A Toxicity Assessment of Sludge Fluid Associated with Tar Sands Tailings

by Mostafa Abdel Warith

A Thesis submitted to the Faculty of Graduate Studies
and Research in partial fulfilment of the requirements
for the Degree of Master of Engineering.

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August 1983

Abstract:

This study was conducted in an effort to assess the toxicity of fluid emanating from potential sludges produced as a result of the "hot water extraction process" employed in extracting oil from the tar sands in Alberta. A further attempt was made to identify specific components, factors, and/or properties which might be responsible for any toxicity observed in the emanating sludge fluid. Fluids emanating from 14 different potential sludges resulting from various proposed treatment processes were considered. All emanated fluids were observed to be highly toxic to the green alga, Selenastrum capricornutum, with LC 50 range varying from 5-33% (v/v) and an average LC 50 value of 25.8% (v/v). After activated carbon treatment the LC 50 range for the same samples ranged from 17-47% (v/v) with

an average LC 50 value of 34.8% (v/v). All emanated fluids were observed to exhibit much less toxicity to the marine bacterium resembling <u>Photobacterium</u> <u>phosphoreum</u> with an LC 50 range varying from 40% to 100% (v/v) and with an average LC 50 value of 85% (v/v). Oxygen uptake curves obtained from samples inoculated with microorganisms of industrial origin also indicated a relatively low toxicity in the emanated sludge fluid.

Differences of toxicity not greater than 5% between samples with added EDTA and samples without EDTA indicated that metals are not accountable for the high toxicity observed with respect to <u>S</u>. <u>capricornutum</u>. The toxicity observed appeared to be associated with the ammonia content of the samples which found to be above the safe limits for receiving waters. The samples toxicity was decreased by 25% during 25 week period of exposure to the laboratory environmental conditions.

RESUME

Cette étude fut conduite dans le but de déterminer la toxicité du fluide émanant des boues produites résultant du"procédé d'extraction d'eau chaude" utilisé dans l'extraction de l'huile des sables bitumineux de l'Alberta. Un effort fut mené vers l'identification de composantes spécifiques, facteurs et/ou propriétés qui pourraient causer la toxicité observée. Les fluides émanant de 14 boues différentes, résultant de procédés de traitement variés, furent considérés. Tous les fluides émanants ont démontré une forte toxicité envers l'algue de Selenastrum capricornutum avec une concentration létale variant de 5-33% (v/v) et avec une moyenne de 25.8% (v/v). Après un traitement au carbone activé, les concentrations létales des mêmes échantillons s'échelonnaient entre 17-47% (v/v) avec une valeur moyenne de 34.8% (v/v). Tous les échantillons de fluides émanants étudiés ont démontré une toxicité moindre envers la bactérie aquatique similaire au Photobacterium phosphoreum, avec une concentration létale variant de 40% à 100% (v/v) avec une moyenne de 85% (v/v). Des courbes de consommation d'oxygène obtenues à partir d'échantillons de fluides ensemencés avec des microorganismes d'origine industrielle, ont comme l'essai précédent, démontré une faible toxicité. Des différences de toxicité

ne dépassant pas 5% entre les échantillons enrichis d'EDTA et ceux sans EDTA ont indiqué que les métaux présents ne sont pas responsables de la forte toxicité observée avec le S. capricornutum. La toxicité des fluides émanants semblait être associée avec le contenu en ammoniaque des échantillons, qui après analyse se sont montrés être au-dessus des limites sécuritaires pour les eaux réceptrices. La toxicité des échantillons fut décrue de 25% après exposition de ces mêmes échantillons pendant 25 semaines à des conditions d'environnement du laboratoire.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation and gratitude to:

Dr. R. N. Yong, William Scott Professor of Civil Engineering & Applied Mechanics, and Director of the Geotechnical Research Center at Mc Gill University for his guidance and continued encouragement throughout this study.

Dr. A. Sethi, for invaluable suggestions and helpful advice and discussion.

Mr. N. Birmingham, C. Blaise and the laboratory staff for technical assistance (Environmental Canada, Environmental Protection Service, Chemistry Laboratory, Montreal, Canada).

Mrs. M. Mourato for typing the thesis.

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CHAPTER I

Introduction

INTRODUCTION

1.1. Statement of the problem

The Athabasca Oil Sands deposits in Northeastern Alberta, are a unique deposit requiring recovery methods entirely different from those of conventional oil deposits. Although a multitude of companies have spent large amounts of money in research on alternate methods of oil recovery, only one process namely the hot water recovery method, is presently employed (Appendix A). Large scale development using this method in the forseasable future will require disposal of large quantities of wastewater or tailings. These tailings consist principally of water with an approximately 3% solids content comprised mainly of sand and clay-sized particles. Because the hot water recovery method is only 90-95% efficient in extracting the oil, a small fraction of the oil or bitumen is also incorporated into the tailings. Present technology has dealt with this problem by retaining the majority of these waste-waters or tailings in ponds. The tailings ponds, due to the settling properties of the solid fraction of the tailings, consist of a large volume of sludge covered by a relatively thin layer of surface water (solid concentration ≃ 1.0%). Eventually, the environmental implications of these large tailings ponds must be considered. To date, environmental impact studies associated with the tailing ponds have been rather limited. Hrudey et al (1976) conducted a toxicity study on a dyke drainage from the tailings ponds employing Salmo gairdneri (Rainbow trout) and observed a high degree of toxicity with an extrapolated 96 hour LC 50 value of 11% (by volume). (The LC 50 value refers to the median lethal concentration or that concentration which is expected to kill 50% of exposed organisms over a specific time of observation). This study by Hrudey et al indicates that the contents of the tailings ponds may indeed pose a serious threat to surrounding aquatic ecosystems(**1976**).

Another effort was carried out by Ludwig (1983) to assess the toxicity of the tar sands tailings with respect to other indices of toxicity (the fresh water, green algae, S. capricornutum, glucose degrading microorganisms from the Athabasca River and the marine bacterium resembling Photobacterium phosphoreum). The results obtained indicate that fresh tailings water (i.e. tailings water produced from extraction process and no more than one week old) is substantially more toxic to all indices of toxicity than aged tailings water (i.e. tailings produced from extraction process and approximately one year old).

Fresh tailings water is also more toxic than emanated sludge fluid to 2 of the selected indices of toxicity (i.e. glucose degrading microorganisms and marine bacterium resembling Photobacterium phosphoreum).

The present study is another phase of the overall program of studing the toxic effect of the tailings water on aquatic organisms and it focused on the fluid emanating from potential sludges produced as a result of the hot water extraction process. Several treatments for enhancing the dewatering and the consolidation of the sludge in the tailings ponds are presently being considered. Consequently, the characteristics of the sludge and therefore the characteristics of the emanating fluid may vary depending on the treatment process employed. It is therefore desirable to determine the relative toxicity of the sludge fluids resulting from the various treatment processes and their relative potential environmental impact.

1.2. Purpose and scope of the present study

This study was conducted in an effort to assess the toxicity of emanating or released fluid, which accumulate from the settling process of both treated and untreated tailings sludge produced as a result of the "hot water extraction process" employed in extracting oil from the tar sands in Alberta. A further attempt was made to identify specific components, factors, and/or properties which might be responsible for any toxicity observed in the emanating sludge fluid. Identification of any factors which may contribute to potential toxicity may provide options for treatment processes which might eventually be employed in rendering the contents of tar sands non-toxic.

The test program consisted of.-

1) a physical and chemical characterization of the emanating sludge fluid.

2) prediction of the growth of the green alga, *Selenastrum capricornutum* with different samples concentration (100, 50, 25 and 12.5% by volume).

3) a toxicity assessment of the emanating sludge fluid using three indices of toxicity. The indices of toxicity employed are:
   a) *Selenastrum capricornutum* (employed using the Algal Assay Bottle test).
   b) marine bacteria resembling *Photobacterium phosphorum* (using Microtox system).

c) mixed culture of bacteria (using oxygen uptake as mesured by the Electrolytic Respirometer).

4) measurement of the variation in toxicity pH, and COD with time, and subsequent prediction of the effect of time on toxicity.

5) determination of the time required for the surface water layer, in an initially non-toxic state, to reach a toxic state. A flow chart outlining the scope of the present study is provided in Figure 1.1.

Chemical and physical analysis of the test samples provide information on the quantity of toxic materials and nutrients present, but supplies no knowledge of their availability for aquatic organisms in the ecosystem. The study of heavy metals interaction in the wastewater is complicated by uncertainty of the metal. Thus with few exceptions, the chemically analyzed heavy metals content of the test samples may not reflect the resultant biological interaction and productivity in the emanating fluid. To measure the toxicity level Algal Assay Bottle test (employing S. capricornutum green alga) and Micotox system (using marine bacteria resembling Photobacterium phosphorum) were used to calculate the Median tolerance

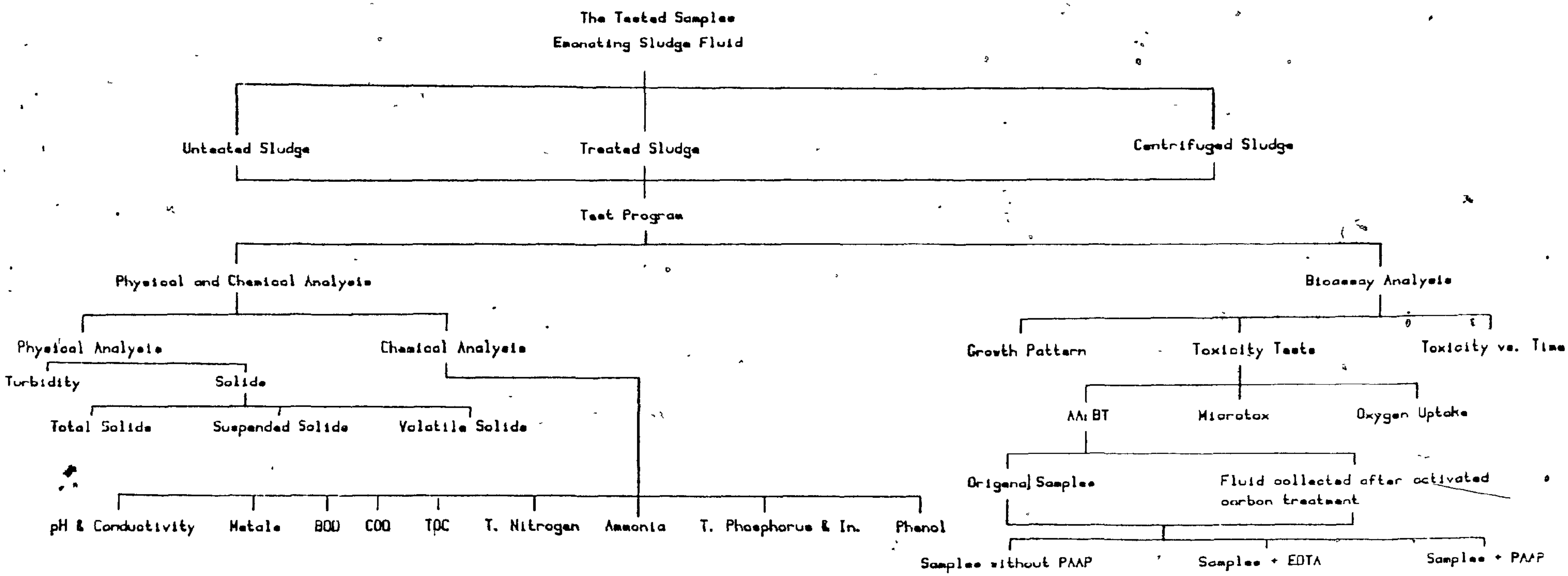


FIG. 1.1 Schematic diagram of the present study

limit (TLm or LC 50), which is defined as the concentration of the toxicant in which just 50% of the test organisms are able to survive for a specific period of exposure. This lethal concentration value is only an estimate of the acute toxicity, under laboratory conditions (Leith, 1973).

The Algal Assay Bottle test, Microtox technique and the oxygen uptake by mixed culture of bacteria were preferred to the 96 hour LC 50 test using rainbow trout. This later test has some limitations, namely, the use of a small number of test organisms, and the relatively short exposure time by the organisms to the test samples. Also the fish toxicity tests are relatively more expensive than the algal bioassays. The significance of the Algal Assay Bottle test is that a differentiation can be made between the toxic constituents determined by chemical and physical analysis, from the interactions of the constituents which actually regulate the algal growth. The Microtox technique is a rapid method for toxicity assessment. It can be used to give quick and rough estimate of the toxicity of the samples.

1.3. Organization of the Thesis

The thesis consists of seven chapters and three appendices. The contents of the chapters are as follow:-

Chapter 1: of which this section is a part, is an introductory chapter which presents the problem and the purpose of the present study.

Chapter 2: consists of a literature review of the experimental work carried out on the tailings water and of the toxicity of oil components to different aquatic biota.

Chapter 3: provides a complete description of the experimental methods, materials and techniques used.

Chapter 4: contains the experimental results obtained with a discussion of these results, in an attempt to predict the causes of the toxicity demonstrated.

Chapter 5: presents a more extensive discussion of the results.

Chapter 6: calculation of retoxification time.

Chapter 7: conclusions.

The second part of this thesis includes the following appendices:

Appendix A:- presents a brief review of the "hot water extraction process" and describes the oil sands content and formation.

Appendix B:- gives the description of the synthetic Algal Nutrient Medium and sample of calculations.

Appendix C:- contains some of the test results.

CHAPTER 2

Literature Review

LITERATURE REVIEW

Oil is not a single chemical but a collection of hundreds of substances of widely different properties and toxicants. Also oil in water should be regarded not as an ordinary pollutant but as a dynamic system actively reacting with environment.

2.1. The chemical constituents in the Tar sands tailings

The organic constituents of wastewaters from the Athabasca extraction plant were characterized and quantified by Strosher and Peak (1976). Twenty chemical parameters were determined on a total of 10 samples taken from three locations: the tailing pond dike filter drainage system, the upgrading plant final effluent to the Athabasca River, and the intake pond waters.

A mass balance of extracted versus recovered organic constituents is contained in Table (2.1) (Strosher and Peak, 1976).

Table (2.1) Mass Balance of Organic Constituents of the tailings of waters (mg/l) (Strosher and Peak, 1976)

Sample No.	Extracted organic carbon	Recovered Organic Constituents				
		Hydro-carbons	Oxygenated compounds	Nitrogen compounds	Sulfur compounds	Others
Upgrading plant effluent						
1	14	0.03	3.64	1.15	2.5	0.73
2	13	0.97	3.45	1.00	2.1	0.72
5	17	1.04	3.22	0.96	2.9	0.68
8	15	1.00	3.38	0.98	2.6	0.87
Tailing Pond-Dike filter drainage						
3	70	0.03	63.5	0.79	3.0	0.20
4	68	0.03	61.0	0.84	3.3	0.19
6	73	0.03	66.1	0.78	4.2	0.21
9	89	0.04	76.8	0.98	5.4	0.33
Intake Waters						
7	4	0.02	1.37	0.63	0.4	0.01
10	3	0.01	0.89	0.62	0.1	0.05

On the basis of these findings, as well as previously measured water flow date, Strosher and Peak (1976) found that an average of 198 kg of organic carbon was released to the river daily from the tailing pond dike filter system, and 1470 kg per day discharged from the upgrading plant effluent. These amounts corresponded to about 0.8% of the natural organic load of the river.

Hrudey et al (1976) conducted a gas-chromatographic (GC) and Mass - spectrometric (MS) analysis on two wastewaters streams from an oil sands extraction and upgrading plant. The main effluent stream from the upgrading plant was not acutely toxic, in flowthrough bioassay while the construction drainage from tailings pond dyke was acutely lethal in static bioassay with an extrapolated LC 50 of 11% (by volume). Analysis by GC-MS of the sample permitted to identify four compounds: 2,6-di-tert-butyl-p-cresol, (BHT); di-n-butyl phthalate, (DBP); bis (2-ethylhexyl) adipate, (BEHA), and bis (2-ethylexyl) phthalate, (BEHP). 96 hour static bioassays with rainbow trout indicated LC 50 values of 540 mg/l for BEHP, 3 mg/l for BHT, 1.2 mg/l for DBP, and an approximate range of 54-110 mg/l for BEHA. Table (2.2) shows the acute toxicity of (DBP) to four species of fish at different time intervals.

Table (2.2) Acute toxicity of Di-n-butyl Phtalate (DBP) to four species of fish (Hrudey et al, 1975).

Species	Temperature °C	LC 50 (mg/l)		
		24 hr	48 hr	96 hr
Falhead minnow (<u>Pimephales promelas</u>)	17 °C	-	1.49	1.3
Bluegill (<u>Lepomis marchirus</u>)	17 °C	1.23	0.73	0.73
Channel catfish (<u>Ictalurus punctatus</u>)	17 °C	3.72	2.91	2.91
Rainbow trout (<u>Salmo gairdneri</u>)	12 °C	-	-	6.47

Burchfield and Hepler (1979) carried out quantitative analysis of dissolved sodium, potassium, calcium, magnesium, chloride, carbonate, bicarbonate, alkalinity sulphate, aluminum, iron and silicon in tailings water originating from the Great Canadian Oil Sand (GCOS) tailings pond and from the Syncrude pilot plant. Results of the chemical analysis are summarized in table (2.3).

Table (2.3) Quantitative analysis of tailing water (Burchfield and Hepler, 1979)

	GCOS	Syncrude
Sodium	260	207
Potassium	11	7
Calcium	7	16
Magnesium	4	5
Chloride	11	9
Carbonate + bicarbonate	700	280
Alkalinity	540	230
Sulfate	73	226
Aluminum	<1.0	21
Iron	<2.0	2
Silicon	6	14

* All these analytical results are reported in terms of mg/L at 23°C.

Table (2.4) shows the results of the infrared spectra of organic material extracted from GCOS pond water (Burchfield and Hepler, 1979).

Table (2.4) Summary of infrared spectra of organic chemical extracted from GCOS pond water. (Burchfield and Hepler, 1979)

Absorbtion frequency	Group assignment
1040 cm-1	sulphur - oxygen
1455 cm-1	C-H
1703 cm-1	carbonyl
2850- 3000 cm-1	C-H
3000- 3500 cm-1	H-bonded and phenolic oH

The general picture of water soluble organic substance in tailing water derived from Burchfield and Hepler (1979) is similar to the picture provided by Strosher and Peak (1976).

2.2. Review of the toxicity effect of oil and its compounds

The toxicity of an oil is usually closely related to its content of aromatic hydrocarbons (Zitko and Carson, 1970). Kauss and Hutchinson (1975) studied the effect of benzene, toluene, o-xylene and naphlalene on the growth of Chlorella cells. They noted that final cell densities, as well as the duration of the lag period, were generally proportional to the initial hydrocarbon concentration. Statistical analysis of cell counts, after one day of incubation, showed that the inhibition of growth by benzene, o-xylene and naphthalene at all concentrations, and by 50-500 ppm of toluene was significant at the 5% level. (Ottaway, 1971).

The toxic effects of nitrile compounds on minnows and bluegills have been evaluated (Henderson et al, 1960). Lactonitrile is the most toxic of the nitrile compounds in the acute toxicity studies. The data observed during chronic toxicity tests indicate that the 20-day TLm (Median tolerance limit) for lactonitrile is 0.66- 0.77 mg/L, with almost no evidence of chronic toxicity. However, acrylonitrile exhibited chronic toxicity with a 30- day TLm of 2.4 to 3.0 mg/L.

Korzep (1962) studied the toxicity of various organic chemicals to a species of green algae Chlorella pyrenoidosa, under controlled laboratory conditions. The data indicated that organic acids are least toxic to these alga, followed by alcohols and amines, alkyl halides, aldehydes and ketones, with compounds of the benzene series exhibiting the highest toxicity. The assay indicates that nitrobenzene and cupferron were toxic at concentrations of 0.01 mg/L or less. Ethylbenzene, formaldehyde, furfural, cersol and alpha-naphthylamine-hydrocholoride exhibit a certain degree of toxicity at concentrations above 0.1 mg/L. In concentrations above 1.0 mg/L xylene and ethylene dichloride were toxic to these alga.

Nelson - Smith (1972) concluded that the relatively high soluble components such as benzene and toluene are very toxic. Wallen et al (1957) reported that benzene is toxic to different varieties of fish at different concentrations, thus at a concentration of 386 mg/L, it is toxic to Mosquito fish, and at a concentration of 6 mg/L it is toxic to bluegill sunfish. Toluene is toxic at a concentration range from 50 to 65 mg/L to bluegill sunfish and also toxic to Mosquito fish at a concentration of 1.18 mg/L.

The highly volatile and more soluble lighter fractions are believed to be chiefly responsible for the high initial (acute) toxicity of crude oils. These fractions include the low-boiling paraffinic and aromatic hydrocarbons. The low-boiling aromatics are considered to be potentially the most toxic to aquatic life (Blumer, 1969).

Reynolds et al (1975) observed that phenol is the controlling inhibitor to the growth of green alga, <u>Selenastrum</u> <u>capricornutum</u>. Also, they observed that phenol was more toxic at 24°C than at either 20°C or 28°C. Phenol appears to be less toxic towards lower aquatic organisms than are sulfides or ammonia. A review of the more intensive bioassay tests with phenol, at 20°C, indicate a 96 hour LC 50 value between 10 and 20 mg/L for most fish. Concentrations of 40 mg/L (Mckee and Wolf, 1963) cause threshold toxic effects on the algae <u>Scendensums</u>. Kastyaev (1969) reported that phenol concentrations of 10-40 mg/L stimulated the growth of <u>Scendensums</u> while concentrations greater than 50 mg/L retarded the growth. He also found that phenol concentrations greater than 500 mg/L prevented growth altogether; however, this concentration is highly unlikely to be found in refinery effluents.

Ammonia is primarily generated from crude oil hydrocracking. The toxic action of ammonia and ammonium salts to aquatic organisms depends on the hydrogen ion (H^+) concentration (pH) and therefore on the undissociated ammonia (Pace, 1974). For example, Nelson Smith (1972) found that the poisoning action of an ammonium salt is increased by 200% when the pH was raised from 7.40 to 8.0. Carbon dioxide decreases the concentration of the toxic NH3 by lowering the pH value. The lowest reported lethal (i.e.

96 hour LC 50) concentration to fish was 0.2 mg/L un-ionized ammonia for <u>Salmo</u> <u>gairdneri</u> (Rainbow trout).

Hrudey et al (1976) reported a concentration of ammonia in the range of 0.2 - 0.5 mg/L in the effluent from the bitumen upgrading plant and 1.3 mg/L in the drainage from the tailing pond dyke. He also found that ammonia concentration of 0.41 mg/L is toxic to Rainbow trout.

Mironov, (1968) reported that many toxic components of refinery effluents, even if present in sublethal concentrations, may influence aquatic organisms synergistically. Heavy metals behave in this way. Zinc in combination with copper, and zinc, lead, copper and phenols in combination with low oxygen, have been reported to react synergistically. These same metals can concentrate in food-chain organisms and thus may have implications for human health. Zinc occurs in all Canadian refinery effluents studied, at concentrations of 0.05 - 0.17 mg/L and is toxic to trout at levels as low as 0.1 mg/L (Mckee and Wolf, 1963).

The results of various heavy metals analysis (Strosher and Peak, 1976) indicated that vanadium was the most abundant of the metals, occuring in the upgrading plant wastewaters in amounts of 0.25 to 0.6 mg/L. Tailing pond dike drainage effluent and intake waters contained considerably lower amounts of vanadium - 0.001 to 0.002 and less than 0.001 mg/L respectively.

2.3. How oil compounds cause mortality in aquatic organisms:

The effects of oil on aquatic organisms fall into two categories. The first category includes effects associated with coating or smothering of an organism with oil. Such effects are associated primarily with the higher molecular weight, water insoluble hydrocarbons, and the various tarry substances that coat and cover the organisms (Nelson-Smith, 1972). The second category of toxic effects involves disruption of an organism's metabolism due to the ingestion of oil and the incorporation of hydrocarbons into lipid or other tissue in sufficient concentration to upset the normal function of the organism. With respect to this second category of effects, it is generally agreed that aromatic hydrocarbons are the most toxic, followed by cycloalkanes, then olefins, and lastly alkanes (Nelson-Smith, 1972).

Laws (1981) reported that aromatic and other toxic hydrocarbons apparently exert their effect in part by becoming incorporated into the lipid layer that makes up the interior of cell membranes. As a result, the membrane is disrupted, and ceases to properly regulate the exchange of substances between the interior and exterior of the cell. In extreme cases, the cell membrane may lyse, allowing the contents of the cell to spill out and obviously destroying the cell. Although the low molecular weight alkanes and cycloalkanes were once considered to be harmless to aquatic life, it is now known that these compounds can cause narcosis and anesthesia in a variety of lower animals. Such effects are probably due in part to disruption of cell membrane (Blumer, 1969). In addition to disrupting cell membranes, there is evidence that hydrocarbons interact with proteins in a variety of plants and animals (Nelson - Smith, 1972). Both enzymes and structural proteins appear to be affected.

In summary, petrochemicals may cause mortality in aquatic organisms in various ways:

1) produce a toxic reaction,
2) depletethe dissolved oxygen,
3) increase the temperature,

4) change the pH value, and

5) increase the salinity and upset the osmotic pressure.

2.4. What concentration of oil are toxic to aquatic organisms:

Much experimental work has been done to predict the concentration of oil toxic to aquatic organisms. The results depend on the type of oil or combination involved. Adult fish are rather resistant to oil pollution, since their bodies, including the mouth and gill chambers, are coated with slimy mucus that resists wetting by oil. Incipient lethal levels of crude oil for adult fish are of the order of 10 - 100 parts per thousand. However, incipient lethal levels of individual aromatics for the same fish are roughly a factor of 1000 lower (Nelson - Smith, 1972). Crude oil and fuel oil have been found to be toxic to fish eggs at concentrations as low as 0.5 - 10 ppm (Longwell, 1977). Other types of plankton may be even more sensitive. Tests on phytoplankton involving fuel oil, kerosene, and crude oil have generally revealed toxic level to be of the order of 50 - 200 ppb, but growth rates are noticeably depressed at concentrations of about 1 ppb (Nelson - Smith, 1972). Blue-green alga are apparently quite resistant to oil pollution. In general, macroscopic plants are relatively resistant to oil toxicity, and even if extensively damaged tend to recover more rapidly than many other organisms.

CHAPTER 3

Materials and Experimental Techniques

Materials and Experimental Techniques

3.1. Samples description

Four different groups of samples were used in this investigation. The description of these samples is found in table (3.1) and is given below.

(1) Group No. (1): group no. (1) includes samples 1, 2, 6 and 7. The source of this group was the emanating fluid from the settling process of untreated (normal settling). Tar sands sludge.

(2) Group No. (2): group no. (2) includes samples 3, 4 and 5. The origin of this group was the amanating sludge. (settling by using some flocculanted agents).

(3) Group No. (3): group no. (3) includes samples no. 11 to 19 taken from the sedimentation tanks # 1 to # 9 respectively (Suncor Inc. Resources Group at McMarry, Alberta on 31/3/82).

(4) Group No. (4): group no. (4) includes samples no. 8 and 9. These two samples were taken from the supernatent fluid collected after centrifugation of Terminal sludge samples (total solids = 30.6%) at 10000 r.p.m. over a 30 minute, and they are considered as fresh-

ly emanated sludge fluid samples. (This group was used only in the toxicity analysis using S. capricornutum).

The system of chemical and physical analysis employed in this study is similar to that employed by Ludwig (1983).

All samples for physical and chemical analysis were preserved in accordance with the preservation regimen of table (3.2).

3.2. Inorganic and Organic Characterization of Samples

The physical and preliminary analysis involved the determination of the following parameters: pH, turbidity, specific conductivity, total solids, suspended solids, and volatile solids. The chemical analysis involved the determination of parameters of potential significance in the assessment of toxicity. The selected chemical analysis (inorganic and organic) included the following parameters: metals, total nitrogen, ammonia, total organic carbon (TOC), biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Also phenols concentrations were determined.

Table (3.1) Description of the tested samples

Sample No.	Source of the sample	Date sample taken
1	6" ϕ column # 1 (Macdonald)	Feb. 3, 1982
2	TA # 1 (plant #3) (Macdonald)	" " "
6 *	Tailing water (Macdonald)	March 1, 1982
7	Tailing water (Macdonald)	" " "
3	6" ϕ column # 2 (Macdonald)	Jan. 13, 1982
4	6" ϕ column # 3 (Macdonald)	" " "
5	Tank # 1 (Macdonald)	" " "
11 **	Tank # 1, Suncor Inc. Ft.McMurray	March 31, 1982
12	Tank # 2, " " "	" " "
13	Tank # 3, " " "	" " "
14	Tank # 4, " " "	" " "
15	Tank # 5, " " "	" " "
16	Tank # 6, " " "	" " "
17	Tank # 7, " " "	" " "
18	Tank # 8, " " "	" " "
19	Tank # 9, " " "	" " "
9 }	Supernatant fluid collected after centrifugation of terminal sludge at 10000 r.p.m. at 30 min (solid concentration = 30.6%)	Oct. 2, 1982
8 }		" " "

* sample 6,7 reached Mc Gill laboratory on October 27, 1981.

** sample 11, 19 reached Mc Gill laboratory on April 20, 1982.

Table (3.2) Preservation Regimen Used

Parameters	Preservation
Total solids Suspended solids Volatile solids BOD Algal Assay Microtox	No preservation refrigerated
COD TOC	2 ml. $H_2 SO_4$ refrigerated
Lead, Manganese Nickel, Ferric Copper, Cadmium Zinc, Arsenic Vanadium	3 ml. $H NO_3$ refrigerated
Phenols	1 gm $CuSO_4$, pH=4 with $H_3 PO_4$, refrigerated
Total phosphate	2 ml $H_2 SO_4$, PH = 2 refrigerated
Ammonia	20 mg - $Hg Cl_2$ refrigerated

3.2.1. pH:

The pH or hydrogen ion activity was determined electrometrically, using a Fisher pH meter Model 29. The pH meter was calibrated for each measurement with two buffer solutions. Sodium interference was not considered since all samples had pH value below 10 (Standard Methods, 1980).

3.2.2. Turbidity:

This measurement is based on the comparison of the intensity of light scattered by the sample with that scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. Formazin polymer was used as a reference turbidity standard suspension. "Hach Laboratory" turbid meter model 1860-A was used for ~~all~~ the measurements.

3.2.3. Conductivity:

Conductivity is the capacity for transmitting electricity, and is a function of ion content. The conductivity for all samples was determined using a Radiometer Copenhagen conductivity meter. A standardized cell was used and the conductivity measurements in all samples are reported as the specific conductivity (mho /cm) (Standard Methods 1980).

3.2.4. Total Solids:

Total solids or the total residue remaining in a vessel after a sample is dried at 105°C, was determined for all samples, gravimetrically. Procedure and interference in the Standard Methods (1980).

3.2.5. Suspended Solids:

Suspended solids is the residue remaining on "Whattman Glass" fiber paper (GF/A) after filtration of the samples. Procedure and interference in the Standard Methods (1980).

3.2.6. Volatile Solids:

Volatile solids is the loss of weight of the total solids content after ignition at 550°C ± 50°C for 15 minutes in a muffle furnace. It was determined for all samples gravimetrically. Determination of volatile solids provides a rough approximation of the quantity of organic matter present in the samples (Standard Metholds 1980).

3.2.7. Metals:

Aquatic biota are generally only affected by metals in their dissolved form, i.e. the dissolved metals content are much more important on a toxicity point of view than the total metals content. As mentioned in table (3.2), samples used for dissolved metals analysis were preserved by addition of concentrated nitric acid ($H\ NO_3$) to pH = 2.0 in order to prevent loss of the metals by adsorption and/or precipitation in the sample container.

The analysis for metals were conducted by spectrophotometry using the "Perkin-Elmer" Model 503 Atomic Absorption spectrophotometer.

The atomic absorption investigation was carried out on nine heavy metals (copper, lead, manganese, iron, nickel, cadmium, zinc, vanadium and arsenic), and four other metals (calcium, potassium, magnesium and sodium).

3.2.8. Total Nitrogen

The 703C Chemiluminescent Nitrogen System (CNS), Antek Instruments Inc., was used in the analysis of total nitrogen. The method of analysis involves a high oxidation temperature of the entire sample, the stabilization temperature was 1050°C, converting any chemically bound nitrogen to nitric oxide (NO). The NO is placed in contact with ozone (O_3) to produce metastable nitrogen dioxide (NO_2).

As the NO_2 relaxes, it chemiluminesces and this light emission is sensed by a photomultiplier, this light emission is proportional to the amount of nitrogen in the sample.

3.2.9. Ammonia:

The ammonia concentration was determined by the Automated Berthelot Reaction method No. 307-74 WM. The automated procedure is based on the formation of a blue-colored compound, believed to be closely related to indophenol, when the solution of an ammonium salt is added to sodium phenoxide followed by the addition of hypochlorite. The intensity and the speed of development of the resulting color were enhanced by addition of sodium nitro prusside. A solution of sodium citrate was added to the sample stream to eliminate the precipitation of hydroxides of calcium and magnesium. The intensity of indophenol blue was measured at 630 nm (EPS, 1979).

3.2.10 Nitrates - Nitrites:

Nitrate - nitrite concentration were measured by using a Technicon Auto-analyzes II system. Nitrates are reduced to nitrites by passing the sample through a column containing cadmium granules coated with a layer of copper. Total nitrites were determined, under acidic condition, by the reaction of sulfanilic acid with nitrite ions, to form a diazo compound. The diazo compound subsequently combines with α-napthylamine to form an intense red ozodye with maximum absorption at 520 nm (EPS, 1979).

3.2.11. Phosphorus:

Phosphorus content in the samples is classified in two general categories, total phosphorus and inorganic phosphorus. These two categories were determined using a Technicon Auto Analyzer II System, in which orthophosphates react with molybdate ion to yield heteropoly acids which are reduced with ascorbic acid to form a blue molybdophosphoric acid color. Absorbance was measured at 885 nm (EPS, 1979).

3.2.12. Total organic carbon (TOC):

Total organic carbon was determined for all samples using a Beckman Model 915A Total Organic Carbon Analyzer. Total carbon is determined from the oxidation of the organic and inorganic carbon fractions to CO_2 at 950°C. Total inorganic carbon determination is based on the fact that inorganic carbon will oxidize to CO_2 at lower temperatures than will the organic carbon (150°C). In both combustion tube the CO_2 produced quantitatively measured by the infra-red analyzer (Standard Methods, 1980).

3.2.13. Biochemical Oxygen Demand (BOD):

In order to determine the BOD progression, a set of BOD bottles were incubated in a standard BOD air incubator and the sample temperature was maintained at 20 ± 2°C by appropriate adjustments on the incubator. The samples were diluted with specially prepared dilution water so that adequate growth factors and oxygen could be available during the incubation period. The dilution water was "seeded" with a bacterial culture that had been acclimated to the organic matter present in the water for 48 hr. The seed culture used was a mixed culture, containing large number of saprophytic bacteria and other organisms that oxidize the organic matter, in addition to certain autotrophic bacteria that oxidize non carbonacous matter. After a five day incubation period, the content of the bottles tested by the Winkler method for dissolved oxygen (Standard Methods, 1980).

3.2.14. Chemical Oxygen Demand (COD):

The Chemical Oxygen Demand was determined by the dichromate reflux method (Standard Methods 1980). COD was measured in terms of the amount of potassium dichromate ($K_2 Cr_2 O_7$) reduced by the sample during 2 hr of reflux in a medium of boiling H_2SO_4, and in the presence of $Ag SO_4$ catalyst. Chloride interference was prevented by adding mercuric sulfate ($Hg SO_4$) (Standard Methods, 1980).

