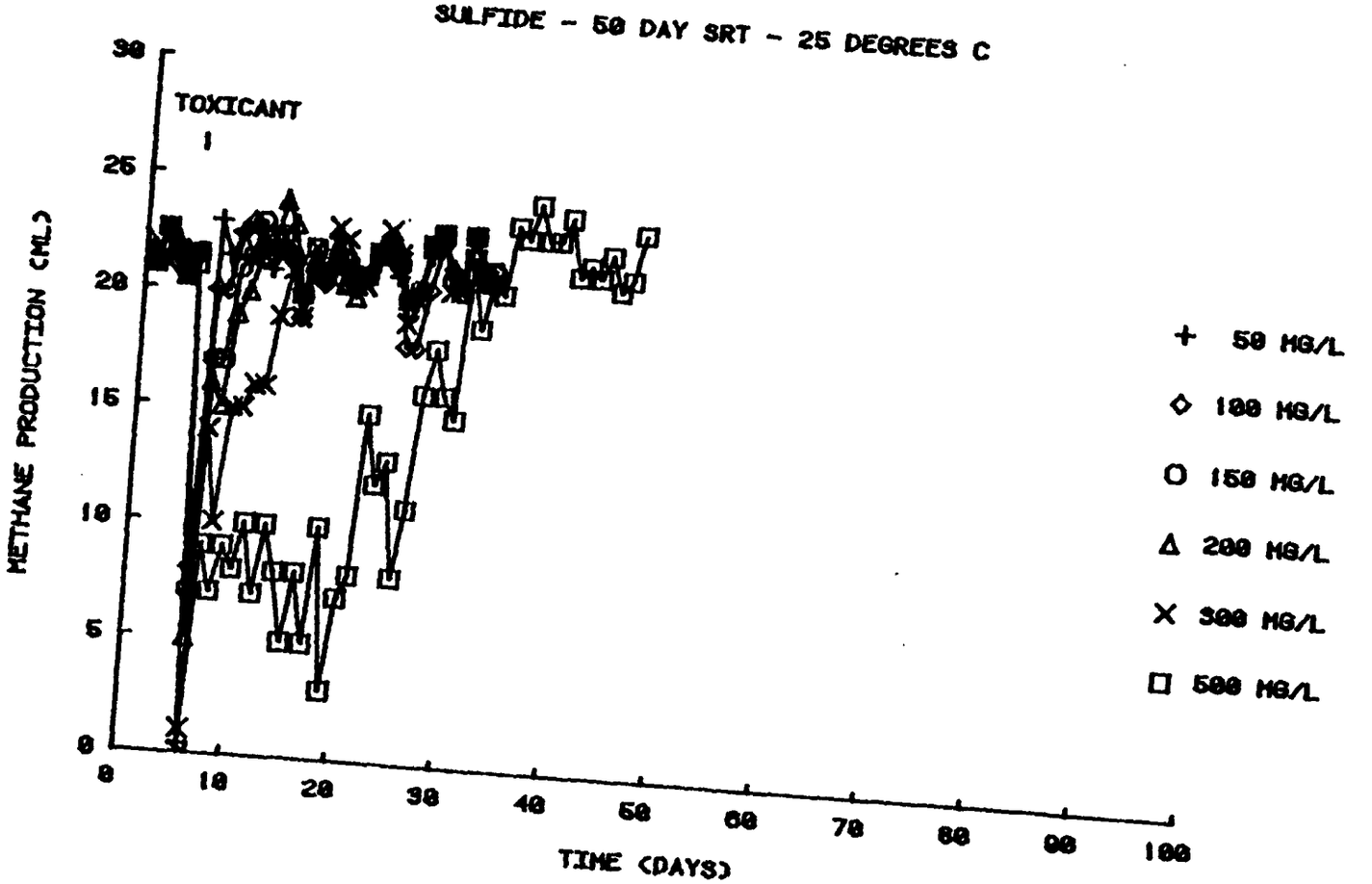


FIGURE 69. RESPONSE OF METHANOGENS TO SLUG DOSES OF SULFIDE

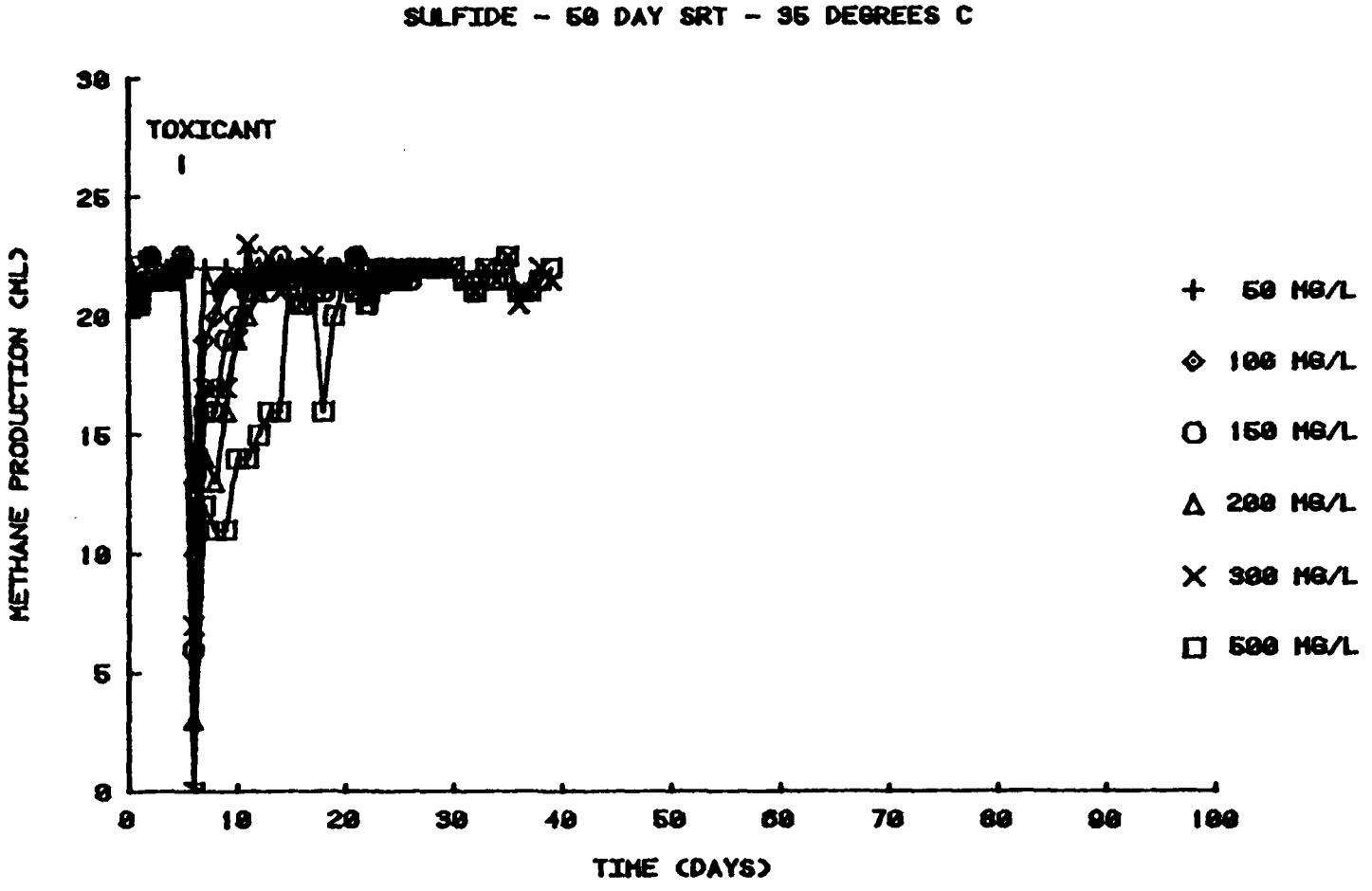


FIGURE 70. RESPONSE OF METHANOGENS TO SLUG DOSES OF SULFIDE

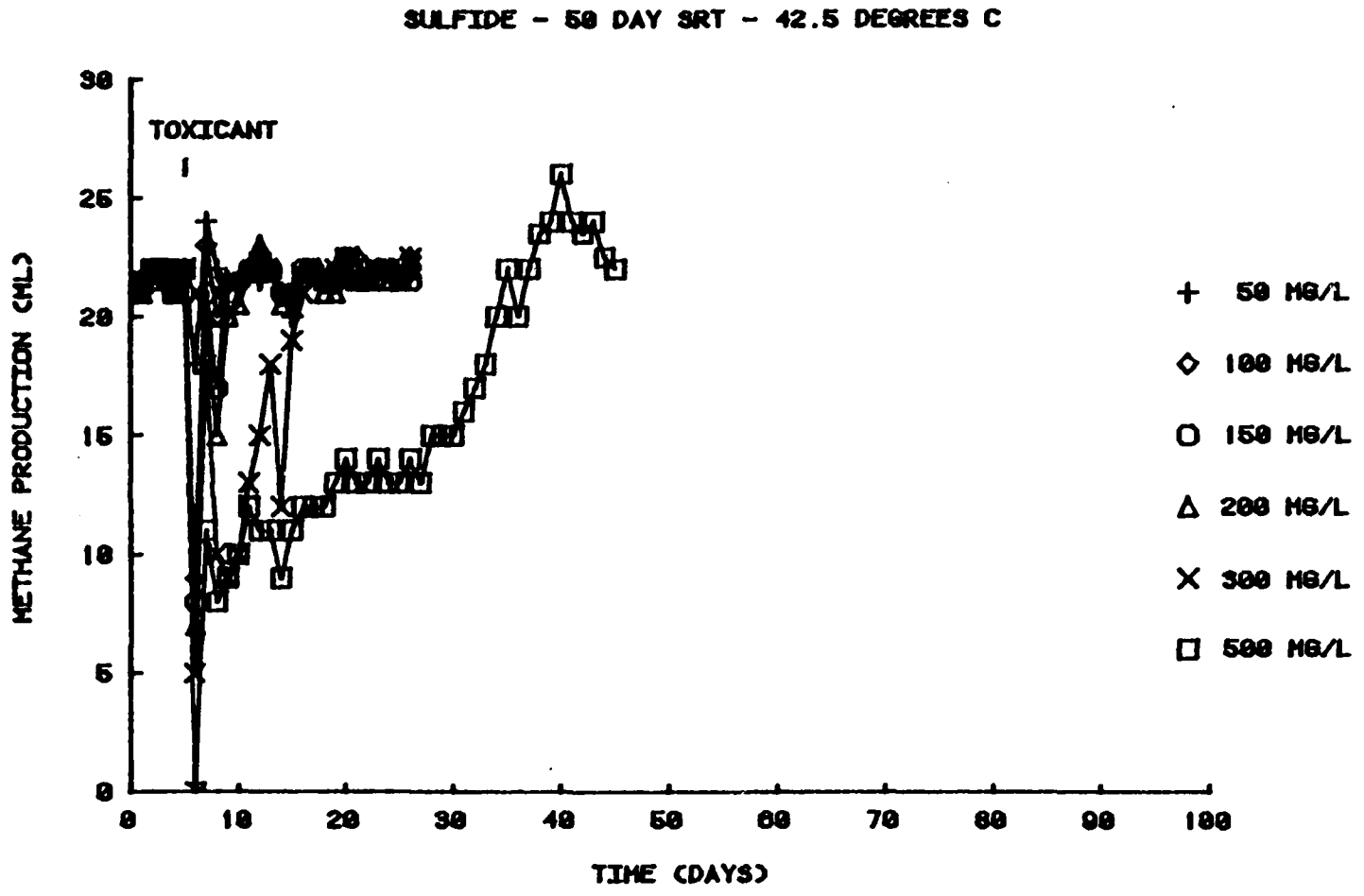


FIGURE 71. RESPONSE OF METHANOGENS TO SLUG DOSES OF SULFIDE

response at 42.5°C was a little less severe, and minimum sensitivity to sulfide was exhibited by 35°C serum bottles.

Acclimation to sulfide in the concentration range of 100 mg/l to 800 mg/l was not indicated under any set of conditions (Figures 72 to 78). Responses to sulfide slug doses appear to be independent of previous exposure to the toxicant for the concentrations tested.

Chloroform ($CHCl_3$)

Enrichment cultures were exposed to slug-dose concentrations of chloroform of 0.25, 0.5, 1.0, 2.5, 5.0 and 7.5 mg/l. Chloroform is volatile and as such will partition between the liquid and gas phases after the slug addition is made to the serum bottle. Using the technique developed by Yang (1981), it is estimated that the concentration of chloroform in the liquid is approximately 70 percent of the desired level.

After 24 hours of toxicant exposure, dramatic decreases in methane generation were recorded for all concentrations causing a measurable response. Recovery from the lower concentrations began on the second day, and recovery was generally rapid, with the exception of bottles exposed to 5.0 and 7.5 mg/l (Figures 79 to 85).

Systems maintained with a 25-day SRT were consistently better able to cope with the chloroform than those at a 50-day SRT and the 15-day, 35°C system was the least affected, an unexpected observation. This aspect will be addressed in more detail in the DISCUSSION section.

Variation in toxicant response due to changing SRT and temperature appears to be interdependent. At a 25-day SRT, the 25°C and 35°C serum bottles were much more capable of tolerating chloroform exposure than the 42.5°C cultures (Figures 80 to 82). A 50-day SRT resulted in a slightly more severe response by 35°C bottles compared to the 25-day SRT (Figure 84) and the 42.5°C responses were generally independent of SRT (Figures 82 and 86). However, increasing the SRT resulted in much more severe responses by 25°C cultures (Figure 83).

Considerable acclimation was demonstrated at all temperatures and SRTs (Figures 86 to 92).

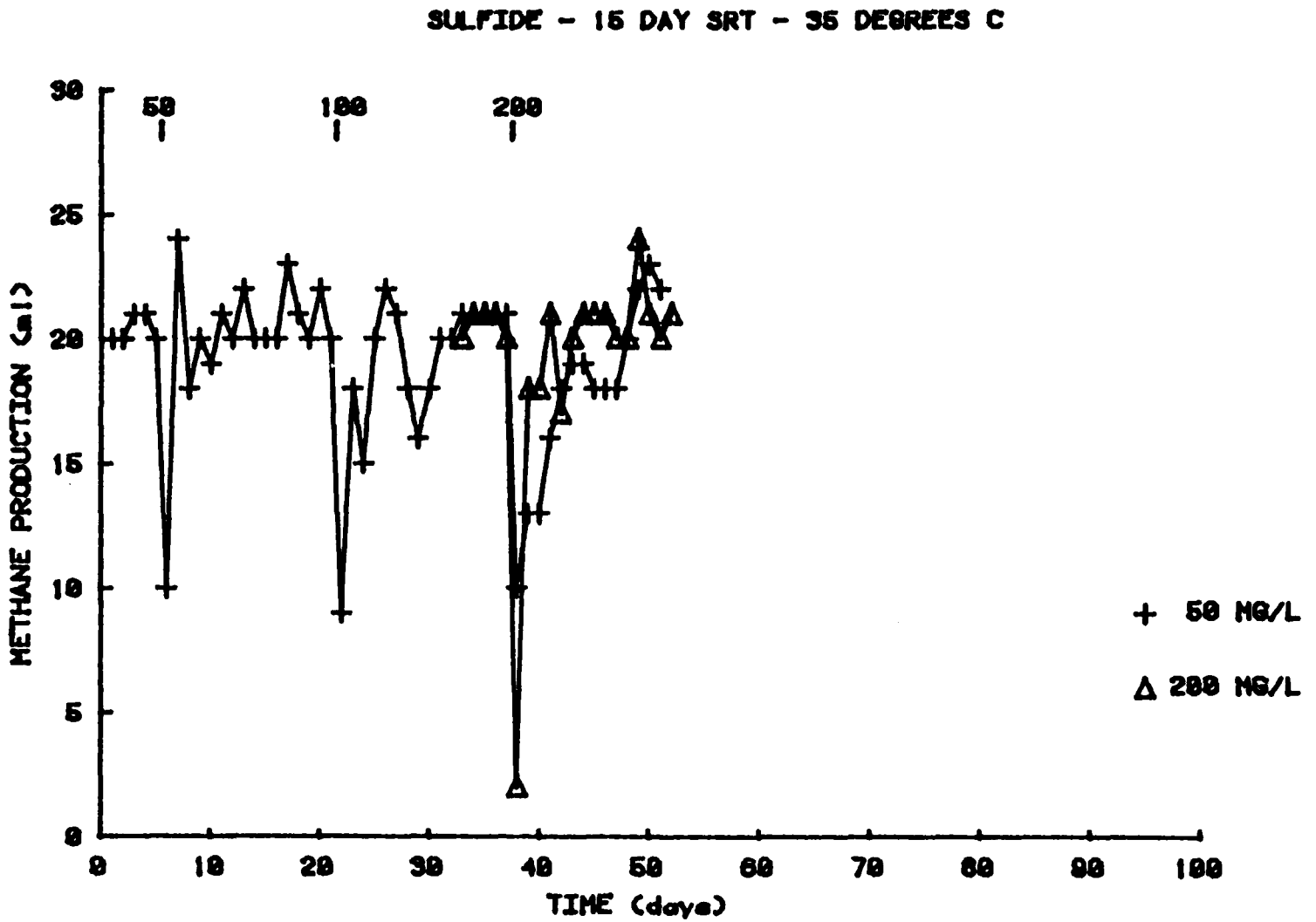


FIGURE 72. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF SULFIDE

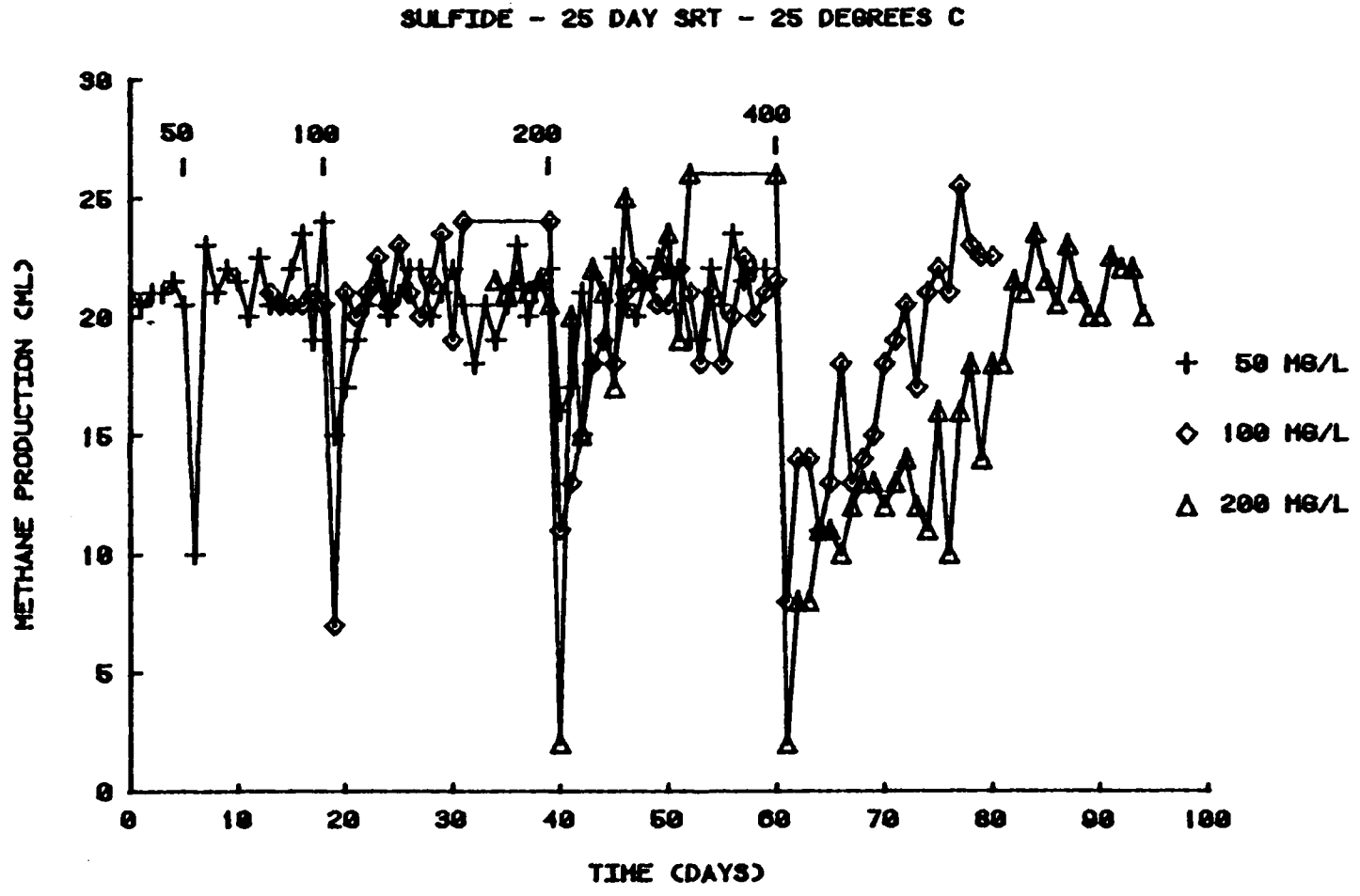


FIGURE 73. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF SULFIDE

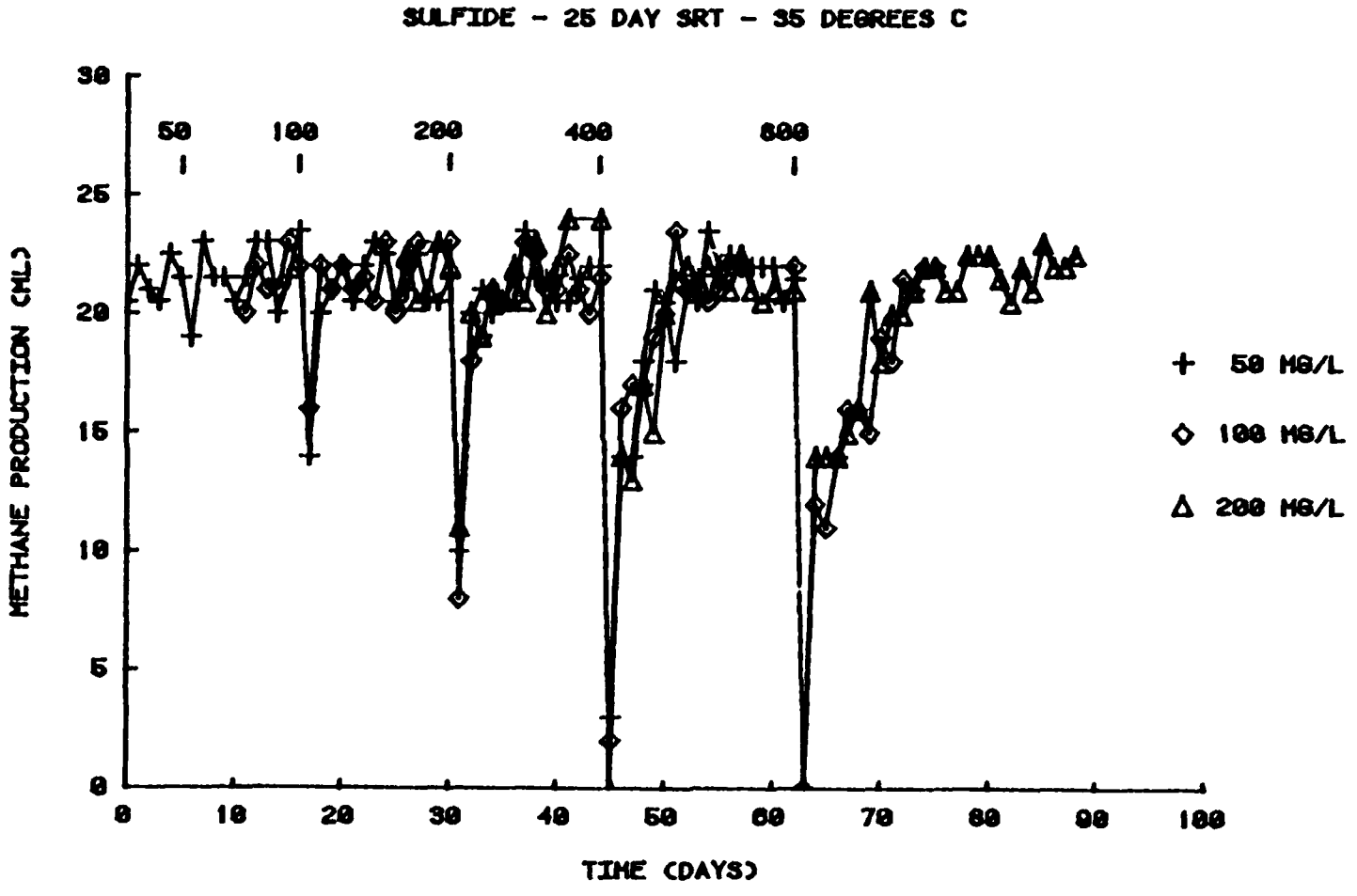


FIGURE 74. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF SULFIDE

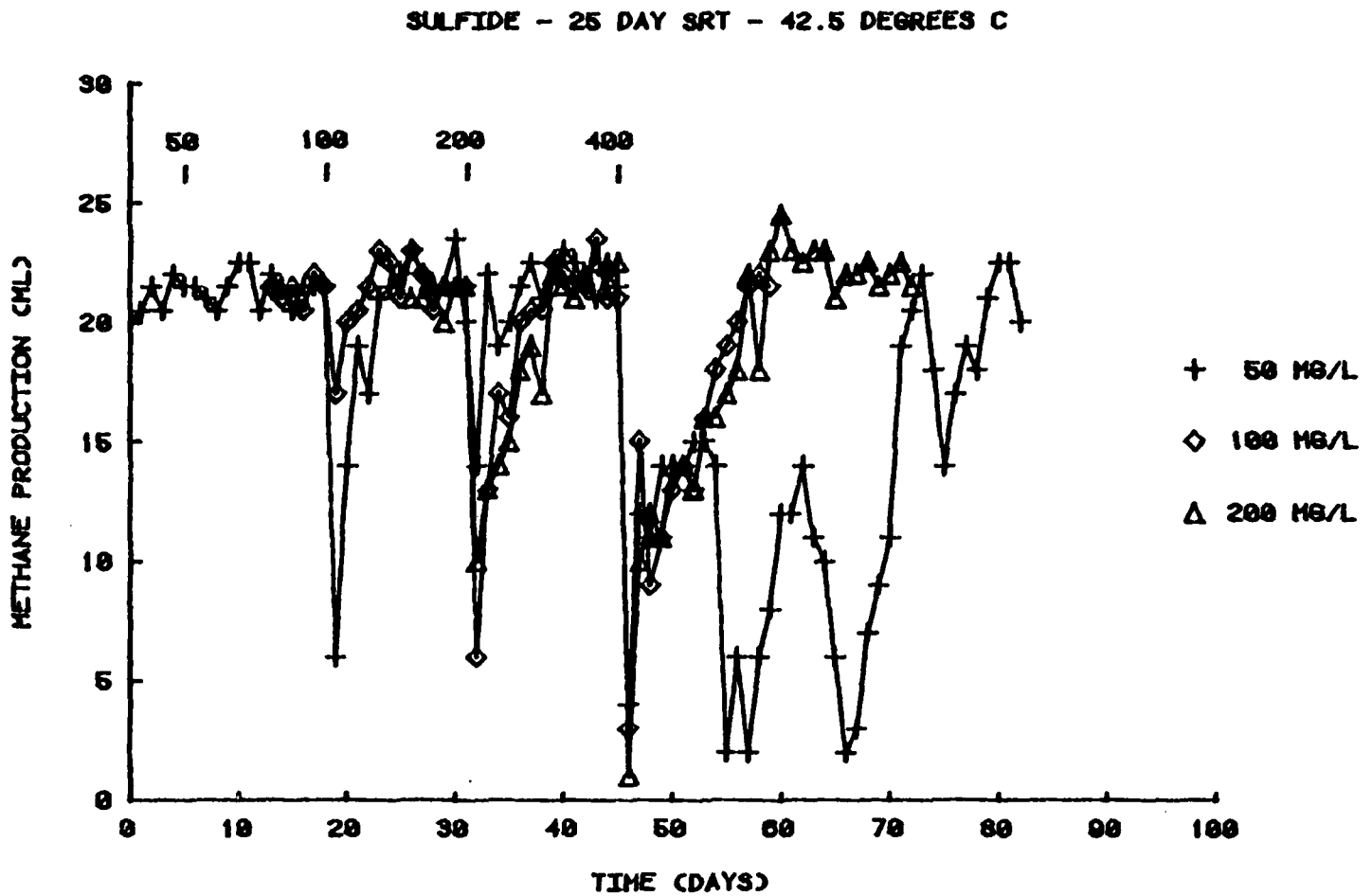
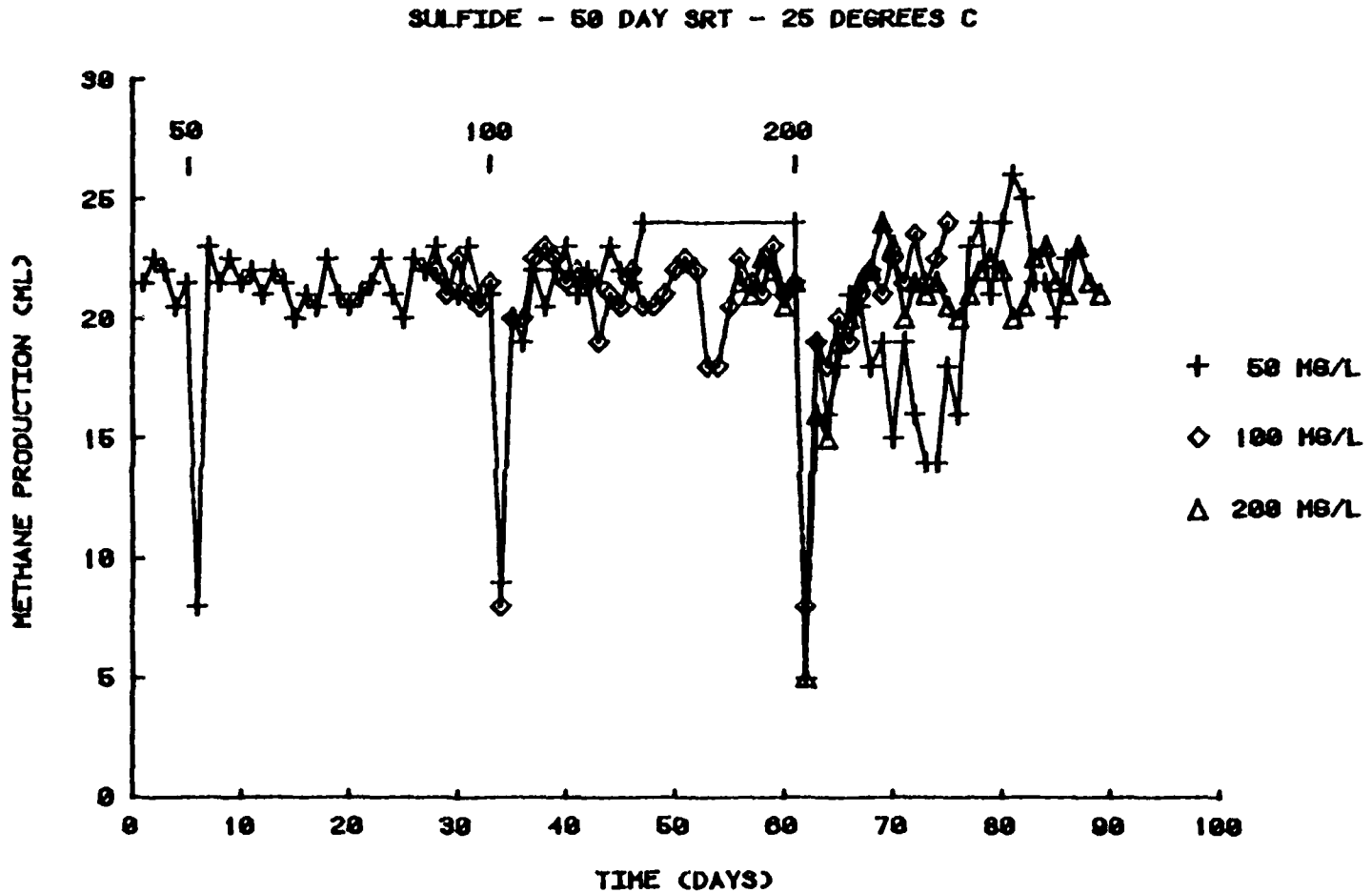


FIGURE 75. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF SULFIDE



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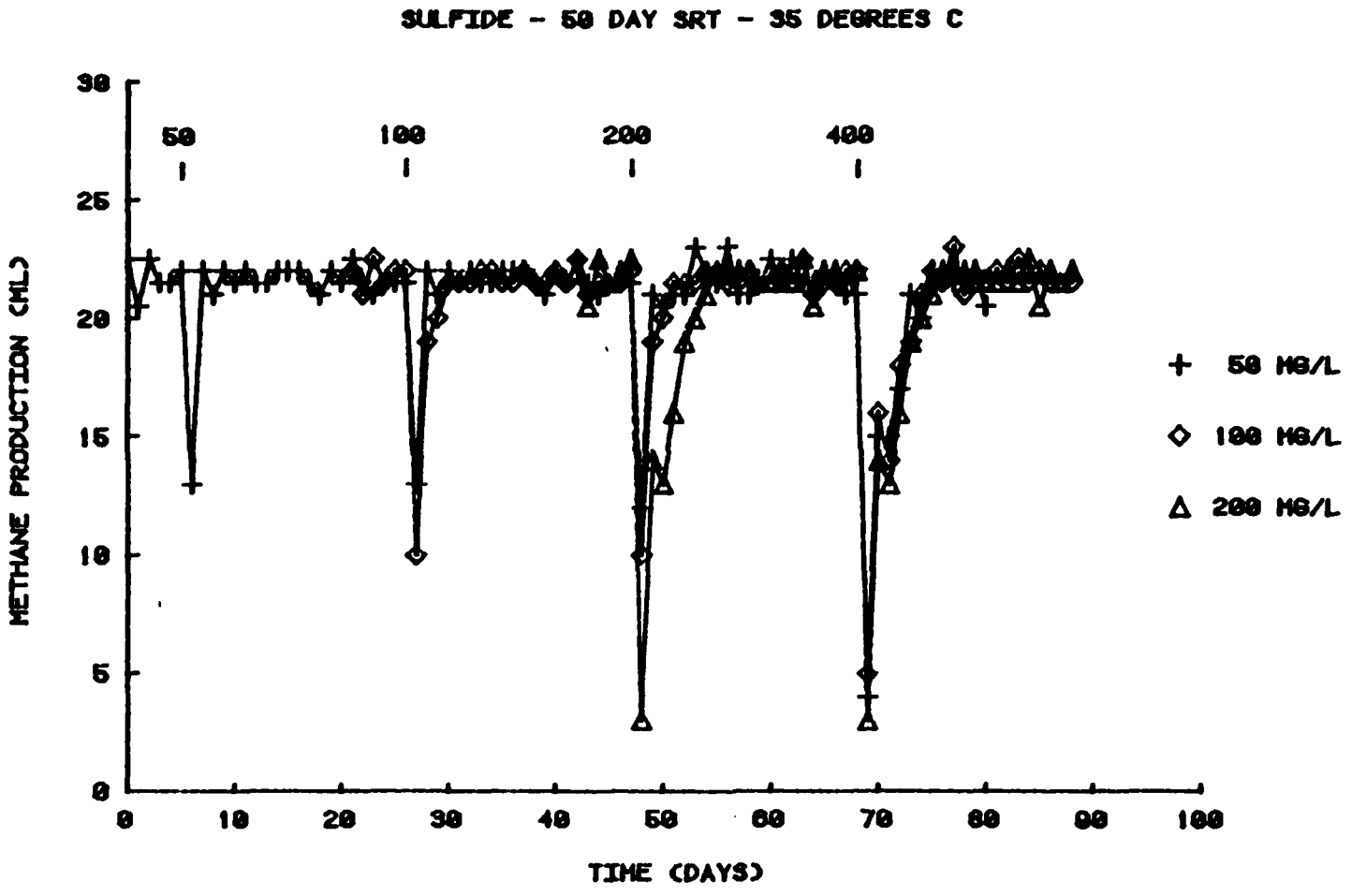