3.2.15. Phenols:

A Technicon Auto-Analyzer system was used for all determinations. Phenols are removed from the sample by steam distillation under acidic conditions, and collected in alkali conditions at a pH of 10 using an ammonium hydroxide buffer. The reaction of phenol with 4- amino-antipyrine, under oxidizing conditions, provided by ammonium persulfate at a constant pH of 10, yields a red color, the intensity of which is measured spectrophotometrically (EPS, 1979).

To remove the organic contaminants from the samples, a powder-activated carbon column was used. The column is 3 cm in diameter and 50 cm in length.

To measure the dissolved organic carbon, samples were extracted with benzene, and after evaporated by a rotary vacuum apparatus, to remove traces of solvent. The samples were analyzed again for the organic carbon content by carbon analyzer. (Backman - Model 915A).

3.3. Biological Indices of Toxicity:

The following tests were used to predict the toxicity of the emanated sludge fluid and to calculate the lethal concentration (LC 50).

3.3.1. Algal Assay: Bottle Test (AA:BT):

The significance of measuring the algal growth potential of the tested water samples is that a differentiation can be made between the nutrients that are in the sample, as determined by chemical analysis, and the nutrients that are actually available for algal growth. Also, if algal growth remains limited when nutrients are insufficient supply and the physical conditions for growth exist, the presence of a toxicant is indicated (Miller et al, 1974 ; Green et al

1976). The principle of the algae test is based on a modification of "Liebig's Law" of the minimum, which states that "growth is limited by the substance that is present in minimal quantity in respect to the needs of organism".

The recommended test alga which has been the principal "workhorse" during the development of the Algal Assay: Bottle test is the green alga Selenastrum capricornutum. Selenastrum belongs to the order chlorococcales and the family Selenastraceae which have a wide tolerance towards environmental conditions (Rodhe, 1978). This algae was selected because of its case of culture and maintenance, it is characterized by its unicellular or colonial habit in which the cells are in a non-mobile condition either for their entire life history or for the greater part of it. These attributes allow this algae to be enumerated by an electronic-particle counter on a daily basis or at any time during the incubation period.

Basically, the assay consists of the inoculation of 40,000 cells (1000 cell lml) of S. capricornutum into test water which is incubated in a constant temperature control at 24 ± 2°C. The incubation period was 8 days, in the presence of illumination - cool white fluorescent lighting to provide 4304 lumens (400 ± 10% ft-c) measured at the liquid level. The incubator is provided with culture closure - Foam plugs which must be used to permit good gas exchange and prevent contamination. The samples were shaken at least once daily to prevent settling and give an adequate supply of CO_2 (Skulberg, 1965). The assays were carried out in 200-ml Erlenmeyer flasks containing 40 ml of the algal assay medium (AAM). Bibasic potassium phosphate (K_2HPO_4) and Sodium nitrate ($NaNO_3$) salts were used as sources of phosphorus and nitrogen respectively. All assays were conducted in triplicate and each flask was inoculated from a seven-day-old culture of S. capricornutum grown in AAM.

The samples were filtered using 0.45 microns membrane filter. Filtration is essential to eliminate unwanted biological contaminants which would invalidate the growth response of the test organism.

An electronic particle - counter was used for all counting (Model-TA II Coulter Electronic Inc.) (USA, EPA, 1978). To calculate the inhibition percentage, a computer program operated on a Hewlett Packard 9830A programmable calculator was used. The LC 50 was estimated by plotting tested concentrations versus inhibition percent on a probits-log-scale paper, and extrapolating the best fit straight line.

3.2.2. Microtox Technique:

The second method for estimating the toxicity level of the tested samples was the Microtox technique, using a selection of marine bacteria ressembling Photobacterium phosphoreum as an index of toxicity. This bacteria was recommended based on its response to a wide spectrum of toxicants and on the fact that its response compares well with 96 hour LC 50 fish test (Bekman Instruments, Inc. Bulletin 6894).

This bacterium are of marine origin, and therefore, require a minimum level of NaCl as well as osmotic protection. Photobacterium phosphoreum display a good light stability in the presence of 2% ± 0.2 NaCl. Significant departure from this tolerance will cause measurement error due to the osmotic shock. Also, the light output of these bacteria is very dependent on temperature.

Several dilutions (usually between 3 and 5 dilutions) of the emanating fluid were tested, to assure a reliable estimation of toxicity. When preparing dilutions, the sample was first adjusted to 2%. NaCl, using the Microtox Diluent (Beckman, 1979). For all measurements, the Microtox Toxicity Analyzer - Beckman - Model 2055 was used, with 5 minutes exposure time of the marine bacteria resembling Photobacterium phosphoreum to the emanating fluid samples at 15°C. The effective lethal concentration was calculated by a program operated on a Hewlett Packard 9830A programmable calculator (Sprague, 1969). It predicted the inhibition percent of different sample concentrations. The LC 50 was estimated by plotting tested concentrations versus inhibition percent on a probits-log scale paper and extrapolating the best fit straight line.

3.3.3. Oxygen uptake:

The third toxicity index was the oxygen uptake by bacteria seeded into the tested samples. A 5 ml of wastewater (with microorganism of industrial origin) was used as a bacterial seed to which was added 10 ml of growing solution, containing all salts required for maintenance and reproduction of bacteria, this mixture was added to the samples, and oxygen uptake was measured frequently (every 2 hours) over a period of 24 hr using the Electrolytic Respirometer. Model ER-101, which uses closed reaction vessels. On top of each vessel is an electrolysis cell. The cell makes use of the pressure change due to the biochemical consumption of oxygen gas to sense the need for more oxygen. The necessary oxygen is electrolytically generated into the reaction vessel. As the reaction proceeds, an internal clock adds time to one of the two memories of each reactor. As oxygen is consumed and replanished, these data are added to the remaining memory for each reactor. Data are then printed out automatically at 2 hour periods.

CHAPTER 4

Results and Discussion

Results and Discussion

In the following paragraphs, the results are presented as average values for the three groups of samples (as shown in table 3.1), as well as highest and lowest values. The three groups of samples originate from different potential sludges:

a) The first figure always refers to group No. 1 samples, i.e. untreated sludge.

b) The second figure always refers to group No. 2 samples, i.e. treated sludge.

c) The third figure always refers to group No. 3 samples, i.e. sludge taken from sedimentation tanks (Suncor Inc., McMarry, Alberta) of different treatment processes.

The results for various tests mentioned in the previous chapter are provided in this chapter. The results are accompanied by brief discussion concerning their possible significance in the toxicity study.

4.1. Physical and chemical analysis:

The results of the physical and chemical analysis are summarized in Tables (C-1, C-2, C-3, and C-4). The obtained results will be presented and discussed below.

4.1.1. pH:

The pH values were in the range 7.5 to 8.20 with averages of 8.10, 7.9 and 7.8 for the three tested groups respectively (Table C.1). The pH values for all samples fall within the range 5 to 9 (pH units), which is the range generally tolerated by most aquatic organisms. However, the toxicity of chemicals change significantly with pH changes. For example, the toxicity of un-ionized ammonia increases markedly as pH increases from 7 to 8 (for the same ammonia concentration). Also the ionization and toxicity of hydrogen sulfide (H_2S) are similarly dependent on pH (Saweyer and Mc Carty, 1978).

4.1.2. Turbidity:

Turbidity values varied from 13 to 65 JTU in all samples and this difference is attributed to the addition of dewatering additives to some samples. The average turbidity values were 31, 37 and 44 for the three groups respectively (Table C.1). These turbidity values measured the light scattering properties due to the presence of suspended matter that interferes with the passage of light through the samples.

4.1.3. Specific Conductivity:

Specific conductivity values varied from 0.44 mmho/cm up to 1.570 mmho/cm. The average values for the three groups are 1.10, 0.887, and 0.725 mmho/cm respectively (Table C.1). This test measured the samples capacity for the transmitting of electric current as a function of the presence of ions, ions concentration, mobility, valence, and the temperature (Standard Methods, 1980). The measured specific conductivity values fall within the range 0.05 - 1.5 mmho/cm which generally characterizes potable waters in U.S.A. and thus the overall ionic strength of the samples would not have a direct effect on the toxicity observed.

4.1.4. Total Solids:

The total solid content varied from 737 mg/L to 4085 mg/L. The average values of the total solids in the three groups were 1500, 1093, and 2814 mg/L respectively (Table C.1). These values represent the material left in a vessel after sample evaporation in an oven at 105°C. The total solids results were considered to be above the permissible limit of potable water (500 mg/L) (EPA, 1978) and also compared to the previously determined values by Hrudey (1975) are considered high. This discrepancy between the values obtained here and those of Hrudey are attributed to the fact that these samples were taken from supernatant water coming from sludge settling processes while Hrudey's samples were from the upgrading plant— effluent stream following retention in the effluent pond and tailings pond dyke drainage.

4.1.5. Volatile Solids:

The volatile solids range from 204 up to 410 mg/L. The average volatile solids for the three groups are 350, 354, and 264 mg/L respectively (Table C.1). The determination of volatile solids offers a rough approximation of the amount of organic matter present in the solid fraction of the tested samples. However, it should be noted that this is only a rough measure, as results may also reflect loss of water crystallization, loss of volatile organic matter before combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

4.1.6. Suspended Solids:

Suspended solids varied from 34.0 up to 396 mg/L for all samples; the average suspended solids values are 197, 265, and 108 mg/L for the three groups respectively (Table C.1). These results are similar to those obtained by Hrudey (1975). The suspended solids can retard the photosynthetic process, blanket the benthic communities and also contribute to the biochemical oxygen demand. There is no evidence that concentrations of suspended solids less than 25 mg/L have any harmful effect on fish, and it should be possible to maintain good or moderate populations in water containing up to 80 mg/L suspended solids (Côté, 1976).

The high values obtained in this work indicate that the emanating fluid would be harmful to aquatic organisms, especially fish.

4.1.7. Metals:

a) Heavy Metals: Dissolved heavy metals concentrations, i.e. those metals able to pass through 0.45 micron membrane filter are given in table (C.2).

The most abundant metal was iron whose concentration averaged 2.13, 3.49, and 2.2 mg/L for the three groups respectively, and ranged from 1.029 up to 5.0 mg/L (Table

C.2). Concentration of iron greater than 0.2 mg/L have been reported to be lethal to 3 types of fish, but Wetzel (1972) found that iron concentrations as high as 40 mg/L was not lethal to channel catfish. The measured iron values are nevertheless a possible source of toxicity. Tests which determine whether or not iron and other metals are in fact a direct source of toxicity in the samples, are considered in section 4.2.2.

Manganese was the next most abundant metal, with concentration levels of 0.032 up to 0.612 mg/L (Table C.2). High concentrations of manganese (greater than 1.0 mg/L) commonly are very inhibitory to blue-green algae (Gertoff and Skoag, 1957; and Pateric et al, 1969). On the other hand, an antagonistic response was demonstrated in which increasing calcium (calcium average concentration 15.8 mg/L, table C.2) reduced the inhibitory effect of high manganese concentration. Manganese concentrations of less than 0.05 mg/L were found to inhibit the development of green and blue-green algae in streams, and to strongly favor diatomic growth (Wetzel, 1975). Both iron and manganese however, are essential micronutrients of microflora, plants and animals (Wargersky, 1963 and Coughlan, 1971) and are toxic at very high concentrations. Concentrations of manganese measured in the samples are clearly lower than levels observed to exhibit toxicity.

Copper was the third most abundant metal, with concentration levels of about 0.09 mg/L. Copper concentrations varying from 0.1 mg/L up to 1.0 mg/L have been reported to be non-toxic to most fish by many investigators (Wetzel, 1975; and Patric et al, 1969) while others have reported copper concentrations ranging from 0.015 - 3.0 mg/L to be toxic to fish and ZOO plankton (Wetzel, 1975). Therefore, copper levels in the samples may possibly constitute to a source of toxicity.

Zinc was measured in the test samples at an average value of 0.09 mg/L and within a range varying from 0.062 up to 0.137 mg/L (Table C.2). Zinc concentrations of 0.01 mg/L have been reported by Mckee and Wolf (1963) to be lethal to Rainbow trout, although concentrations less than 0.1 mg/L are generally observed not to be toxic to many aquatic organisms. Zinc therefore may or may not be a source of toxicity in the emanating sludge fluid samples. Another aspect in the toxic effect of zinc is the simultaneous presence of copper. Wetzal (1972) observed that fish could survive at zinc concentrations of 8 mg/L for 8 hr but in the presence of 0.025 mg/L of copper and only 1 mg/L zinc most of the fish died after 8 hr.

Cadmium concentrations were less than 0.02 mg/L. Purves (1977) reported that, cadmium toxicity is increased by the presence of other chemicals, including copper and zinc, which are present in significant concentration in the tested samples.

Concentration of the other metals such as nickel, vanadium, lead, and arsenic are undelectable in the tested samples, indicating that these metals (if present) are present at concentrations below toxic levels.

The heavy metals are toxic to microorganisms because of their ability to tie up the proteins in the key enzyme systems. The heavy metallic salts prevent the proteins from reacting normally and even change the charge at the molecular level, at certain points from negative to positive, causing repelling reaction rather than attracting reactions. If the concentration of heavy metals is increased further, the surface of the cells become completely coated preventing materials from entering the cell. Precipitation of the cellular protein may even occur.

It is difficult to establish safe standards of concentration of toxic elements in the environment, for account has to be taken of the fact that in organic combination they may cause a greater hazard than when present in inorganic form. For example, organic coumpounds of lead are more toxic than the metal itself. The ionic state may also affect the level of toxicity, for example chromium is more toxic in the hexavalent than in the trivalent state.

b) Other Metals: The emanating sludge fluid samples were examined for calcium, magnesium, sodium and potassium. The results for these metals are summarized in table (C.2). These four metals are measured to examine the ability of the samples to provide macronutrients and micronutrients to the aquatic organisms.

Calcium is found in the tested samples with an average concentrations of 8.5, 14.1, and 24.8 mg/L, with a range varying from 7.5 up to 43 mg/L. Calcium has been implicated in numerous ways in the growth and population dynamics of flora and fauna. It is required by green algae and is considered to be a basic inorganic element of alga cell. Calcium is essential for maintenance of the structural and functional integrity of cell membranes in ion absorption and retention (Wetzal, 1975).

Magnesium concentrations averaged 8.2, 6.3, and 12.4 mg/L in the three tested groups respectively, and the magnesium range varied from 7.7 up to 45 mg/L. Magnesium is required as a micronutrient in the enzymatic transformation of organisms, especially in transphosphorylation of alga, fungi, and bacteria. The demand for magnesium in metabolism is minor in comparison to the quantities present in the test samples.

The monovalent cations, sodium and potassium are involved primarily in ion transport and exchange. Sodium content ranged from 151 up to 215 mg/L with averages of 184.5, 181.3, and 184.6 mg/L for the three tested groups respectively. Potassium concentration ranged from 8.07 up to 14.20 mg/L, with averages of 12.02, 10.37 and 13.0 mg/L.

Sodium requirements are particularly high in some species of blue-green alga (Allen, 1952 and Gerloff et al, 1952). A threshold level of 4 Na mg/L is required for near optimal growth of several species (Kratz and Myers, 1954). Maximal growth of several blue-green alga was found at 40 mg Na/L.

4.1.8. Biochemical Oxygen Demand (BOD)

The BOD values for the three tested groups averaged 32.0, 35.7 and 49.7 mg/L respectively (Table C.3). The BOD range varied from 22.0 up to 64.25 mg/L. The BOD results reflect the presence of toxic compounds and/or complex organic materials. Theoretically, the BOD values obtained for different dilutions for a given sample should have been equal. That is, multiplication of the dilution factor by the difference in dissolved oxygen between the blank on day 5 and the given sample on day 5 should have yielded the same BOD for each dilution of the given sample. This is not the case as shown in Figures (4.1 to 4.6). In all samples, smaller dilutions yield lower BOD values apparently indicating that the ability of microorganisms to degrade organic constituents in the samples decreases with increasing concentrations of sample. So that the use of the BOD test in the conventional sense (i.e. determination of the amount of oxygen required by microorganisms while stabilizing decomposable organic matter under aerobic conditions) was, therefore not possible. (This observation was also observed by Ludwig (1983) using the Fresh tailings water and Aged tailings water).

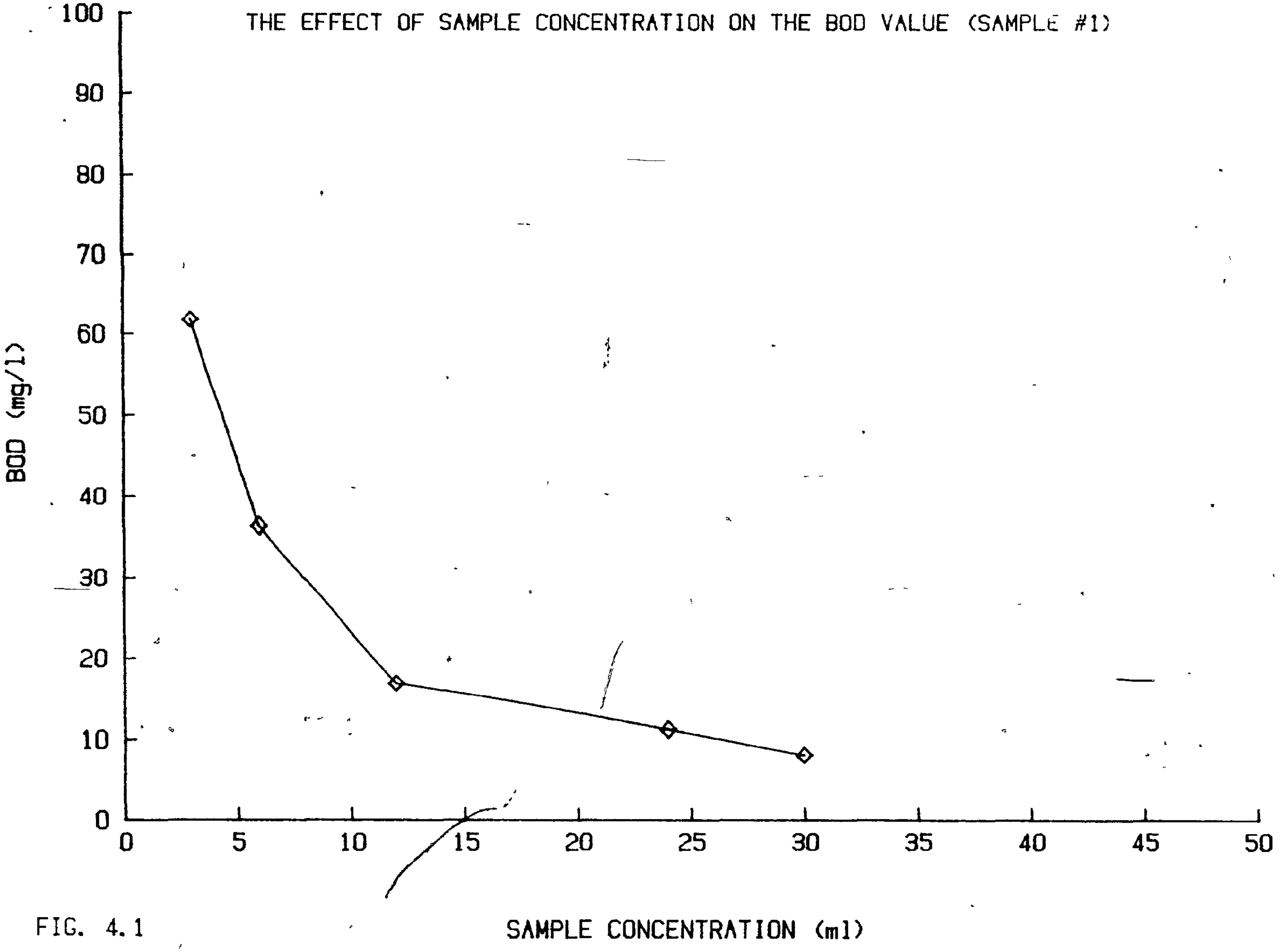

THE EFFECT OF SAMPLE CONCENTRATION ON THE BOD VALUE (SAMPLE #1)
BOD (mg/l)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
SAMPLE CONCENTRATION (ml)

FIG. 4.1

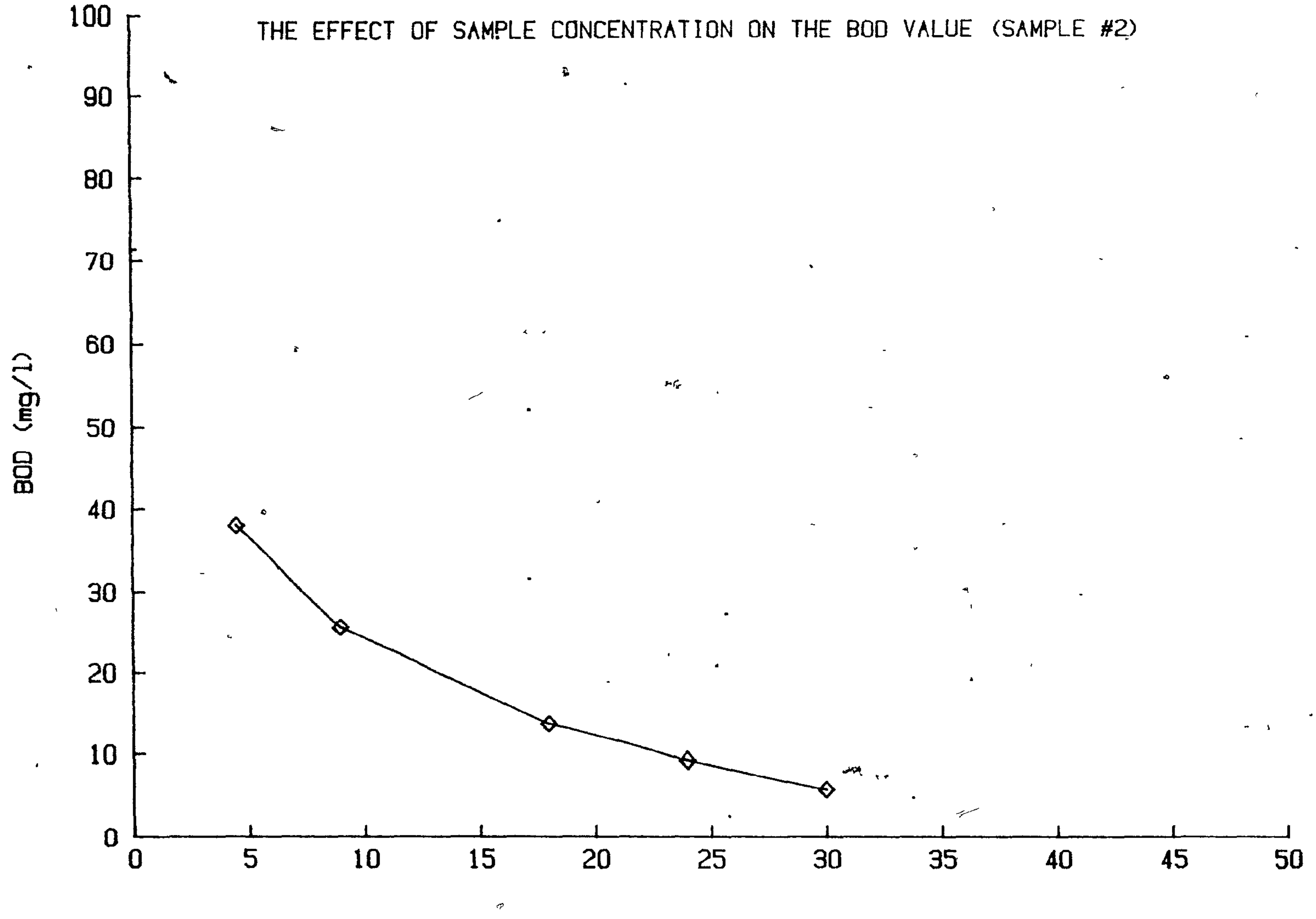

THE EFFECT OF SAMPLE CONCENTRATION ON THE BOD VALUE (SAMPLE #2)
BOD (mg/l)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
SAMPLE CONCENTRATION (ml)

FIG. 4.2

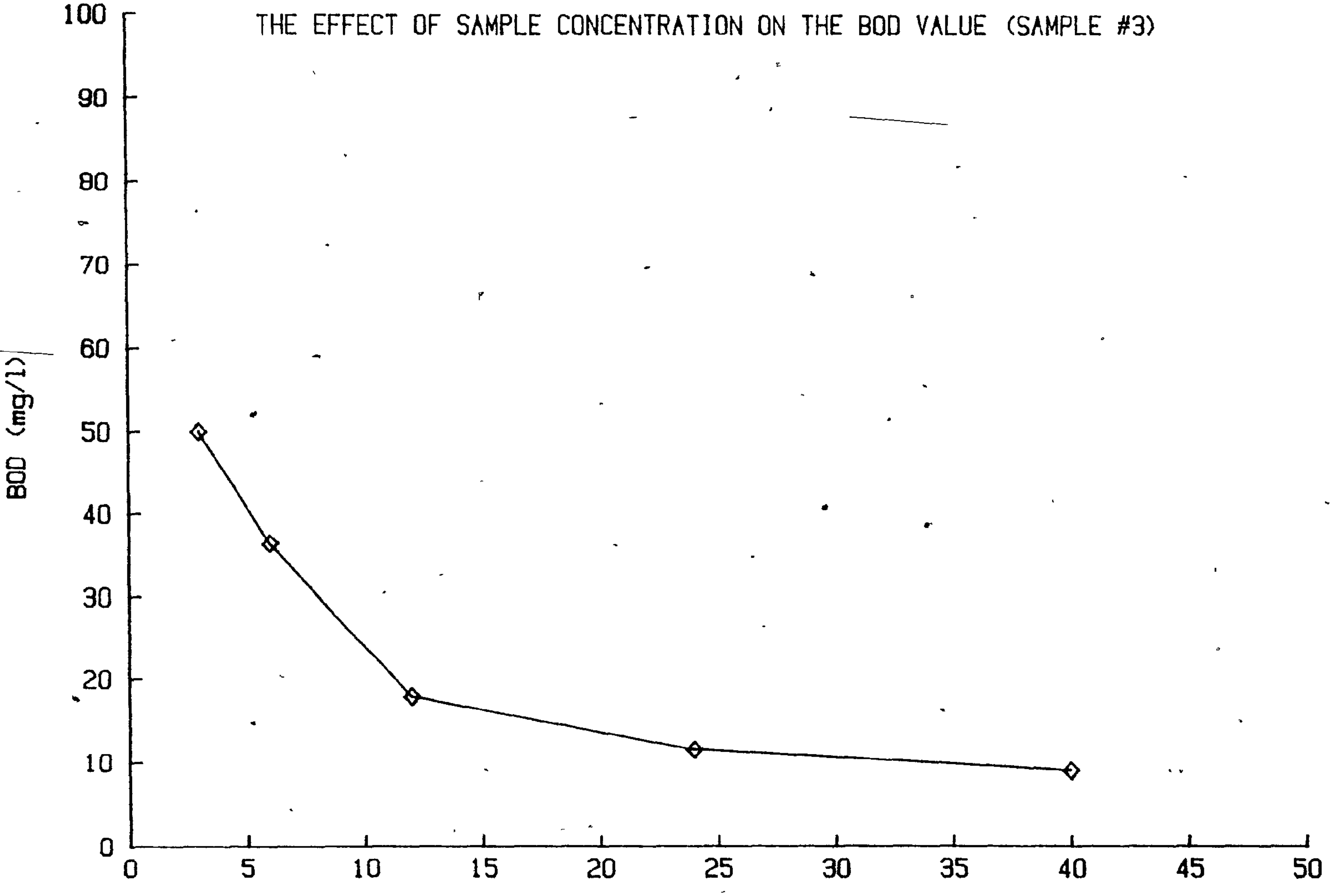

THE EFFECT OF SAMPLE CONCENTRATION ON THE BOD VALUE (SAMPLE #3)
BOD (mg/l)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
SAMPLE CONCENTRATION (ml)

FIG. 4.3

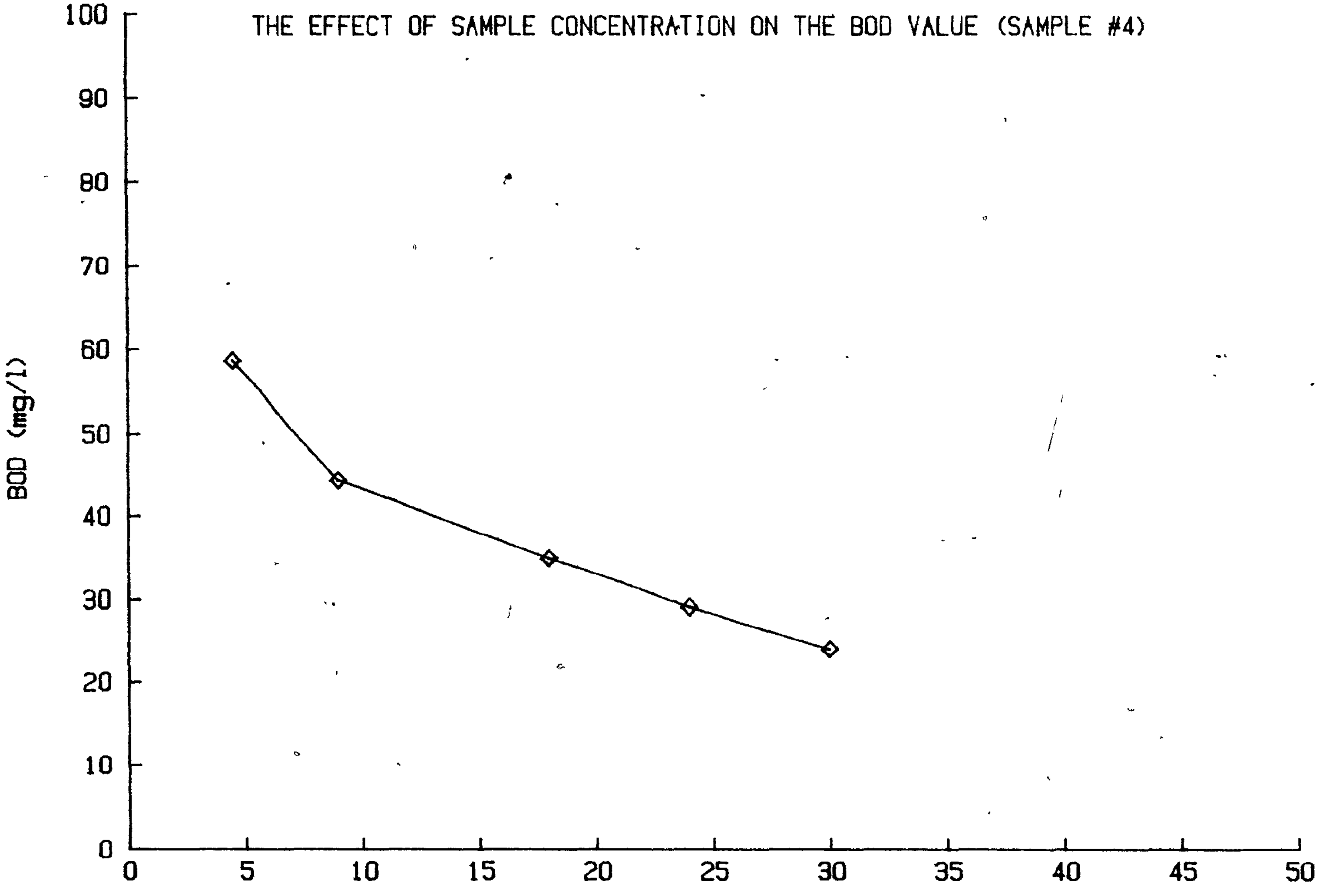


FIG. 4.4

SAMPLE CONCENTRATION (ml)

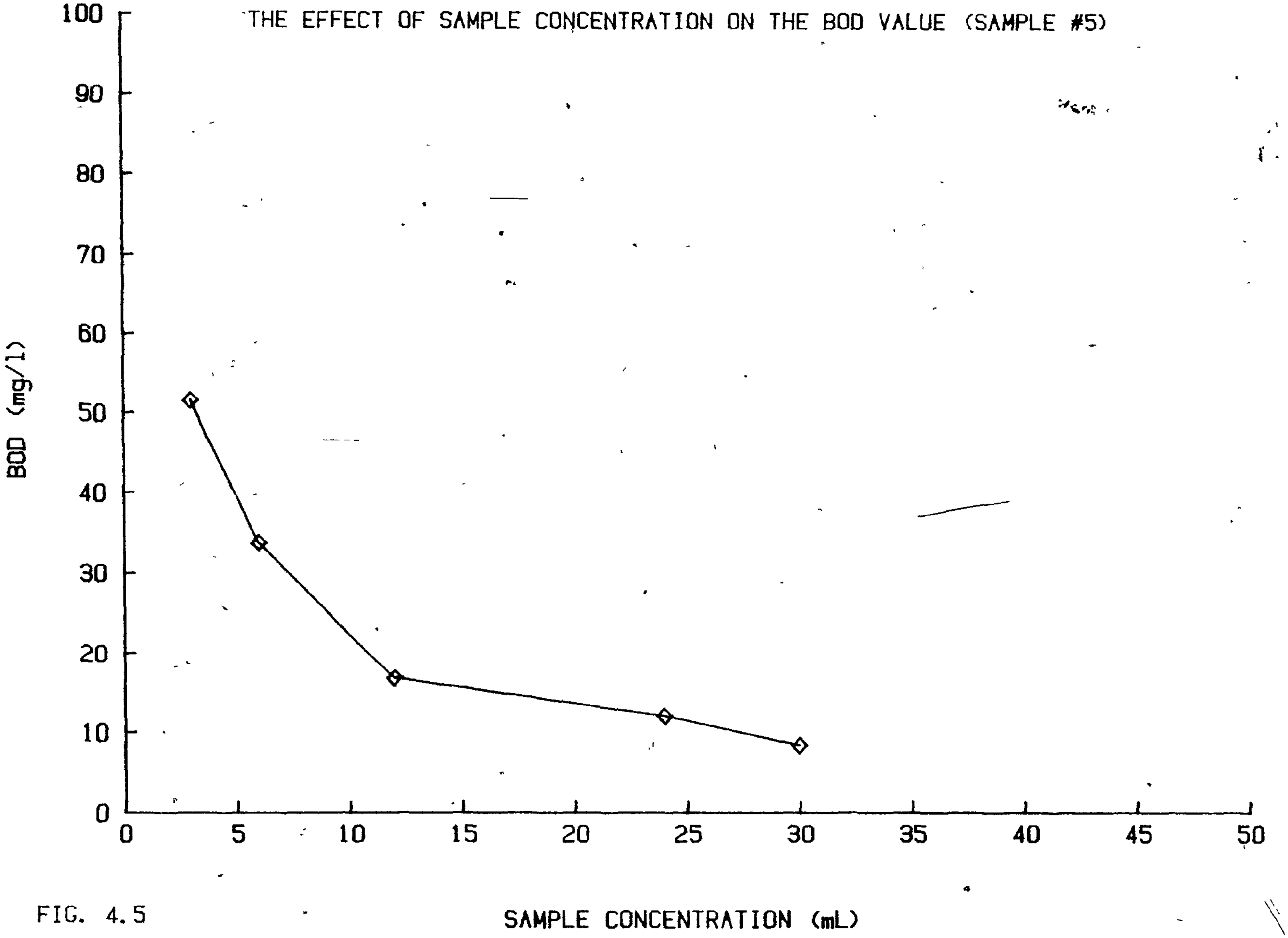

THE EFFECT OF SAMPLE CONCENTRATION ON THE BOD VALUE (SAMPLE #5)
BOD (mg/l)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
SAMPLE CONCENTRATION (mL)

FIG. 4.5

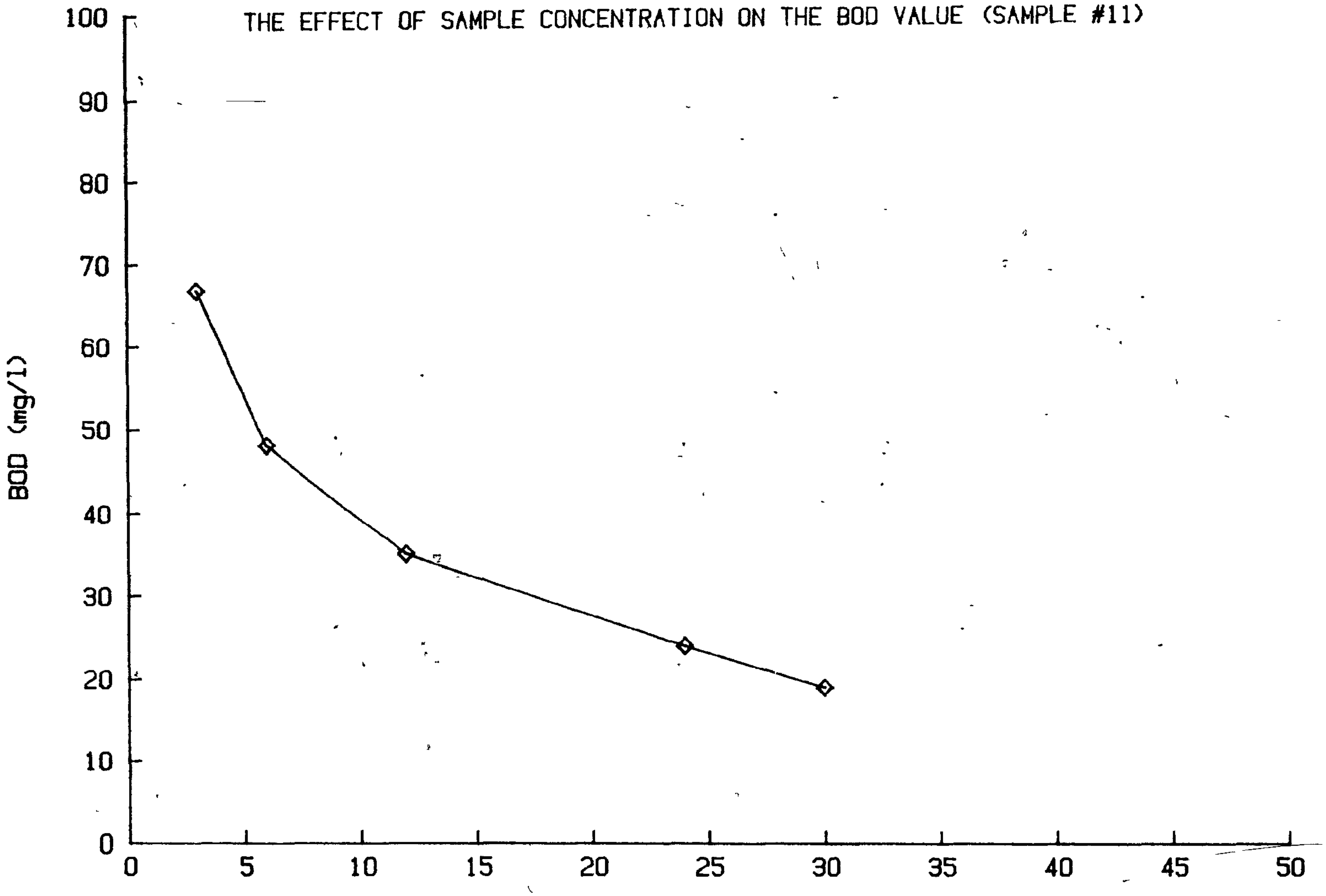

THE EFFECT OF SAMPLE CONCENTRATION ON THE BOD VALUE (SAMPLE #11)

FIG. 4.6

4.1.9. Chemical Oxygen Demand (COD):

The results for chemical oxygen demand (COD) averaged 423, 353, 354 mg/L for the three groups respectively (Table C.3). COD varied from 216 up to 490 mg/L. The COD results agree with the volatile solids results (see Table C.1). Also the general picture of water soluble organic substances derived from this study is similar to that of previous investigations (Strosher and Peak, 1976; Hrudey, 1975; and Ludwig, 1983). These values are substantially higher than the BOD values obtained for the same samples (Sec. 4.1.8), thereby suggesting that the samples may either contain large quantities of non-biodegradable organic matter or, that the samples are toxic to microorganisms used as a toxicity index.