FIGURE 77. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF SULFIDE

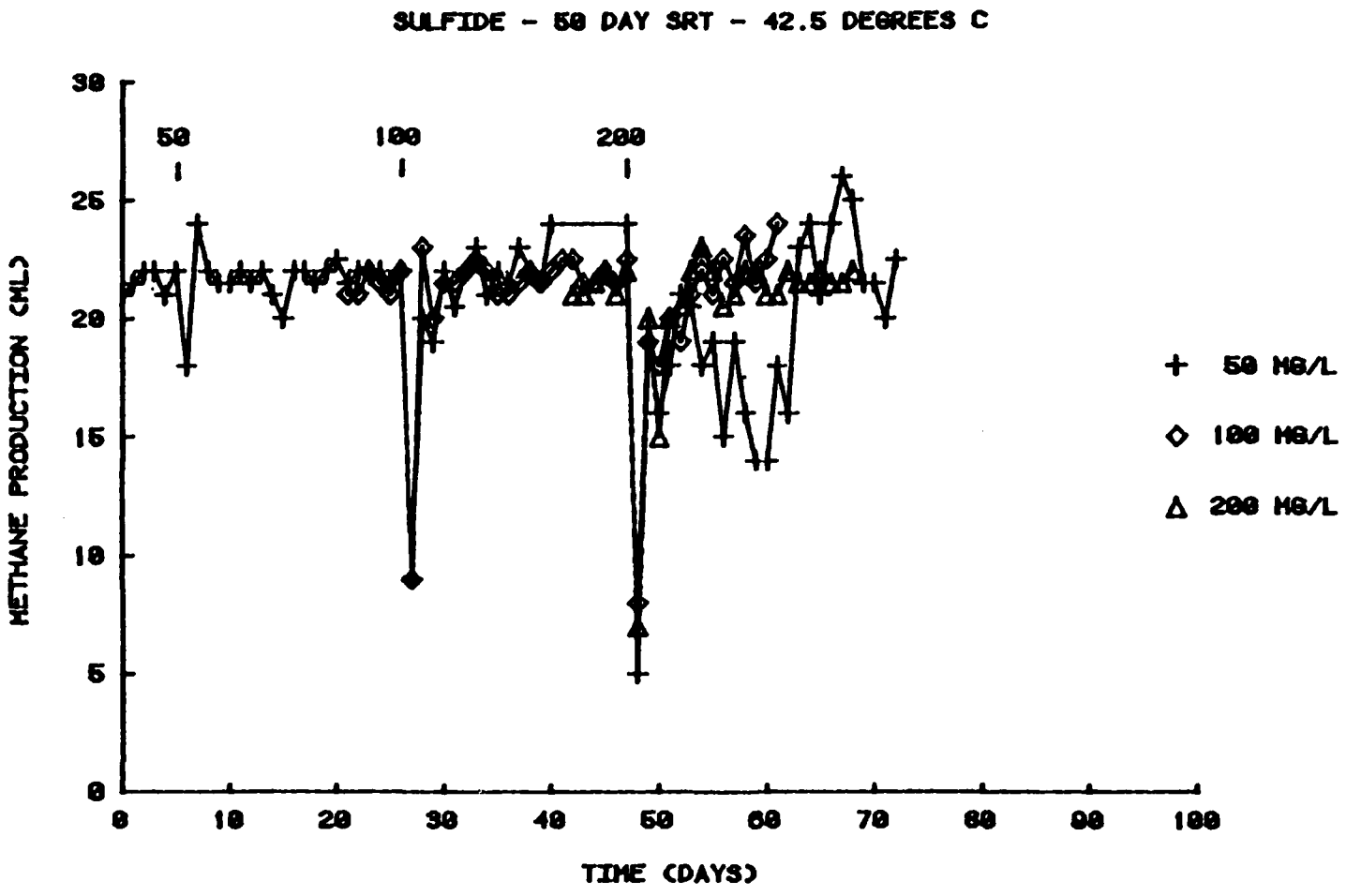

FIGURE 78. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF SULFIDE

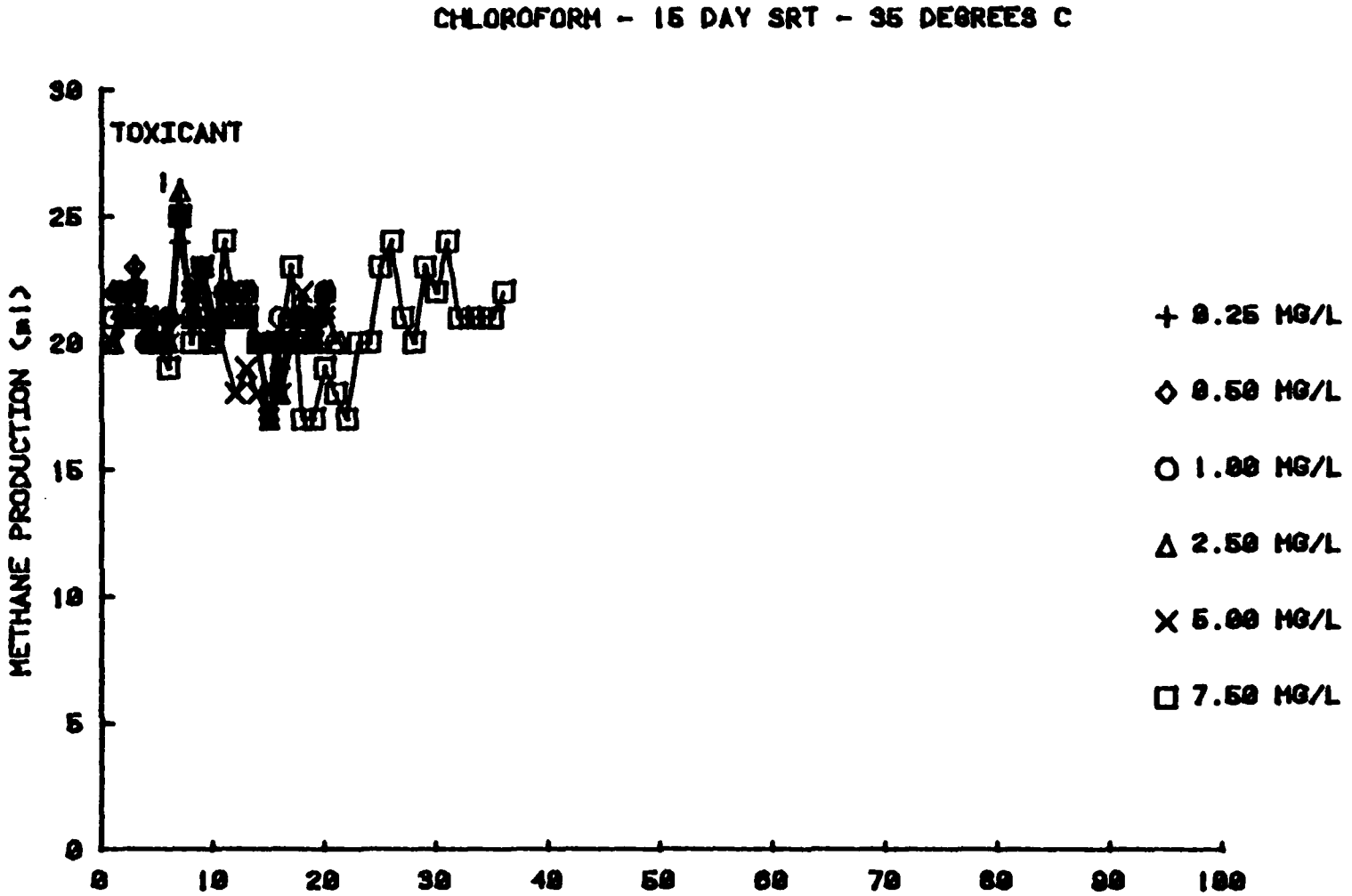

FIGURE 79. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

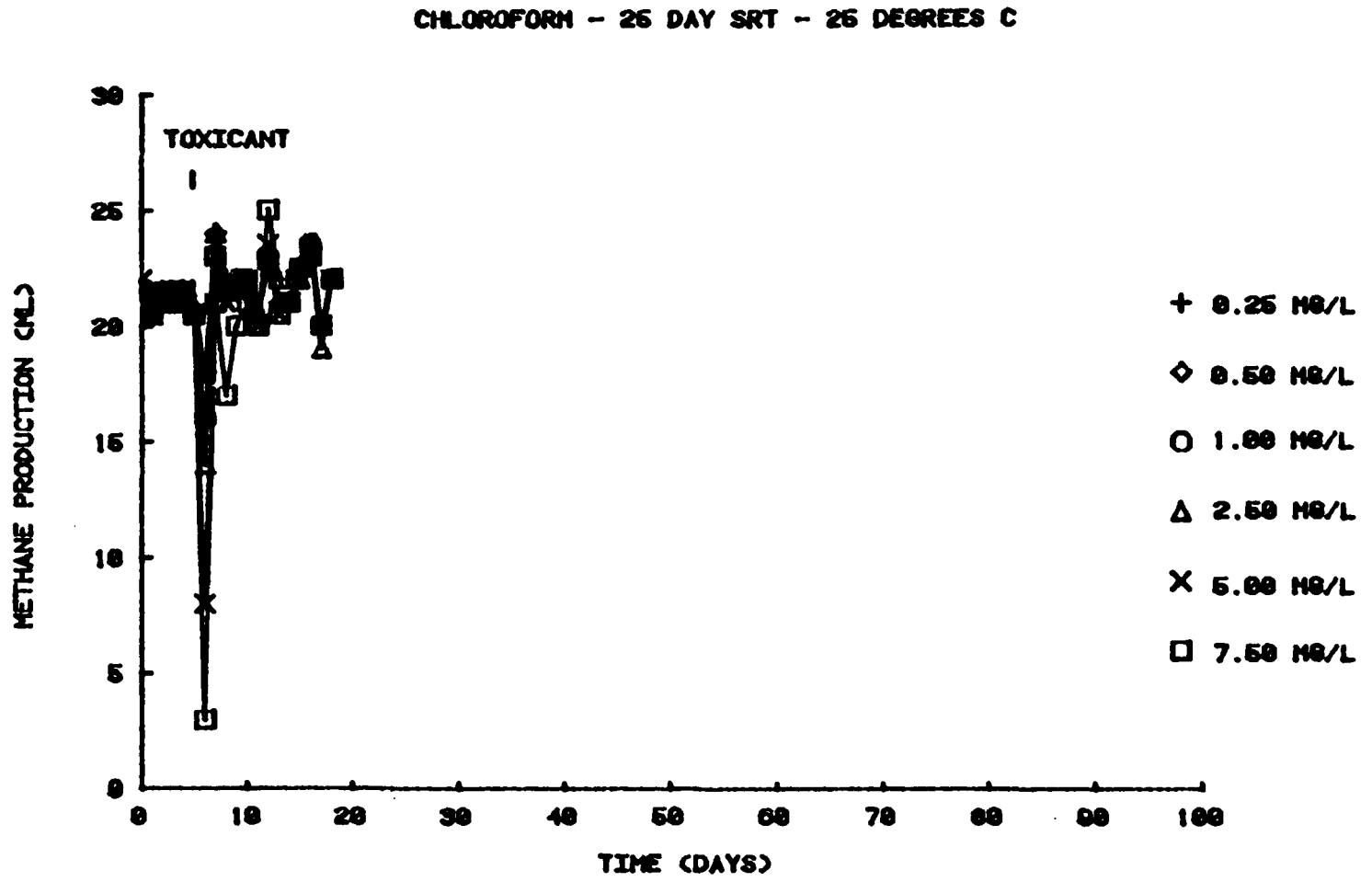

FIGURE 80. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

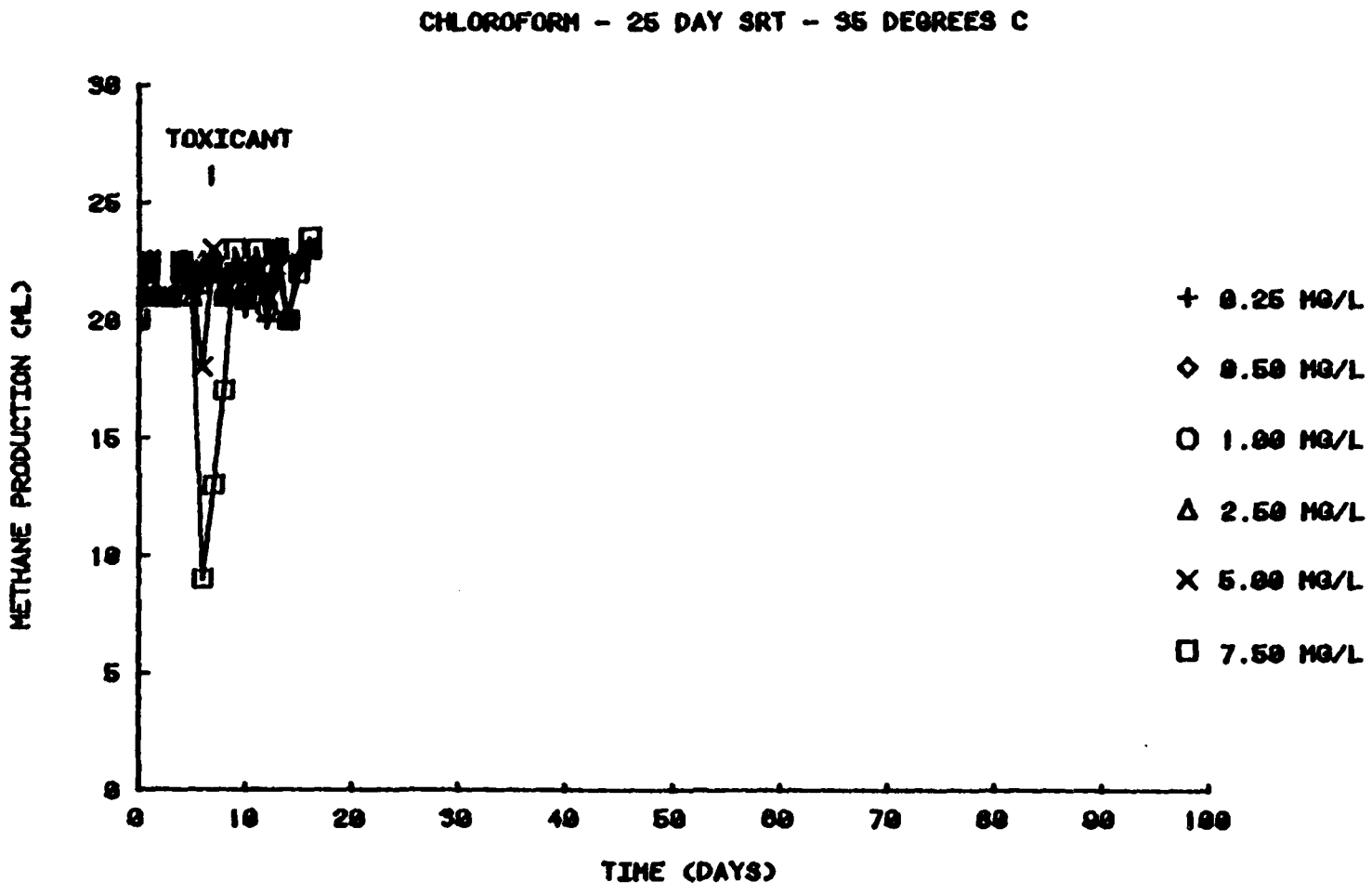

FIGURE 81. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

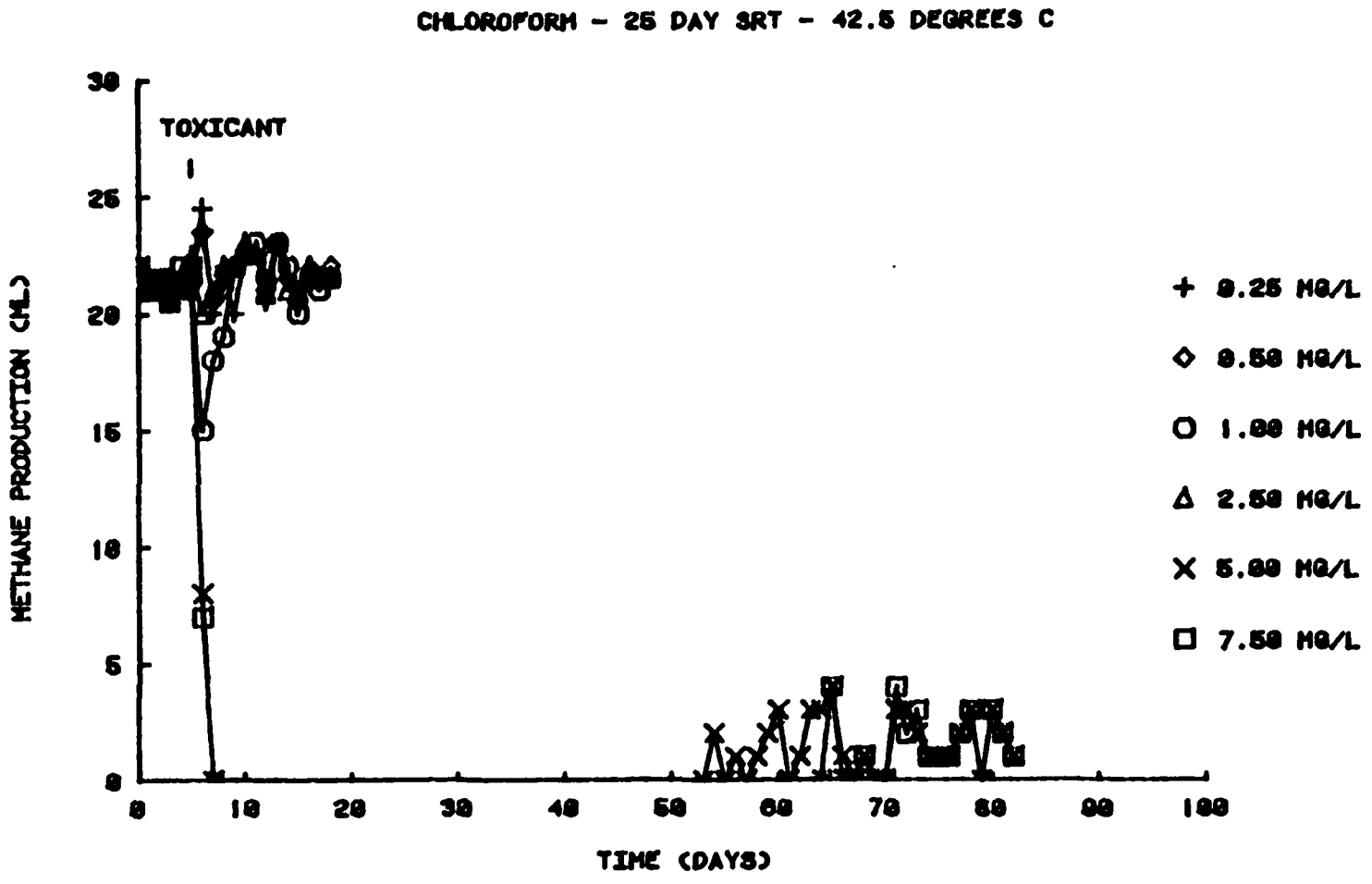

FIGURE 82. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

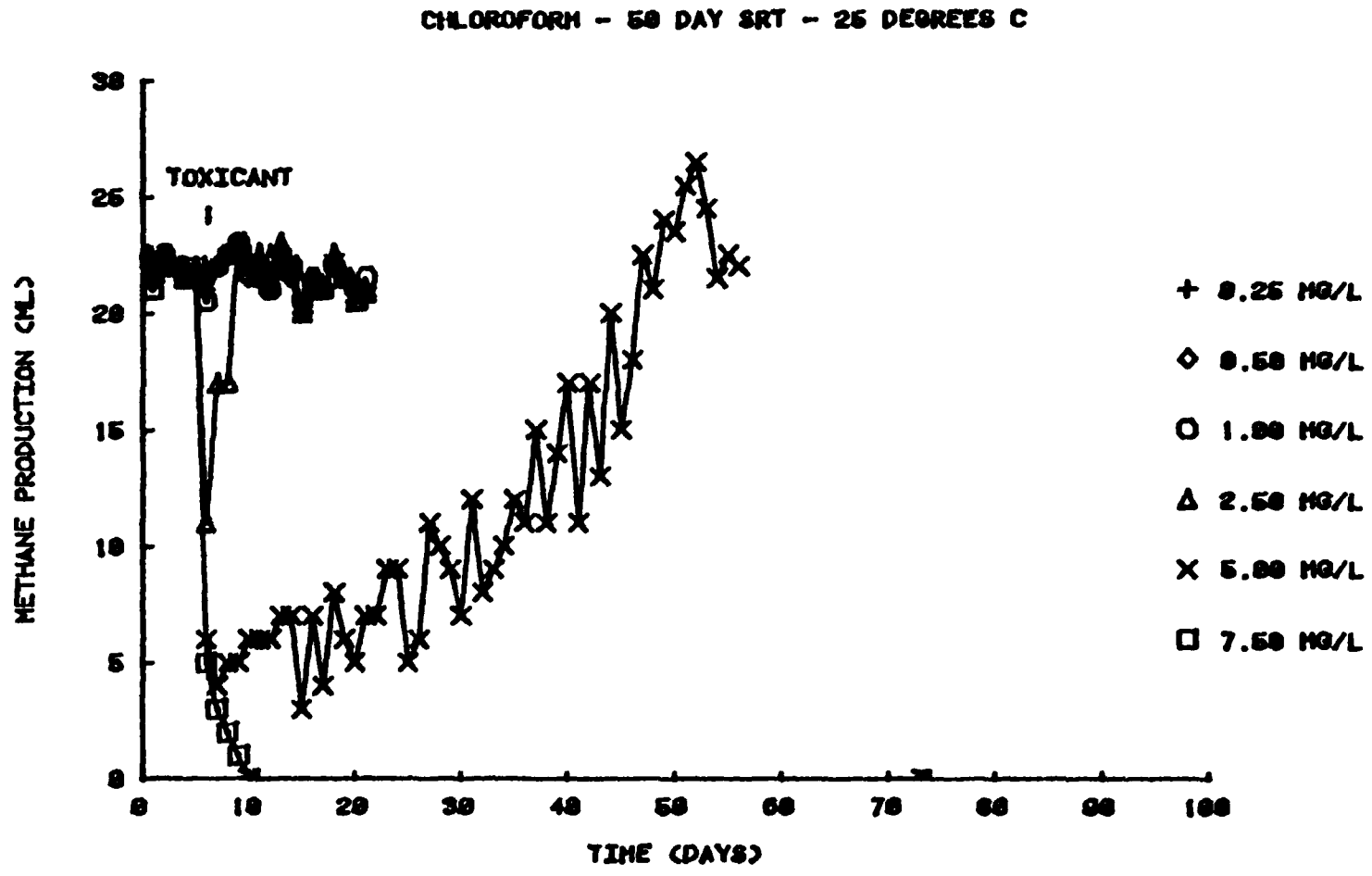

FIGURE 83. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

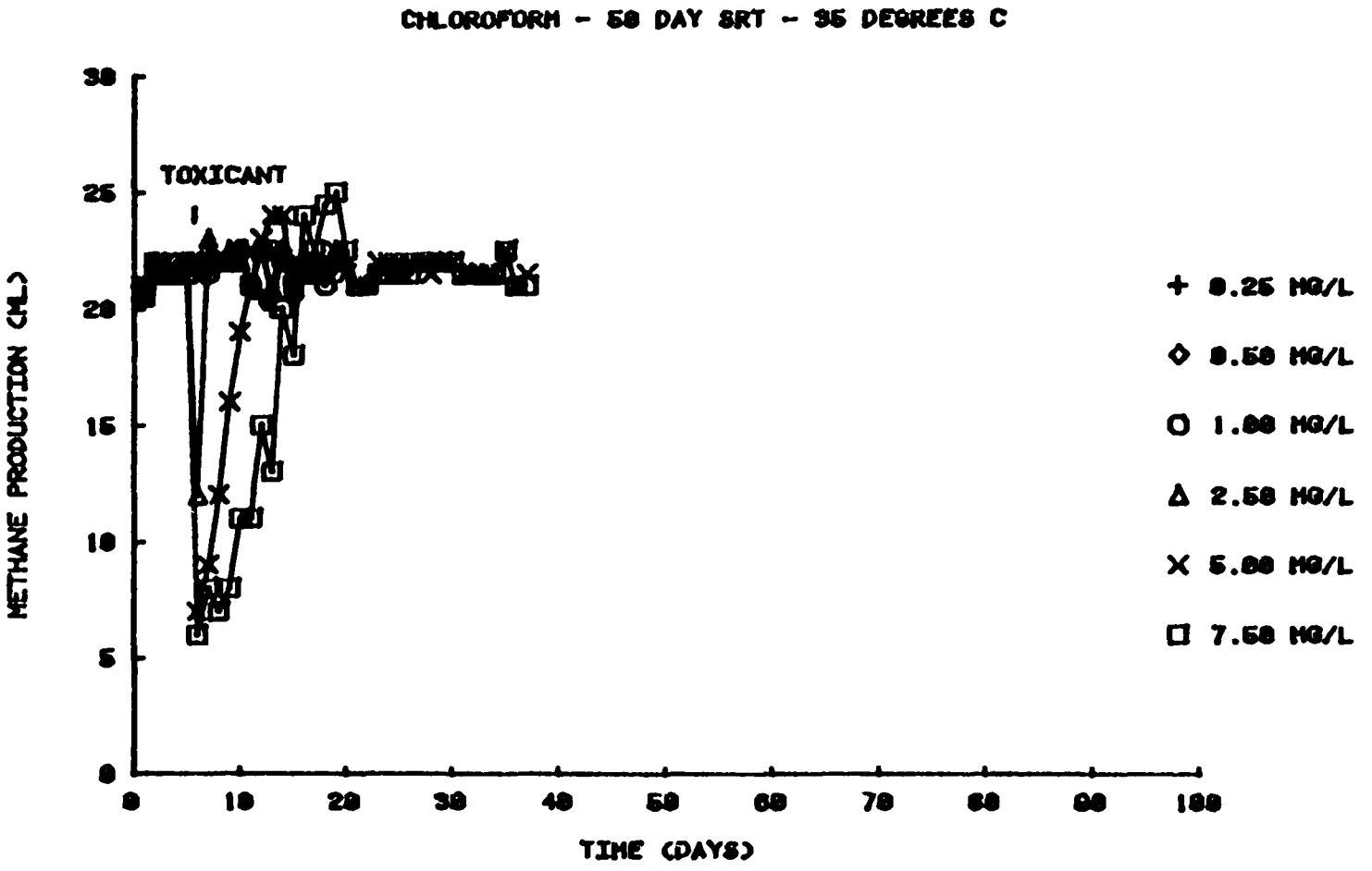

FIGURE 84. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

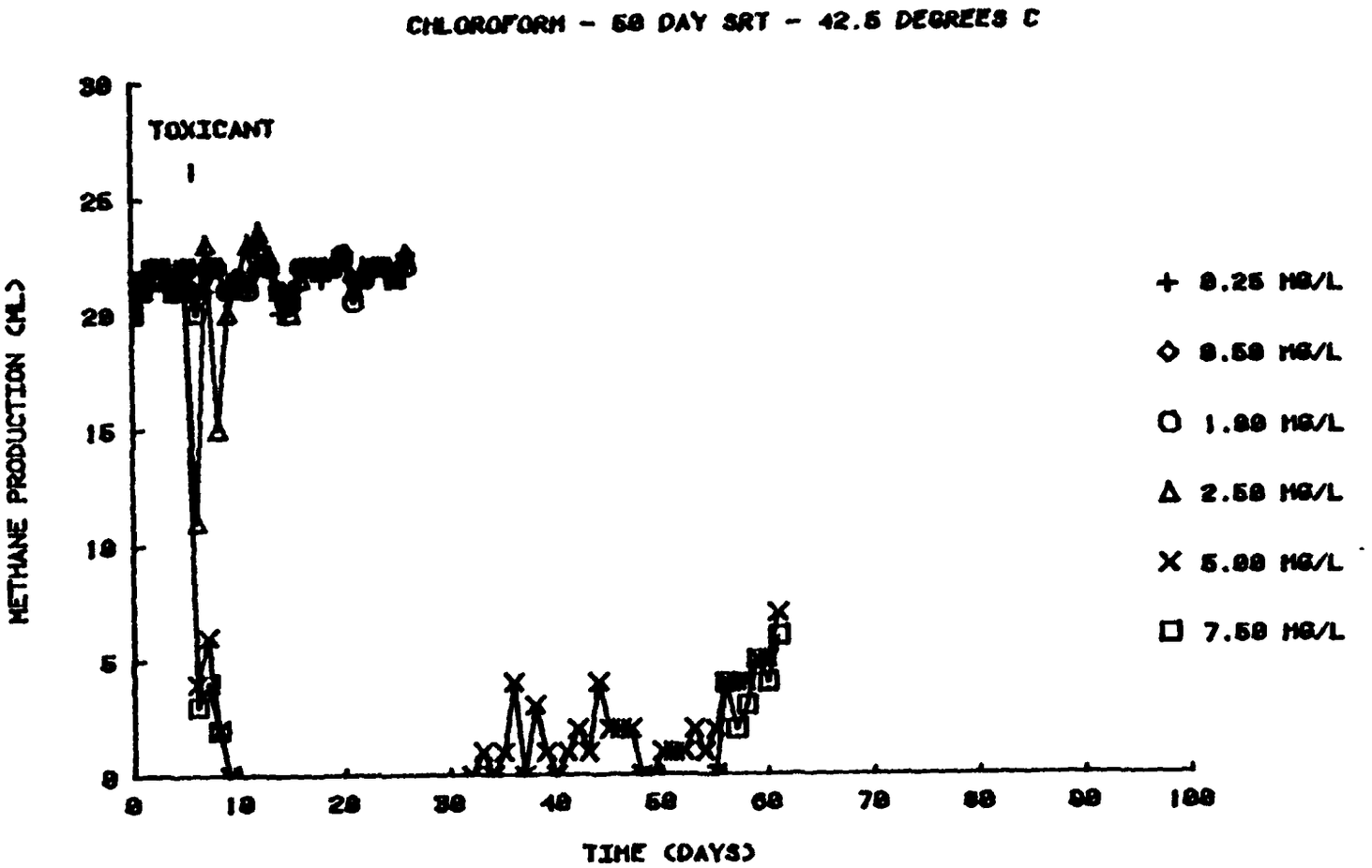

FIGURE 85. RESPONSE OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

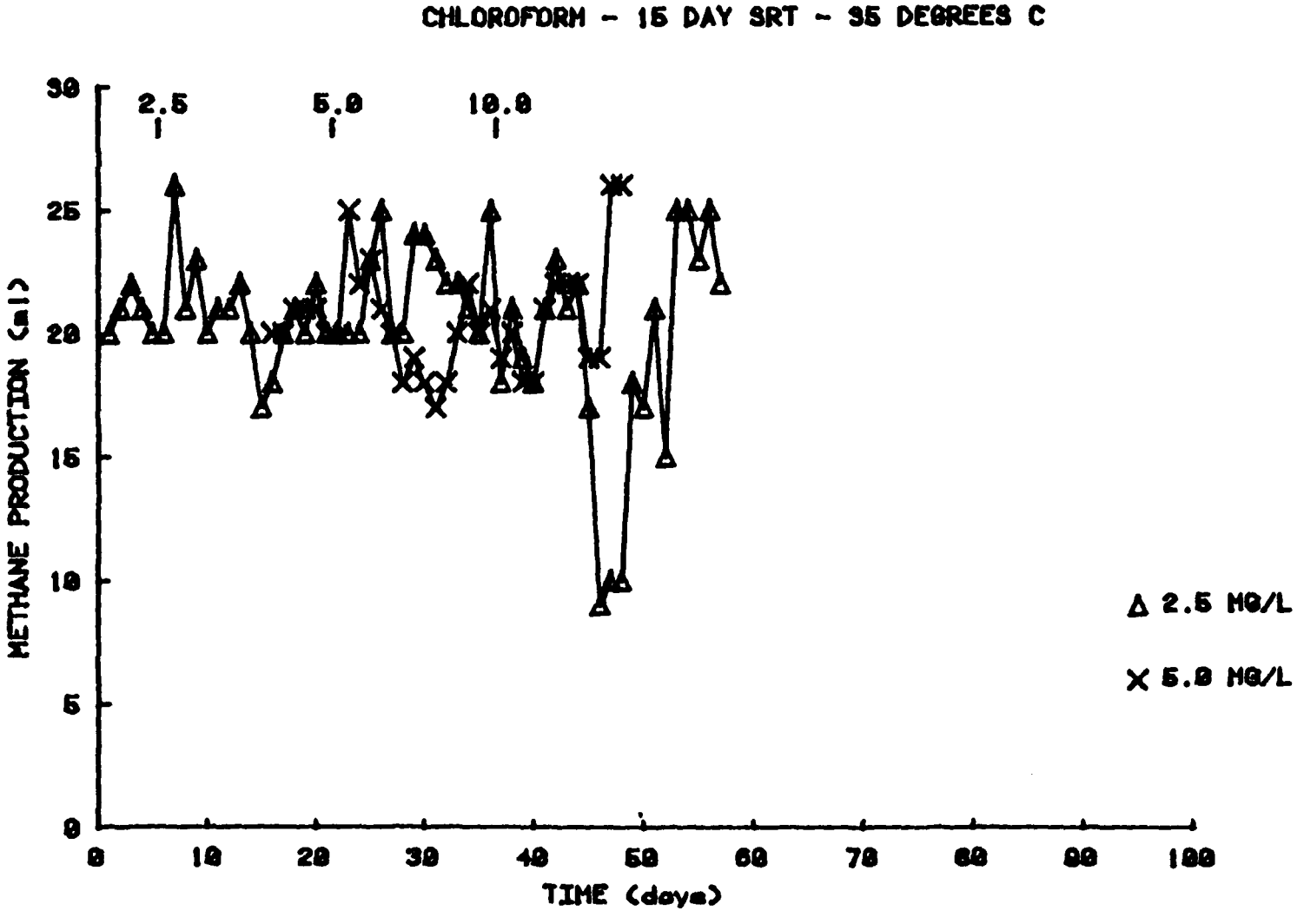

FIGURE 86. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

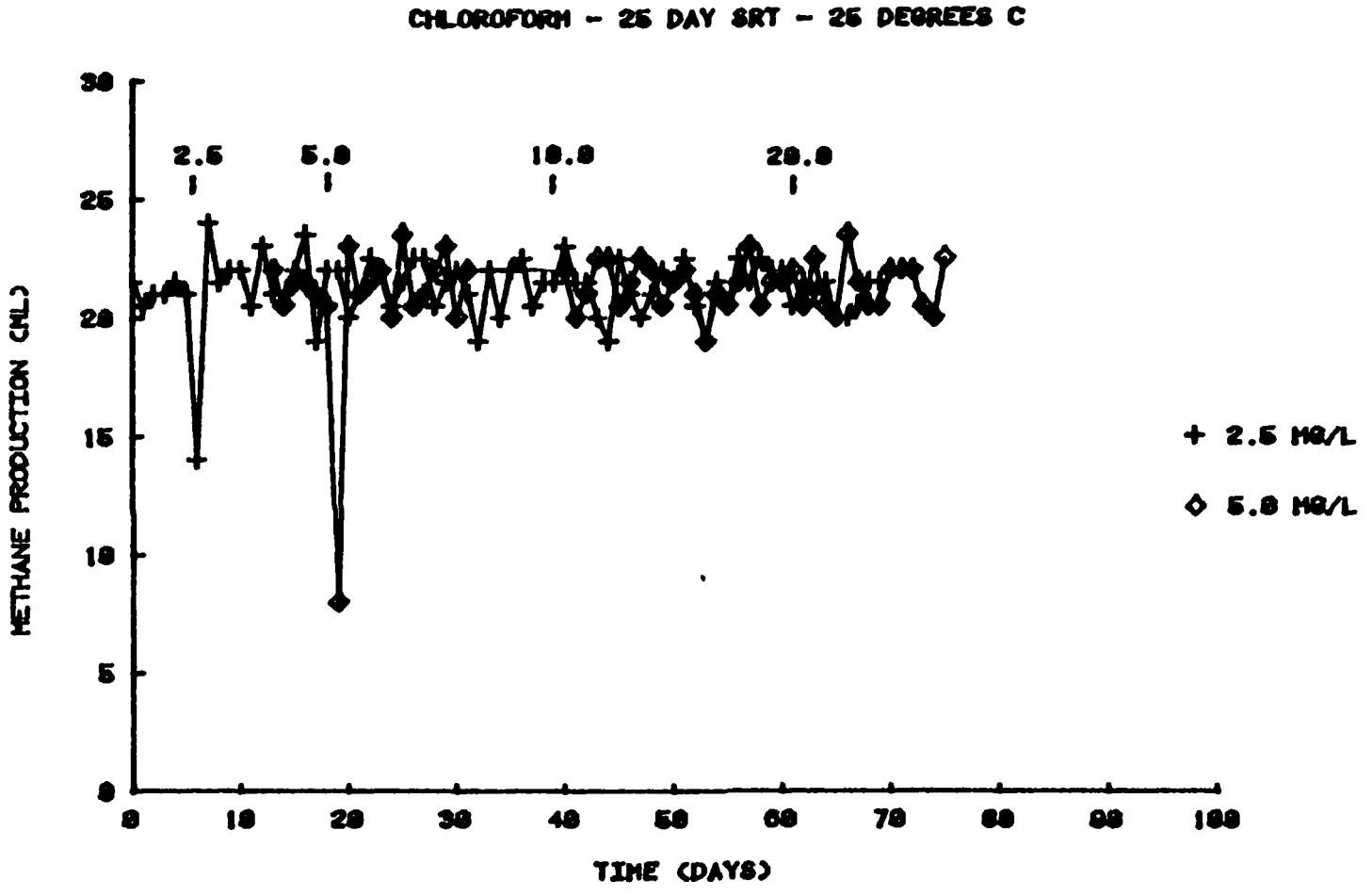

FIGURE 87. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

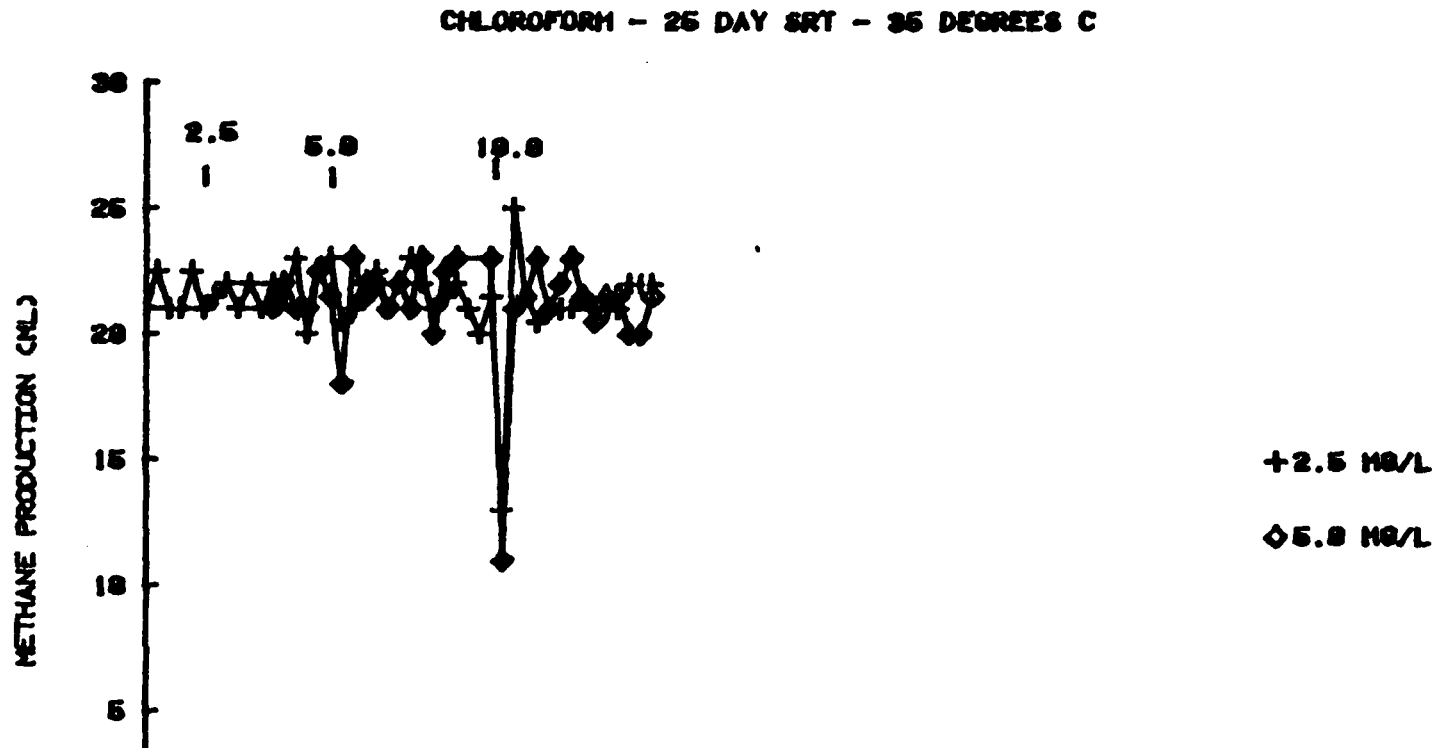

FIGURE 88. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

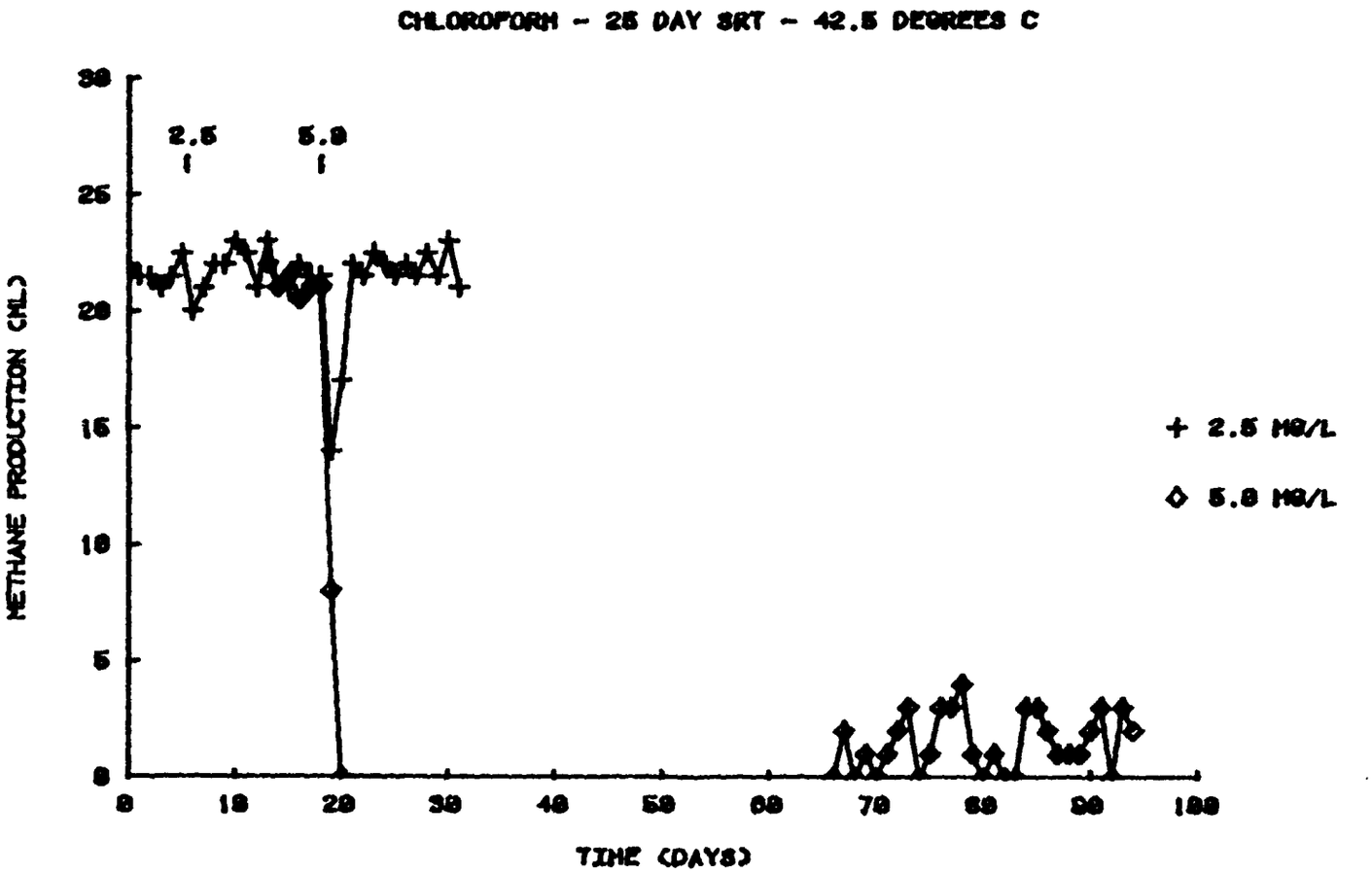

FIGURE 89. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

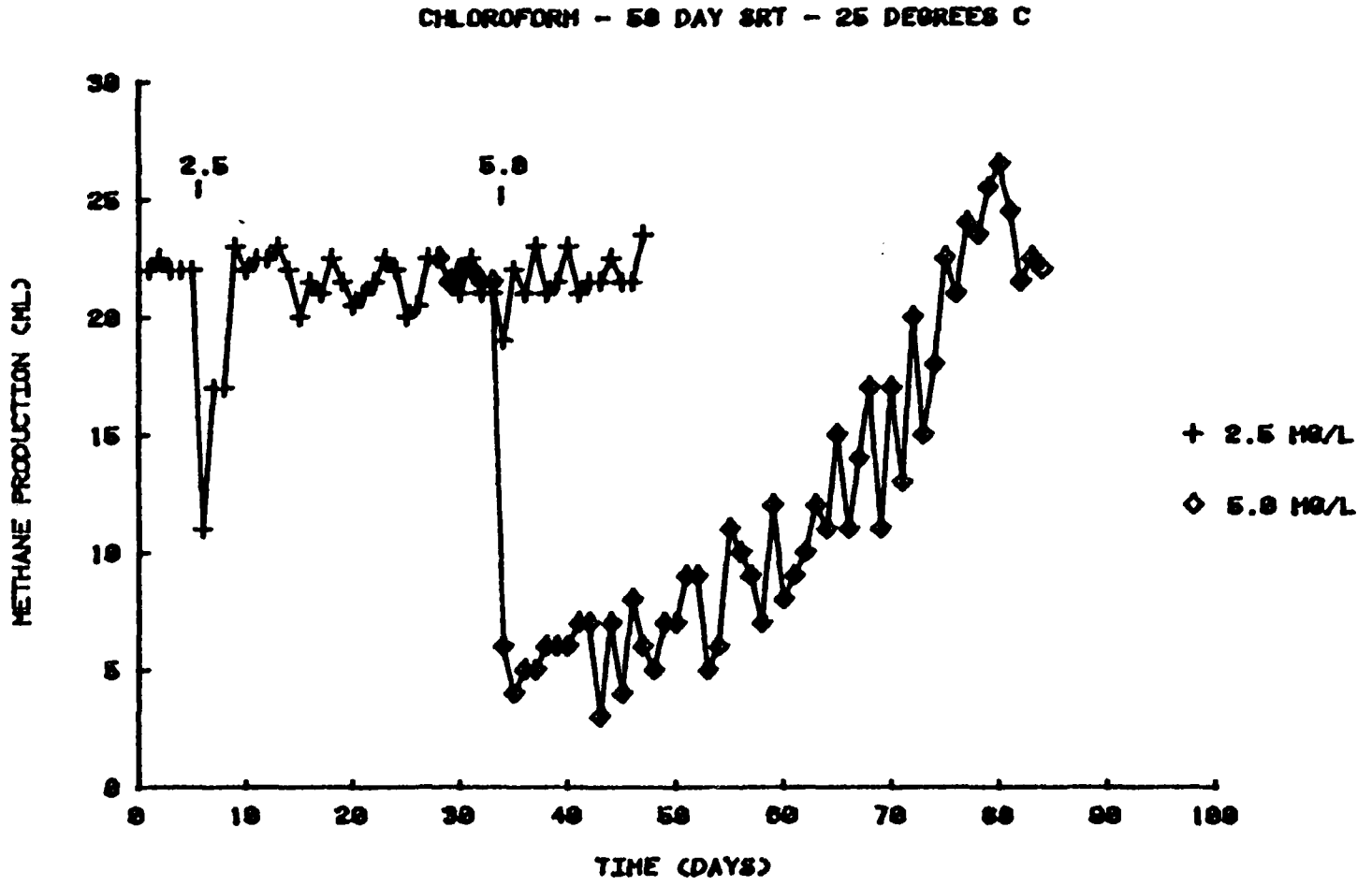

FIGURE 90. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

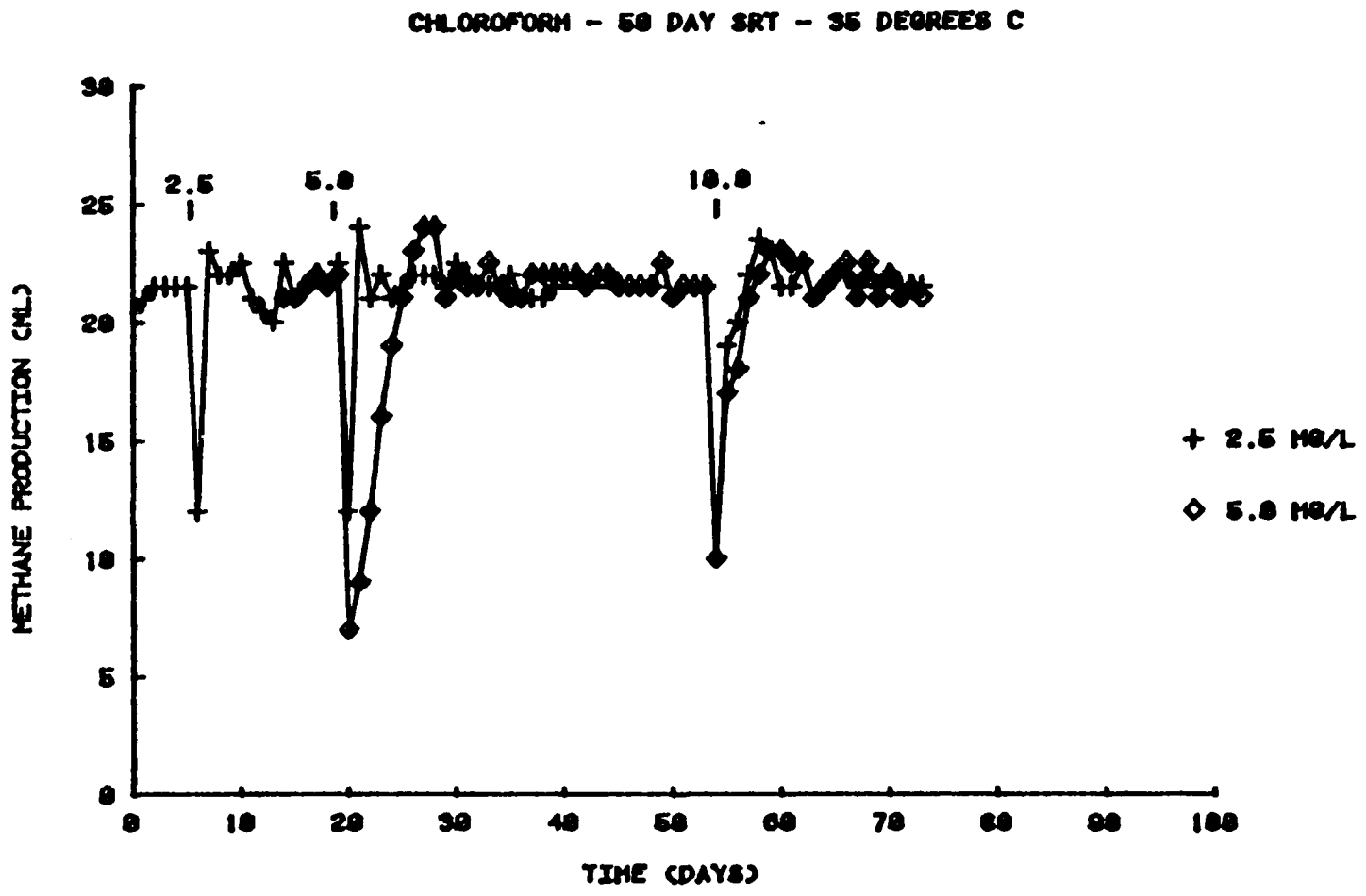

FIGURE 91. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

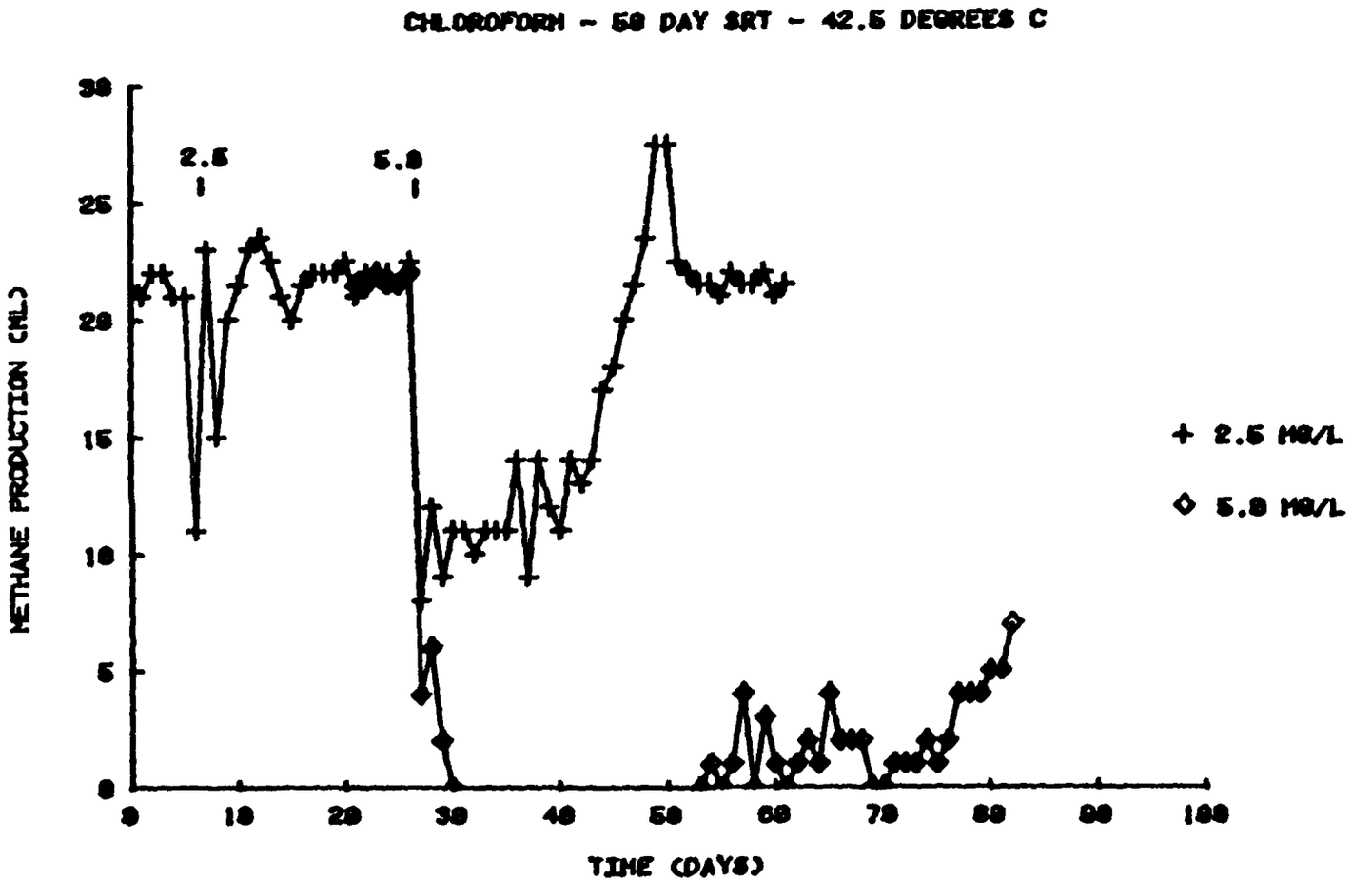

FIGURE 92. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF CHLOROFORM

Dichloroethylene (ClHC=CHCl)

Slug dose concentrations of 25, 50, 100, 250, 500 and 1000 mg/l were added to serum bottles using undiluted dichloroethylene as stock. Toxicant exposure caused immediate and dramatic decreases in methane production. Recovery was erratic at all three temperatures (Figures 92 to 95).

Severity of responses increased with increasing temperature. The optimum temperature appears to be 25°C (Figure 92); severity of responses to 25 and 50 mg/l dichloroethylene increased significantly at 35°C (Figure 94), with a similar increase in response severity by 42.5°C cultures (Figure 95). Acclimation to dichloroethylene was not observed (Figures 96 to 98).

Trichloroethylene ($Cl_2C=CHCl$)

Concentrations of 25, 50, 100, 250, 500 and 1000 mg/l trichloroethylene were added to serum bottles as slug doses. Responses to trichloroethylene were generally immediate, although 1-day delays occurred with some lower concentrations. Recovery was erratic, similar to dichloroethylene recovery patterns (Figures 99 to 101).

Minimal responses to trichloroethylene were observed at 25°C (Figure 99). The severity of responses increased as temperatures were increased to 35°C and 42.5°C (Figures 100 and 101). Acclimation to trichloroethylene did not occur with the conditions tested (Figures 102 to 104).

Ethyl Benzene ($C_6H_5-CH_2-CH_3$)

Using undiluted ethyl benzene as stock solution, slug doses of 25, 50, 100, 250, 500 and 1000 mg/l were added to serum bottle cultures. Responses to ethyl benzene exposure were usually immediate and rapid. Recovery patterns were generally steady, although some residual toxicity was noted (Figures 105 to 107).

Effects of temperature on ethyl benzene toxicity were similar to temperature effects on inhibition by dichloroethylene and trichloroethylene. The 25°C incubation resulted in the least severe responses for exposure to the five lower toxicant concentrations; however, exposure to 1000 mg/l ethyl benzene at 25°C caused the most severe response of all conditions tested

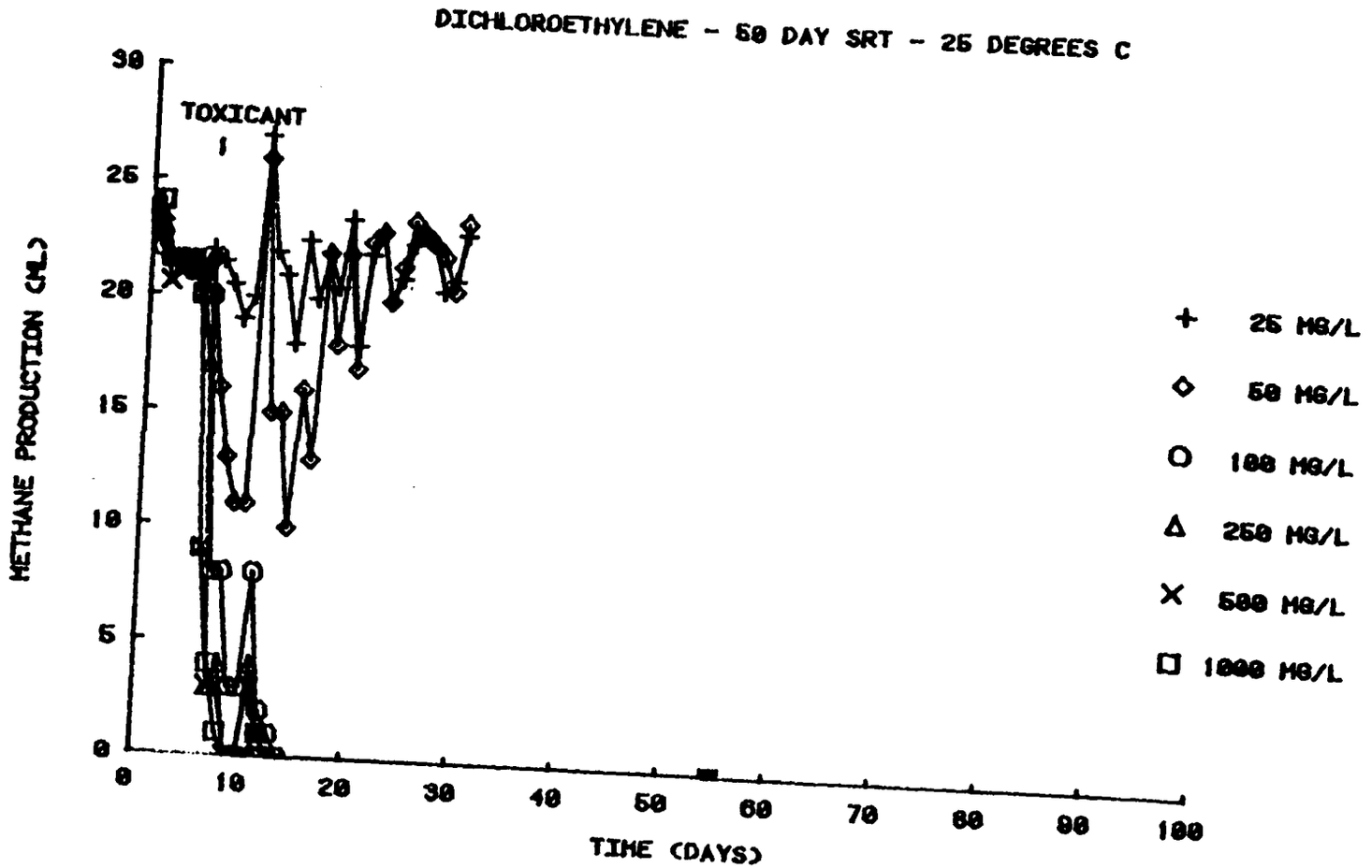

FIGURE 93. RESPONSE OF METHANOGENS TO SLUG DOSES OF DICHLOROETHYLENE

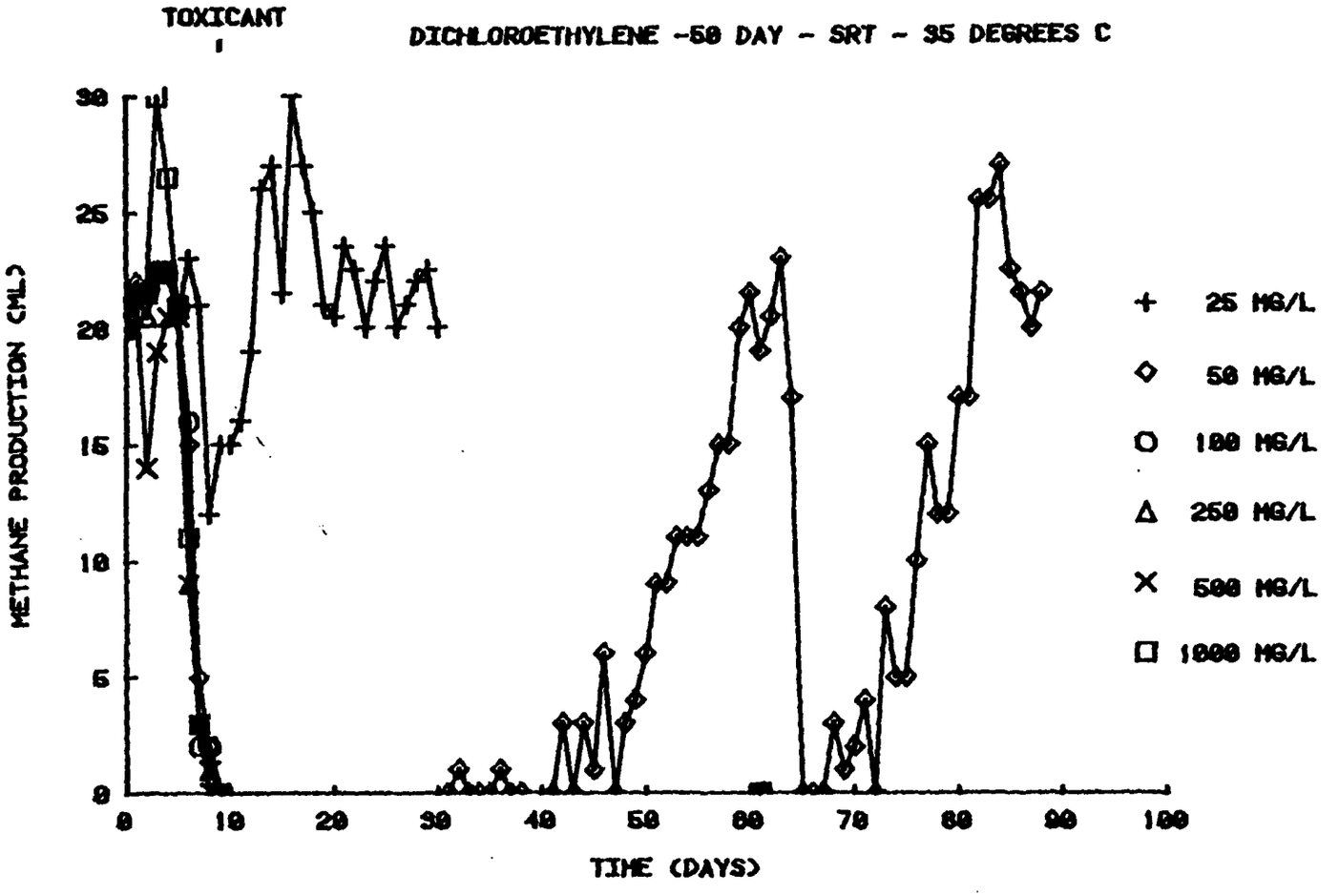

FIGURE 94. RESPONSE OF METHANOGENS TO SLUG DOSES OF DICHLOROETHYLENE

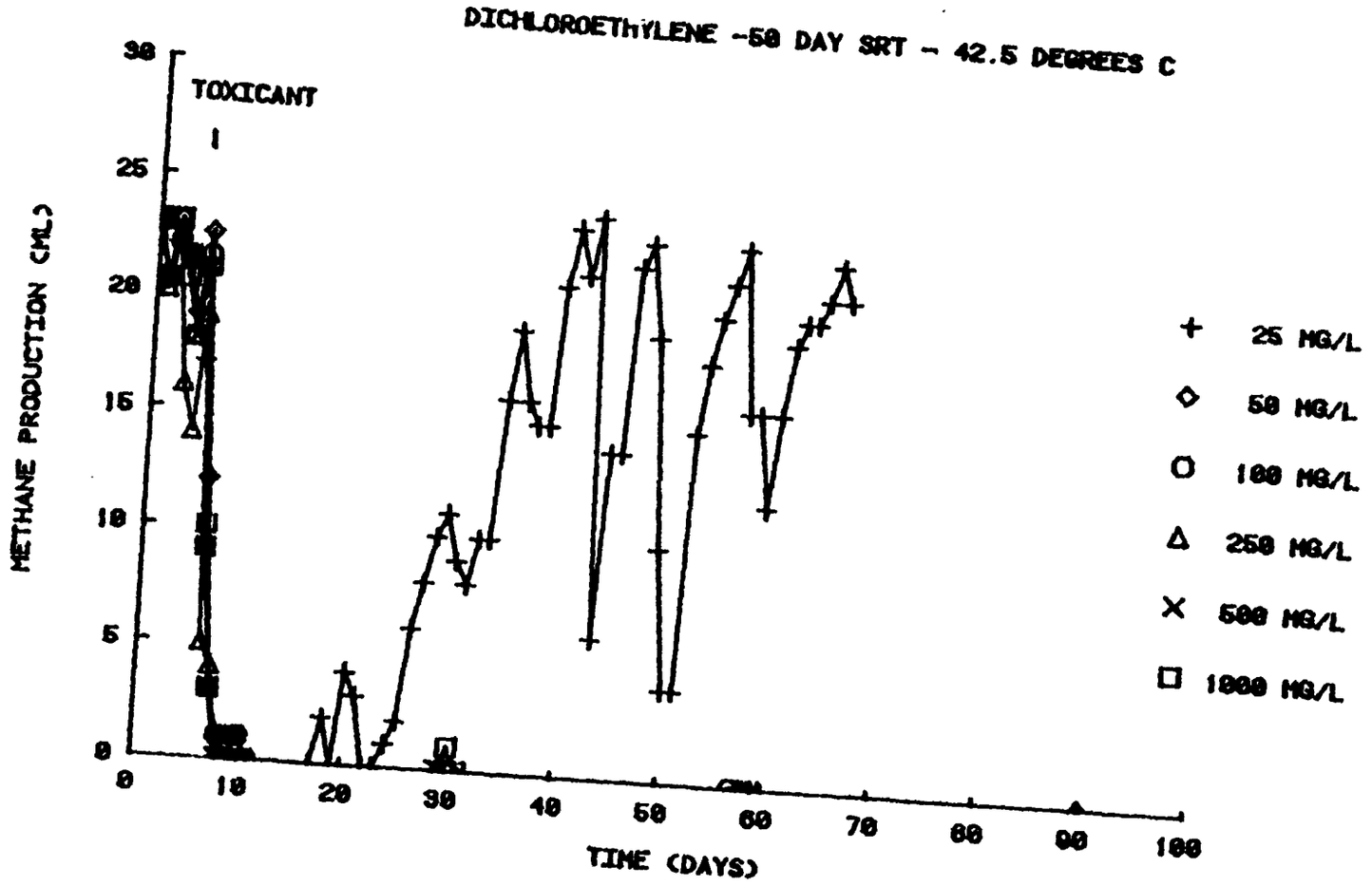

FIGURE 95. RESPONSE OF METHANOGENS TO SLUG DOSES OF DICHLOROETHYLENE

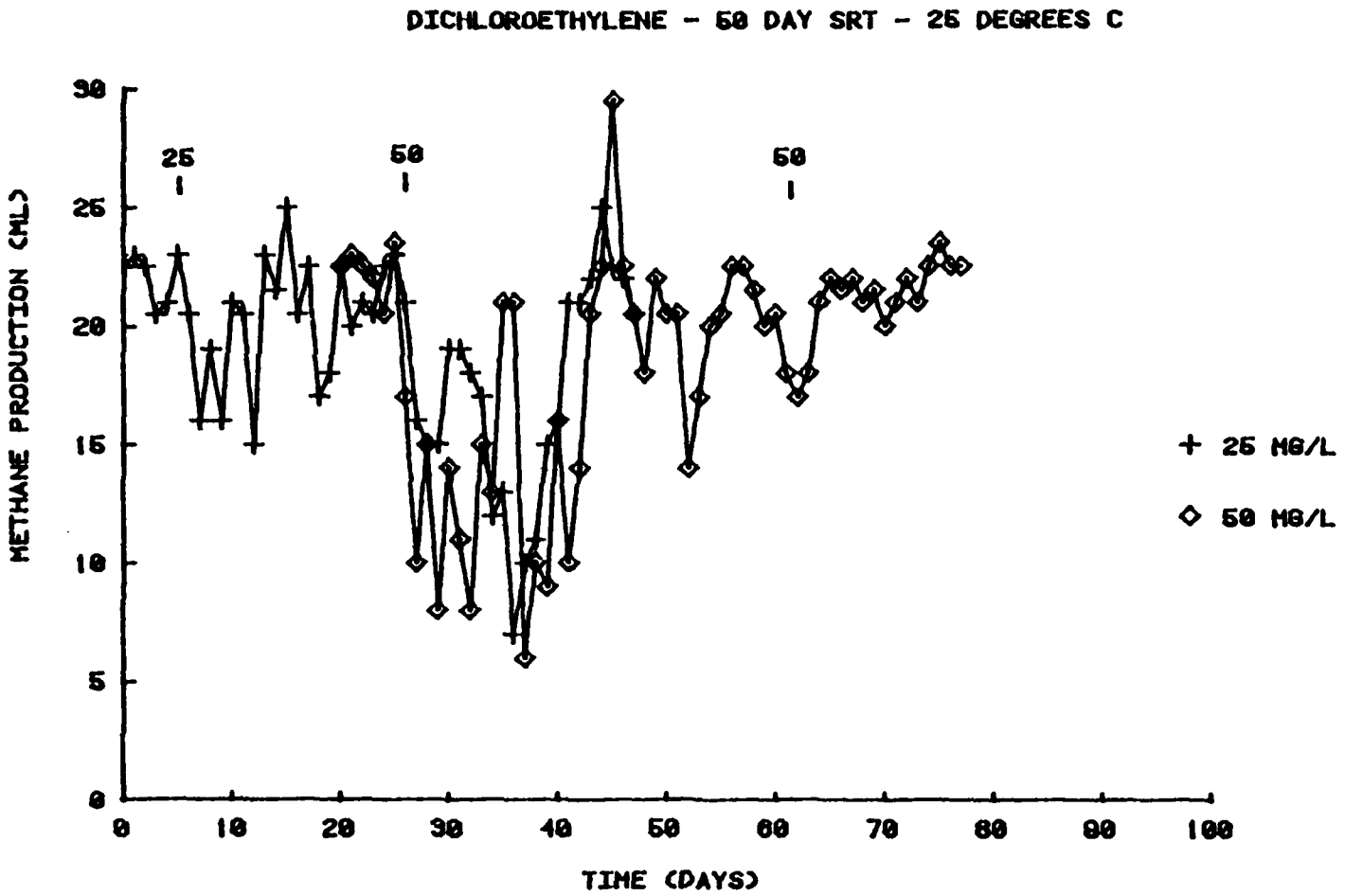

FIGURE 96. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF DICHLOROETHYLENE

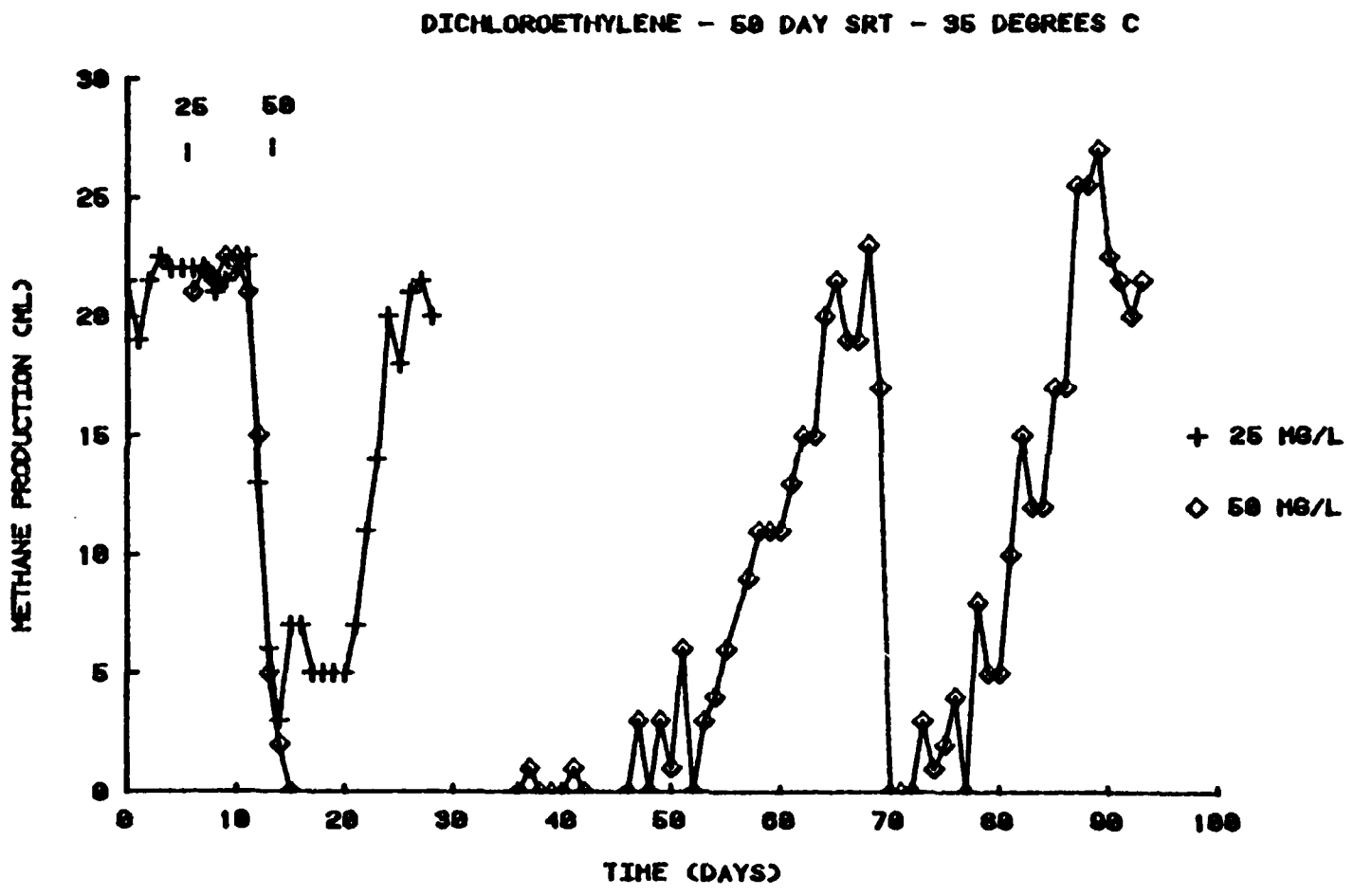

FIGURE 97. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF DICHLOROETHYLENE

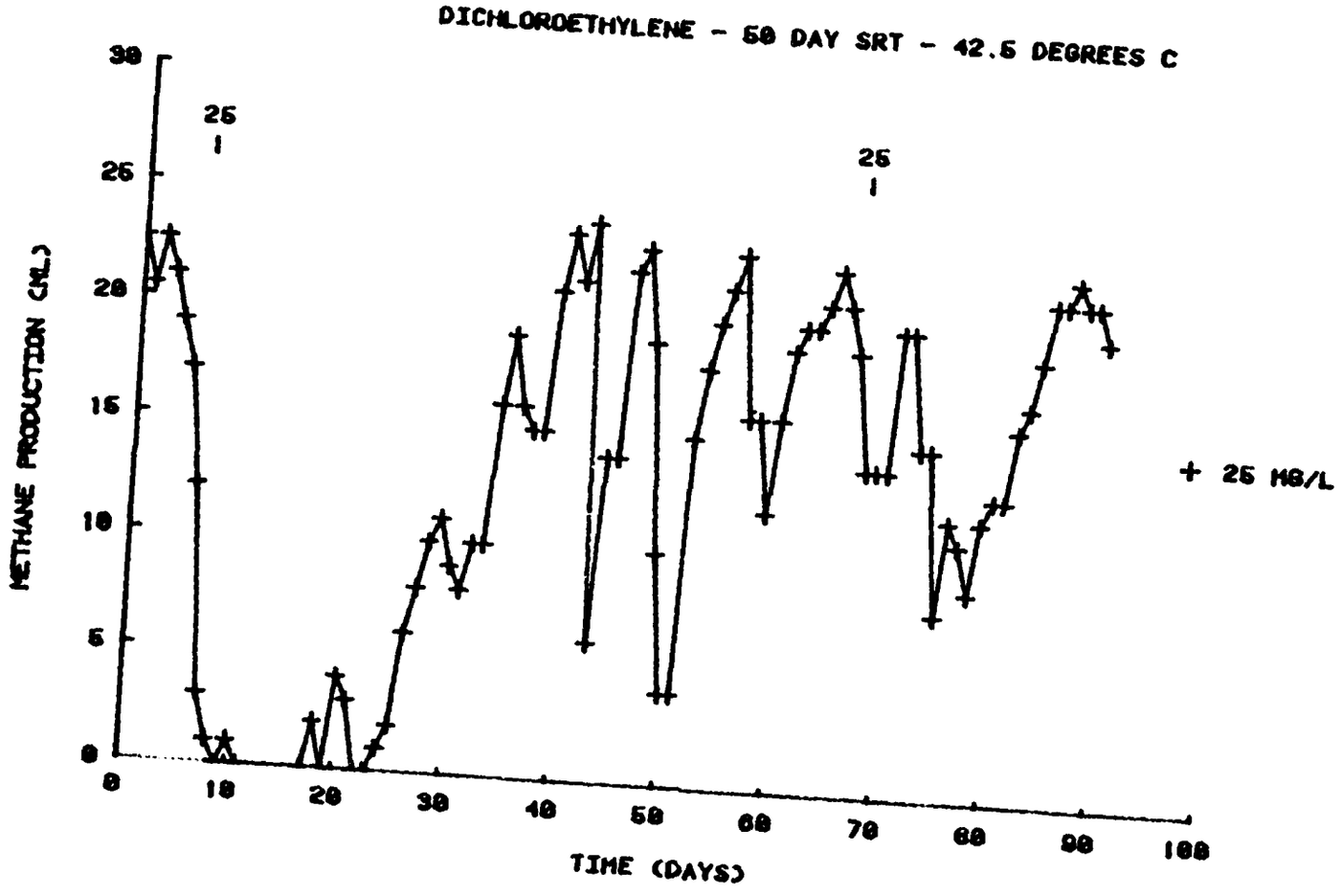

FIGURE 98. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF DICHLOROETHYLENE

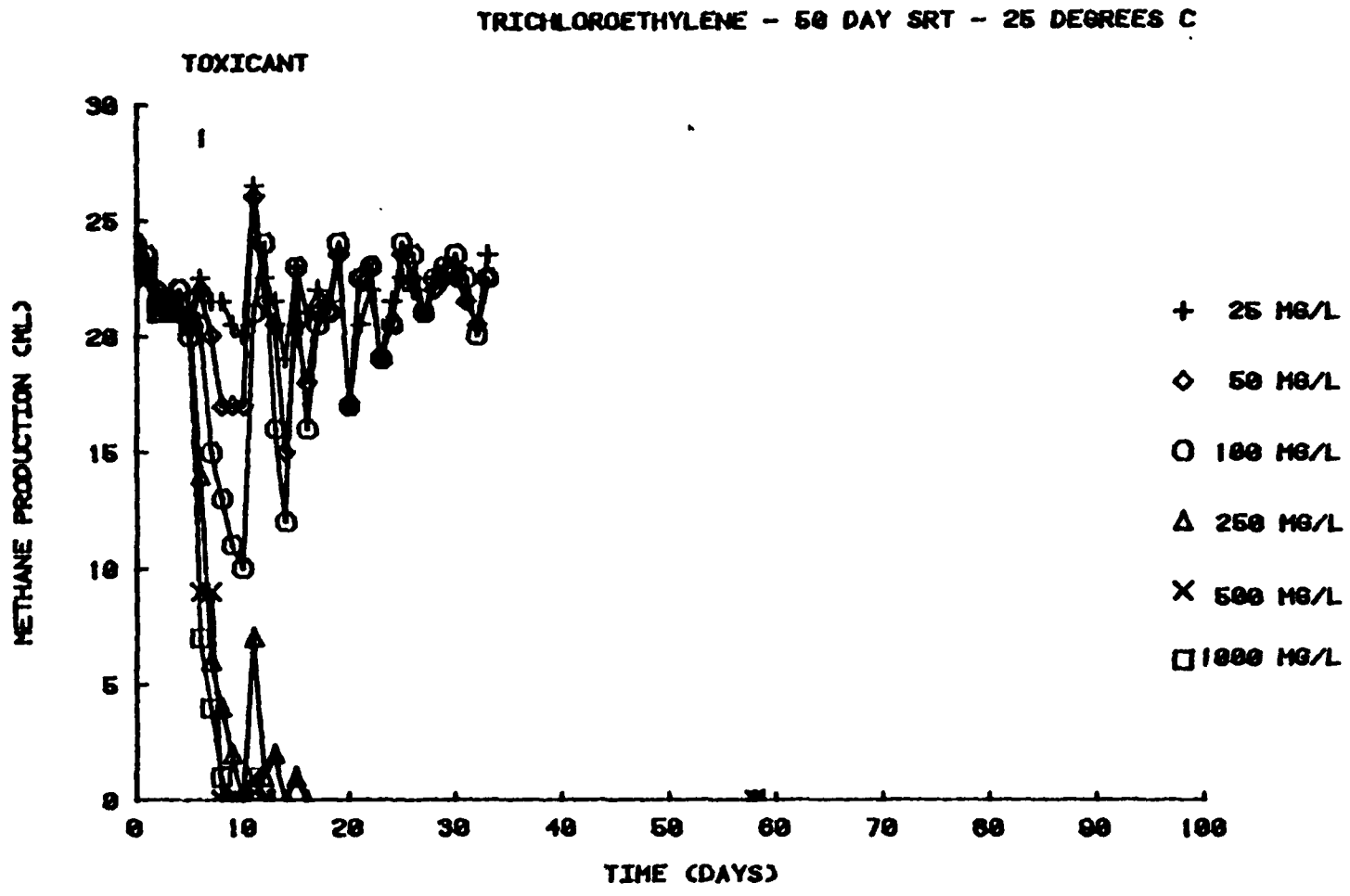

FIGURE 99. RESPONSE OF METHANOGENS TO SLUG DOSES OF TRICHLOROETHYLENE

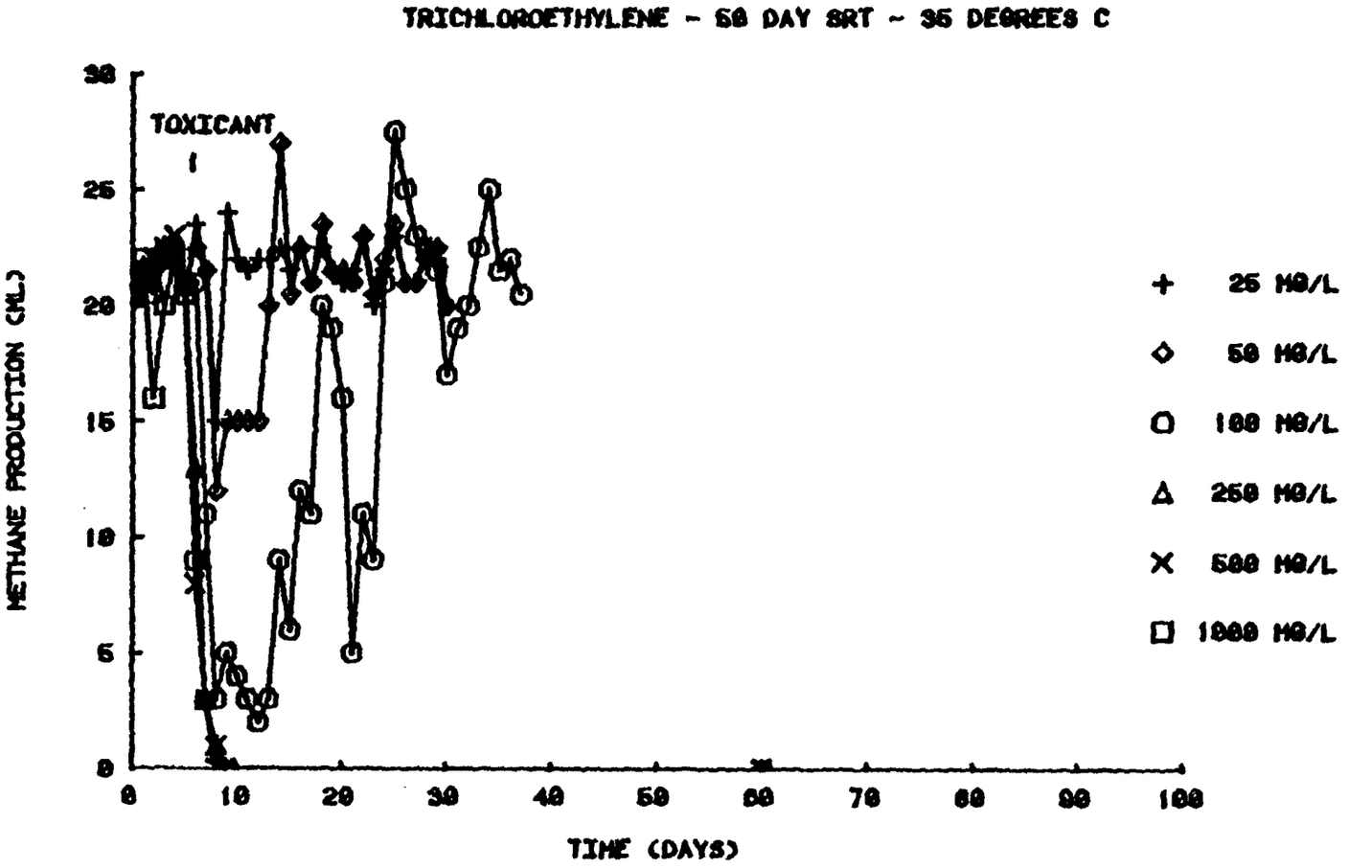

FIGURE 100. RESPONSE OF METHANOGENS TO SLUG DOSES OF TRICHLOROETHYLENE

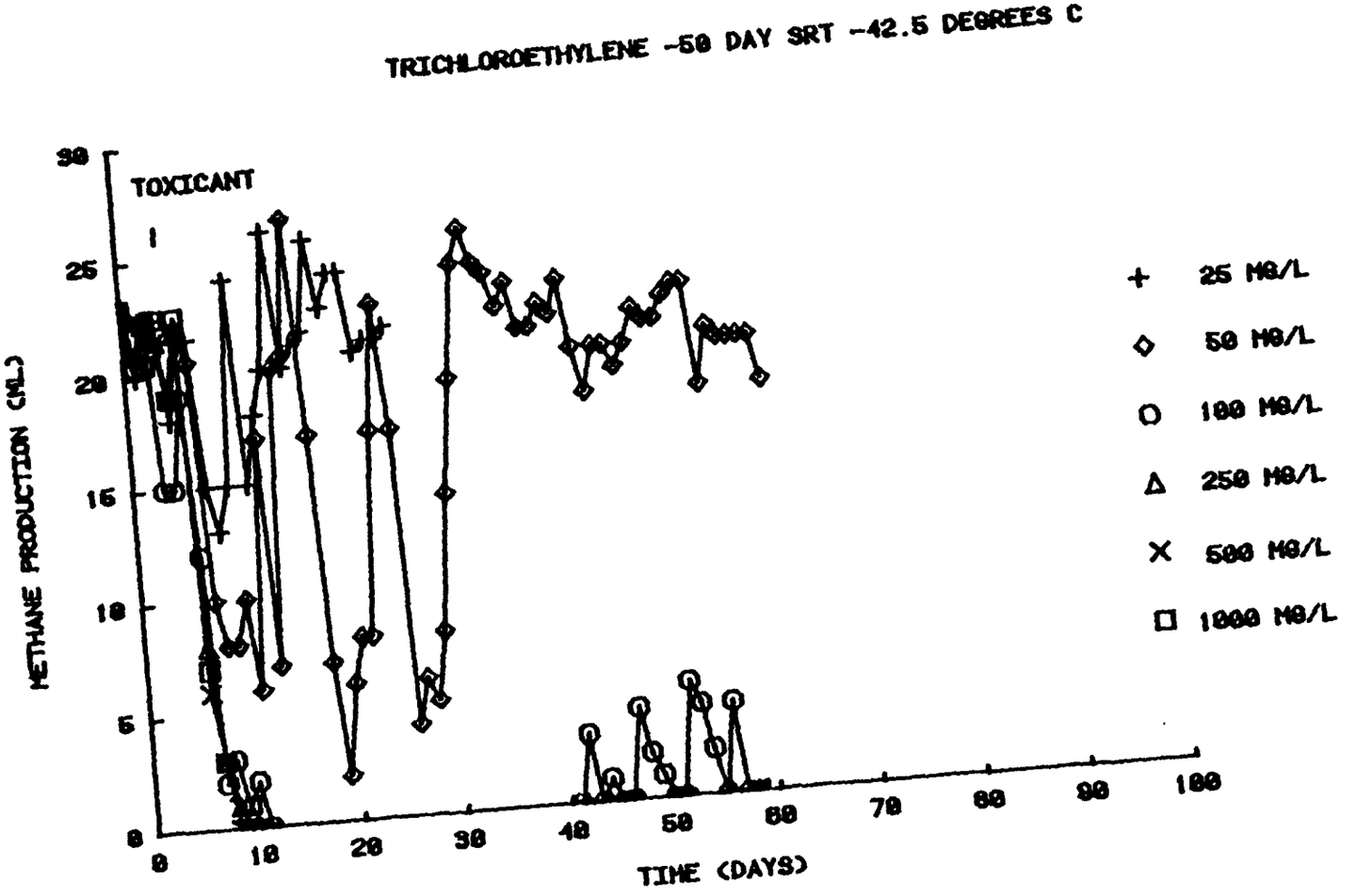

FIGURE 101. RESPONSE OF METHANOGENS TO SLUG DOSES OF TRICHLOROETHYLENE

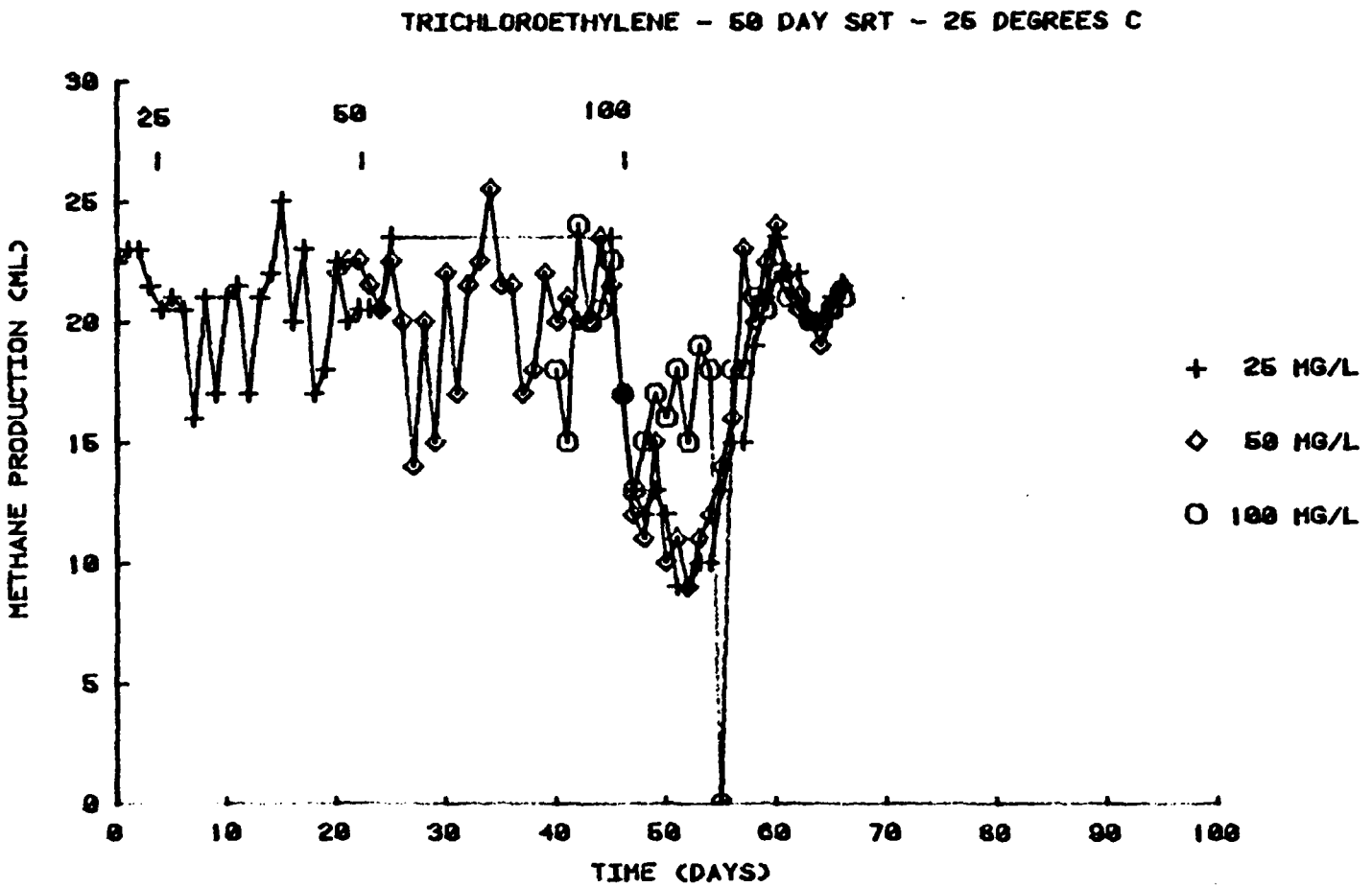

FIGURE 102. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF TRICHLOROETHYLENE

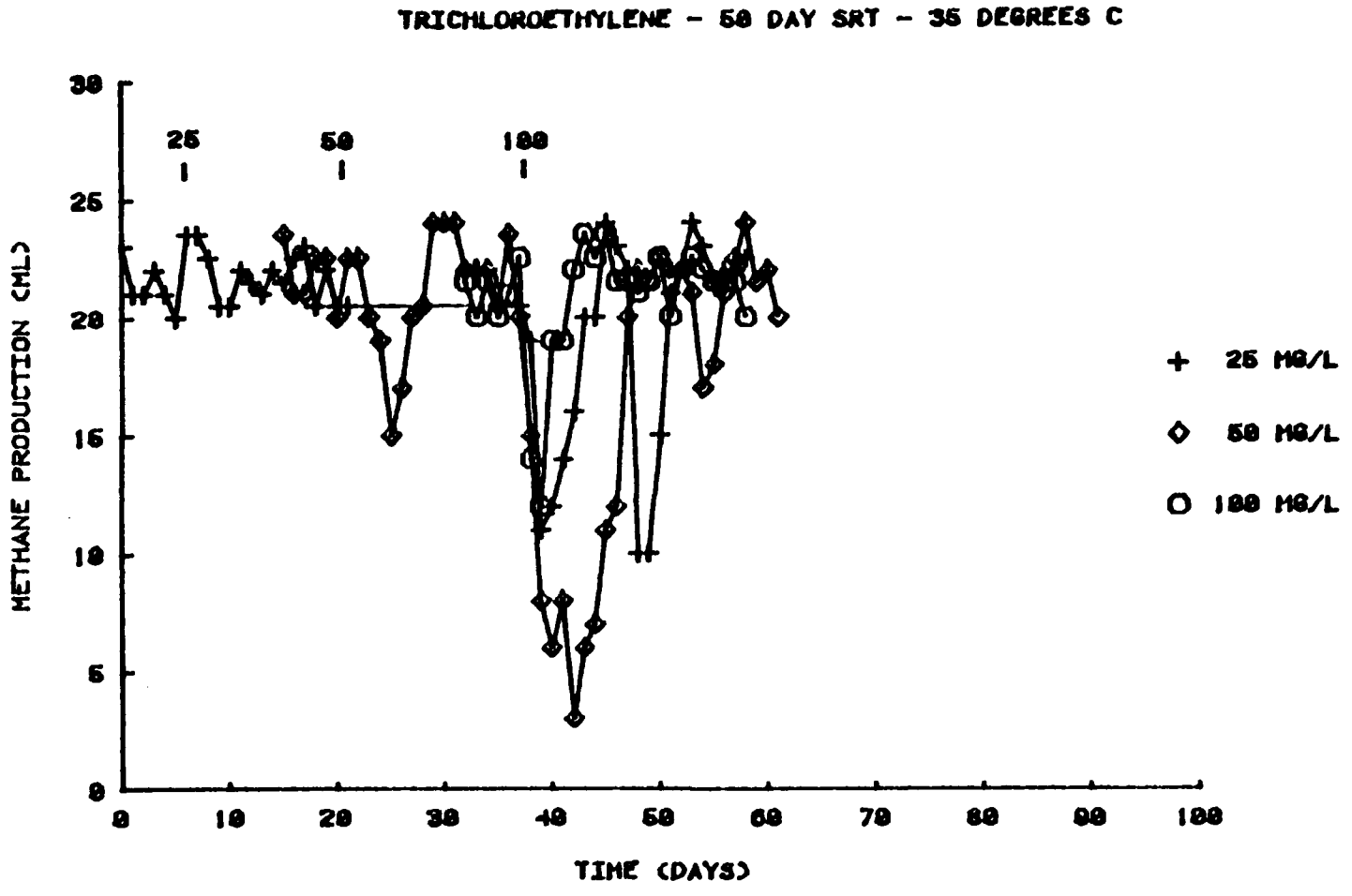

FIGURE 103. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF TRICHLOROETHYLENE

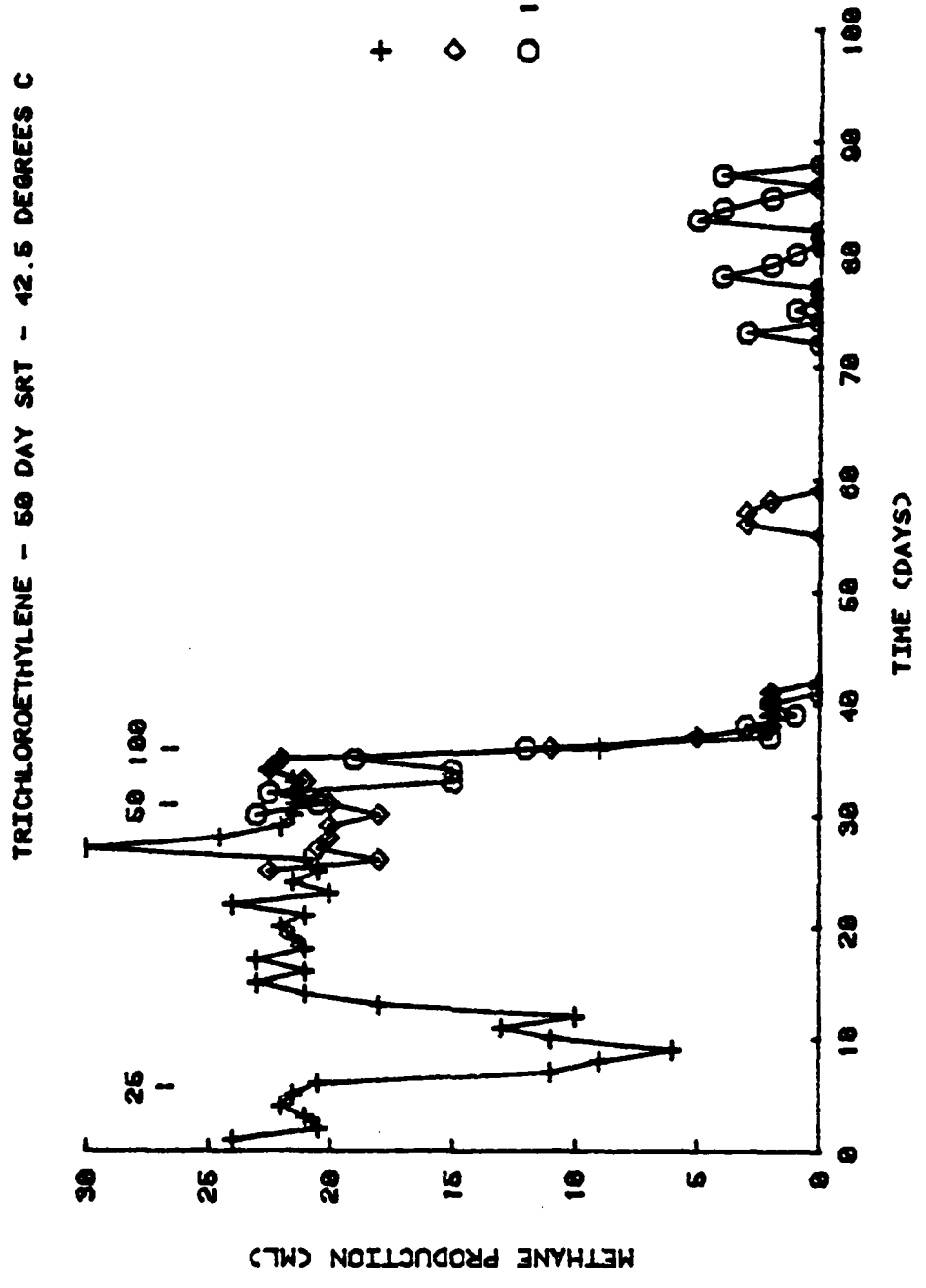

FIGURE 104. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF TRICHLOROETHYLENE