COD data cannot be expected to correlate well with BOD data primarily because the two tests measure different aspects of oxidizable constituents of tested samples. To explain the difference between COD and BOD results, the Ultimate Biochemical Oxygen Demand (BODu) test (which is expected to approximately reproduce the COD value in the absence of toxic compounds) was carried out on five samples. The BODu average value was 200 mg/L, with an average difference of about 170 mg/L from the COD average value (Table C.3). This large difference implies the existence of toxic compounds and/or biologically resistant organic matter.

Certain organic compounds, particularly low - molecular weight fatty acids are not oxidized by dicromate unless a catalyst is present (it has been found that silver ion acts effectively in this capacity). Aromatic hydrocarbons and pyridine are not oxidized under any circumstances. Because of all these limitations, the results do not necessarily reflect the right amount of organic matter present in the tested samples.

4.1.10 Total Organic Carbon (TOC):

The TOC average values for the three tested groups were 107, 127.9, 239.8 mg/L respectively. The TOC values varied from 52.30 up to 281.90 mg/L for all samples (Table C.3). The ratio between COD/TOC is equal to 2.30 (while the theoretical ratio equals to $\frac{32}{12} = 2.66$, in the case where all organic matter present is potassium dicromate oxidizable). This difference is attributed to the presence of organic carbon that did not respond to either BOD or COD tests.

The TOC values obtained in this study are higher than those of Hrudey (1975), since the samples originating from emanated sludge fluid have a high content of aromatic hydrocarbons and pyridine, not oxidized by the COD process. (Table C.3). So, conclusions based on a comparison between COD results and TOC results are rather precarious.

4.1.11. Inorganic Carbon:

The total inorganic carbon averaged 73.10, 56.20 and 144 mg/L for the three tested groups respectively. The total inorganic carbon values varied from 36.72 up to 183.40 mg/L and were of the order of 55% of TOC (Table C.4).

4.1.12. Extractable Carbon:

Samples were extracted with benzene and, evaporated by a rotary vaccuum apparatus, to remove traces of solvent, they were then analyzed for the total organic carbon content. The results indicate that the ratio of extractable carbon to original carbon is in the range of 63 to 74% (Table C.4).

Organic materials may exist in the test samples in several forms: as dissolved organic compounds, as dispersed or micellar materials, as suspended carbon particles, and as materials adsorbed on the surface of suspended inorganic particles. Dissolved organic compounds and dispersed organic material may be readily extracted from the samples with solvents such as benzene; compounds adsorbed on the surface of inorganic particles are not readily extracted and carbon particles are not extractable from the water at all. The fact that over 30% of the organic carbon present in the test samples is not extractable indicates that it either occurs as discrete carbon particles or that it is strongly adsorbed on the surfaces, or that it is found in the form of unextractable humic and fulvic acids. (Strosher and Peak, 1976).

4.1.13. Total Nitrogen:

The total nitrogen values indicate that most of the nitrogen is in the ammonium form. The total nitrogen content values for the three groups averaged 38.5, 44.6 and 44.1 mg/L respectively. The total nitrogen values varied from 18.0 up to 72.0 mg/L for all samples (Table C.5). The tested ammonia concentrations (ionized and un-ionized) of the three groups were 26.5, 27.6 and 38.3 mg/L respectively, with a range of 12.6 up to 63.0 mg/L for all samples (Table C.5). The results for total nitrogen and total ammonia are similar to the results of Barbour et al, 1977 and Ludwig, 1983. Total nitrogen concentrations of the samples indicate that the nitrogen would not likely be a limiting factor in the growth processes of aquatic organisms such as algae (Miller et al, 1976).

4.1.14. Phosphorus:

Total and inorganic phosphorus concentrations are shown in table (C.5). The concentrations of the total phosphorus varied from 0.02 to 0.05 mg/L and the values of inorganic phosphorus were in the range of 0.01 to 0.02 mg/L. Thus, phosphorus may not be considered sufficient to support substantial algal growth and could be considered a limiting nutrient (Miller et al, 1976).

The phosphorus results may be affected by the delay of the analysis which can alter the relationship between dissolved and particulate forms of phosphorus. Also, the presence of nitrile compounds and arsenic lowered by abount 3% the phosphorus results (Standard Methods, 1980).

Usually, phosphorus is considered by most investigator's to be the key nutrient in controlling algal growth, since both nitrogen and carbon dioxide exist in the atmosphere and reach equilibrium concentration in water.

4.1.15. Phenol:

Phenol concentration (and its steam - distillable ortho - and meta substituted derivatives) was measured at an average range of 0.03 mg/L (Table C.5). This value is far below the EPA estimated safe phenol concentration for receiving waters of 0.1 mg/L and thus it would not be expected to have a direct toxic effect on the biological indices employed. The possible synergistic effects of phenols must, however, not be neglected (Sawyer and Mc Carty, 1978).

4.2. Bioassay Analysis:

4.2.1. Growth Pattern:

Figures (4.7 - 4.12) show comparison of the growth of Selenastrum capricornutum exposed to sample concentrations (v/v) of 12.5, 25, 50 and 100% with the growth of the control. The initial cell density in all samples was 1000cell/mL. The rate of growth of S. capricornutum with different emanating fluid concentrations follow a growth pattern similar to that given by Knaysi,(1951), Lamanna,(1955), Kauss et al, (1975) and Graudy and Graudy, (1980).

These curves shown in Figures (4.7 - 4.12) may be divided into five defined phases:

(1) Lag phase: due to adaptation to a new environment, and characterized by a long generation time, a null growth rate, a maximum cell size and a high rate of metabolic activity.

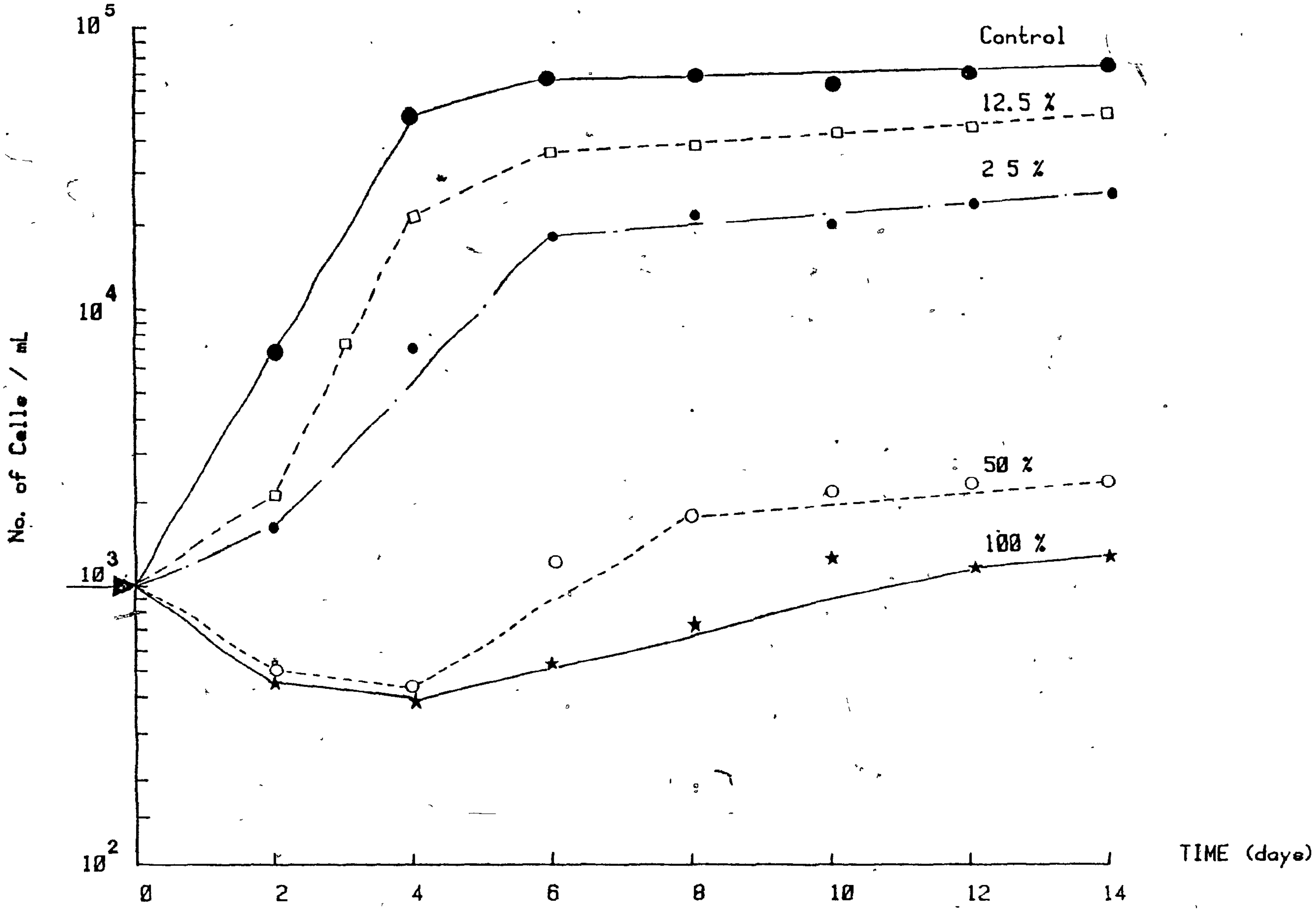


FIG. 4.7 Growth of S. *capricornutum* in medium containing sludge fluid (Sample # 1)

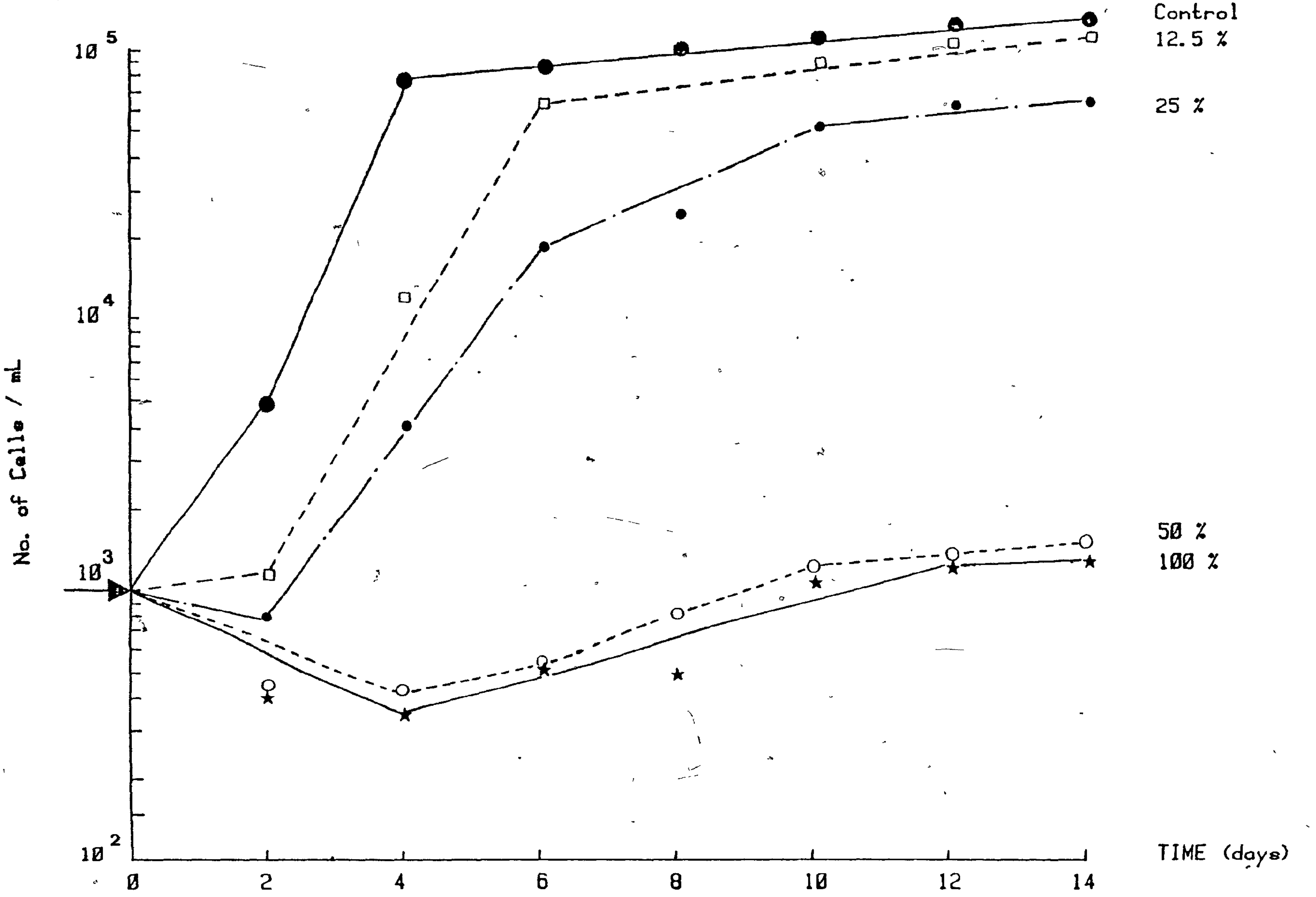


FIG. 4.8 Growth of S. capricornutum in medium containing sludge fluid (Sample # 3)

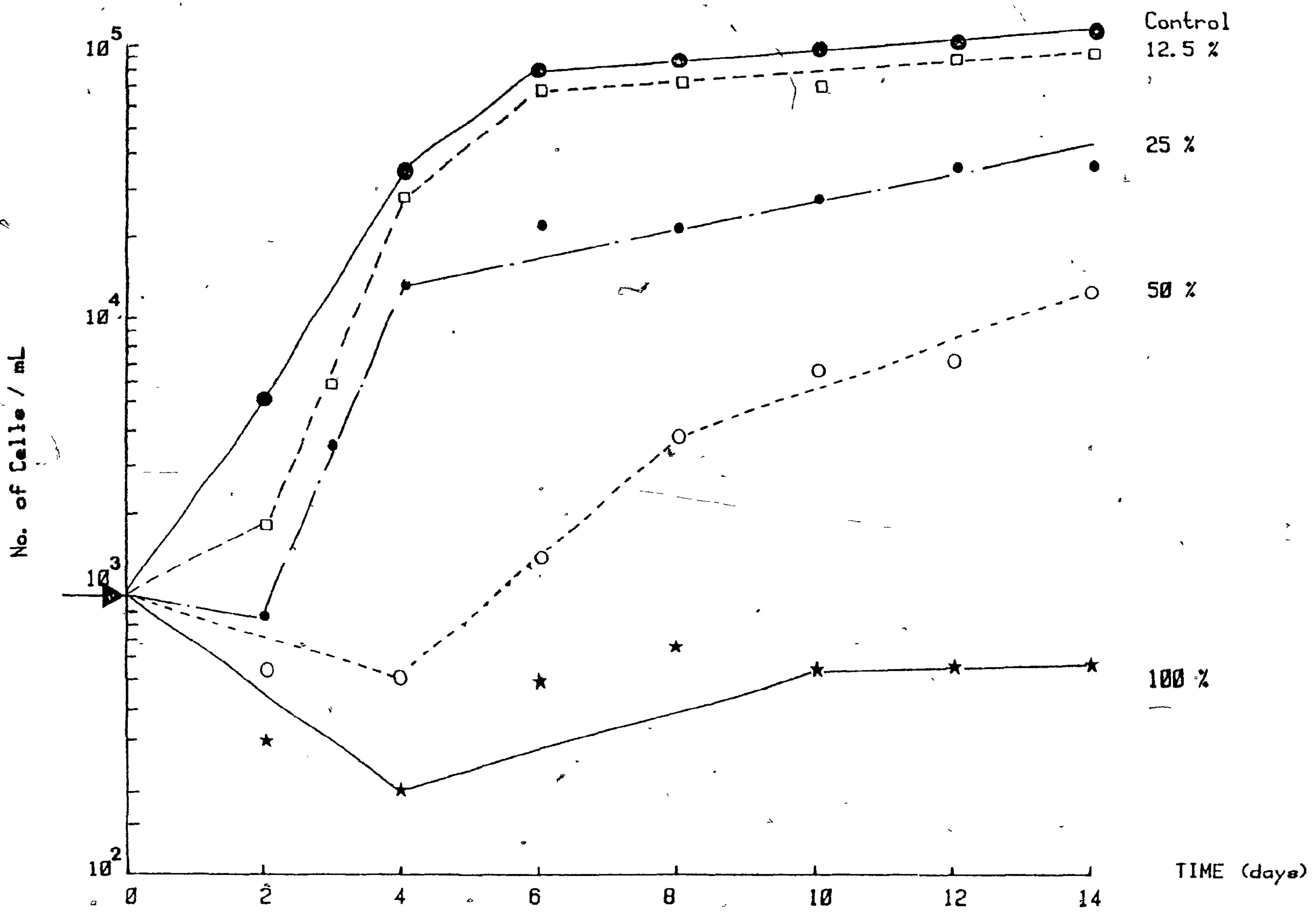


FIG. 4.9 Growth of S. capricornutum in medium containing sludge fluid (Sample # 11)

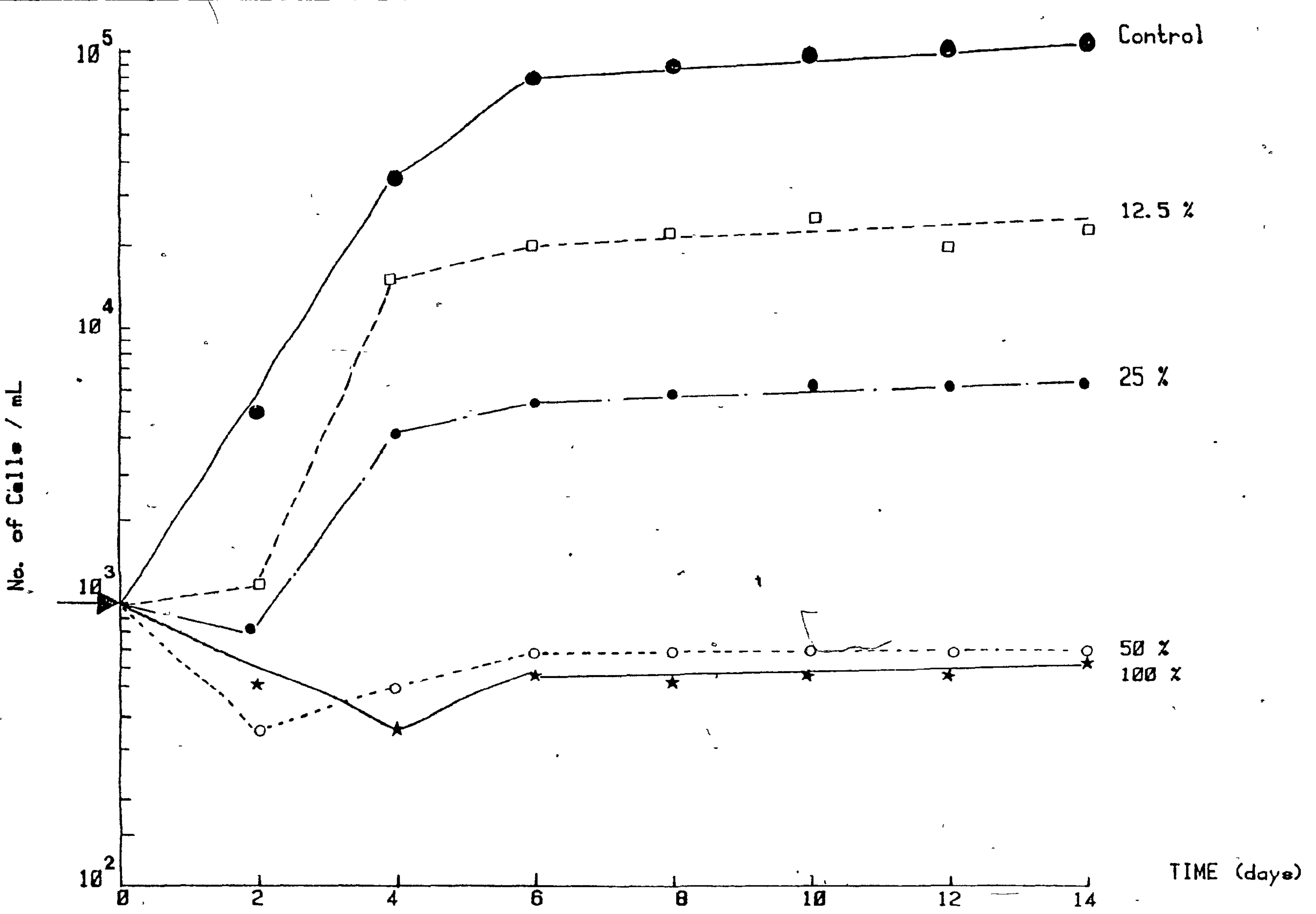


FIG. 4.10 Growth of S. capricornutum in medium containing sludge fluid (Sample # 13)

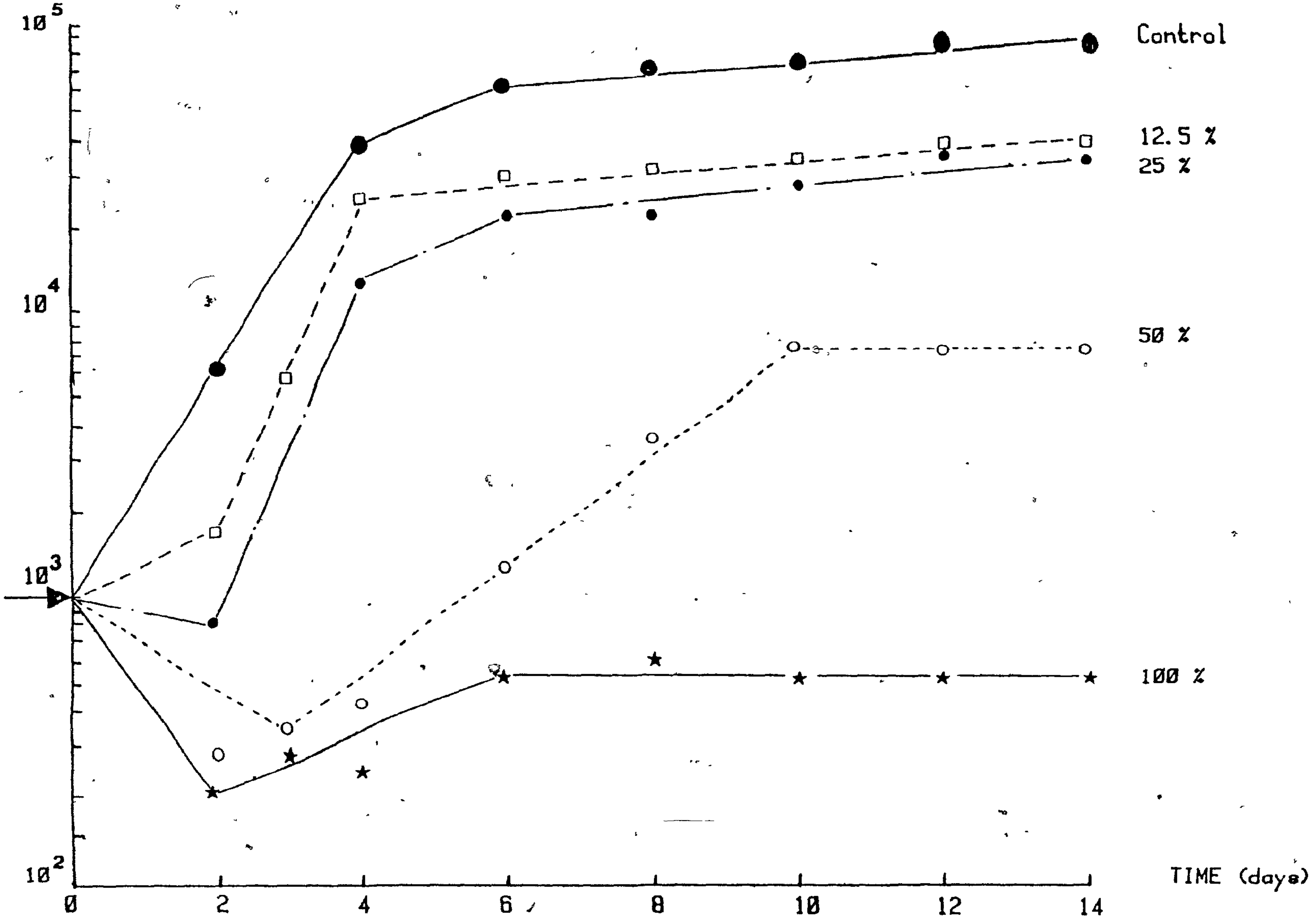


FIG. 4.11 Growth of S. capricornutum in medium containing sludge fluid (Sample # 16)

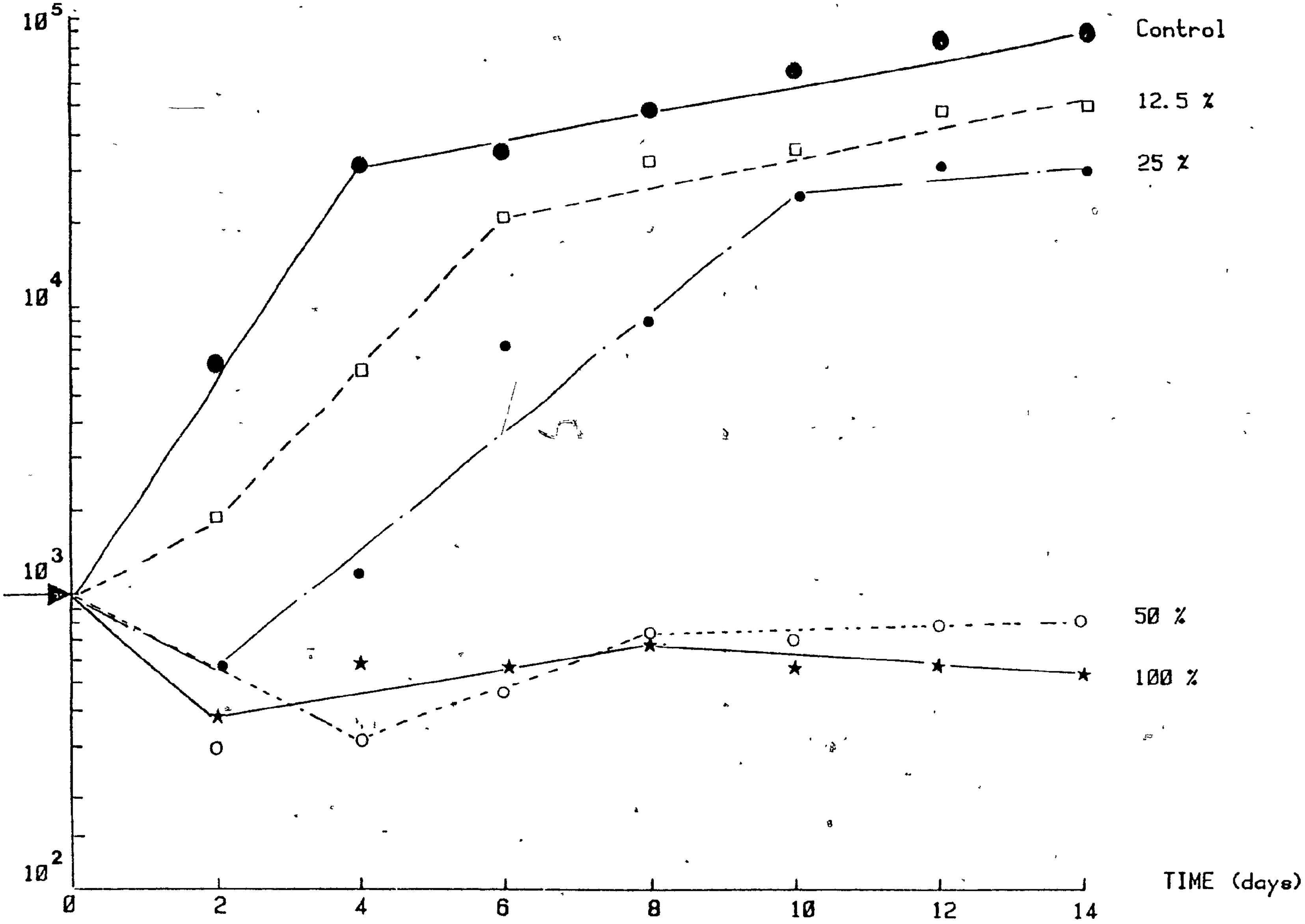


FIG. 4.12 Growth of S. *capricornutum* in medium containing sludge fluid (Sample # 19)

(2) Acceleration phase: characterized by decreasing generation time and increasing growth rate.

(3) Exponential phase: characterized by minimal and constant generation time, maximal and constant specific growth rate and increasing cell density.

(4) Declining growth phase: characterized by increasing generation time and decreasing specific growth rate, due to gradual decrease in substrate concentration, and increased accumulation of toxic metabolites.

(5) Stationary phase: characterized by exhaustion of nutrients, high concentration of toxic metabolites and maximum physical crowding.

The results of the bioassay analysis employing the green algae S. capricornutum as a test organism are given in Table (C.6). The average cell densities recorded at one or two-day intervals have been expressed as a percentage of those in the control flasks. The initial toxicity of the different concentrations (100, 50, 25 and 12.5% by volume) varied considerably. After two days of exposure cell densities for 100% and 50% samples concentrations (by volume), were from 3.3% to 11.6% and from 5% to 12.6% lower than in the controls respectively. For the higher dilutions (25% and 12.5% by volume) cell densities were from 13% to 22% and from 24% to 38% lower than in the controls respectively. Comparison with controls revealed that the lag period between inoculation and commencement of growth progressively increased with the initial sample concentration. Recovery was less rapid at higher concentrations. In 100% samples concentration, no recovery of growth occurred during the test period. The slope of the curves $\frac{dx}{dt}$ (X = no of cell/mL) increase in phase 2 and 3 (acceleration phase and exponential phase), and thereafter decrease until the maximum concentration of cell number is obtained and the population enters the stationary phase in which $\frac{dx}{dt} = 0$.

There are many reasons why cells do not grow indefinitely, including exhaustion of nutrients, depletion of the dissolved oxygen supply, crowding, growth-induced changes in the chemical environment, and/or presence of toxic substances (Gaudy and Gaudy, 1980).

4.2.2. Algal Assay Bottle test (original samples)

The S. capricornutum Algal Assay Bottle test results are summarized in Table (C.7) and Figures 4.13 & C.1 - C.13 The figures are presented in the form of histograms in which the height of each individual histogram for any given sample is equal to the algal density as a percent of control. The algae growth in the control flasks (distilled water plus nutrient (PAAP) solution only), is considered to be the theoretically expected algae growth, thus the growth in the other flasks are compared with that in the control, (i.e. the growth in the control is considered as 100% growth). Sample concentrations ranging from 100% to 3.13% (by volume) were examined. Three different cases are considered in this study:

1) 100% (by volume) samples concentration only.