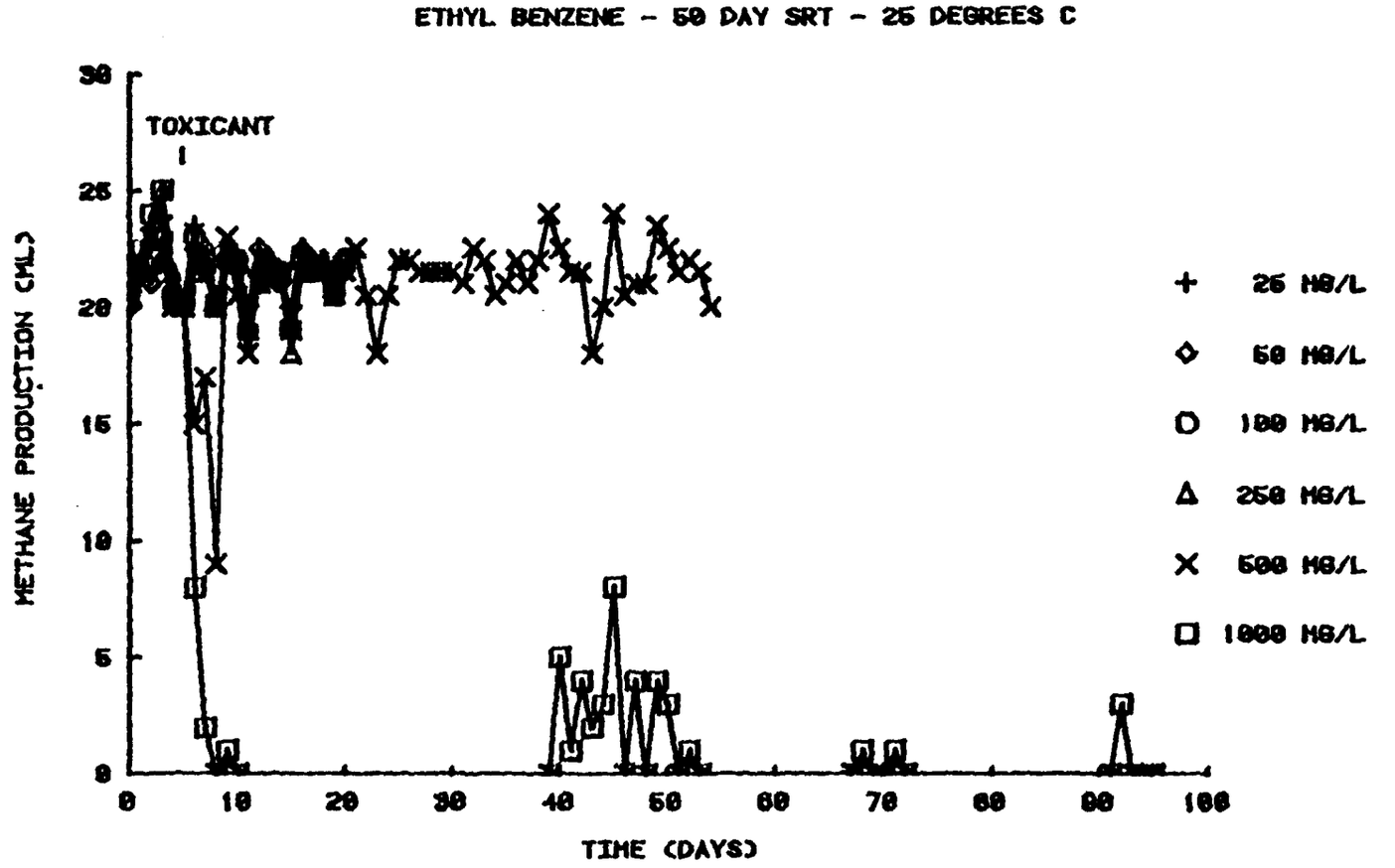

FIGURE 105. RESPONSE OF METHANOGENS TO SLUG DOSES OF ETHYL BENZENE

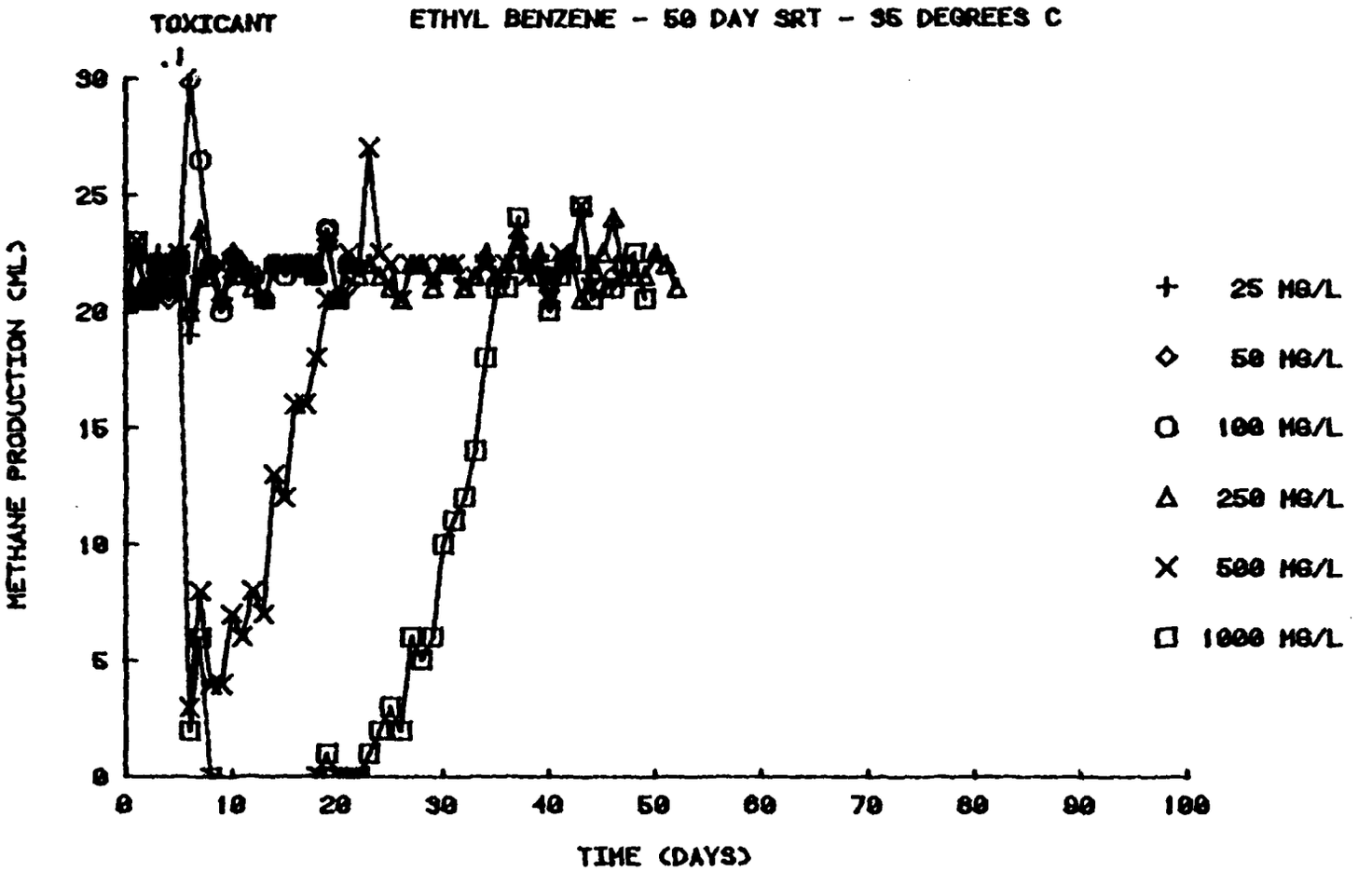

FIGURE 106. RESPONSE OF METHANOGENS TO SLUG DOSES OF ETHYL BENZENE

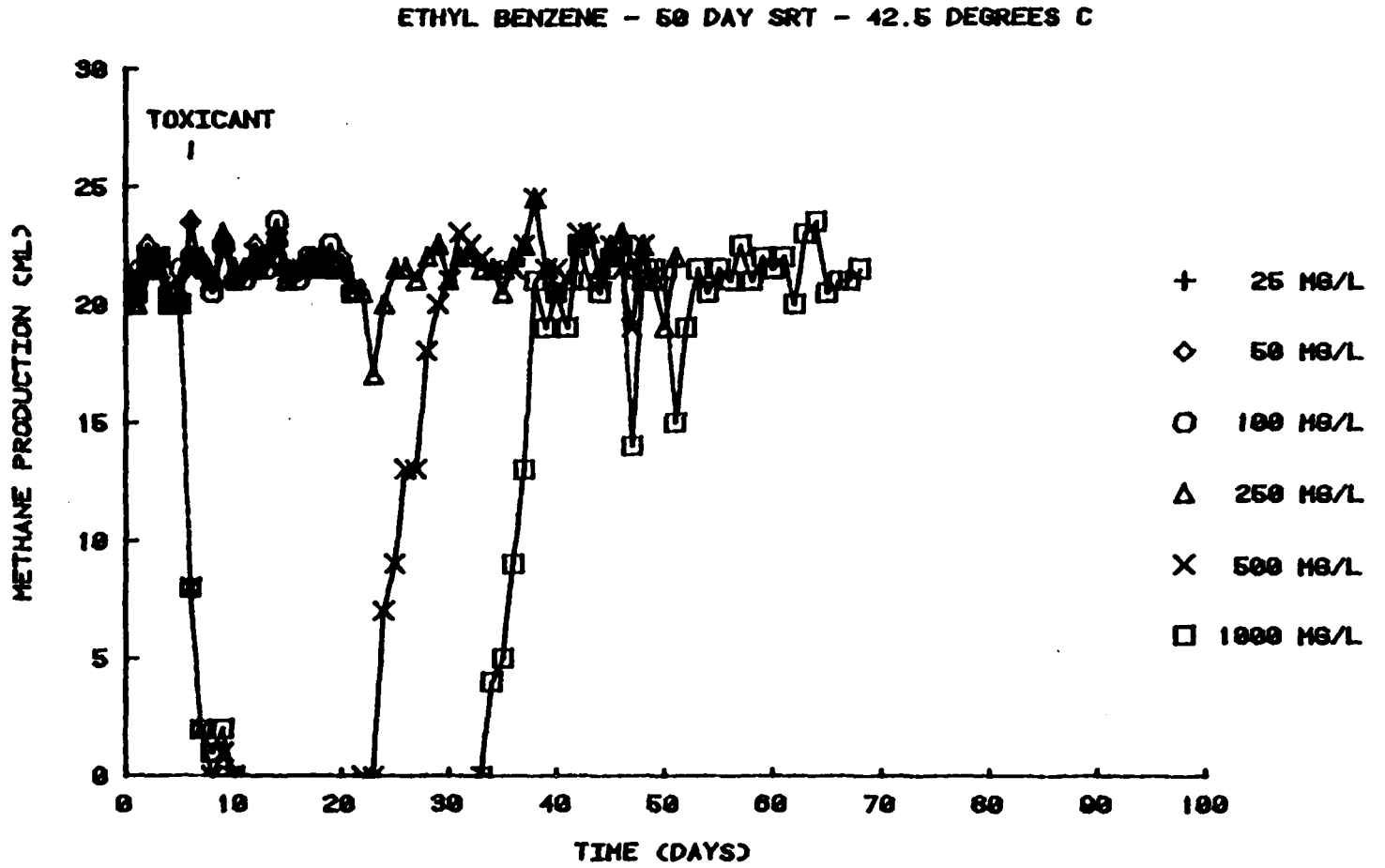

FIGURE 107. RESPONSE OF METHANOGENS TO SLUG DOSES OF ETHYL BENZENE

(Figure 105). At 35°C, the cultures were more severely affected by 500 mg/l than at 25°C, although the response to 1000 mg/l was much less severe (Figure 106). A temperature of 42.5°C caused toxicant responses to be significantly more severe (Figure 107).

There were some indications that acclimation to 500 mg/l and 1000 mg/l ethyl benzene may have occurred, however the data do not permit a definite conclusion (Figures 108 to 110).

Hyamine 1622 (a cationic surfactant)

Slug dose concentrations of 1, 5, 10, 20, 50 and 100 mg/l were added to serum bottles using dilutions of Hyamine 1622 as stock. Exposure to this cationic surfactant caused a very sharp and immediate response as expressed by methane production. Recovery occurred at slow rates and was noticeably erratic (Figures 111 to 113).

Comparison of the 25°C and 35°C bottles must be based on the two largest slug doses (50 and 100 mg/l) since the lower concentrations did not cause a measurable response. Recovery from the larger slug doses occurred faster at 35°C (Figure 112) than at 25°C (Figure 111). Those cultures maintained at 42.5°C showed the quickest recovery from the 50 and 100 mg/l concentrations, but they also were significantly affected by 10 and 20 mg/l Hyamine 1622 (Figure 113).

Acclimation to Hyamine 1622 was not evident from the data collected (Figures 114 to 116).

Hyamine 3500 (a cationic surfactant)

Stock solutions were prepared by diluting Hyamine 3500 and were used to add slug doses of 1, 5, 10, 20, 50 and 100 mg/l. Dramatic and immediate decreases in methane production resulted from exposure to the larger slug doses (Figures 117 to 119), although a delay in response to the toxicant did occur in a culture maintained at 25°C with a 50-day SRT (Figure 117).

Responses to the higher concentrations (20, 50 and 100 mg/l) were most severe in bottles kept at 25°C (Figure 117). Cultures incubated at 35°C displayed the minimum response to all toxicant concentrations (Figure 118). At 42.5°C, responses to the higher slug doses were comparable to the 35°C responses, but those bottles exposed to 10 mg/l or less of Hyamine 3500

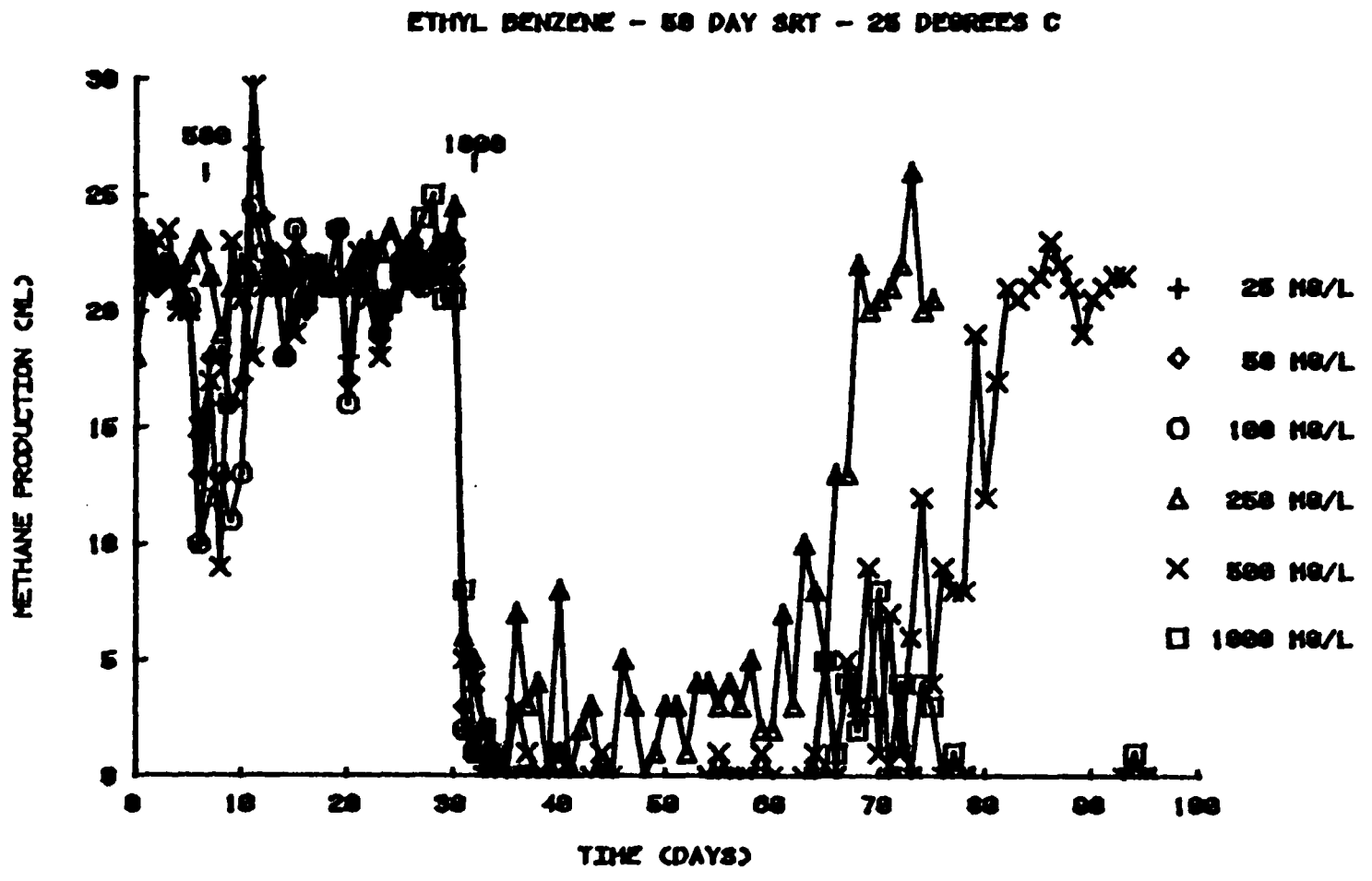

FIGURE 108. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF ETHYL BENZENE

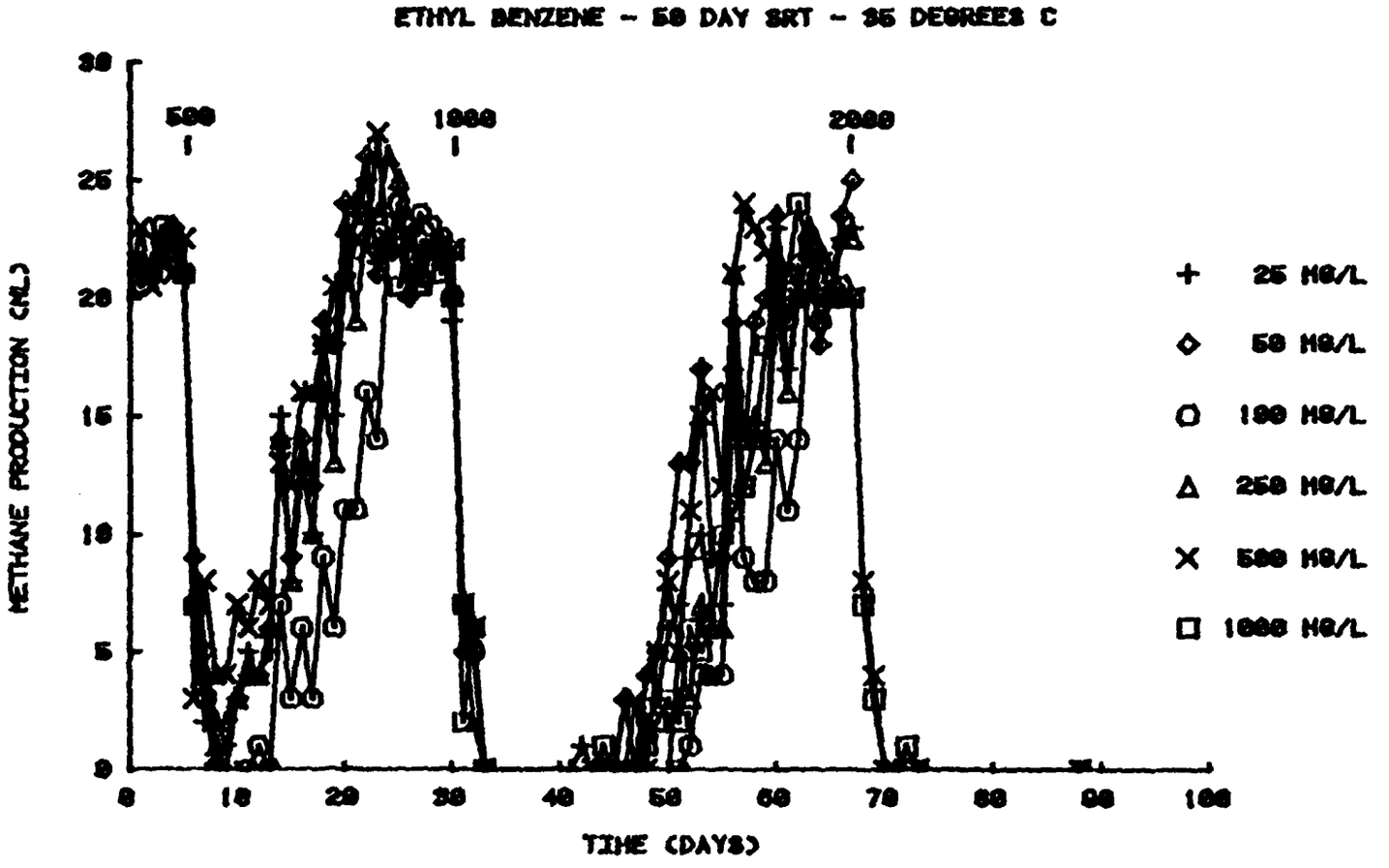

FIGURE 109. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF ETHYL BENZENE

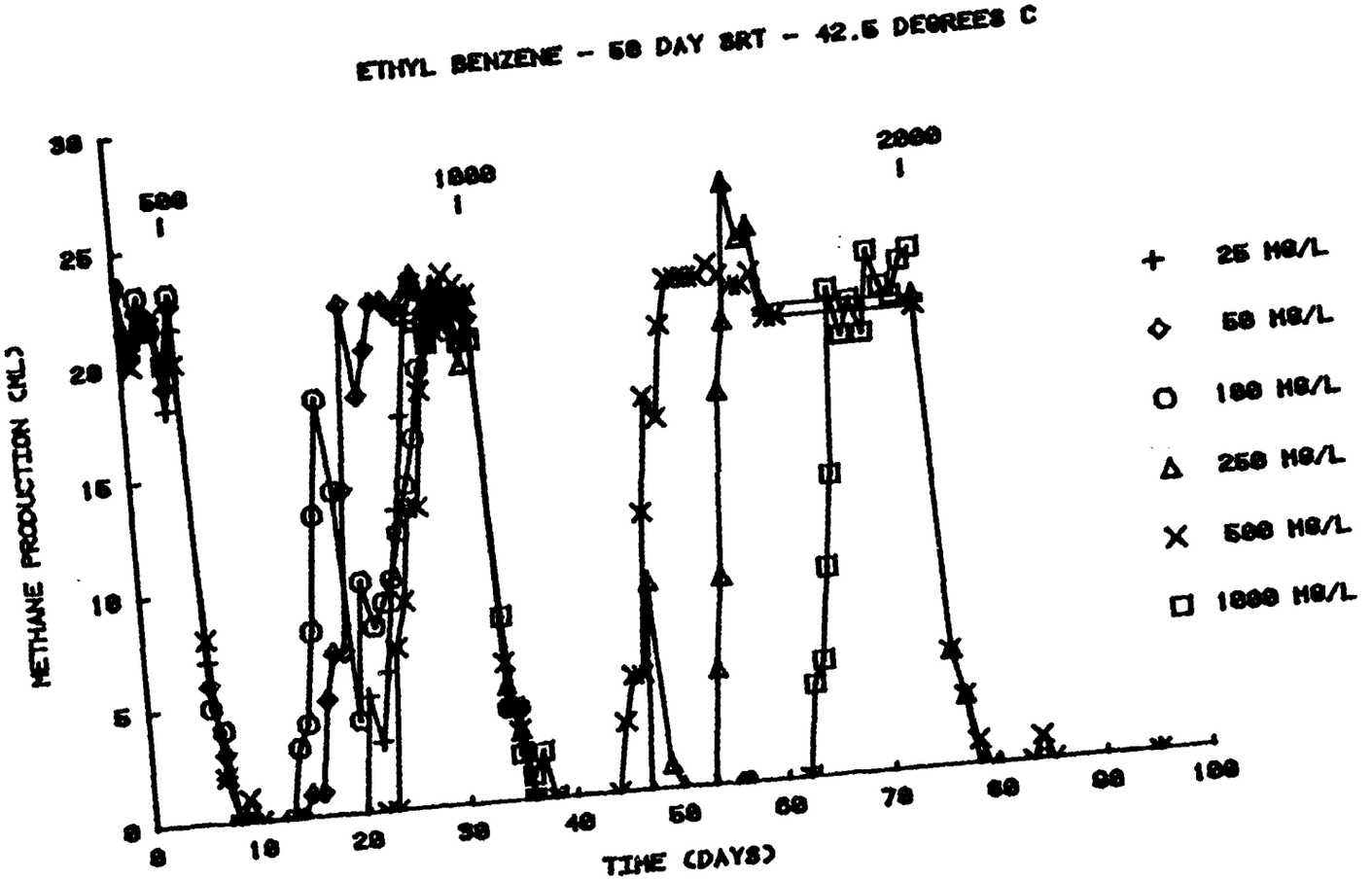

FIGURE 110. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF ETHYL BENZENE

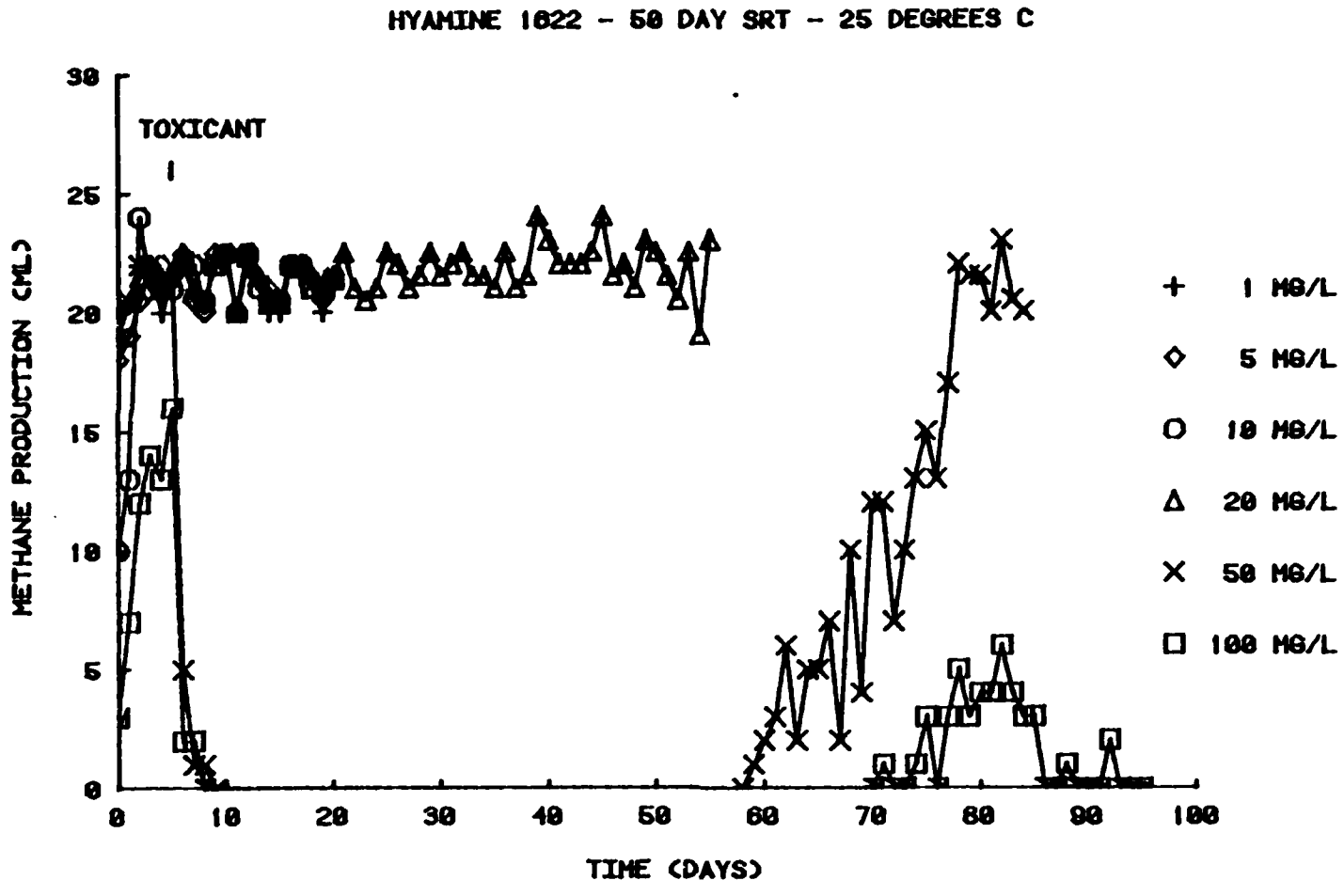

FIGURE 111. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYAMINE 1622

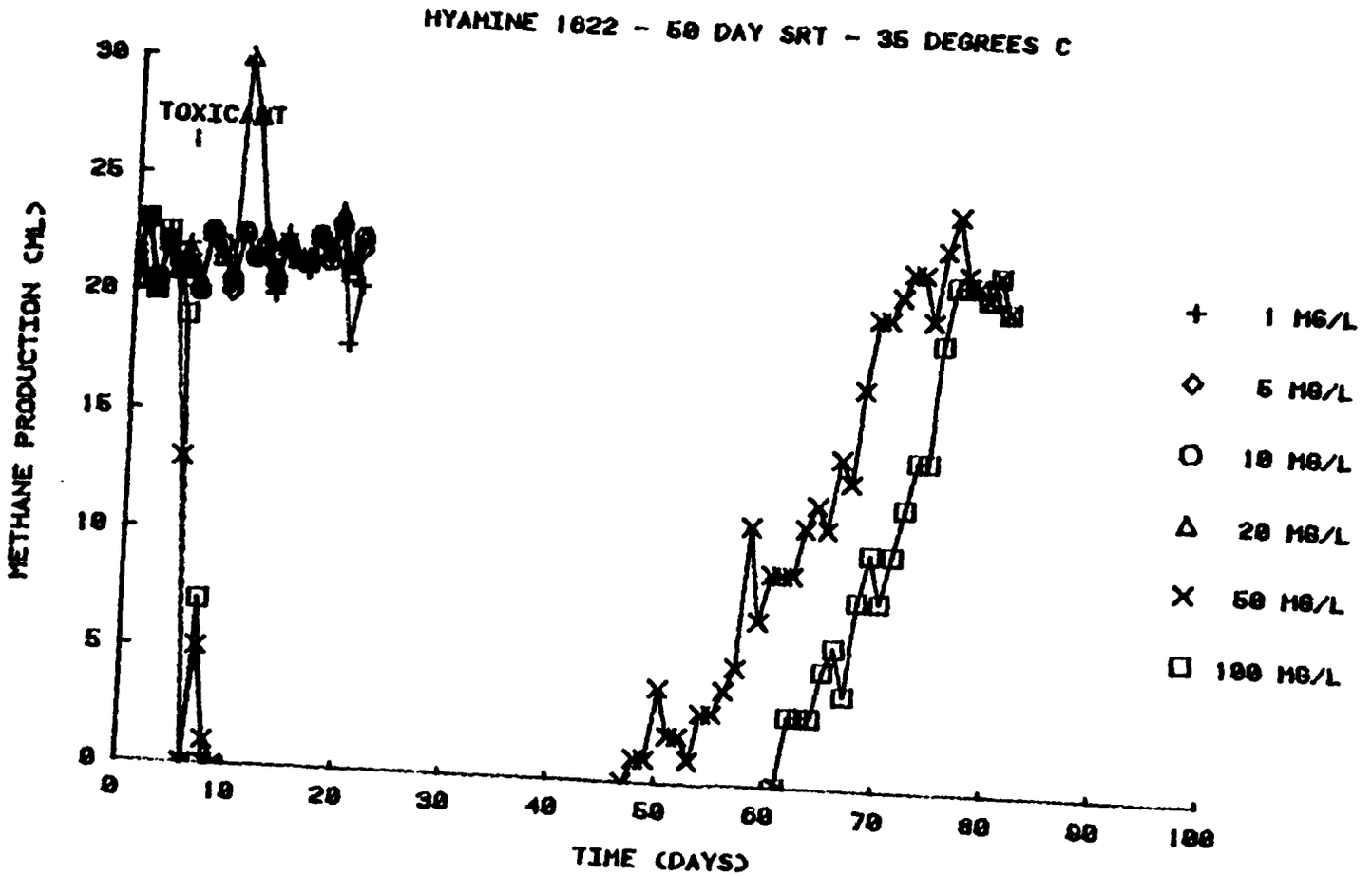

FIGURE 112. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYAMINE 1622

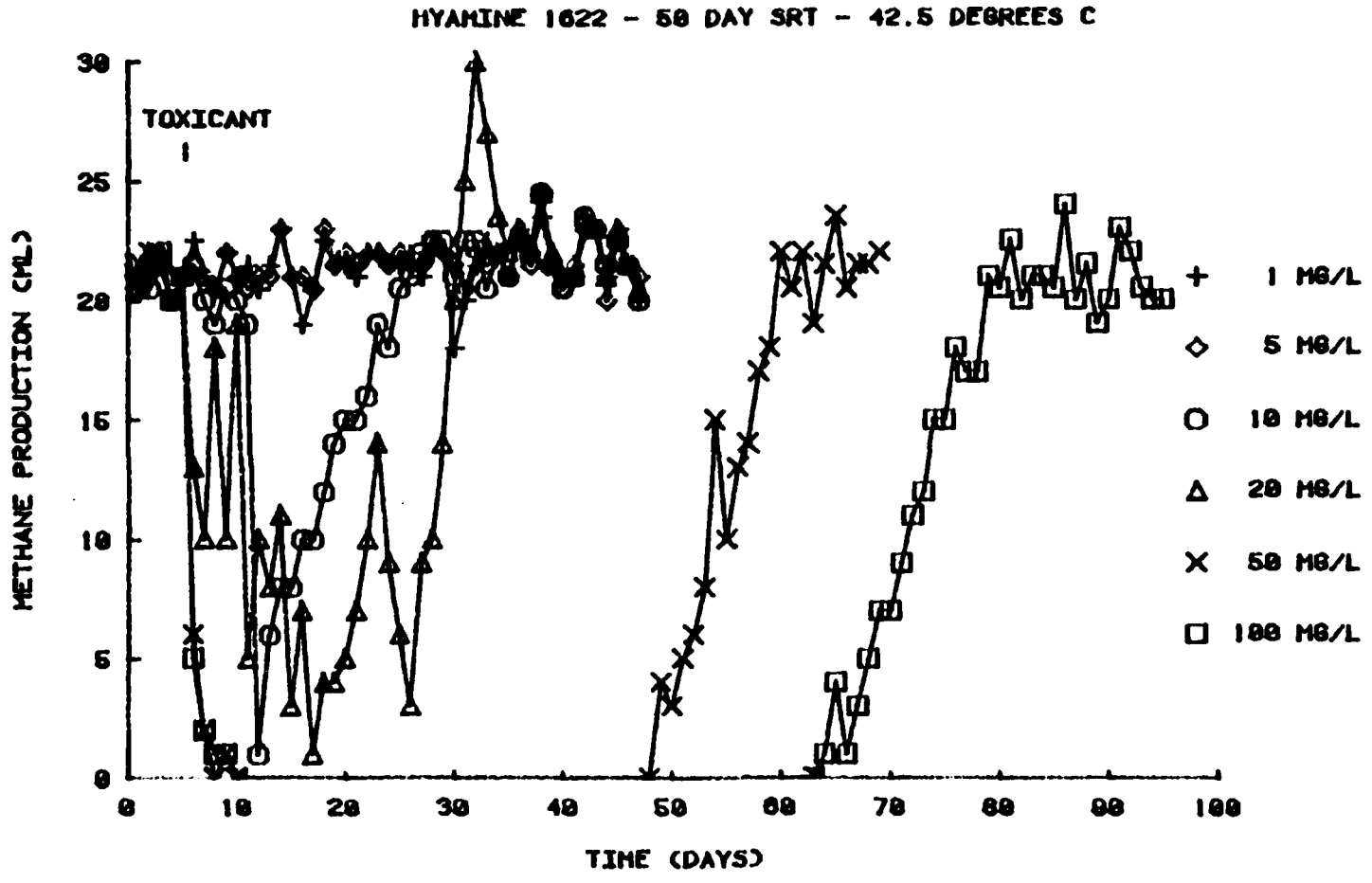

FIGURE 113. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYAMINE 1622

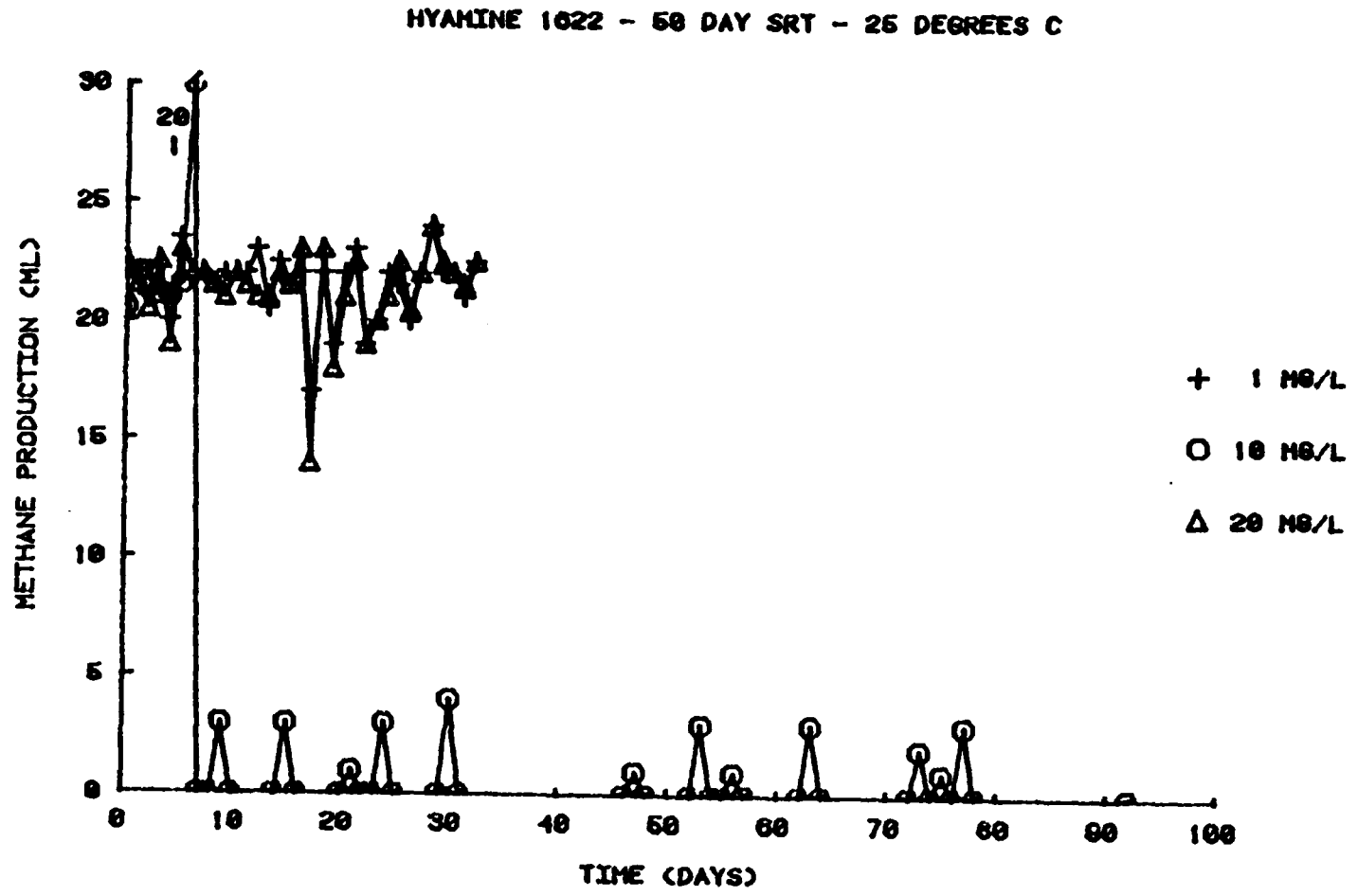

FIGURE 114. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYAMINE 1622

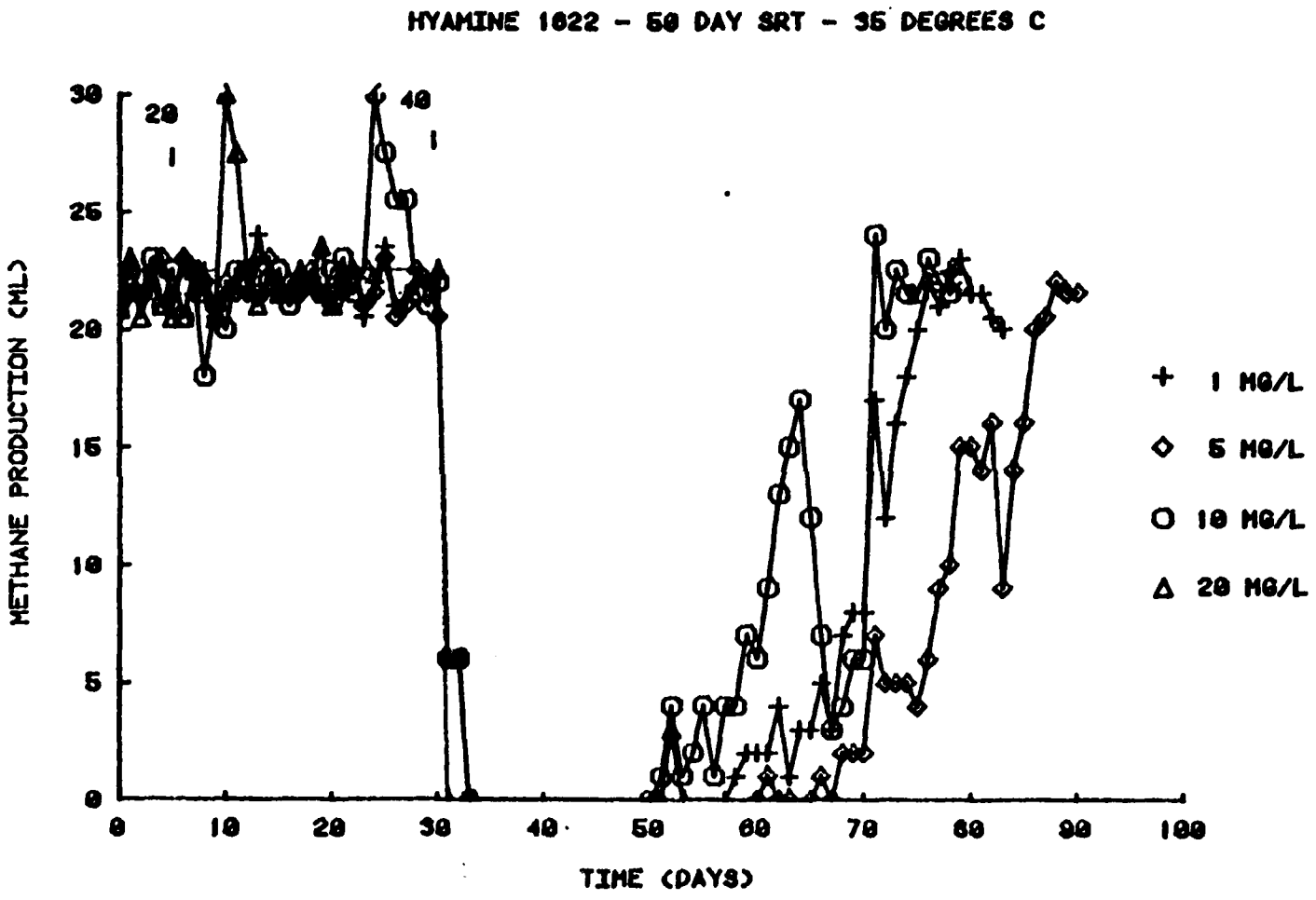

FIGURE 115. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYAMINE 1622

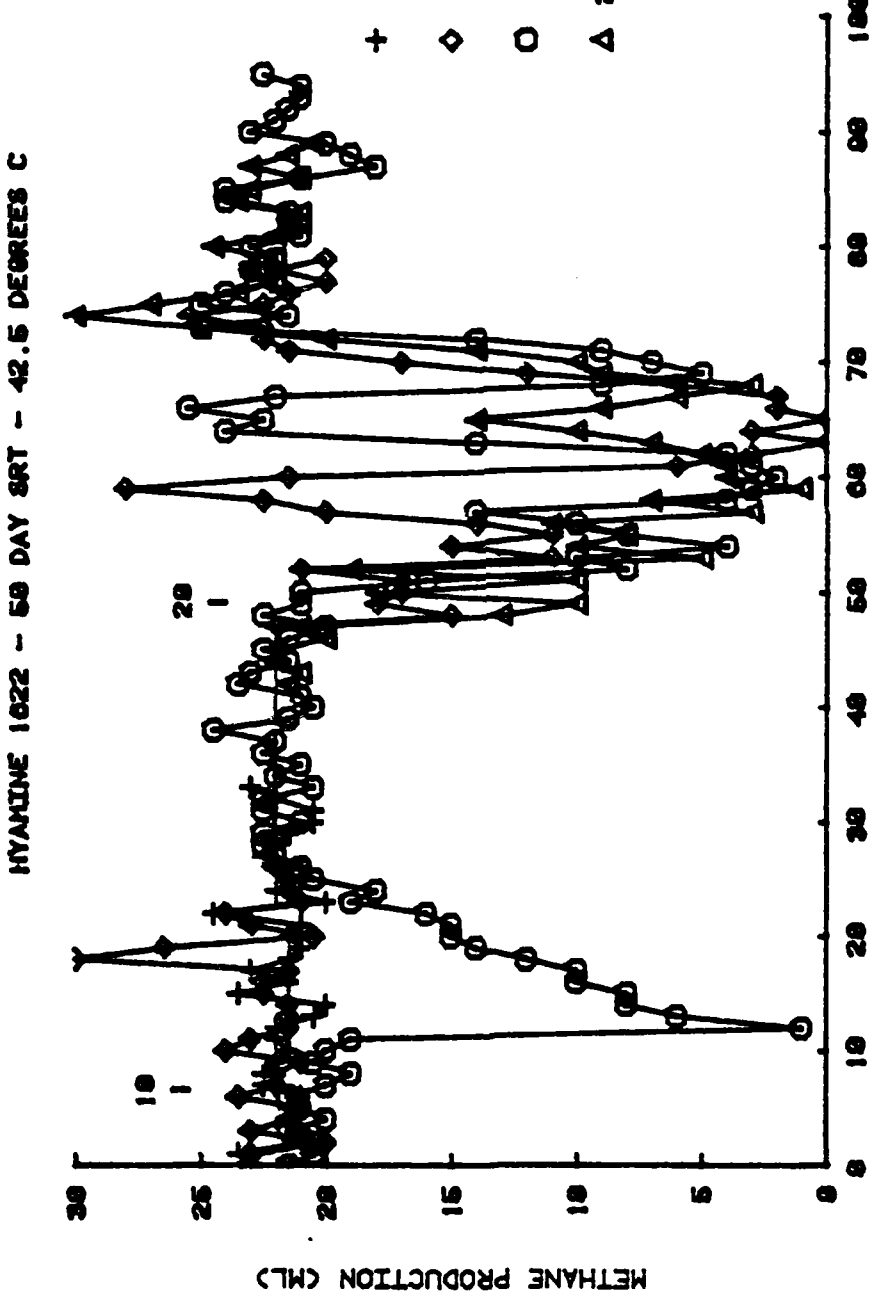

FIGURE 116. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYAMINE 1622

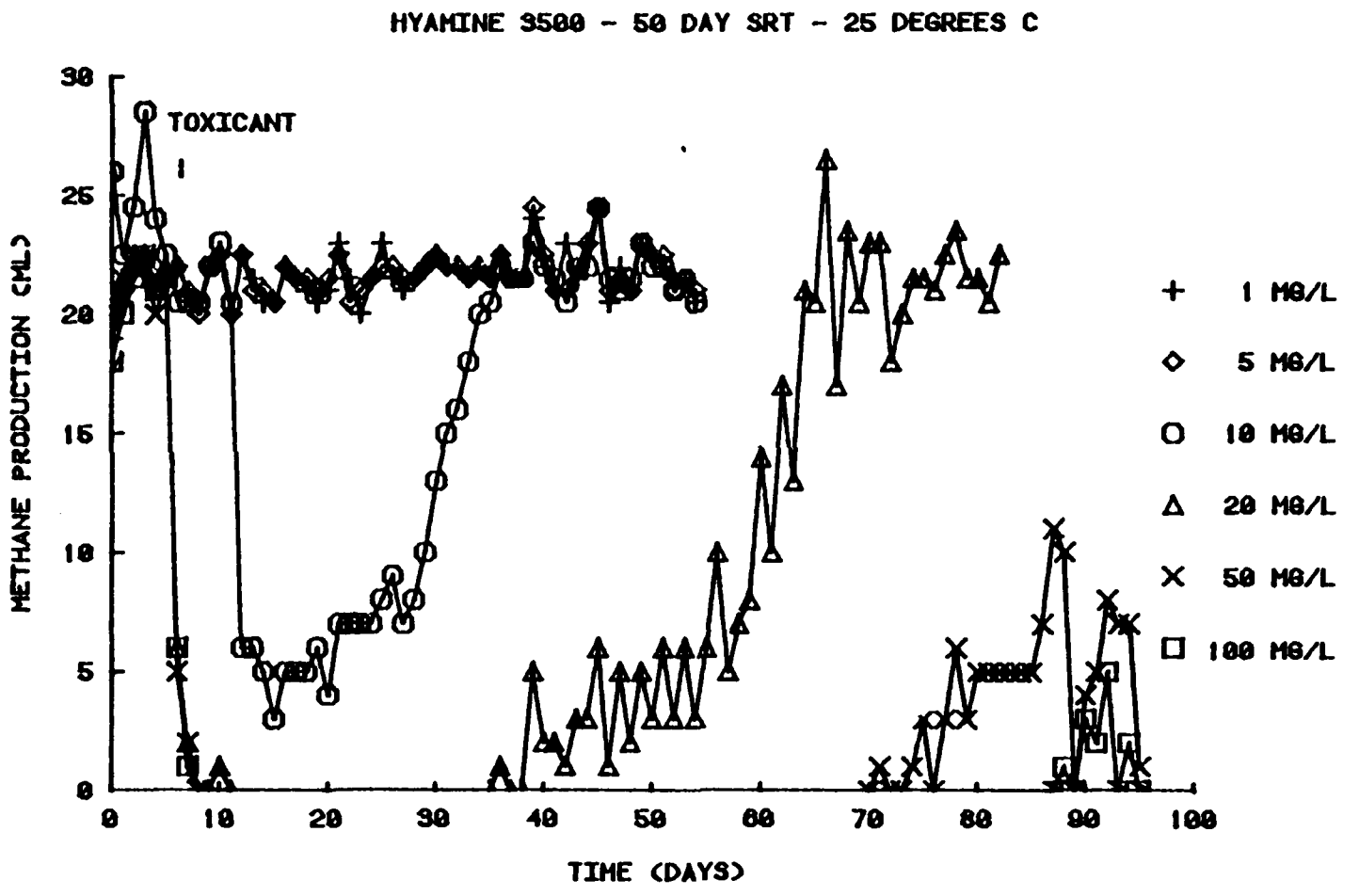

FIGURE 117. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYAMINE 3500

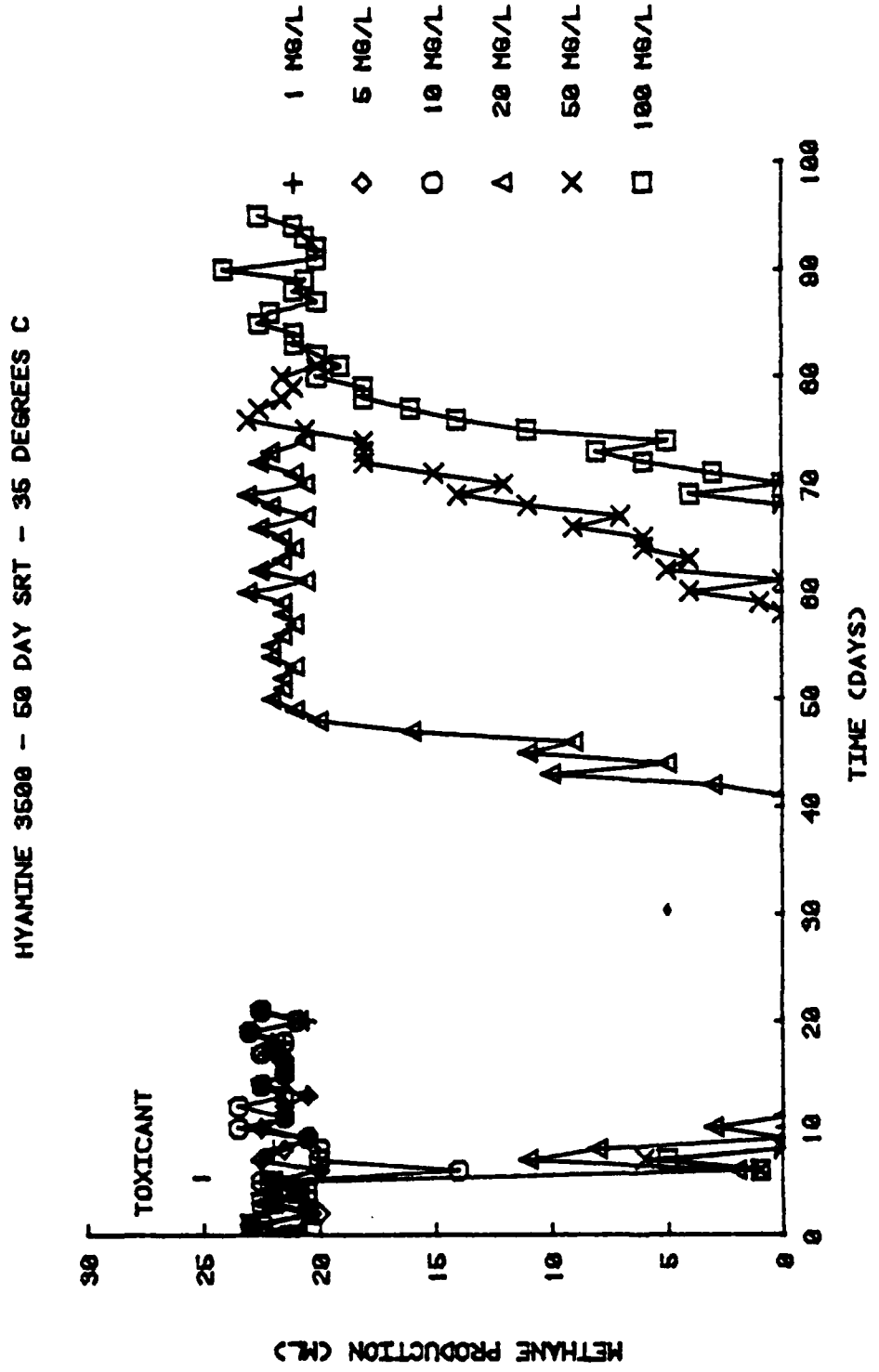

FIGURE 118. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYAMINE 3500

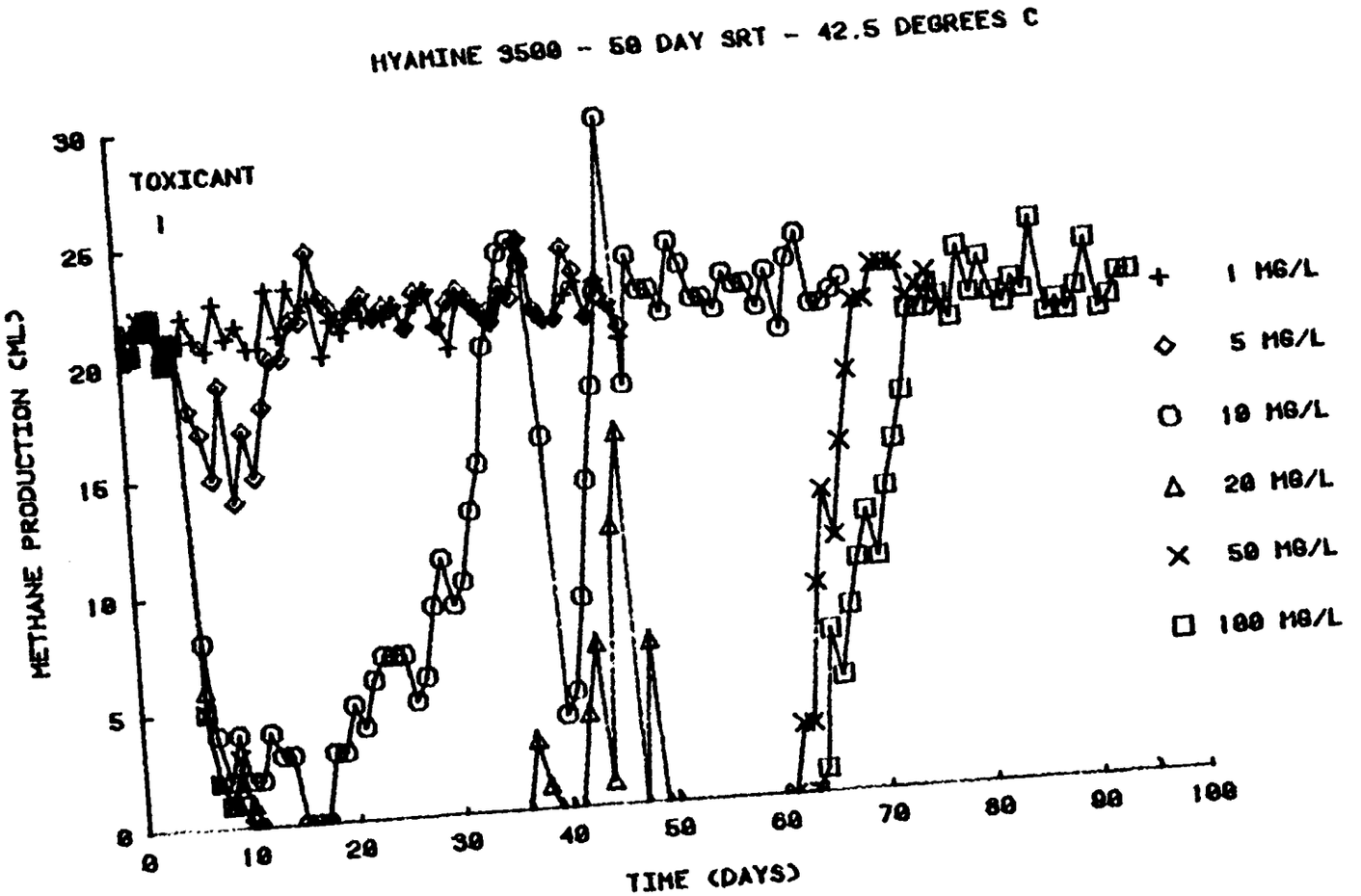

FIGURE 119. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYAMINE 3500

were significantly more affected than cultures at 25°C or 35°C (Figure 119).

There is some evidence of acclimation to low concentrations of Hyamine 3500 although acclimation characteristics do not appear to be very consistent (Figures 120 to 122).

Gasoline (regular, leaded)

Gasoline was added, undiluted, to serum bottles at initial slug doses of 25, 50, 100, 250, 500 and 1000 mg/l. Concentrations were calculated based on the measured density of the gasoline. However, since no responses resulted from exposures to these concentrations, additional slug doses were injected. The bottle with the highest initial dose (1000 mg/l) was not given a second dose, but the other bottles exposed to 500, 250, 100, 50 and 25 mg/l were exposed to slug doses of 2500, 5000, 7500, 10,000 and 15,000 mg/l, respectively.

The toxicant caused immediate and very sharp responses, followed by a period of zero or low gas production and a slow, erratic recovery (Figures 123 to 125).

Those cultures maintained at 42.5°C were the most sensitive to lower concentrations of gasoline (2500 mg/l or less), but displayed the fastest recovery from higher concentrations (Figure 125), comparable to the recovery pattern at 35°C (Figure 124). The 25°C cultures were most severely affected by these higher slug doses (Figure 123).

Acclimation characteristics were not clearly defined (Figures 126 to 128), although there were indications of acclimation to 5000 mg/l gasoline at 35°C and 42.5°C (Figures 127 and 128). It appears that some degree of acclimation is possible.

Jet Fuel (JP-4)

As with gasoline, initial slug doses of 25, 50, 100, 250, 500 and 1000 mg/l jet fuel did not cause sufficient responses. Therefore, additional toxicant was introduced to the cultures exposed to the five lower concentrations. Injections of 2500, 5000, 7500, 10,000 and 15,000 mg/l jet fuel were added to initial slug doses of 500, 250, 100, 50 and 25 mg/l, respectively.

Responses to jet fuel were generally delayed a few days and decreases in gas generation were erratic and occurred at an unusually slow rate

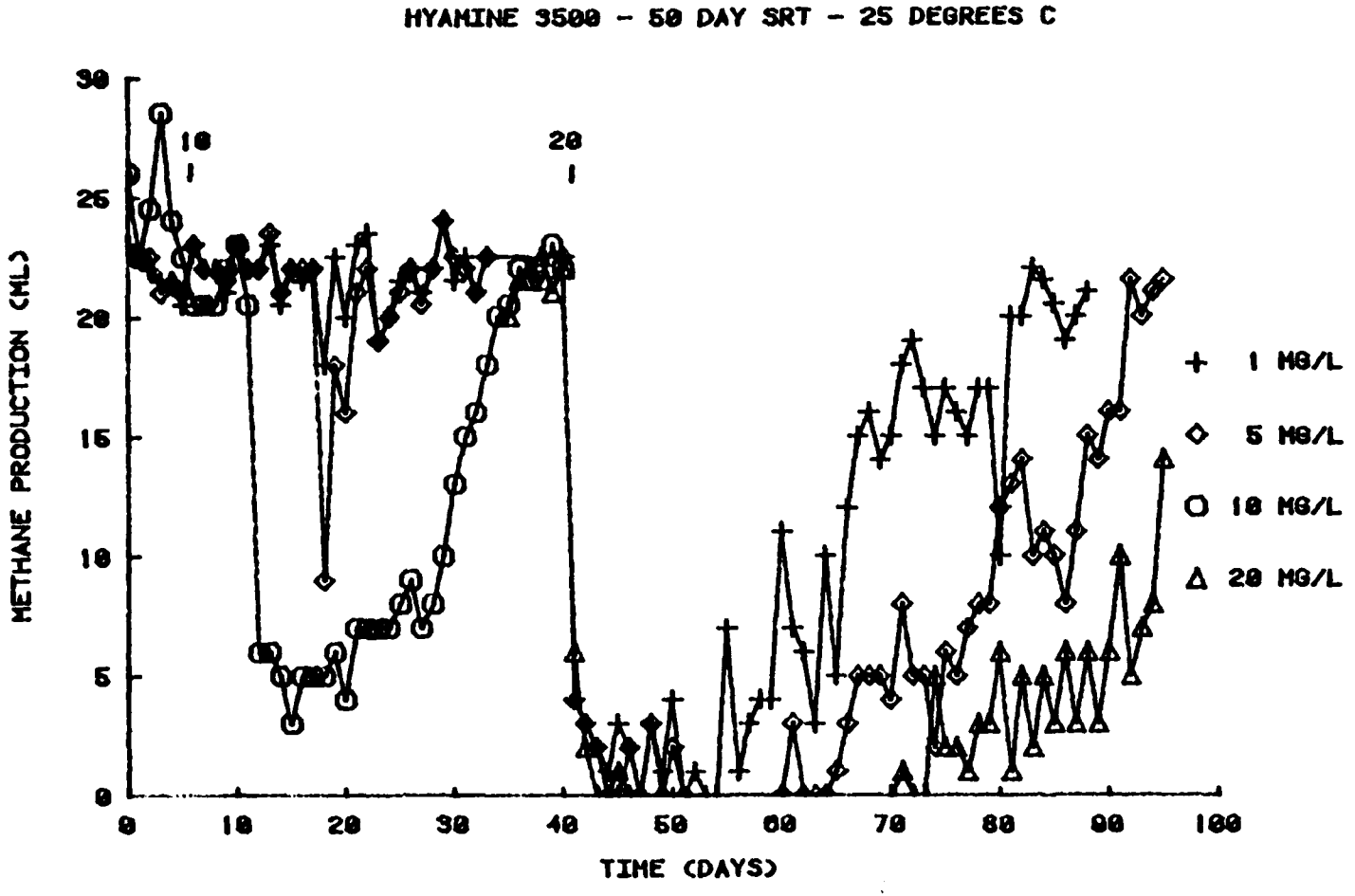

FIGURE 120. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYAMINE 3500

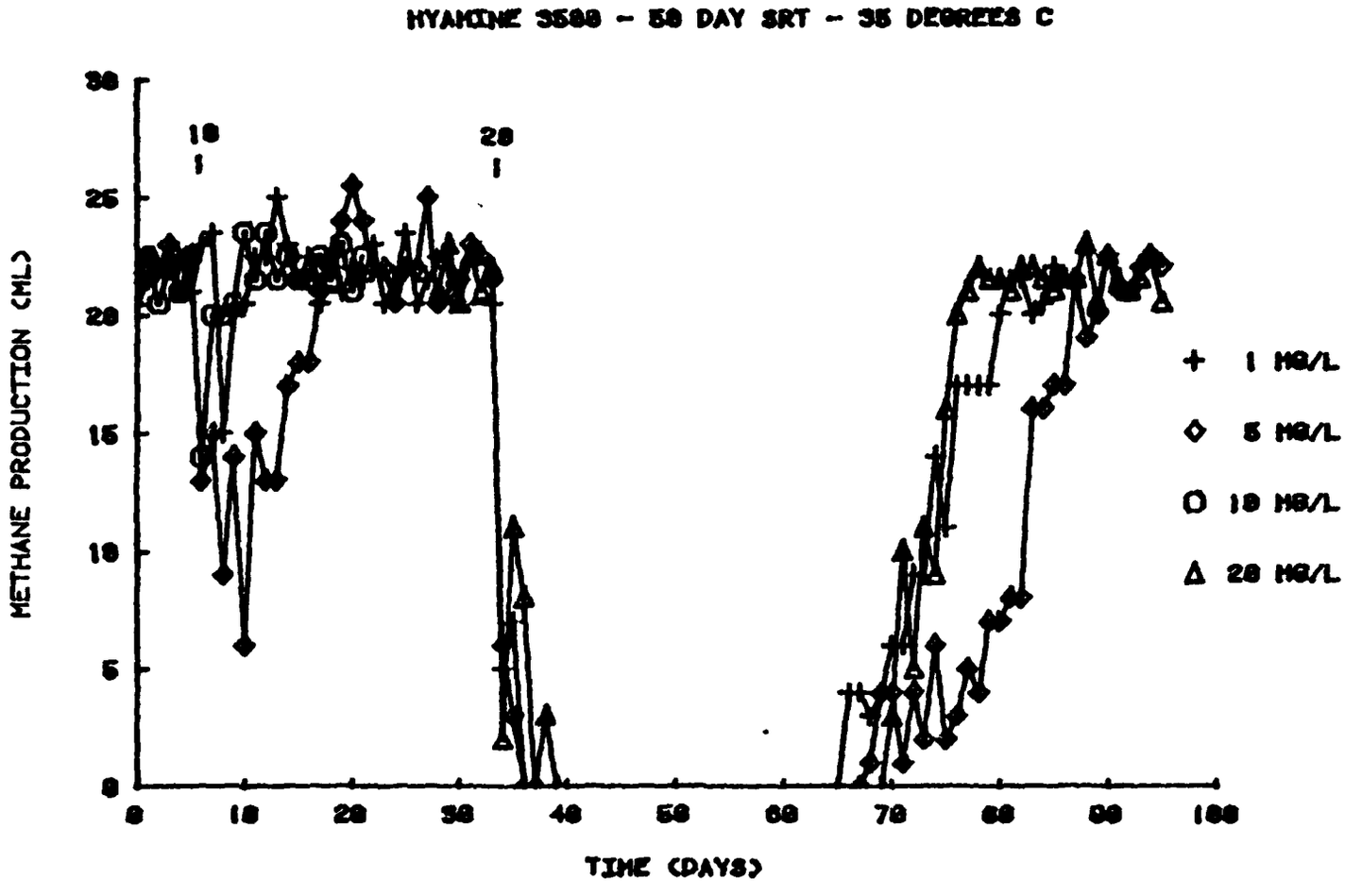

FIGURE 121. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYAMINE 3500

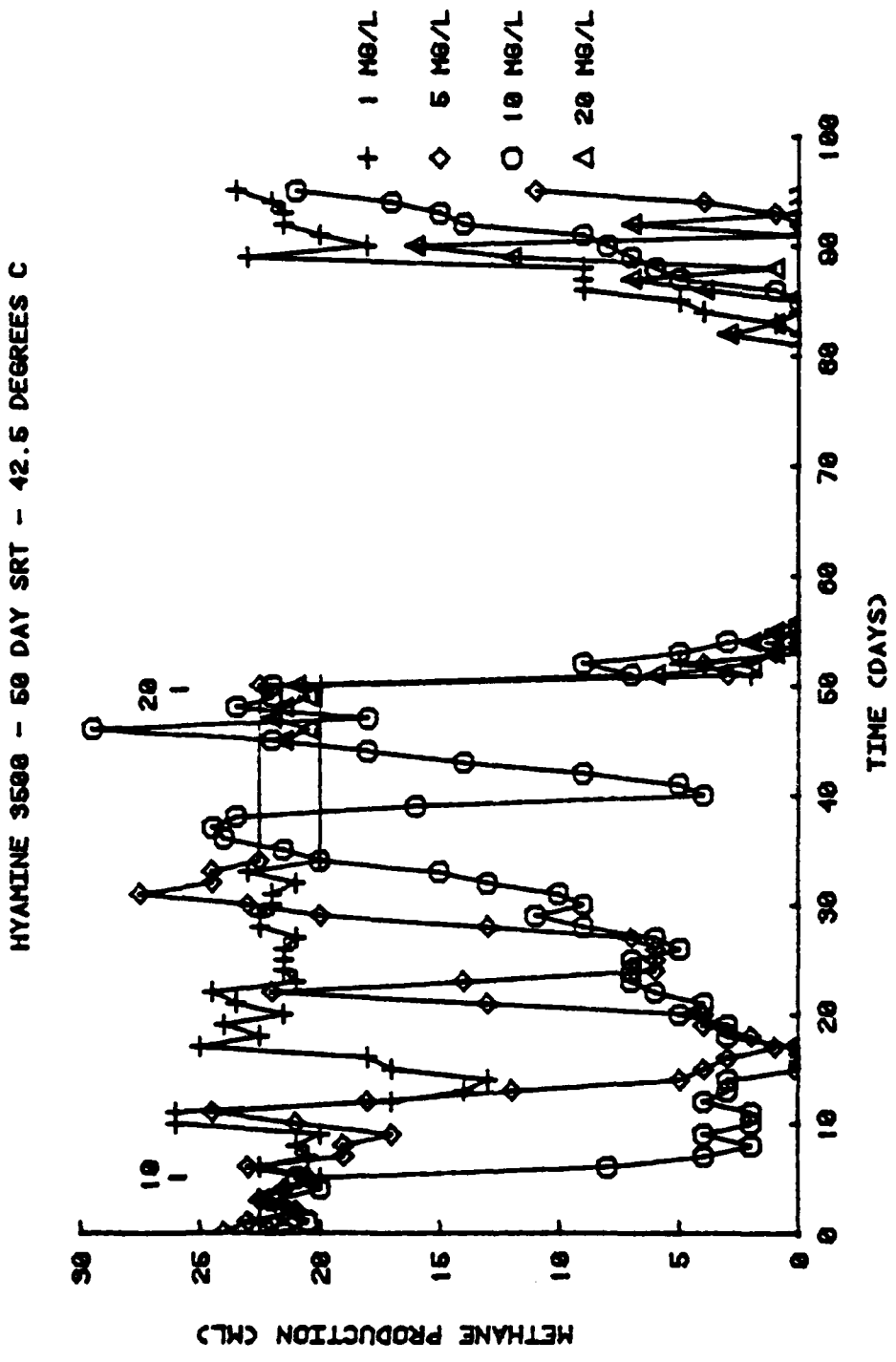

FIGURE 122. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYAMINE 3500

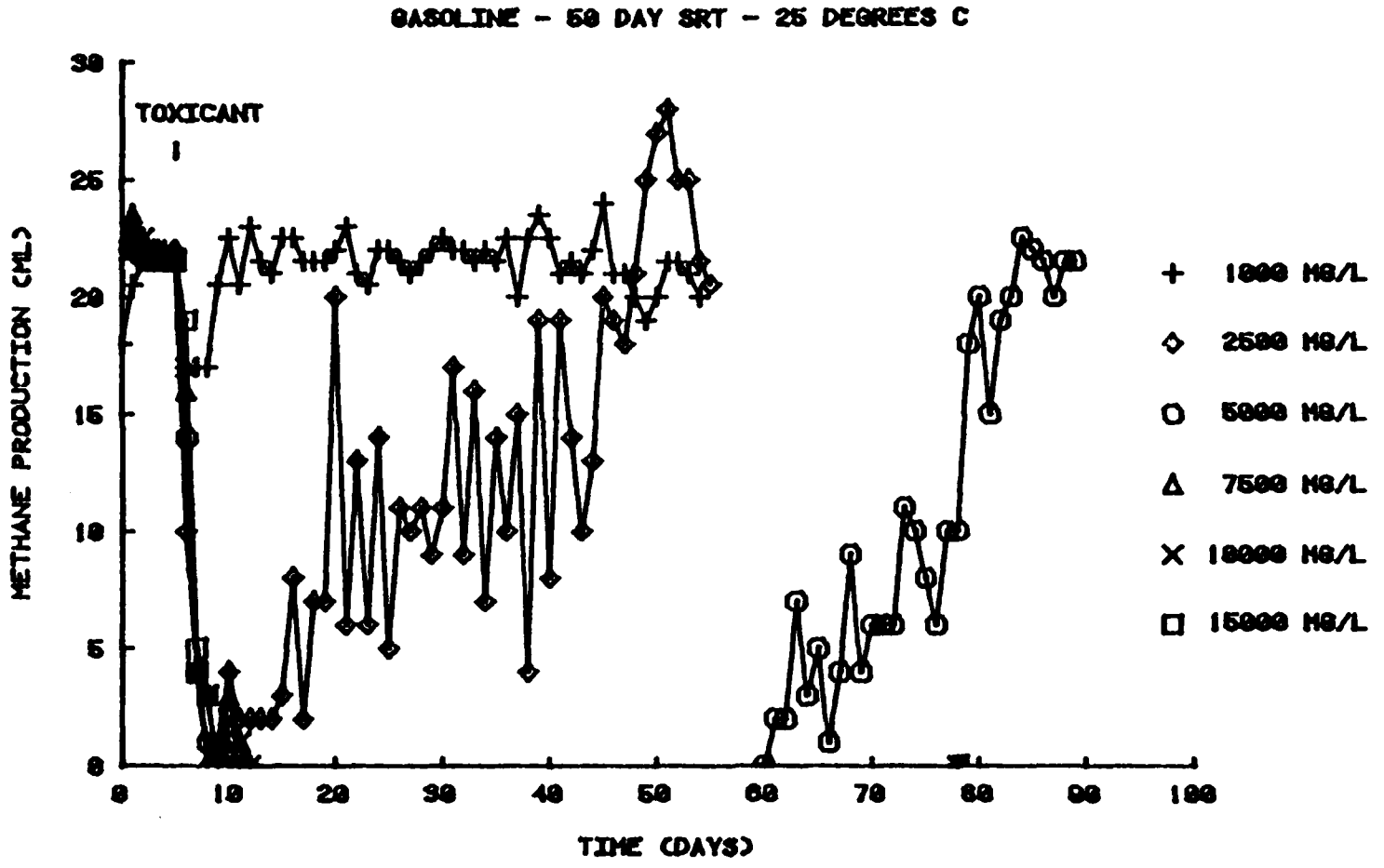

FIGURE 123. RESPONSE OF METHANOGENS TO SLUG DOSES OF GASOLINE

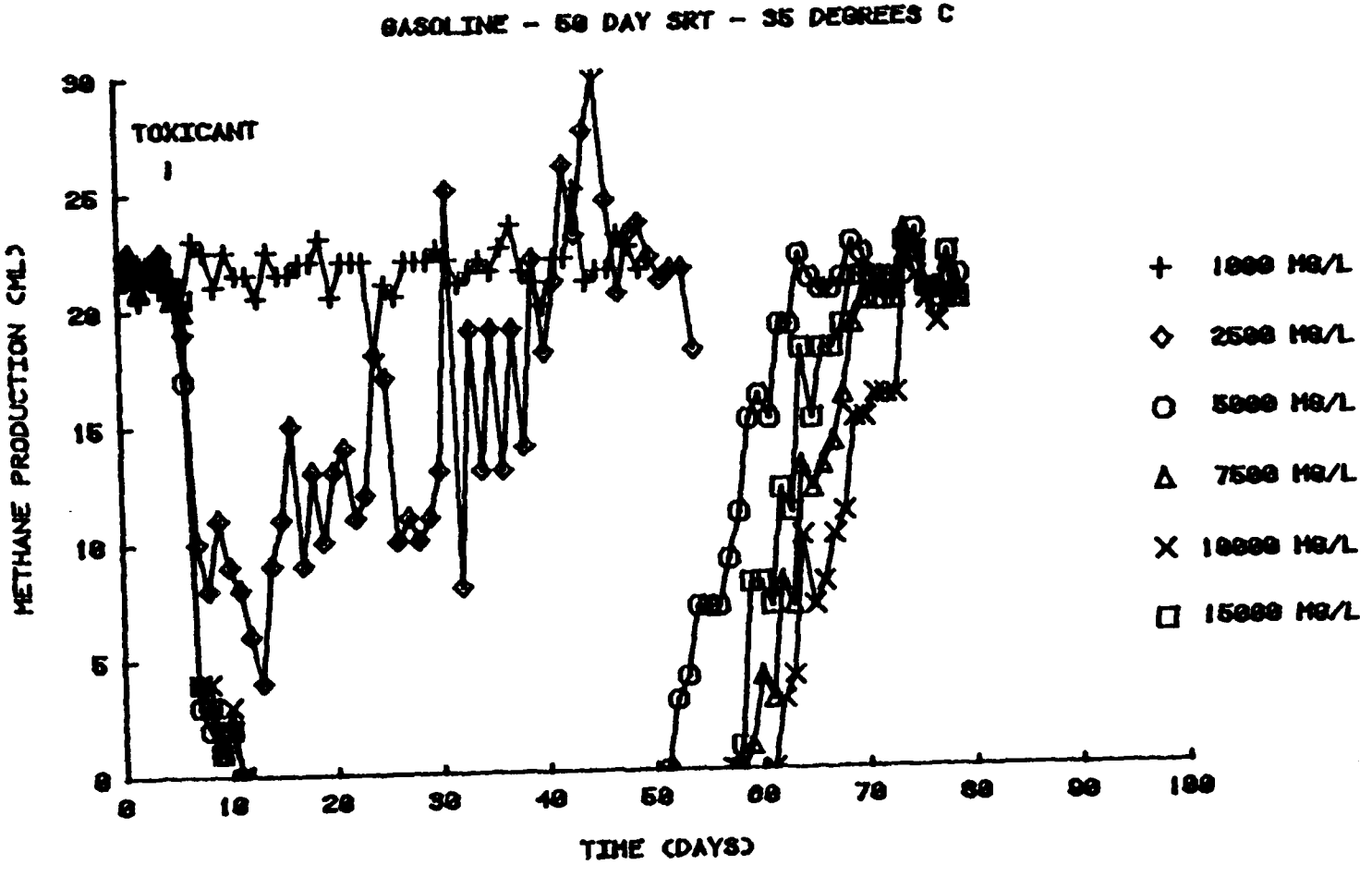

FIGURE 124. RESPONSE OF METHANOGENS TO SLUG DOSES OF GASOLINE

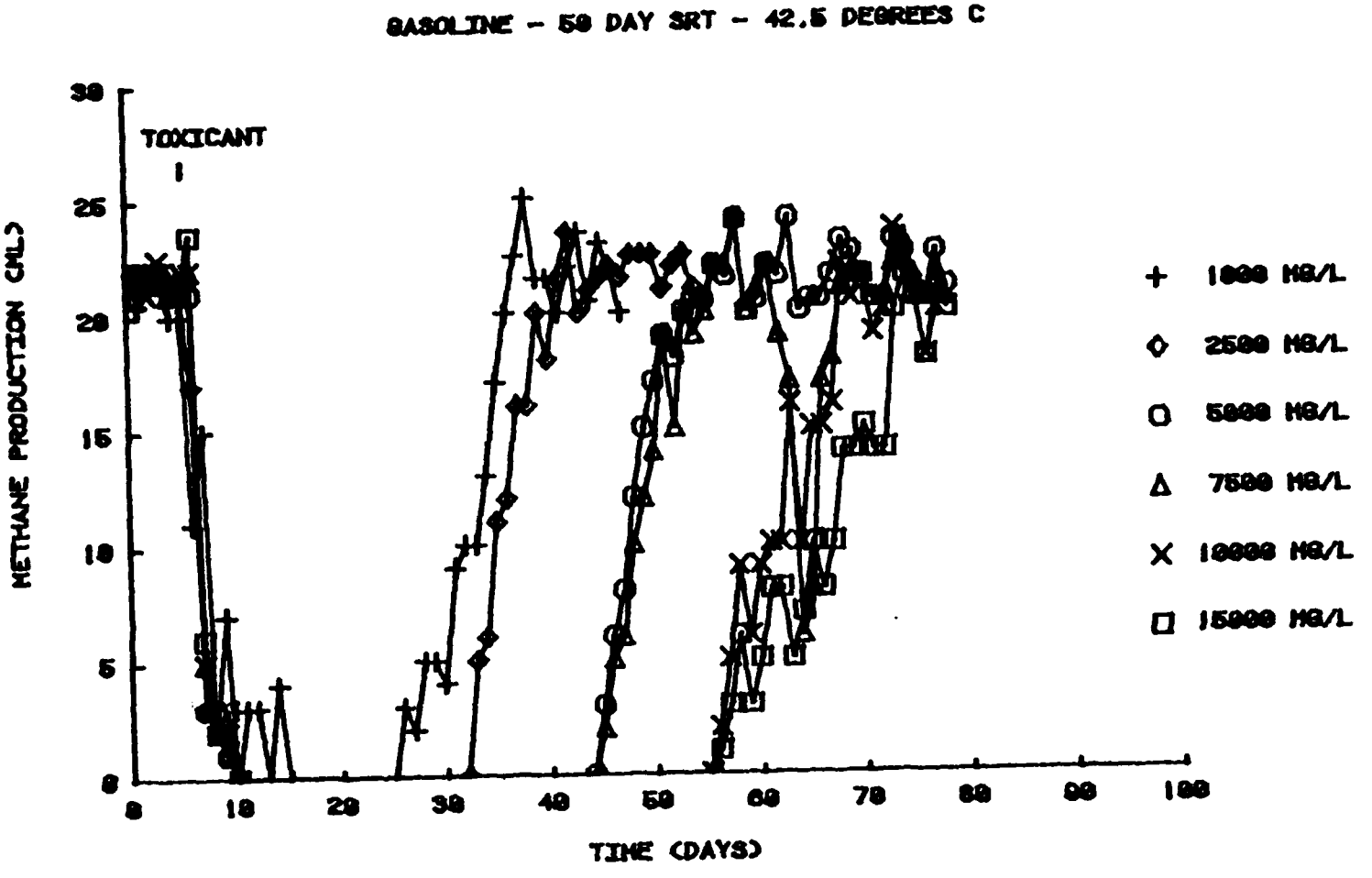

FIGURE 125. RESPONSE OF METHANOGENS TO SLUG DOSES OF GASOLINE

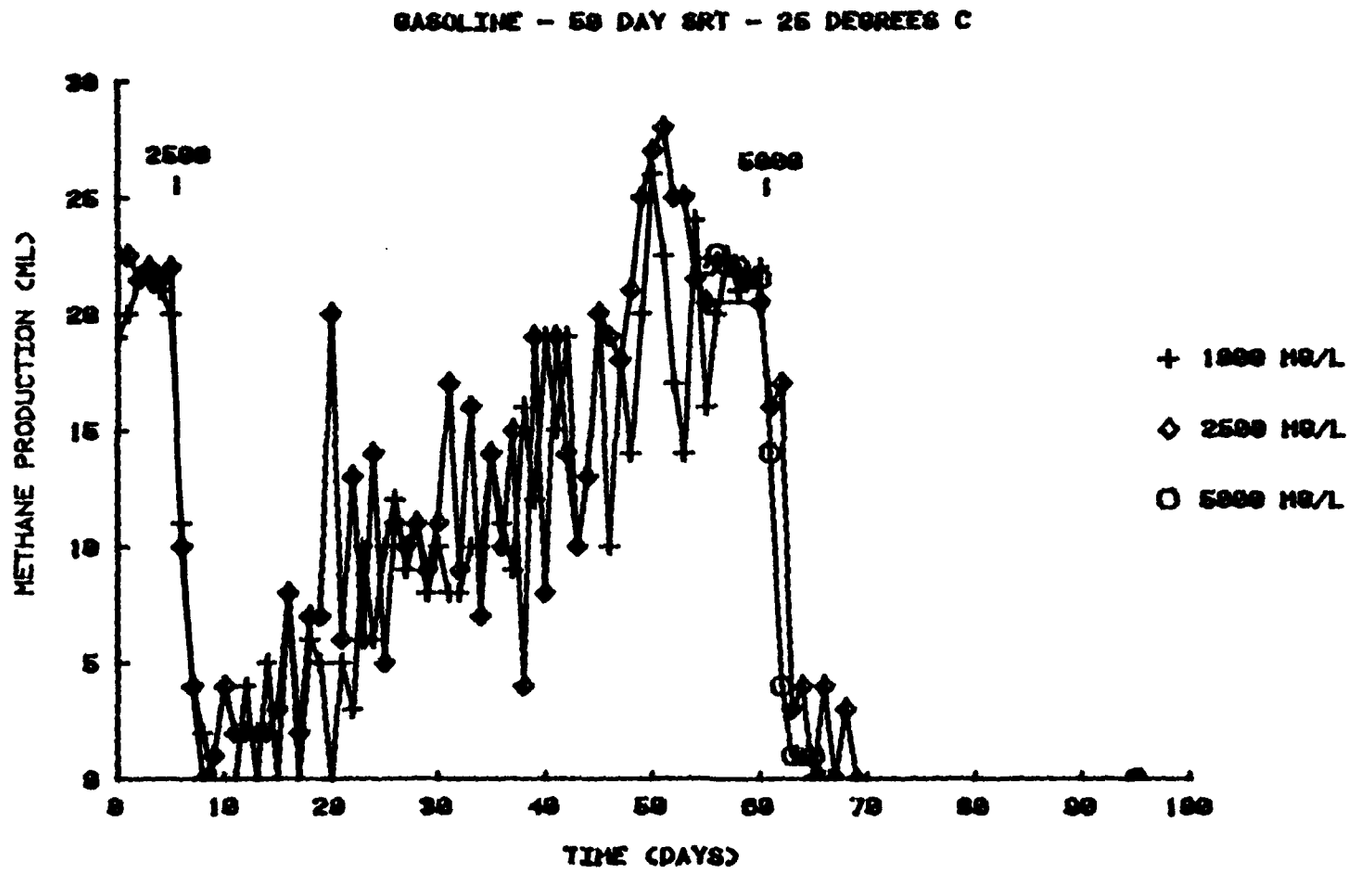

FIGURE 126. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF GASOLINE

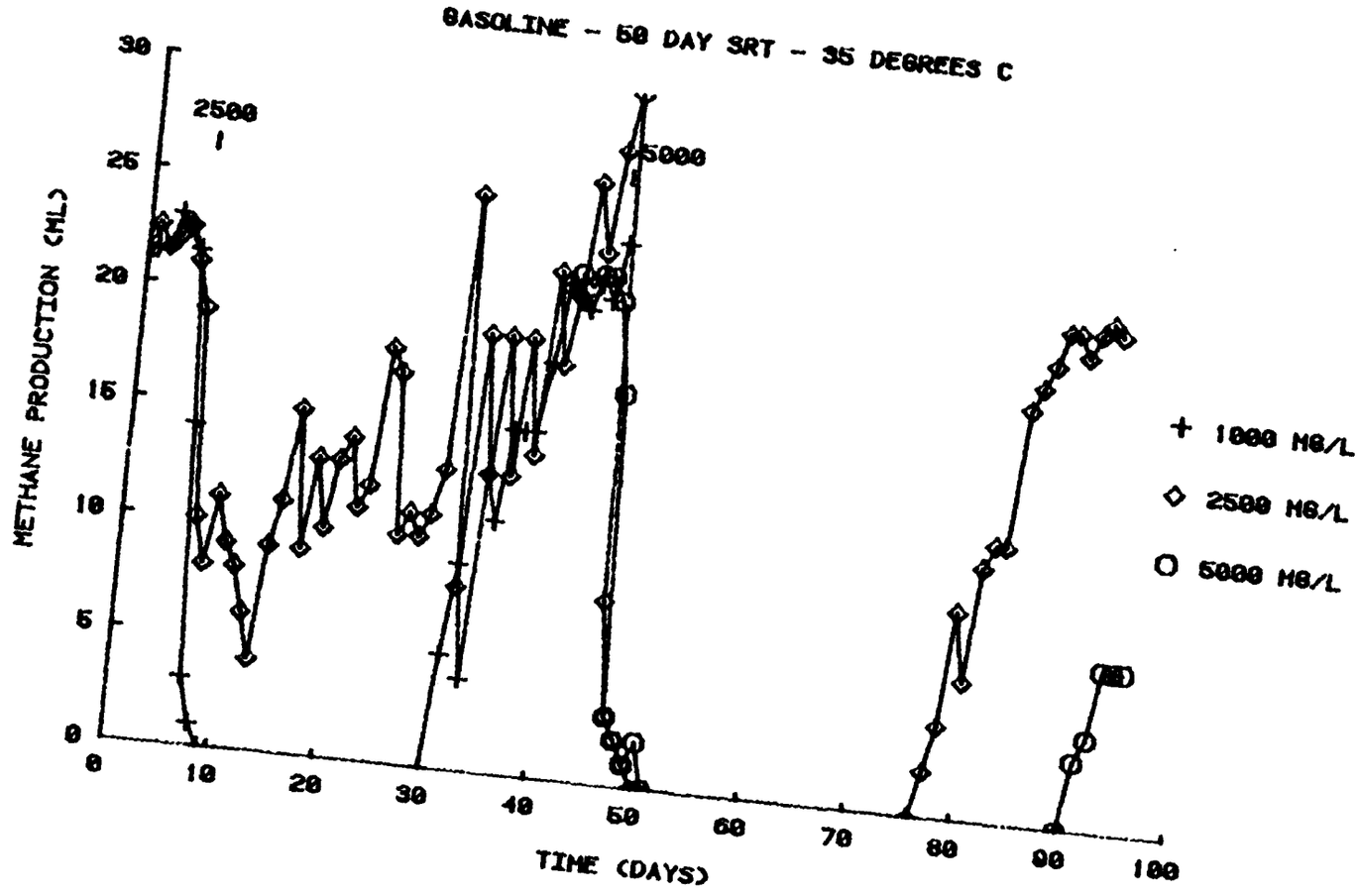

FIGURE 127. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF GASOLINE

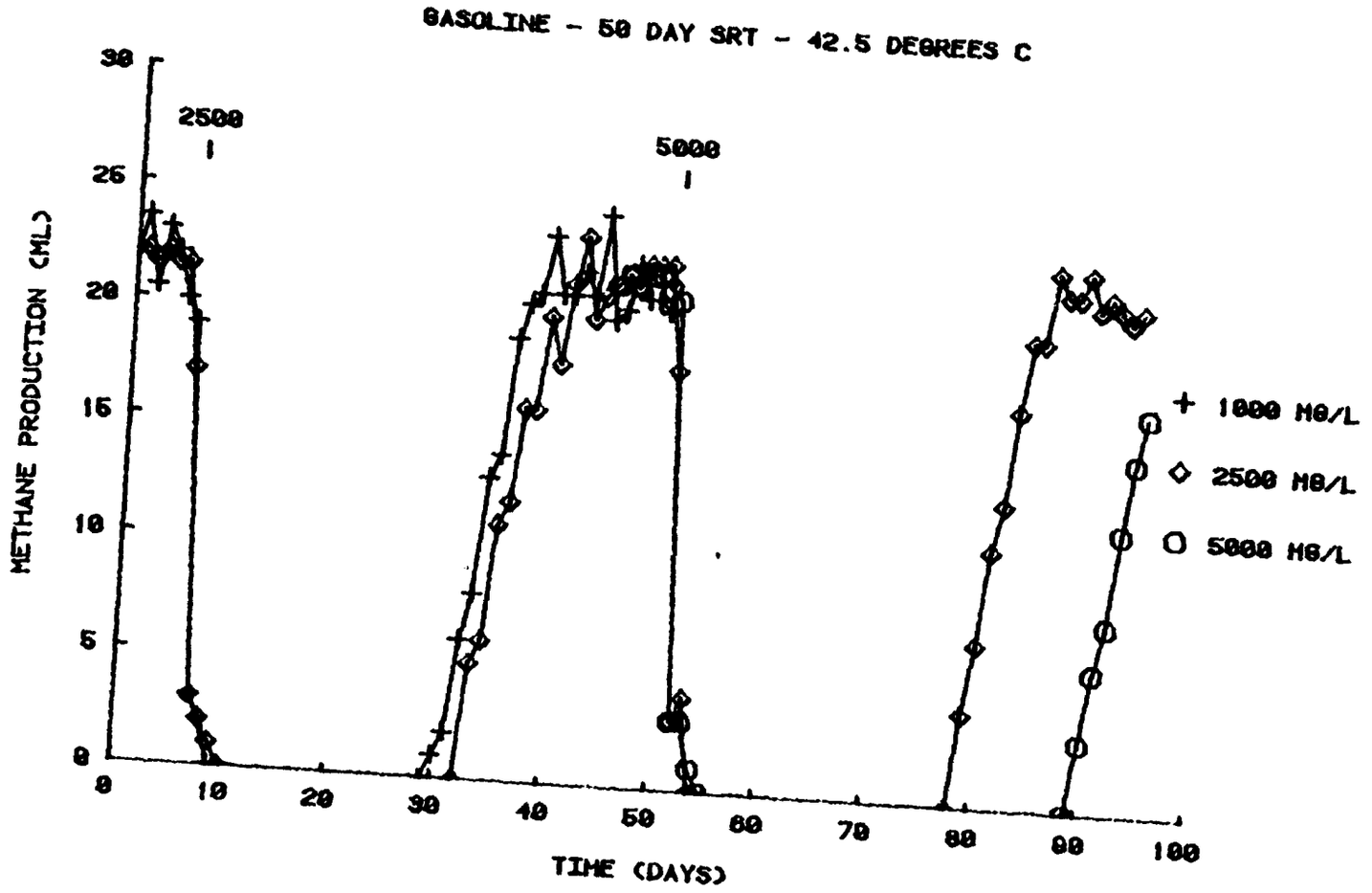

FIGURE 128. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF GASOLINE

compared to responses to other toxicants (Figures 129 to 131). Recovery rates were even slower and more erratic, with residual toxicity frequently being observed.

Temperatures of 25°C and 35°C resulted in similar response patterns (Figures 129 and 130). However, at 42.5°C, the responses were much more severe and considerable residual toxicity was demonstrated (Figure 131).

Cultures did clearly demonstrate acclimation to 5000 mg/l jet fuel (Figures 136 to 138). However, the additional slug doses also caused residual toxicity as concentrations reached 10,000 mg/l in 25°C and 35°C bottles and 2500 mg/l in 42.5°C cultures.

Hydrazine (N_2H_4)

Slug dose concentrations of 10, 25, 50, 75, 100 and 150 mg/l hydrazine were added to serum bottle cultures, making use of stock solutions of $N_2H_4(H_2SO_4)$.

The rate of decrease in methane production resulting from hydrazine exposure was much lower than that for other toxicants (Figure 135 to 137).

Cultures maintained at 25°C demonstrated the longest periods of low gas production (Figure 135). At 35°C, the serum bottles were most capable of tolerating hydrazine exposure (Figure 136). Responses by bottles kept at 42.5°C were more severe than those by 35°C cultures, but were less severe than 25°C responses (Figure 137).

Acclimation was not clearly demonstrated at any temperature (Figures 138 to 140).

CONTINUOUS ADDITION OF TOXICANTS

Daily methane production by serum bottles operated in the semi-continuous mode was recorded. The continuous addition of nickel, chloroform, and hydrazine was begun after the serum bottles had stabilized at quasi steady-state methane generation levels. Each of the three toxicants was tested at three concentrations, three solids retention times (15, 25, and 50 days) and three temperatures (25°C, 35°C, and 42.5°C).

Statistical analysis of the control serum bottles for each set of

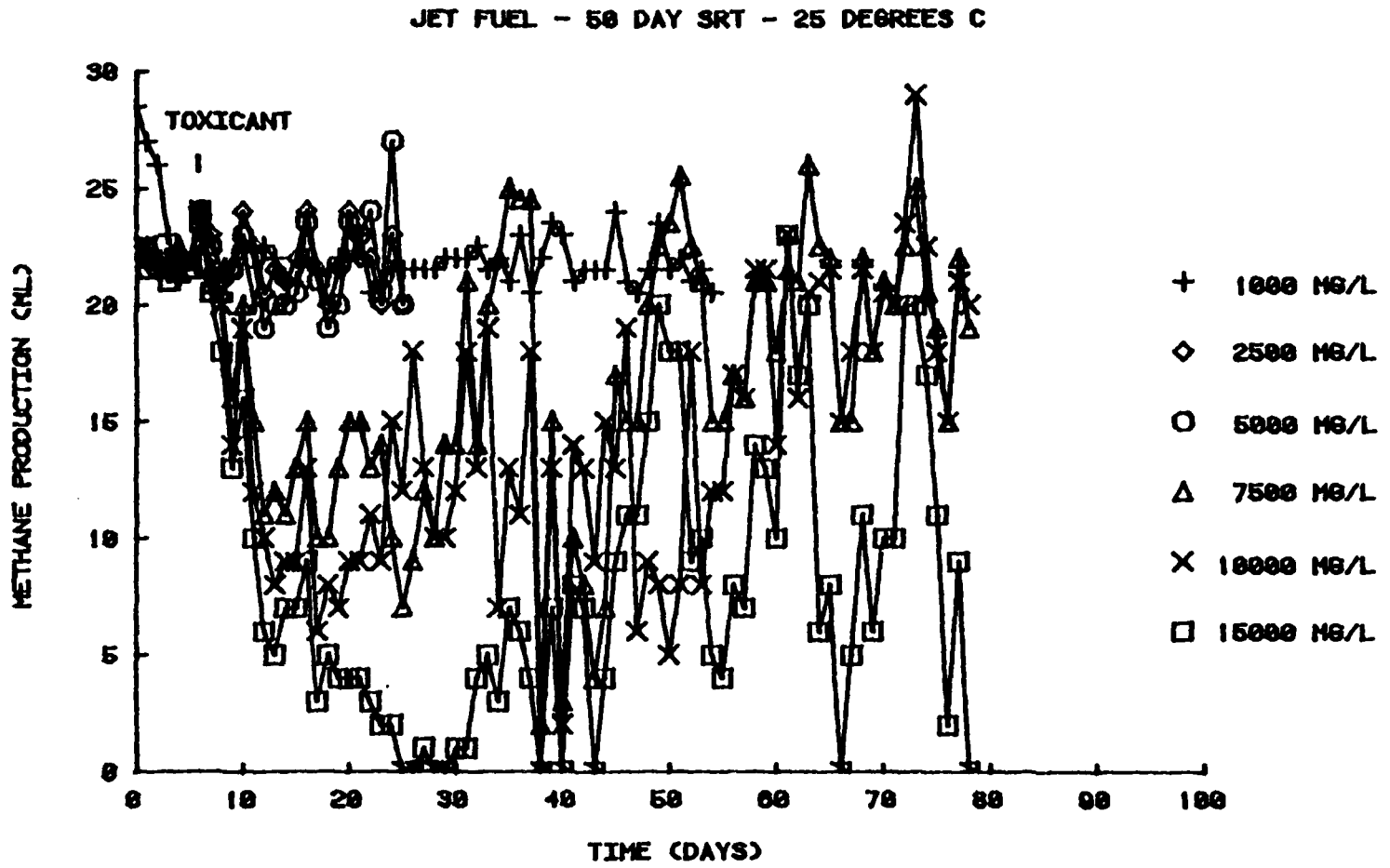

FIGURE 129. RESPONSE OF METHANOGENS TO SLUG DOSES OF JET FUEL

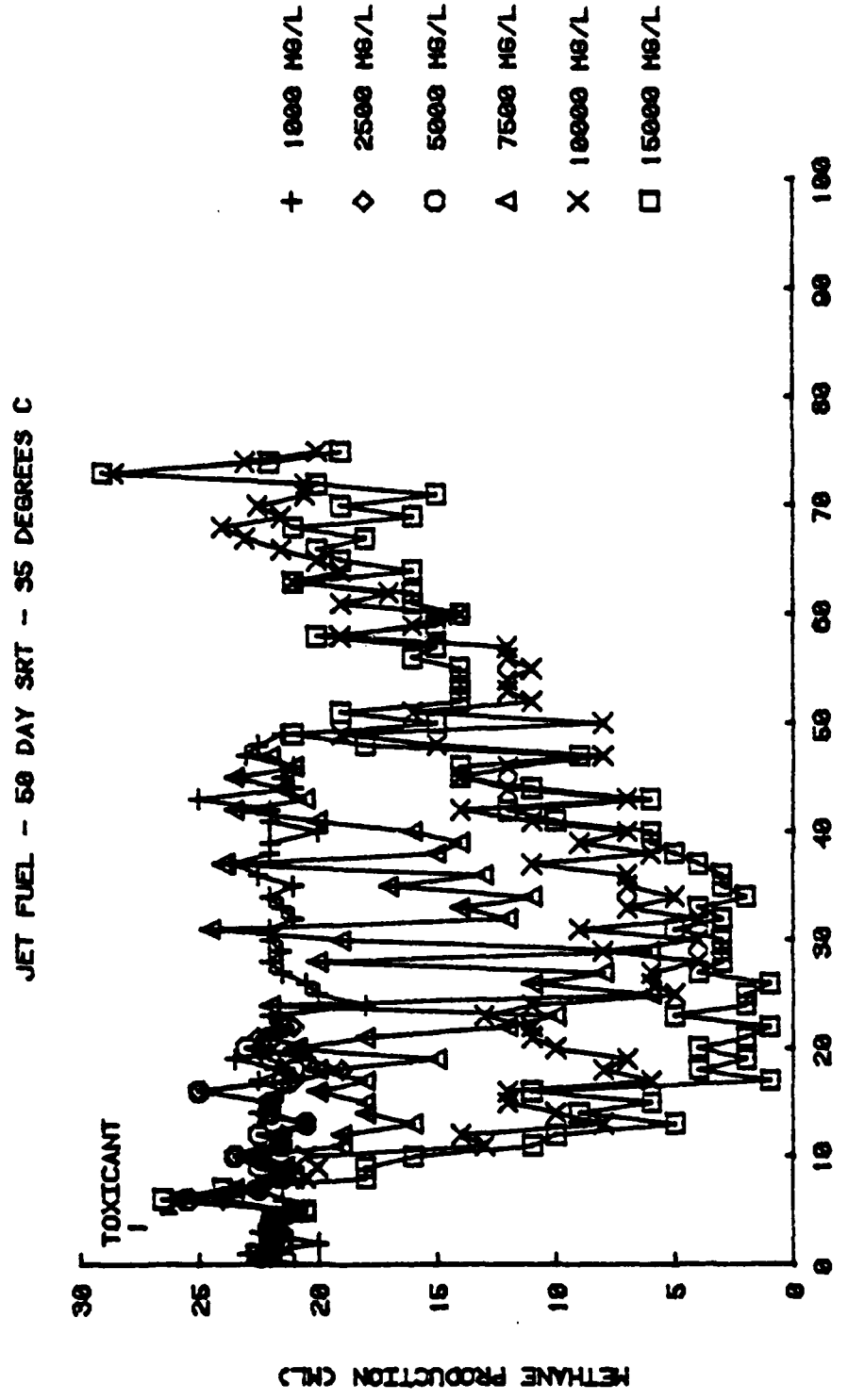

FIGURE 130. RESPONSE OF METHANOGENS TO SLUG DOSES OF JET FUEL

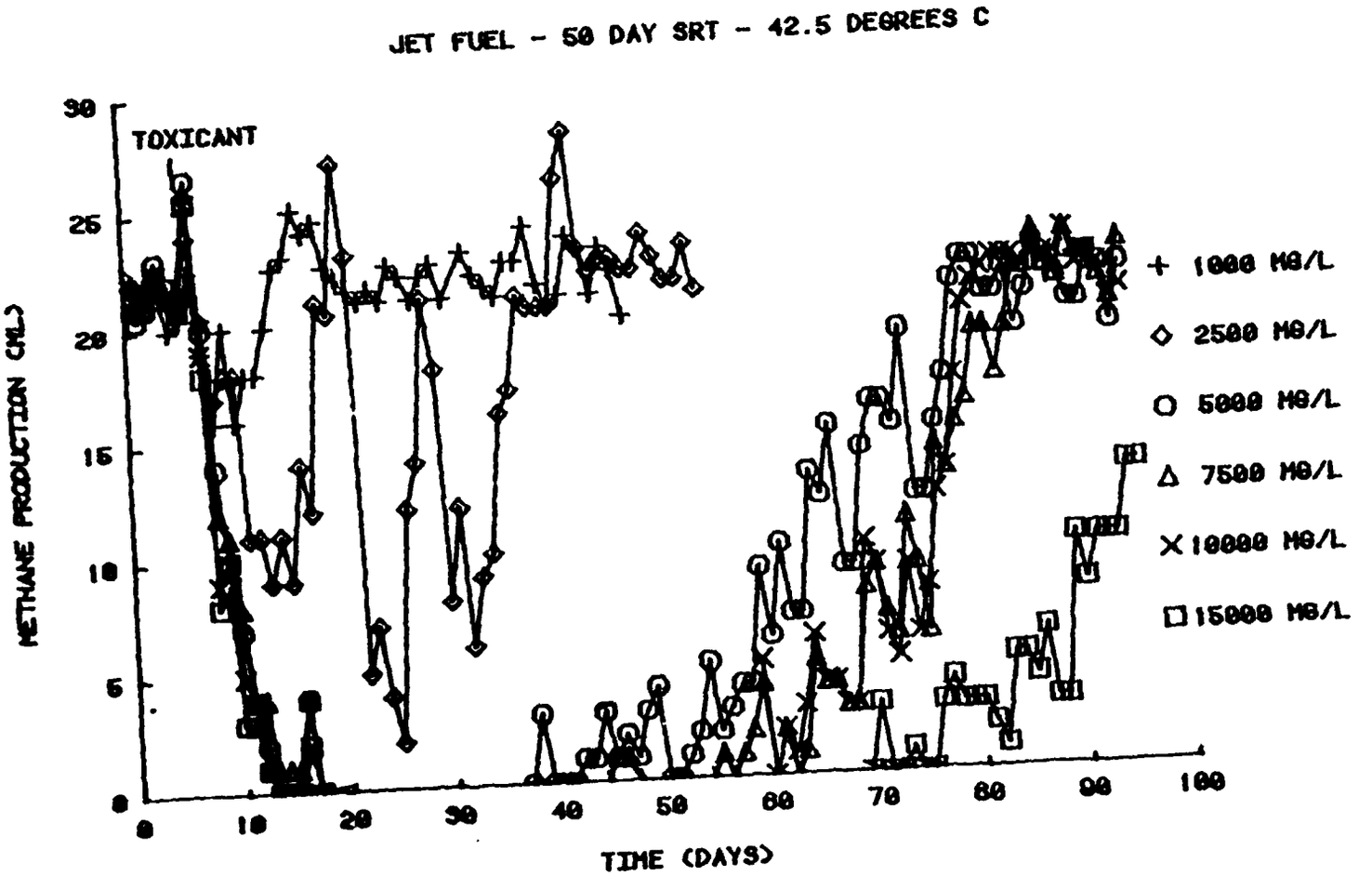

FIGURE 131. RESPONSE OF METHANOGENS TO SLUG DOSES OF JET FUEL

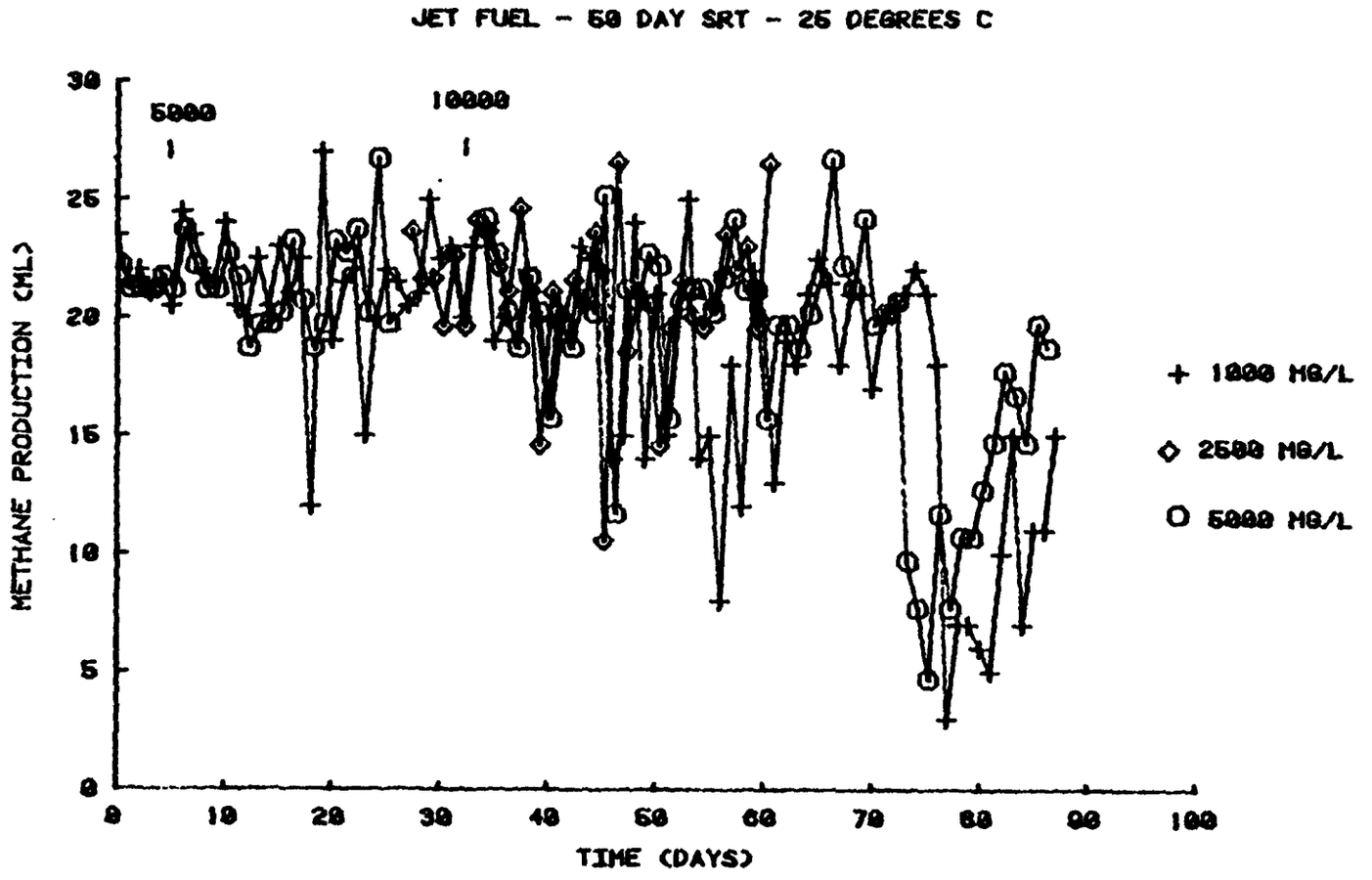

FIGURE 132. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF JET FUEL

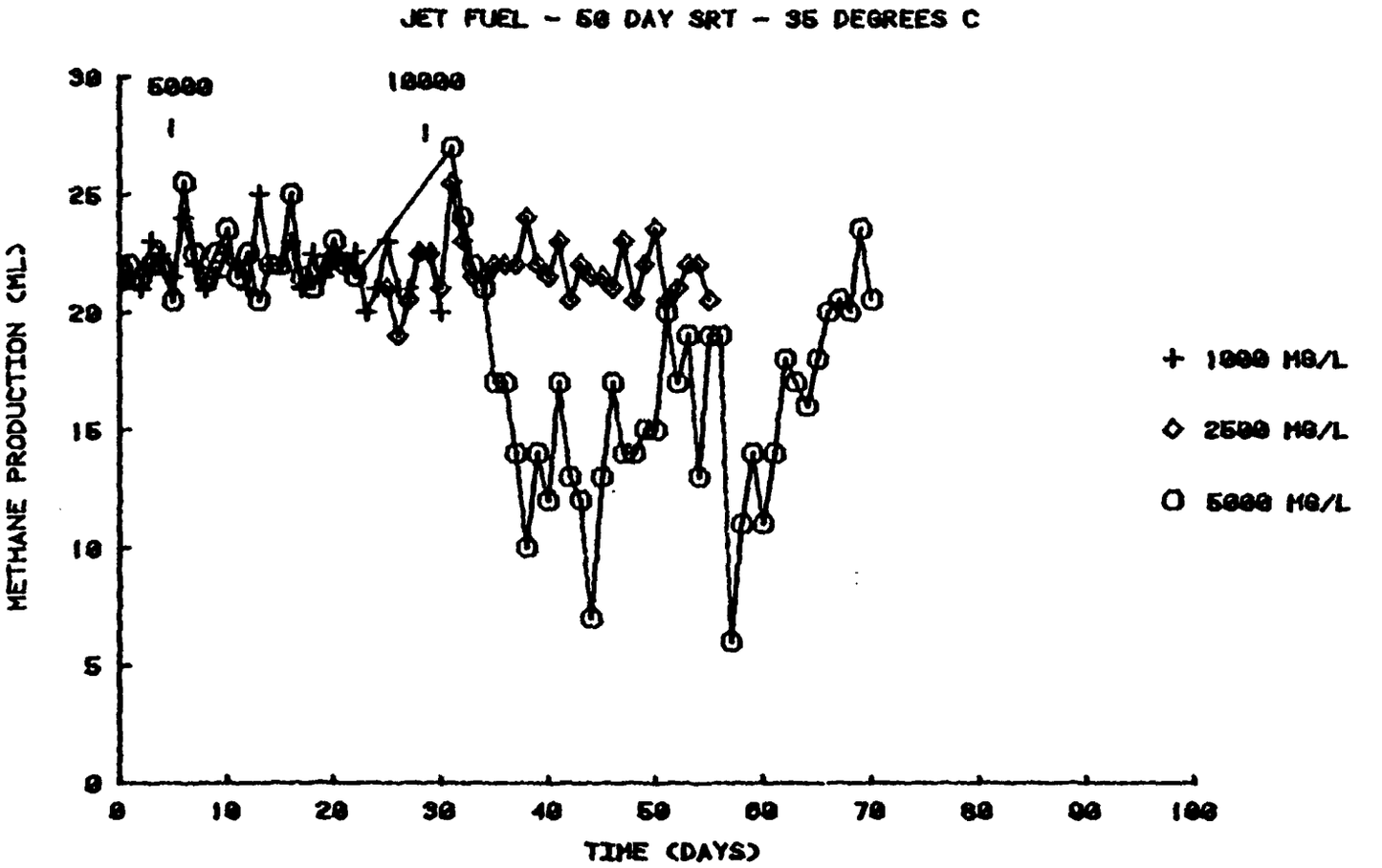

FIGURE 133. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF JET FUEL

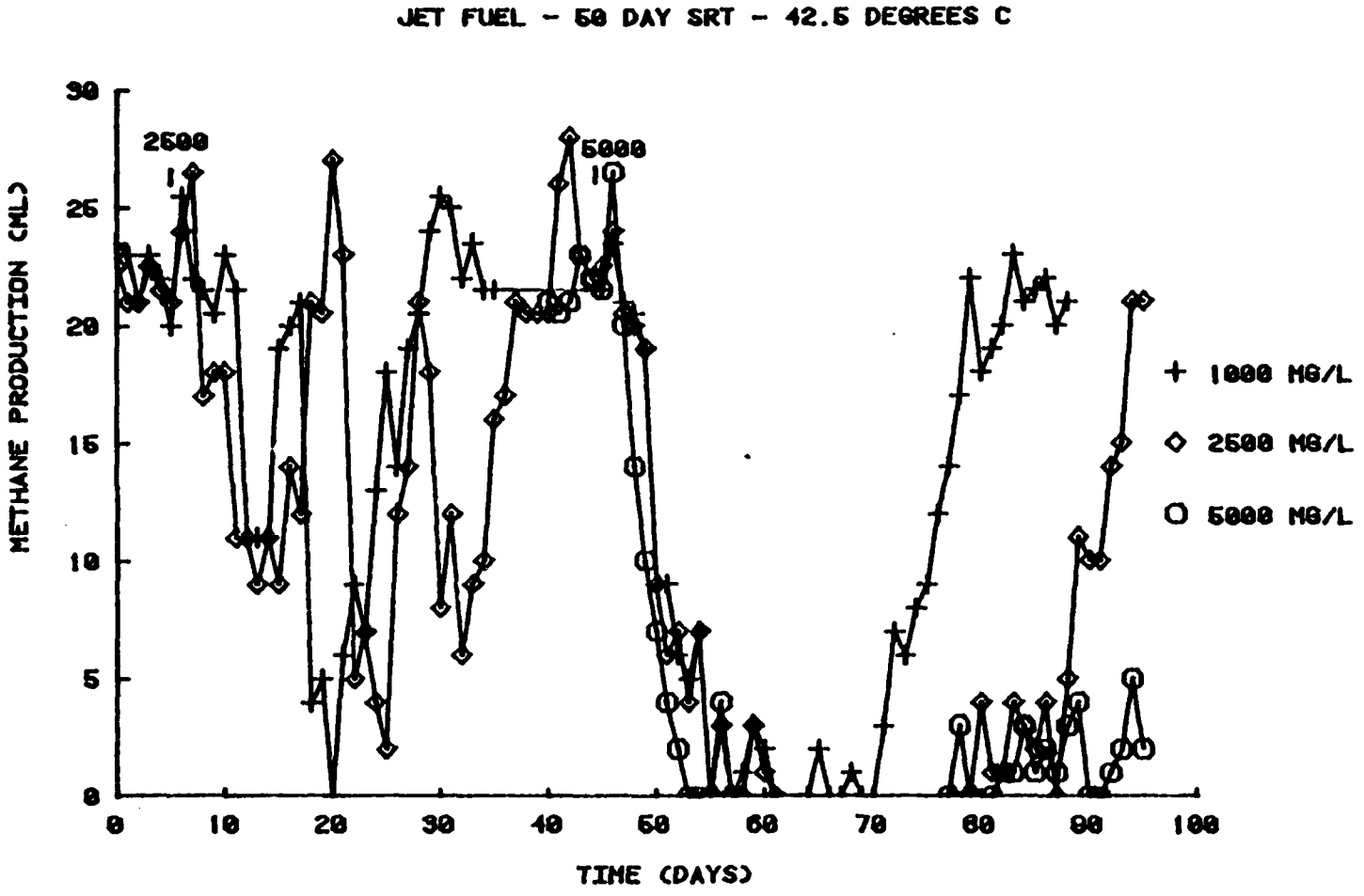

FIGURE 134. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF JET FUEL

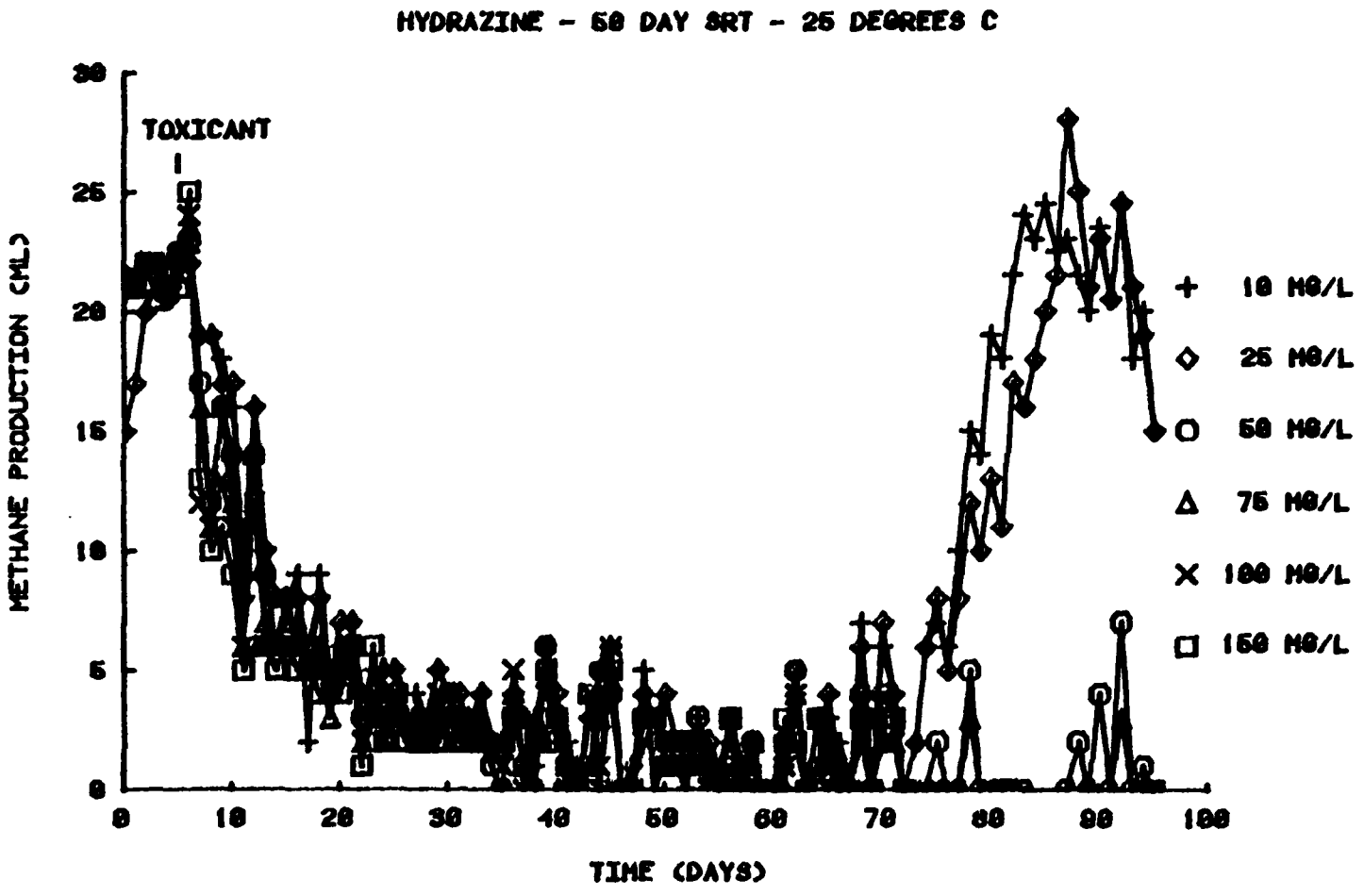

FIGURE 135. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYDRAZINE

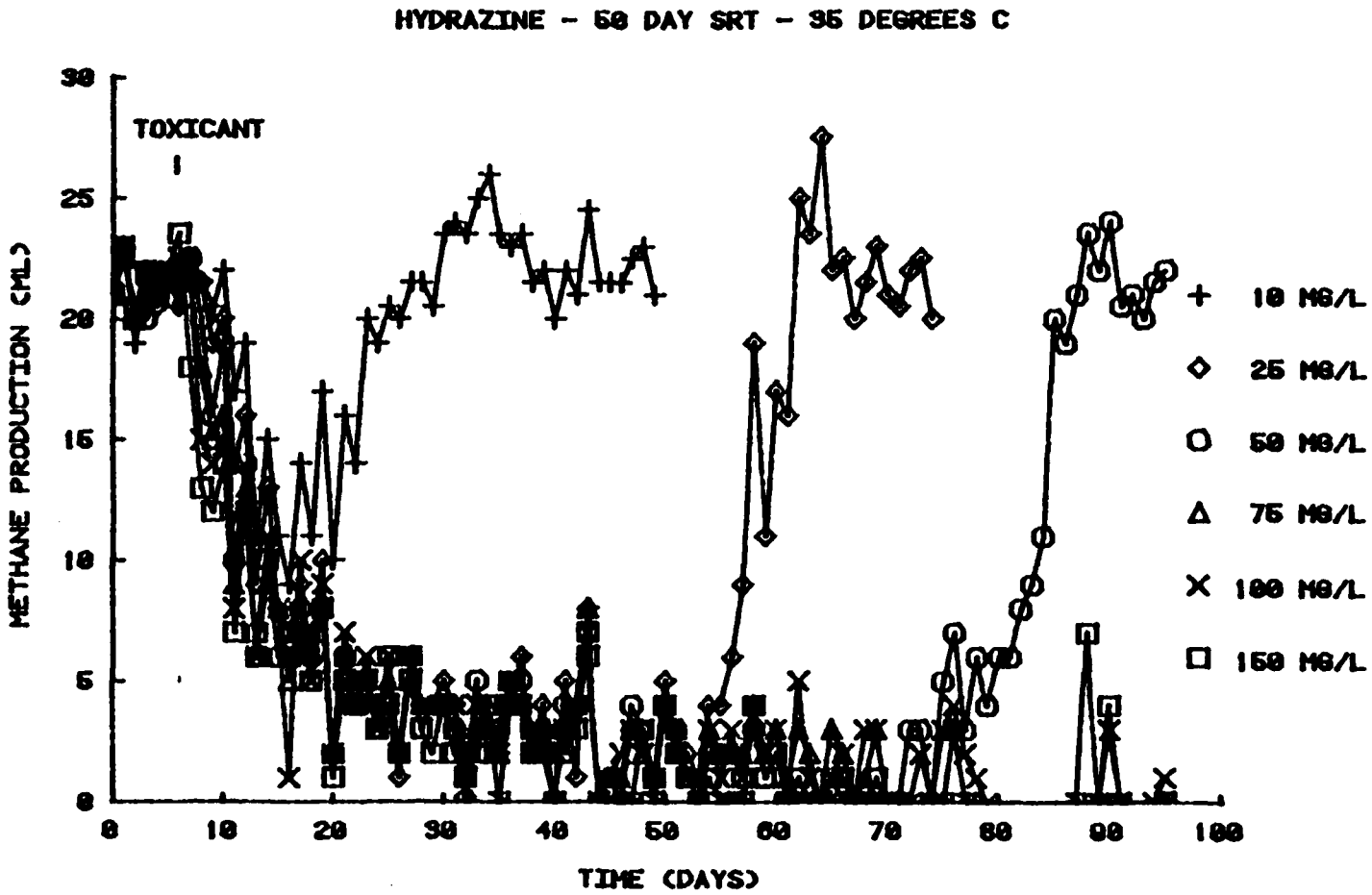

FIGURE 136. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYDRAZINE

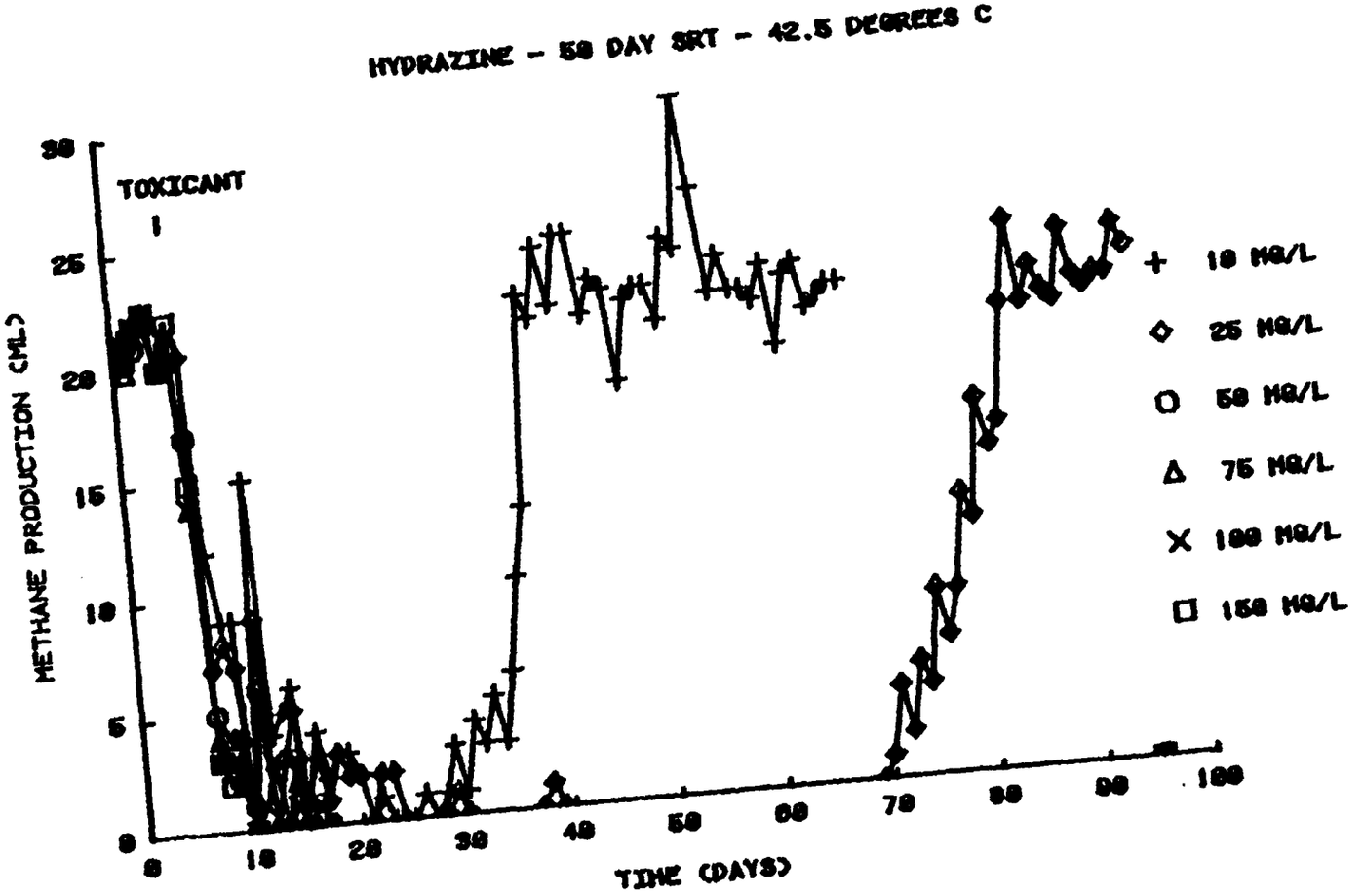

FIGURE 137. RESPONSE OF METHANOGENS TO SLUG DOSES OF HYDRAZINE

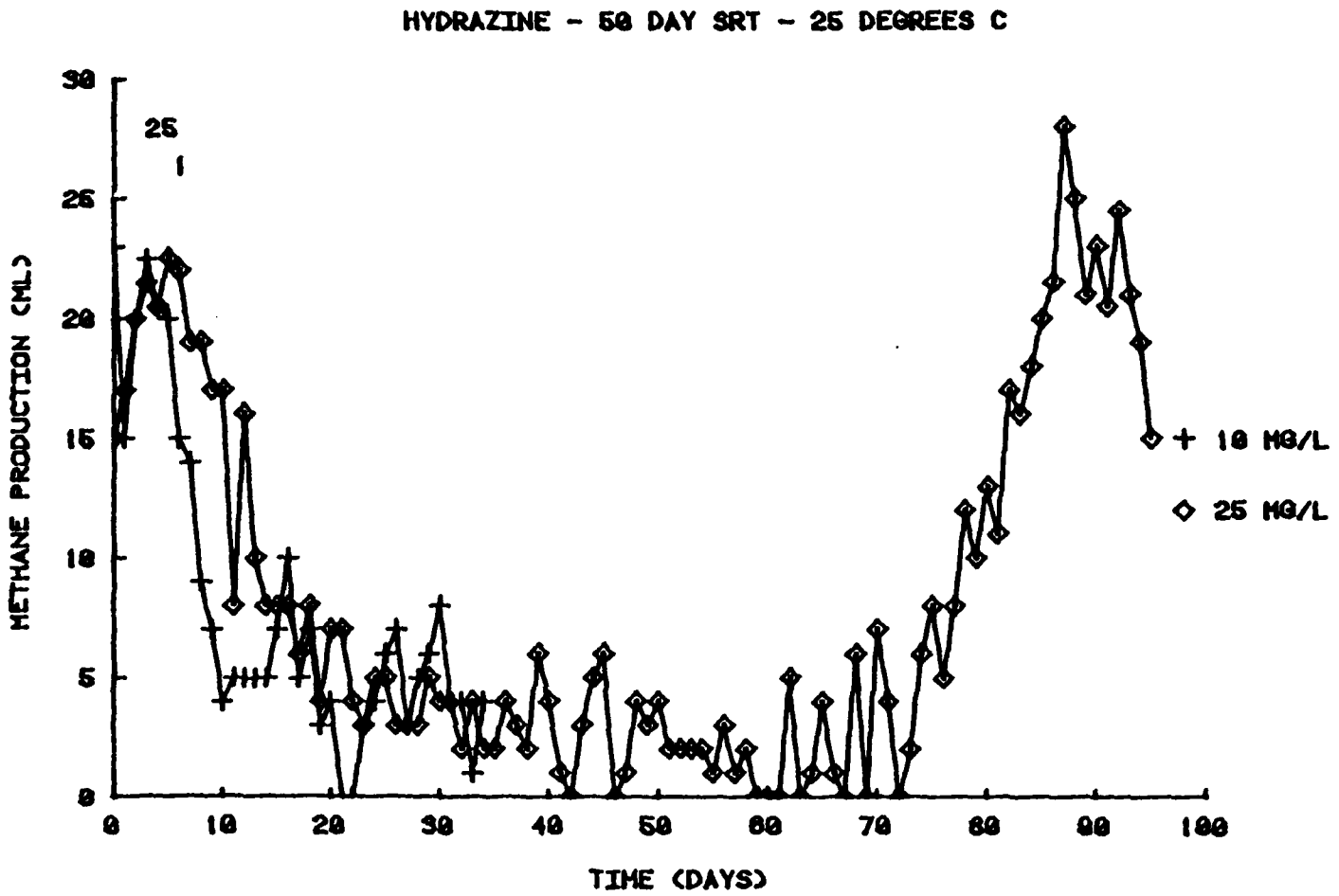

FIGURE 138. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYDRAZINE

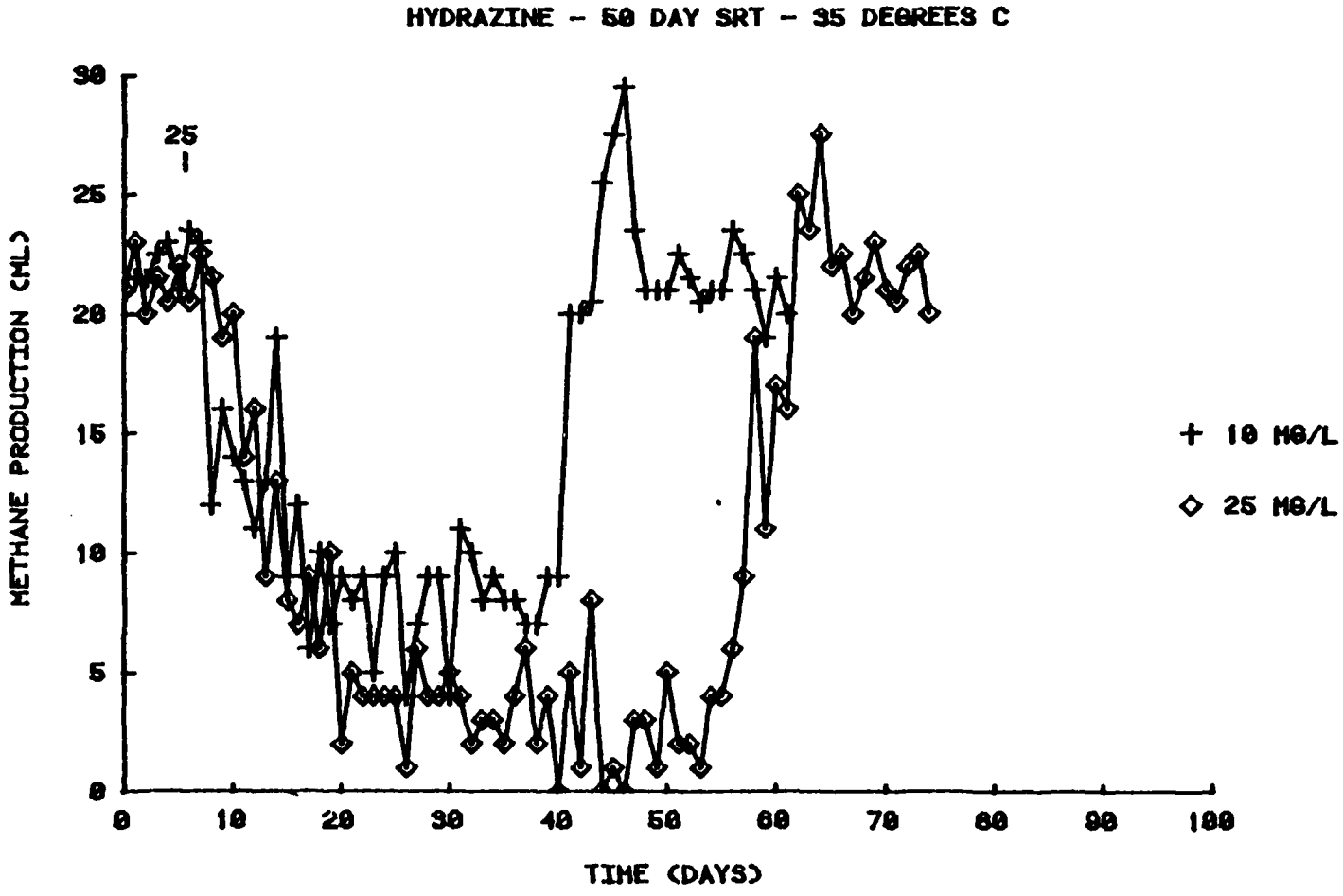

FIGURE 139. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYDRAZINE

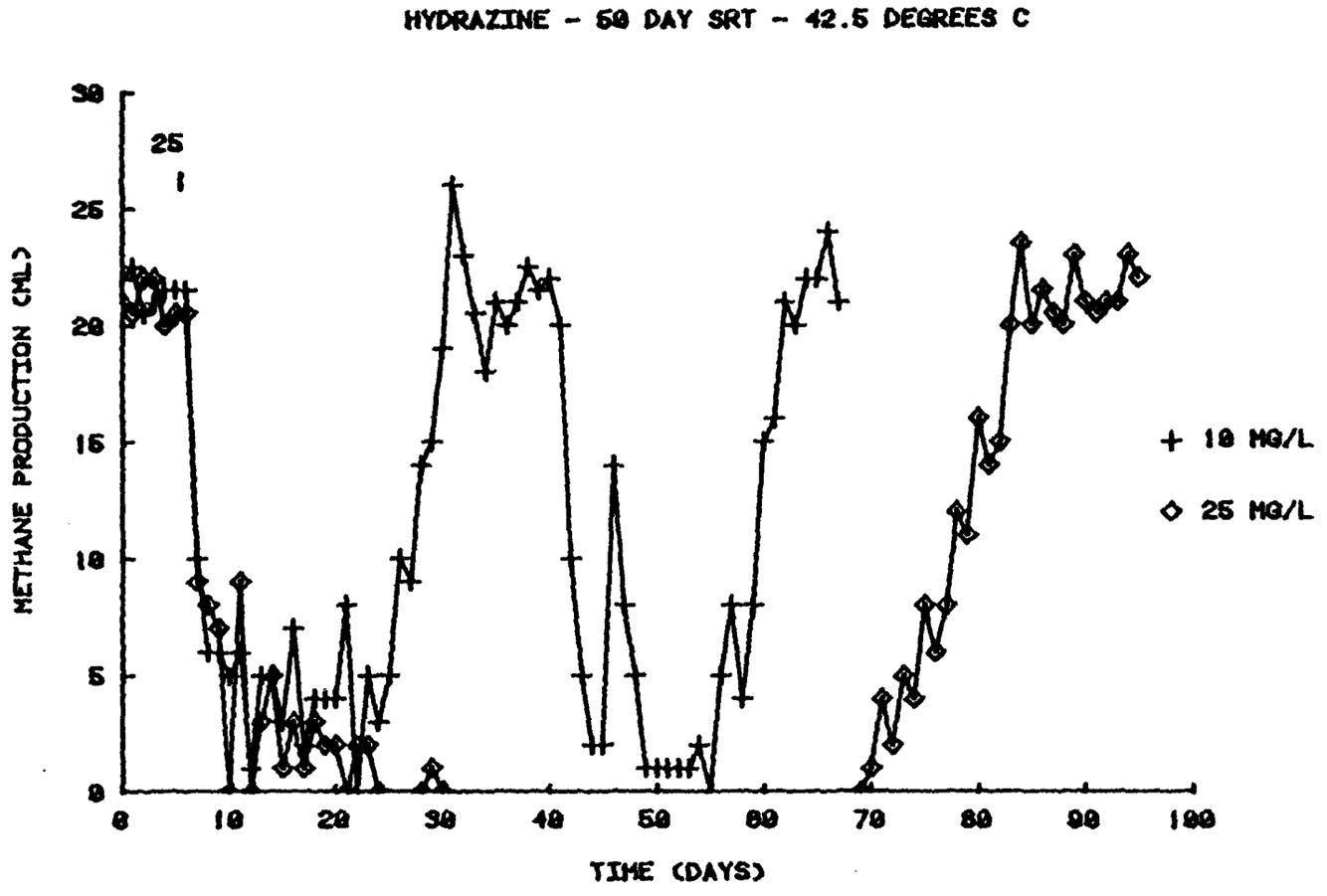

FIGURE 140. ACCLIMATION OF METHANOGENS TO SLUG DOSES OF HYDRAZINE

environmental conditions revealed that the mean daily methane gas production ranged from 20.2 to 22.0 ml/day with standard deviations ranging from 0.15 to 0.91 ml. Temperature fluctuations were similar in all three incubators, normally ± 0.5°C. No significant differences were noted for any of the various SRTs.