2) 100% (by volume) samples concentration plus EDTA.

3) samples concentrations ranging from 100% to 3.13% (by volume) plus (PAAP) solution, (a nutrient solution, see Appendix B).

The inhibition percent of S. capricornutum green alga with 100% sample (first case) ranged from 92.75% to 99.70% which means that the emanating fluid is acutly toxic. This can be attributed to the presence of highly toxic trace elements, either organic or inorganic, or the deficiency of the samples to present the required nutrients for the growth and reproduction of the test organisms.

To examine if the toxicity of the emanating fluid was caused by the heavy metals or not, the synthetic organic ligands, ethylenediamineteraacetic acid (EDTA), considered as a chelating agent, was added principally to collect Fe and Mn which are present in significant amounts in the test samples as observed in the heavy metals analysis and also collect Zn (see table C.2). The toxicity results in this case provided an inhibition percent ranged from 96.05 to 99.75%, i.e. the emanating fluid supported only approximately 3% of the theoretically expected algae growth. The difference in algae growth in the presence and absence of EDTA is very small (in the order of 1 or 2%), and these results indicate that the metals appear not to be the only cause of the observed toxicity. Nevertheless, if metals do contribute to toxicity, their contribution appears to be relatively insignificant in the toxicity results presented in this study.

Up to this stage, the conclusion is that the samples are toxic to the green alga S. capricornutum and this acute toxicity is not attributed only to metals, but either to the deficiency of the nutrients or the presence of dissolved hydrocarbons.

The N:P ratios of the test samples indicate that phosphorus is a growth limiting factor. To make sure if the deficiency of the nutrients may or may not cause the growth limit of the test organisms, 100% samples concentrations plus (PAAP), a nutrient solution containing Dibasic potassium phosphate ($K_2 HPO_4$) and sodium nitrate ($NaNO_3$) as sources of phosphorus and nitrogen respectively, were examined. The inhibition percentage of S. capricornutum algae gained from these series of experiments was of the range 94.97 to 99.77%, i.e. the samples supported only approximately 4% of the theoretically expected algae growth. These results indicate that, not only the deficiency of the phosphorus alone is the reason of the growth limiting condition. So that the toxicity observed may be attributed to the presence of dissolved hydrocarbons, and this will be discussed in the next section (4.2.3.).

To estimate the lethal concentration (LC 50), the inhibition percentage with different concentrations of emanating fluid (100, 50, 25, 12.5, 6.25, and 3.13% by volume) were measured and plotted against concentrations in a probits - log scale graph paper (Standard Methods, 1980). The best fit straight line was drawn and the lethal concentration (LC 50) was determined. The lethal concentration is defined as the concentration of toxicant in which just 50% of the test organisms are able to survive for specific period of exposure . The lethal concentrations LC 50 were of an average for the three groups tested (Table 3.1) of 24, 30 and 13.5% (by volume). Hrudey (1976) employing Rainbow trout (Salmo gairdneri) got a result of 96 hour - LC 50 of 11% (by volume) for Tailings pond dyke drainage, which is lower than the above results (Table C.7), taking into consideration the difference in the test organisms (the lower the organisms the less sensitive to the toxicant). Figure 4.13 (a representative graph) shows the relation between sample concentrations (by volume) and algal cell density as percent of that observed in the control (the theoretically expected algae growth). As shown the less the sample concentration, the higher the algal growth, i.e. the less the inhibition percent (Figures C-1 - C-13 : see Appendix C).

4.2.3. Algal Assay Bottle test results (for emanating fluid samples of the passing through activated carbon column):

Another set of bioassay results employing *S. capricornutum* green algae as a test organism was carried out. The samples examined in this set were the same samples used before but were passed through an activated carbon column. The activated carbon column was used to remove a portion of the dissolved organic matter, and as mentioned

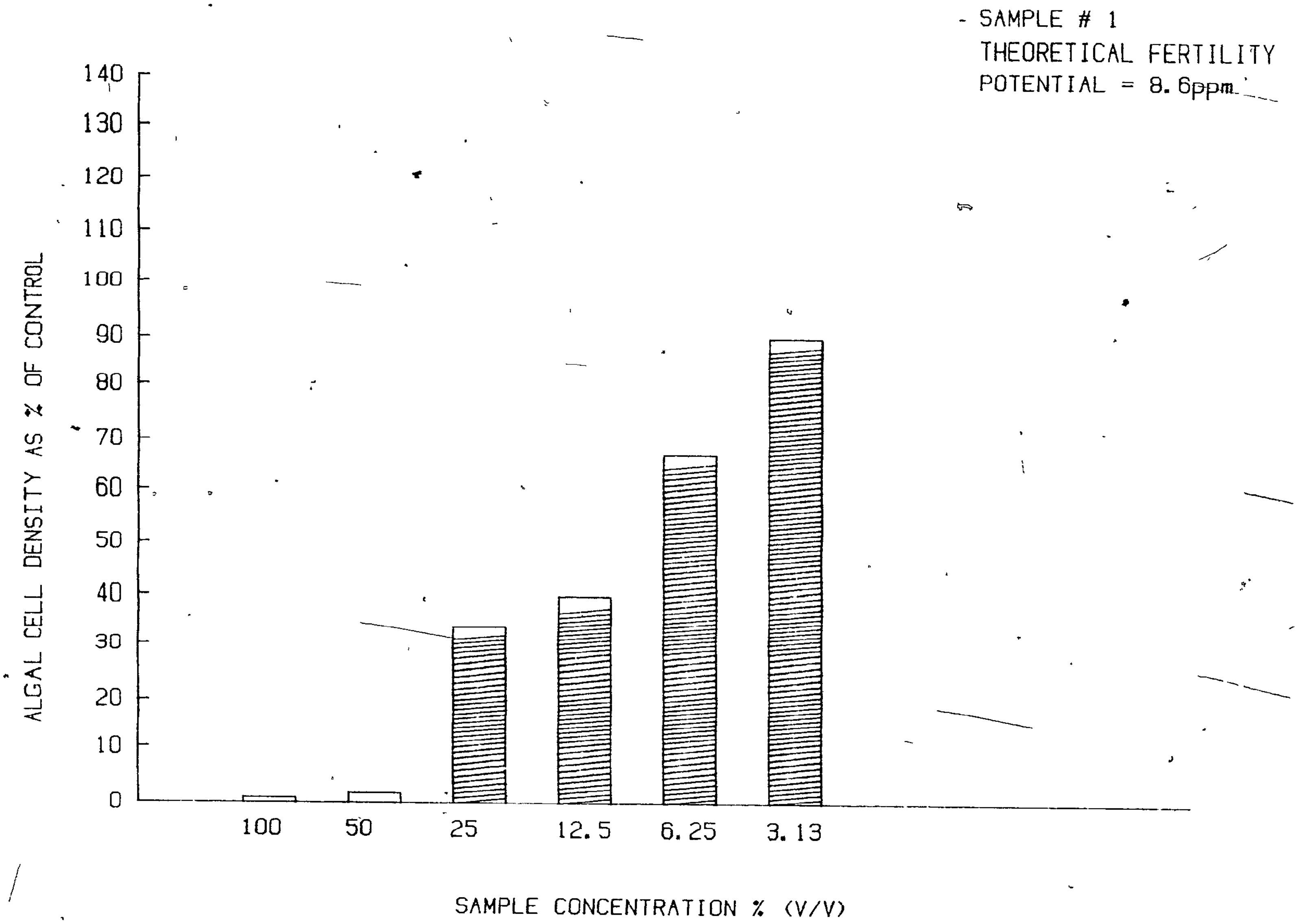


FIG. 4.13 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

in section 4.1.12, the ratio of extractable carbon to original carbon is in the range of 63% to 70% (Table C.4) which may indicate the presence of high percentage of dissolved hydrocarbons. The emanating fluid samples was applied to the top of the column and withdrawn at the bottom. The results of this set are summarized in table (C.8). The same steps taken in the previous section (section 4.2.2.) was followed in testing this group of samples.

The inhibition percentage results of S. capricornutum with 100% (by volume) sample concentrations with or without the nutrient solution (PAAP) consisting of macro and micro nutrients (see Appendix B) were in the range of 90 up to 95% (by volume) i.e. the emanating fluid samples supported only from 5 to 10% of the theoretically expected algae growth. The results of inhibition percentage are lower than that of the original sample by only 5%, which is relatively small, and may be attributed to the presence of high percentage of the dissolved hydrocarbons which include the most toxic components of oil, and are also the most soluble in water (Laws, 1981).

The effects of dissolved hydrocarbons fall into two general categories. The first category includes effect associated with coating or smothering of an organism. The second category of toxic effects involves disruption of an organism metabolism due to the ingestion of oil and the incorporation of hydrocarbons into lipid or the tissue in sufficient concentration to upset the normal functioning of the organism (Nelson - Smith, 1972).

Another 100% samples concentration set were examined for acute toxicity in the presence of the chelating agent (EDTA). The inhibition percentage was in the range from 90.27 up to 99.66%, i.e. the samples supported only approximately 1% up to 10% of the theoretically expected algae growth, and this is the same as shown in section 4.2.4., where the metals would not solely cause the observed toxicity.

Table (C.8) shows that the lethal concentration (LC 50) averaged 40, 36, and 28.5% (by volume) for the three groups tested respectively. The difference between the lethal concentration in the original samples and those passed through the activated carbon column was of the average of 30%, and this result reflects the ability of the activated carbon column to adsorb and remove a portion of the dissolved hydrocarbons in the tested samples. Figure 4.14 (a representative graph) shows the relation between sample concentrations (by volume)

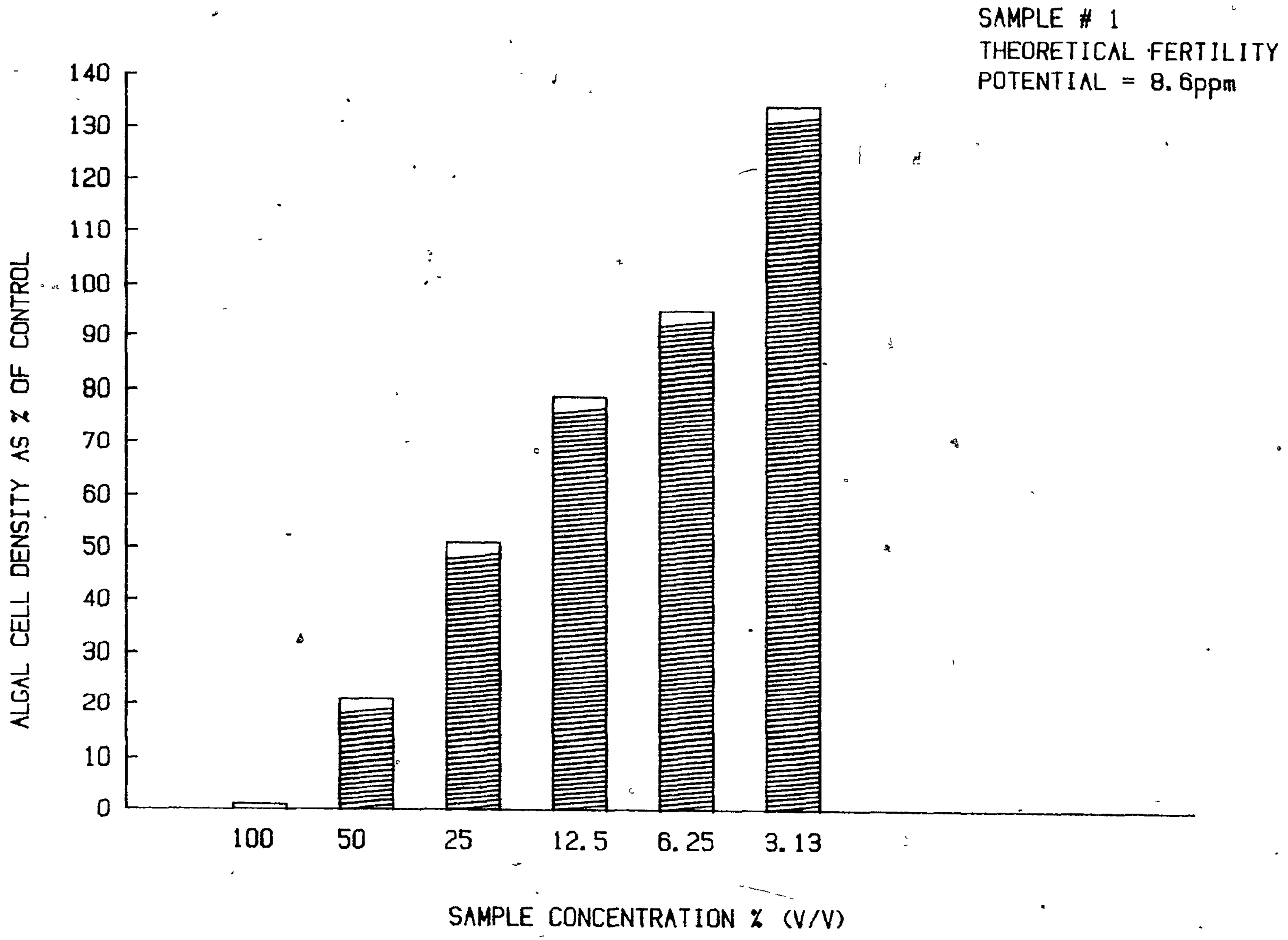


FIG. 4.14 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

and the algal cell density as percentage of control in the form of a histogram. As the sample concentration decreases, the algal cell density approaches that in the control or even more. (Figures C.14 to C.26, see Appendix C).

4.2.4. Microtox Bioassay:

The toxicity results using the marine bacterium resembling Photobacterium phosphoreum as shown in Table (C.9) and Figures 4.15 & C.27 - C.39. In this toxicity test, the quantity of light emitted by the bacteria five minutes after inoculation into the samples relative to the quantity of light emitted by the bacteria immediately after inoculation into the samples is considered to be a reflection of the toxicity of the samples.

The inhibition percent of 100% sample concentration (by volume) measured in the preliminary test showed an acutely toxic effect for the bacterium resembling Photobacterium phosphoreum. To estimate the lethal concentration (LC 50) and the concentration lethal to 20%, a series of samples concentration were examined (50, 25, 12.5, 6.25, and 3.15% by volume). The LC 50 are of an average of 94.5, 90.8 and 70% by volume for the three

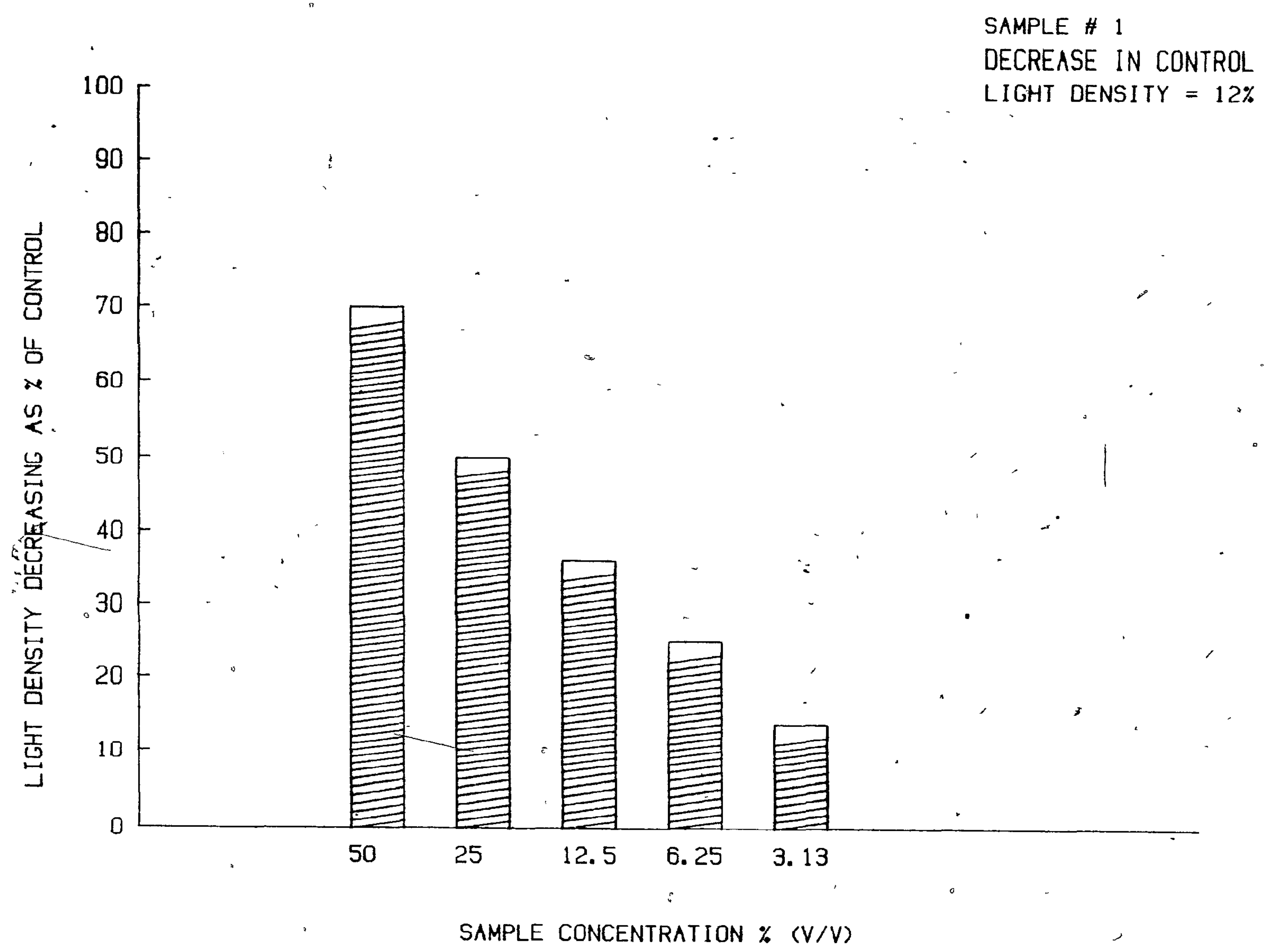


FIG. 4.15 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

groups respectively. The average LC 20 values are 29.3, 32.5, and 26% by volume for the three groups respectively. Figures 4.15 & C.27 are provided in the form of histograms where the height of each individual histogram for any given sample is equal to the decrease in light intensity (emitted by the bacterium resembling Photobacterium phosphoreum) as percentage of control (which is considered as the theoretically expected light emission after 5 minutes). (Figures C-27 to C-39, see Appendix C).

The results of LC 50 indicate that the emanated fluid was not highly toxic to the marine bacterium resembling Photobacterium phosphoreum. Also, the results of LC 50 (S. capricornutum) and LC 20 (Photobacterium phosphoreum) are in the same order. This observation attributed to the fact that the lower organisms are less sensitive to the toxicants. It is also of importance to note that the same compound exhibits varying degrees of toxicity for different species of the test organisms.

4.2.5. Toxicity Results Using the Electrolytic Respirometer:

The oxygen uptake by the bacteria, in the presence of a growing solution containing all the salts required for their maintenance and reproduction, was measured every two hours for a 24 hour period. The results are summarized in Table (C.10). The cumulative oxygen uptake in mg/L are shown in figures (4.16 to 4.23).

The method is based on the fact that, in a closed system, consisting of gaseous and liquid phases, and operating at constant temperature (20°C) and volume, any change in the amount of gas can be gauged by the accompanying change in pressure. The flasks holding the tested samples were held at constant temperature (20°C) by immersion in a water bath, they were shaken at a known speed and amplitude to agitate the liquid surface in the flask.

The cells grew using the oxygen dissolved in the liquid, which causes oxygen from the gaseous phase to diffuse into the liquid. Aerobic metabolism produces large amounts of carbon dioxide which escapes into the gas phase. The cell fixed at the top of the flasks makes use of the pressure change due to the biochemical consumption of oxygen gas to sense the need for more oxygen. The necessary oxygen is electrolytically generated into the reaction flasks.

The results indicates that growth in the control flasks is higher than that on the other flasks holding

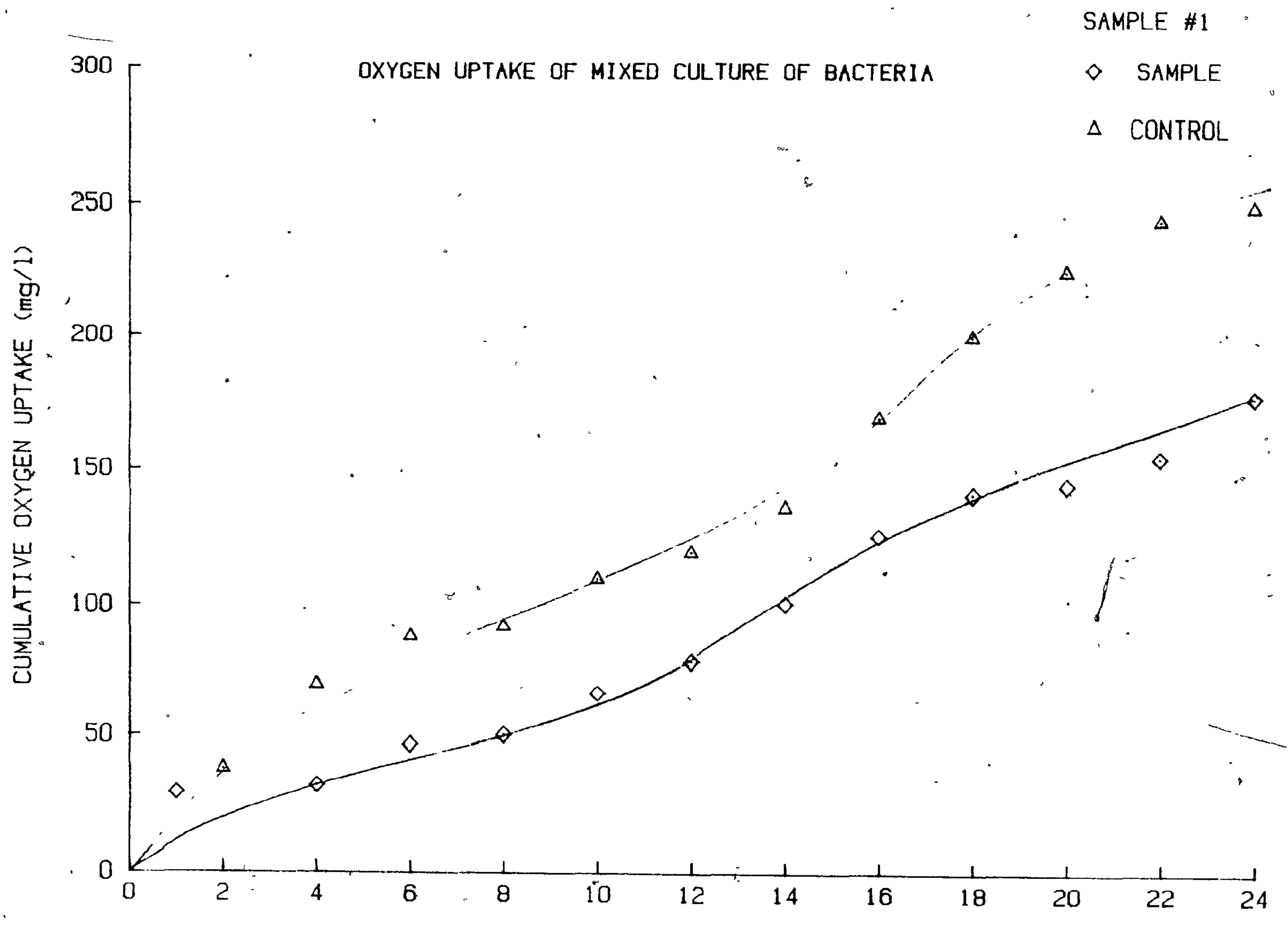
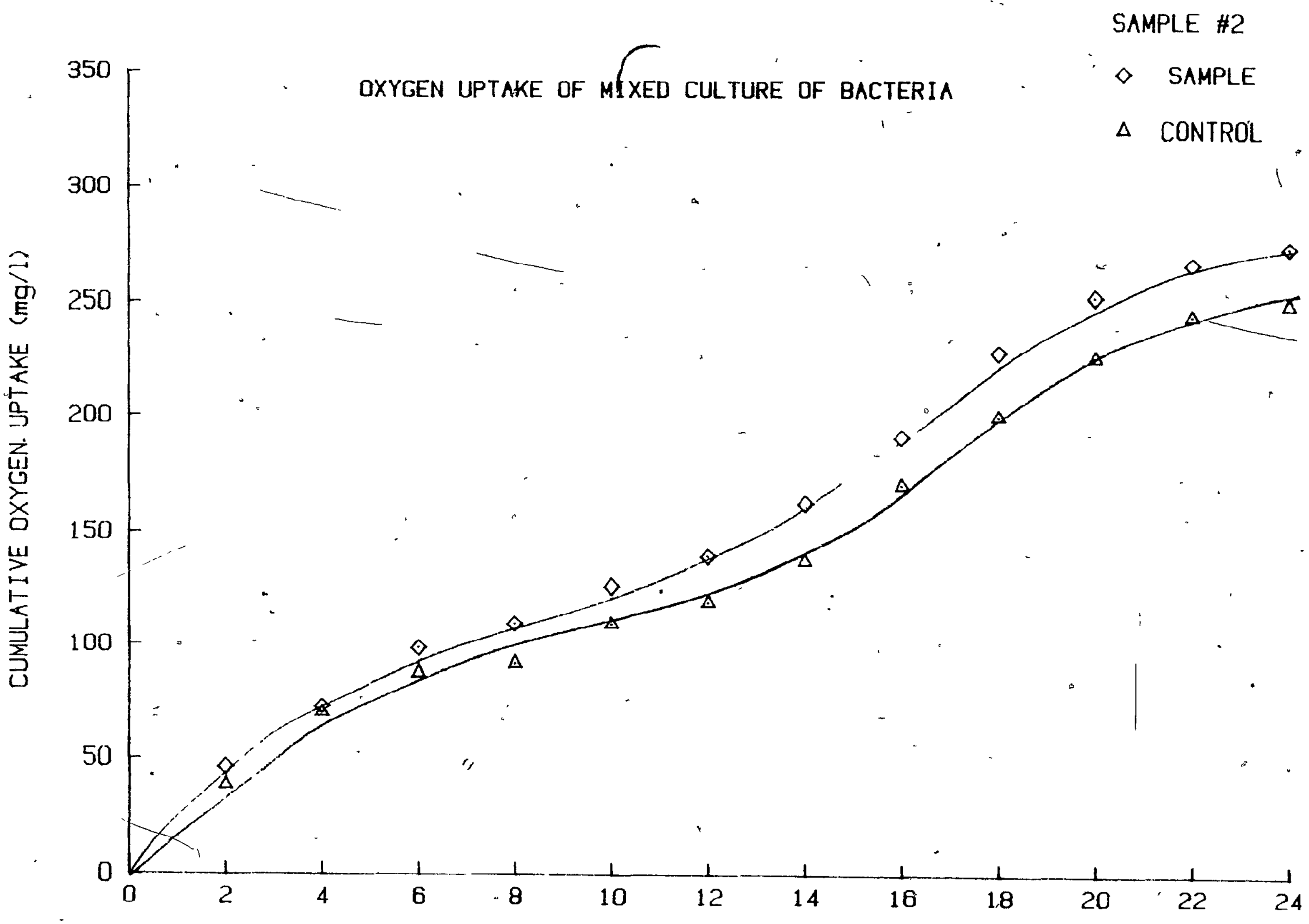

OXYGEN UPTAKE OF MIXED CULTURE OF BACTERIA
SAMPLE #1
◇ SAMPLE
△ CONTROL
CUMULATIVE OXYGEN UPTAKE (mg/l)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hr)

FIG. 4.16



OXYGEN UPTAKE OF MIXED CULTURE OF BACTERIA
SAMPLE #2
◇ SAMPLE
△ CONTROL
CUMULATIVE OXYGEN UPTAKE (mg/l)
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hr)

FIG. 4.17

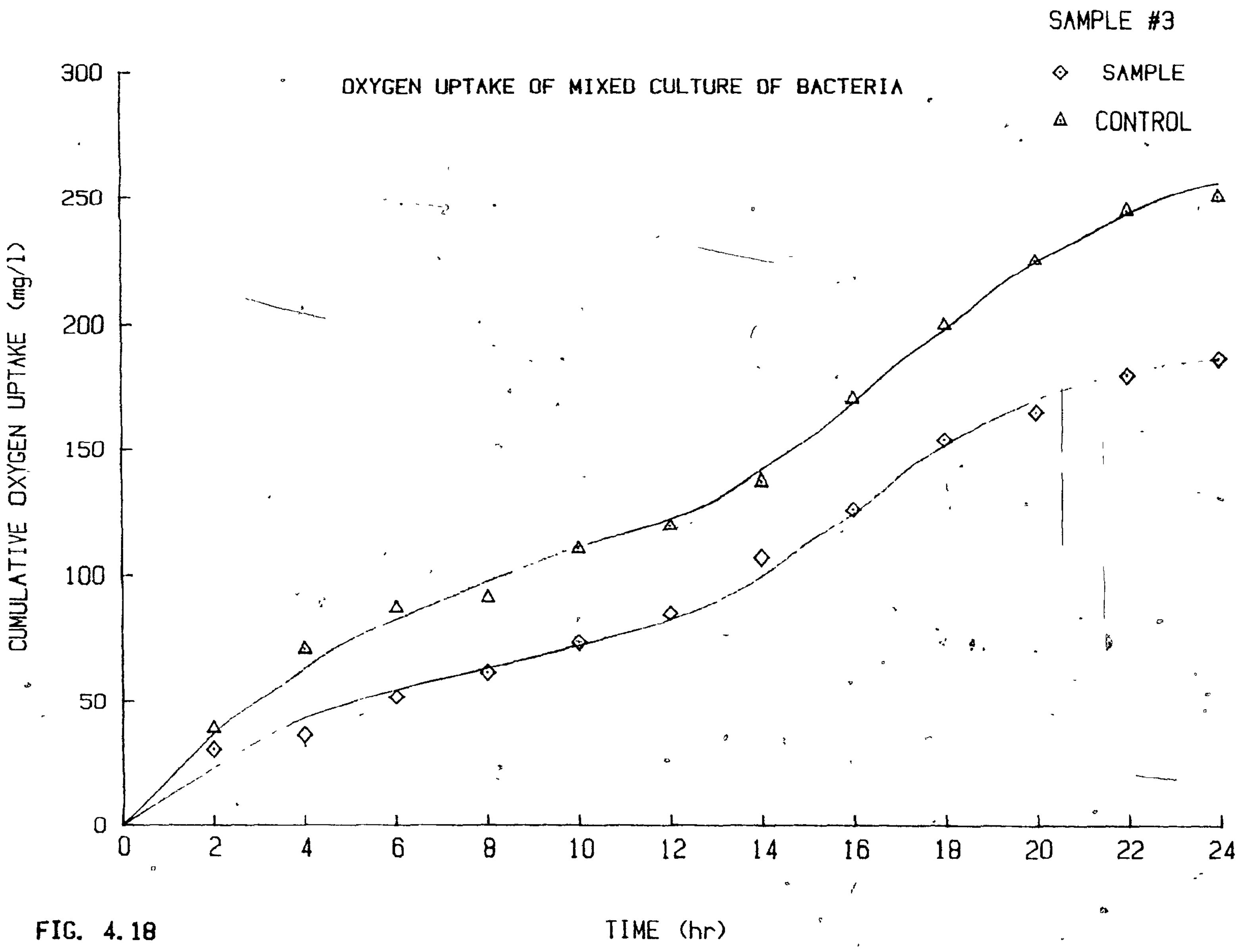

OXYGEN UPTAKE OF MIXED CULTURE OF BACTERIA
SAMPLE #3
◇ SAMPLE
△ CONTROL
CUMULATIVE OXYGEN UPTAKE (mg/l)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hr)