Nickel (Ni^{2+})

The final desired concentrations resulting from the continuous addition of nickel to the serum bottles were 100, 200 and 400 mg/l as Ni^{2+}. Nickel may form a precipitate with sulfide, thus reducing the soluble concentration in the serum bottle. The maximum sulfide available from the nutrient solution was estimated to be 97 mg/l S^{2-}. Using the appropriate solubility calculations (Sawyer and McCarty, 1978), the maximum possible nickel that could be precipitated is calculated to be 178 mg/l. It should be noted again that it is unlikely that all the sulfur in the serum bottle will be available for precipitation. Soluble Ni^{2+} was not measured.

The highest concentration, 400 mg/l as Ni^{2+}, could not be tolerated at any of the SRTs or temperatures (Figures 141 to 149). In less than 10 days after the initial introduction of nickel, gas production dropped to zero and did not resume again before the study.

The 15-day SRT was the most unstable system (Figures 141, 144 and 147). At 25°C, both the 200 mg/l and 400 mg/l concentrations could not be tolerated (Figure 141). The methanogenic bacteria were only able to acclimate to the 100 mg/l concentration, although gas production was irregular at times. At 35°C gas production for the 100 mg/l system dropped to zero for two days and returned to about 9 ml/day for the remainder of the study (Figure 144). Although gas dipped for the 200 mg/l concentration, it returned to the level of the control within a few days. The reason for this apparent anamoly is unknown. Gas production at 42.5°C was erratic for both 200 mg/l and 400 mg/l Ni^{2+} (Figure 147).

The 50-day SRT tolerated nickel much better than the 25-day SRT in the long run, but the 25-day SRT seemed to recover faster (Figures 142, 143, 145, 146, 148, and 149). There was not much difference in the results for these two SRTs. The preferred temperature was 35°C, with 25°C next and 42.5°C the most irregular. Significant acclimation potential was exhibited at 25°C and

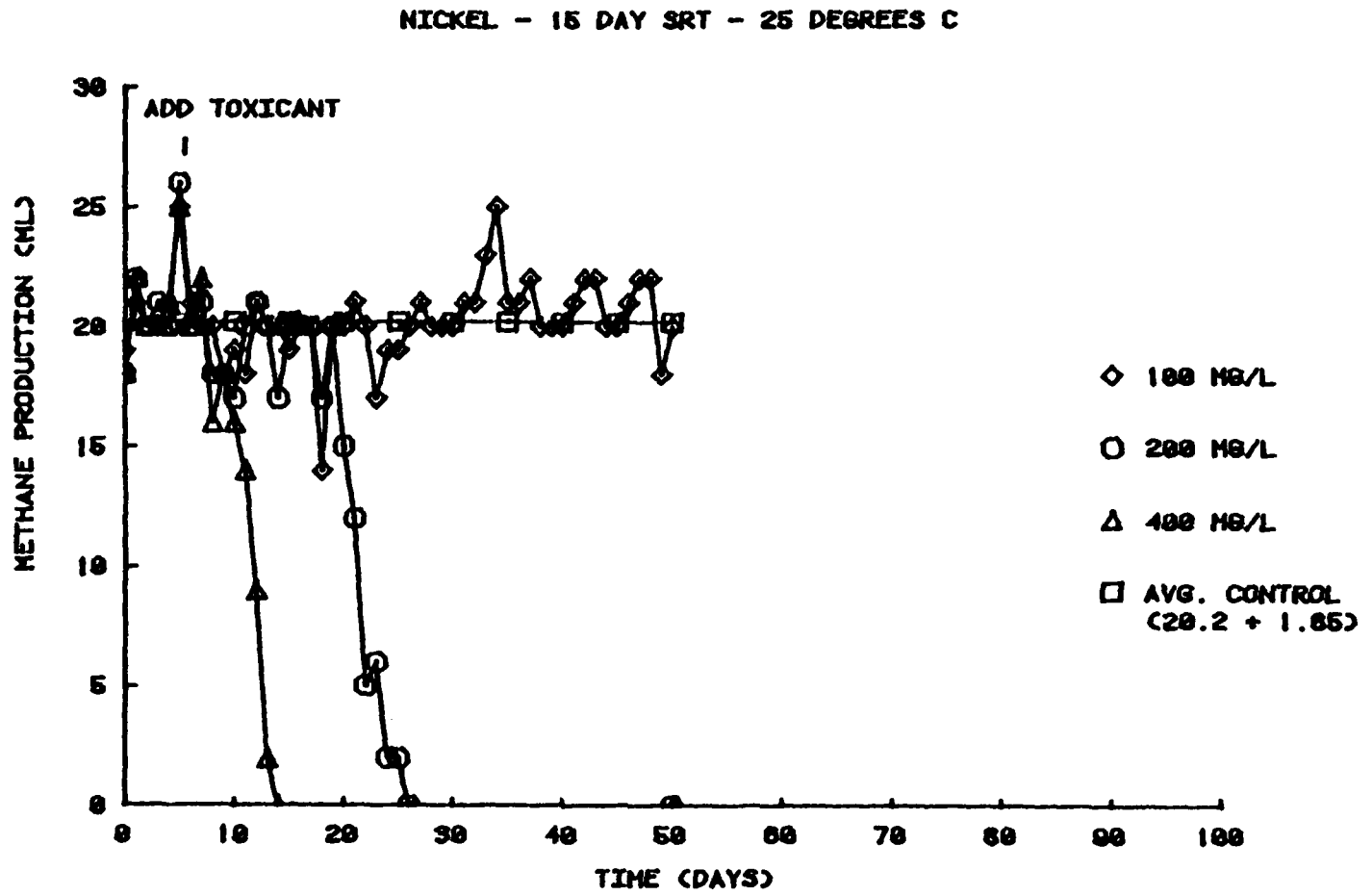

FIGURE 141. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

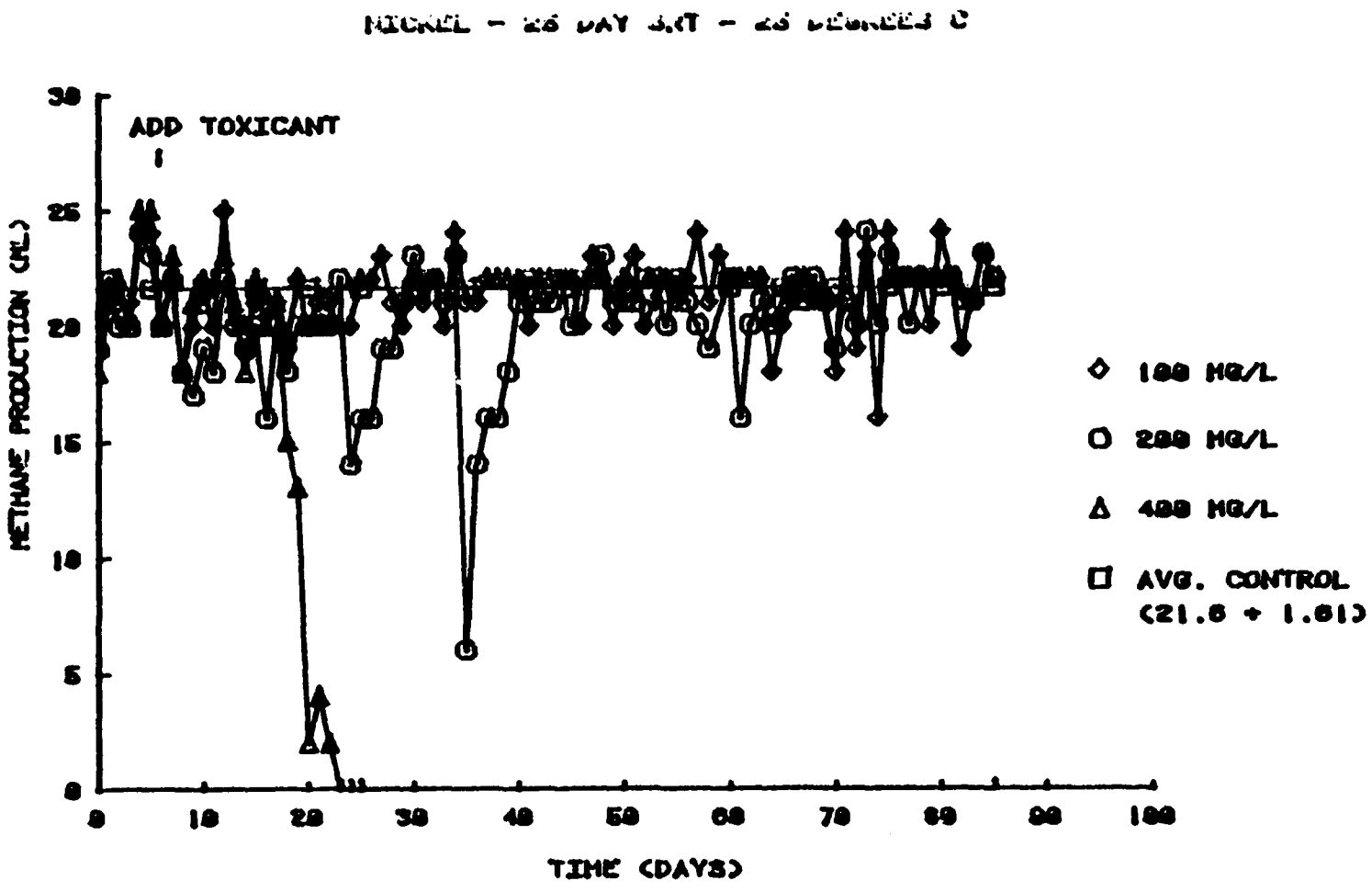

FIGURE 142. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

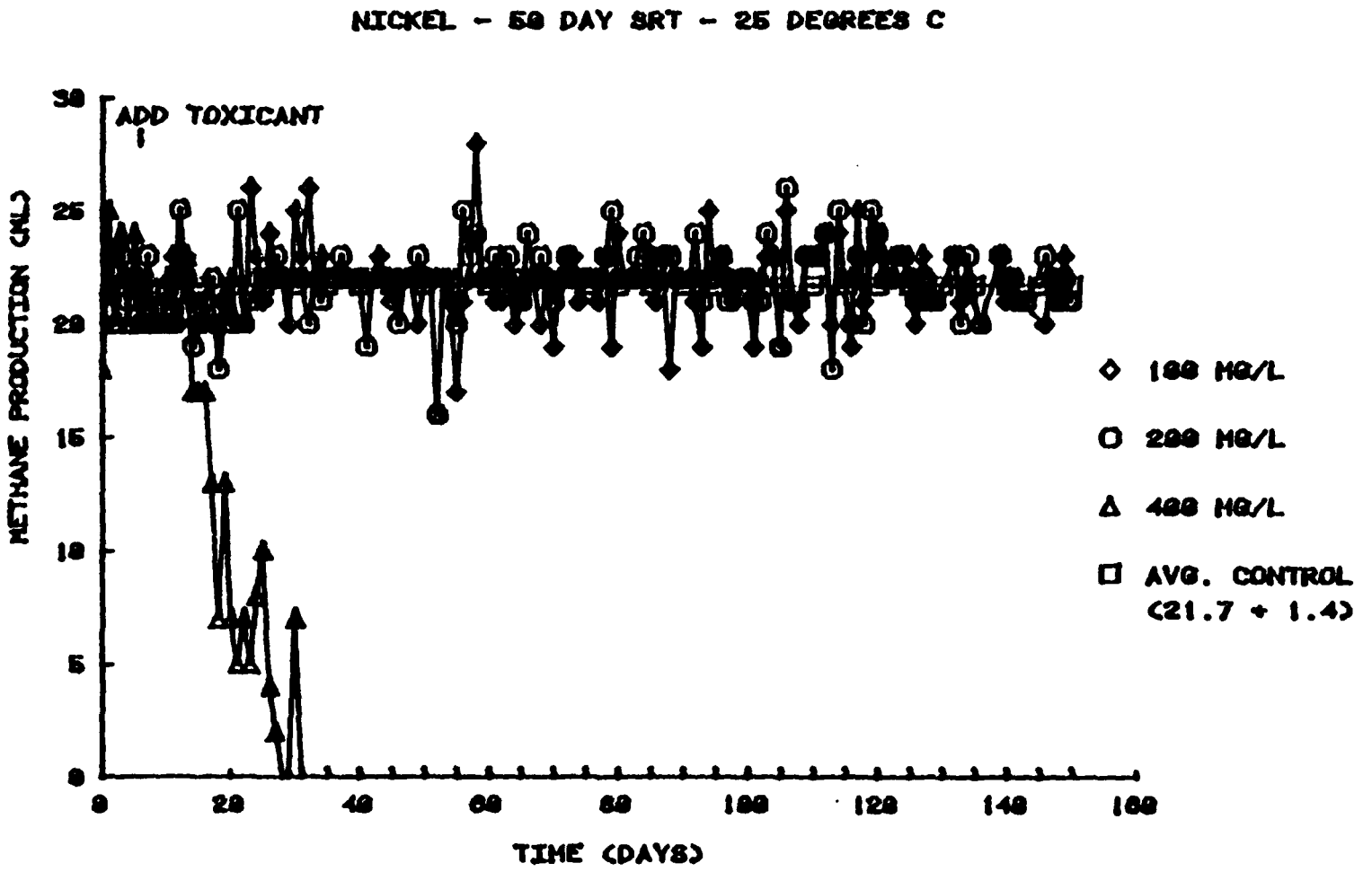

FIGURE 143. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

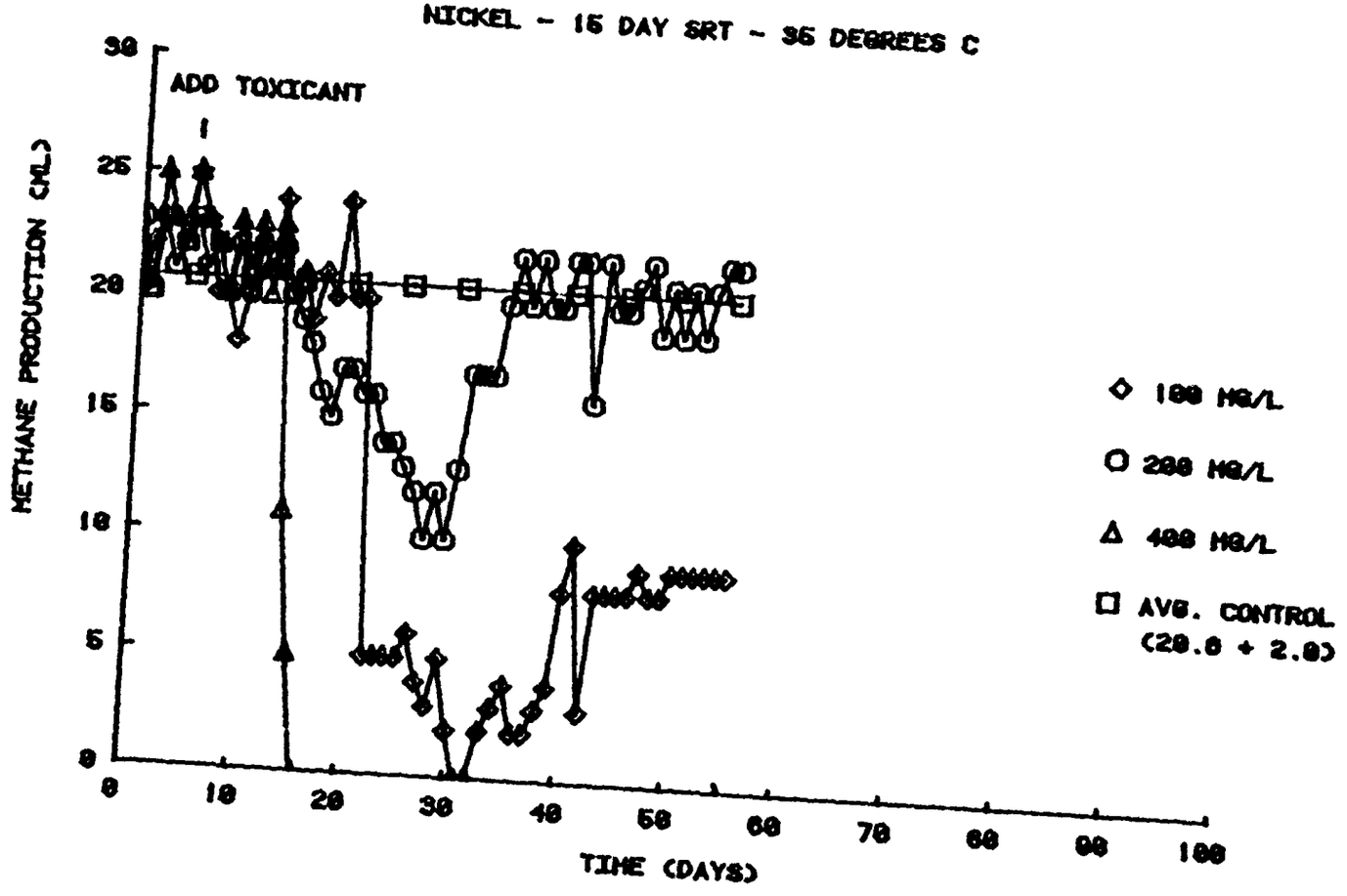

FIGURE 144. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

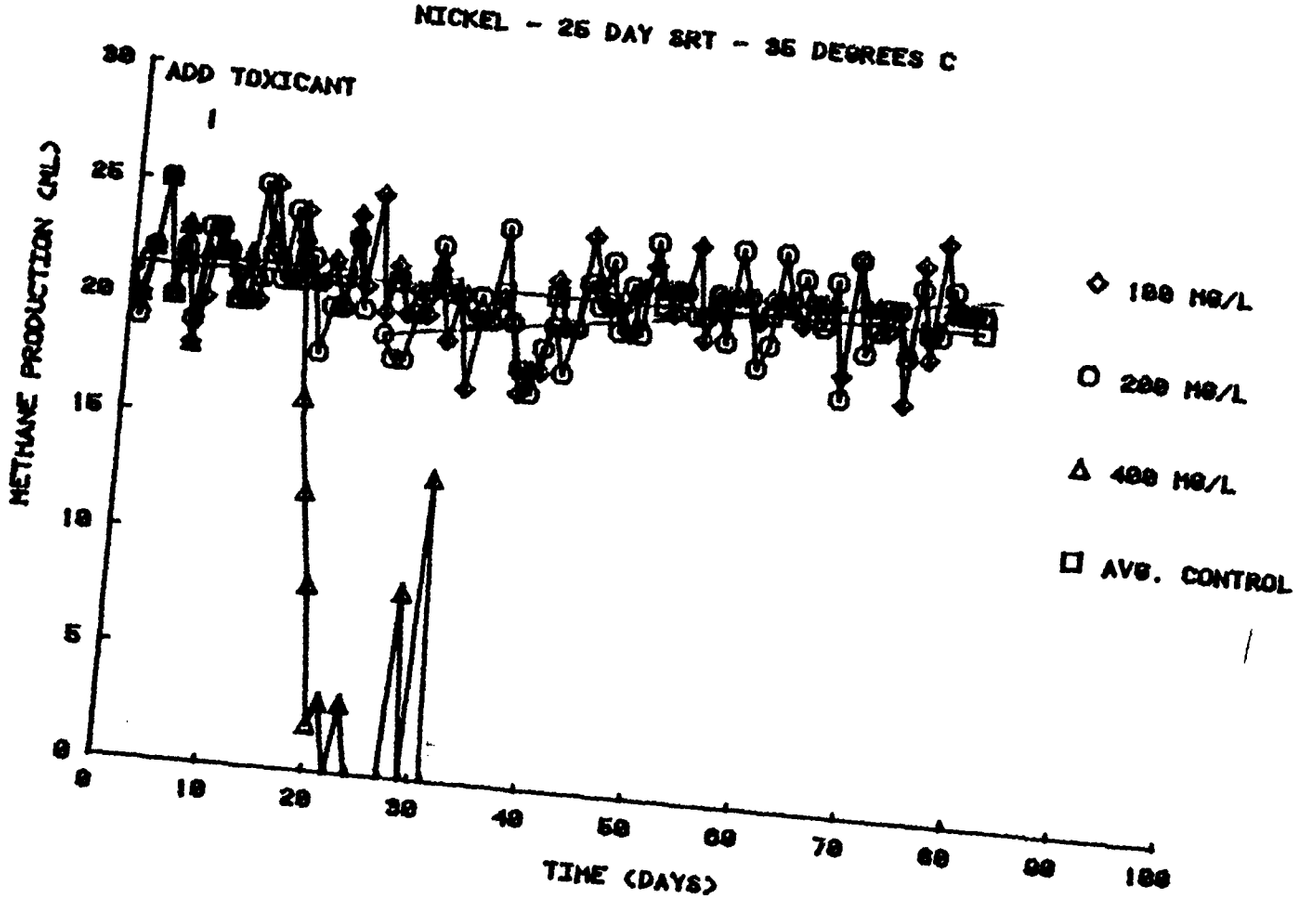

FIGURE 145. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

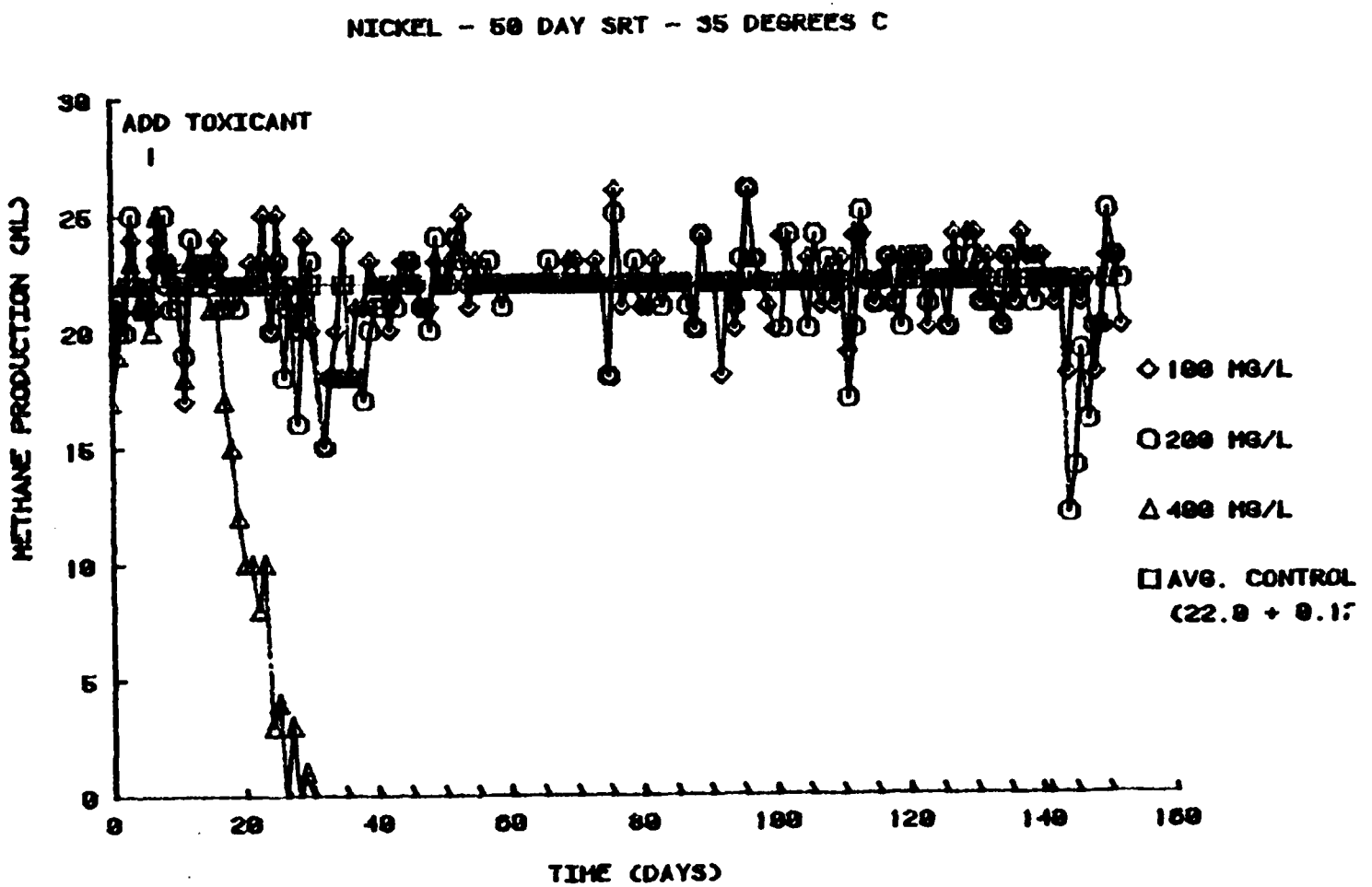

FIGURE 146. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

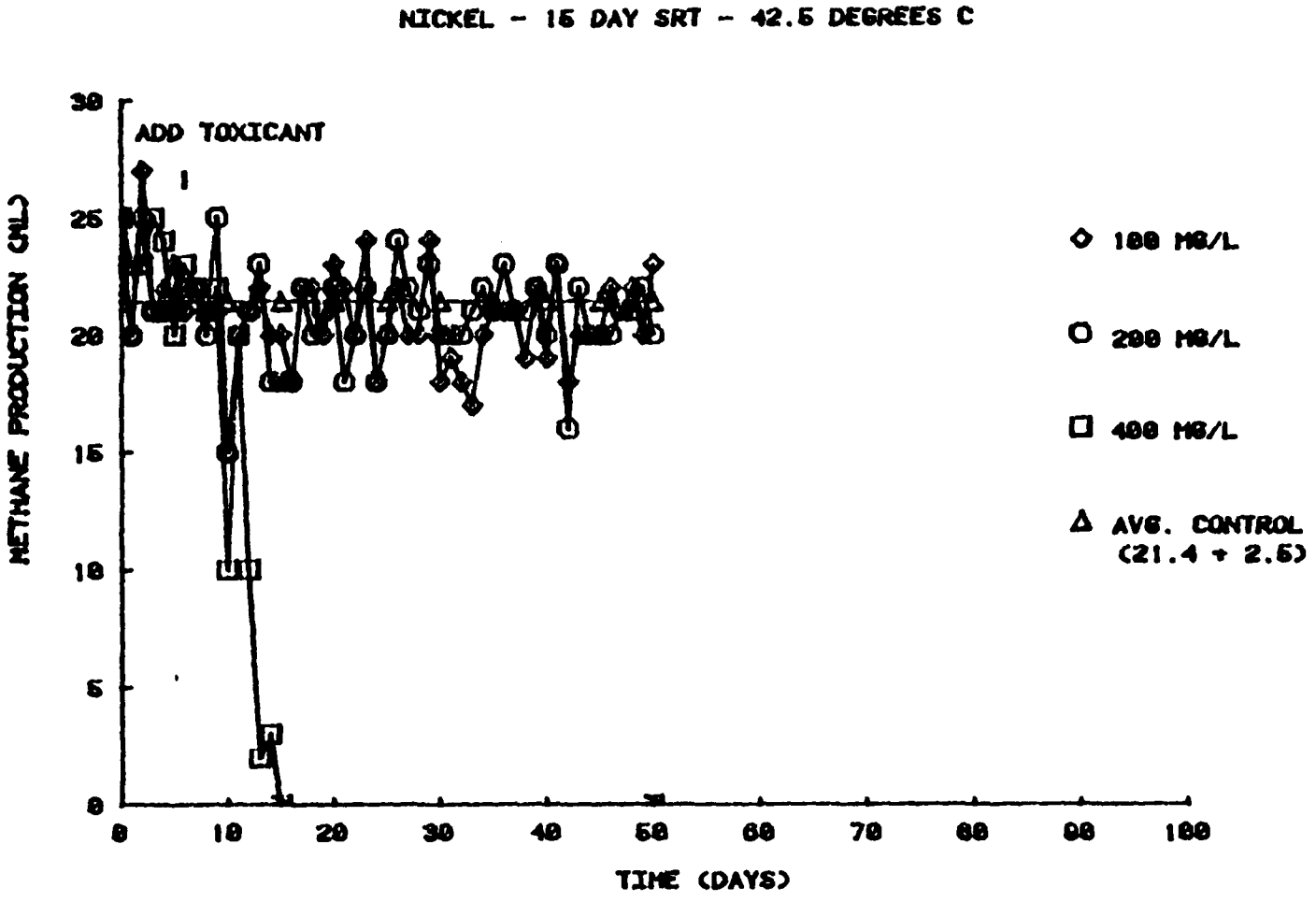

FIGURE 147. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

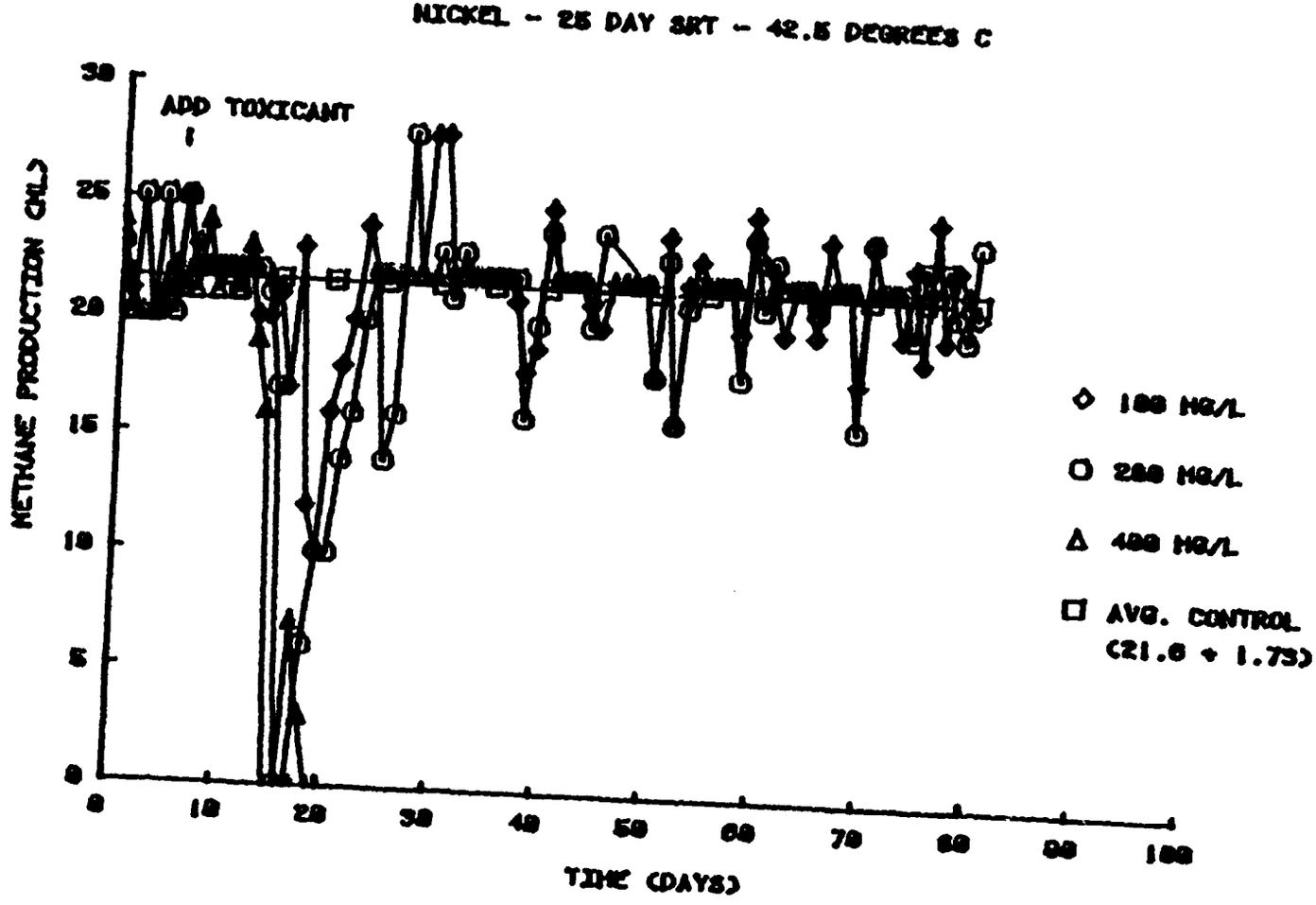

FIGURE 148. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

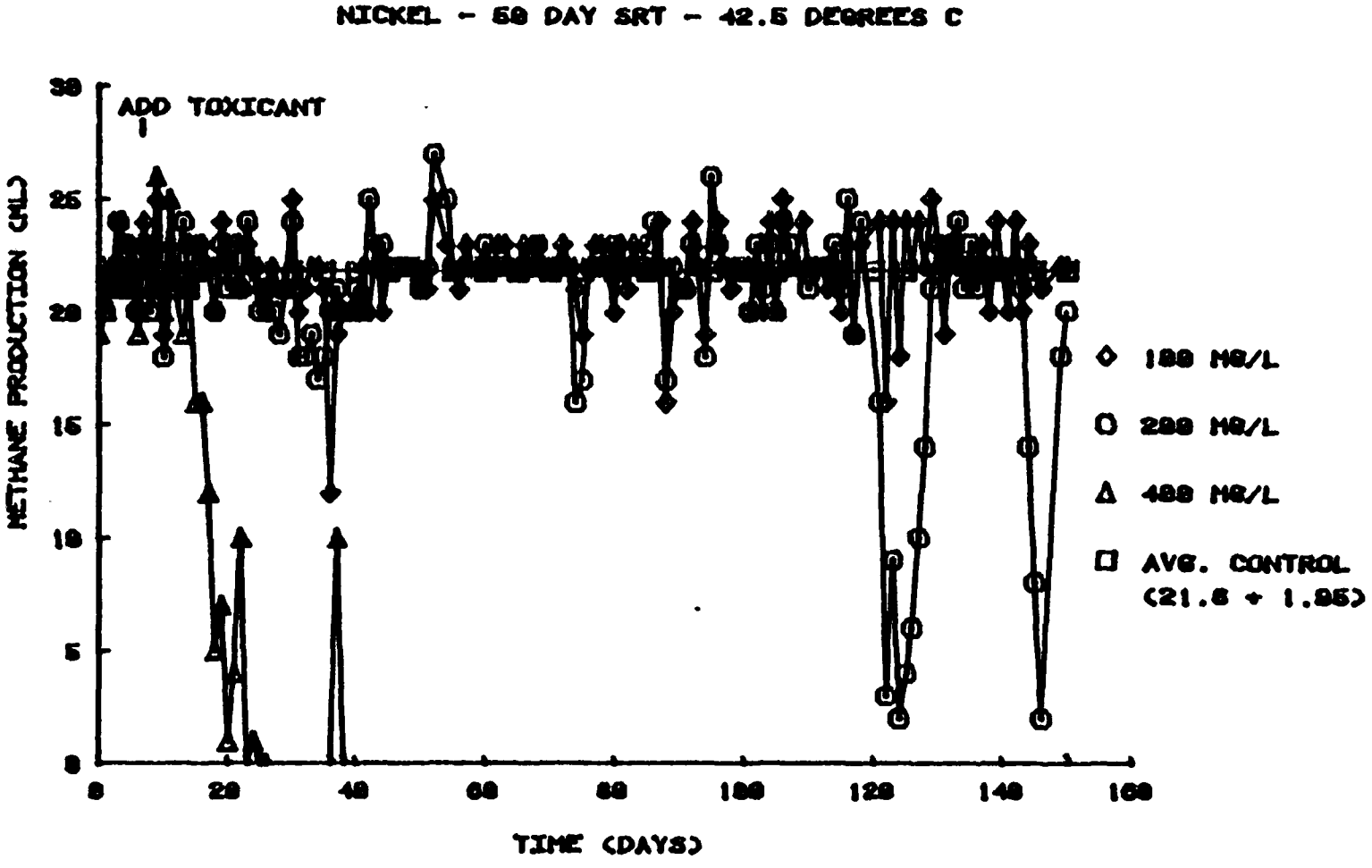

FIGURE 149. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF NICKEL

35°C and at 25-day and 50-day SRT for nickel concentrations of 100 mg/l and 200 mg/l.

Chloroform ($CHCl_3$)

The final desired concentrations resulting from the continuous addition of chloroform to the serum bottles were 5, 10, and 20 mg/l. It may be noted, however, that due to the effects of partitioning of chloroform between the gas and liquid phases (investigated by Yang (1981)), the final concentrations are calculated to be approximately one-half of those desired. No analytical measurements were performed to confirm this. Results with chloroform are shown in Figures 150 to 158.

At 25°C, the 15-day SRT system yielded one of the most irregular gas productions of the study (Figure 150). All concentrations experienced a drop in gas production, with the smallest concentration of 5 mg/l descending the furthest, to zero, before recovering to near the control level. A similar response was experienced by the 50-day SRT (Figure 152). Again the low concentration was the most severely affected. Gas production dropped to zero before ascending to the control level. The two higher concentrations exhibited no change. Only the 25-day SRT was unaffected, except for a temporary drop in gas production that may be attributed to experimental error (Figure 151).

The 15-day SRT was the only system affected at 35°C (Figure 153). Gas production dropped to zero for the 10 mg/l concentration and did not resume before the end of the study. The two remaining concentrations, 5 mg/l and 20 mg/l, fell below the control level, to 10 to 12 ml, respectively and remained near that level until the end of the study. Both the 25-day and 50-day SRTs showed little deviation from the control over the length of the study (Figures 154 and 155).

The serum bottles at 42.5°C showed the least acclimation potential (Figures 156 to 158). Only at the low concentrations did the systems remain unaffected. The higher concentrations caused fluctuations above and below the control level.

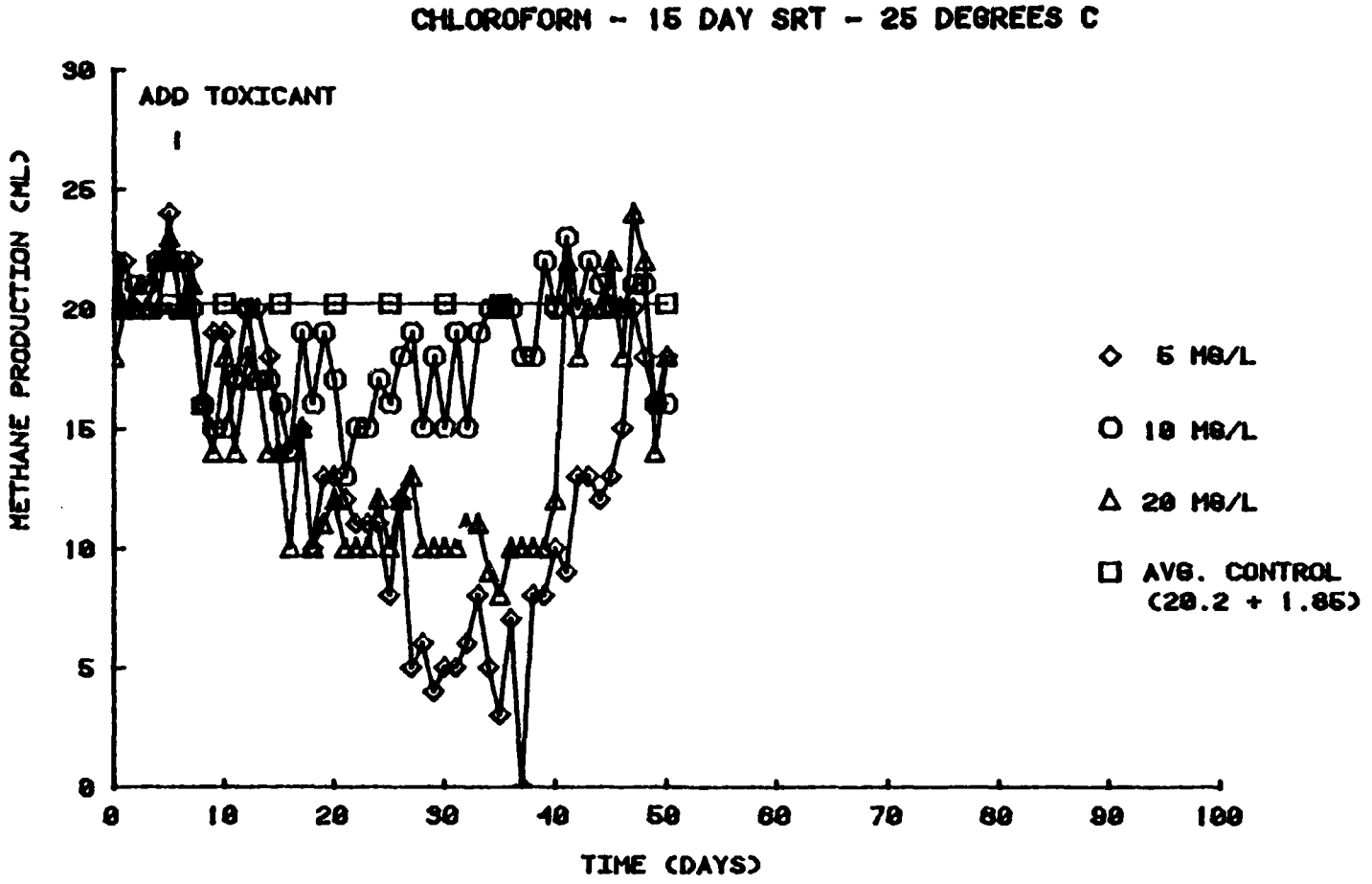

FIGURE 150. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

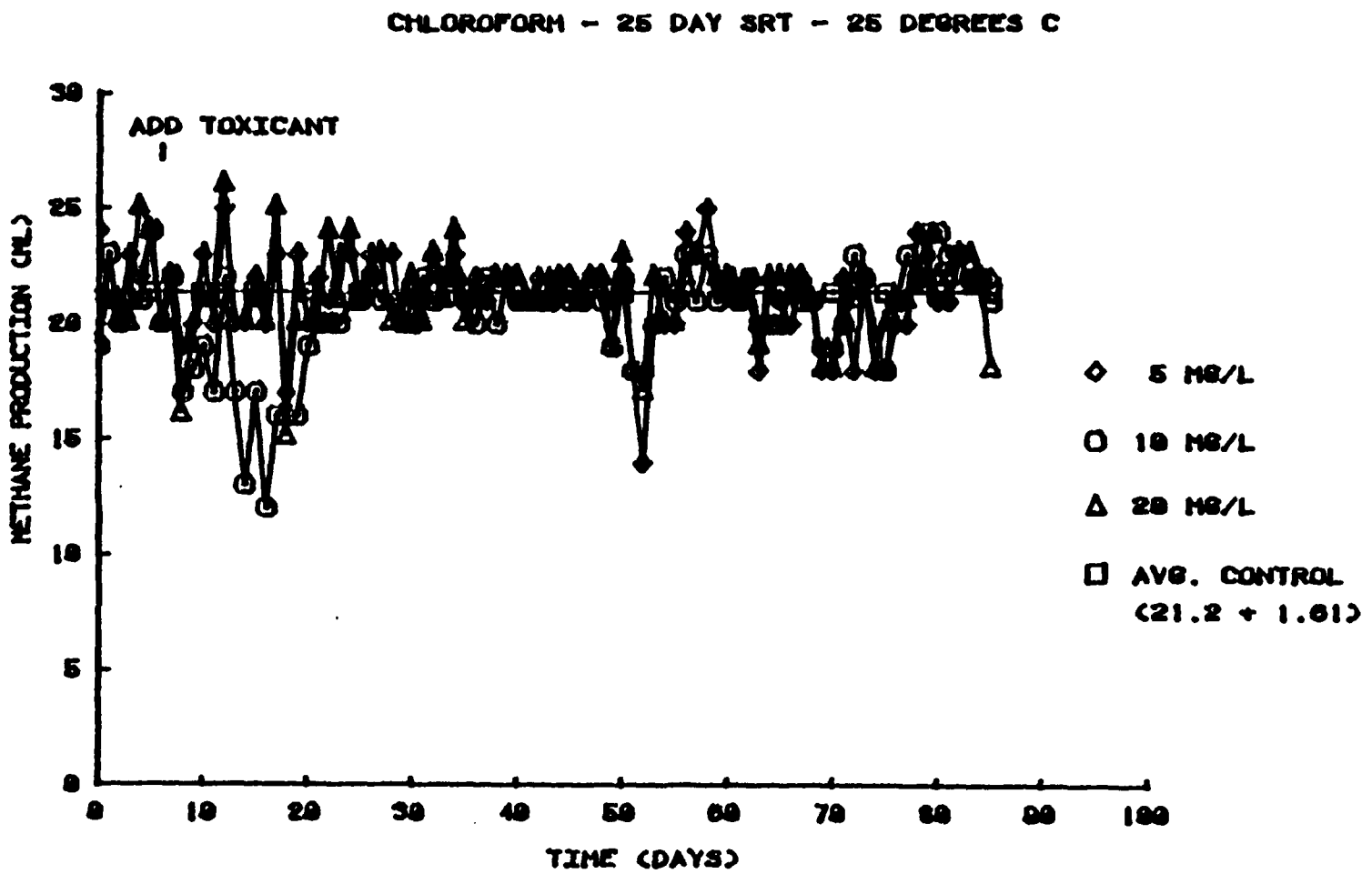

FIGURE 151. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

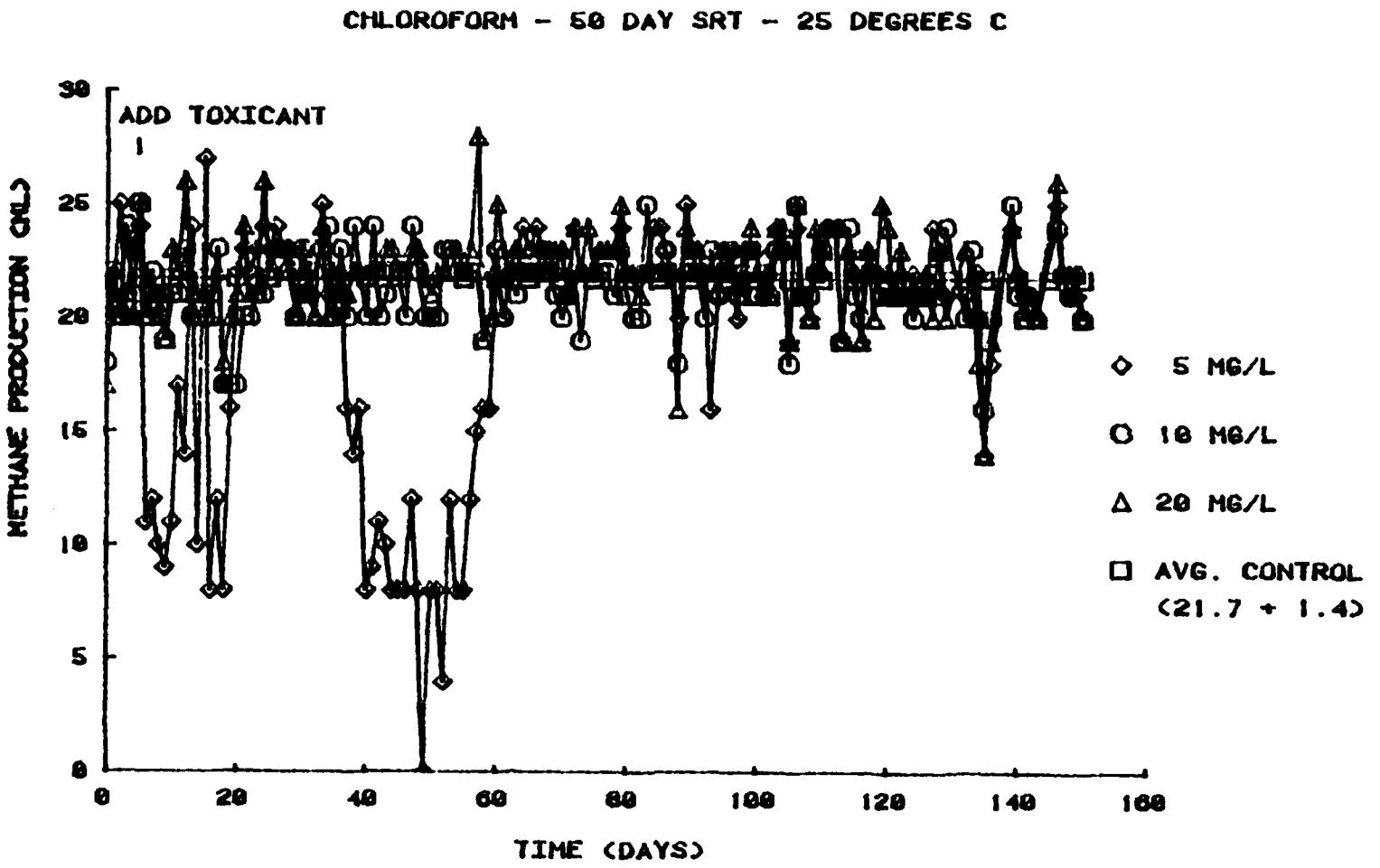

FIGURE 152. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

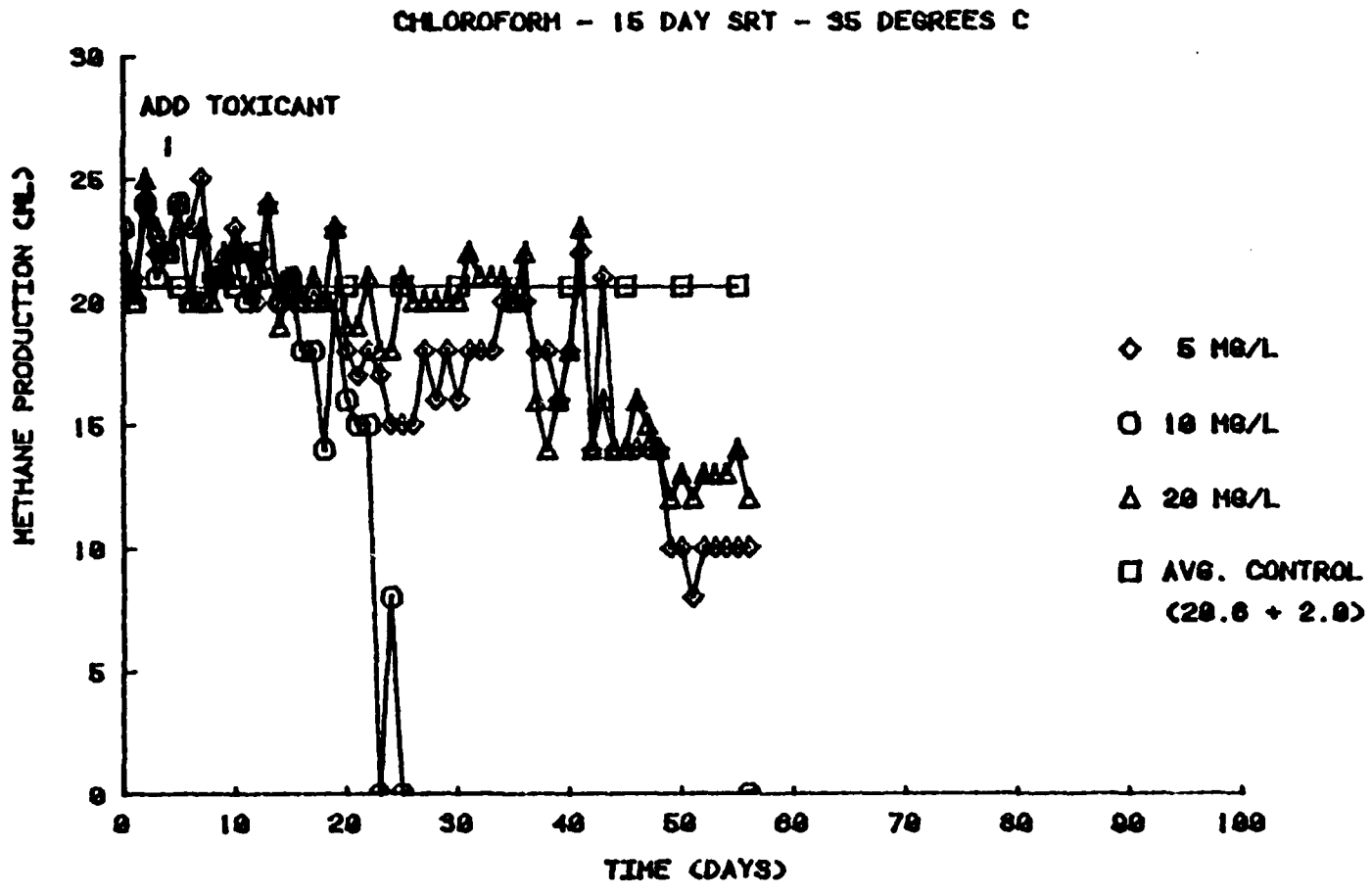

FIGURE 153. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

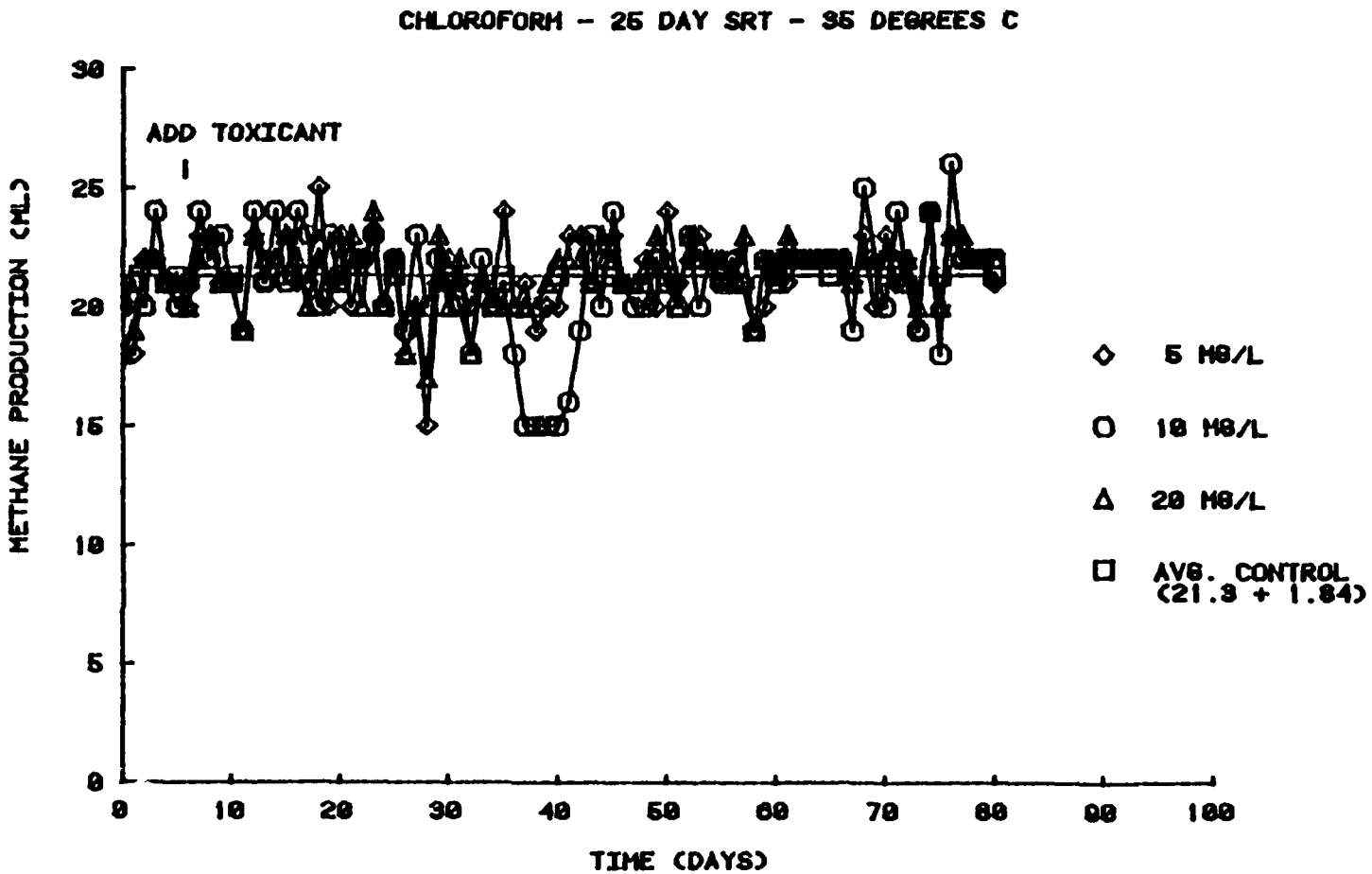

FIGURE 154. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

AD-A104 868 DREXEL UNIV PHILADELPHIA PA ENVIRONMENTAL STUDIES INST F/G 6/13
MICROBIAL METHANE FERMENTATION KINETICS FOR TOXICANT EXPOSURE.(U)
AUG 81 G F PARKIN, W M KOCHER, S W MILLER F49620-79-C-0190
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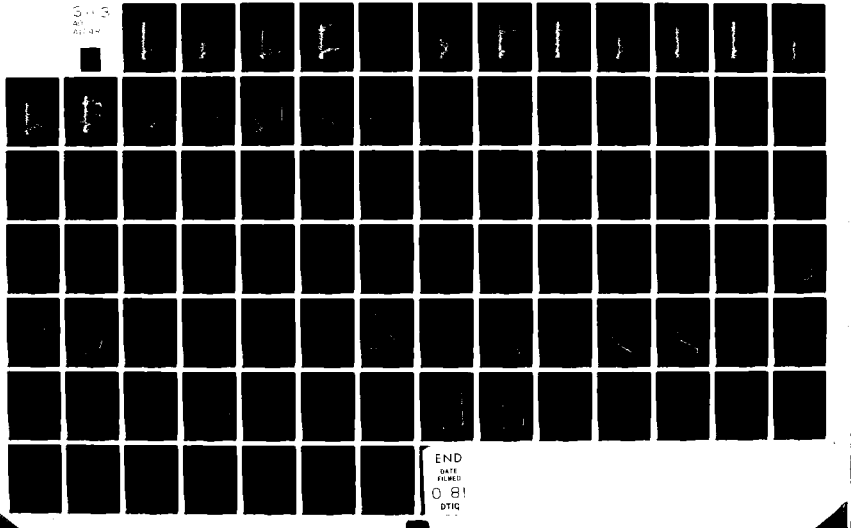

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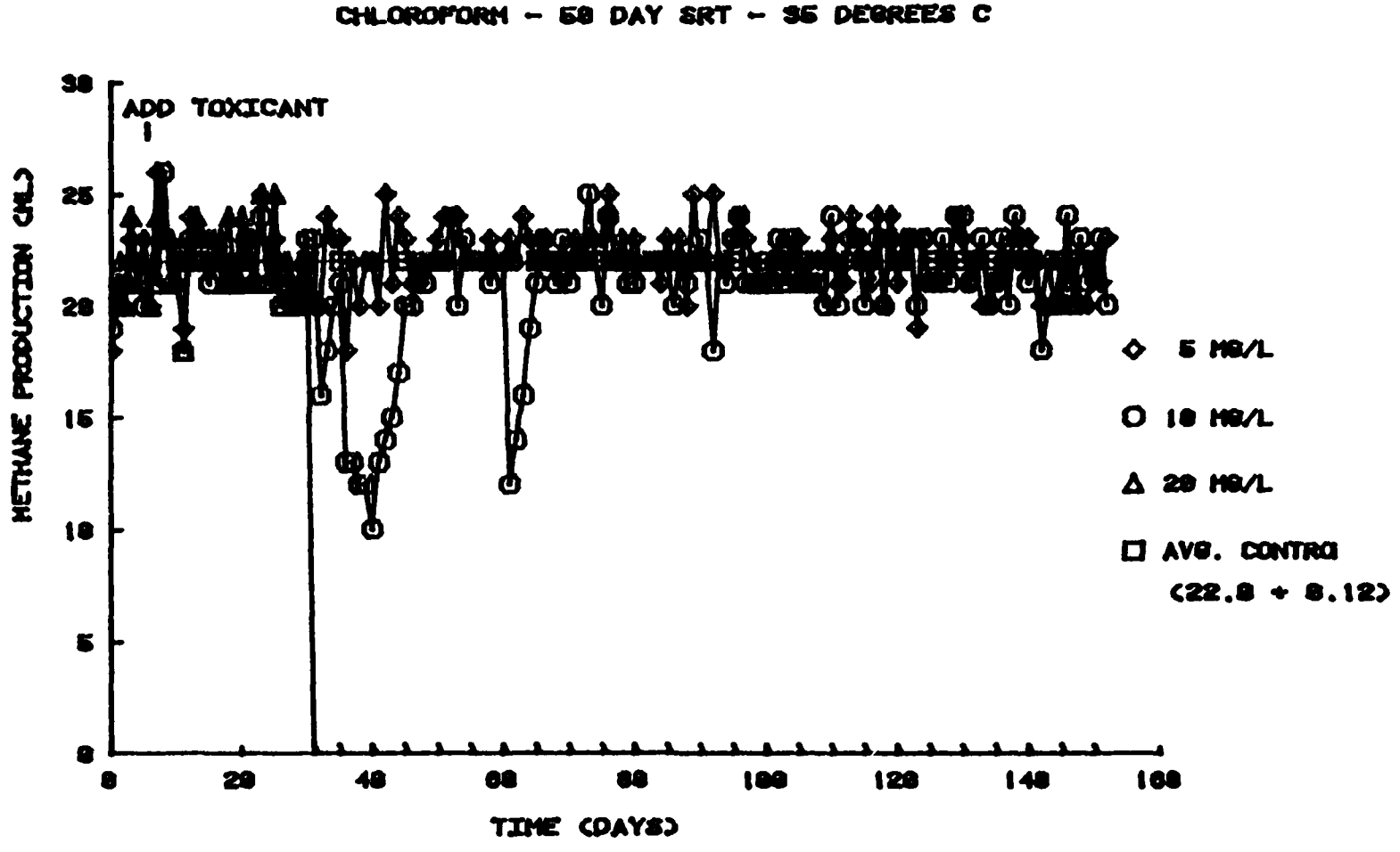


FIGURE 155 RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

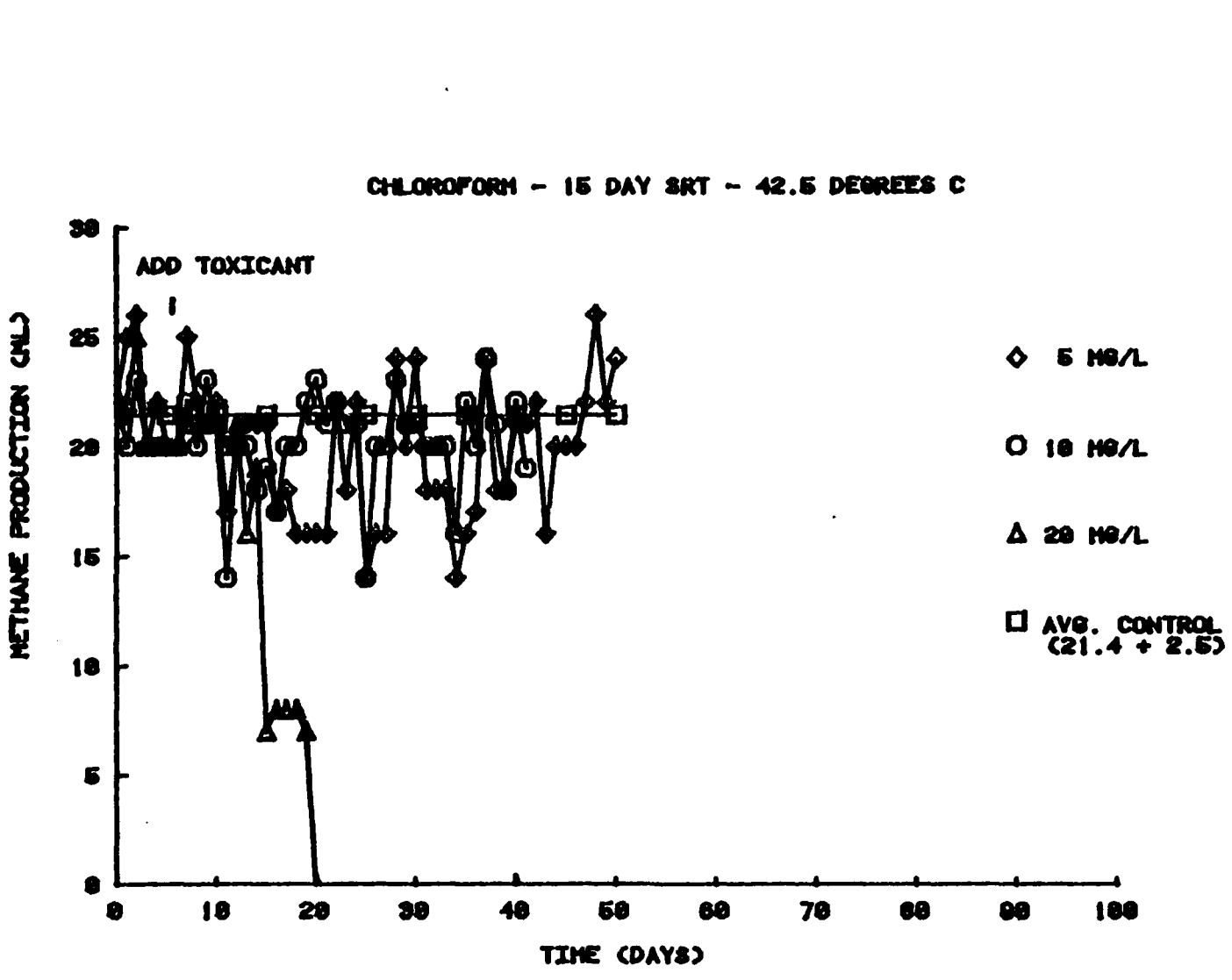


FIGURE 156. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

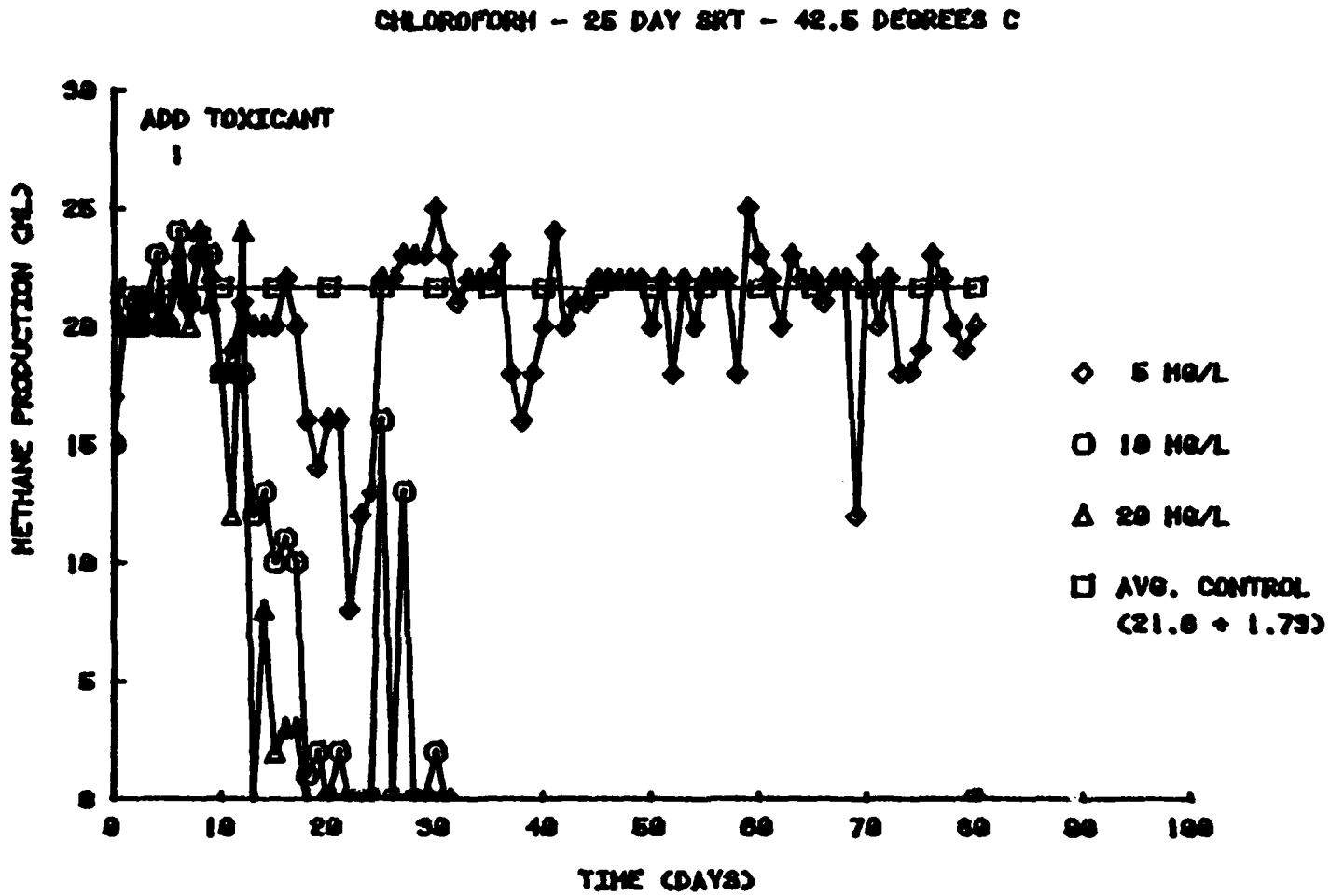


FIGURE 157. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

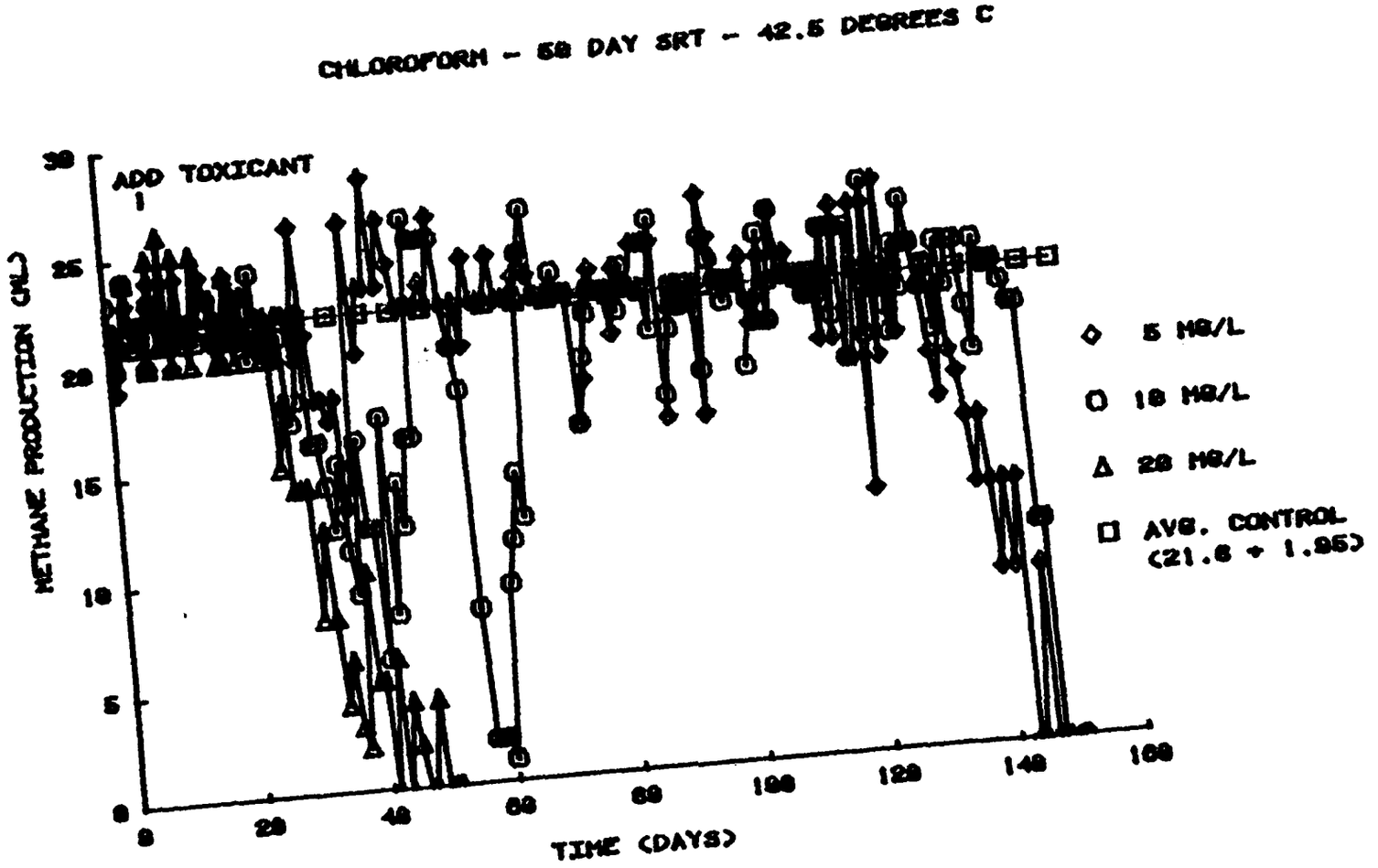


FIGURE 158. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF CHLOROFORM

Hydrazine (N_2H_4)

The desired final concentration resulting from the continuous addition of hydrazine to the serum bottles were 5, 25, and 50 mg/l as N_2H_4. Results are shown in Figures 159 to 167.