FIG. 4.18

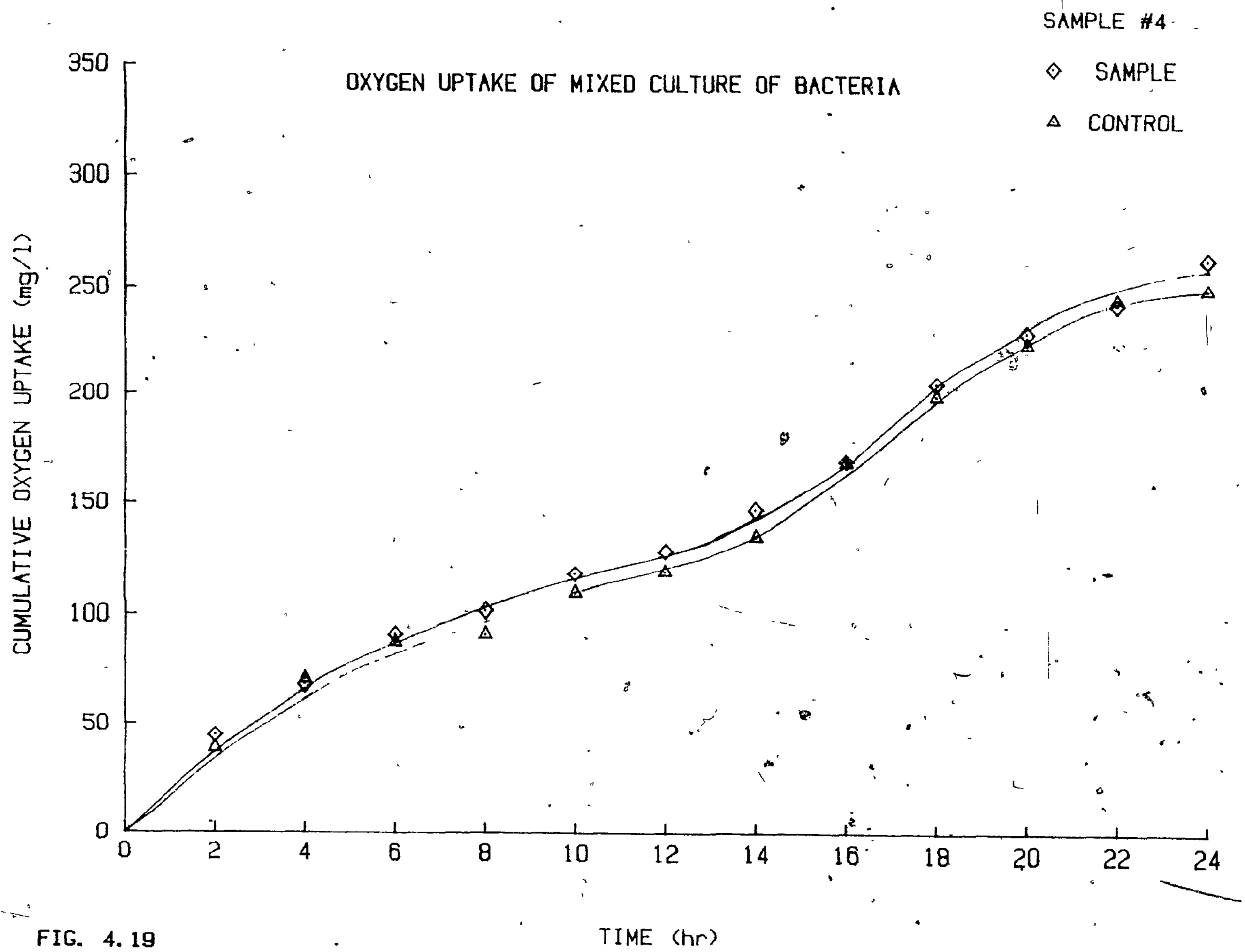
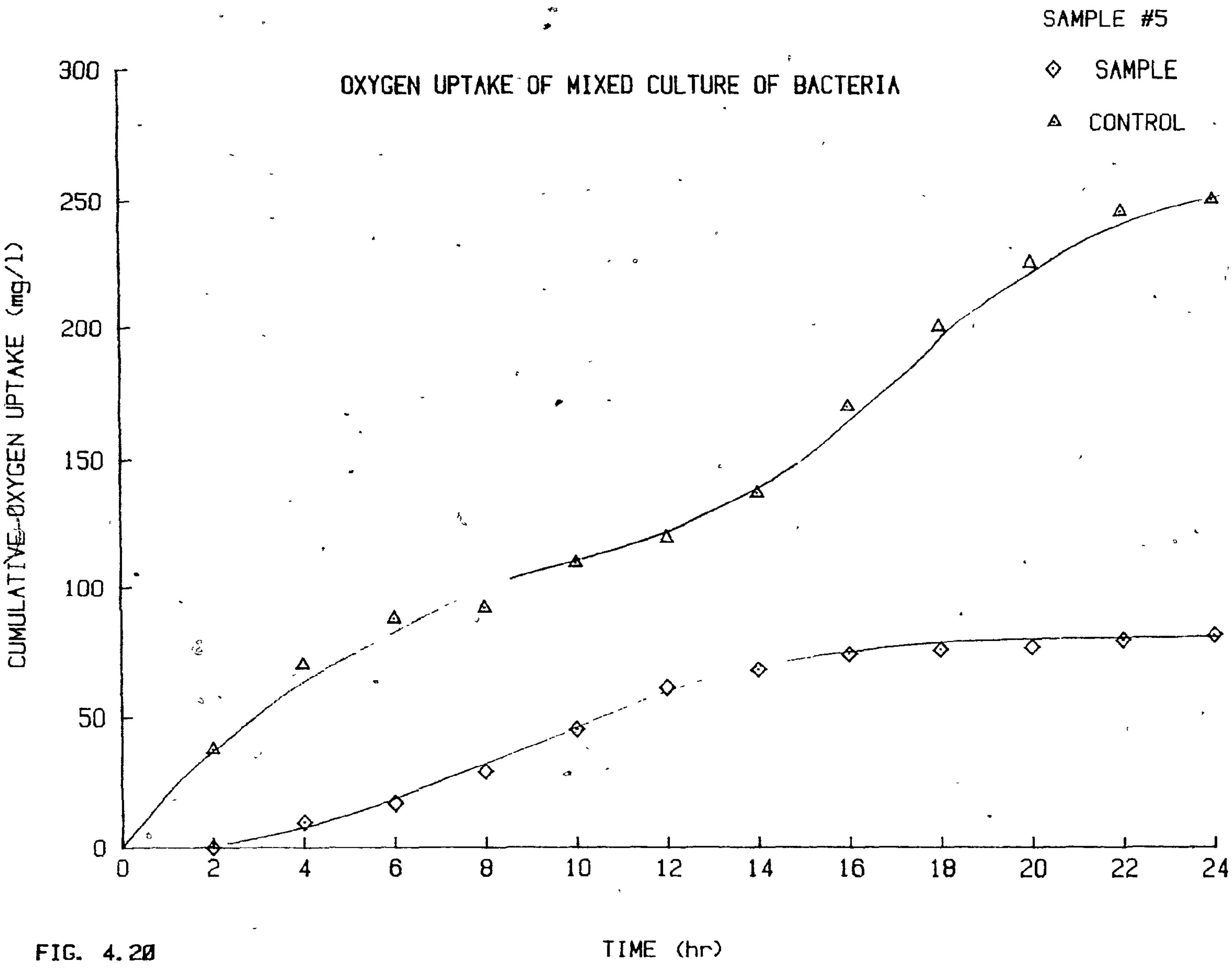

SAMPLE #4
◇ SAMPLE
△ CONTROL
OXYGEN UPTAKE OF MIXED CULTURE OF BACTERIA
CUMULATIVE OXYGEN UPTAKE (mg/l)
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hr)

FIG. 4.19



OXYGEN UPTAKE OF MIXED CULTURE OF BACTERIA
SAMPLE #5
SAMPLE
CONTROL
CUMULATIVE OXYGEN UPTAKE (mg/l)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hr)

FIG. 4.20

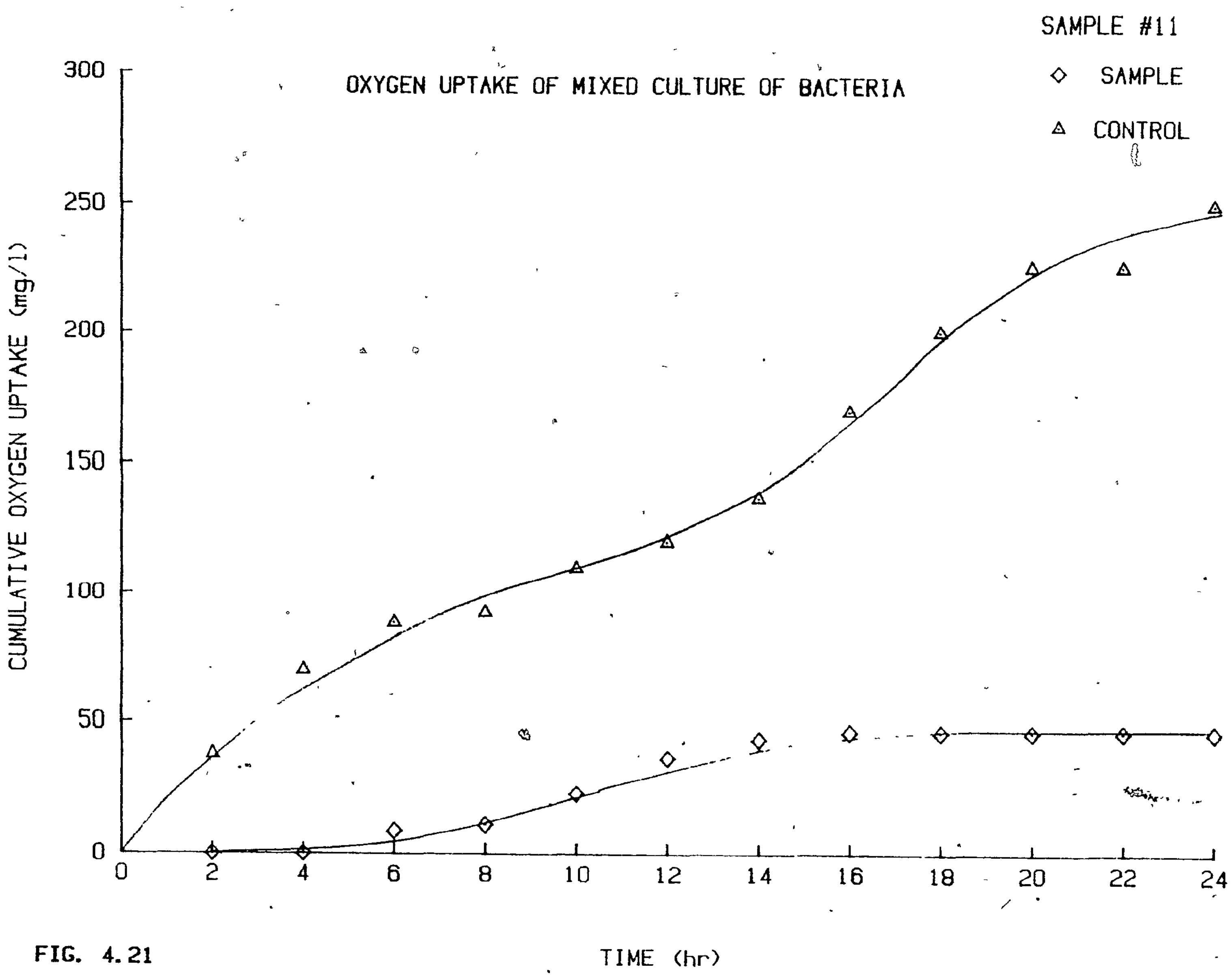

SAMPLE #11
◇ SAMPLE
△ CONTROL
OXYGEN UPTAKE OF MIXED CULTURE OF BACTERIA
CUMULATIVE OXYGEN UPTAKE (mg/l)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hr)

FIG. 4.21

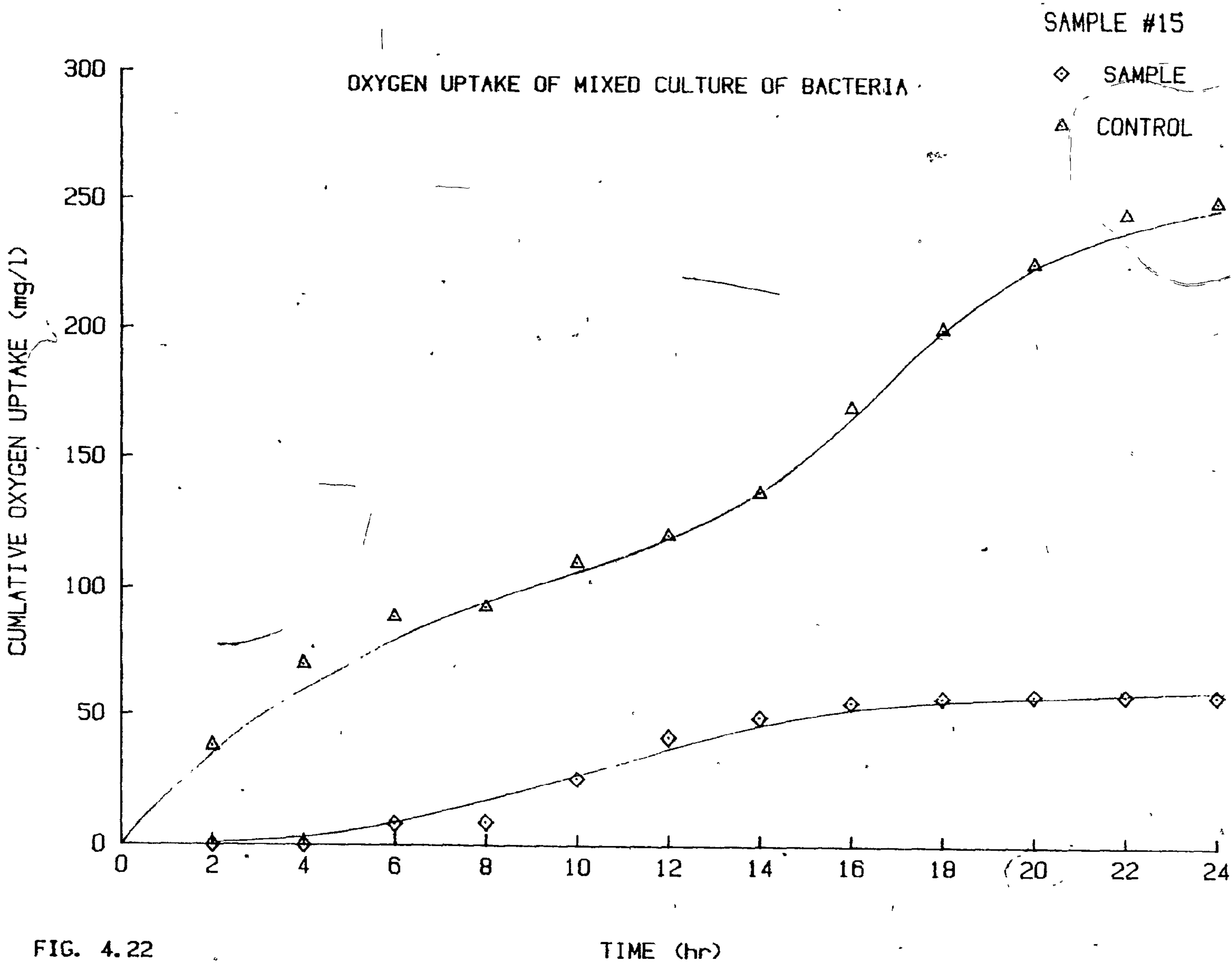

SAMPLE #15
◇ SAMPLE
△ CONTROL
OXYGEN UPTAKE OF MIXED CULTURE OF BACTERIA
CUMLATIVE OXYGEN UPTAKE (mg/l)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hr)

FIG. 4.22

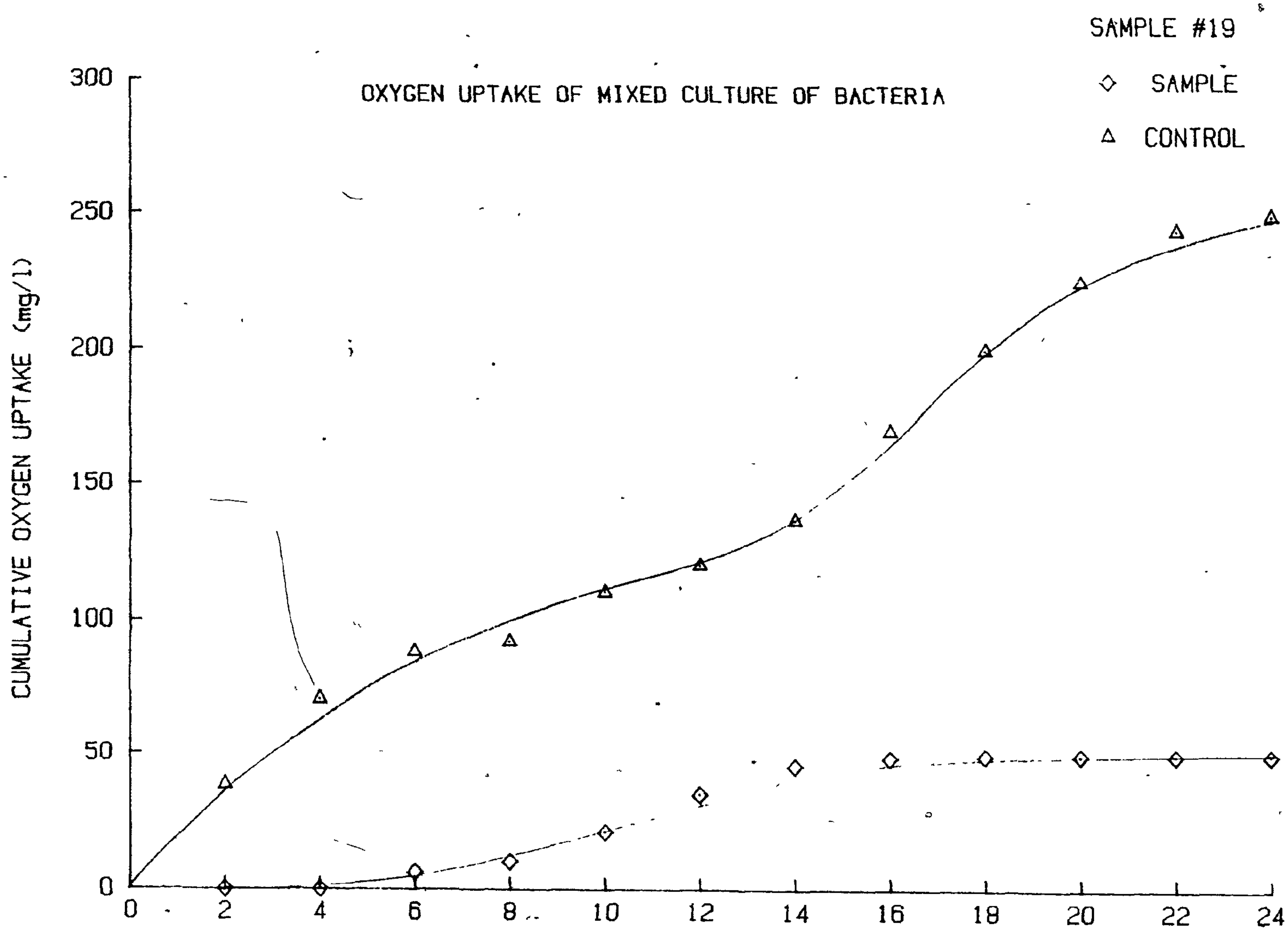

SAMPLE #19
◇ SAMPLE
△ CONTROL
OXYGEN UPTAKE OF MIXED CULTURE OF BACTERIA
CUMULATIVE OXYGEN UPTAKE (mg/l)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24

FIG. 4.23

TIME (hr)

the tested samples. These results correlate well with the BOD and COD or TOC results, and in fact the micro-organisms present in the mixed culture used as a seed could acclimate to the nutrients present or added to the samples in growth and reproduction, but the dissolved hydrocarbons and/or the complex organic coumpounds which are hardly degradable, present in the samples, limits the growth. Two of the tested samples were of an oxygen uptake of more than that at the control flask , which indicates the ability of this mixed bacteria culture to adapt to the emanating sludge fluid. There is no doubt from these results that some bacteria present in the inoculum can metabolize petroleum hydrocarbons, but no single bacterium can metabolize all different hydrocarbons in crude oil (EPA, 1972). The amount of oxygen required to completely degrade petroleum hydrocarbons (to CO_2 and H_2O) is enormous; the oxidation of one liter of crude oil would require all the oxygen in about 320,000 liter of seawater (Ellis, 1973).

Another fact to be taken into account here, is that the oxygen demand of effluent samples reduces the available oxygen in the sample, thus causing an additional stress on living organisms.

The oxygen stress can be noted by an increase in the respiration rate to compensate for the lower oxygen level.

In summary, oxygen uptake curves obtained from samples inoculated with microorganism of industrial origin indicated a relatively low toxicity in the emanated sludge fluid.

4.3. Variation of Toxicity with time:

Two untreated emanating sludge fluid samples were used to study the variation of toxicity with time, as well as the variation of pH and chemical oxygen demand (COD). The results are summarized in tables (C.11, C.12 and C.13).

Growth bioassays with S. capricornutum, using cell numbers as an index of growth with different sample concentrations ranging from 100% to 6.25% (by volume) (Fig. 4.26 and 4.27) showed a rapid decreasing of the inhibition percentage with sample concentrations of 6.25%, 12.5% and 25% (by volume) during the first 17 weeks of exposure to laboratory environmental conditions. The variation of lethal concentration (LC 50) with time was represented by a straight line as shown in Fig. (4.24

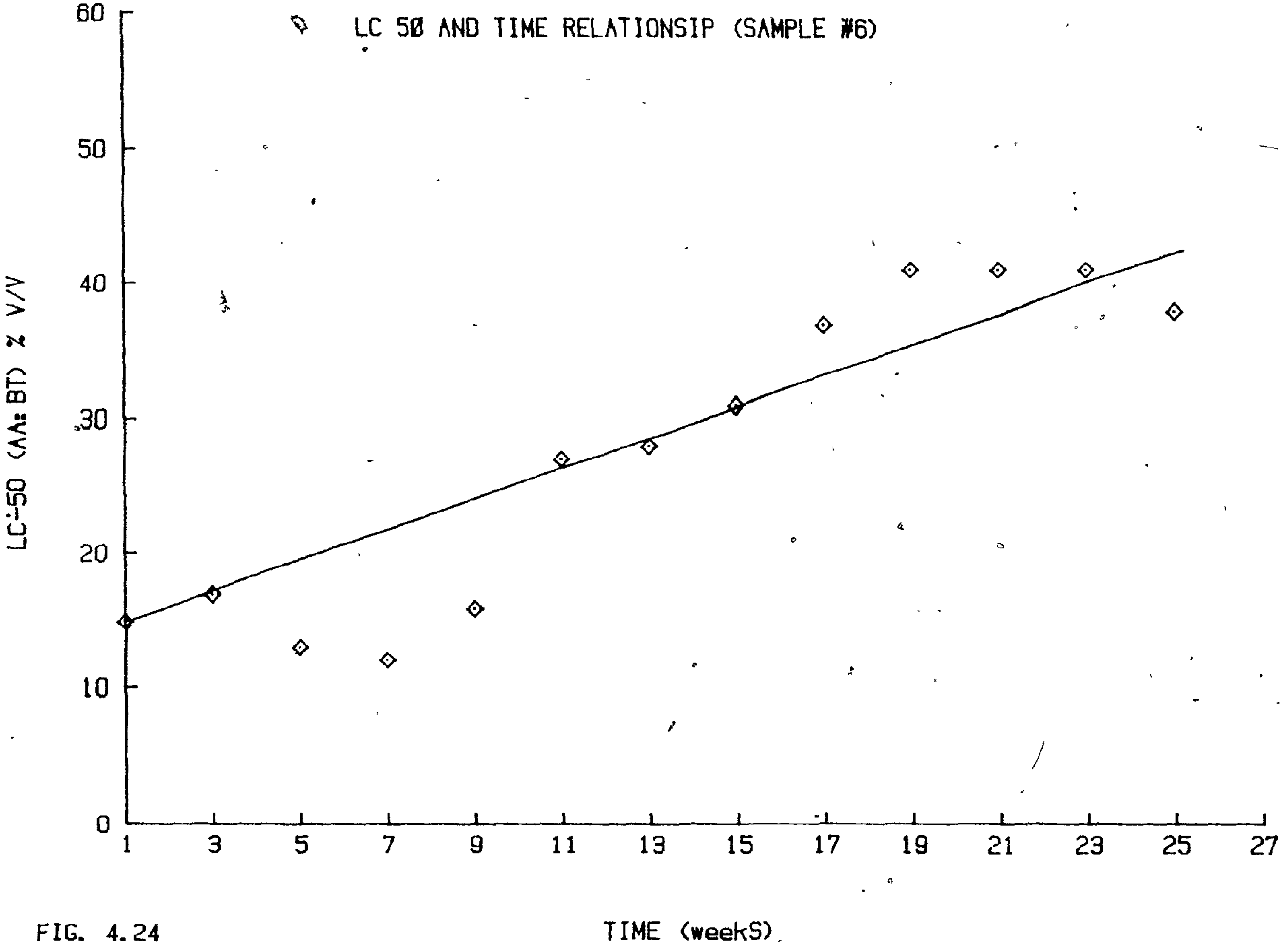

LC 50 AND TIME RELATIONSIP (SAMPLE #6)
LC-50 (AA: BT) % V/V
60
50
40
30
20
10
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
TIME (weekS)

FIG. 4.24

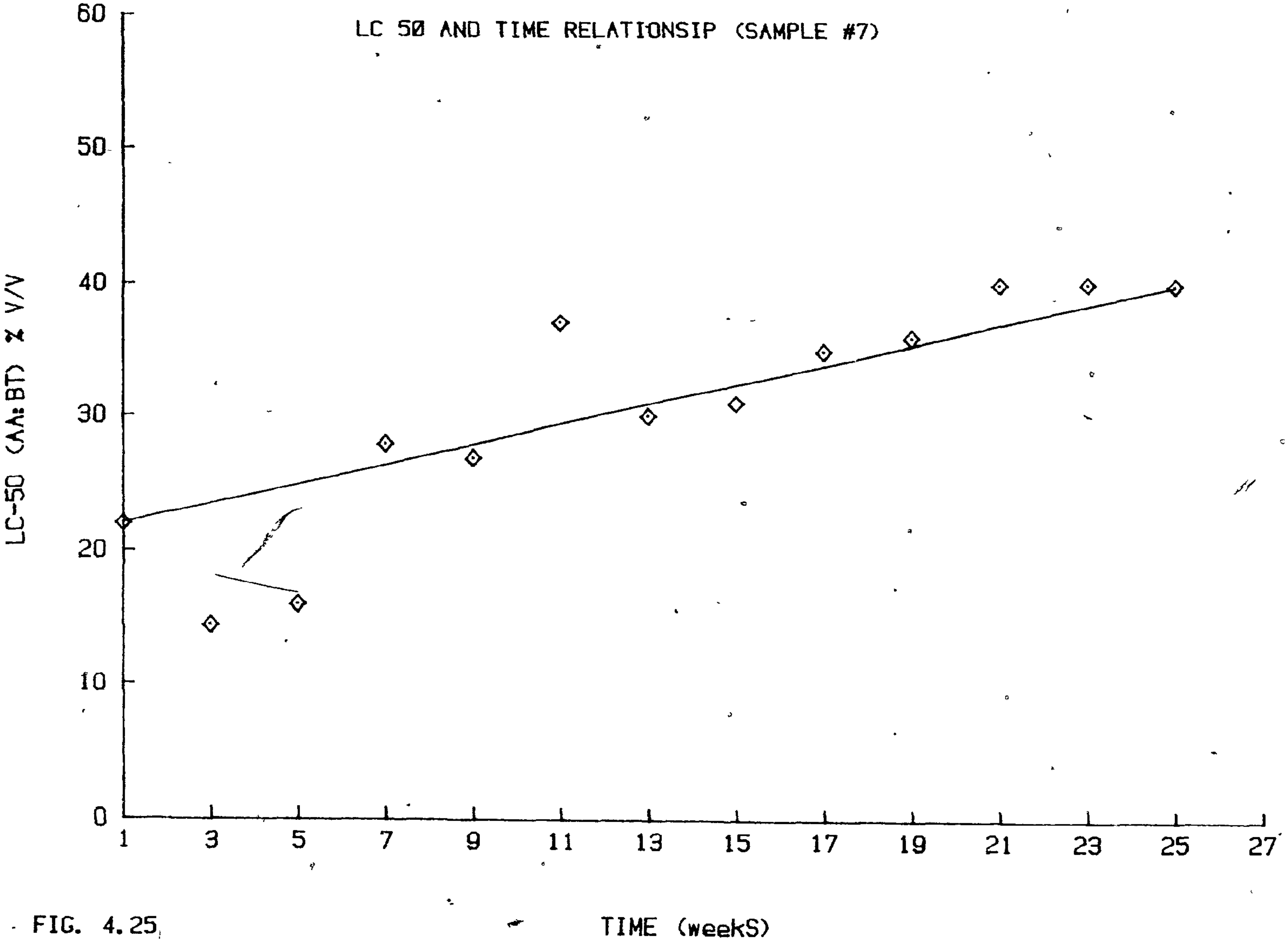

LC 50 AND TIME RELATIONSIP (SAMPLE #7)
LC-50 (AA:BT) % V/V
60
50
40
30
20
10
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
TIME (weekS)

FIG. 4.25

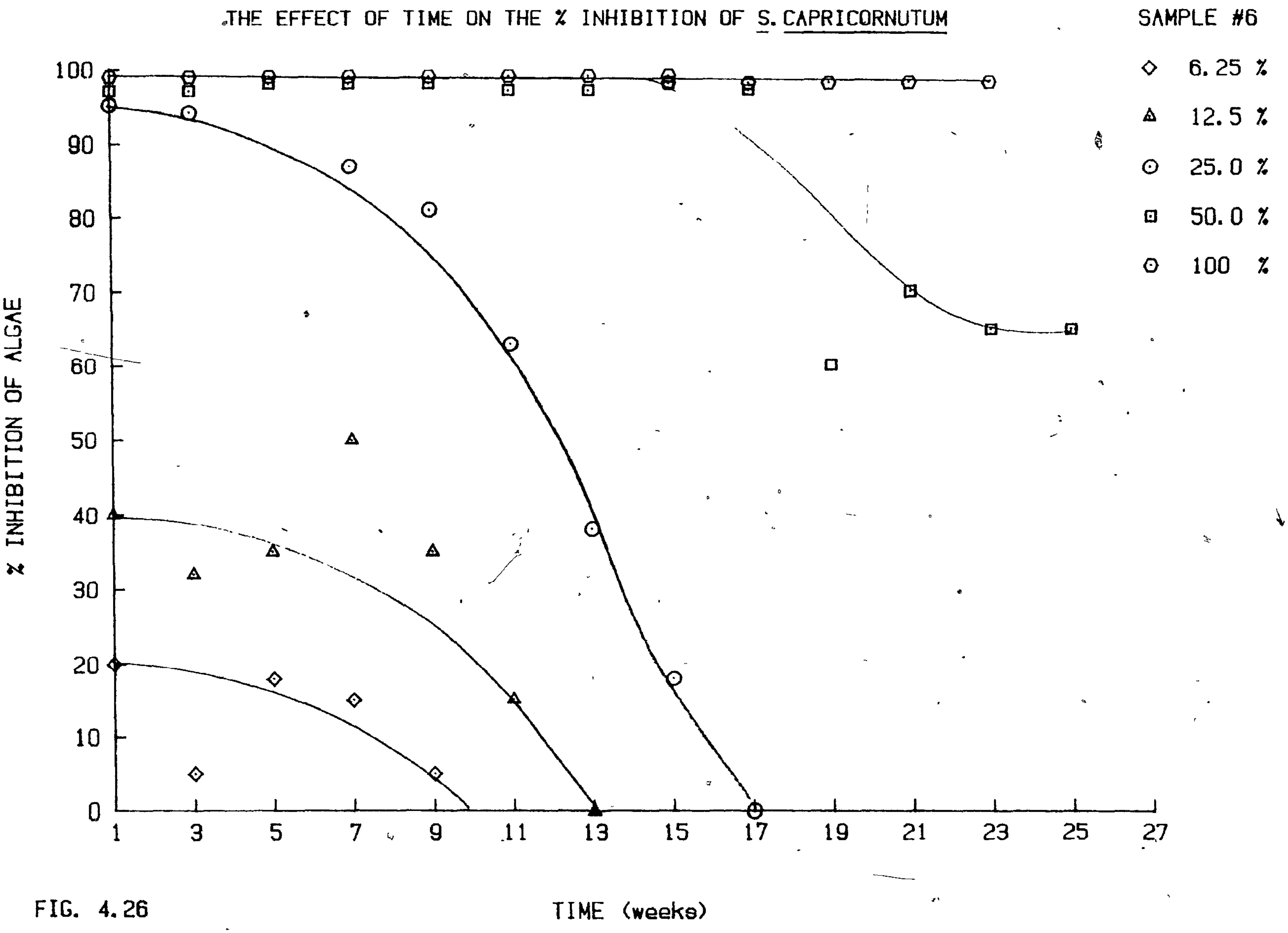

THE EFFECT OF TIME ON THE % INHIBITION OF S. CAPRICORNUTUM
SAMPLE #6
◇ 6.25 %
△ 12.5 %
⊙ 25.0 %
⊡ 50.0 %
⊙ 100 %
% INHIBITION OF ALGAE
0
10
20
30
40
50
60
70
80
90
100
1
3
5
7
9
11
13
15
17
19
21
23
25
27
TIME (weeks)

FIG. 4.26

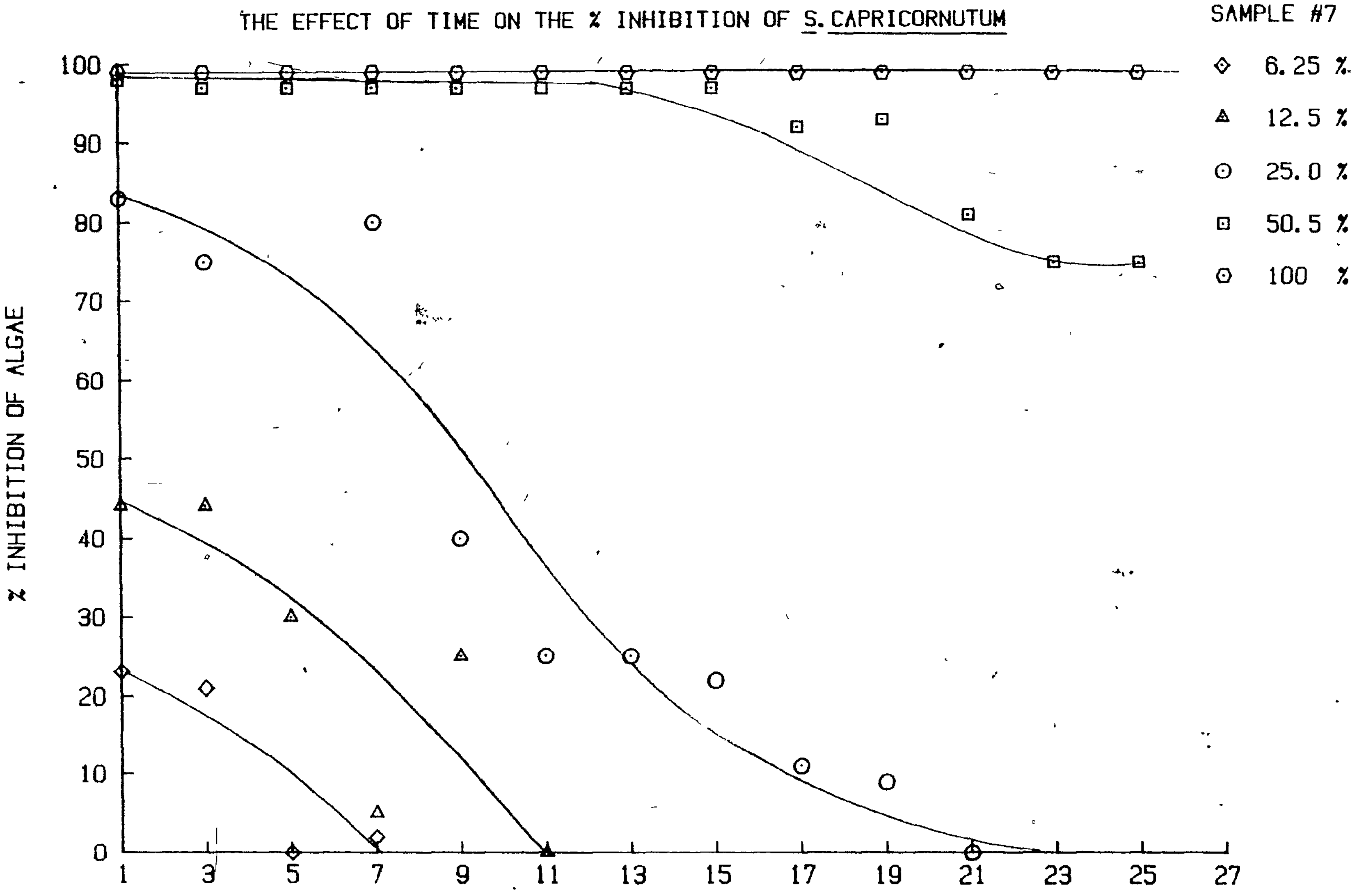

THE EFFECT OF TIME ON THE % INHIBITION OF S. CAPRICORNUTUM
SAMPLE #7
◇ 6.25 %
△ 12.5 %
⊙ 25.0 %
⊡ 50.5 %
○ 100 %
% INHIBITION OF ALGAE
100
90
80
70
60
50
40
30
20
10
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
TIME (weeks)

FIG. 4.27

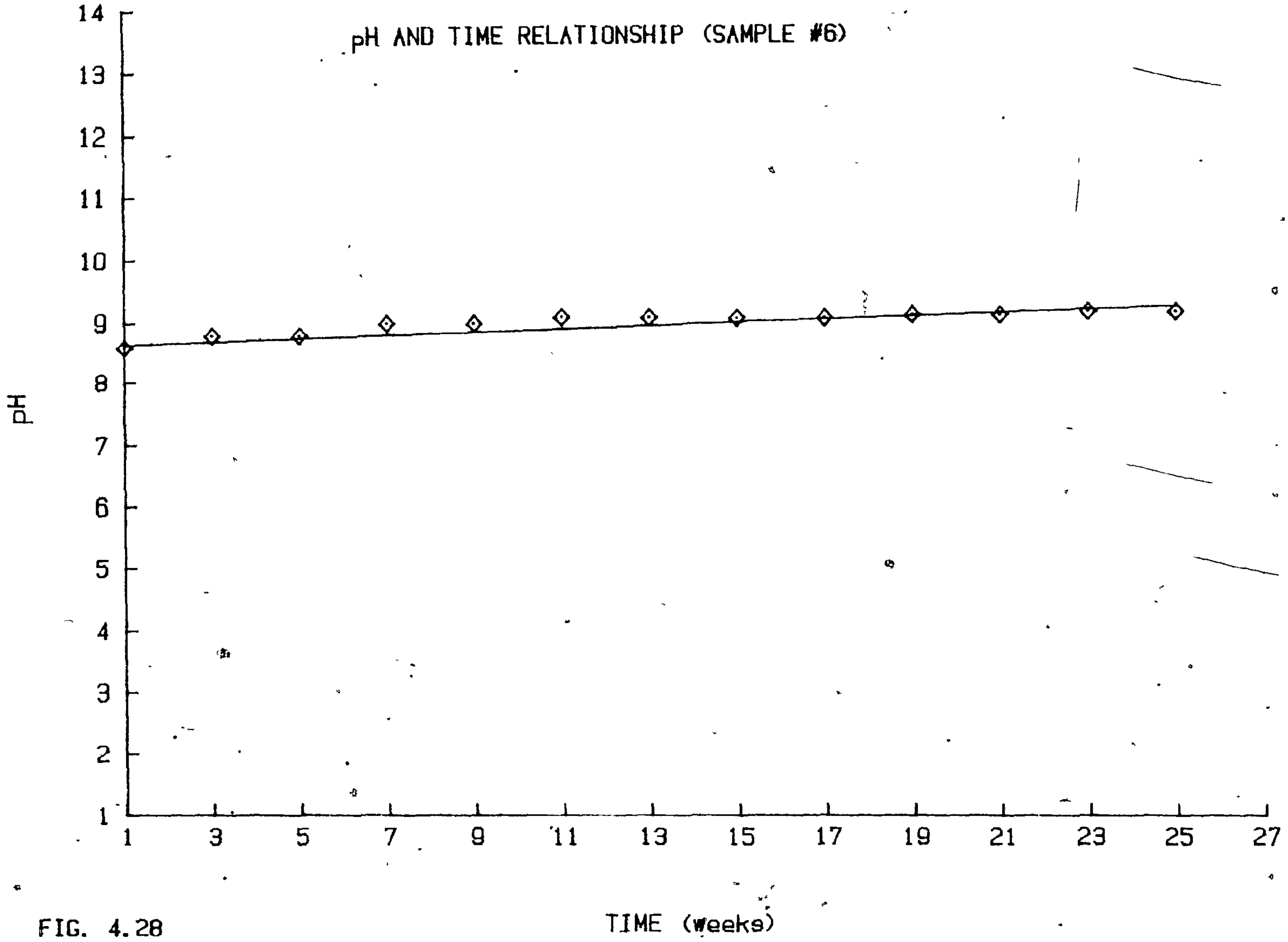

pH AND TIME RELATIONSHIP (SAMPLE #6)
pH
14
13
12
11
10
9
8
7
6
5
4
3
2
1
1
3
5
7
9
11
13
15
17
19
21
23
25
27
TIME (weeks)

FIG. 4.28

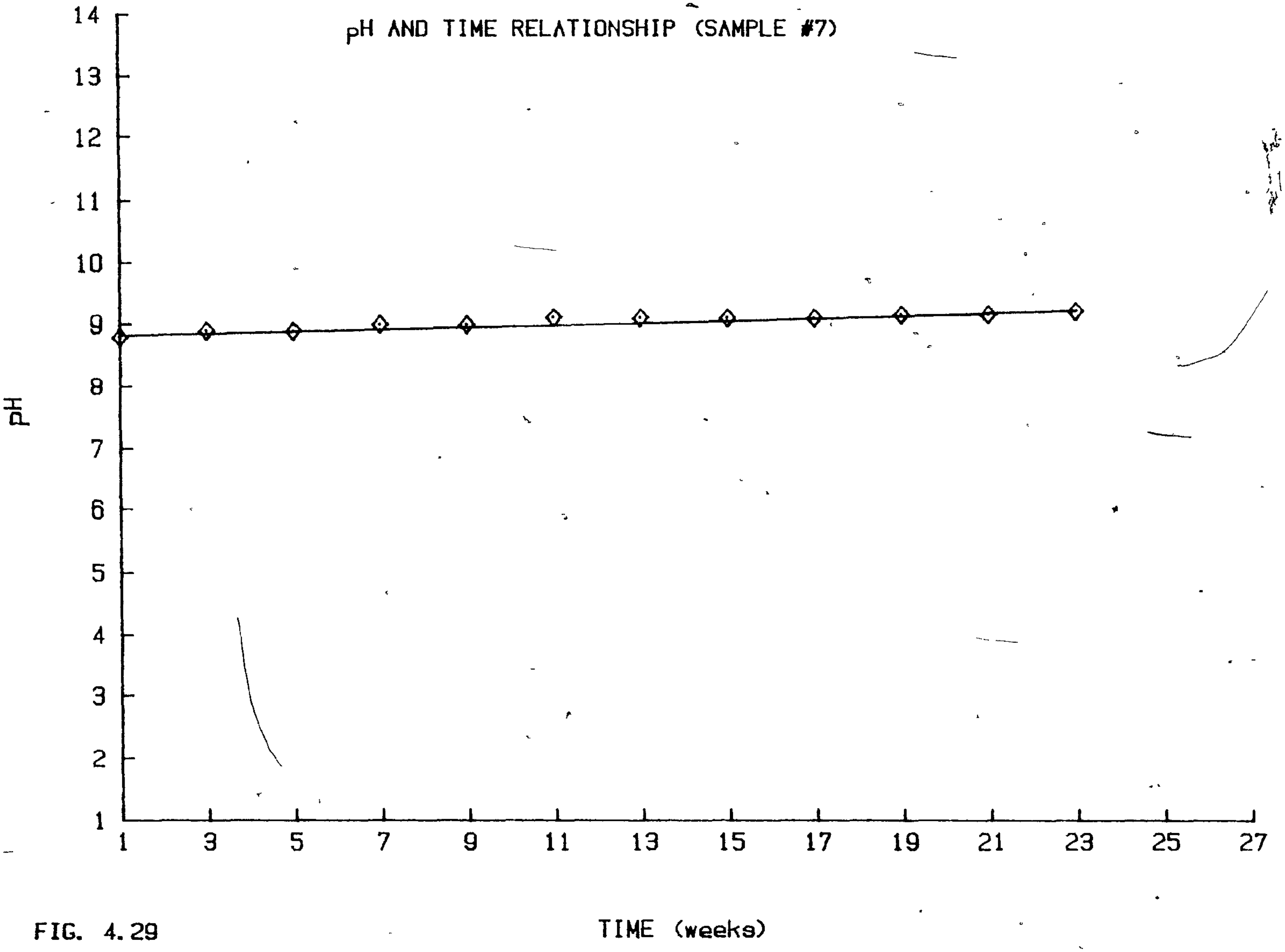

pH AND TIME RELATIONSHIP (SAMPLE #7)
pH
14
13
12
11
10
9
8
7
6
5
4
3
2
1
1
3
5
7
9
11
13
15
17
19
21
23
25
27
TIME (weeks)

FIG. 4.29

and 4.25). The regression analysis indicates a degree of correlation (r =0.92 and 0.83 for sample 6 and 7 respectively),

The second parameter whose variation was observed with time was the pH value. The pH values varied from 8.6 to 9.20 during the study (25 weeks). This variation with time can be due to any or all of the following: interaction of the aqueous solution with the minerals, hydrolysis of the components of bitumen, oxidation of bitumen by air, transfer of CO_2 between solution and atmosphere, and bacteriological processes (Burchfield and Hepler, 1979). Figures 4.28 and 4.24 show the variation of pH with time and this relationship is represented by a straight line of slope (0.064 and 0.065 for samples 6 and 7 respectively).

The chemical oxygen demand (COD) values decreased by 37%, during the period of the study. The decrease of COD may be attributed to the reasons mentioned above (with pH variation) or may be caused by the evaporation of the volatile hydrocarbons (the evaporation rate was observed to be equal to 0.5 $ml/cm^2/day$), which includes the most toxic components and also the most soluble in the samples (Laws, 1981). The relations of COD variation with time are given in figures 4.30 and 4.31 .

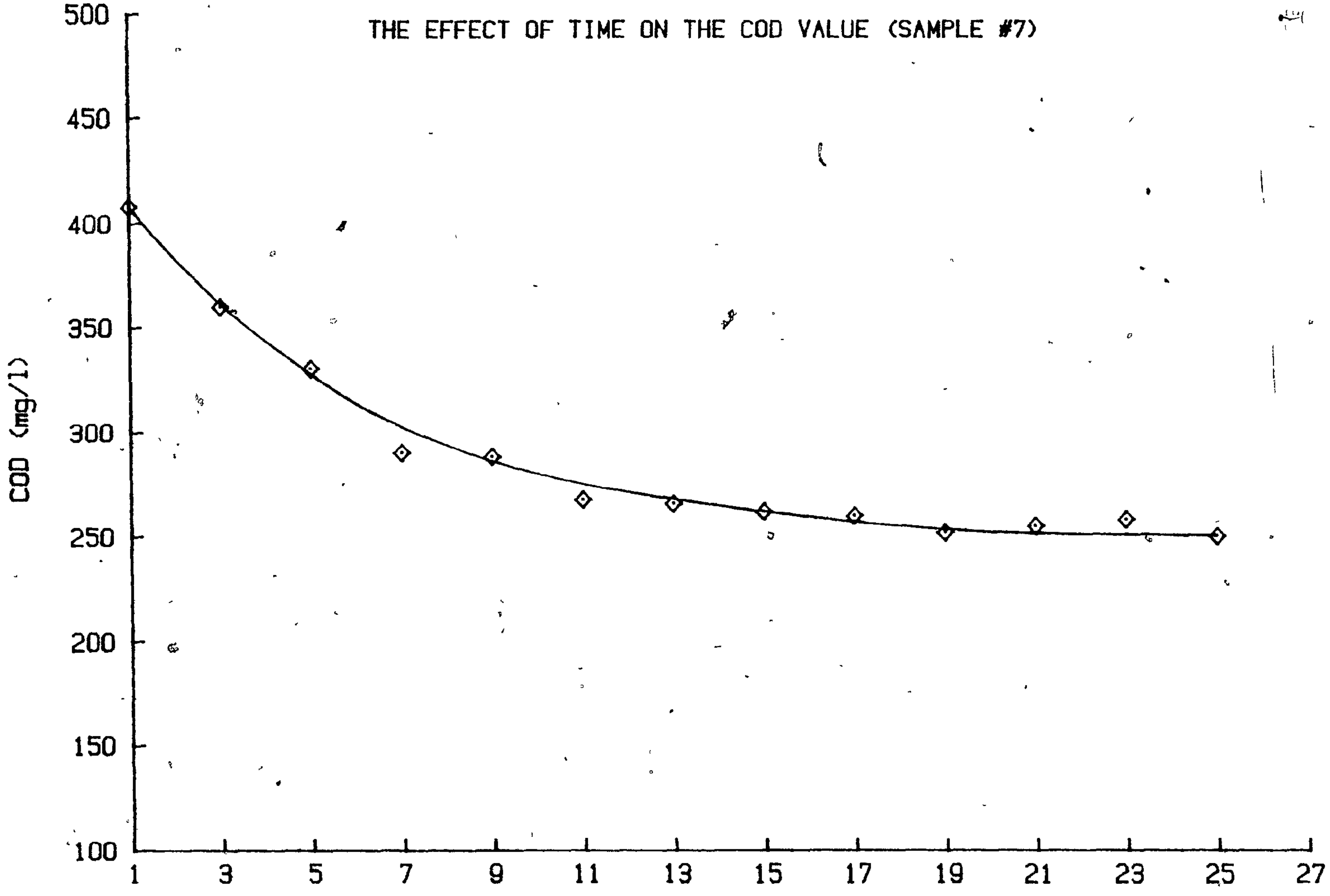


FIG. 4.30

TIME (weeks)

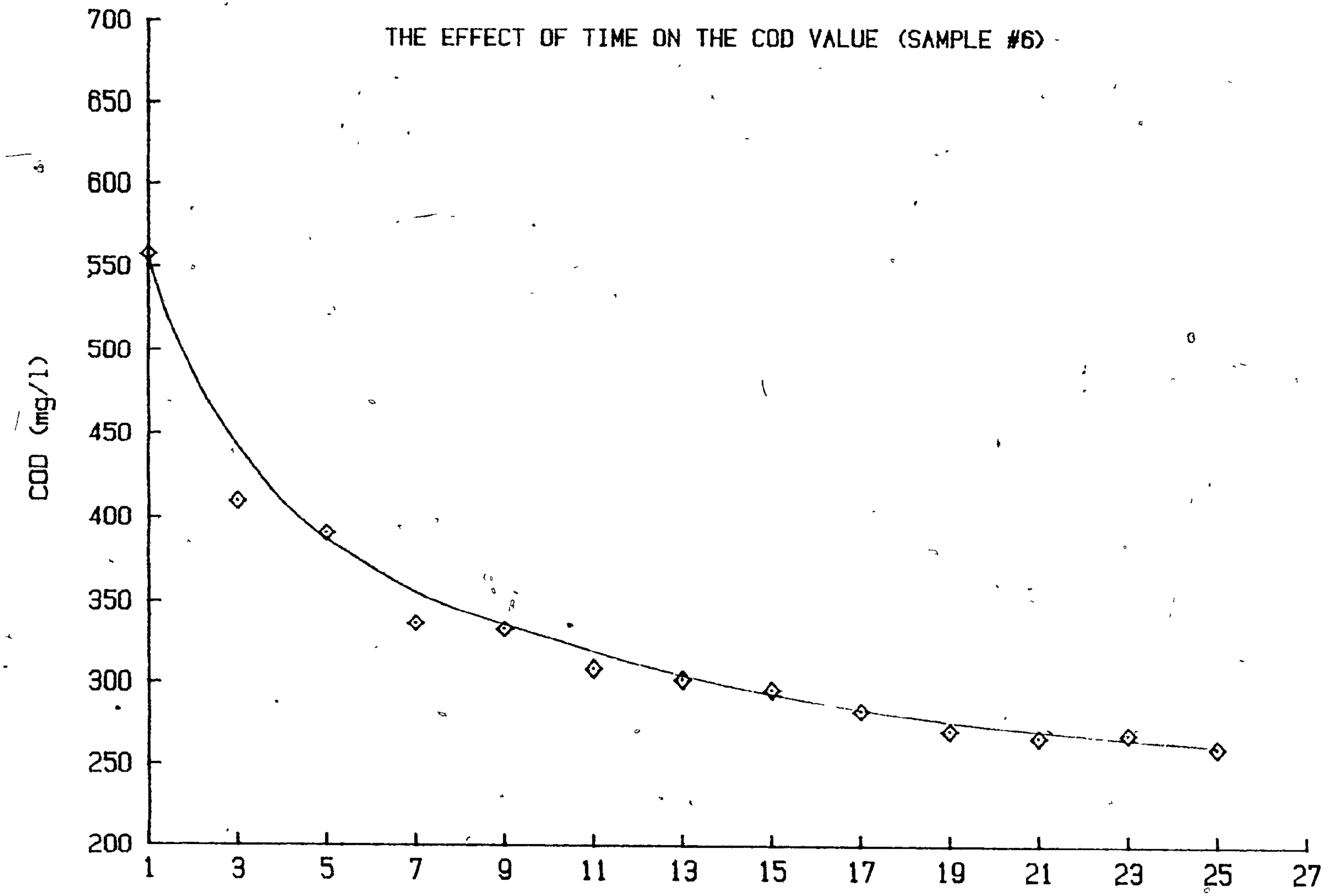


FIG. 4.31 TIME (weeks)

CHAPTER 5

Discussion

Discussion

The results obtained indicate that the fluid emanating from the sludge produced as a result of the "hot water extraction process" is toxic to the fresh water, green algae Selenastrum capricornutum and less toxic to both the marine bacterium resembling Photobacterium phosphoreum and the mixed bacteria culture of industrial origin. The results also show that certain components are present in relatively high concentrations. Thus, it would be appropriate to carefully consider the different components and factors characterizing all samples and thereby attempt to determine the source(s) of toxicity in the samples.

5.1. pH:

The high pH values observed (≃ 8.0) (Table C.1) and the variation in the pH values between the samples, may be due to any or all of the following: interaction of the aqueous solution with the minerals, hydrolysis of the components of bitumen, oxidation of bitumen by air, transfer of CO_2 between solution and atmosphere and bacteriological processes. These pH values may be partially due to the presence of COO^-, as several previous investigators (Moschopedis et al, 1976; Burchfield et al, 1979; and Strosher et al, 1976) have shown that bitumen contains compounds that will hydrolyze to carboxylates.

The pH value plays an important role in determining the degree of toxicity of some components such as ammonia. It is well known that the molecular ammonia is toxic but that the ammonium ion is not and the relationship between the two is pH dependent,

$$NH_3 + H^+ \rightleftharpoons NH_4^+$$

(Sawyer and McCarty, 1978). Although pH is relatively high, it falls within the range 5-9 (pH units) which is generally tolerated by organisms. In this study, pH is not considered a factor directly influencing the survival of the test organisms.

5.2. Solids and Specific Conductivity:

In the analysis of total solids and suspended solids, an observed high dissolved solids content is noticed. Although the specific conductivity values fall within the range 0.05 - 1.5 m`mhos/cm (Table C.1), which generally characterizes potable water in U.S.A.. Thus it would not be expected that the overall ionic strength of the samples would have a direct effect on the toxicity observed.

The relatively high values of volatile solids, for all samples (Table C.1), point out the presence of a high organic content, but as mentioned before, the volatile solids are sometimes a misleading measurement because they may reflect a loss of water of crystillization, a loss of volatile organic matter before combustion, incomplete oxidation of certain complex organics, and/or decomposition of mineral salts during combustion.

The suspended solids with the turbidity results fall within the harmful range to aquatic organisms (Table C.1). The suspended solids can retard the photosynthetic process by preventing light penetration , blanket the benthic communities and also contribute to the biochemical oxygen demand. Côté (1976) reported that there is no evidence that concentrations of suspended solids less than 25 mg/L have any harmful effect on fish and it should be possible to maintain good or moderate fish populations in water containing up to 80 mg/L suspended solids.

5.3. Metals

The analysis of heavy metals indicated that most of the metals (Cu, Cd, Mn, Ni, Pb, As, Zn and V) were below the toxic levels in public water supplies as recommended by the

EPA (1972), except for iron which was present at an average concentration of 2.6 mg/L (Table C.2) while the maximum permissible concentrations is 0.3 mg/L (EPA, 1972). During Algal bioassays tests using the green alga S. capricornutum as a test organism, EDTA was added as a chelating agent to complex iron and any other metals in a potentially toxic state. The results of this series of experiments point out that the inhibition percentage does not change EDTA. Thus the effect of the heavy metals as a direct source of toxicity appears to be insignificant as compared to the other sources of toxicity. However in the presence of organics, the toxicity of metals increases. For example, organic compounds of lead are much more toxic than the metal itself. The ionic state of the metal may also affect the level of toxicity. For example, chromium is more toxic in the hexavalent than in the trivalent state. Furthermore, heavy metals, even if present in sublethal concentrations, may react with other metals or components synergistically or antagonistically **(Wetzel,1975)**.

5.4. BOD, COD, and TOC:

The BOD results indicate the presence of toxic compounds and/or complex organic materials difficult to degrade by the microorganisms of industrial waste origin. As mentioned before (see 2.2.) hydrocarbons are quite toxic to aquatic microorganisms (especially the low molecular weight hydrocarbons) and this could explain the quantitative difference between BOD and both TOC and COD results. In all samples (Table C.3), higher dilutions yield lower toxicity values (lower inhibition percentage), and this observation was supported by the following results:

1) The higher the sample concentration in the BOD bottles, the less the value observed after 5 days (with respect to the dilution factors). (see Fig. 4.1 - 4.6.).

2) With decreasing sample concentration, the rate of reproduction and growth of S. capricornutum and the marine bacterium resembling Photobacterium phosphoreum increased significantly (Tables C.7, C.8 and C.9).

The ratio TOC/COD (both are representative of the organic content of the samples) is equal to 2.30 on the average for all samples, while the theoretical value should be 2,66 (in the case where all organic matter present is potassium dichromate oxidizable, TOC/COD = 32/12 = 2.66). This difference may be due to the presence of organic compounds that are not accounted for in either BOD or COD tests. Certain organic compounds, particularly low molecular weight fatty acids, are not oxidized by dicromate unless a catalyst is present. Aromatic hydrocarbons and pyridines are not oxidized under any circumstances.

As the organic content of the samples decreased the toxicity also decreased, as observed in the study of toxicity with time (see sec. 4.3.). The organic content likely decreased either by gradual decomposition and hydrolysis or by evaporation of the low molecular weight hydrocarbons. The same results were observed in studying the toxicity of the samples after passage through an activated carbon column. (sec. 4.2.3.). The toxicity decreased due to the removal of a portion of dissolved organics by an average value of 30% for all samples. This result may be due to the presence of high hydrocarbon contents dissolved in the samples. The emanating sludge fluid contained an average of 170 mg/L of organic carbon of which 70% was extractable organic material (Table C.4). Organic material may exist in the samples in several forms; as dissolved organic compounds, as dispersed or micellar material, as suspended carbon particles, and as material adsorbed on the surface of suspended inorganic particles. Dissolved organic compounds and dispersed organic material may be readily extracted from the samples with solvents such as benzene: compounds adsorbed on the surface of inorganic particles are not readily extracted and carbon particles can not be extractable from the samples. The fact that over 70% of the organic carbon present in the samples is extractable indicates either that it occurs as dissolved organic compounds or dispersed organic material.

5.5. Total Nitrogen and Ammonia:

The total nitrogen and ammonia results (Table C.5) indicated that the emanating fluid samples were high in ammonia content and low in nitrate and nitrite content. Miller et al (1976) reported that the high ammonia concentration was observed to be toxic to the green alga S. capricornutum. The observed ammonia content is above the safe limit for receiving water (1.0 mg/L) (Sawyer and Mc Carty, 1978).

The presence of high ammonia and low nitrate and nitrites likely indicated that oxidation of ammonia to nitrate and nitrites is not complete. Similarly other oxidiation processes may also not be complete. For example, hydrocarbons which are highly toxic may be oxidized to less toxic compounds, eventually to carbon dioxide and water.

The toxic action of ammonia depends on the H^+ concentration (pH). Carbon dioxide decreases the concentration of the toxic NH_3 by lowering the pH value. Ammonia, as well, observed to be toxic to Rainbow trout (i.e. 96 hour LC 50) at 0.41 mg/L of un-ionized ammonia (Hrudey et all, 1976).

The high ammonia content may be a direct source of toxicity to S. capricornutum, however, it is very difficult to confirm this conclusion because of the difficulty of isolating the ammonia in the toxicity test employed.

5.6. Phenols:

Phenols in the emanating fluid samples may originate from the hydroxy derivatives of benzene and its condensed nuclei . Phenols observed at low concentrations far below the EPA estimated safe phenol concentration for receiving waters (0.1 mg/L), and this can be attributed to the acidic properties of phenols and the caustic extraction process utilized by the present oil sands extraction plant. Thus it would not be expected that phenols would have a direct toxic effect on the toxicity indices used in this study (S. capricornutum and the bacterium resembling Photobacterium phosphoreum). Also, phenols appear to be less toxic towards lower aquatic microorganisms. These results were similar to those of Strosher and Peak (1976) for tailing pond dike filter drainage and that of Ludwig (1983).

5.7. Inorganic phosphorus:

The inorganic phosphorus results indicated that none of the samples contained enough inorganic phosphorus to support the growth of S. capricornutum in the Algal Assay: Bottle test analysis. The inorganic phosphorus is believed to be a limiting nutrient in the tested samples.

CHAPTER 6

Calculation of the re-toxification time

Calculation of the re-toxification time

Introduction

As mentioned in the introduction, this study focused on the fluid emanating from the potential sludges produced as a result of the hot water extraction process, and as shown in the previous discussion, the emanating fluids are highly toxic to the fresh water green alga S. capricornutum with LC 50 range varying from 5- 33% (v/v) and of an average value of 25.8% (v/v). These results agree with that of Ludwig (1983) who concluded that the fresh tailing water and the emanated fluid are highly toxic to S. capricornutum with LC 50 values of 24.5% and 29.5% (v/v) respectively. Ludwig (1983) also observed that the aged tailings water was slightly toxic to the alga S. capricornutum in the presence of added nutrients (i.e. PAAP solution, see Appendix B).

The following is a schematic drawing for the tailings pond which shows the non-toxic clear water surface layer and the sludge layer which is the source of the contaminated emanating fluid. It is required to calculate the time of re-toxification of the surface layer.

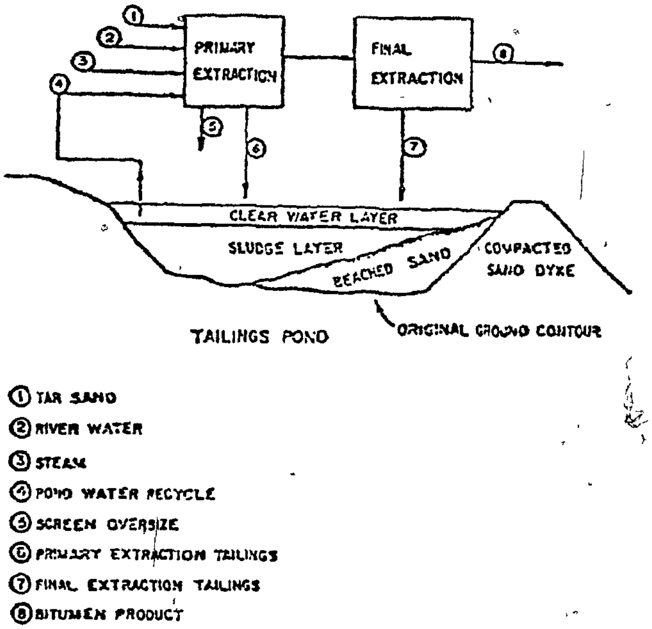


FIG. 6.1 Schematic diagram of hot water extraction and Tailing disposal (Frederick, 1977)

Calculation of the time required for sludge water layer in an initially non-toxic state, to reach a toxic state

The time required for re-toxification (i.e. 50% inhibition in algal growth) of the surface water layer in the tailings ponds, due to the influence of the released fluid, (in the event that the surface water layer is initially rendered "non-toxic" detoxified) is dependent on at least two important factors, namely:

1) the initial volume of the surface water layer,

2) the rate of sludge fluid released at any given time.

All tests were conducted in the laboratory supernatant fraction (samples 8, and 9), collected after centrifugation of terminal sludge samples (total solids concentration = 30.6%) at 10,000 rpm over a 30 minute period of time. Such a sample is considered to be equivalent to freshly released sludge fluid. Distilled water was considered equivalent to surface water in a "non-toxic" state. The toxicity, i.e. the percentage inhibition in algal growth of mixtures of varying proportions of supernatant fraction and distilled water was subsequently determined. The results indicated that the supernatant fraction at a concentration of only 14.8% by volume, exhibited toxicity (i.e. inhibited 50% of algal growth). This means that the surface water layer (when initially "non-toxic" becomes toxic once the emanating fluid is incorporated into the surface water layer, at a concentration of 14.8% by volume.

Sample calculation

Assumption:

(1) the toxicity of the emanating sludge does not further decrease with time, except for the decrease occuring over an initial 8 days period of time.

(2) Other sources of fluid input into the water layer (aside from the emanating sludge fluid) are not present.

(3) The potentially toxic influence of emanating sludge fluid on the detoxified water layer is the same as that observed on distilled water.

A typical example for the calculation of a predicted re-toxification time is provided below:

1) Assume initial surface water layer volume =

200 million Cubic feet (McF)

2) Date for de-toxification of surface layer

(any arbitrary date) = January, 1, 1984.

3) Toxic concentration = 14.8% (v/v) **(measured)**

$$= \left[\begin{array}{l}\text{volume of sludge} \\ \text{fluid released}\end{array}\right] \Big/ \left[\begin{array}{l}\text{Initial volume of} \\ \text{surface water layer}\end{array}\right]$$

Volume of released sludge fluid incorporated into surface water layer required to re-toxify.

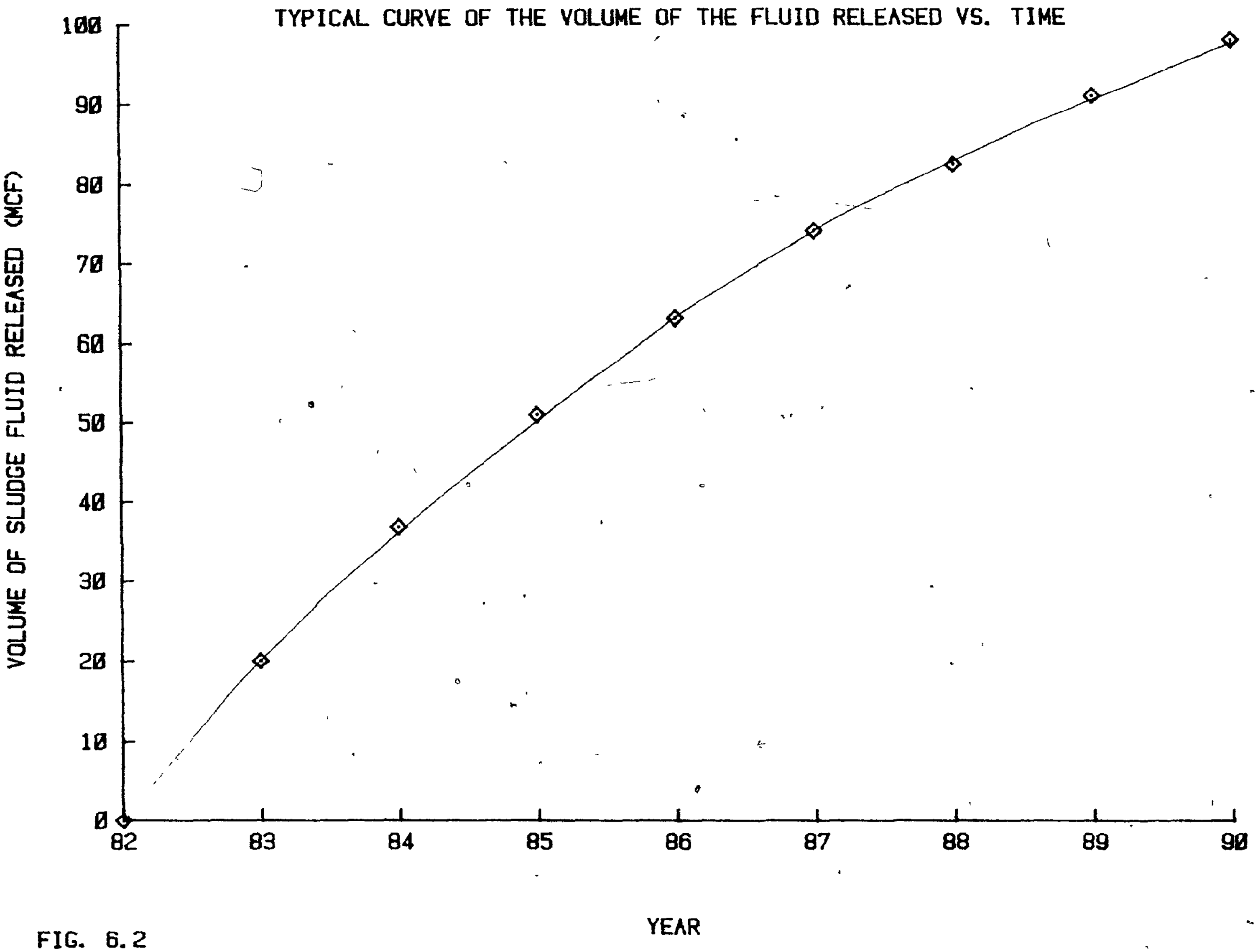
= (toxic concentration) (initial volume of surface water layer)

= (14.8%) x 200 MCF

= 29.6 million cubic feet (MCF)

From Fig (6.2) it can be observed that from the commencement of the year 1984, it would take 28½ months for the release of 29.6 million cubic feet of sludge fluid. Therefore: toxicity in the detoxified surface water layer would be exhibited after 28½ months.

In fact, indications are that the released sludge fluid does decrease in toxicity with time and hence, the time values given by the above analysis represent the minimal times expected for re-toxification of the surface water layer.



TYPICAL CURVE OF THE VOLUME OF THE FLUID RELEASED VS. TIME

FIG. 6.2

CHAPTER 7

CONCLUSIONS

Conclusions

This study was conducted in an effort to assess the toxicity of fluid emanating from potential sludges produced as a result of the "hot water extraction process" employed in extracting oil from the tar sands in Alberta. A further attempt was made to identify specific sources which might be responsible for any toxicity observed in the emanating sludge fluid. Conclusions concerning the toxicity of emanated sludge fluid are as follows:

1) The emanated sludge fluid is highly toxic to the fresh water, green alga, Selenastrum capricornutum and this result was observed for all different potential sludges (both treated and untreated sludges), with extrapolated average LC 50 values for all samples of 22.5% (v/v), in the presence of added nutrients (i.e. PAAP).

2) The emanated fluid is less toxic to the marine bacterium resembling Photobacterium phosphoreum with an average lethal concentration LC 50 value of 85% (by volume).

3) Oxygen uptake curves obtained from samples inoculated with microorganisms of industrial origin also indicated a relatively low toxicity in the emanated sludge fluid (treated and untreated).

4) Metals were not a significant source of toxicity in the samples. Difference of no greater than 5% between samples with added EDTA (metal complexing agent) and samples without EDTA, indicated that metals are not solely accountable for the high toxicity observed with respect to <u>S. capricornutum</u>.

5) Ammonia content of the emanated fluid was observed to be above the safe limits for receiving waters, which indicates that ammonia may exert toxicity directly and/or synergistically on the green alga, <u>S. capricornutum</u>. Also the presence of high ammonia content and low nitrites and nitrates indicate that the oxidation of ammonia to both nitrites and nitrates is not complete. Similarly, other oxidation processes for some hydrocarbons, which may oxidize ultimately to carbon dioxide and water, may not be complete.

6) The dissolved organics are observed to be one of the main sources of the acute toxicity. After the activated

carbon treatment to remove a portion of dissolved organics, the toxicity of all samples decreased by an average value of 30% from that of the original samples. The emanating sludge fluid contained an average of 170 mg/L of organic carbon of which 70% was extractable organic material (dissolved organic compounds and dispersed organic material may be readily extracted from samples with solvent such as benzene).

7) The inorganic phosphorus results indicated that none of the samples contained enough inorganic phosphorus to support the growth of the green alga <u>S. capricornutum</u>.

8) Toxicity was observed to decrease by 25% during a 25 week period of exposure of the samples to the environmental conditions.

BIBLIOGRAPHY

Bibliography

1. Albert Oil Sands Technology & Research Authority (1980) "5th annual report and 5 year review". Edmonton, Alberta.

2. Allen, M. B. (1952). "The cultivation of myxophyceae". Arch. Microbial., 17: 34-53.

3. Ali, L. H. (1977). "Surface - active agents in the aqueous phase of the hot water flotation process for oil sand". Fuel, 56: 359-367.

4. Bailey, N.J., Jabson, A.M. and Rogers, M.A. (1973). "Bacterial degradation of crude oil". Chemical Geology, 11: 202-220.