The 15-day SRT was more capable of tolerating hydrazine at 42.5°C than 35°C, which was better than 25°C (Figures 159, 162 and 165). The 25-day SRT, 35°C system was more capable of tolerating hydrazine than a 50-day SRT system at either 35°C or 42.5°C. (Figures 163, 164, and 167).

Serum bottles maintained at 25°C demonstrated the longest periods of low gas production (Figures 159 to 161) and the serum bottles maintained at 35°C were in general the most stable (Figures 162 to 164).

REVERSIBILITY

Chloroform, nickel, and sulfide were studied for reversibility at the concentrations listed below:

Chloroform:	2, 4, 12 mg/l
Nickel:	400, 800, 2400 mg/l
Sulfide:	250, 500, 1500 mg/l

The most significant results are presented in Figures 168 to 171.

All toxicants exhibited reversible toxicity, but all showed some residual toxicity in the form of a slow, as opposed to rapid, return to full gas production. For comparative purposes, Figure 172 shows the very rapid recovery to full gas production after exposure to 24,000 mg/l NH_4^+ (Parkin et al, 1980). The residual effect noted with chloroform (Figure 168) may be due to chloroform associated with biomass, since only chloroform present in the centrifugate was discarded. Removal of chloroform from the liquid phase after one hour exposure resulted in a more rapid recovery to full gas production, as evidenced by comparison with control curves.

Data on nickel toxicity (Figures 169 and 170) indicate that recovery, and thus observed reversibility, was dependent on concentration and exposure time. Concentrations less than 800 mg/l and exposure times less than one day resulted in rapid recovery once contaminated liquid was removed from the system. However, a concentration of 2400 mg/l (one hour exposure) and an exposure of four days (800 mg/l) resulted in zero gas production with no recovery during the testing period.

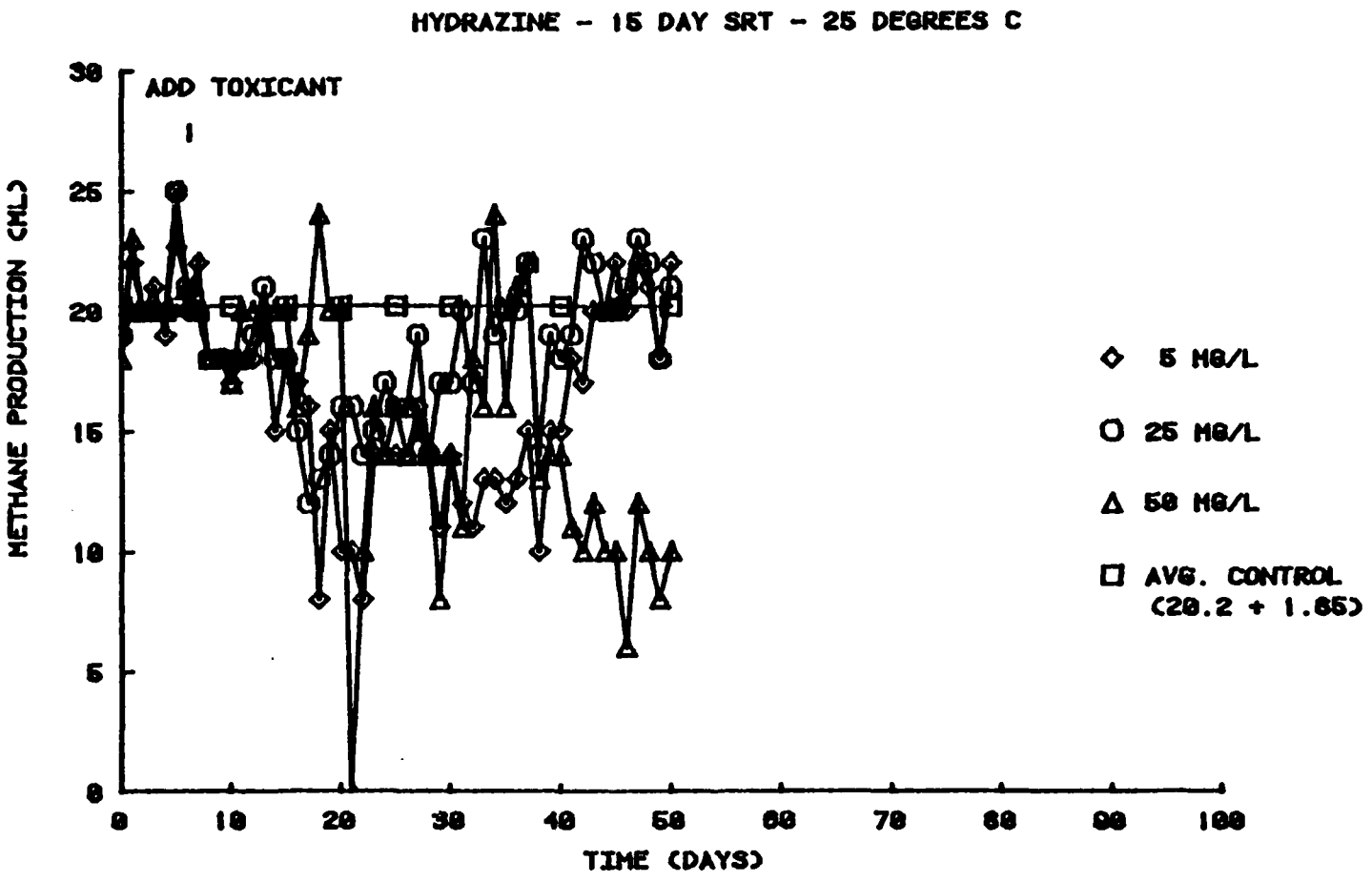


FIGURE 159. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

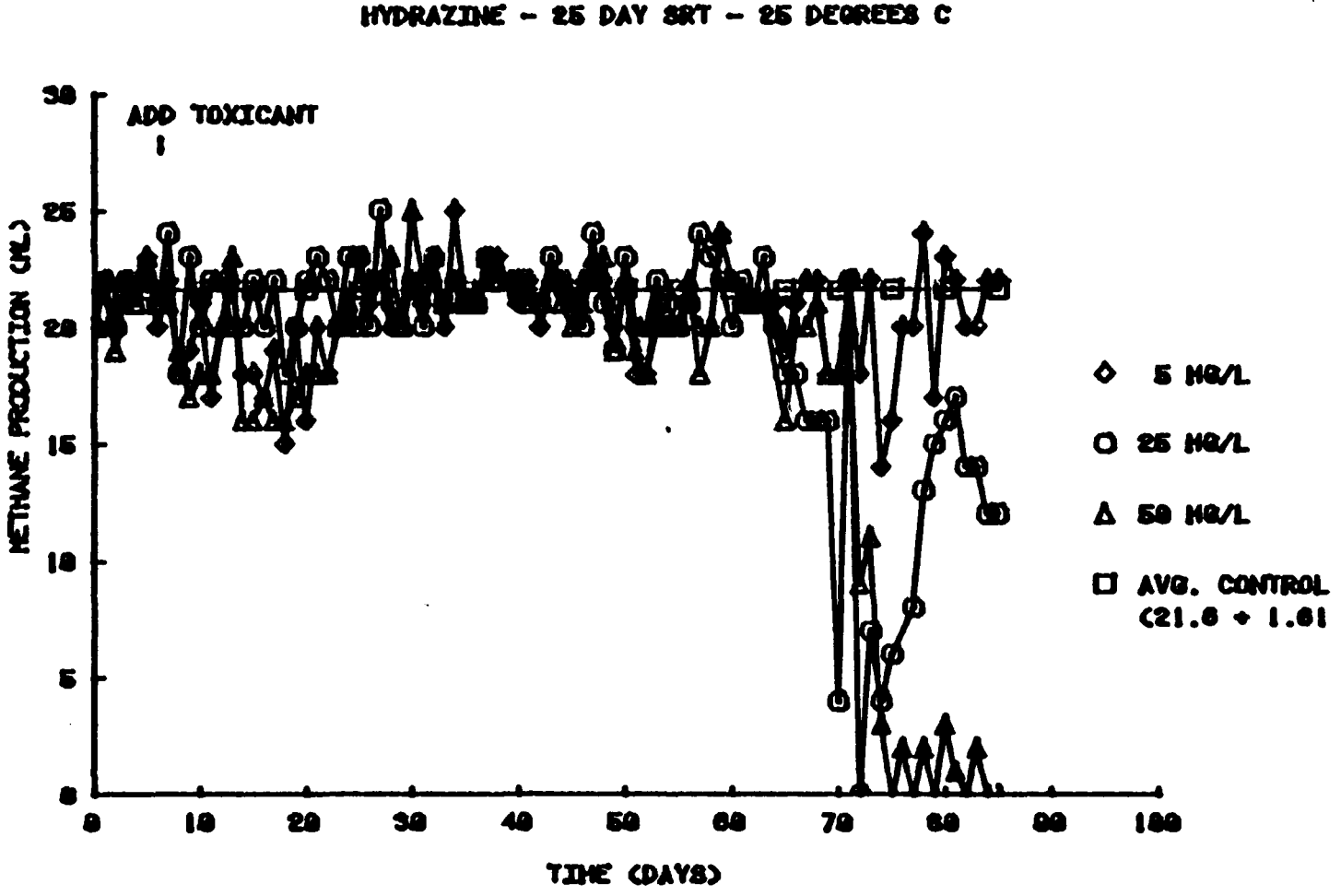


FIGURE 160. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

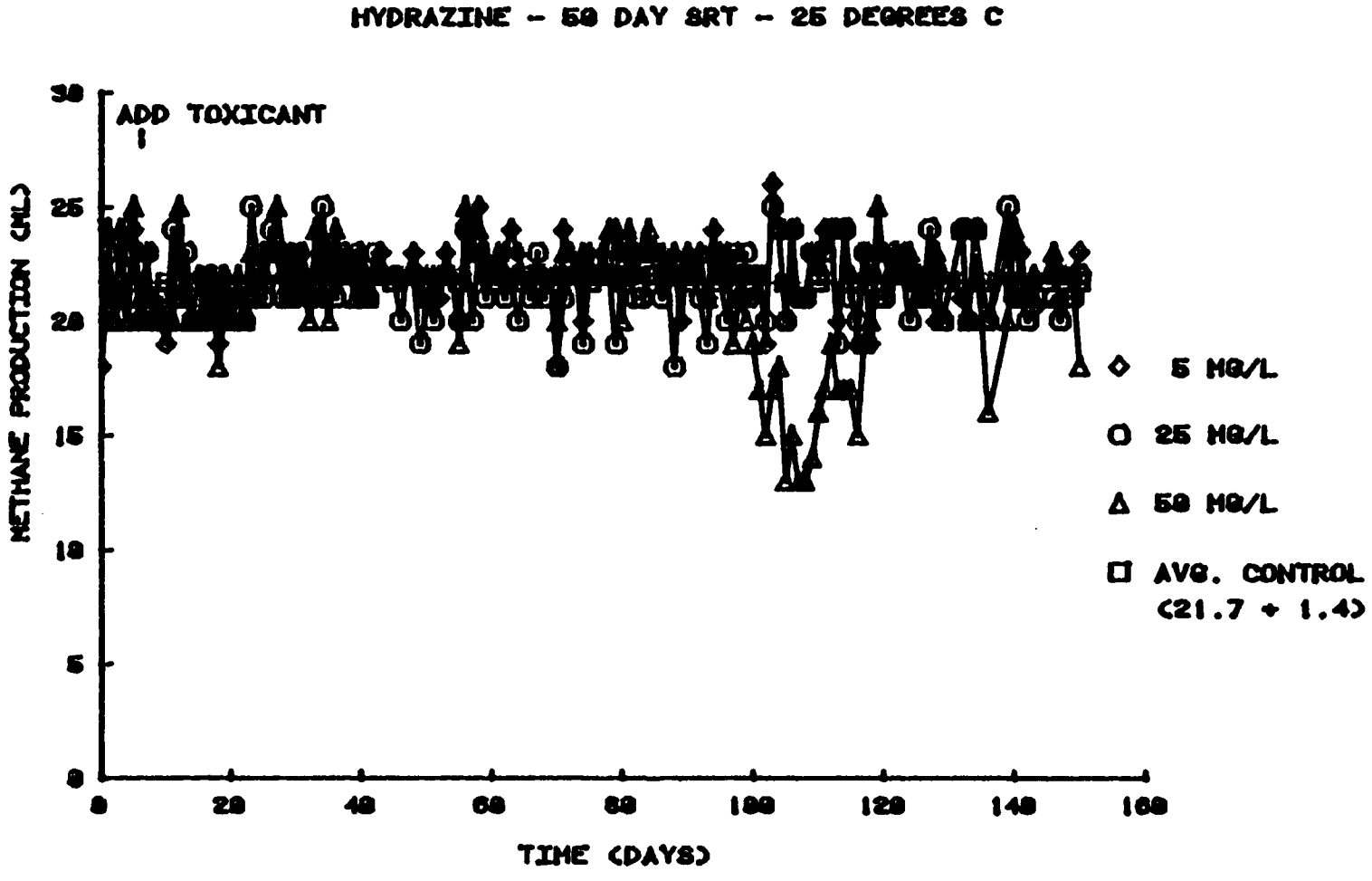


FIGURE 161. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

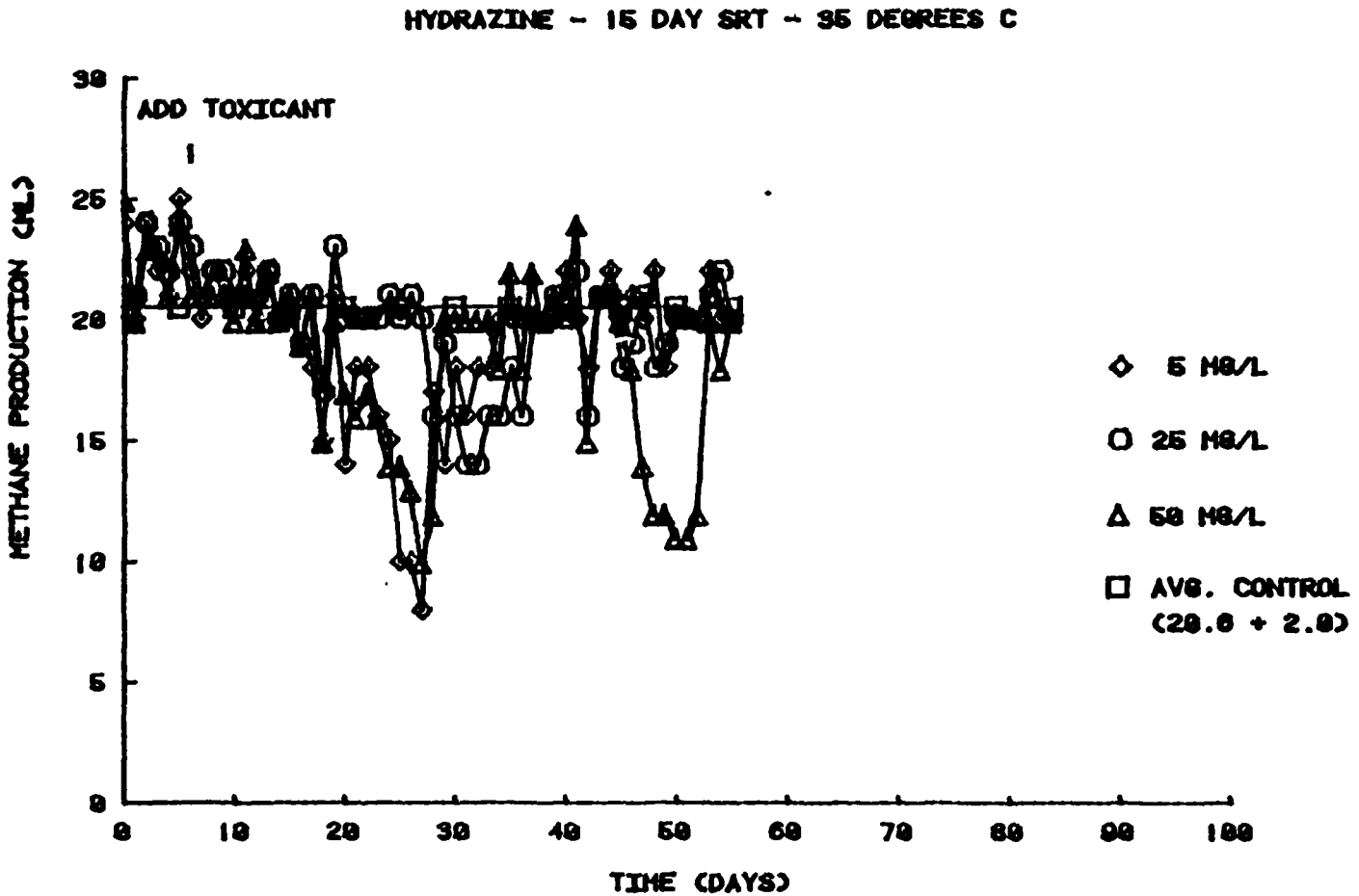


FIGURE 162. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

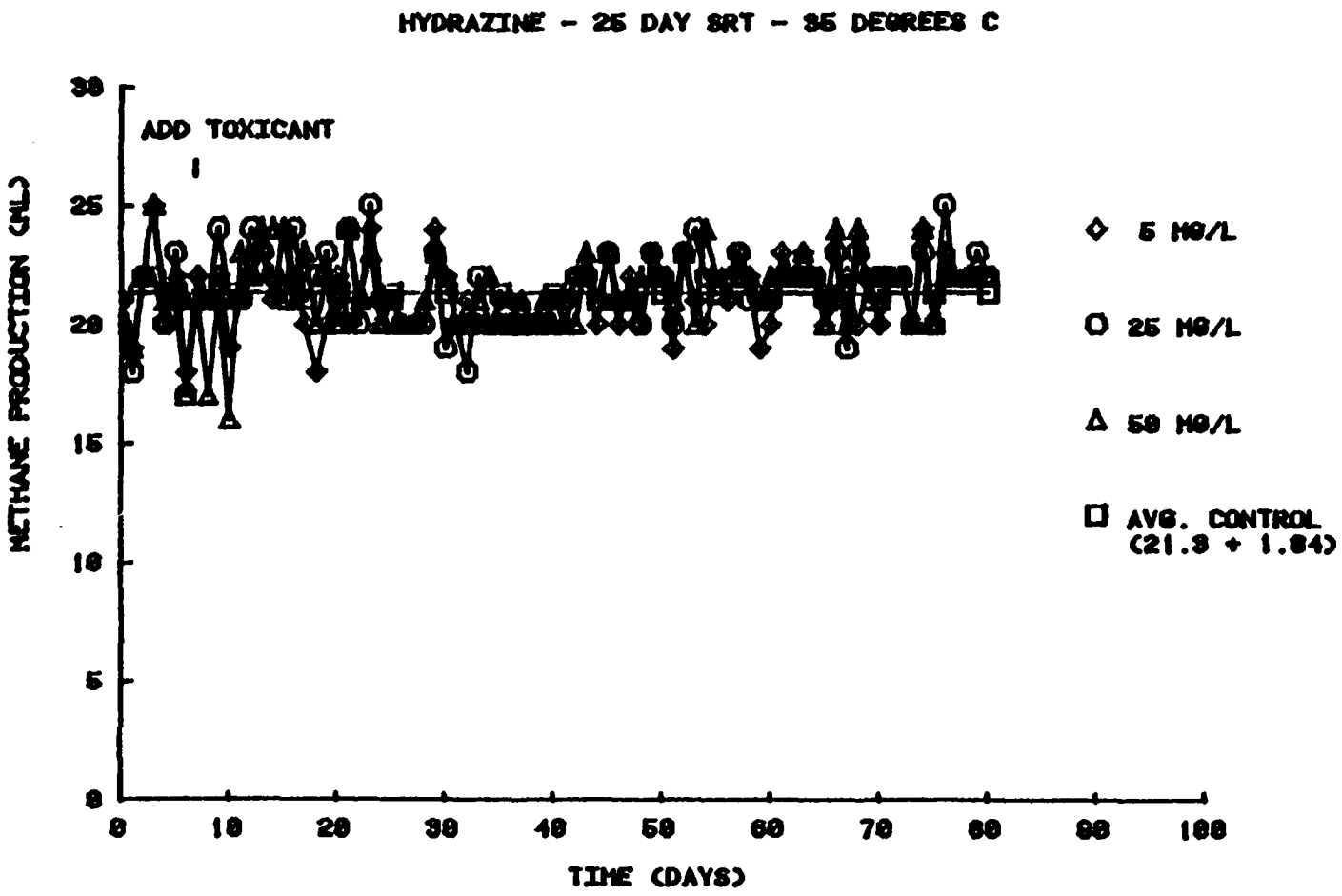


FIGURE 163. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

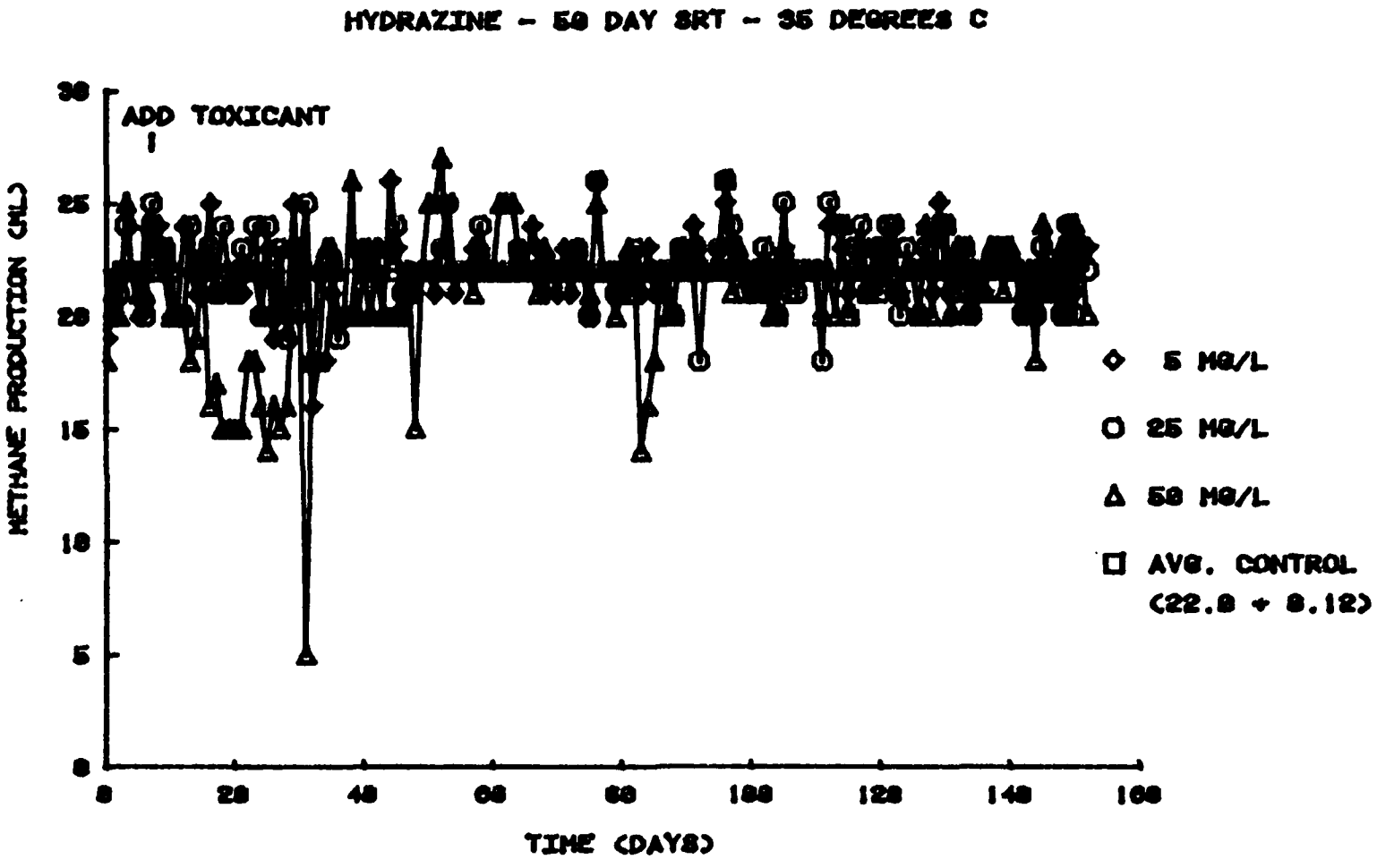


FIGURE 164. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

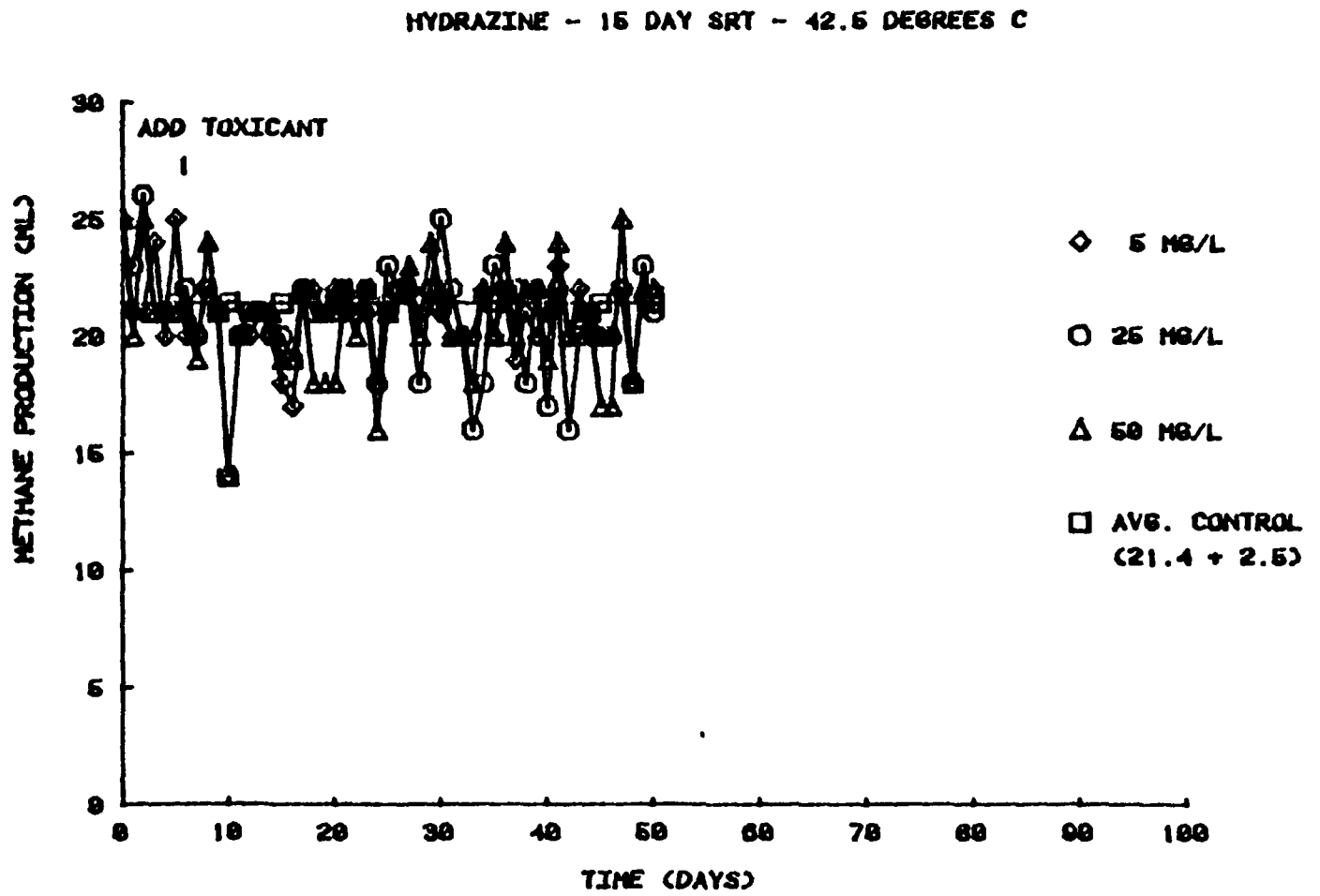


FIGURE 165. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

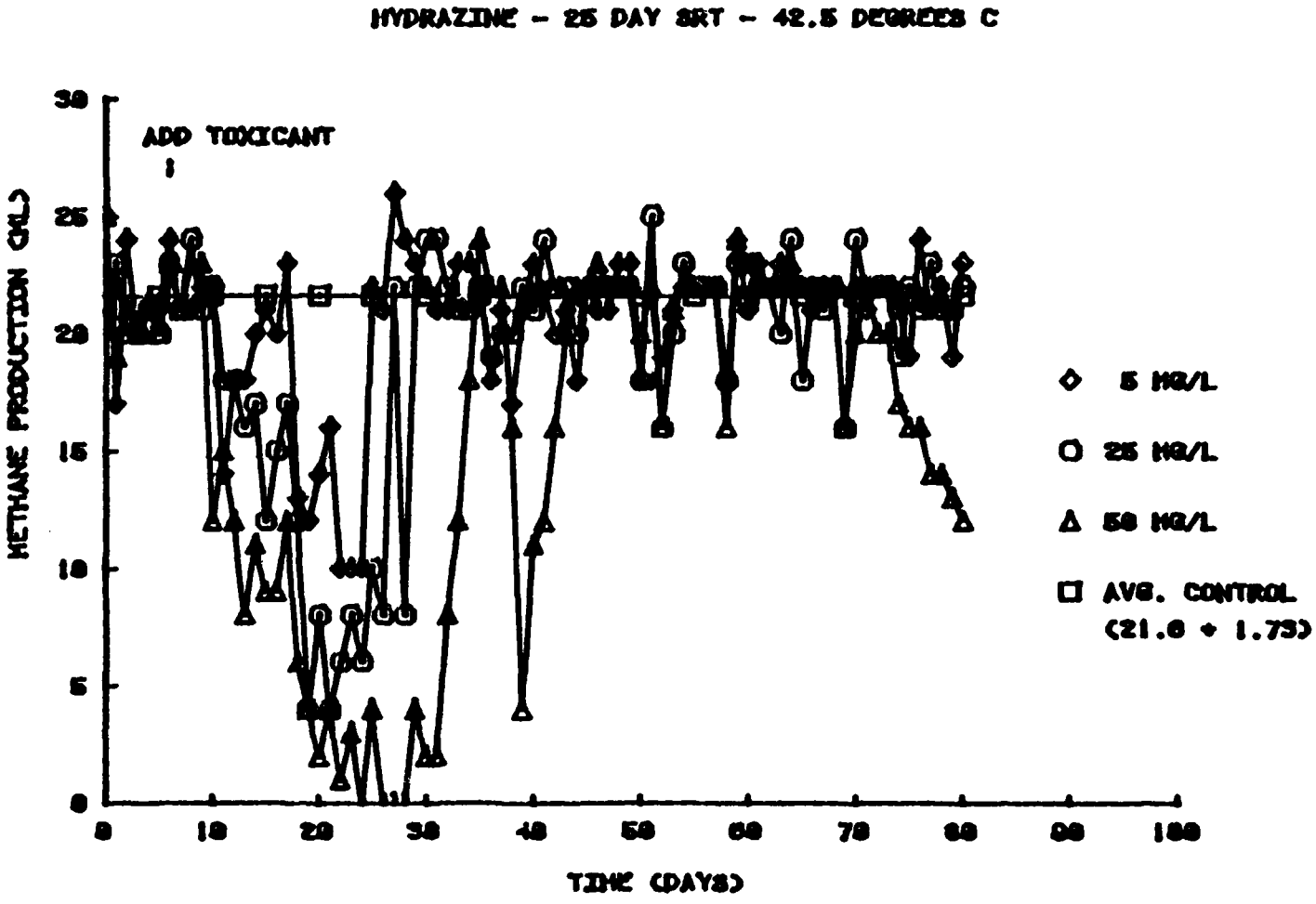


FIGURE 166. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

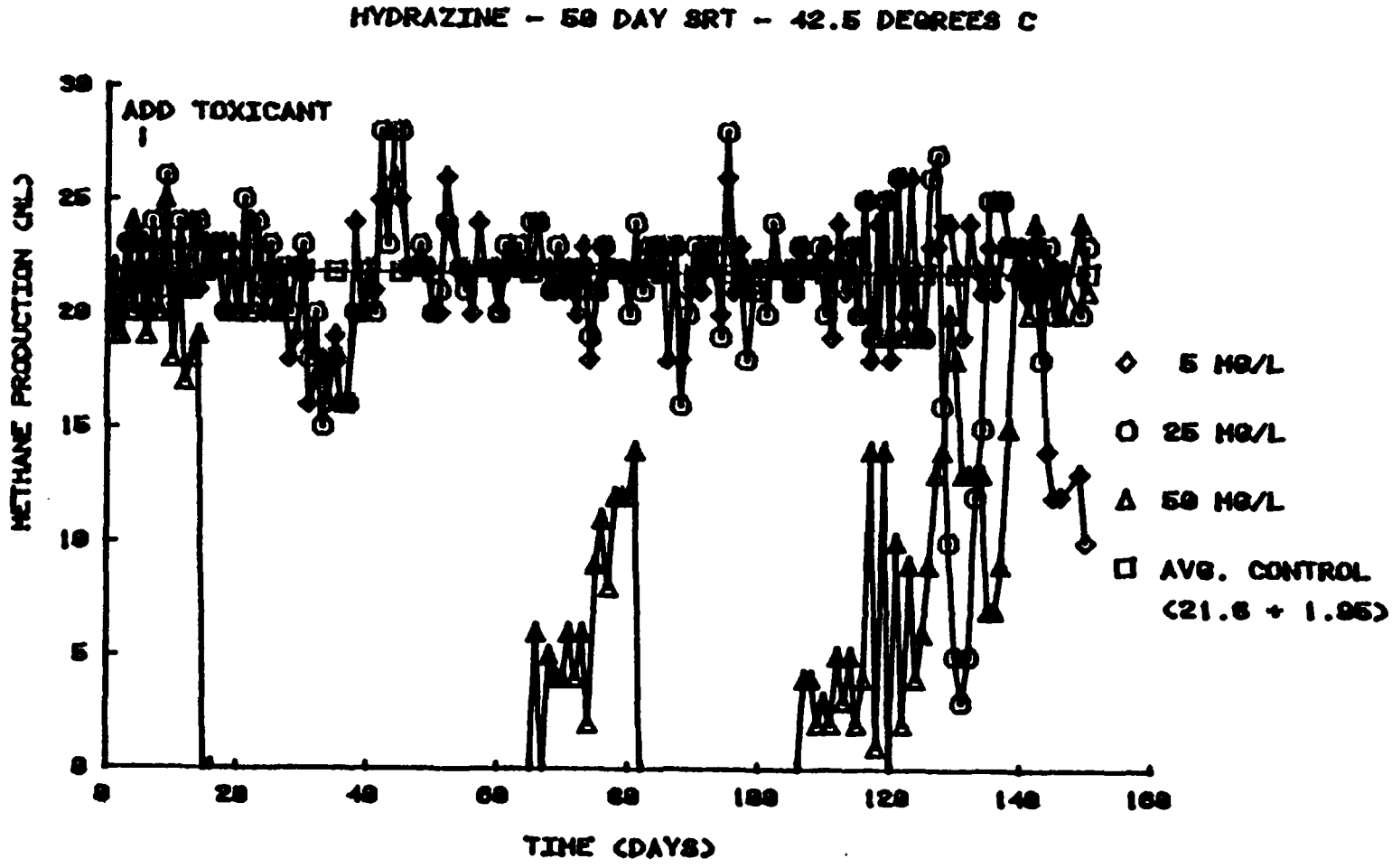


FIGURE 167. RESPONSE OF METHANOGENS TO CONTINUOUS ADDITION OF HYDRAZINE

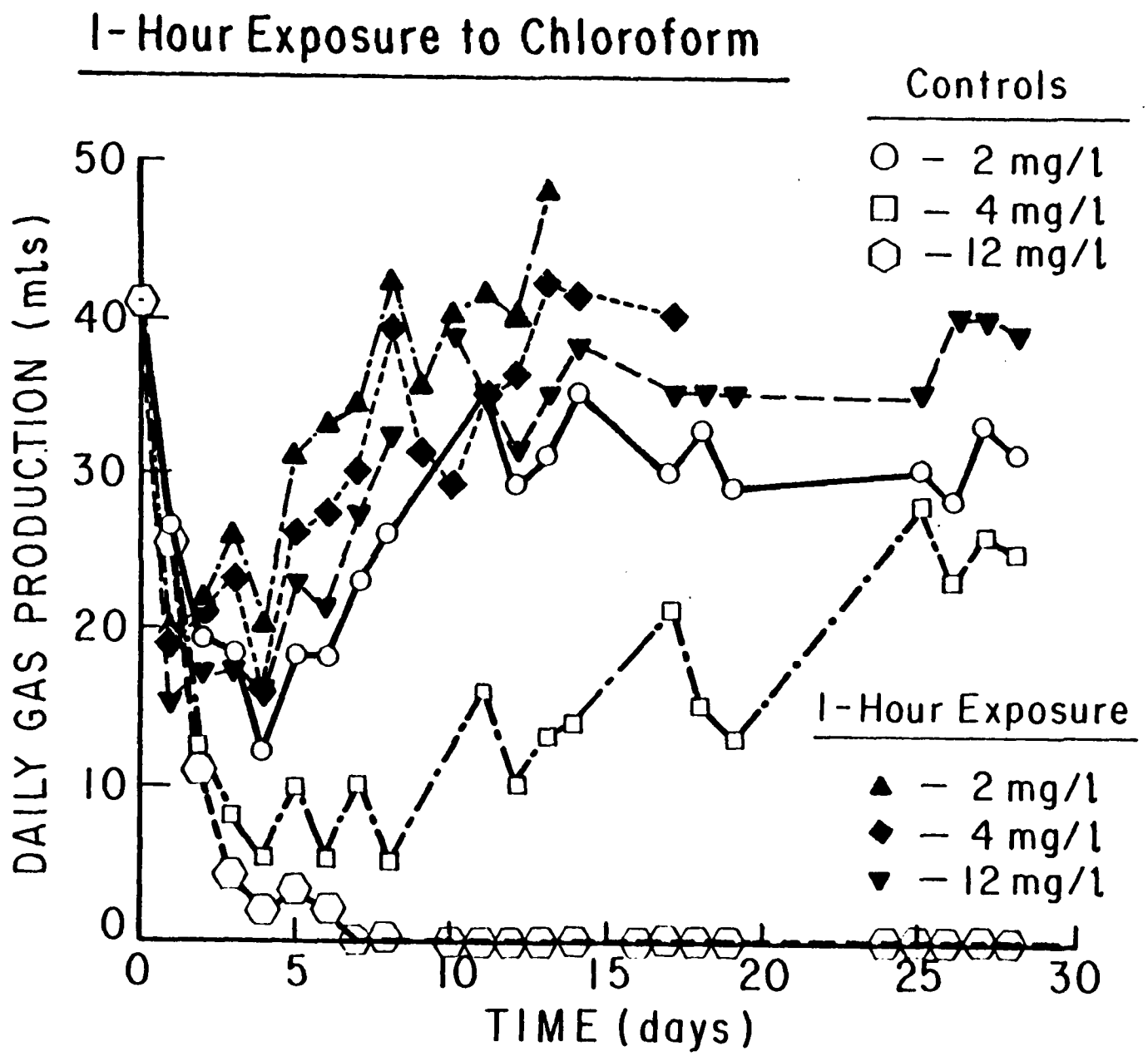


Fig. 168 Effect of Exposure Time on Toxicity Response: Chloroform

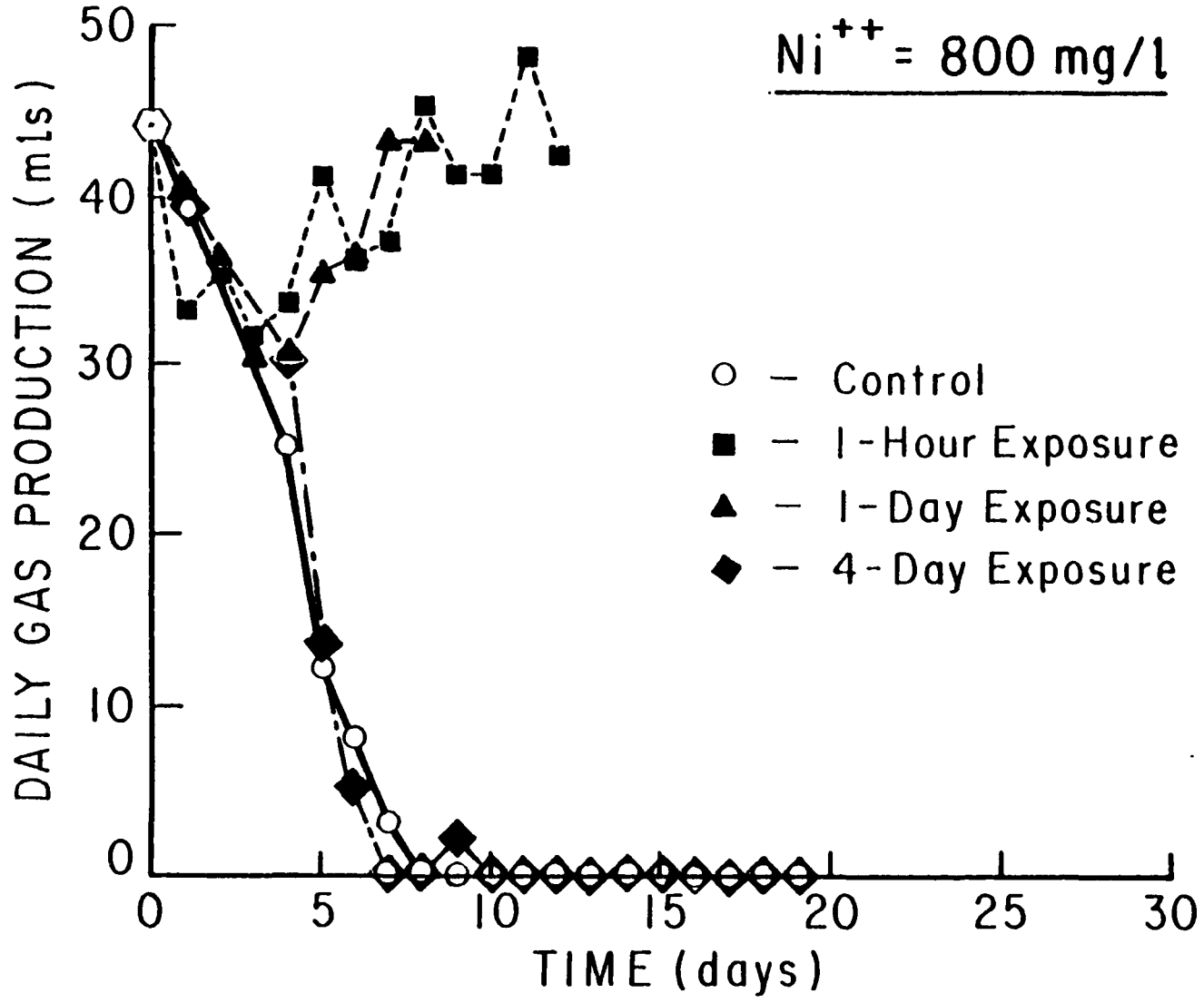


Fig. 169 Effect of Exposure Time on Toxicity Response: Nickel

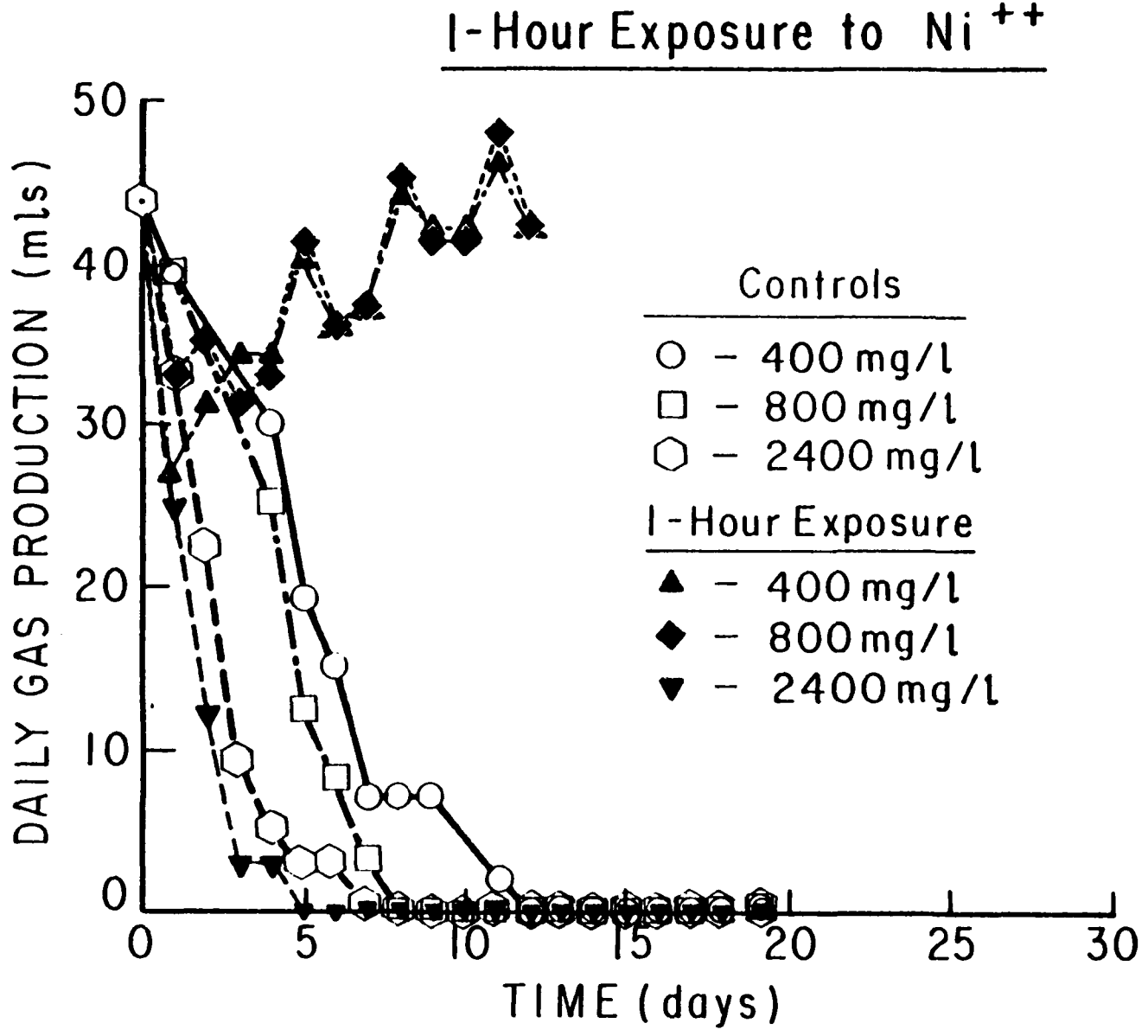


Fig. 170 Effect of Exposure Time on Toxicity Response: Nickel

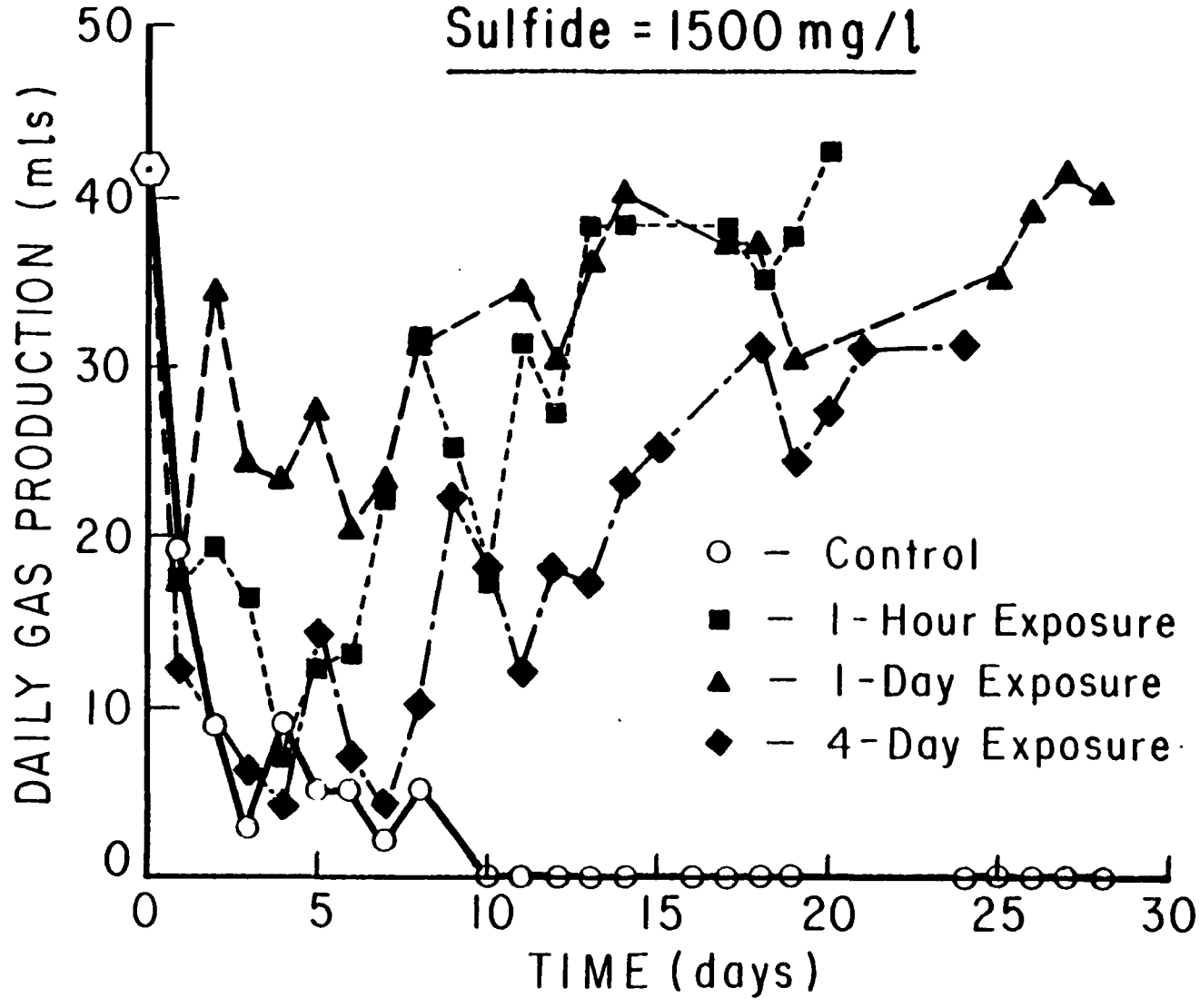


Fig.171 Effect of Exposure Time on Toxicity Response: Sulfide

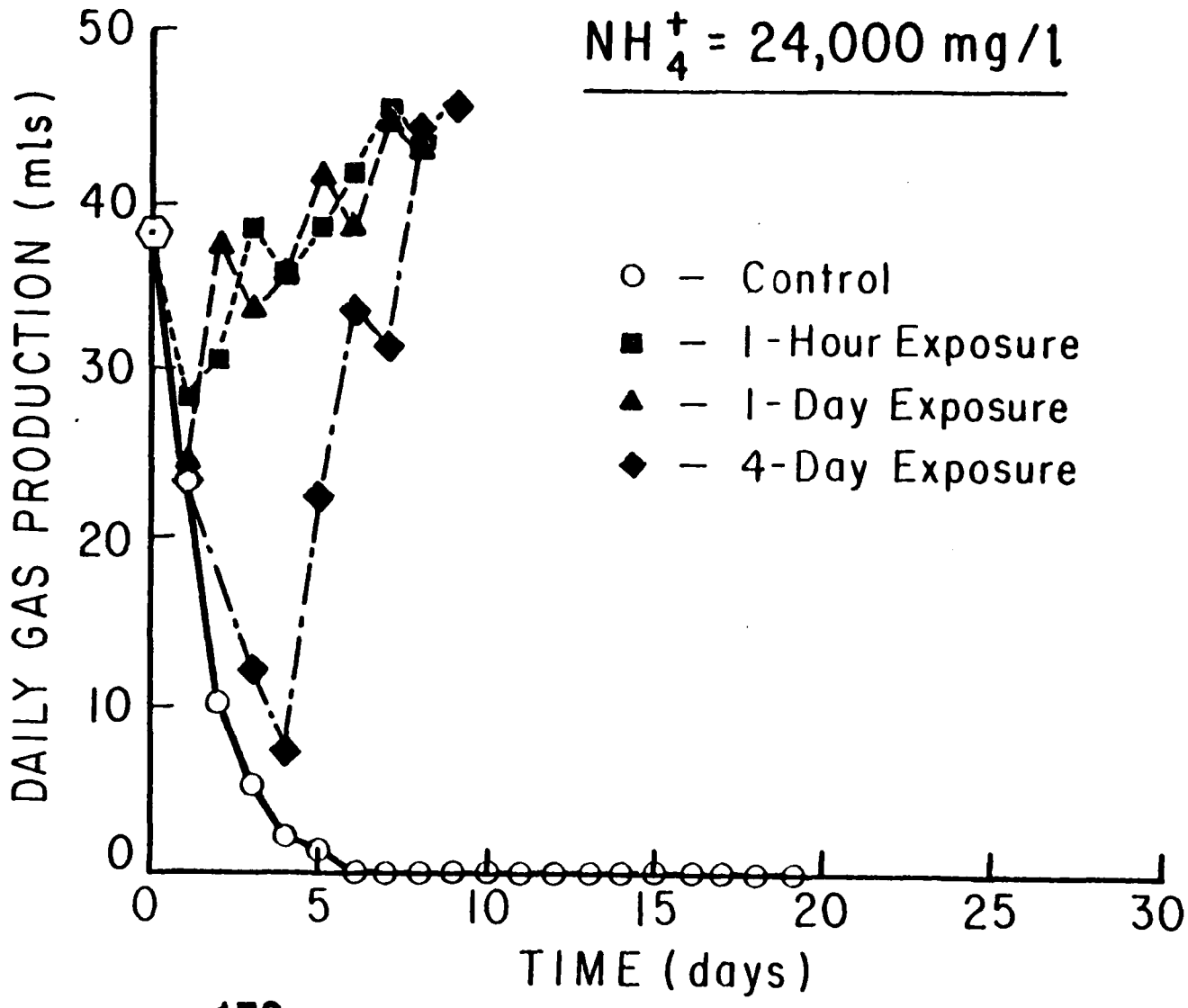


Fig. 172 Effect of Exposure Time on Toxicity Response: NH_4^+

Studies with 1500 mg/l sulfide again demonstrated the effect of exposure time on the 'speed' and degree of reversibility (Figure 171).

INFINITE DILUTION TECHNIQUE

A typical curve of substrate concentration versus time is shown in Figure 173. An initial increase in substrate concentration occurred due to the lag period induced by transferring the bacteria from one environment to another. After this initial increase, the substrate concentration decreased rapidly towards a steady-state value. The data in Figure 173 confirm that a steady-state value was obtained within five to six hours. In later experiments the rising and falling portions of the substrate concentration versus time curve were not monitored. At least two samples were drawn from each reactor for each experiment at approximately five and ten hours from the start.

The assumption that the organism concentration does not vary within the time-frame of the experiments was verified by measurement of VSS, which indicated changes of less than six percent. The steady state substrate concentration was coupled with a measured organism concentration at the end of the experiment, which corrected for growth which may have occurred during the experiment.

A linear regression applied to the data obtained from the infinite dilution technique gave a slope, $\frac{K_s}{k}$, of 5.478 $\frac{\text{mg-VSS-day}}{\ell}$ with 95 percent confidence limits calculated as $\pm$ 0.617 $\frac{\text{mg-VSS-day}}{\ell}$. The intercept, 1/k, was calculated as 0.299 day with a 95 percent confidence limit calculated as $\pm$ 0.086 day. A range of values, with 95 percent confidence, were calculated for K_s as 12.6 to 28.7 $\frac{\text{mg-Ac}^-}{\ell}$ and for k as 2.6 to 4.7 day^{-1}. K_s was calculated as 18.3 $\frac{\text{mg-Ac}^-}{\ell}$ and k as 3.3 day^{-1} with a coefficient of determination of 0.97. A plot of the obtained data is shown in Figure 174.

Data obtained from the serum bottle experiments gave k values that fall within the range of k values that were calculated by the infinite dilution technique. The k-values were calculated from the slopes of gas production versus time plots as 3.0, 3.5, 4.0, and 4.4 day^{-1}. A regression analysis was performed to compare the four calculated slopes. The null hypothesis, that the slopes were all equal, was evaluated for a 95 percent confidence level and was rejected.

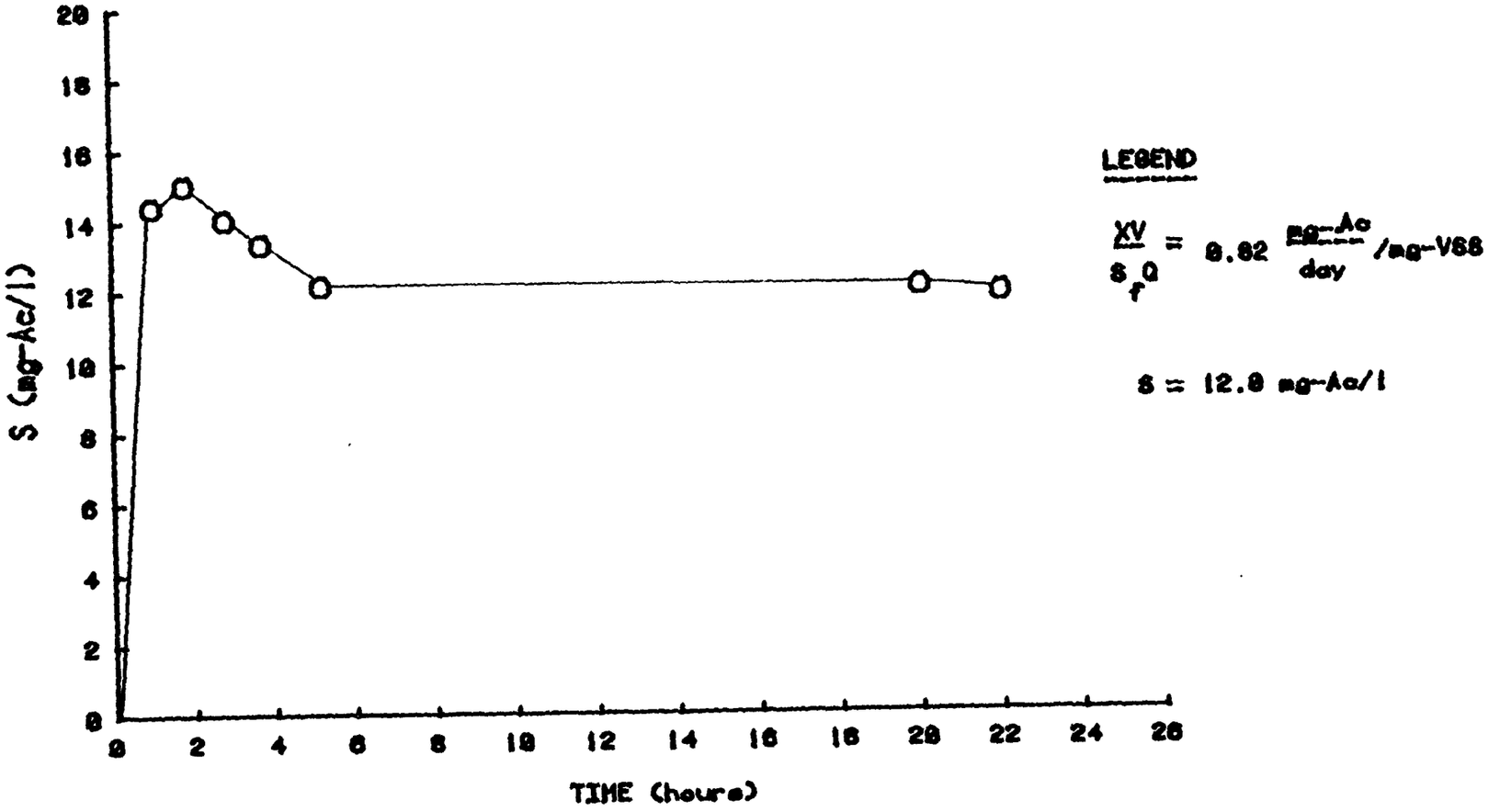


FIGURE 173. TYPICAL EXPERIMENTAL RESULTS FROM INFINITE DILUTION EXPERIMENT

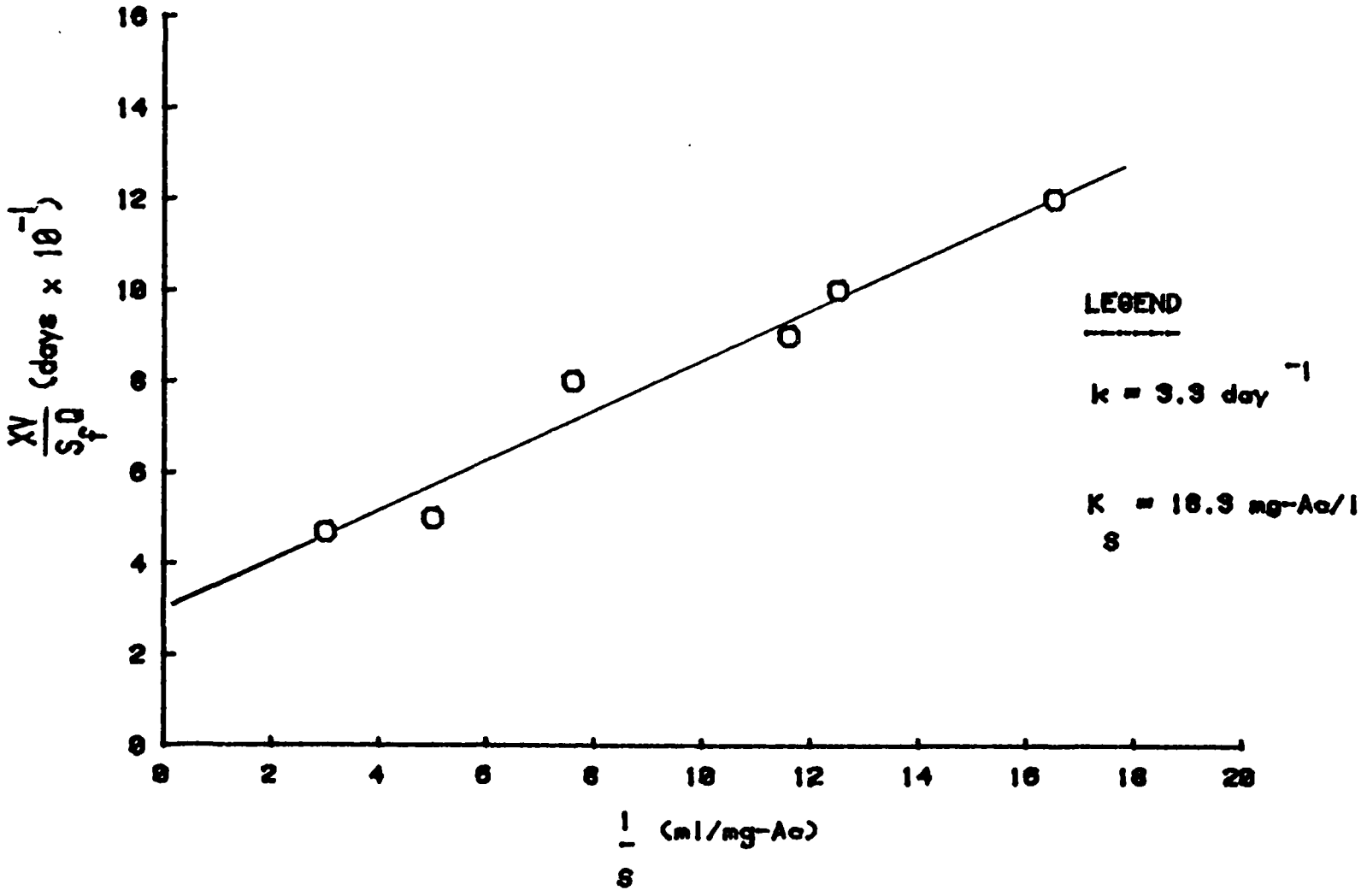


FIGURE 174. LINEWEAVER-BURKE PLOT OF INFINITE DILUTION DATA

DISCUSSION

RESPONSE PATTERN - SLUG DOSE

Although the initial toxicant concentration in each serum bottle could be accurately determined, the actual concentration available to act on the methanogens could be altered in several ways. Daily wasting for SRT control decreases the concentration of toxicant the microbes 'see.' Other phenomena include chemical precipitation (Ni^{2+} precipitation with the sulfide present in the nutrient salts), complexation (heavy metals), ionization equilibria ($S^{2-}/HS^{-}/H_2S$), and metabolism (chloroform, organics in general).