5. Barbour, F.A., Guffey, F.D., and Dorrence, S.M. (1977). "Preliminary Examination of waste waters produced during an in-situe Rotaring of Tar Sand". J. Can. Petrol. Tech., 50: 110-115.

6. Beckman Instruments, Inc. (1979). "Microtox Model 2055 Toxicity Analyzer System ". Bulletin 6984.

7. Blumer. M. (1969). "Oil pollution of the ocean". In D.P. Hoult (Ed) Oil on the Sea. Plenum Press, New York, pp 5-13.

8. Blumer, M., Sanders, H.L. and Grassie, J.F. Grassie (1971). "Asmall oil spill", Environment 13 (2): 2-12

9. Burchfield, T.E. and Hepler L.G. (1979). "Some chemical and physical properties of produced water from an in-sit Oil sands plant". Fuel, 58: 745-747.

10. Côté, R.P. (1976). "The effects of petroleum refinery liquid wastes on aquatic life, with special emphasis on the Canadian environment". Natial Research, Council, Canada.

11. Coughlan, M.P. (1971). "The role of iron in microbial metabolism". Sci. Progress Oxford, 59: 1-23.

12. Dewaison, G.J. (1977) "Tar sand and supergiant Oil Fields". The oil sands of Canada, pp. 9.

13. Environmental Protection Agency (EPA) (1972). Water quality criteria. EPA-R3-73-033. Washington D.C.

14. Environmental Protection Service, (1978). Laboratory Manual, Government of Canada, Department of Environment.

15. Frederick, W.C. (1977). "Great Canadian Oil Sands - Tailing Disposal" Can. J. Chem. Eng., 55: 581-591.

16. Gallup, W.B. (1974). "The geological history of McMurray, - clear water deposit in the Athabasca oil Sands area". Can. Soc. Petroleum Geologists, 3: 100-114.

17. Gerloff, G.C., Fitzgerald, G.P. and Skoog, F. (1952). "The mineral nutrition of microcystis aeruginosa". Amer. J. Bot., 39: 26-32.

18. Gerloff, G.C. and Skoog, F. (1957). "Availability of iron and manganese in Southern Wisconsin lakes for the growth of microcystis aeruginosa. Ecology, 38: 551-556.

19. Green, J.C., Soltero, R.A., Miller, N.E. and Gasperino, A.F. (1976). "The relationship of laboratory algal assays to measurements of indigenous phytoplankton in long lake", Washington, p. 93-126. Ann. Arbor Sci.

20. Henderson, C.P. Pickering, Q.H. and Lemke, A.E. (1960). "The effect of some organic cyanides (Nitriles) on fish". Proc. 15th Ind. Wast Conf., Purdue University, 120.

21. Hrudey, S.E. (1975) "Characterization of waste waters from the Great Canadian Oil Sands Bitumen extraction and upgrading plant". Water pollution control section, Environmental Protection Service, Northwest Region, Environment Canada, Report no. EPS-5-NW-WR 75-6, September (1975).

22. Hrudey, S.E., Sergy, G.A. and Trackray, T. (1976). "Toxicity of oil sands plant wastewaters and Associated organic contaminants". Water Pollution Research no. 11.

23. Hrudey, S.E. and Scott, J.D. (1981). "Water reuse needs of oil sands industry in Alberta". AWWA.

24. Hutchinson, T.C. and P.M. Stokes (1975). "Heavy metal toxicity and algal bioassays, in water quality parameters". ASTMSTP 573, American Society for Testing and Materials pp 320-343.

25. Jardine, D. (1974), "Cretaceous oil sands of Western Canada", in Hills, L.V. (ed.) Oil Sands, Fuel of the Future, Calgary, Alberta Can. Soc. Prf. Geol. p. 59-67.

26. Kauss, P.S. and Hutchison, T.C. (1975). "The effects of water-soluble Petroleum components on the growth of chlorella Vulgaris Beijerinck" Environ. Pollut. (9).

27. Kanysi, G. (1951). "Elements of bacterial cytology" 2nd ed. Cornell University press, Ithaca, N.Y.

28. Korzep, D.A. (1962) "Toxicity of organic compound, M. Thesis, The University of Texas, Austin.

29. Kostyaev, V.Y. (1969). "Effects of phenol on Scenedesmus acuminatus". Chem. Abstr. 72: 114 (Abstr. no. 108, 273-5).

30. Kratz, W.A., and Myers, J. (1955). "Nutrition and growth of several blue green algae". Amer. J. Bot., 42: 282-287.

31. Lamanna, C. and Mallette, M.E. (1951). "Basic Bacteriology", The Williams and Wilkins Co., Baltimore, Md.

32. Laws, E.A. (1981). "Aquatic Pollution" Jhon Willy pp. 370-403.

33. Leith, W. (1973). "The analysis of organic pollutants in water and waste water". part (9). Phenols 114-127.

34. Ludwig, R. (1983). "A toxicity study on tar sands tailing". Master thesis, Mc Gill University.

35. Mckee, J.E. and Wolf, H.W. (1963). "Water quality Criteria", publication no. 3-A, 2nd edition - State Water Quality Board, Sacramento, California, pp 548.

36. Miller, W.E. and Maloney, T.E. and Green, J.C. (1974). "Algal productivity in 49 lake waters as determined by algal assays". Water Res. 8: 667-679.

37. Miller, W.E., Green, J.C. and Shiroyama, T. (1976). "Use of algal assays to define trace - element Limitation and heavy metal toxicity". Environmental Protection Agency. Corvallis, OR 97330.

38. Miller, W.E., Green J.C. and Shiroyama, T. (1978). "Algal bioassay techniques for pollution evaluation", p. 9-16 in toxic materials in the aquatic environment. Seminar orgon St. Univ. Water Res. Res. Inst. Spring (1978), Corvallis, SEMN WR 024-78.

39. Mironev, O.G. (1968). "The effect of oil pollution on flora and fauna of the Black Sea". FAO Technical Conference on Marine Pollution and its Effect on living Resources and Fishing, 9-18 December (1968), Rome.

40. Moschopedis, S.E. and Speight, J.G. (1976). "Water Soluble derivatives of Athabasca Asphaltenes". Fuel 50, 34.

41. Moschopedis, S.E., Fryer, J.F. and Speig ht, J.G. (1976). "Investigation of the carbonyl functions in a resin fraction from Athabasca Bitumen". Fuel 55, 184.

42. Moschopedis, S.E. Fryer, J.F., and Speight, J.G. (1977). "Water - Soluble Constituents of Athabasca Bitumen". Fuel 56, 109.

43. Nagendran. J. and Hrudey, S.E. (1980). "Oil recovery and recycle water treatment for insides oil sand production". Proc. 7th Nat. Conf. on Energy of Environment. AICHE Phoenix.

44. Nelson- Smith A. (1972). "Effects of the oil industry on shore life in estuaries". Proc. R. Soc. B. 180, 487-96.

45. Nelson - Smith A. (1973). "Oil pollution and Marine Ecology". Eleke Science, London.

46. Nicholls, J.H. and Luhning, R.W. (1977). "Heavy Oil Sand in-situ pilot plants in Alberta (Past and Present)". J. Can.Petrol. Techn., July - September, 50.

47. Ottaway, S. (1971). "The comparative toxicities of crude oils in the ecological effects of oil pollution on littoral communities - ed. by E.B. Cowell, 172-80 London, Institute of Petroleum.

48. PACE, (1974). "Petroleum refinery technology and pollution control seminar. Petroleum Association for conservation of the Canadian Environment, Toronto.

49. Page, H.W. (1974). "The Environmental impact of Alberta's Tar Sands Industry" Oil sands fuel of the Future. Editor L.V. Hills.

50. Patric, R., Crum, R. and Coles, J. (1969). "Temperature and manganeas determining factors in the presence of diatom or blue green-alga floras in streams." Proc. Nat. Acad. Sci 64:472-478.

51. Purves, D. (1977), "Trace - Element contamination of the Environment". Ann Arb. Science, pp 19-27.

52. Reynolds, J.H., Middlebrooksard Grenny, W.J. (1975). "Effects of temperature on oil refinery waste toxicity". J. WPCF. 47(11): 2674-2693.

53. Rodhe, W. (1978). "Algae in culture and nature" Mitt. Int. Ver. Limnol.

54. Sawyer, C.N. and Mc Carty, P.L., (1978). "Chemistry for Environmental Engineering" third edition, McGraw - Hill Company.

55. Skulberg, O.M. (1966). "Algal culture as a means to assess the fertilizing influence of pollution". Advanced in water pollution Research volume 1.

56. Sprague, J.B. (1969). "Measurement of pollutant toxicity to fish I Bioassay method for acute toxicity". Water Research, 3: 793-821.

57. Sprague, J.B. (1970). "Measurement of pollutant toxicity of fish II. utilizing and applying bioassay results". Water Research, 4: 3-32.

58. Standard Methods for the Examination of Water and Wastewater (1980), edited by Taras, M.J., 15th edition, American Public Health Association, Washington, D.C.

59. Stephan, C.E. (1977). "Methods for calculating an LC 50". Aquatic Toxicology and Hazard Evaluation, ASTM, STPG34, F.L. Mayerand, J.L. Haneling, Gds, American Society for Testing and Materials, pp. 65-84.

60. Strosher, M.T. and Peak, E. (1976). "The Evaluation of wastewaters from an oil sands extraction plant". Alberta Oil Sand Environ. Res. Prag. HY 3-1 (Dec. 1976).

61. Takamura, K. (1982). "Microscopic Structure of Athabasca Oil Sand", The Canadian J. of chemical Eng. 60:538-545.

62. United State Environmental Protection Agency (1978). "The Selenastrum capricornutum Prints Algal Assay Bottle test". Environmental Research Lab. Corvallis Organ. 97330 - July, 1978.

63. Wallen, I.E., Geer, W.C. and Lasater, R. (1957). "Toxicity to Gambusia Affinis of certain pure chemicals in turbid waters". Sewage and Ind. Wastes 29, 695.

64. Wangersky, P.J. (1963). "Manganese in ecology" In v. Schultz and A. W. Klement, Jr. eds. Radio ecology First National Symposium on Radioecology. New York. Rein hold Publishing Corp. pp 499-508.

65. Wetzel, R.G. (1972). "The role of carbon in hard-water marnakes. In G.E. likens, ed. Nutrients and Eutrophication. The limiting Nutrient controversy - Special Symposium, Amer. Soc. limnol oceanogr., 1: 84-91.

66. Wetzel, R.G. (1975)."Salinity of inland waters", Limnology, Jhon Willy, pp 153-58.

67. Williams, J.A. and Winters, J.C. (1969). "Microbial alteration of crude oil in the reservoir". Symp. Petroleum Transformations in Geologic Environments. Am. Chem. Soc. Nat. Meet. 158th New York, N.Y. (1969), Paper no. PETR 86: E.22. E.31.

68. Zitko, V. and Carson, W.V. (1970). "The characterization of petroleum oils and their determination in the aquatic environment. Tech. Rep. Fish. Res. Bd. Can., no. 217.

APPENDIX A

APPENDIX A

This appendix presents a brief review of the "hot water extraction process" and describes the oil sands content and formation.

A.1 - General:

Alberta is blessed with enormous quantities of petroleum in the ground - at least 2.15 X10^{11} cubic meters (Takamura, 1982), most of which are contained in oil sands and heavy oil deposits. As Nicholls and Luhning (1977) have pointed out, only about 8% of this is covered by less than 46 meters of overburden and is therefore suitable for economic recovery by surface mining. The majority of bitumen reservoir 78% is estimated to occur under more than 150 meters of overburden and should be recovered by some form of in-situ process.

The best known Canadian deposit is the Athabasca deposit in Northestern Alberta, which contains approximately 1.4x10^{11} cubic meters of bitumen-in-place. The Athabasca Deposit is also the only one currently under commercial exploitation, by Suncor and Syncrude (page, 1974 and Jardian, 1974). The three other oil sand deposits are

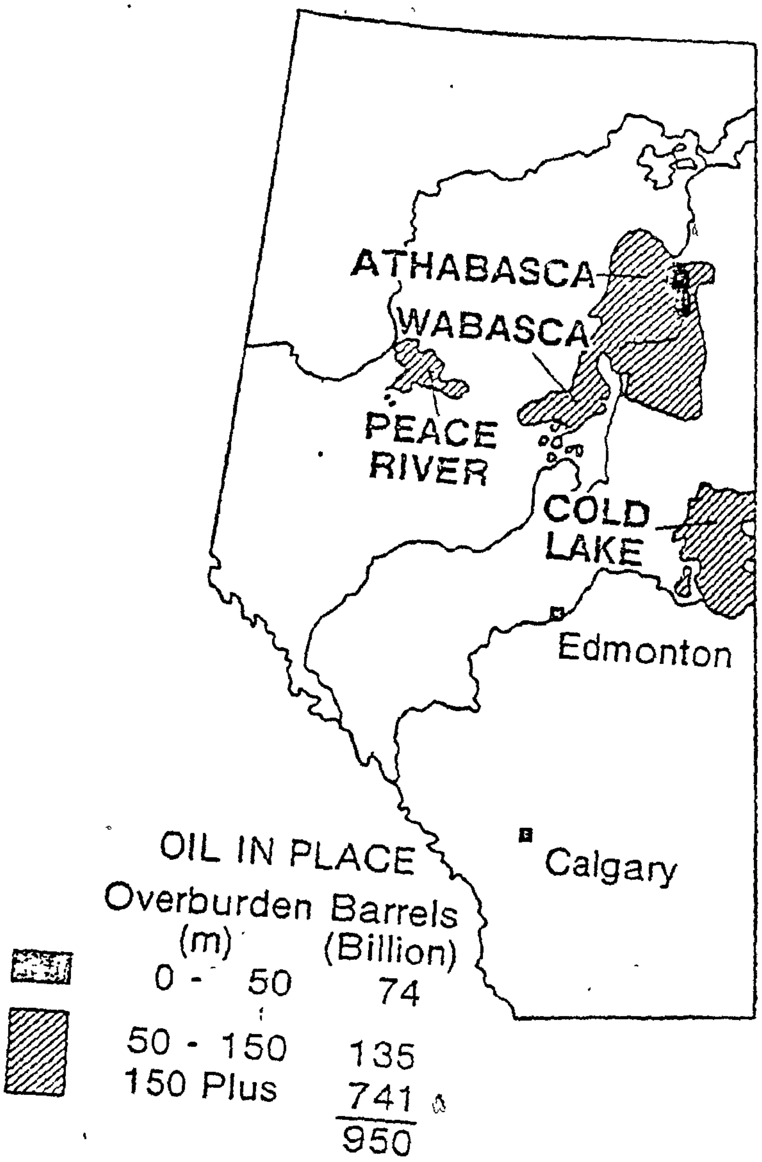


FIG. A.1 Location of Tar Sands deposits in Alberta.

Wabasca, Cold Lake, and Peace River (fig. A.1). The amount of bitumen-in-place at Wabasca is estimated to be $6x10^9$ cubic meters at Cold Lake $25x10^9$ cubic meters and at Peace River $10x10^9$ cubic meters. Table (A.1) summarizes some properties of Alberta oil Sand.

A.2 What are Oil Sands ?

The oil sands are composed of sand, bitumen and water. The bitumen and water occur in the pore space among the grains of sand. There is no direct contact between sand grains because of a thin film of water which surrounds them

Table (A.1)

Alberta Oil Sand & Formation Properties (Nicholls et al, 1977)

	Athabasca	Cold Lake	Wabasca	Peace River
Depth (ft)	0-2000	1000-2000	250-1000	1000-2500
Average Oil in Place (bbl/acre ft)	1500	1240	1100	1150
Porosity %	30-40	30-35	30-35	25-30
Gravity (°A PI)	7-8	10-12	6	8.5-9.0

(Nagendran et al, 1980 and AOSTRA, 1980). The bitumen content is variable, averaging 12% wt. of the deposit. The bitumen, at the in-situ temperature of about 4°C, is a viscoussemi-solid which is slightly heavier than water. Water typically runs 3% to 6% of the mixture, increasing as the bitumen content decreases. The meneral content is relatively constant ranging from 84% to 86% (fig. A.2.).

The origin of tar sand has long been a subject of specilation among geologists. Tar sands have been considered by some as locally generated "immature-oil". Others, such as Gallup (1974), have proposed that some tar occurences are "sedimentary oil", that is surface seepage oil reworked into younger sediments. The modern concept, resulting from major advances in organic geochemistry is that tar accumulations are created by water-washing and bacterial degradation of fluid medium gravity crude oil (Williams and Winters, 1969 and Bailey et al, 1973).

The Athabasca oil sand is a dense sand with in-situ densities as high as 2.25 gm/cm^3, which corresponds to a porosity of about 0.30. The grains, which are almost entirely quartz, are not cemented together. The sand has

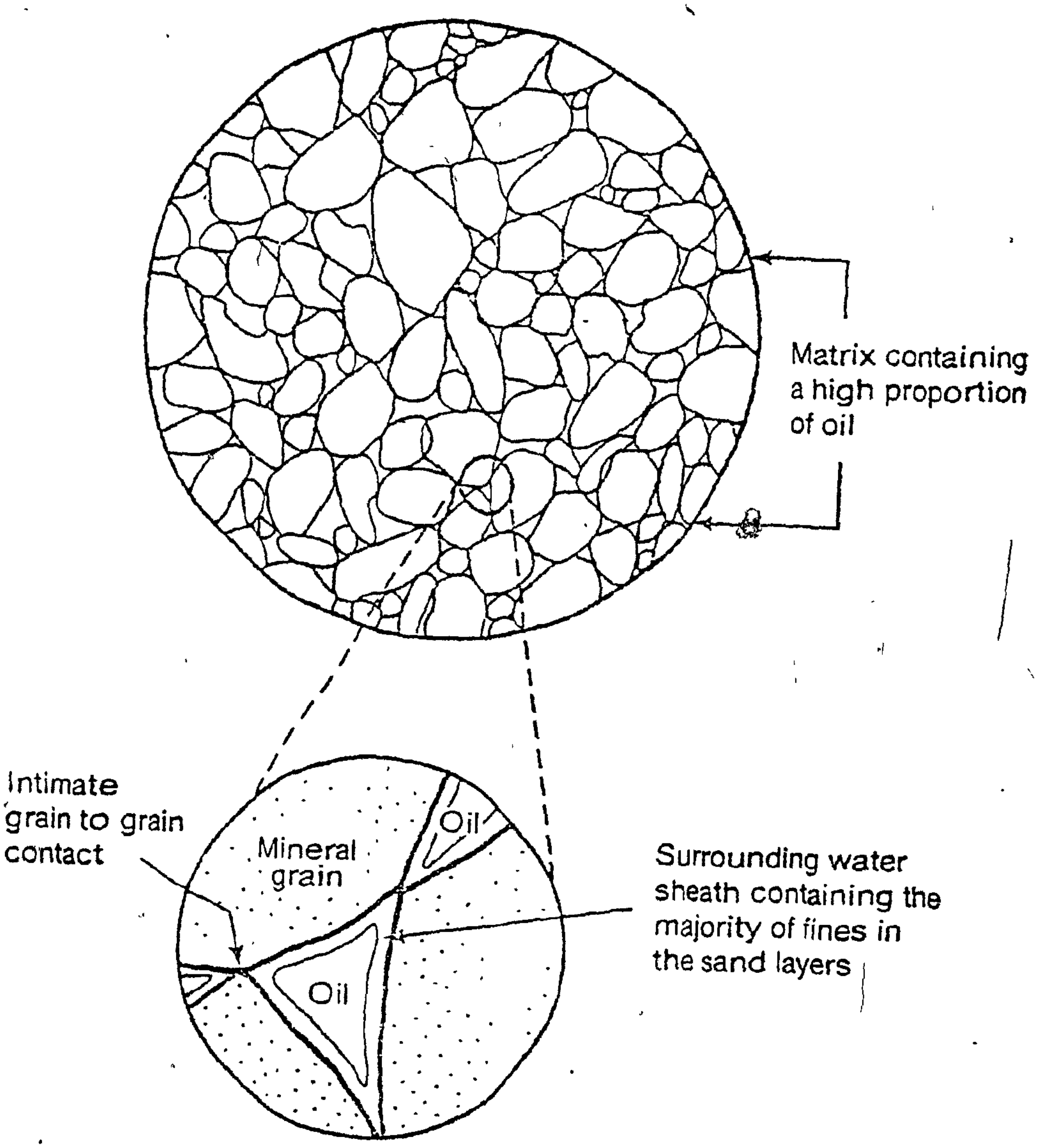


FIG. A.2 Tar Sand Formation (Hruday , 1975) a uniform, fine to medium grain size distribution, with less than 10% of material being silt and clay size (Hrudey, 1981). The in-situ density is greater than what can be obtained in the laboratory when disturbed samples are compacted. This high in-situ density is possible because of the considerable interlocking of the sand grains, caused by its geological history..

A.3 The hot water extraction process:

The hot water process for primary extraction of bitumen from Tar Sands consists of three major process steps outlined schematically in (fig. A.3) (Hrudey, 1975). A fourth step, final extraction, is used to clean up the recovered bitumen for downstream processing.

In the first step, conditioning, tar sand is mixed with water, and treated with open steam to form a pulp of 70 to 85 wt.% solids. Sodium hydroxide is added as required to maintain a pH value in the range 80-85. Under these conditions, bitumen is stripped from the individual sand grains, and mixed into pulp in the form of discrete droplets of a particle size of the same order as that of the sand grains. It turns out that the same process conditions are also ideal for accomplishing

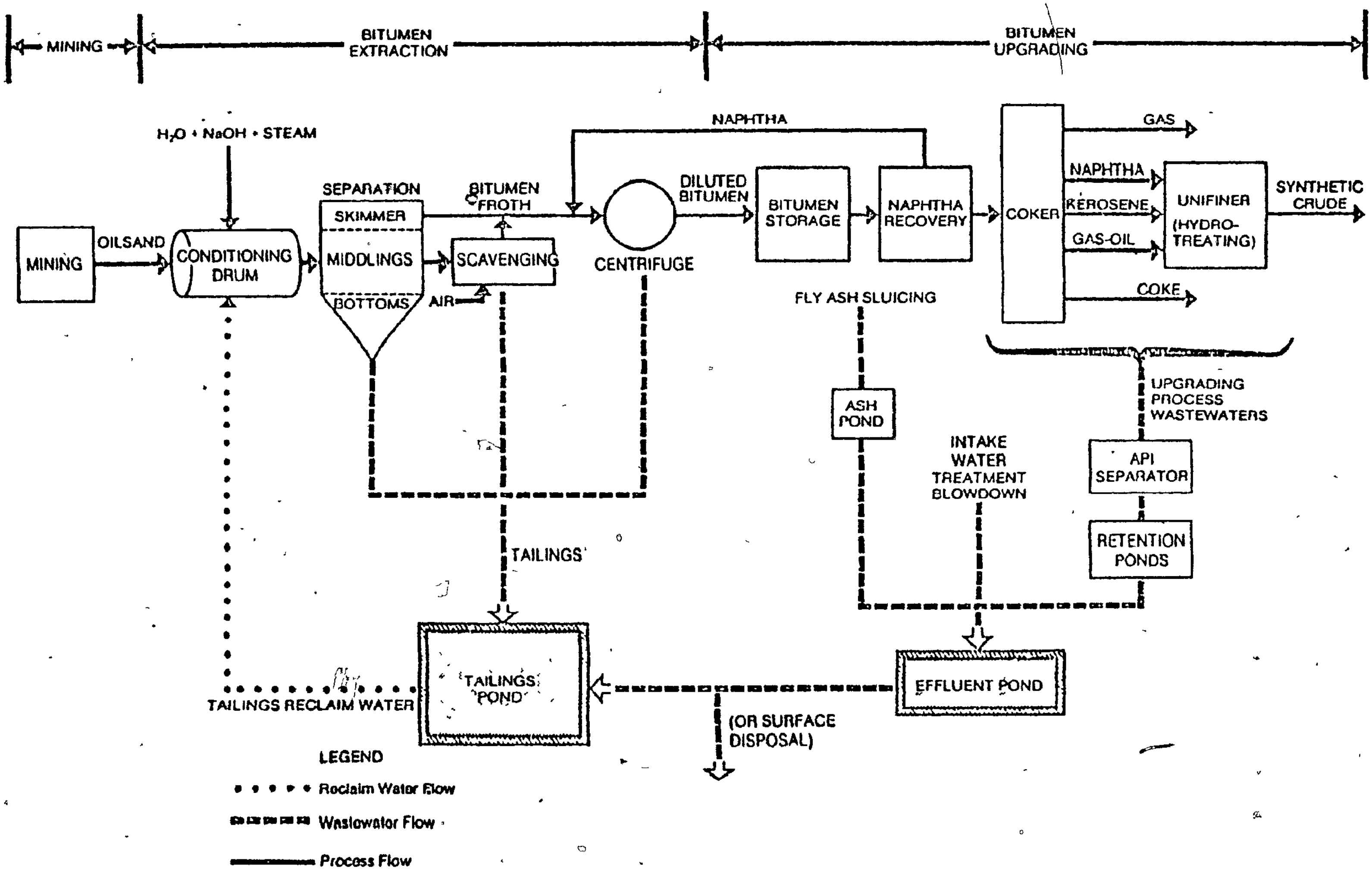


FIG. A.3 Schematic diagram of hot water extraction process (Hrudey, 1975) defloculation of the clay which occur naturally in the tar sand feed. Defloculation, or dispersion, means breaking down the naturally occuring aggregates of clay particles, producing a slurry of individual particles. Thus, during conditioning, a large fraction (some 60-80%) of the clay becomes well-dispersed and mixed throughout the pulp.

In the separation step, the conditioned pulp is diluted further, so that settling can take place. The bulk of the sand (particle sinze 744 pm) rapidly settles, and is withdrawn as sand tailings. Most of the bitumen rapidly floats (settles upward) to form a coherent mass known as froth, which is recovered by skimming the settling vessel. The medium through out these two settling processes takes place is called the middlings and consists primarily of water, with suspended fine mineral and bitumen particles.

As feed fines content increase, more water must be used in the process to maintain the middling viscosity within the operable range (Frederick, 1977).

The third step of the hot water process is scavening. The feed fines content sets the process water requirement, through the need to control middlings viscosity (which is governed by the clay/water ratio). Depending on the net process water input, it may or may not be necessary to withdraw a dragstream of middlings to maintain separation cell material balance. If it is necessary to do so, this stream of middlings can be "scavenged" for recovery of incremental amounts of bitumen.

Air flotation is an effective scaving method and is used by Great Canadian Oil Sands Limited and Suncor.

Final extraction, or froth clean up, is accomplished by centrifugation. Froth from primary extraction is diluted with naphtha. The diluted froth then is subjected to two-stage centrifugation. This process yields an oil product of essentially pure bitumen. Water and mineral removed from the froth constitute an additional tailing stream which must be disposed of.

Finally, the hot water added to the conditioning drum is discharged with sand and clay fines to produce the tailing stream. The stream and hot water can dissolve various minerals present in the deposit. Several recent investigations (Moschopedis, et al, 1976 and Ali, 1977) have shown that reaction of bitumen with steam or hot aqueous solution can be expected to lead to water soluble organics.

APPENDIX B

APPENDIX B

B.1. Synthetic Algal Nutrient Medium:

The culture medium is prepared as follows: add one mL of each stock solution (in 1 through 7 in the order given below) to approximately 900 mL of distilled or de-ionized water, and then dilute to one liter. Adjust final medium pH to 7.5 ±0.1 with 0.1 normal sodium hydroxide or hydrochloric acid as appropriate. Immediately filter the adjusted medium through 0.45 micron membrane at a vacuum not to exceed 380 mm mercury or at a pressure not to exceed 1/2 atmosphere.

1) Sodium nitrate stock solution: Dissolve 12.750 g $NaNO_3$ in 500 mL distilled water.

2) Magnesium chloride stock solution: Dissolve 6.082 g MgCl2. $6H_2O$ in 500 mL distilled water.

3) Calcium chloride stock solution: Dissolve 2.205 g $CaCl_2$ $2H_2O$ in 500 mL distilled water.

4) Micronutrient stock solution: Dissolve in 500 mL distilled water:

a) 92.760 mg H_3 BO_3

b) 207.690 mg Mn cl_2- $4H_2O$

c) 1.635 mg Zn Cl_2

d) 79.880 mg Fe Cl_3 $6H_2O$

e) 150.000 mg Na_2 EDTA. $2H_2O$

f) 0.714 mg $COCl_2$ - $6H_2O$

g) 3.630 mg Na HO_4. $2H_2O$

h) 0.006 mg Cu Cl_2 - $2H_2O$

5) Magnesium sulfate stock solution: Dissolve 7.350 g Mg SO_4. $7H_2O$ in 500 mL distilled water.

6) Potassium phosphate stock solution: Dissolve 0.522 g K_2 HPO_4 in 500 mL distilled water.

7) Sodium bicarbonate stock solution: Dissolve 7.50 g Na HCO_3 in 500 mL distilled water.

The final concentrations of macronutrients as salts and elemental conentration mg/L in distilled or de-ionized waters are shown in table (B.1)

Final concentration of micronutrients as salt and elemental concentration micro g/L in distilled or de-ionized water are shown in table (B.2).

Table (B.1) Final concentration of macronutrients as salt and elemental concentration (mg/L) of distilled water in algal culture medium.

Compound	Concentration mg/L	Element	Concentration mg/L
Na NO_3	25.5	N	4.20
$MgCl_2$ $6H_20$	12.164	Mg	2.904
CaC/2. $2H_20$	4.410	Ca	1.202
Mg SO_4 $7H_20$	14.70	S	1.911
K_2 HPO_4	1.044	P	0.186
$NaHCO_3$	15.0	Na	11.001
		K	0.469
		C	2.143

Storage of culture medium:

The culture medium must be stored in the dark at 4°C to avoid any (unknown) photo-chemical changes.

Table (B.2) Final concentration of micronutrients concentration as salts and elemental concentration (mg/L) in distilled water in algal culture medium

Compound	Concentration (mg/L)	Element	Concentration mg/L
$H3\ BO3$	185.52	B	32.460
$Mn\ Cl_2\ 4H_2O$	415.610	Mn	115.374
$Zn\ Cl_2$	3.271	Zn	1.570
$CO\ Cl_2\ 6H_2O$	1.428	Co	0.354
$Cu\ Cl_2\ 2H_2O$	0.012	Cu	0.004
$Na\ MoO4\ 2H_2O$	7.260	MO	2.878
$Fe\ Cl_3\ 6H_2O$	160.00	Fe	33.051
$Na\ EDTA\ 2H_2O$	300.00	-	-

B.2 Test Algae:

The recommended test alga Selenastrum capricornutum Printz is a green algae (chlorophyceae) of the order chlorococcales. This algae was isolated from the River Nitelva, in the County of Akershus, Norway, by Olav M. Skulberg, Norwegian Institute for Water Research, 1959.

Many green algae, such as Chloralla, Scenedesmus, and Ankistrodesmus occur in waters of the most diversified composition. Selenastrum belongs to this group of ubiqutous algae which have a wide tolerance towards environmental conditions (Rodhe, 1978). Selenastrum capricornutum is characterized by its unicellular habit in which the cells are in a non-mobile condition throughout their entire life cycle. These attributes allow these alga to be enumerated by the electronic particle counter.

Source of test alga: Available from the Environmental Protection Agency. Corvallis Environmental Research Laboratory, Special studies Branch, 200 SW 35th street, Corvallis, Oregon 97330.

B.3. Preparation of inoculum:

Rince algal inoculum free of culture medium as follows: Fill centrifuge tube with 7-10 day stock culture, and centrifuge at 1000 xg for 5 minutes. Decant the supernatant and resuspend the cells in sodium bicarbonate solution ($Na HCO_3$). Repeat the centrifugation and decantation step and resuspend the cells in $NaHCO_3$ prior to determining the initial cell concentration.

After determining the initial algal cell counts, the following equation can be used to prepare the inoculum:

$$\frac{\text{Final volume of inoculum} \times \text{Final concentration of inoculum in test flask (cell / mL)} \times \text{volume of solution in test flask}}{\text{Initial Algal Cell Counts (Cells/mL) in the stock culture}} = Q$$

where Q is the initial stock culture suspension to be added to the volumetric flask before bringing the solution up to volume. This inoculum solution should contain a final concentration of 100.000 ± 10% celles/mL.

B.4. Calculation sample:

a) The actual number of algae present in the sample was obtained by using the following equation:

$$N = \left(\frac{n}{1000}\right)^2 \times 0.9261 + n$$

where

N = actual algae count

n = initial algae count

b) Fertility potential of all samples was determined from the phosphorus and nitrogen concentrations by using the following relationships:

$$(\text{inorganic phosphours}) \times 430 = \text{fertility potential due to phosphours alone} \quad \_\_\;(1)$$

$$\left[\text{nitrites} - \text{nitrates}\right] + \text{ammonia} \times 38 = \text{fertility potential due to nitrogen alone} \quad \_\_\;(2)$$

The smallest value obtained from the above relationships represents the fertility potential of the sample, or in other words the limiting nutrient.

c) By using the computer program operated on a Hewlett Packard 9830 A programmable calculator, the inhibition percentage was obtained.

d) The LC 50 (lethal concentration) was estimated by plotting test concentration versus inhibition percentage on a probits-log scale paper (EPA, 1978).