A general comment on the response patterns is warranted. Responses for the majority of toxicants was remarkably similar; a rapid decrease in methane production was noted followed by periods of decreased or zero methane production, the length of which was dependent on initial concentration and finally, a rapid return to full gas production. Calcium and jet fuel seemed to exhibit some residual toxicity after an initial return to full gas production. The rate of recovery was much greater than could be explained by bacterial regrowth meaning that the bacteria were not 'dead,' but only adjusting to the presence of the toxicant. Whether this adjustment took the form of developing enzyme systems to metabolize the toxicant or alterations of existing enzymes to tolerate the toxicant is largely unknown and most definitely dependent on the nature of the toxicant. For example, chloroform and jet fuel may be metabolized while it is likely that nickel will not.

Relative Toxicity

The relative toxicity of the fifteen toxicants, made by comparing the threshold and lethal slug doses of each, is summarized in Table 5. Threshold dose is defined as the slug dose resulting in onset of decreased gas production (versus a control) and lethal dose is the slug dose resulting in zero methane production for at least one detention time following toxicant addition.

Chloroform was the most toxic of the fifteen toxicants, demonstrating a threshold dose near or less than 2.5 mg/l and lethal doses close to or above 7.5 mg/l. The highest threshold and lethal doses observed were for calcium toxicity. Threshold doses varied between 5,000 and 15,000 mg/l

Ca^{++}, while slugs of 15,000 to 25,000 mg/l were necessary to exhibit lethal effects.

Due to the unique response pattern to cadmium exposure, comparison to the other heavy metals is difficult. All four heavy metals exhibited toxicity in the same general range of slug dose concentrations; threshold doses were all below 100 mg/l and lethal doses were near or above 100 mg/l. Chromium toxicity was clearly more severe at the lower temperature (25°C), responses to nickel became increasingly severe as the temperature was increased, and cadmium did not demonstrate large differences in response due to changing temperature. Chromium (VI) appears to be more toxic than chromium (III) and nickel; nickel seems to be the least toxic of the heavy metals tested. Ranking cadmium is difficult because of its unique response pattern.

Dichloroethylene and trichloroethylene demonstrated toxicity similar to the heavy metals. Threshold doses for the two toxicants were identical, but trichloroethylene lethal doses were considerably higher than those for dichloroethylene. The threshold slug doses of ethyl benzene were higher than the lethal doses of trichloroethylene, and lethal doses of ethyl benzene exceeded 1000 mg/l.

Toxic effects of the two cationic surfactants from Rohm and Haas, Hyamine 1622 and Hyamine 3500, were both increased by increasing the temperature. Hyamine 1622 exhibited toxicity in the same concentration range as dichloroethylene, and Hyamine 3500 was slightly more toxic.

Concentrations of gasoline and jet fuel (JP-4) required to cause toxicity were about an order of magnitude higher than for ethyl benzene. Increasing the temperature dramatically reduced the toxic effects of gasoline, while the toxic effects of the jet fuel were more severe at the higher temperature. Jet fuel was less toxic than gasoline only at 25°C and 35°C.

Hydrazine toxicity also became more severe when the temperature was increased to 42.5°C. Threshold doses were comparable to those of Hyamine 3500, but at 25°C and 35°C the lethal doses were closer to those of the heavy metals.

Threshold doses for sulfide were similar to those for the heavy metals, however, the lethal doses appear to be much higher than for the heavy metals.

Table 5. Relative Toxicity for Slug Addition of Toxicants

Toxicant: Conditions		Threshold Dose (mg/l)	Lethal Dose (mg/l)
Calcium			
15-day SRT	35 °C	10,000-15,000	>30,000
25-day SRT	25 °C	5000	20,000-25,000
	35 °C	10,000-15,000	20,000-25,000
	42.5°C	5,000-10,000	20,000
50-day SRT	25 °C	5,000-10,000	15,000-20,000
	35 °C	5,000-10,000	15,000-20,000
	42.5°C	5,000-10,000	20,000
Cadmium			
50-day SRT	25 °C	<50	75-100; >200
	35 °C	<50	>200
	42.5°C	<50	100; >200
Chromium (III)			
15-day SRT	35 °C	5	>100
25-day SRT	25 °C	10-20	20
	35 °C	40-60	>100
	42.5°C	40-60	>100
50-day SRT	25 °C	5-10	60
	35 °C	40-60	>100
	42.5°C	20-40	>100
Chromium (VI)			
15-day SRT	35 °C	<5	>100
25-day SRT	25 °C	<5	40- 60
	35 °C	5-10	60-100
	42.5°C	5-10	40-100
50-day SRT	25 °C	<5	40
	35 °C	<5	40- 60
	42.5°C	5-10	40- 60
Nickel			
15-day SRT	35 °C	90-100	>100
25-day SRT	25 °C	90-100	>100
	35 °C	60- 70	>100
	42.5°C	<50	>100
50-day SRT	25 °C	70- 80	>100
	35 °C	70- 80	>100
	42.5°C	<50	>100

Table 5. Relative Toxicity for Slug Addition of Toxicants (Contd.)

Toxicant: Conditions		Threshold Dose (mg/l)	Lethal Dose (mg/l)
Sulfide			
15-day SRT	35 °C	<50	>500
25-day SRT	25 °C	<50	>500
	35 °C	<50	>500
	42.5°C	50-100	>500
50-day SRT	25 °C	<50	>500
	35 °C	<50	>500
	42.5°C	<50	>500
Chloroform			
15-day SRT	35 °C	2.5-5.0	>7.5
25-day SRT	25 °C	<0.25	>7.5
	35 °C	2.5-5.0	>7.5
	42.5°C	.5-1.0	2.5-5.0
50-day SRT	25 °C	1.0-2.5	5.0-7.5
	35 °C	1.0-2.5	>7.5
	42.5°C	1.0-2.5	5.0-7.5
Dichloroethylene			
50-day SRT	25 °C	25-50	50-100
	35 °C	<25	50-100
	42.5°C	<25	25- 50
Trichloroethylene			
50-day SRT	25 °C	25-50	100-250
	35 °C	<25	100-250
	42.5°C	<25	100-250
Ethyl Benzene			
50-day SRT	25 °C	250-500	500-1000
	35 °C	250-500	>1000
	42.5°C	250-500	>1000
Hyamine 1622			
50-day SRT	25 °C	20- 50	50
	35 °C	20- 50	50-100
	42.5°C	5- 10	50-100
Hyamine 3500			
50-day SRT	25 °C	5- 10	20- 50
	35 °C	5- 10	50
	42.5°C	1- 5	10- 50
Gasoline			
50-day SRT	25 °C	<1000	2,500- 5,000
	35 °C	1000-2500	5,000- 7,500
	42.5°C	<1000	10,000-15,000
Jet Fuel			
50-day SRT	25 °C	5000-7500	>15,000
	35 °C	5000-7500	>15,000
	42.5°C	<1000	7,500
Hydrazine			
50-day SRT	25 °C	<10	>150
	35 °C	<10	>150
	42.5°C	<10	10-25

There does not seem to be any definite trend due to either SRT or temperature on threshold or lethal doses.

The observed threshold values are generally higher than those commonly reported in the literature. The implication is that a wider variety of industrial wastewaters, those containing relatively high concentrations of toxicants, can be treated by methane fermentation.

Effect of SRT

Caution must be exercised when examining the effects of SRT because the biomass concentration was not determined for each of the serum bottle cultures. Measurements of volatile suspended solids (VSS) for the seed digesters at each SRT was observed to vary significantly over a period of several weeks. For most experiments, initial VSS levels were 500-700 mg/l for the 50-day SRT systems, 300-500 mg/l for the 25-day systems, and near 300 mg/l for the 15-day SRT systems.

Six toxicants were evaluated for the effect of SRT on severity of response to slug additions of toxicants: Ca^{2+}, Cr^{3+}, Cr^{6+}, Ni^{2+}, S^{2-}, and chloroform. Table 6 lists the "best" SRT, the one giving the least-severe response, for each toxicant and temperature tested. The term "best" is a relative term and its selection was necessarily arbitrary and meant only to serve as a basis for observing trends. There were no clear trends.

Table 6. "Best" SRT : The SRT Giving the Least-Severe Response to Slug Addition of Toxicant

	Temperature		
Toxicant	25°	35°	42.5°
Calcium	25	15	25 ≈ 50
Chromium III	50	25 ≈ 50	25 ≈ 50
Chromium VI	25 ≈ 50	15	25
Nickel	25	15 ≈ 25 ≈ 50	25 ≈ 50
Sulfide	50	25 ≈ 50	50
Chloroform	25	15 ≈ 25 ≈ 50	25 ≈ 50

The observed effects of SRT on toxicity varied with the nature of the toxicant and temperature. A 15-day SRT was only investigated at 35°C and

proved to be the "best" system for exposure to Ca^{2+} and Cr^{6+}. All three SRTs gave similar responses to nickel at 35°C. Only for sulfide exposure did the 50-day system yield the least severe response for all three temperatures, although for Cr^{3+} the 25-day and 50-day systems were not much different. For the remaining toxicants, the SRT resulting in the least-severe response was temperature dependent.

The less-severe responses observed for the lower SRT systems can be explained by several factors. In the lower SRT systems (SRT and the hydraulic retention time (HRT) were equal), the toxicant was washed out at a faster rate than for the higher SRT systems. Recoveries by 15-day and 25-day SRT cultures frequently occurred in less time than required by the 50-day systems, indicating that washout rates of toxicant may have as much influence on toxicity responses as washout of the biomass.

Other factors that may affect response include biomass concentration (VSS) and cell age (controlled by SRT). Yang (1981), in related studies conducted in our laboratory, has shown that increasing VSS levels at constant SRT reduced the time required for systems to recover from slug additions of cyanide and chloroform. This researcher also showed that for the same VSS levels, younger cells (lower SRT) were much better able to cope with cyanide exposure and slightly better able to handle chloroform additions. Baresi et al (1978) also reported that younger cells were better able to respond to toxicants such as chloroform. Thus the effect of SRT is much more complex than originally envisioned.

Effect of Temperature

All the seed cultures were grown at 35°C and thus bottles maintained at 25°C and 42.5°C may not indicate the true responses of bacteria grown at those temperatures. However, observations can be made on the effect of decreases or increases in temperature, which may be a more common occurrence.

Although a clear pattern of temperature effects on all toxicants is not evident, there are some noticeable trends. At 25°C, the rate of decrease in gas production following toxicant introduction and frequently the recovery rates were significantly slower than at the higher temperatures. However, the most severe responses to the toxicants did not necessarily occur at 25°C. For chromium (III), chromium (VI), gasoline and sulfide, the cultures

incubated at 25°C were clearly the most affected. Increasing the temperature generally increased recovery rates, however, going from 35°C to 42.5°C caused increases in recovery times from exposure to nickel, chloroform, jet fuel, hydrazine, and sulfide. Trends were not observed for the other toxicants. 35° was definitely the "preferred" temperature.

Effect of Initial Toxicant Concentration (I_o)

By assuming that the concentration of toxicant was not reduced biochemically or chemically, the concentration of toxicant remaining in a serum bottle (I) after t days of exposure can be calculated from:

$$I = I_o((SRT - 1)/SRT)^t$$

where I_o is the initial slug-dose toxicant concentration. Using this equation, the concentration of toxicant remaining in the system when recovery starts (termed I_{sr}) and when recovery is complete (I_{cr}) can be calculated. Comparison of the difference between I_{sr} and I_{cr} indicates the rate of system recovery as a function of I_o. By comparing I_{cr} with the threshold dose concentrations listed in Table 5, one can speculate on the effect of I_o on the reversibility, acclimation, and recovery of the methane bacteria. Calculated values for I_{sr} and I_{cr} are listed in Tables 7 and 8.

It should be emphasized that for toxicants that may react chemically or biochemically (for example, calcium, nickel, chloroform), actual concentration may be less than calculated I_{sr} and I_{cr} values. Thus, some caution must be exercized when interpreting the data. However, trends can be noted, preliminary conclusions offered, and potential significance discussed.

Table 7. Effect of Initial Toxicant Concentration as a Function of SRT and Temperature

TOXICANT Temperature	I_o^1 (mg/l)	I_{sr}^2 (mg/l)			I_{cr}^3 (mg/l)		
		15-day SRT	25-day SRT	50-day SRT	15-day SRT	25-day SRT	50-day SRT
CALCIUM							
25°C	5,000	-	NR[4]	NR	-	NR	NR
	10,000	-	9,600	9,604	-	9,600	9,604
	15,000	-	14,400	10,014	-	14,400	6,882
	20,000	-	6,642	<4,577	-	3,318	4,577
	25,000	-	<1,911	<5,722	-	<1,911	<5,722
	30,000	-	<2,293	<6,866	-	<2,293	<6,866
35°C	5,000	NR	NR	NR	NR	NR	NR
	10,000	NR	NR	8,858	NR	NR	8,007
	15,000	6,554	10,388	12,762	2,173	8,823	10,640
	20,000	3,819	5,878	6,994	1,669	4,240	6,196
	25,000	3,622	3,118	6,591	1,379	<2,075	5,172
	30,000	3,534	<2,490	6,594	1,094	<2,490	5,520
42.5°C	5,000	-	NR	NR	-	NR	NR
	10,000	-	5,421	8,007	-	4,796	6,812
	15,000	-	9,972	9,237	-	6,365	6,822
	20,000	-	2,818	7,986	-	1,728	6,452
	25,000	-	<1,125	9,105	-	<1,125	<8,065
	30,000	-	<1,350	<9,678	-	<1,350	<9,678
CHROMIUM (III)							
25°C	5	-	NR	NR	-	NR	NR
	10	-	NR	5.8	-	NR	5.0
	20	-	3.1	11.5	-	<1.5	9.1
	40	-	<3.0	11.6	-	<3.0	<10.3
	60	-	<4.5	24.1	-	<4.5	16.8
	100	-	<7.6	31.6	-	<7.6	<25.8
35°C	5	NR	NR	NR	NR	NR	NR
	10	3.3	NR	NR	2.9	NR	NR
	20	5.8	NR	NR	3.6	NR	NR
	40	10.1	NR	NR	5.1	NR	NR
	60	14.1	30	40.0	4.7	26.5	36.2
	100	27.0	52	65.4	7.8	36.0	59.1

Table 7 (Contd.)

TOXICANT Temperature	I_o^1 (mg/l)	I_{sr}^2 (mg/l) 15-day SRT	I_{sr}^2 (mg/l) 25-day SRT	I_{sr}^2 (mg/l) 50-day SRT	I_{cr}^3 (mg/l) 15-day SRT	I_{cr}^3 (mg/l) 25-day SRT	I_{cr}^3 (mg/l) 50-day SRT
CHROMIUM III (Contd.)							
42.5°C	5	-	NR	NR	-	NR	NR
	10	-	NR	NR	-	NR	NR
	20	-	NR	NR	-	NR	NR
	40	-	NR	26.7	-	NR	25.1
	60	-	33.9	40.9	-	31.2	37.7
	100	-	61.3	66.8	-	48.0	60.4
CHROMIUM (VI)							
25°C	5	-	4.8	4.9	-	4.8	4.9
	10	-	9.6	9.8	-	9.6	9.6
	20	-	19.2	18.8	-	17.7	18.4
	40	-	6.4	25.6	-	<3.1	10.3
	60	-	<4.6	<15.5	-	<4.6	<15.5
	100	-	<7.6	<25.8	-	<7.6	<25.8
35°C	5	4.7	NR	4.9	4.4	NR	4.9
	10	9.3	NR	9.8	8.7	NR	9.8
	20	18.7	NR	19.2	16.3	NR	19.2
	40	37.3	34.0	28.4	10.1	10.0	17.1
	60	9.3	13.2	20.6	2.9	7.9	16.5
	100	5.9	<8.6	20.7	4.2	<8.6	<18.4
42.5°C	5	-	NR	NR	-	NR	NR
	10	-	9.6	9.8	-	8.8	9.8
	20	-	16.3	19.6	-	9.6	18.8
	40	-	16.3	18.6	-	11.3	14.6
	60	-	10.0	20.6	-	<2.6	<19.4
	100	-	7.0	<32.3	-	<3.5	<32.3
NICKEL							
25°C	50	-	NR	NR	-	NR	NR
	60	-	NR	NR	-	NR	NR
	70	-	NR	NR	-	NR	NR
	80	-	NR	68.1	-	NR	68.1
	90	-	NR	69.2	-	NR	62.6
	100	-	88.6	65.4	-	85.1	49.3

Table 7 (Contd.)

TOXICANT Temperature	I_o[1] (mg/l)	I_{sr}[2] (mg/l)			I_{cr}[3] (mg/l)		
		15-day SRT	25-day SRT	50-day SRT	15-day SRT	25-day SRT	50-day SRT
NICKEL (Contd.)							
35°C	50	NR	NR	NR	NR	NR	NR
	60	NR	NR	NR	NR	NR	NR
	70	NR	65.9	NR	NR	64.6	NR
	80	NR	75.3	64.0	NR	72.3	64.0
	90	NR	79.7	67.8	NR	76.6	63.8
	100	70.8	90.4	72.4	43.7	81.7	68.1
42.5°C	50	-	NR	47.1	-	NR	47.1
	60	-	57.6	50.0	-	54.2	48.0
	70	-	65.9	52.8	-	62.0	46.7
	80	-	73.8	57.9	-	68.1	45.4
	90	-	75.0	58.9	-	66.5	44.4
	100	-	73.9	56.8	-	60.4	34.3
SULFIDE							
25°C	50	-	48	49	-	48	49
	100	-	96	98	-	96	98
	150	-	144	147	-	133	141
	200	-	192	196	-	157	184
	300	-	288	294	-	199	255
	500	-	480	490	-	57	290
35°C	50	47	48	49	49	48	49
	100	-	96	98	-	96	98
	150	140	144	147	131	144	147
	200	187	192	196	152	177	192
	300	280	288	294	131	225	277
	500	467	-	490	102	-	425
42.5°C	50	-	NR	49	-	NR	49
	100	-	96	98	-	96	98
	150	-	144	147	-	127	141
	200	-	192	196	-	150	186
	300	-	288	294	-	150	245
	500	-	480	490	-	141	278

Table 7 (Contd.)

TOXICANT Temperature	I_o^1 (mg/l)	I_{sr}^2 (mg/l) 15-day SRT	I_{sr}^2 (mg/l) 25-day SRT	I_{sr}^2 (mg/l) 50-day SRT	I_{cr}^3 (mg/l) 15-day SRT	I_{cr}^3 (mg/l) 25-day SRT	I_{cr}^3 (mg/l) 50-day SRT
CHLOROFORM							
25°C	0.25	-	0.24	NR	-	0.24	NR
	0.50	-	0.48	NR	-	0.48	NR
	1.0	-	0.96	NR	-	0.96	NR
	2.5	-	2.4	2.3	-	2.4	2.3
	5.0	-	4.8	4.0	-	4.8	2.1
	7.5	-	7.2	<1.9	-	6.4	<1.9
35°C	0.25	NR	NR	NR	NR	NR	NR
	0.50	NR	NR	NR	NR	NR	NR
	1.0	NR	NR	NR	NR	NR	NR
	2.5	NR	NR	NR	NR	NR	NR
	5.0	3.1	4.8	4.7	2.2	4.8	4.4
	7.5	3.1	7.2	7.0	2.2	6.4	4.0
42.5°C	0.25	-	NR	NR	-	NR	NR
	0.50	-	NR	NR	-	NR	NR
	1.0	-	0.96	NR	-	0.85	NR
	2.5	-	NR	NR	-	NR	NR
	5.0	-	0.70	2.9	-	<0.21	<1.6
	7.5	-	0.67	2.7	-	<0.32	<2.4

[1] I_o = Initial slug-dose concentration of toxicant.

[2] I_{sr} = Calculated toxicant concentration when system starts to recover.

[3] I_{cr} = Calculated toxicant concentration when recovery is complete.

[4] NR = 'No Response' to toxicant.

Table 8. Effect of Initial Toxicant Concentration as a Function of Temperature at a 50-day SRT

TOXICANT Temperature	I_o^1 (mg/l)	I_{sr}^2 (mg/l)	I_{cr}^3 (mg/l
CADMIUM			
25°C	50	40.9	23.2
	75	21.9	14.6
	100	29.2	18.5
	125	47.4	24.3
	150	72.5	41.2
	200	102.7	68.6
35°C	50	44.3	34.8
	75	31.5	26.8
	100	42.0	33.6
	125	61.6	48.4
	150	80.2	61.7
	200	106.9	89.1
42.5°C	50	38.5	32.7
	75	29.6	23.2
	100	32.3	28.0
	125	54.6	44.6
	150	72.5	60.4
	200	120.7	104.8
DICHLOROETHYLENE			
25°C	25	NR	NR
	50	45.0	37.7
	100	< 36.4	< 36.4
	250	< 91.0	< 91.0
	500	<182.0	<182.0
	1000	<364.0	<362.0
35°C	25	23.5	21.3
	50	24.2	9.5
	100	< 32.0	< 32.0
	250	< 81.0	< 81.0
	500	<161.0	<161.0
	1000	<323.0	<323.0
42.5°C	25	19.6	7.9
	50	< 1.7	< 1.7
	100	< 34.0	< 34.0
	250	< 86.0	< 86.0
	500	<171.0	<171.0
	1000	<343.0	<343.0

Table 8. (Contd.)

TOXICANT Temperature	I_o^1 (mg/l)	I_{sr}^2 (mg/l)	I_{cr}^3 (mg/l)
TRICHLOROETHYLENE			
25°C	25	NR	NR
	50	46.1	44.3
	100	90.4	78.5
	250	< 86.0	< 86.0
	500	<171.0	<171.0
	1000	<343.0	<343.0
35°C	25	23.5	23.5
	50	47.1	42.5
	100	86.8	65.4
	250	< 82.0	< 82.0
	500	<165.0	<165.0
	1000	<329.0	<329.0
42.5°C	25	23.5	21.3
	50	44.3	29.0
	100	47.4	< 35.0
	250	< 87.0	< 87.0
	500	<175.0	<175.0
	1000	<350.0	<350.0
ETHYL BENZENE			
25°C	25	NR	NR
	50	NR	NR
	100	NR	NR
	250	NR	NR
	500	471.0	471.0
	1000	503.0	<166.0
35°C	25	NR	NR
	50	NR	NR
	100	NR	NR
	250	NR	NR
	500	490.0	377.0
	1000	769.0	546.0
42.5°C	25	NR	NR
	50	NR	NR
	100	NR	NR
	250	NR	NR
	500	348.0	308.0
	1000	568.0	493.0
HYAMINE 1622			
25°C	1	NR	NR
	5	NR	NR
	10	NR	NR
	20	NR	NR
	50	17.1	11.4
	100	26.9	< 16.6

Table 8. (Contd.)

TOXICANT Temperature	I_o^1 (mg/l)	I_{sr}^2 (mg/l)	I_{cr}^3 (mg/l)
HYAMINE 1622 (Contd.)			
35°C	1	NR	NR
	5	NR	NR
	10	NR	NR
	20	NR	NR
	50	21.4	14.0
	100	32.3	23.8
42.5°C	1	NR	NR
	5	NR	NR
	10	8.7	6.7
	20	15.7	12.1
	50	21.0	16.5
	100	31.0	22.4
HYAMINE 3500			
25°C	1	NR	NR
	5.	NR	NR
	10	8.2	5.6
	20	10.9	6.1
	50	13.5	< 8.3
	100	19.1	<16.6
35°C	1	NR	NR
	5	NR	NR
	10	9.8	9.8
	20	9.7	8.4
	50	17.1	12.2
	100	28.0	22.0
42.5°C	1	NR	NR
	5	4.5	4.2
	10	7.8	5.6
	20	10.7	< 3.3
	50	16.1	13.7
	100	31.0	24.8
GASOLINE			
25°C	1,000	960	941
	2,500	2,353	1,049
	5,000	1,646	1,056
	7,500	<1,716	<1,716
	10,000	<2,289	<2,289
	15,000	<3,433	<3,433
35°C	1,000	NR	NR
	2,500	2,127	1,208
	5,000	1,974	1,518
	7,500	2,571	2,018
	10,000	3,226	2,481
	15,000	5,246	4,118

Table 8. (Contd.)

TOXICANT Temperature	I_o^1 (mg/l)	I_{sr}^2 (mg/l)	I_{cr}^3 (mg/l)
GASOLINE (Contd.)			
42.5°C	1,000	668	535
	2,500	1,449	1,208
	5,000	2,274	1,896
	7,500	3,411	2,732
	10,000	3,642	2,801
	15,000	5,463	3,798
JET FUEL*			
25°C	1,000	NR	NR
	2,500	NR	NR
	5,000	NR	NR
	7,500	5,007	4,260
	10,000	7,847	3,428
	15,000	9,815	4,839
35°C	1,000	NR	NR
	2,500	NR	NR
	5,000	NR	NR
	7,500	5,007	3,624
	10,000	6,283	2,976
	15,000	9,831	3,875
42.5°C	1,000	904	851
	2,500	1,669	1,310
	5,000	2,620	1,144
	7,500	2,787	1,520
	10,000	3,428	2,243
	15,000	4,118	<2,490
HYDRAZINE			
25°C	10	4.3	2.1
	25	8.2	5.0
	50	9.5	< 8.3
	75	13.6	<12.5
	100	<16.6	<16.5
	150	<25.0	<25.0
35°C	10	8.0	6.7
	25	11.4	7.9
	50	14.3	9.9
	75	<12.5	<12.5
	100	<16.6	<16.6
	150	<25.0	<25.0

* Not including residual toxicity

Table 8. (Contd.)

TOXICANT Temperature	I_o^1 (mg/l)	I_{sr}^2 (mg/l)	I_{cr}^3 (mg/l)
HYDRAZINE (Contd.)			
42.5°C	10	6.8	5.1
	25	6.9	5.2
	50	< 8.3	< 8.3
	75	<12.5	<12.5
	100	<16.6	<16.6
	150	<25.0	<25.0

[1] I_o = Initial slug-dose concentration of toxicant

[2] I_{sr} = Calculated toxicant concentration when system starts to recover

[3] I_{cr} = Calculated toxicant concentration when recovery is complete

[4] NR = 'No Response' to toxicant

Rates of recovery are most definitely a function of I_o; in general, higher I_o values resulted in reduced rates of recovery. In some cases, the decrease in rate of recovery was quite drastic (for example, ethyl benzene at 25°C, 50-day SRT, I_o = 1000 mg/l).

The general pattern for all toxicants seemed to be that as I_o was increased, I_{sr} and I_{cr} both increased initially, leveled off, and finally decreased as I_o continued to increase. The implication here is that there exists a maximum, or critical, value of I_o (termed I_o^{crit}) beyond which the ability of the methane bacteria to recovery (acclimate and/or reverse the toxicity), as measured by I_{cr}, is drastically reduced. A tentative definition for I_o^{crit} would be that value of I_o which results in value of I_{cr} below the threshold concentration (I_{th} - Table 5). Once I_o^{crit} is present, I_{cr} is reduced below I_{th}, which results in a longer recovery period. On a practical level, this means that the operator of a system must plan on a protracted recovery time, or alternately, must plan on diluting down to I_{cr}. Perhaps with proper planning, dilution to I_{th} would result in better economics. A careful program of acclimation would also be possible with many of the toxicants.

This 'standard' pattern can be observed in Tables 7 and 8 for calcium, chromium (VI), nickel, chloroform and ethyl benzene. The pattern was not observed for the remainder of the toxicants for two reasons. First, I_o^{crit} was apparently larger than the highest slug doses of cadmium, chromium (III), sulfide, Hyamine 1622, Hyamine 3500, gasoline and jet fuel. In the cases of dichloroethylene, trichloroethylene and hydrazine, it is not clear whether I_o^{crit} was or was not reached. Since several slug doses were an order of magnitude greater than threshold doses, the cultures would have to have been operated for much longer periods of time before washout would have decreased the toxicant concentrations to threshold levels.

It is interesting to note that although the less-severe responses to calcium occurred in 15- and 25-day SRT systems, the values of I_{sr} and I_{cr} are generally larger at a 50-day SRT. This indicates that recovery from the toxicant is not completely controlled by the concentration at any given time, but exposure time must also be considered, perhaps reflecting acclimation characteristics.

RESPONSE PATTERN - CONTINUOUS ADDITION

In general, three types of response patterns were exhibited after the initial additions of toxicant occurred. The first was no effect, in which no decrease in gas production was observed. Apparently, the methanogenic bacteria were able to 'acclimate' to some toxicants (under some conditions) without a reduction in gas production, except for some experimental variation as previously noted, for all SRTs, temperatures and selected concentrations.

The second type of response produced a period of decreased gas production. Shortly after the introduction of toxicants, a decrease in gas production was observed before return to the level of the control. One example of this type of response is the pattern shown for chloroform (Figures 150 to 158). Lowered gas production was observed for from several days to several weeks before returning to the control level in most cases. The magnitude of the decrease in gas production was dependent upon the concentration of the toxicant (i.e., the greater the concentration, the more severe the response). The methanogens were able to 'acclimate' to the toxicants after a brief period of decreased gas production.

There are two hypotheses proposed to explain this apparent acclimation. The first is that chloroform and hydrazine may have been metabolized or

volatilized to a reduced level more acceptable to the methanogenic bacteria. In the second case, for nickel, it is unlikely that it was metabolized. Instead it was thought that the slow introduction of the toxicant allowed the bacterial enzyme system to adjust to tolerate the toxicant without detrimental effects. This second hypothesis could also apply to acclimation to chloroform and hydrazine.

The third type of response was that experienced by the methanogens exposed to toxicant concentrations that were too high to be tolerated and the methanogens were unable to acclimate at these concentrations. Methane production went to zero and remained there.

Relative Toxicity

Table 9 lists the estimated threshold and lethal doses from continuous addition of nickel, chloroform, and hydrazine. The threshold dose is defined as the calculated concentration at which methane production goes to 15 mls or below. (The 15 ml value was arbitrarily selected as approximately 2 standard deviations (worst case) below the control level -- a 95% confidence level. This procedure should yield a somewhat conservation estimate of the threshold dose.) The lethal dose is defined as the concentration at which gas production reaches zero and remains there. These doses were calculated assuming no chemical or biological reaction occurs in the serum bottle using the following:

$$I_t = I_o \left[1 - \left(\frac{SRT-1}{SRT}\right)^t\right]$$

where: I_t = calculated concentration of toxicant in the reactor after t days;

I_o = concentration of toxicant in the feed solution.

The range of values in Table 9 represent values for different I_o.

In addition, Table 9 lists threshold doses from slug-dose studies (Table 5) for comparison. For most conditions, it may be seen that continuous addition resulted in higher values for threshold dose, indicating possible acclimation. Lethal doses are also higher for continuous addition, definitely indicating acclimation.

Table 9. Estimated Threshold and Lethal Doses (mg/l) for Continuous Addition of Toxicants

Toxicant-Condition	Estimated Threshold Dose		Estimated Lethal Dose from Continuous Addition
	Slug Addition	Continuous Addition	
Ni^{2+}			
15-day SRT			
25°	-	129-136	153-185
35°	90-100	67-170	213
42.5°	-	29-117	199
25-day SRT			
25°	90-100	108-165	208
35°	60- 70	155	200
42.5°	<50	39-123	134
50-day SRT			
25°	70- 80	86	149
35°	70- 80	42- 92	138
42.5°	<50	80- 90	122
Chloroform			
15-day SRT			
25°	-	2- 5	>20
35°	2.5-5.0	4- 18	>20
42.5°	-	4- 9	13
25-day SRT			
25°	<0.25	3- 4	>20
35°	2.5-5.0	3- 7	>20
42.5°	0.5-1.0	2- 4	4-8
50-day SRT			
25°	1.0-2.5	1- 19	>20
35°	1.0-2.5	5- 8	>20
42.5°	1.0-2.5	2- 7	10
Hydrazine			
15-day SRT			
25°	-	2- 33	>50
35°	-	3- 30	>50
42.5°	-	> 50	>50
25-day SRT			
25°	-	2- 47	23-47
35°	-	> 50	>50
42.5°	-	1- 9	27
50-day SRT			
25°	< 10	43	>50
35°	< 10	12	>50
42.5°	< 10	5- 11	9

An interesting observation was made when calculating the threshold dose. For each feed concentration (I_o in the equation above), a corresponding threshold dose was calculated. The general pattern was that as I_o increased, the estimated, calculated threshold dose increased. This was especially true with hydrazine. This indicates that acclimation was occurring and makes it difficult to estimate the "true" threshold dose. For continuous addition of toxicants, the threshold dose would seem to be a function of feed concentration.

It does seem that for continuous addition, threshold doses is a function of both SRT and temperature, although the nature of the relationship is not clear.

Effect of SRT

It must be again noted that when examining the effects of SRT some caution must be exercised because biomass concentration was not determined for each serum bottle. Measurements of volatile suspended solids (VSS) for the inoculum digesters during continuous addition studies remained at fairly stable levels. Initial VSS levels were between 500-700 mg/l for the 50-day systems, 400-500 mg/l for the 25-day systems and 350-400 mg/l for the 15-day systems.

The observed effects of SRT on toxicity response varied with the nature of the toxicant and temperature. The "best" SRT was estimated for the conditions tested as was done for slug additions (Table 6). These values are presented in Table 10.

Table 10. "Best" SRT : The SRT Giving the Least-Severe Response to Continuous Addition of Toxicant

	Temperature		
Toxicant	25°	35°	42.5°
Nickel	25 ≃ 50	25 ≃ 50	15 ≃ 25
Chloroform	25	25	25 ≃ 50
Hydrazine	50	25 ≃ 50	15

There was little difference observed for nickel with 50-day and 25-day SRTs, and both were better than the 15-day SRT, except at 42.5°C. The 25-day system seemed to give best results with chloroform exposure while response to hydrazine varied widely with temperature.

The results are similar to the SRT effect noticed for slug-addition studies. The question again arises, however, as to why in some cases did the shorter SRTs perform better than the longer SRTs? Although this study did not examine this question directly, reference again is made to the work of Yang (1981). He examined this phenomenon for chloroform and cyanide. Results from his studies indicated that for the same SRT, as VSS concentration decreases, time for recovery increases. Also, for the same initial VSS, older cells (larger SRT) were more sensitive to cyanide and a bit more sensitive to chloroform, although the difference for chloroform was not statistically significant. Studies by Baresi, et al (1978) also found evidence that younger cells were better able to handle toxicants like chloroform. Washout of toxicant, thought to be a major factor in slug-addition studies, was not a factor here. Thus, to really determine the effect of SRT on continuous addition of toxicants, precautions must be taken to keep VSS reasonably constant. However, in a real CSTR, longer SRTs result in lower VSS levels. This is an area worthy of further study.

Effect of Temperature

As was the case with the slug-addition studies, although there was no clear pattern of temperature effects for all toxicants, there were some noticeable trends. 35°C was found to be the best overall temperature with the least-severe responses for most of the toxicants. However, with a 15-day SRT, the 42.5°C systems showed the least-severe responses while at 25- and 50-day SRTs, 25°C gave better results than 42.5°C. Perhaps the higher temperature affords a measure of safety at the lower SRTs. It is not expected that temperature will be a major factor in response to continuous addition of toxicants as long as the temperature remains relatively constant and the system is not organically overloaded (all systems in this study were loaded at 1120 mg COD/l-day (70 lb COD/1000 ft^3-day)).

ACCLIMATION

Acclimation to Slug Addition

The potential of the methane bacteria to acclimate to slug doses of the various toxicants was evaluated semi-quantitatively. After the systems recovered from the initial slug dose (this recovery itself demonstrates acclimation), a second slug dose was administered to yield twice the initial concentration. Thus the bottles now contained toxicant at twice the concentration of the initial slug dose plus whatever toxicant remained from the initial slug dose. Acclimation Potential (AP) was then estimated by comparing the gas production response of an unacclimated system (no prior toxicant exposure) with that of the above-described 'double-dosed' system using the following equation:

$$AP = \frac{\text{Area under curve without prior exposure}}{\text{Area under curve with prior exposure}}$$

For systems that showed no decrease in gas production following a second slug addition, the area was arbitrarily taken to be 1.0. If this wasn't done, AP would be reported as infinity. An AP value of 1.0 indicates no calculated potential for acclimation at the concentrations tested. Table 11 contains a summary of calculated values of AP for the various toxicants and experimental conditions studied.

Firm conclusions regarding the effect of temperature on AP are not possible since the methanogens were grown at 35°C prior to experiments at 25° and 42.5°C. The bacteria had only 10 to 20 days exposure to these new temperatures before addition of toxicants. The data do suggest, however, that temperature may profoundly affect the acclimation potential of methanogens. The practical implication here is the importance of temperature fluctuations when starting up a methane fermentation system or when coping with a sudden, slug addition of toxicant.

Focusing primarily on the 35°C systems, data in Table 11 indicate that the potential of methanogens to acclimate is very dependent on toxicant type and perhaps on SRT. Acclimation was evident for calcium, nickel, and chloroform at a 50-day SRT, but only for chloroform at a 25-day SRT. At 15-day SRT, acclimation to chromium III, nickel, and chloroform was evident. Chromium VI and sulfide gave little indication of acclimation at any of the

Table 11. Estimated Acclimation Potential (AP) of Methanogens to Repeated Slug Additions of Toxicants

Toxicant	Concentration	Values of AP for: SRT = 50 days T=25°	SRT = 50 days T=35°	SRT = 50 days T=42.5°	SRT = 25 days T=25°	SRT = 25 days T=35°	SRT = 25 days T=42.5°	SRT = 15 days T=35°
Calcium	10,000	25	96	1	1	1	2.4	1
Cadmium	100	1.7	1.6	1.7	-	-	-	-
Chromium (III)	40	1	1	1	1	1	1	2.1
Chromium (VI)	20	1	1	1	1	1	1	1
Nickel	100	25	2.3	1.9	33	1	1.5	2.4
Sulfide	200	1	1	1	1.2	1.2	1.2	1
Chloroform	5.0	495	4.5	6.6	8	4	>138	8
Dichloroethylene	50	1.4	6.8	-	-	-	-	-
	25	-	-	5.9	-	-	-	-
Trichloroethylene	50	1	1	1	-	-	-	-
Ethyl benzene	1,000	>1.4	1.2	2.2	-	-	-	-
Hyamine 1622	20	1	1	1	-	-	-	-
Hyamine 3500	20	1.3	1	1	-	-	-	-
Gasoline	5,000	1	1.4	1.4	-	-	-	-
Jet Fuel	10,000	1	208	-	-	-	-	-
	5,000	1	1	1.4	-	-	-	-
Hydrazine	25	1	2	1.9	-	-	-	-

SRTs investigated. The ability to acclimate to cadmium, dichloroethylene, ethyl benzene, gasoline, jet fuel, and hydrazine was demonstrated in 50-day cultures. In some cases (chromium VI results, for example), repeated injection of toxicants resulted in more-severe responses than for one-time addition, indicating a cumulative effect of toxicity. Recognizing that the present definition of acclimation potential is somewhat arbitrary and definitely a function of the concentrations selected, the data definitely show that methanogens can acclimate to concentrations of toxicants heretofore thought to be lethal.

The practical significance of these results and those described under RESPONSE PATTERN - SLUG DOSE is that with proper attention paid to acclimation, wastewaters periodically containing relatively high concentrations of toxicants (transient toxicity -- slug addition) can be effectively treated by methane fermentation. Extended periods of zero gas production are not indicative of ultimate process failure. These periods of zero gas production can be decreased, and in some cases eliminated, with acclimation. The key is not to wash still-viable biomass from the system. The engineering design and operating variable most important for insuring acclimation is SRT (Speece et al, 1980; Yang et al, 1980). By inspecting the response pattern curves presented in the RESULTS (slug addition studies) section, the importance of SRT is obvious. If active bacteria are not washed from the system, it will recover. Some systems were down for periods in excess of 40 days prior to recovery. Sufficient SRT insures retention of active bacteria. The anaerobic filter and fluidized bed, inherently giving SRT values in excess of 100 days, provide an advantage over the more conventional suspended growth systems in this regard.

Acclimation to Continuous Addition

To obtain a more reasonable estimate of the acclimation potential of the acetate-utilizing methanogens, selected toxicants were continuously added to the serum bottles. The concentration of inhibitory material in the system is thus increased gradually, allowing for acclimation to occur. Acclimation potential here is defined as the ratio of the maximum concentration giving no effect in the continuous addition systems to the estimated threshold dose. The selection of the maximum concentration is limited to those concentrations tested.

As was discussed previously, estimation of threshold dose from continuous addition studies is difficult because of potential acclimation as the toxicant is slowly added to the systems in the daily feed. Thus the threshold dose used here is taken from slug-dose and batch results. Results from slug-addition studies showed no clear trend in the effect of SRT or temperature on threshold dose. Values for nickel ranged from less than 50 to 100 mg/l; for chloroform, from less than 0.25 (probably an experimental anomaly) to 5 mg/l; and for hydrazine, studied only at a 50-day SRT, less than 10 mg/l. Batch studies with nickel (see Figure 187 under KINETICS) gave an estimated threshold dose of less than 50 mg/l at 35°C and with chloroform (Yang et al, 1980) gave a threshold dose of less than 0.5 mg/l. From this data, it was decided to use estimated threshold doses of 50 mg/l for nickel, 1.0 mg/l for chloroform, and 10 mg/l for hydrazine. Although these choices are somewhat arbitrary, the experimental data does warrant their selection. Calculated acclimation potentials for continuous addition are listed in Table 12.

Values for estimated acclimation potential listed in Table 12 demonstrate conclusively that the methanogens can acclimate to unexpectedly high concentrations of the three toxicants tested. With chloroform, except for the 42.5° systems, it appears that the methanogens can acclimate to 20 times the concentration causing inhibition to unacclimated methanogens, with nickel the values are 2-4, and with hydrazine the values range from 2.5 to 5. The chloroform data agree well with the estimate of 25 offered by Yang et al (1980). The anomaly observed for the 25-day, 25° hydrazine system was most likely due to the selection of concentrations used for the continuous addition studies (5, 25, and 50 mg/l).

There does not seem to be a clear pattern as a function of SRT, although in general, the 50-day systems gave consistently higher values for AP. That the effect was not clear may be due to the range of toxicant concentrations selected. If higher concentrations had been selected, perhaps the 50-day systems would have acclimated while the 15- and 25-day systems would not. The fact remains that SRT is very important in assuring or optimizing acclimation potential since if still-viable organisms are wasted from the system prior to acclimation (SRT too low), the system may fail.

Table 12. Estimated Acclimation Potential (AP) of Methanogens to Continuous Addition of Selected Toxicants

Toxicant-Condition	Maximum Concentration[1] with No Effect	Estimated Acclimation Potential
Nickel		
15-day SRT		
25°C	100	2
35°C	200	4
42.5°C	200	4
25-day SRT		
25°C	100	2
35°C	200	4
42.5°C	100	2
50-day SRT		
25°C	200	4
35°C	200	4
42.5°C	100	2
Chloroform		
15-day SRT		
25°C	20	20
35°C	<5*	<5*
42.5°C	10	10
25-day SRT		
25°C	>20	>20
35°C	>20	>20
42.5°C	<5	<5
50-day SRT		
25°C	>20	>20
35°C	>20	>20
42.5°C	<5	<5
Hydrazine		
15-day SRT		
25°C	25	2.5
35°C	25	2.5
42.5°C	>50	>5
25-day SRT		
25°C	5	0.5<AP<2.5**
35°C	>50	>5
42.5°C	25	2.5
50-day SRT		
25°C	50	5.0
35°C	50	5.0
42.5°C	25	2.5

[1] mg/l

* probably an experimental anomaly (Figure 153) -- estimate is probably low.

** see text for explanation

Comparison of Slug and Continuous Addition

Comparing estimated values of acclimation potential listed in Tables 11 and 12, it is evident that the acetate-splitting methanogens have significant potential for acclimation regardless of application pattern or nature of toxicant. It must be borne in mind that an AP of 1.0 doesn't necessarily indicate no potential for acclimation, it just means that none was observed at the concentrations tested. This is especially true for the slug addition studies.

The significantly large AP values for chloroform may suggest metabolism. However, experiments conducted in our lab (Yang, 1981) have tentatively concluded that the enrichment culture of methanogens does not have the ability to metabolize chloroform. With that in mind, it would seem highly unlikely that any other toxicant tested would be metabolized. Thus the mechanism most likely involve adaptation of enzyme systems, chemical/physical reduction of toxicant concentration (e.g. precipitation), or a combination of both.

There seems to be no clear pattern with respect to SRT, although for both slug and continuous addition, the larger SRT gives somewhat higher values for AP. With little exception, 42.5°C seems to result in the least potential for acclimation.

REVERSIBILITY

The magnitude of the decrease in methane production and rate of return to full gas production was a function of toxicant type and concentration, and duration of exposure to toxicant in the liquid phase. Data presented in the RESPONSE TO SLUG ADDITION section showed that all toxicants were reversible to a certain degree since for each toxicant, at least some systems recovered to full methane production. Results from specific reversibility studies (Figures 168 to 171) showed that chloroform, nickel, and sulfide exhibited some residual toxicity. Other studies (Parkin et al, 1980 -- Figure 172) showed that ammonium and cyanide toxicity were very reversible. Thus it seems that the mechanism of toxicity is definitely toxicant-type dependent.

The practical implications of these results lies in the bearing they may have on selecting the process configuration used to treat industrial wastewaters containing toxic substances. Previously it was thought to be an advantage of a complete-mix hydraulic regime (CSTR) that toxicants would be rapidly diluted, thus lessening their impact on anaerobic treatment efficiency. However, the toxicant slug would also be diluted from the system relatively slowly, resulting in protracted recovery periods as shown by the control systems used in this study. The submerged anaerobic filter, anaerobic fluidized bed, and the upflow anaerobic sludge blanket process, on the other hand, operate predominantly in a plug-flow mode, resulting in no initial dilution of the toxicant slug, but rapid elution of the toxicant from the system. For certain types and concentrations of toxicants, it may be better to elute toxic substances quickly with no dilution (the filter) than to rapidly dilute but elute toxicants slowly (CSTR). The above-mentioned process configurations provide for separation of HRT (liquid throughput) and SRT (biomass retention), thus allowing for acclimation (high SRT required) and reversibility. Provisions for recycle shifts the flow mode from the plug-flow towards CSTR. In view of the presented results, such activity may result in decreased process efficiency.

An additional implication is that an operator of an anaerobic treatment system need only remove the adulterated liquid from the system to accelerate recovery from toxicity. If the biomass is removed, start-up will be protracted due to the slow growth rate of the methane bacteria, thus making the operation very expensive.

KINETICS OF TOXICITY

As described in the proposal, data from serum bottles (125-150 ml capacity) were to be used to develop kinetic models. Unfortunately, the distinct advantage of using serum bottles to catalogue response patterns, acclimation potential, reversibility, and the effect of SRT and temperature becomes a disadvantage when attempting to develop fundamental kinetics. Hundreds of serum bottles can be operated daily without much trouble, but analysis of serum bottles is necessarily limited to gas production due to time and available sample size constraints.

It was proposed to use the method of Halwachs (1978) for analysis of serum bottle data. Unfortunately, this technique was much too sensitive to allow for adequate determination of requisite coefficients. Other methods (Sundstrom and Klei, 1979; Connotte and Prins, 1979; Lee and Ryn, 1979) were tried unsuccessfully. It was concluded that larger scale, continuous-feed systems would be required to allow collection of sample sizes large enough for measurement of critical parameters (VSS, toxicant, HAc, ATP, etc.). Preliminary trials with the infinite dilution technique of Williamson and McCarty (1974) showed that this method may also permit measurement of toxicity kinetics. Time did not permit us to complete construction and calibration of the systems required for this type of work.

With these comments as background, the following discussion is presented relative to modeling the kinetics of methane fermentation systems exposed to toxicants.

Model of Response to Slug Addition of Toxicants

A model describing the recovery of methane bacteria following a slug dose of toxicant would be helpful in defining system operating strategies and provide insight into the toxicity phenomenon. The model should describe the recovery pattern as a function of toxicant type and concentration, and time. The shape of the observed recovery curve is similar to the classical dissolved oxygen sag curve and thus an equation similar to the D.O. sag equation was used to empirically model system recovery:

$$G_t = Ae^{-k_1 t} + Be^{k_2 t}$$

where G_t = methane production, ml/day

A,B = empirical constants

t = time after addition of toxicant, days

k_1 = "toxicity" rate constant, day^{-1}

k_2 = "acclimation" rate constant, day^{-1}

Values of k_1, k_2, and B were determined from a regression analysis of experimental data. An example of measured versus calculated values of methane production for calcium toxicity is shown in Figure 175. The above equation adequately describes experimental data for most conditions and toxicants.

The constants A and B and rate constants k_1 and k_2 are function of toxicant type and concentration. In order to develop a predictive model, generalized expressions for these constants must be derived. As an initial approach, the following equations were tried:

$$k_1 = C(I_o)^c$$

$$k_2 = D(I_o)^d$$

$$B = E(I_o)^e$$

$$A = \text{Control methane production} - B$$

I_o represents the concentration of toxicant in the serum bottle immediately following slug addition and C, D, E, c, d, and e are constants determined from a regression analysis.

As examples of the model, a comparison of measured versus predicted methane production for chloroform and nickel toxicity is shown in Figures 176 and 177. For a first attempt, the model appears to fit well, allowing for prediction of down time (periods of zero methane production) as a function of initial toxicant concentration, and thus define control strategies for process recovery. In addition, the model could be used to predict threshold toxicant concentrations. The model was applied to other experimental data from our lab and described response to cyanide, formaldehyde, and copper very well (see item 2, LIST OF PUBLICATIONS AND PRESENTATIONS).

As presently constructed, this model is strictly an empirical description of experimental data, although a useful one. The fact that it did not

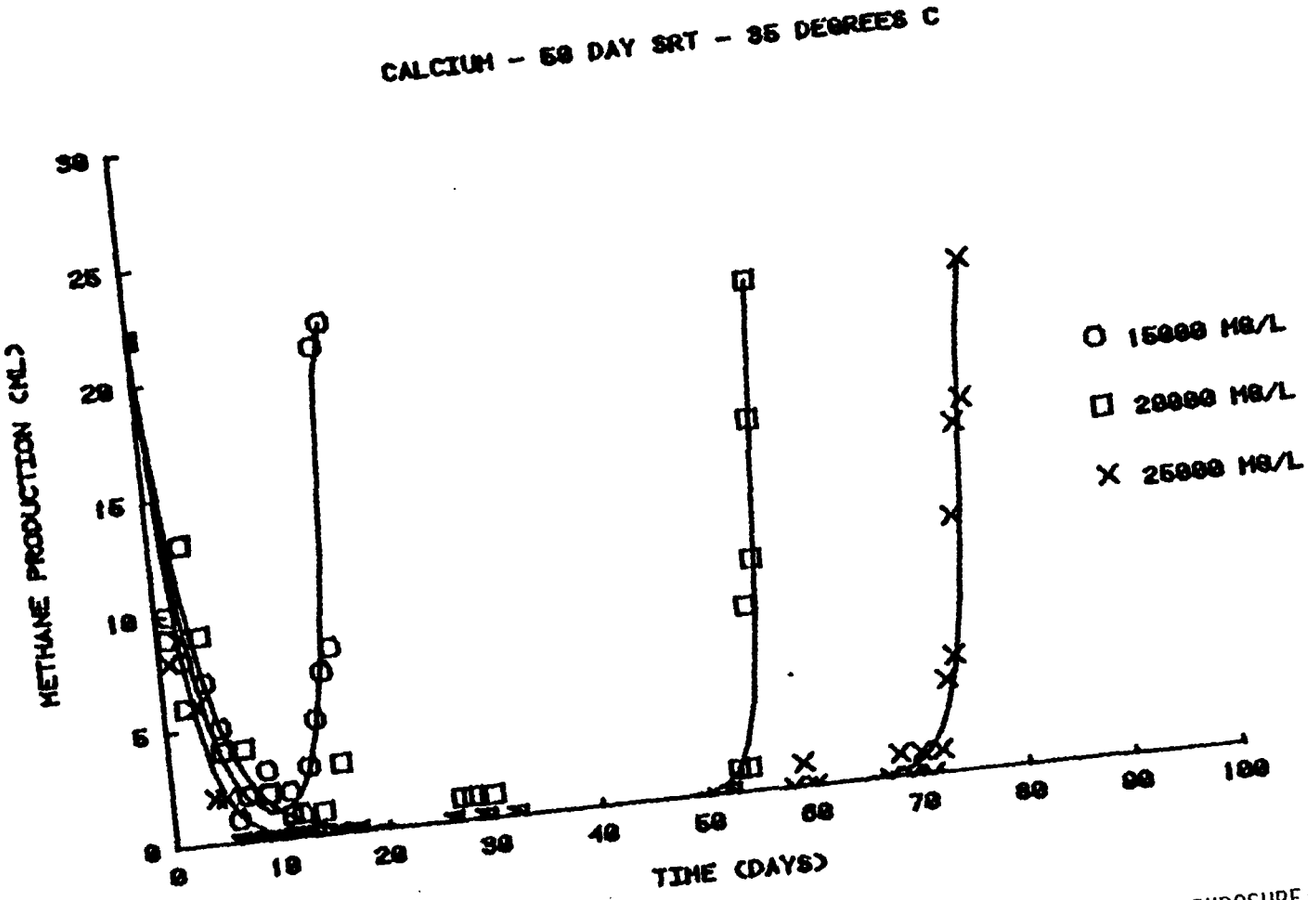


FIGURE 175. MEASURED VS PREDICTED METHANE PRODUCTION FOR TOXICANT EXPOSURE: CALCIUM

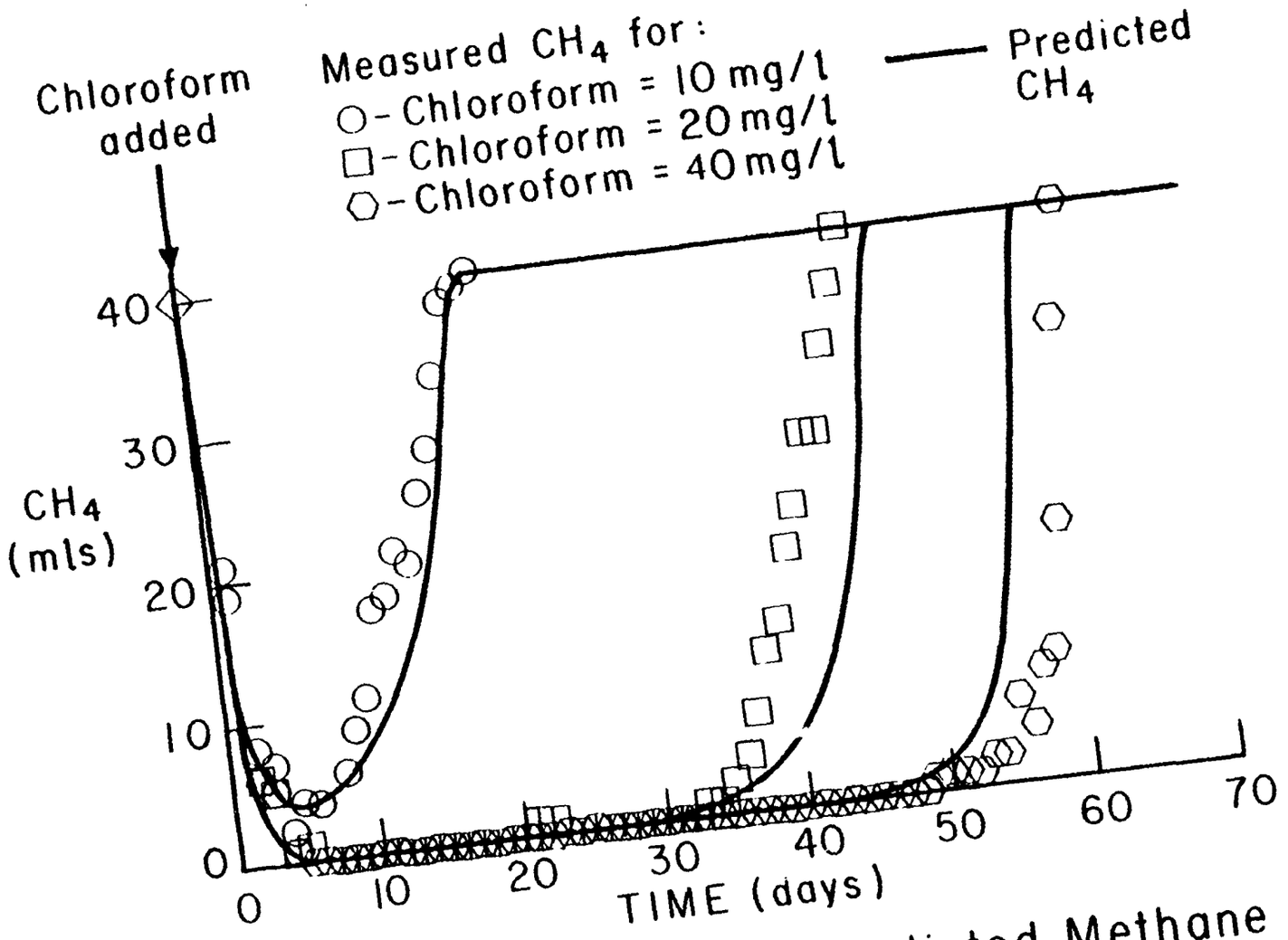


Fig. **176** Measured Versus Predicted Methane Production for Toxicant Exposure: Chloroform

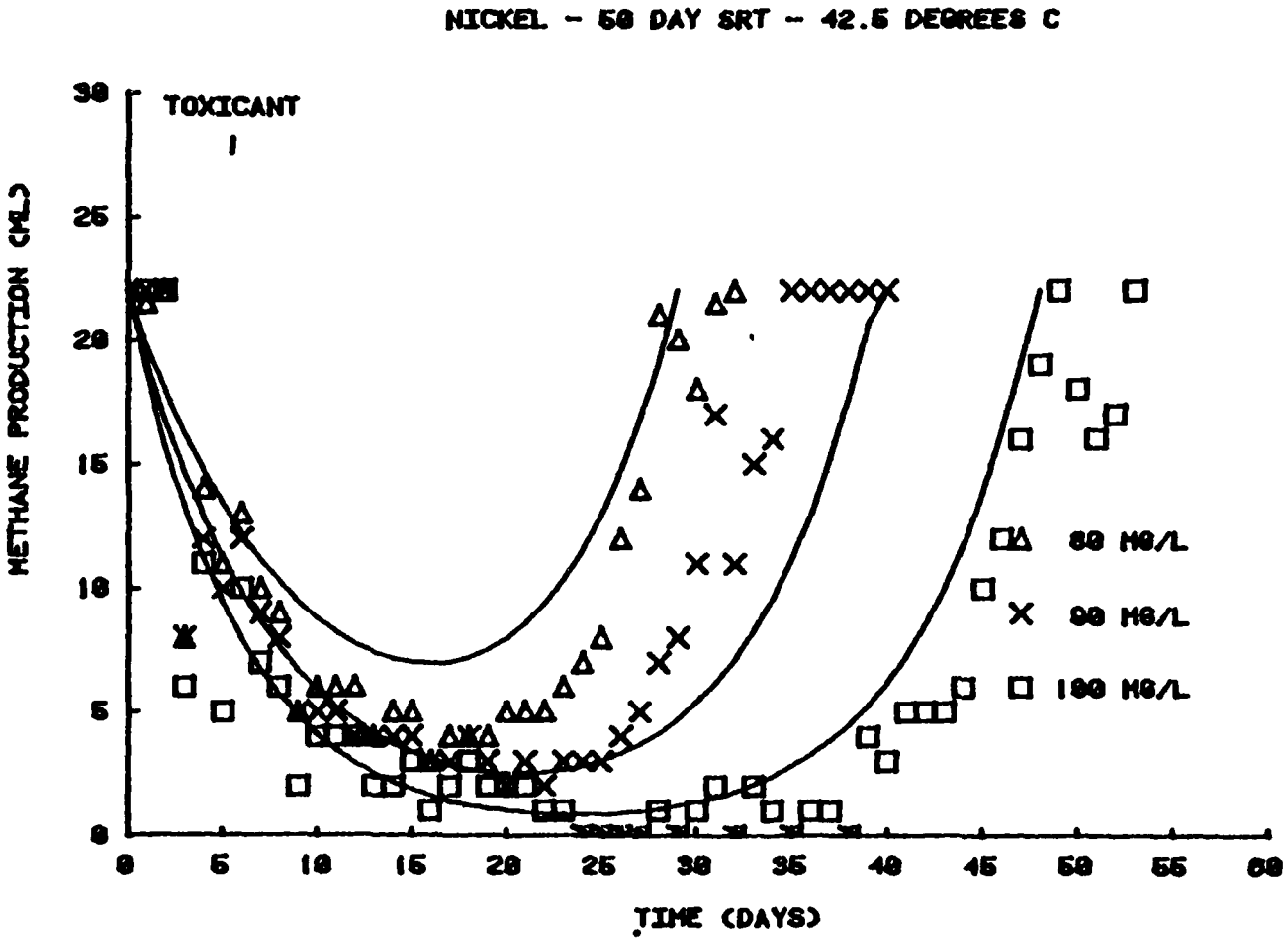


FIGURE 177. MEASURED VS PREDICTED METHANE PRODUCTION FOR TOXICANT EXPOSURE: NICKEL

work well for all toxicants studied could be due to its empirical nature and/or the fact that some experimental results did not follow a pattern that could be used. Some systems responded too quickly to be modeled and for some toxicants, only one or two concentrations resulted in sufficient down time for modeling.

Toxicity Kinetics - Alteration of Monod Equations

The following two models use the traditional Monod-type equation as a starting point for developing a means of describing toxicity kinetics:

$$\frac{-dS}{dt} = \frac{kSX_a}{K_s + S} \qquad (1)$$

Such models should be particularly useful in providing insight into the toxicity phenomena and in defining design strategies. For purposes of this paper, S represents acetate concentration in mg/l, k represents specific substrate utilization rate in mg acetate utilized per day per mg of methane bacteria. X_a represents the concentration of active organisms. In practice, active organism concentration is usually estimated by measuring volatile suspended solids concentration. The product of k and X_a is termed V_{max}. K_s represents the Monod half-velocity constant in mg/l acetate.

Rational design of methane fermentation system usually includes use of the above equation in combination with the bacterial growth equation (Lawrence and McCarty, 1970). As mentioned previously, conversion of acetate to methane is recognized as the rate-limiting step in anaerobic treatment and thus modeling acetate utilization should adequately describe kinetics of methane fermentation of complex organics.

Normal design procedure involves predicting minimum bacterial generation time, θ_c^{lim}, or bacterial washout time, θ_c^{min}:

$$\text{Minimum bacterial generation time} = \theta_c^{lim} = (Yk-b)^{-1}$$

$$\text{Bacterial washout time} = \theta_c^{min} = \left(\frac{YkS_o}{K_s + S_o} - b \right)^{-1}$$

Y represents bacterial yield in mg bacteria synthesized per mg acetate utilized and b is the bacterial decay rate in inverse days. The design solids retention time is calculated by applying a safety factor to θ_c^{lim} or

θ_c^{min}. The effect of design θ_c on process efficiency can be demonstrated by assuming steady-state conditions for a continuously fed, complete-mix reactor (CSTR). Figure 178 contains a family of these design strategy curves.

The effect of changing the specific utilization rate, k, is also demonstrated in Figure 178. Process efficiency is very sensitive to changes in k. (From this curve, the values of θ_c^{min} seem abnormally high. This is an aberration of the values used for Y and b, which are abnormally low and high, respectively. The relative shape as affected by k is the important observation.) Changes in K_s also dramatically affect process performance (Figure 179). It is worth noting that changes in k are associated with non-competitive inhibition and changes in K_s are associated with competitive inhibition (Lehninger, 1976). Process efficiency is much less sensitive to changes in Y and b. These effects will now be developed in more detail. The important point to make here for future reference is that a sufficiently high θ_c should minimize any adverse effect caused by changes in kinetic coefficients.

Algorithms of k and K_s. Addition of toxicants alters the kinetics of methane fermentation. In the first approach altering Monod kinetics for toxicity, the assumption is made that the effect of toxicity is manifested by decreases in k and/or increases in K_s. The response to slug additions of toxicants shown in Figure 180 can be modeled by approximating the unsteady-state behavior using steady-state assumptions. That is, if each day is considered to exhibit steady-state behavior, algorithms for k and K_s can be developed as functions of time after exposure to slug additions of toxicant. Conceptual plots of V_{max}, the product of k times X_a, and K_s versus time are shown in Figure 181. Return of these coefficients to "non-toxic" values is accomplished by a combination of acclimation, metabolism, and toxicant washout.

Determination of kinetic coefficients can be made in several ways. Semi-continuous systems can be considered to be batch systems fed once daily. One general approach is to integrate the basic substrate utilization equation and linearize it. One such linearization was developed by Halwachs (1978) for enzyme kinetics and was tried in this study to determine values for k and K_s. Batch data can be collected each day, or specific batch experiments can be conducted, and values for k and K_s determined. An example of such an analysis is shown in Figure 182 for a batch experiment studying formaldehyde toxicity.

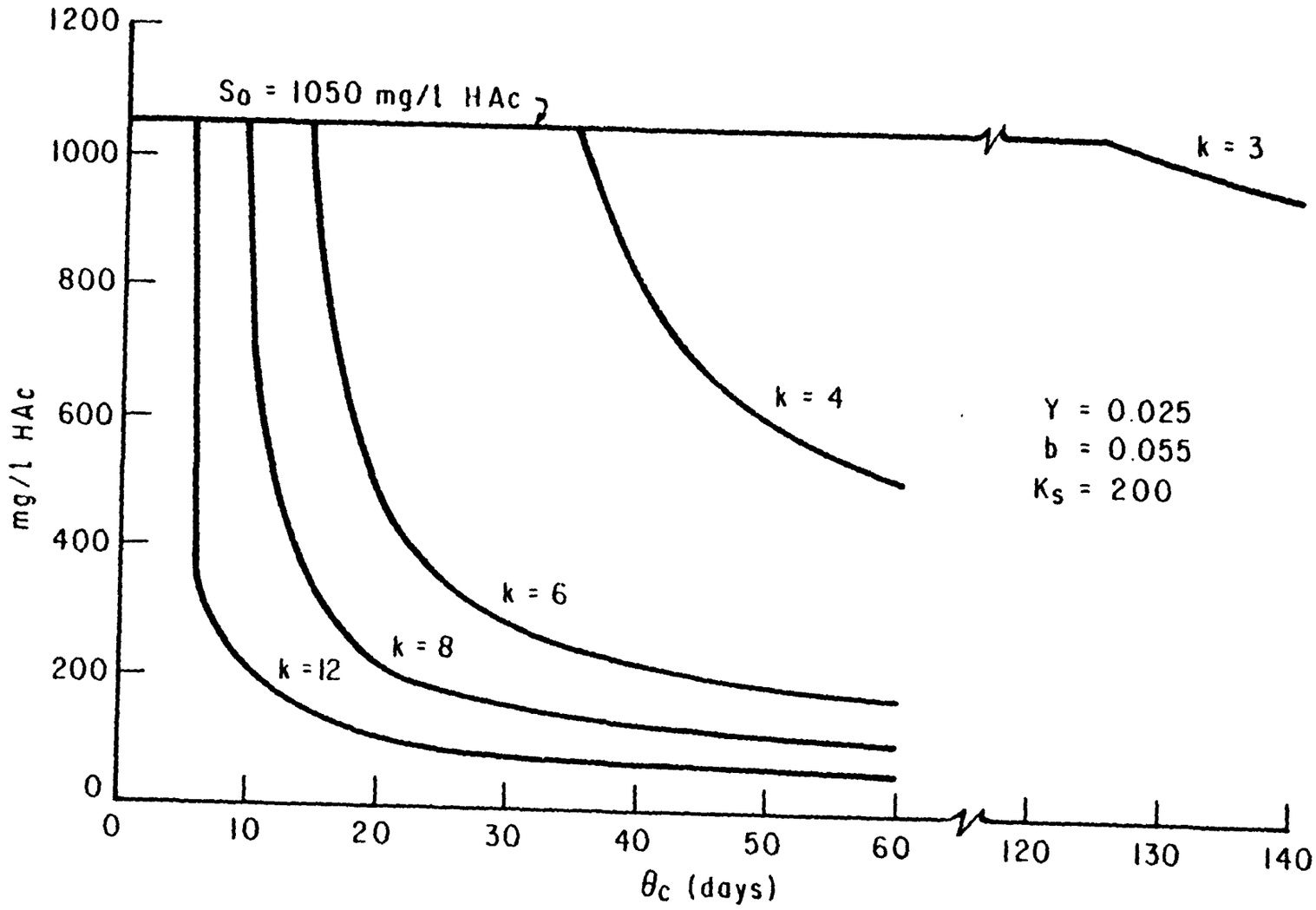


Fig. 178 Effect of k on Effuent Acetic Acid (HAc) Concentration

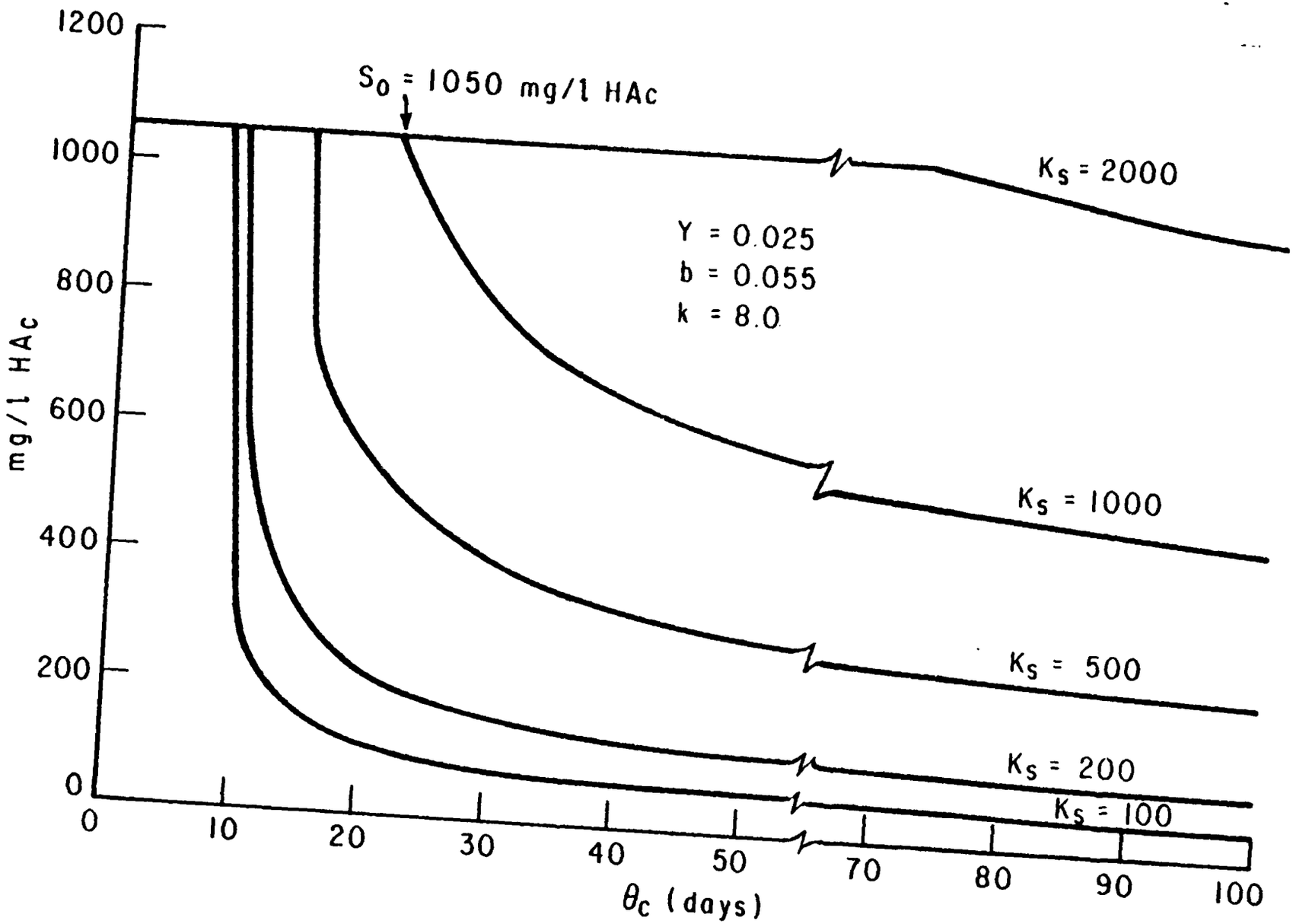


Fig. **179** Effect of K_S on Effluent Acetic Acid (HAc) Concentration

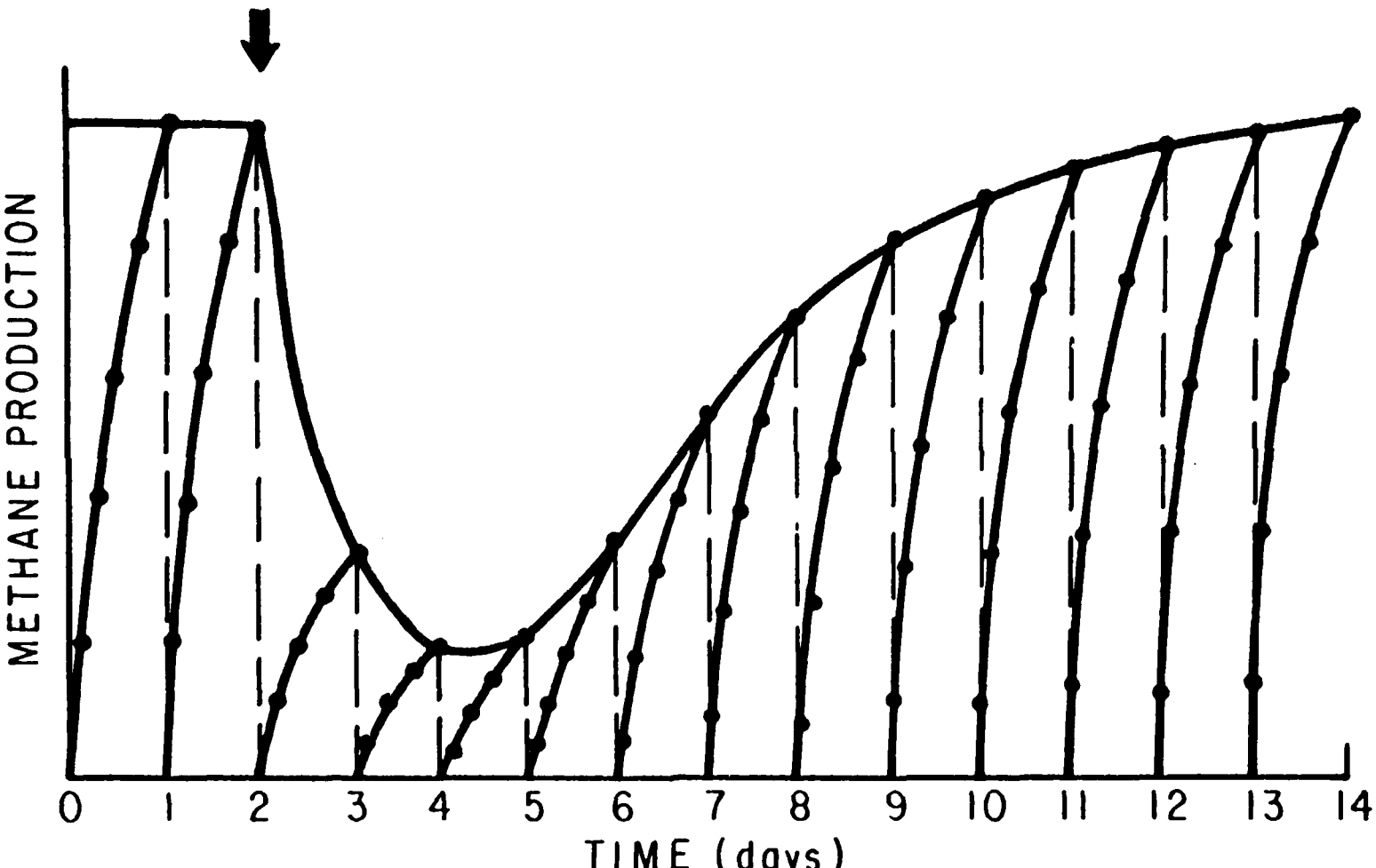
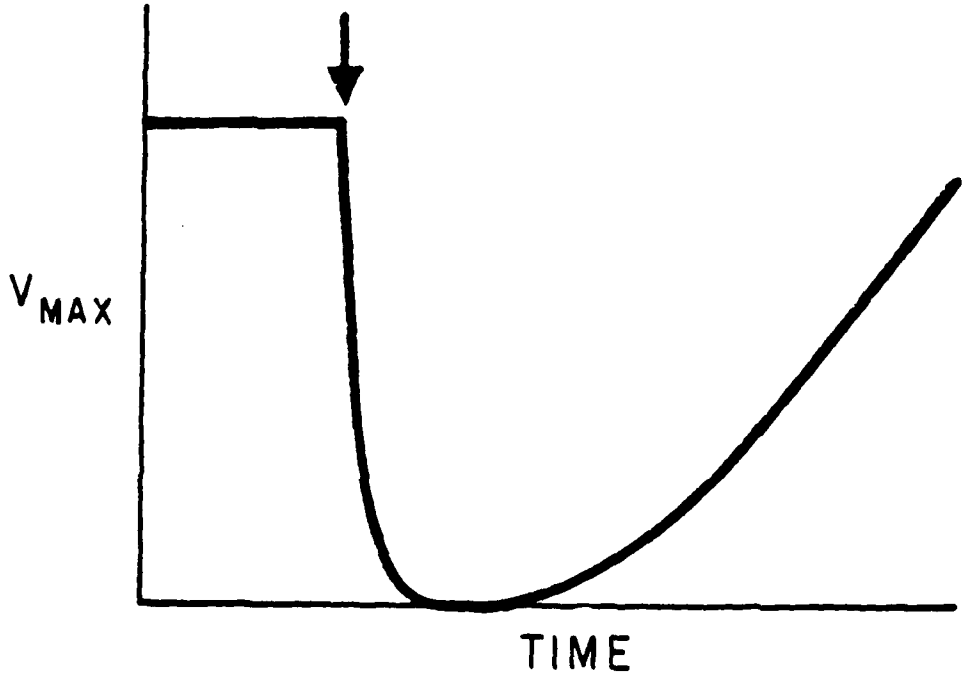


Fig.180.Effect of Slug Addition of Toxicant on Daily Methane Production



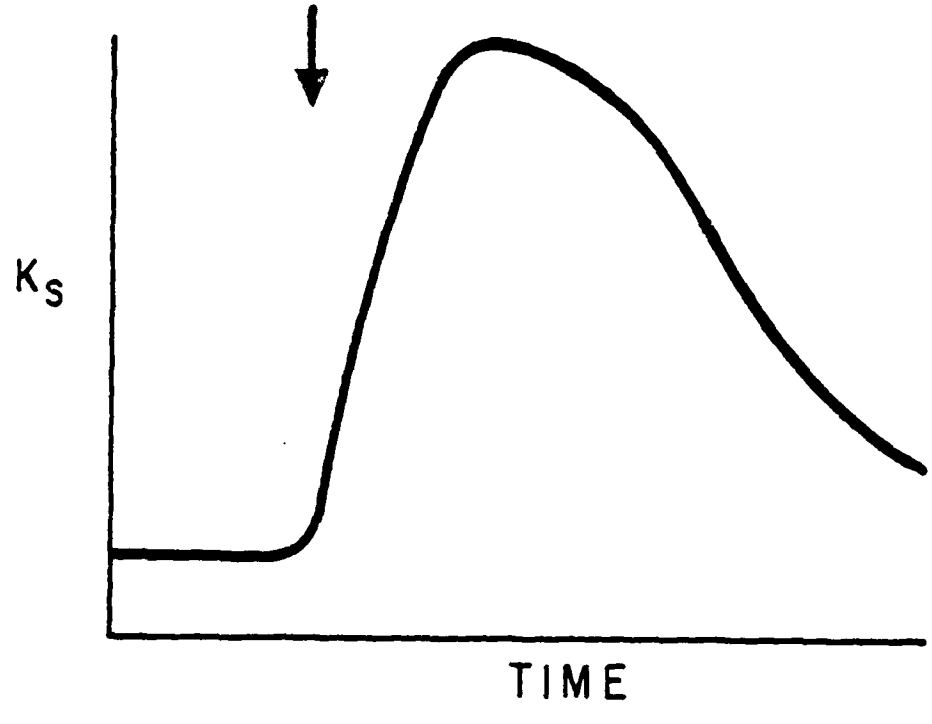


Fig.181 Acclimation Effect of Toxicant on V_{max} and K_S

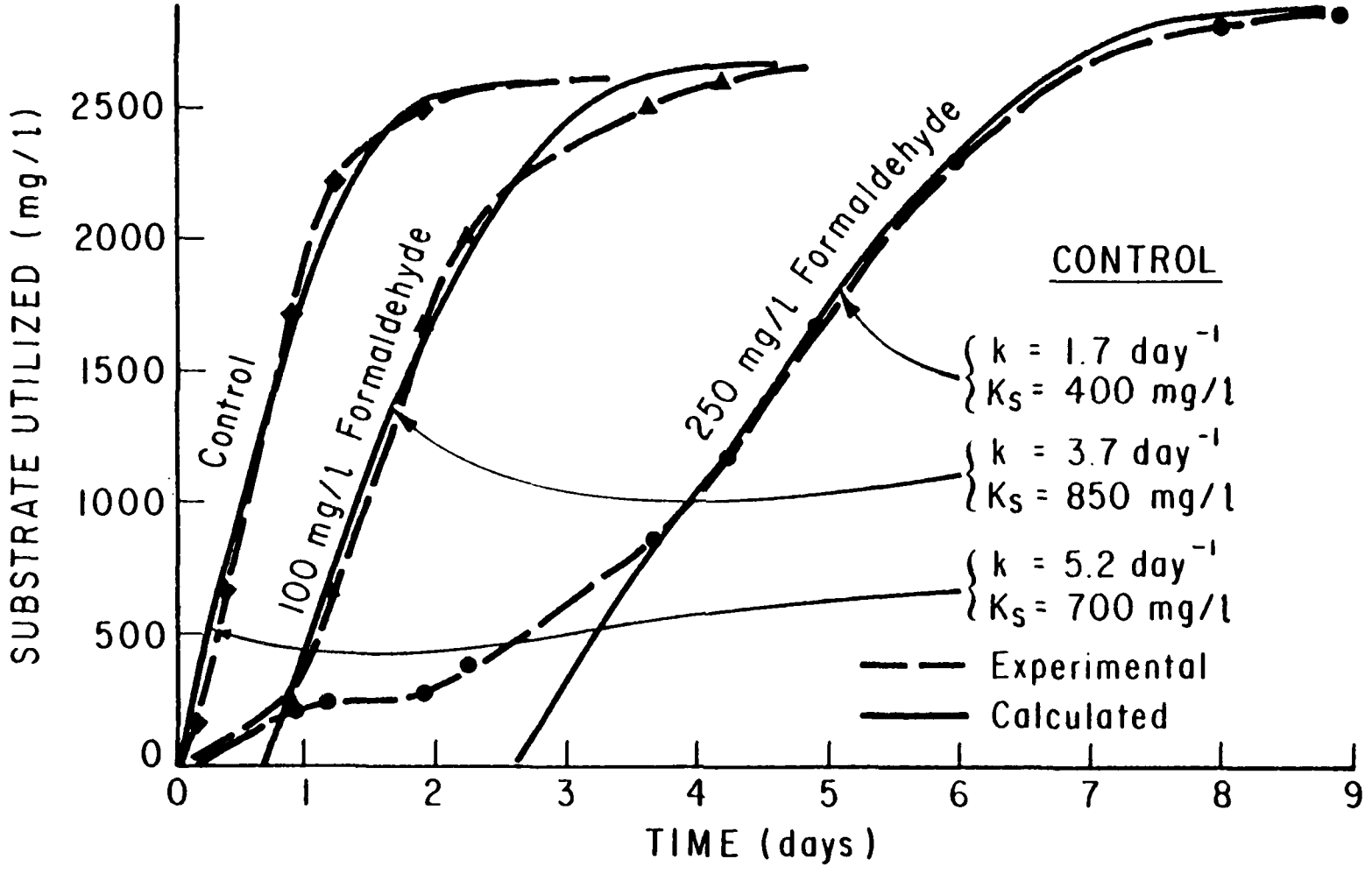


Fig. **182** Experimental and Calculated Fit of Toxicity Kinetics – Formaldehyde

The data in Figure 182 was taken from Speece et al (1979) and is used to demonstrate the usefulness of the model being described. As was mentioned earlier, the Halwachs method, along with several other techniques, did not allow calculation of kinetic coefficients from data generated in this study. For example, data from batch experiments with nickel and sulfide are shown in Figures 183 and 184 could not be analyzed by any of the techniques tried. However, the pattern is similar to that for formaldehyde and thus the concepts used in the model are definitely valid; it is the nature of the experimental data that is deficient.

The effect of adding increasing amounts of toxicant is to dramatically reduce k and significantly alter K_s. That K_s increased for a formaldehyde concentration of 100 mg/l and then decreased for 250 mg/l may not be an experimental anomaly. Noting that K_s is the acetate concentration at which the utilization rate is half the maximum, it is conceivable that the acetate concentration at which the utilization is half the maximum decreases with the addition of toxicants. Of course, experimental error may also explain the behavior.

Assuming steady-state conditions in a CSTR, the effect on process performance of changing values of k and K_s can be seen in Figure 185. A formaldehyde concentration of 250 mg/l temporarily increases the bacterial washout time from about seven days for a control system to near 27 days. Values for Y and b were estimated from other experiments. If the safety factor for design had yielded a design θ_c of 20 days, a fairly typical value, the system would tend toward failure because the "actual" washout time would be exceeded. Whether or not the system fails would also depend on the initial slug-dose concentration, as was demonstrated by the toxicity response curves presented earlier.

Although the curves shown in Figure 185 were generated assuming steady-state conditions, the approach can be used to describe unsteady-state behavior. The "dynamic" nature of θ_c^{min} with toxicant exposure is demonstrated in Figure 186. After addition of the toxicant, θ_c^{min} increases with concomitant decreases in methane production and process efficiency. While drawn as a steady-state response for each of the five conditions, the "elastic" nature of θ_c^{min} is evident. Actual treatment systems are operated at some reasonably fixed θ_c and thus, temporarily at least, the system will be operating under washout conditions after exposure to toxicants. It is also apparent that failure of

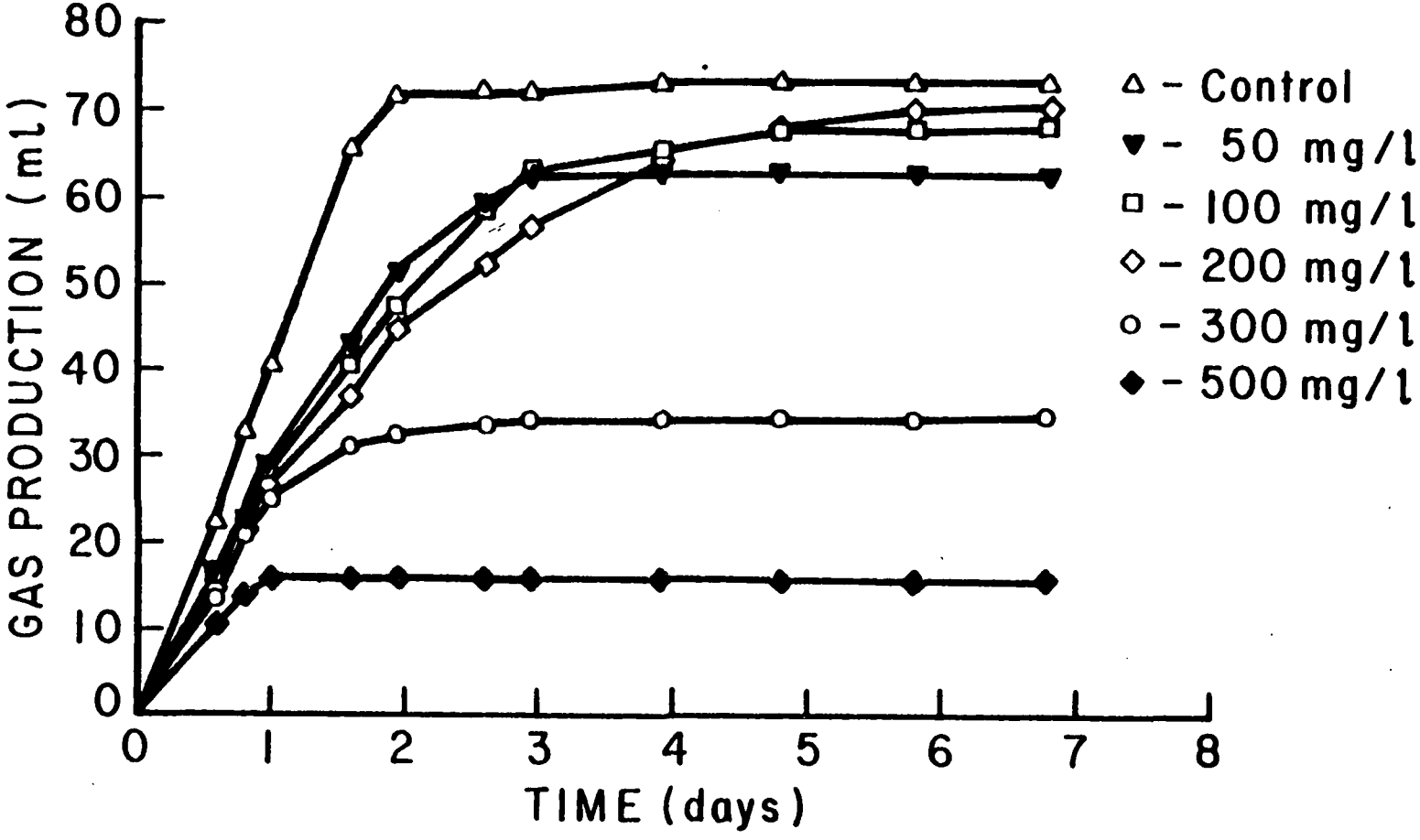


Fig. 183 Response of Unacclimated Methanogens to Nickel

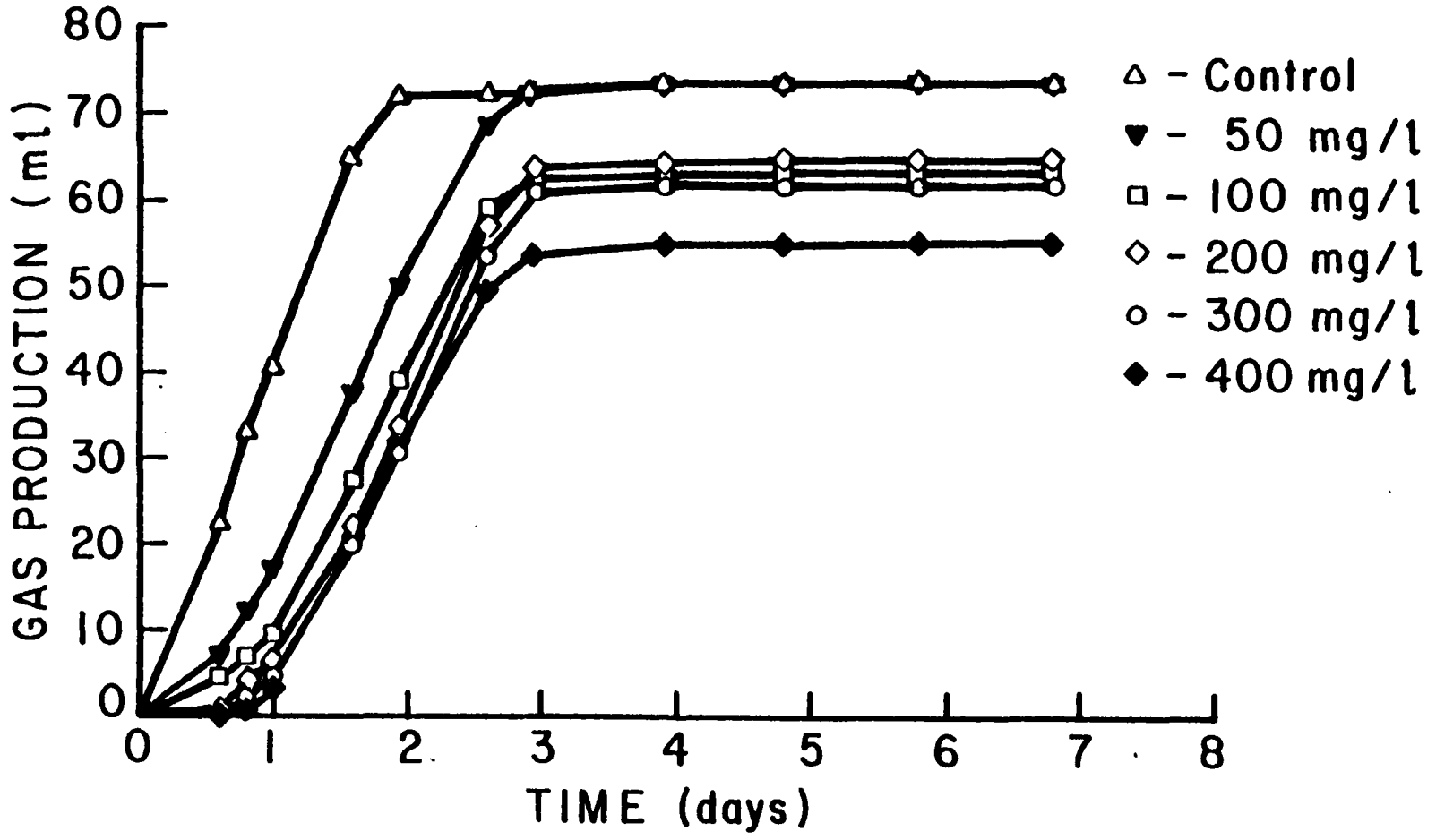


Fig. 184 Response of Unacclimated Methanogens to Sulfide

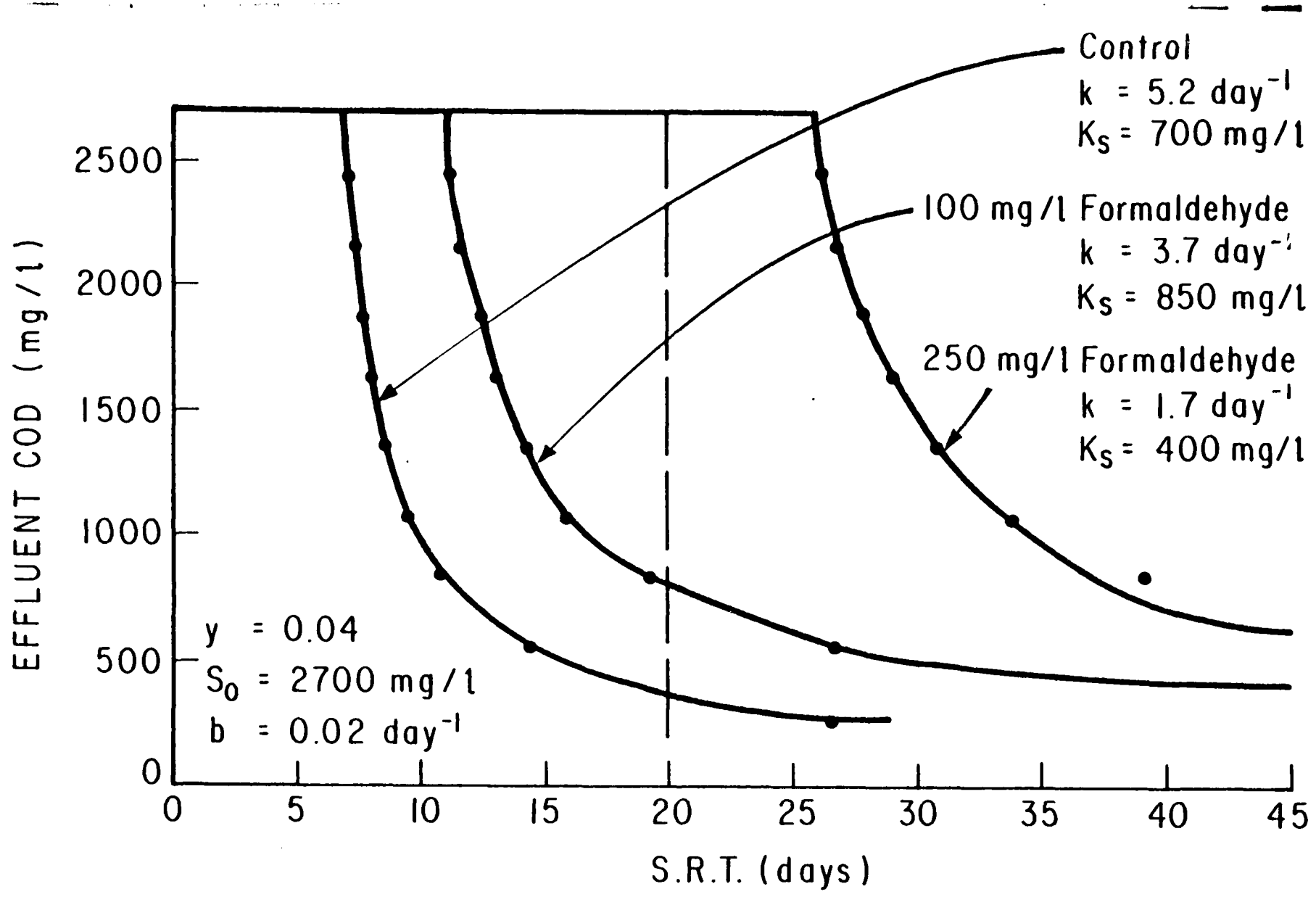


Fig. **185** Steady State Charateristics for Toxicity Kinetic Parameters – Formaldehyde

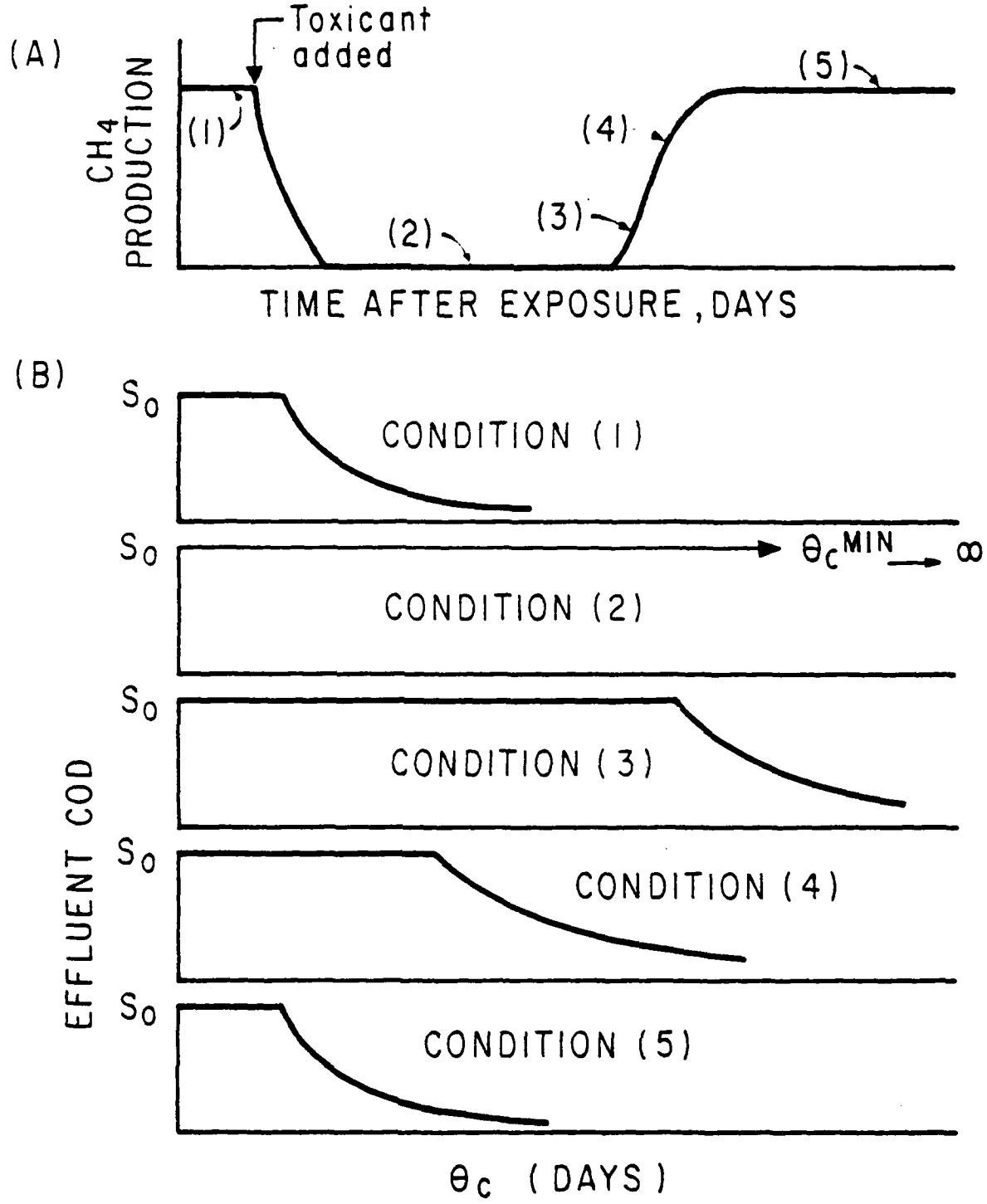


FIG. 186 EFFECT OF TRANSIENT NATURE OF TOXICITY RESPONSE ON PROCESS EFFICIENCY

methane fermentation systems is not instantaneous, but a function of time. A sufficient biological safety factor in the form of a proper design θ_c guards against the above-described failure and allows for acclimation to and/or metabolism of the toxicant.

The practical significance of this model is that it points out the need for sufficiently large values of θ_c when treating wastewaters or sludges containing chronic and transient toxicity. This need has not been sufficiently recognized by design engineers. Not only does proper θ_c insure maximum process stability, it also allows for prolonged operation under temporary washout conditions prior to ultimate failure, a buffer that may give operational personnel adequate time to find and eliminate the cause of toxicity. If a CSTR is used, a large tank is required and/or biological solids recycle may be required, a combination that is very expensive. The submerged anaerobic filter, fluidized bed, anaerobic upflow sludge blanket, and the anaerobic rotating biological contactor (RBC) all offer inherently high values of θ_c at relatively low hydraulic detention times, thus minimizing reactor cost without sacrificing the required high θ_c. These reactors would thus seem to be able to withstand both transient and chronic toxicity better than suspended growth systems for wastewater containing predominantly soluble organics.

We have tried a number of different approaches to determine daily kinetics or batch kinetics for toxicant exposure. For some toxicants like acrylic acid, analysis of the linearization of the basic substrate utilization equation yielded negative values for k and K_s. However, a first-order approximation appears to work well. There is a fundamental basis for using a first-order approach: as K_s gets much larger than S, the basic substrate utilization equation reduces to a first-order equation. However, even if the kinetics reduce to first order, or some other type of expression, the conceptual model shown in Figure 186 still holds: θ_c^{min} is "elastic" and to prevent failure a sufficiently large design or operational θ_c is required.

Inhibition Coefficient Model. A more absolute method of describing inhibition and toxicity kinetics would be desirable. In the following model, the inhibition equations given by Lehninger (1976) in which inhibition coefficients are added to the basic substrate utilization equation will be used. Lehninger describes three types of reversible inhibition: competitive,

uncompetitive, and non-competitive. All of the over 30 toxicants studied in our lab have demonstrated some reversibility. The effect of these types of inhibition on the substrate utilization equation is demonstrated below:

$$\text{Competitive:} \quad -\frac{dS}{dt} = \frac{k\ S\ X_a}{K_s\ (1+T_x/K_I) + S} \tag{2}$$

$$\text{Uncompetitive:} \quad -\frac{dS}{dt} = \frac{k\ S\ X_a}{K_s + S(1+T_x/K_I)} \tag{3}$$

$$\text{Non-competitive:} \quad -\frac{dS}{dt} = \frac{k\ S\ X_a}{(K_s+S)\ (1+T_x/I_I)} \tag{4}$$

T_x represents the concentration of toxicant in mg/l and K_I is the inhibition coefficient in mg/l of toxicant. K_I is a function of toxicant type, time (acclimation), and other factors.

For slug doses of toxicants, the typical unsteady-state response curve shown in Figure 186 can be generated in a manner similar to that used for the preceding model. After addition of toxicant, T_x and K_I will change due to acclimation, metabolism, and washout. Values for K_I can be generated from experimental data by integrating the inhibition equations and solving for K_I. Batch data, daily data from semi-continuous systems, or chemostat data can be used, providing T_x, S, and VSS are measured. The shape of a K_I versus time curve should be identical to the gas production versus time response shown earlier (K_I equals infinity when there is no toxicant present or complete recovery has occurred).

The effect of chronic toxicity on system performance can be seen by assuming a steady-state, CSTR system and varying T_x and/or K_I. Equations resulting from such analysis are:

$$\text{Competitive:} \quad S = \frac{K_s\ (1+b\theta_c)\ (1+T_x/K_I)}{\theta_c\ (Yk - b) - 1}$$

$$\text{Uncompetitive:} \quad S = \frac{K_s\ (1+b\theta_c)}{\theta_c\ [Yk - b\ (1+T_x/K_I)] - (1+T_x/K_I)}$$

$$\text{Non-competitive:} \quad S = \frac{K_s\ (1+b\theta_c)\ (1+T_x/K_I)}{\theta_c\ [Yk - b\ (1+T_x/K_I)] - (1 +T_x/K_I)}$$

Figures 187 and 188 show the effect of T_x and K_I on process performance for non-competitive inhibition. Similar curves can be constructed for competitive

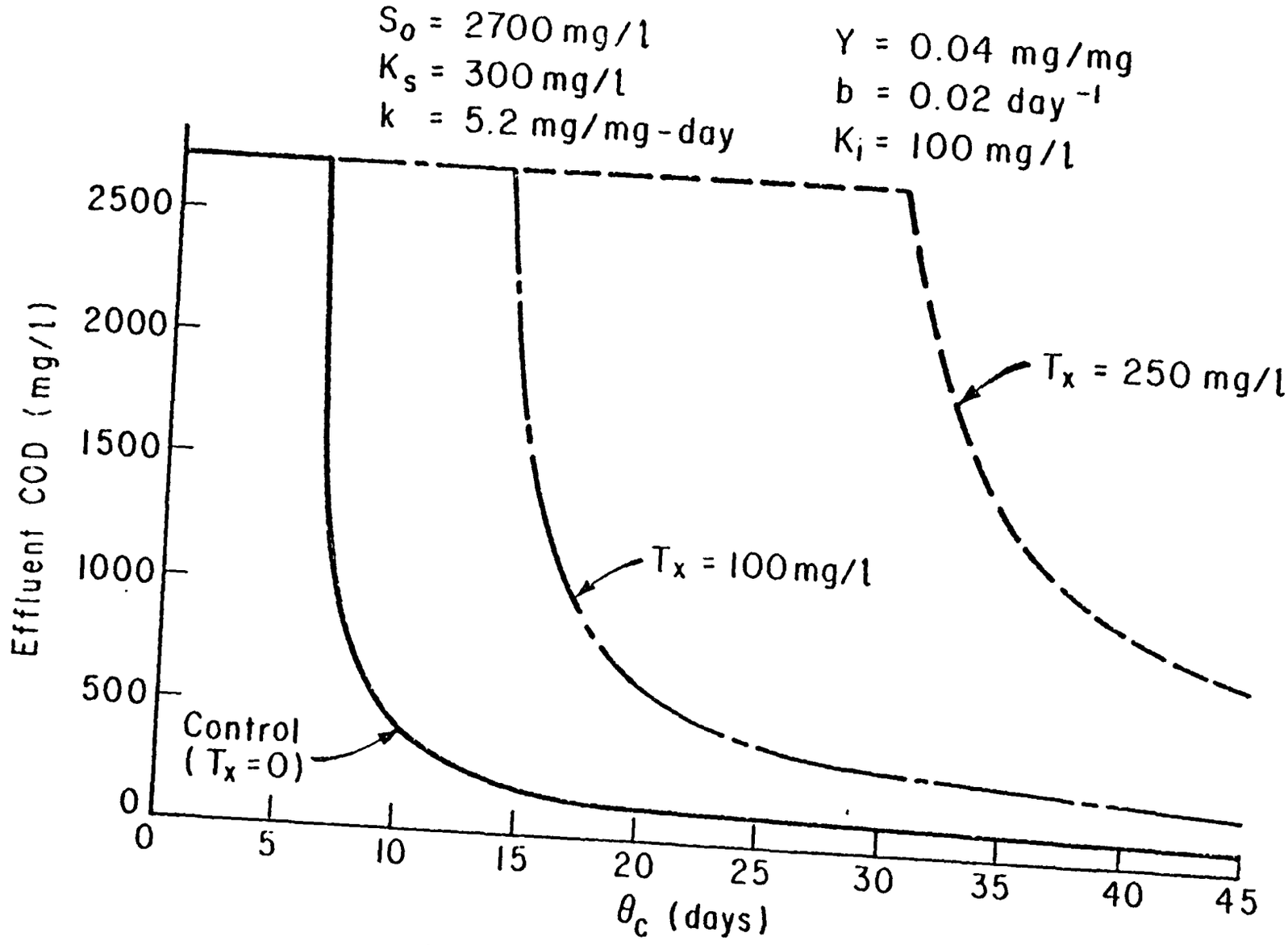


Fig. **187** Non – Competitive Inhibition
Effect of T_x on Effluent COD

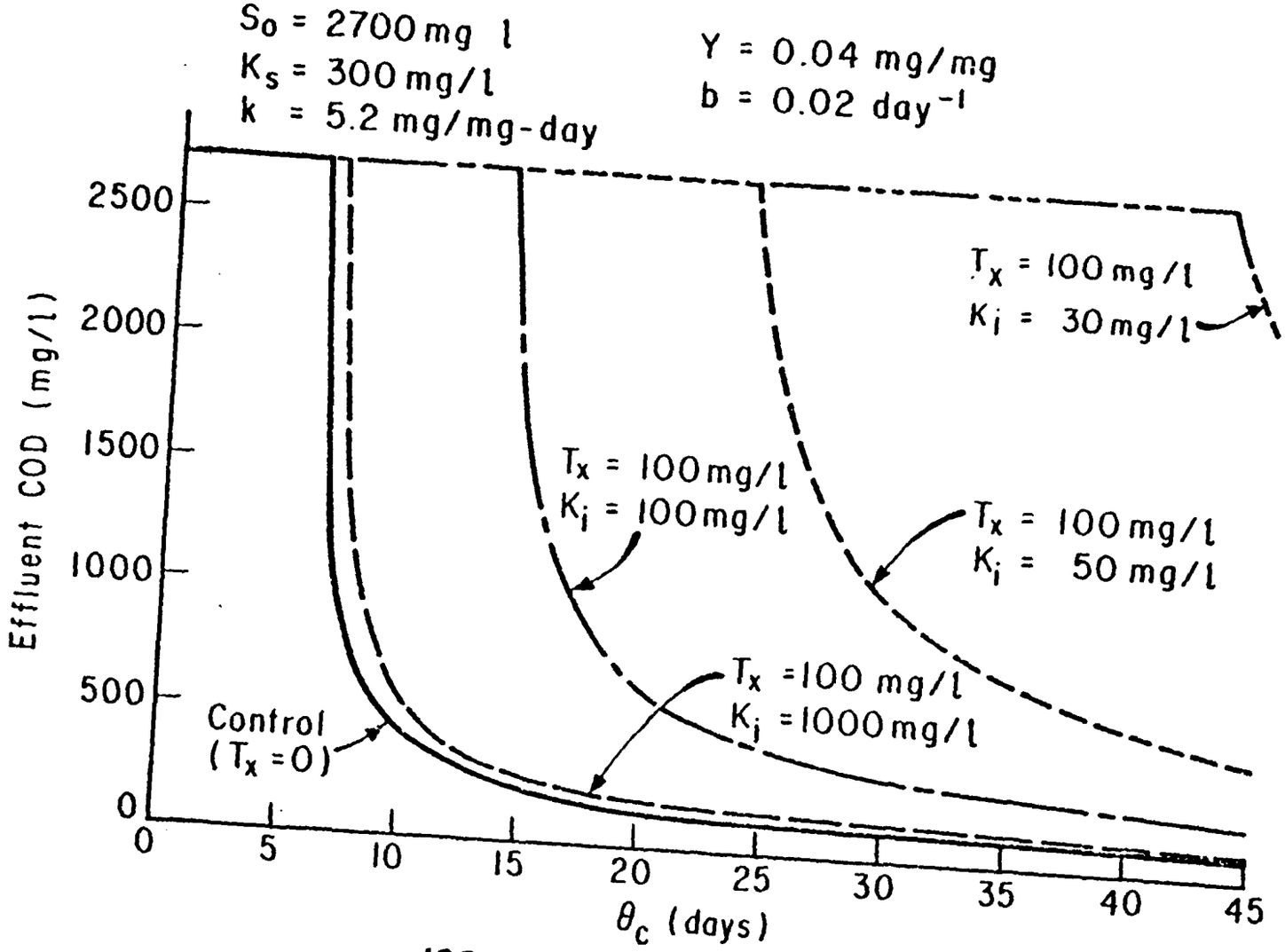


Fig. **188** Non-Competitive Inhibition
Effect of K_i on Effluent COD

and uncompetitive inhibition. Evaluation of K_I can be made from laboratory experiments using chemostats. Nuefeld and Valiknac (1979) have used this type of approach to describe toxicity kinetics for the aerobic treatment of phenol in the presence of thiocyanate.

The important aspect to note once again is the increase in θ_c^{min} caused by toxicant addition; the shape of the process efficiency curve is identical to the one developed from the previous model. If process efficiency is not to be severely decreased, a sufficiently large θ_c is required. For example, with no toxicant present, a design θ_c of 15 days may yield a safety factor of 3, while in the presence of a toxicant, a θ_c of 100 days may be required to give a similar safety factor. To insure against process failure or severe retardation of methane generation, provisions must be made to maintain a high θ_c.

Activity Model. An "activity" model can be developed from Michaelis-Menten enzyme kinetics (Lehninger, 1976) and is an extension of the Monod expression for conditions when toxicant is present. In the non-toxic case, the Michaelis-Menten equation is derived by assuming equilibrium conditions:

$$-\frac{dS}{dt} = V = \frac{kX_a S}{K_s + S}$$

where V is termed the reaction velocity. However, when toxicants are added, equilibrium conditions may no longer exist, temporarily at least.

The "activity" model is obtained by defining activity (A) as the total enzyme concentration available when toxicants are present (non-equilibrium conditions) divided by the total enzyme concentration available under non-toxic (equilibrium) conditions. The equation for reaction velocity then becomes:

$$V = \frac{AkSX_a - \frac{dV}{dt}(1/k_{+1})}{K_s + S}$$

where k_{+1} is the forward reaction rate for the conversion of free enzyme and substrate to enzyme-substrate complex and $\frac{dV}{dt}$ is the rate of change in reaction velocity due to the presence of toxicant. (Note that when no toxicant is present or acclimation has occurred, A is one, $\frac{dV}{dt}$ is zero, and the expression reduces to the Michaelis-Menten equation.)

Since the rate constant (k_{+1}) is not known, a simplifying assumption must be made:

$$k_{+1} >> \frac{\frac{dV}{dt}}{AkSX_a}$$

The reaction rate then becomes:

$$V = \frac{AkSX_a}{K_s + S}$$

(Note that A can be thought of as the quantity $(\frac{1}{1 + T_x/K_I})$ for the non-competitive inhibition case described previously.)

Activity will be a function of stress applied to the system, for example, exposure to toxicants. When sufficient data is not available, an empirical relationship of activity as a function of exposure time can be developed. This activity model can be used to describe the response pattern observed following slug addition of toxicants. Gas production data can be used provided the following assumptions are made.

1. Monod kinetic coefficients used are 3.5 day^{-1} for k, 25 mg/l for K_s (determined during this study), 0.036 mg/mg for Y, and 0.015 day^{-1} for b (adapted from literature values - Metcalf and Eddy, 1979).
2. Since biomass (X_a -usually measured as VSS) was not monitored, it was estimated from mass balances:

$$X_{a2} - X_{a1} = (Y(\Delta S/\Delta t) - (b + 1/\theta_c)X_{a1})(t_2 - t_1)$$

where $X_{a2} - X_{a1}$ is the change in biomass during the time period $t_2 - t_1$ and $\Delta S/\Delta t$ is the HAc utilization in the time period $t_2 - t_1$. Under non-toxic conditions, X_{a2} equals X_{a1}.

3. Since was returned to 1000 mg/l each day, during non-toxic conditions $\Delta S/\Delta t$ was 1000 mg/l-day. During these steady-state conditions 22 ml of methane were produced daily. From this stoichiometry, S can be calculated as:

$$S = 1000 - (1000/22)(\text{daily methane production in ml})$$

for conditions when toxicant is present. Once S is calculated, $\Delta S/\Delta t$ can be determined. V is assumed to equal $\Delta S/\Delta t$ for those data points where S is greater than $4K_s$. When this is the case, the expression for V reduces to:

$$V = AkX_a$$

4. A can now be calculated from the above expression as:

$$A = \frac{V}{kX_a}$$

An empirical relationship was developed for A as a function of time:

$$A = K_1(t_e - t_1)^{k_1} + K_2(t_e - t_2)^{k_2}$$

where: t_e = exposure time;

t_1 = time when toxicity effects begin;

t_2 = time when recovery begins;

K_1, K_2, k_1, k_2 = empirical constants.

Values for t_e, t_1, and t_2 were taken from gas production data while K_1, K_2, k_1, and k_2 were obtained from linear regression analysis of log A vs. $\log(t_e - t_{1 \text{ or } 2})$. This is the same type of expression that was used previously to develop the first model presented in this section.

5. Predicted methane production was then calculated from the pre-predicted activity and estimated X_a:

$$V = AkX_a$$

and

$$CH_4 \text{ production} = \left(\frac{22}{1000}\right)(V)$$

Figures 189 and 190 show results from this "activity" model. Although the model is still empirical due to the lack of requisite data and does not allow for prediction of t_1 and t_2, it does predict the general shape of the response curve and was developed from a more fundamental basis than the first model described in this section. It appears to be a promising starting point for development of a fundamental, dynamic model describing response to toxicant addition. Refinement should allow for handling cases when S is not greater than $4K_s$.

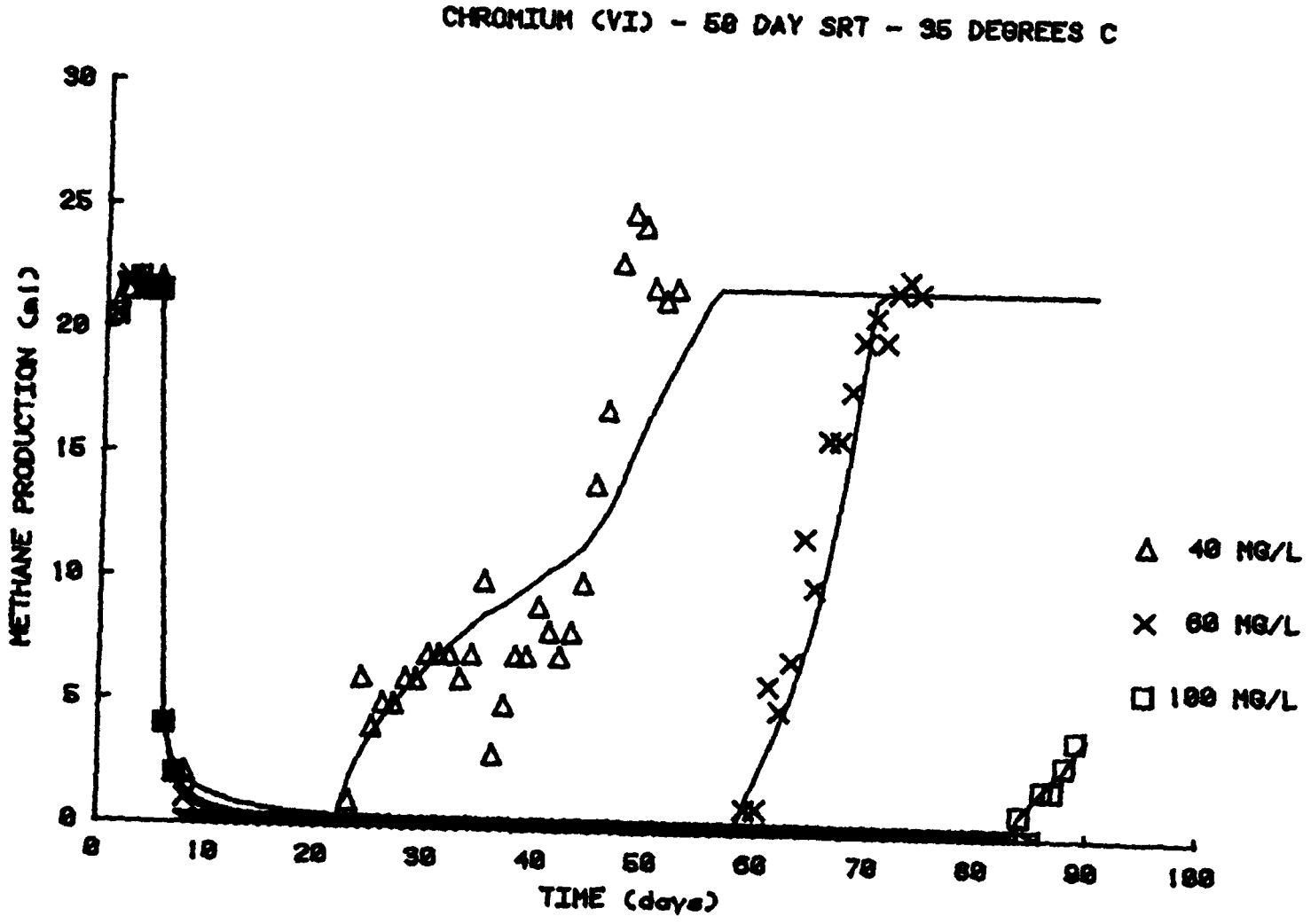


FIGURE 189. MEASURED VS PREDICTED METHANE PRODUCTION FOR ACTIVITY MODEL

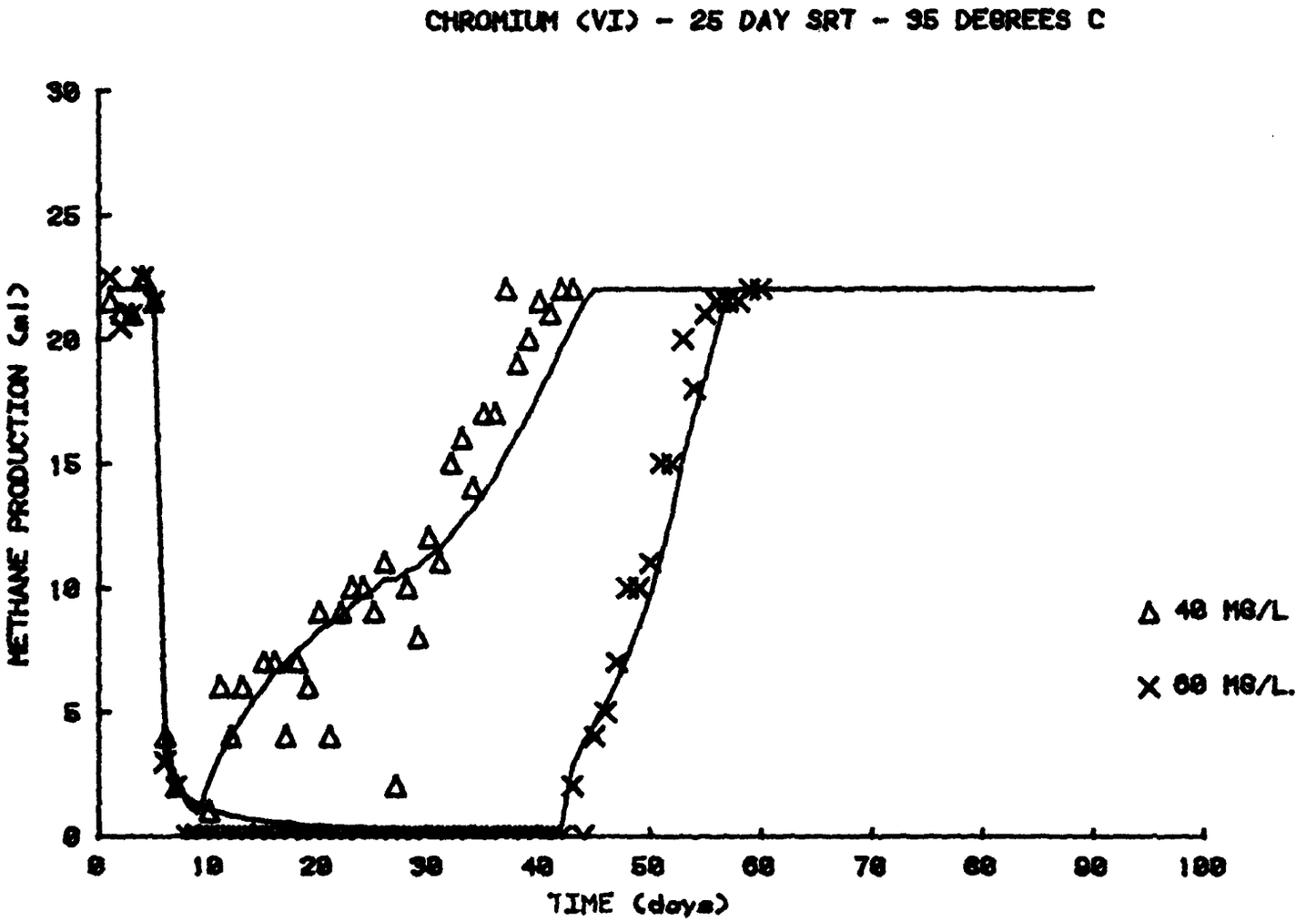


FIGURE 190. MEASURED VS PREDICTED METHANE PRODUCTION FOR ACTIVITY MODEL

RECOMMENDATIONS FOR FUTURE WORK

The major accomplishment of the research has been the development of a catalogue of response patterns, acclimation characteristics, and reversibility properties of acetate-splitting methane bacteria exposed to various common toxic materials. The effects of SRT and temperature have been investigated. Preliminary empirical and conceptual kinetic models have been developed to describe collected data. Use of the serum bottle technique allowed for the collection of massive amounts of very valuable qualitative data. However, only limited information concerning process kinetics and toxicity mechanisms can be extracted. Time and personnel constraints did not permit collection of data required for detailed elucidation of toxicity kinetics and mechanisms.

The next major undertaking is a more fundamental investigation of toxicity kinetics and mechanisms. In order to do this, larger laboratory systems must be used (i.e., 1-2ℓ CSTR instead of serum bottles), a detailed analysis of process behavior must be performed (frequent monitoring of substrate, VSS, toxicant, ATP, F_{420}, etc.), and thus fewer toxicants can be studied. In addition, studies with more complex substrates such as propionate and primary sludge should be undertaken to ascertain if indeed toxicity in anaerobic systems can be adequately described using acetate-splitting methanogens. Potential for acclimation should be investigated farther using a "high SRT, low HRT" system such as the anaerobic filter. Specifically, future research should focus on the following:

1. Investigation of the mechanisms and determination of the kinetics for slug and continuous addition of selected toxicants to acetate-splitting methanogenic cultures.
2. Comparison of the response to toxicant addition of an acetate-splitting methanogenic culture and a system fed a complex organic substrate such as primary domestic sludge.
3. Investigation of the 'ultimate' acclimation potential for acetate-splitting methane bacteria to selected toxicants using the anaerobic filter.

CONCLUSIONS

The following are general conclusions drawn from experiments conducted during this research:

* The effect of SRT on response to toxicant addition is dependent on toxicant type, concentration, and temperature. The effect appears to be a complex interaction of cell age, biomass concentration, and toxicant concentration. In general, the longer SRT (25 and 50 days, compared with 15 days) gave the least severe responses.
* Based on systems tested at 25°, 35°, and 42.5°, the temperature most often resulting in the least severe responses was 35°C.
* All toxicants studied exhibit reversible inhibition, meaning that recovery from toxicant exposure can be accelerated by reducing the toxicant concentration in the liquid.
* Significant acclimation to most all toxicants studied was observed. The degree of acclimation is dependent on toxicant concentration, method of exposure, temperature, and perhaps SRT.

The following are general conclusions drawn from slug-addition experiments:

* Extended periods of zero methane production resulting from toxicant exposure are not necessarily indicative of destruction of methanogenic viability. Bacterial regrowth alone cannot account for the high rates of recovery from zero methane production.
* Responses to slug doses increase in severity as the initial concentrations are increased.
* Recovery from toxicant exposure, as measured by rate of increased methane generation and/or by times of decreased or zero methane production, is a function of toxicant concentration, SRT, and temperature, and can be described by an empirical model similar to the classical dissolved oxygen sag equation.
* The toxicant concentration at which recovery is completed (normal methane production resumes) approaches a maximum as the initial slug-dose concentration is increased until a critical initial concentration is reached. When the initial slug dose exceeds this critical

concentration, recovery time (the period of decreased or zero methane production) is significantly extended and the toxicant concentration remaining at complete recovery is reduced below the maximum. This "complete recovery" concentration may be much lower than a slug dose which causes no response.

* Although the calculated toxicant concentration remaining when recovery is complete is generally higher for the longer SRT systems, there is no clear pattern that applies to all toxicants.

* For the conditions tested, the estimated threshold dose concentration for calcium is between 5000 and 15,000 mg/l Ca^{++}. Significant acclimation is possible.

* The toxicity threshold for cadmium is less than 50 mg/l Cd^{++}. Some acclimation can be achieved.

* Threshold doses for chromium (III) vary between 5 and 60 mg/l, and decreasing the temperature to 25°C significantly increases toxicity. Acclimation was observed only at 35°C with a 15-day SRT.

* Chromium (VI) has threshold slug doses under 10 mg/l, and toxicity is increased by lowering the temperature to 25°C. Acclimation does not appear possible.

* The threshold doses for nickel vary significantly between 100 mg/l to under 50 mg/l, and the toxic effects become more severe with increasing temperature. Significant acclimation can be obtained, depending on environmental conditions. Inhibition by nickel is reversible.

* Sulfide threshold slug doses are near or slightly below 50 mg/l S^{2-} for all conditions tested. Systems at 35°C and the longer SRT can best cope with sulfide addition. Reversible inhibition is demonstrated by sulfide, but significant acclimation does not appear possible, at least at the concentrations tested.

* The toxicity threshold for chloroform is below 5 mg/l. The potential for acclimation to chloroform is very large. Chloroform toxicity is reversible.

* Threshold slug doses of dichloroethylene are less than 50 mg/l. Toxicity is slightly reduced by decreasing the temperature to 25°C. Significant acclimation was not observed.

* Threshold doses for trichloroethylene are very similar to those of dichloroethylene, under 50 mg/l. Dropping the temperature to 25°C again reduced toxicity. Acclimation to trichloroethylene did not occur with the conditions tested.

* Ethyl benzene has threshold slug doses which vary between 250 and 500 mg/l. Acclimation characteristics were not conclusively revealed.

* Hyamine 1622 (a cationic surfactant) threshold slug doses are between 20 and 50 mg/l at 35°C and 25°C, but increasing the temperature causes the threshold doses to decrease to between 5 and 10 mg/l. Significant acclimation does not appear possible.

* The threshold doses for Hyamine 3500 (a cationic surfactant) vary between 1 and 10 mg/l, with a decrease in the threshold dose resulting from a decrease in temperature to 25°C. Some acclimation appears to be possible.

* Threshold doses of regular gasoline are below 2500 mg/l. Toxicity appears to be increased dramatically as the temperature is increased. Acclimation seems to be possible to some degree.

* Jet fuel (JP-4) threshold doses are between 5000 and 7500 mg/l at 25°C and 35°C. However, increasing the temperature to 42.5 significantly increased toxicity, lowering the threshold dose below 1000 mg/l. Acclimation to jet fuel can be obtained.

* Threshold doses of hydrazine are less than 10 mg/l. Toxicity is significantly increased by increasing the temperature to 42.5°C.

* For the six toxicants tested at different temperatures and SRTs, the experimental conditions yielding the least severe responses were: 15-day SRT and 35°C for calcium; 25-day or 50-day SRT and 35°C for chromium III; 15-day SRT at 35°C for chromium VI; 15, 25, or 50-day SRT at 35°C for nickel; 25 or 50 day SRT and 35°C for sulfide; and 15, 25 or 50-day SRT and 35°C for chloroform.

The following are general conclusions drawn from continuous addition experiments:

* Significant acclimation to nickel, chloroform, and hydrazine was possible through continuous addition of these materials. Under optimal conditions, feed concentrations of 200 mg/l nickel, 20 mg/l chloroform, and 50 mg/l hydrazine could be tolerated with no decrease in process performance.

* For experimental conditions tested, estimated lethal concentrations were 120 to 200 mg/l for nickel, 4 to greater than 20 mg/l for chloroform, and 9 to greater than 50 mg/l for hydrazine. The magnitude of the lethal dose was a function of SRT and temperature.

* For experimental conditions tested, conditions yielding the best potential for acclimation were 25 or 50-day SRT at 25°C or 35°C for nickel, 25-day SRT at 25° or 35°C for chloroform, and 25-day SRT and 35°C for hydrazine.

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Walter M. Kocher,	Research Assistant (Ph.D. Candidate)
Steven W. Miller,	Research Assistant (M.S. Candidate), full time summer and fall, 1980 only.
Joseph Hudak,	Research Specialist, half-time from October 1979 to June 1980.
Richard Hergenroeder,	Research Assistant (M.S. Candidate) full time summer 1981 only.

LIST OF PRESENTATIONS AND PUBLICATIONS

Portions of the research were included in the following:

1. Parkin, G.F., Speece, R. E., and Yang, J., "A Comparison of the Response of Methanogens to Toxicants: Anaerobic Filter vs Suspended Growth Systems," Presented at a DOE-sponsored Workshop on Anaerobic Filter Technology, Jan. 1980, Orlando, Florida.

2. Parkin, G.F. and Speece, R.E., "Modeling Toxicity in Methane Fermentation Systems," Presented at ASCE National Conference on Environmental Engineering, July, 1980, New York (submitted to Journal Env. Eng. Div., ASCE for publication).

3. Speece, R.E., Parkin, G.F., Yang, J., and Blumer, L., "Methane Fermentation of Industrial Wastes," VI International Fermentation Congress, London, Ontario, July 1980.

4. Parkin, G.F., Speece, R.E., Yang, C.H.J., and Kocher, W.M., "Response of Methane Fermentation Systems to Industrial Toxicants," Presented at the 53rd Annual Conference of the Water Pollution Control Federation, Sept., 1980, Las Vegas, Nevada (submitted to Jour. Water Pollution Control Federation for publication.

5. Miller, S.W., "The Effect of SRT and Temperature on the Continuous Addition of Toxicants to Methane Fermentation Systems," Unpublished M.S. Thesis, Drexel University, 1981.