APPENDIX C

Test results

Table (C.1) Physical Analysis Results

Sample No.	pH	Turbidity JTU	SP. Conduct. mmoh/cm	T.S. mg/L	V.S. mg/L	S.S. mg/L
1	8.20	45	1.10	2252	410	296.8
2	8.00	17	1.10	747	291	98.5
3	7.80	29	0.976	1538	329.9	158.6
4	7.90	42	1.05	2168	394	303.2
5	8.10	41	0.64	958	329.2	334.3
11	7.80	13	0.65	3895	204	34
12	7.80	42	1.57	1053	340	55
13	7.80	53	0.580	3147	295	143
14	7.50	28	1.10	737	240	156
15	7.90	36	450	2603	265	52
16	7.8	46	440	2462	207	112
17	7.70	41	450	3579	256	69
18	8.00	42	610	3771	285	82
19	7.70	65	680	9085	286	198

Table (C.2) Quantitative analysis of dissolved components of sludge water samples (All these analytical results are reported in term of mg/L)

Sample No.	Cu	Fe	cd	Mn	Ni
1	0.071	5.0	< 0.02	0.45	< 0.02
2	0.071	1.406	< 0.02	0.080	< 0.02
3	0.099	2.99	< 0.02	0.032	< 0.02
4	0.071	2.628	< 0.02	0.048	< 0.02
5	0.085	4.857	< 0.02	0.048	< 0.02
11	0.036	1.234	< 0.02	0.187	0.120
12	0.054	1.029	< 0.02	0.10	0.040
13	0.20	3.851	< 0.02	0.304	0.080
14	0.091	3.851	< 0.02	0.20	0.120
15	0.054	1.087	< 0.02	0.125	0.120
16	0.127	2.116	< 0.02	0.225	0.120
17	0.109	2.557	< 0.02	0.25	0.12
18	0.091	2.146	< 0.02	0.22	0.12
19	0.091	1.911	< 0.02	0.612	0.12

Table (C.2) Cont. Quantitative analysis of dissolved components of sludge water samples
(All these analytical results are reported in terms of mg/L)

Sample No.	Pb	Zn	As	V
1	< 0.1	0.062	< 0.02	< 0.02
2	< 0.1	0.117	< 0.02	< 0.02
3	< 0.1	0.137	< 0.02	< 0.02
4	< 0.1	0.113	< 0.02	< 0.02
5	< 0.1	0.084	< 0.02	< 0.02
11	< 0.1	0.04	< 0.02	< 0.02
12	< 0.1	0.061	< 0.02	< 0.02
13	< 0.1	0.107	< 0.02	< 0.02
14	< 0.1	0.041	< 0.02	< 0.02
15	< 0.1	0.209	< 0.02	< 0.02
16	< 0.1	0.066	< 0.02	< 0.02
17	< 0.1	0.066	< 0.02	< 0.02
18	< 0.1	0.066	< 0.02	< 0.02
19	< 0.1	0.112	< 0.02	< 0.02

Table (C.2) cont. Quantitative analysis of dissolved components of sludge water samples (All these analytical results are reported in terms of mg/L)

Sample No.	Na	Ca	Mg	K
1	196.0	9.50	8.70	13.0
2	173.0	7.50	7.70	11.04
3	190	24.0	18.70	8.07
4	183	10.0	11.90	11.03
5	171	8.30	71.0	12.01
11	179	18.9	45.0	12.06
12	162	30.0	15.0	13.05
13	193	43.0	22.50	14.10
14	151	11.0	7.50	13.5
15	185	15.5	9.0	12.02
16	210	29.0	8.50	11.90
17	178	13.50	11.25	12.08
18	189	17.50	10.5	14.0
19	215	45.0	15.0	14.20

Table (C.3): Results of organic carbon content

(all these results are reported in mg/L)

Sample No.	BOD	BODu	COD	TOC	Total inorganic carbon
1	38.30	230	390	162.50	73.12
2	25.75	210	357	52.30	< 2.0
3	22.0	154	160	91.80	36.72
4	47.0	180	216	129.40	58.23
5	38.30	230	390	162.50	73.12
11	50.0	-	294	223.80	86.20
12	52.10	-	400	281.90	172.40
13	42.0	-	376	209.30	161.40
14	37.0	-	296	191.80	95.40
15	48.50	-	384	232.50	123.40
16	46.25	-	268	246.0	154.10
17	64.25	-	384	225.8	157.70
18	54.40	-	388	267.40	183.40
19	52.90	-	400	250.0	168.70

Table (C.4) Organic carbon content of sludge water samples (All results are reported in mg/L)

Sample No.	Original carbon	Extractable carbon	Residual carbon	Percentage extractable carbon
1	162.5	112.10	50.40	69
2	52.3	37.1	15.20	71
3	91.80	57.80	34.0	63
4	129.40	88.0	41.4	68
5	162.50	113.75	48.75	70
11	223.80	165.60	58.20	74
12	281.90	200.0	81.90	71
13	209.30	150.70	58.60	72
14	191.80	132.30	59.50	69
15	232.50	151.10	81.40	65
16	246.0	176.30	78.70	68
17	255.8	179.0	76.80	70
18	267.40	196.24	71.16	71
19	250.0	167.5	82.5	67

Table (C.5) Chemical analysis results

(all these results are reported in mg/L)

Sample No.	Total N	Ammonia	Total P.	Inorganic P	Phenol
1	40.0	29.0	0.03	0.02	0.030
2	370.0	24.0	0.04	0.04	0.02
3	51.0	30.0	0.04	0.02	0.03
4	38.0	25.0	0.05	0.05	0.04
5	45.0	28.0	0.03	0.02	0.02
11	51.0	45.5	0.02	0.01	0.03
12	30.0	29.0	0.03	0.01	0.02
13	53.0	47.6	0.02	0.01	0.02
14	18.0	12.6	0.02	0.01	0.03
15	41.0	35.7	0.04	0.02	0.03
16	34.0	32.2	0.03	0.01	0.04
17	50.0	42.0	0.02	0.01	0.03
18	48.0	37.8	0.02	0.01	0.03
19	72.0	63.0	0.04	0.02	0.08

Table (C.6) Growth of *S. capricornutum* with different concentrations of samples as % of that in the control

Sample No.	Con.	day-2	day-3	day-4	day-6	day-8
1	100	5.7	2.2	1.0	1.0	1.0
	50	7.1	2.7	1.0	1.9	2.5
	25	22	16	12	29	28
	12.5	30	41	44	54	57
3	100	11.6	2.2	0.4	0.6	0.6
	50	12.6	2.7	0.6	1.0	1.0
	25	16	9	5	25	35
	12.5	24	16	11	78	70
11	100	9	2.5	0.7	0.37	0.44
	50	14	3.5	1.4	1.6	4.5
	25	17	25	37	22	24
	12.5	38	42	80	86	86
13	100	7.0	3.0	1.0	0.68	0.62
	50	10.0	3.2	1.42	0.87	0.77
	25	16.0	12.0	12.0	6.87	6.66
	12.5	26.0	3.0	42.8	25	24
16	100	3.3	2.0	0.87	0.88	0.9
	50	5.0	2.3	1.1	2.10	4.86
	25	13.0	23	35	35	34
	12.5	30.0	40	62.5	46	44
19	100	8	3	1.0	1.0	1.4
	50	12	3	1.4	1.5	1.4
	25	13	6.5	4.2	10	20
	12.5	40	23	17	55	56

Table (C.6) cont. Growth of *S. caprinornutum* with different concentration of samples as % of that in the control

Sample No.	Con.	day-10	day-12	day-14
1	100	1.3	1.7	1.9
	50	3.2	3.3	3.4
	25	2.9	35	37
	12.5	61	67	71
3	100	1.0	1.0	1.0
	50	1.3	1.4	1.5
	25	52	60	65
	12.5	85	93	93
11	100	0.6	0.6	0.6
	50	6	19	14
	25	29	38	55
	12.5	85	90	90
13	100	0.6	0.62	0.60
	50	0.7	0.7	0.7
	25	6.0	6.5	6.0
	12.5	23	25	25
16	100	0.7	0.64	0.61
	50	9.60	8.80	8.8
	25	37.0	41.0	38.0
	12.5	44.8	47.0	44.0
19	100	1.0	0.8	0.7
	50	1.2	1.0	1.0
	25	41	38	40
	12.5	58	57	57

Table (C.7) Algal Assay Bottle test results for sludge water samples (original samples).

Sample no.	Inhibition%				
	100% sample	100% sample +1ml EDTA	100% sample + 1ml PAAP	50% sample + 1ml PAAP	25% sample +1ml PAAP
1	99.25	90.27	99.39	98.32	62.66
2	98.31	98.25	97.36	92.41	94.72
3	92.75	99.52	99.28	79.71	45.83
4	94.31	98.89	94.97	92.45	73.50
5	93.27	96.05	99.62	75.12	76.72
11	99.60	99.49	99.19	98.84	99.20
12	99.44	99.57	99.65	99.45	86.75
13	98.41	99.31	99.37	98.40	86.59
14	99.58	99.75	99.63	90.92	67.63
15	99.45	99.64	99.63	98.67	89.04
16	99.61	99.73	99.66	98.36	96.85
17	99.69	99.75	99.77	98.93	98.67
18	99.37	99.72	99.70	98.81	98.64
19	99.35	99.66	99.62	98.67	96.15

Table (C.7) cont. Algal Assay Bottle test results for sludge water (original samples).

Sample no.	Inhibition %			LC.50 (% by volume)
	12.5% sample +1ml PAAP	6.25% sample + 1ml PAAP	3.13% sample + 1ml PAAP	
1	32.51	12.31	0.00	26
2	17.94	5.06	0.00	23
3	31.23	11.00	0.00	27
4	4.50	0.00	0.00	28
5	4.79	0.00	0.00	33
11	55.79	0.00	0.00	12
12	10.00	0.00	0.00	19
13	73.89	75.14	6.99	11.0
14	19.22	0.00	0.00	20.5
15	85.47	47.65	12.53	8.60
16	38.82	16.34	0.00	18.50
17	71.36	0.00	0.00	9.50
18	95.87	75.84	16.80	5.0
19	67.47	37.74	6.00	8.8

Table (C.8) Algal Assay Bottle test results for sludge water samples offer passing through activated carbon column.

Sample no.	Inhibition %				
	100% sample	100% sample + 1ml PAAP EPTA	100% sample +1ml PAAP	50% sample +1ml PAAP	25% sample + 1ml PAAP
1	97.61	97.65	97.57	54.44	10.0
2	97.78	97.70	97.79	71.48	23.08
3	99.15	99.08	97.34	66.69	36.16
4	98.98	98.92	98.77	55.86	2.91
5	99.05	98.26	98.99	73.03	42.72
11	99.13	98.91	98.38	76.07	53.53
12	99.51	99.52	99.77	99.65	98.52
13	99.26	99.95	99.00	80.14	51.53
14	99.35	99.04	99.00	78.46	59.96
15	99.79	99.74	99.56	98.65	26.94
16	97.93	98.80	98.23	61.28	4.83
17	98.86	99.05	98.82	64.54	31.35
18	98.82	98.83	98.86	67.19	6.02
19	98.58	97.68	98.72	74.07	59.21

Table (C.8) cont. Algal Assay Bottle test results for sludge water samples after passing through activated carbon column

Sample No.	Inhibition %			LC 50% (by volume)	percentage of increase in LC 50
	12.5% sample + 1 ml PAAP	6.25% sample + 1 ml PAAP	3.13% sample + 1 ml PAAP		
1	2.00	0.00	0.00	44	40
2	0.00	0.00	0.00	37	37
3	10.0	0.00	0.00	31	13
4	0.00	0.00	0.00	47	0.32
5	0.00	0.00	0.00	30	-
11	6.85	0.00	0.00	24	50
12	27.38	0.00	0.00	17	-
13	24.52	3.37	0.00	24	54
14	50.86	7.00	0.00	22	6.8
15	0.00	0.00	0.00	30.0	71
16	0.00	0.00	0.00	42	55
17	0.00	0.00	0.00	35	72
18	0.00	0.00	0.00	40	87
19	12.02	0.00	0.00	23	61

Table (C.9) Microtox Toxicity Results

Sample No.	Inhibition %			
	50% sample	25% sample	12.5% sample	6.25% sample
1	30.0	11.30	6.20	2.30
2	27.70	9.0	11.20	5.6
3	29.30	14.70	5.70	1.90
4	28.70	14.70	5.20	0.80
5	30.0	12.60	8.0	4.20
11	57.0	36.0	25.0	11.0
12	53.10	36.10	37.20	17.57
13	35.20	24.70	12.90	6.10
14	38.10	10.70	19.8	0.00
15	36.40	20.0	13.40	7.0
16	22.80	10.0	2.40	0.00
17	36.30	20.90	5.90	0.90
18	34.50	20.60	9.30	4.80
19	35.70	20.10	7.60	2.20

Table (C.9) cont. Microtox Toxicity Results

Sample No.	Inhibition 3.125% sample	LC 20 % by volume	LC 50 % by volume	The % difference between LC 50 AA BT and Microtox Technique
1	0.40	35.55	96.28	73
2	3.70	23.0	92.98	75
3	0.00	33.97	91.59	70
4	0.40	34.57	90.07	46
5	2.20	29.0	N.D.	-
11	2.70	14.0	39.49	70
12	6.34	10.0	40.67	53
13	3.10	20.0	89.02	87
14	0.00	30.0	47.12	57
15	4.20	21.0	93.17	90
16	0.00	45.0	N.D.	-
17	0.00	28.0	64.40	85
18	0.00	25.0	82.84	93
19	0.00	25.40	73.20	88

Table (C.10) oxygen uptake of the sludge water samples in mg/L.

Sample no.	Cumulative oxygen in mg/L					
	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr
1	28.9	31.9	47.0	50.6	66.9	79.1
2	46.2	73.1	98.5	110.	126.6	139.2
3	30.3	35.8	51.9	62.1	72.9	85.5
4	44.4	67.7	91.9	102.4	119.0	130.3
5	0.0	10	17	29	46	61
11	0.0	0.0	8.7	11.0	22.6	37
15	0.0	0.0	8.0	8.7	26	42
19	0.0	0.0	6.0	10.0	21.3	35

Table (C.10) cont. Oxygen uptake of the sludge water sample in mg/L.

Sample no.	Cumulative oxygen uptake mg/L					
	14 hr	16 hr	18 hr	20 hr	22 hr	24 hr
1	101.1	126.4	141.8	145	155	178
2	162.7	190.9	227.4	253	267	291
3	107	127.2	153.6	164.7	178.7	185
4	148.1	171	205.7	230	242	264
5	67.9	74.2	76	77	79.8	81.9
11	44	46.8	46.8	46.8	46.8	46.8
15	49.2	55	57	58	58	58
19	45.2	48.1	49	49.2	49.2	49.2

Table (C.11) Results of toxicity with time.
(Sample # 6)

Time weeks	Inhibition %				
	100% sample	100%sample +1ml EDTA	100%sample +1ml PAAP	50% sample +1ml PAAP	25% sample +1ml PAAP
1	94.09	97.74	99.33	99.29	95.91
3	90.93	99.16	99.37	97.73	94.69
5	94.45	99.28	99.55	99.29	97.66
7	99.43	99.71	99.67	99.70	99.49
9	99.43	98.72	98.45	97.56	83.42
11	98.18	98.89	98.26	98.72	25.67
13	98.43	98.83	99.21	97.86	38.09
15	99.33	98.67	99.63	98.46	18.0
17	99.50	99.52	99.51	98.31	0.00
19	99.21	99.42	98.92	56.21	0.00
21	97.30	97.81	98.02	86.30	0.00
23	97.52	97.35	97.11	65.80	0.00
25	97.10	98.32	97.21	65.60	0.00

Table (C.11) cont.

Sample # 6

Time Weeks	Inhibition %			EC 50% v/v
	12.5% sample +1ml PAAP	6.25% sample +1ml PAAP	3.13% sample +1ml PAAP	
1	39.03	21.08	0.00	15.0
3	31.00	3.00	0.00	17.0
5	47.33	2.50	0.00	13.0
7	51.76	15.30	0.00	12.0
9	36.34	6.66	1.24	16.0
11	0.00	0.00	0.00	27.0
13	0.00	0.00	0.00	28.0
15	0.00	0.00	0.00	31.0
17	0.00	0.00	0.00	37.00
19	0.00	0.00	0.00	41.0
21	0.00	0.00	0.00	41.0
23	0.00	0.00	0.00	41.0
25	0.00	0.00	0.00	38.0

Table (C.12)

Sample # 7: Results of toxicity with time.

Time weeks	Inhibition %				
	100%sample	100%sample +1ml EDTA	100%sample +1ml PAAP	50% sample +1ml PAAP	25% sample +1ml PAAP
1	98.30	99.37	98.27	98.84	64.94
3	90.13	99.41	99.48	99.23	76.27
5	95.40	99.78	99.72	99.68	81.58
7	99.44	99.51	99.40	99.30	28.03
9	97.35	98.41	99.40	97.71	40.36
11	99.30	98.39	98.79	70.84	26.58
13	99.53	99.66	99.66	96.27	26.94
15	99.37	99.76	99.73	99.57	24.91
17	99.48	99.70	99.59	93.73	11.14
19	99.08	99.00	99.51	95.43	10.2
21	99.20	99.00	97.63	82.71	0.00
23	98.60	98.78	98.27	76.30	0.00
25	97.30	96.52	96.33	66.20	0.00

Table (C.12) cont.

Sample # 7 : Results of toxicity with time.

Time Weeks	Inhibition %			EC.50% v/v
	12.5% sample +1ml PAAP	6.25% sample +1ml PAAP	3.13% sample +1ml PAAP	
1	44.88	0.00	0.00	22.0
3	45.00	20.99	0.00	14.50
5	30.02	22.21	2.02	16.0
7	5.00	0.00	0.00	28.0
9	26.09	18.06	1.24	27.0
11	0.00	0.00	0.00	37.6
13	0.00	0.00	0.00	30
15	0.00	0.00	0.00	31.0
17	0.00	0.00	0.00	35.0
19	0.00	0.00	0.00	36.0
21	0.00	0.00	0.00	40.0
23	0.00	0.00	0.00	40.1
25	0.00	0.00	0.00	40.0

Table (C.13) The results of COD of pH with time

Weeks	Sample # 6		Sample # 7	
	pH	COD (mg/L)	pH	COD mg/L
1	8.6	558	8.8	403
3	8.8	410	8.90	360
5	8.8	390	8.90	330
7	9.0	336	9.0	290
9	9.0	332	9.0	288
11	9.10	308	9.10	268
13	9.10	302	9.10	266
15	9.10	296	9.10	262
17	9.10	283	9.10	260
19	9.20	270	9.20	252
21	9.20	266	9.20	255
23	9.20	268	9.20	258
25	9.20	260	9.20	250

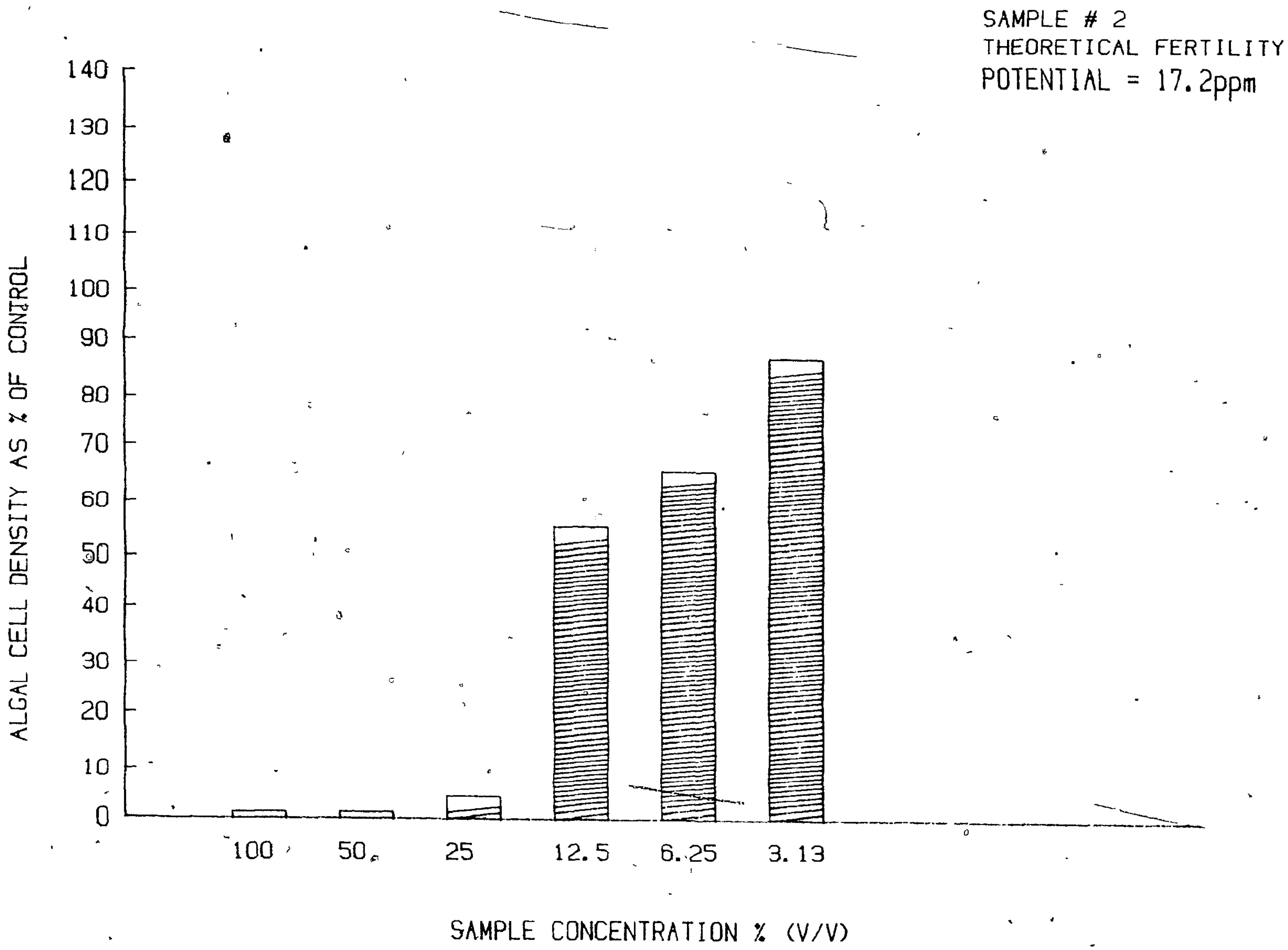


FIG. C.1 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

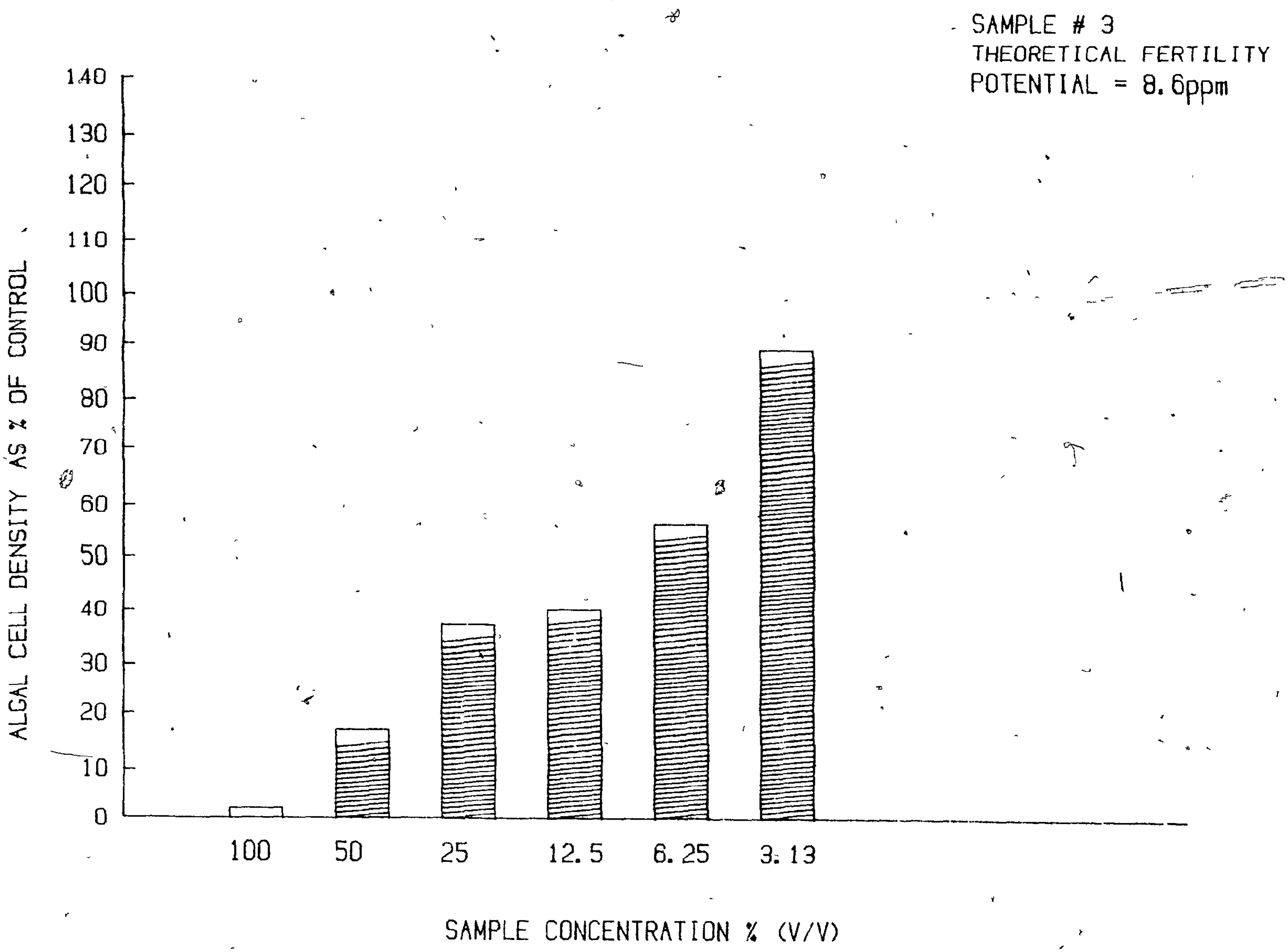


FIG. C.2 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF <u>S. capricoronutum</u>

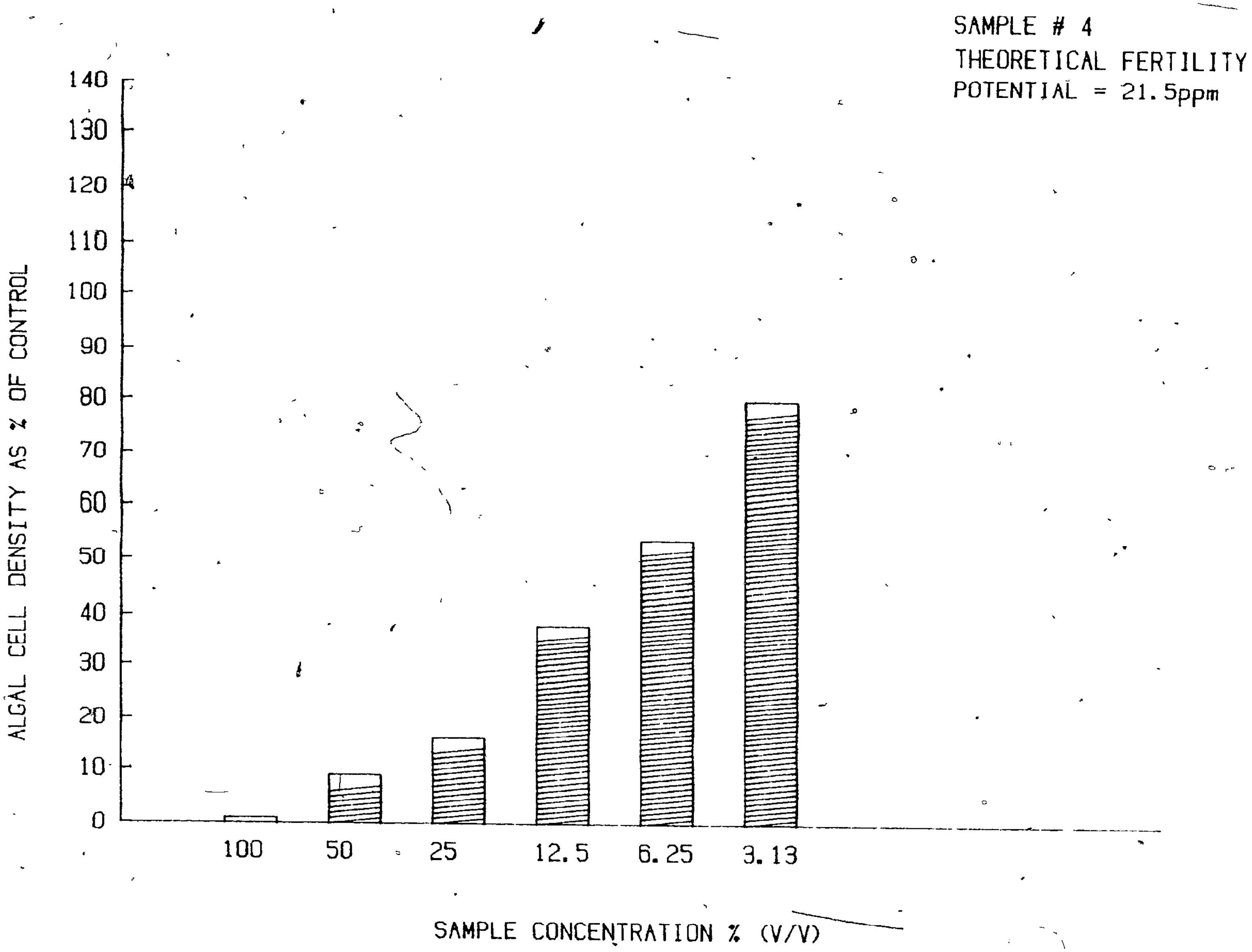


FIG. C.3 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

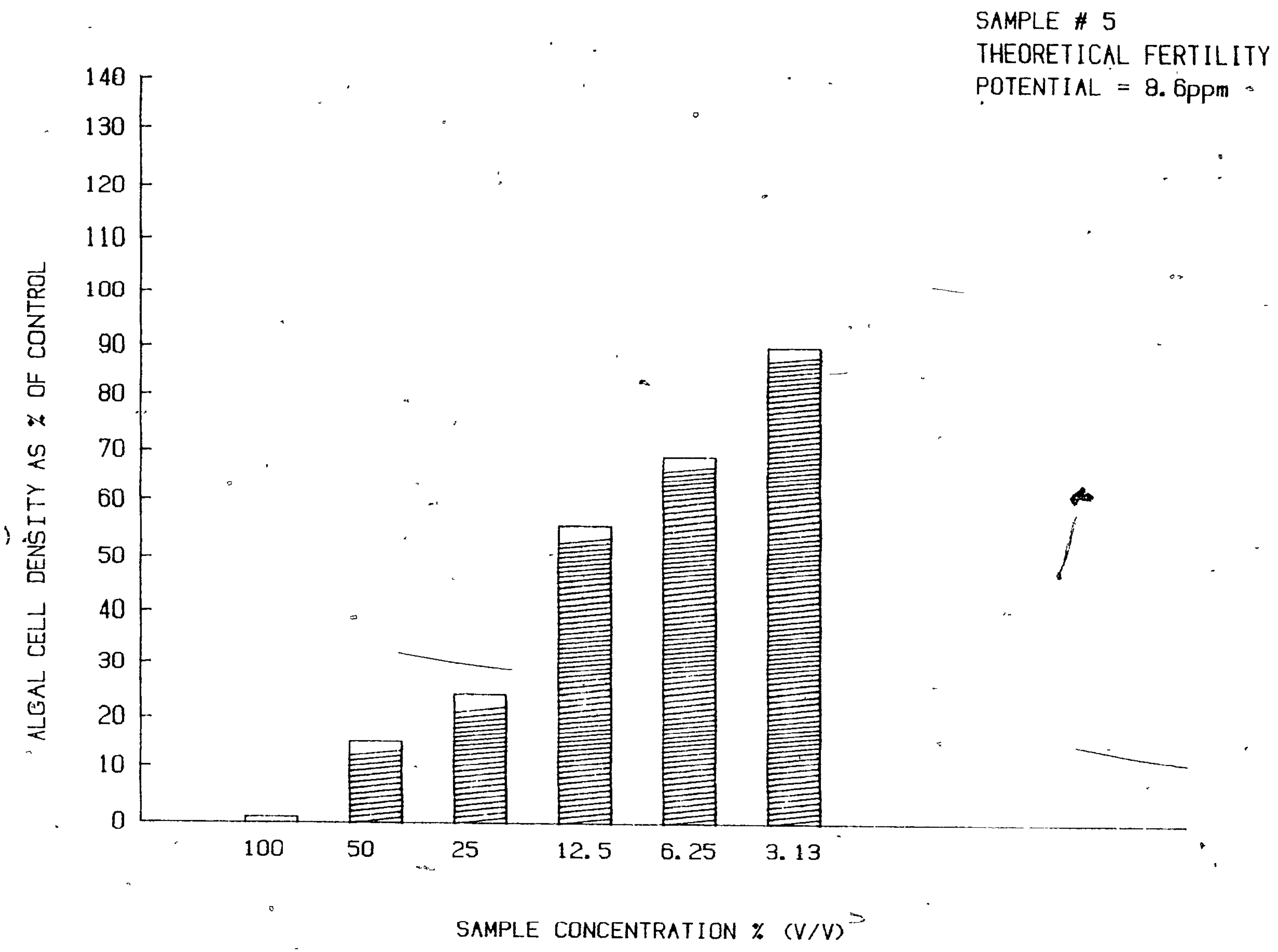


FIG. C.4 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF <u>S. *capricoronutum*</u>

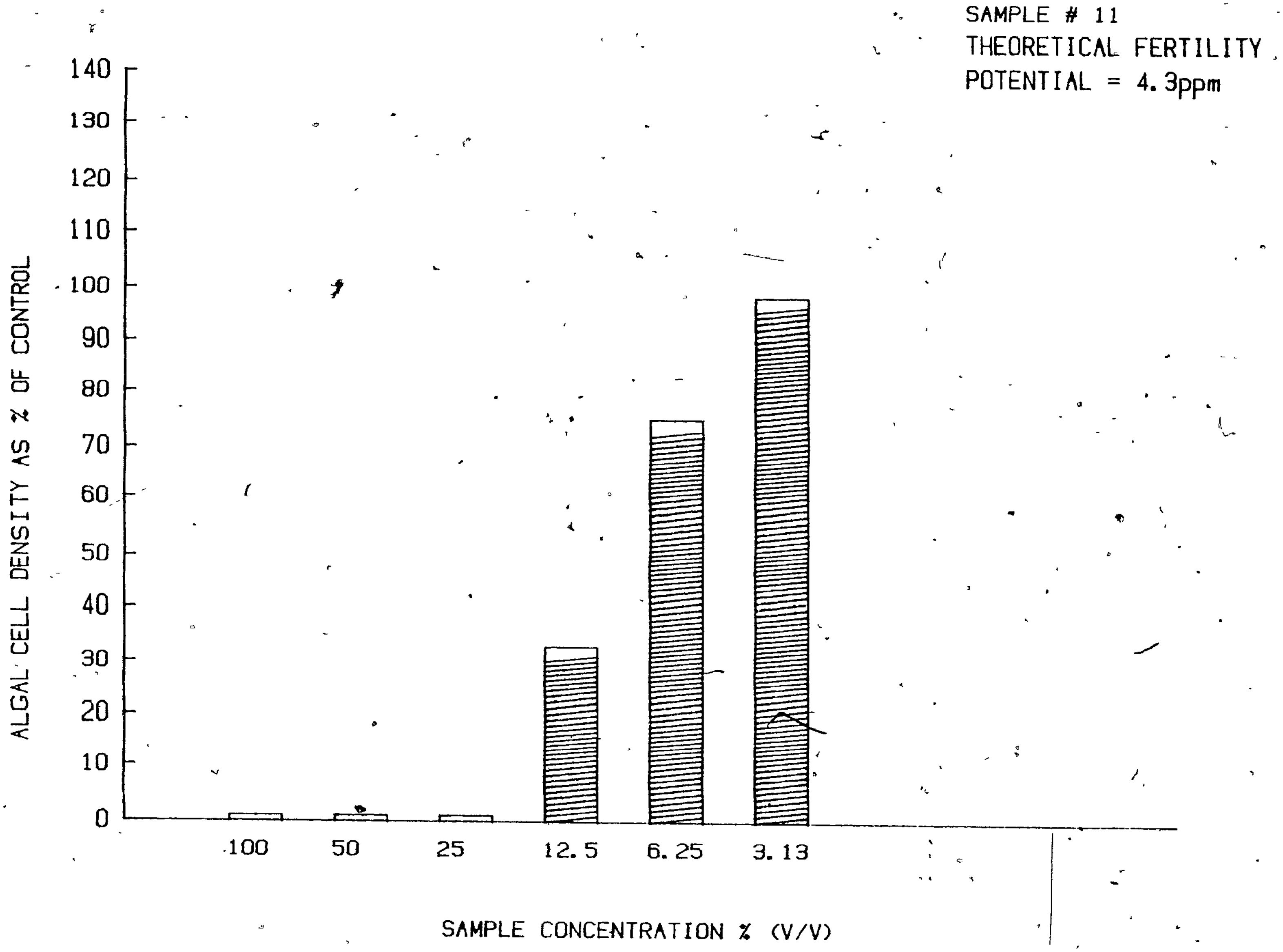


FIG. C.5 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

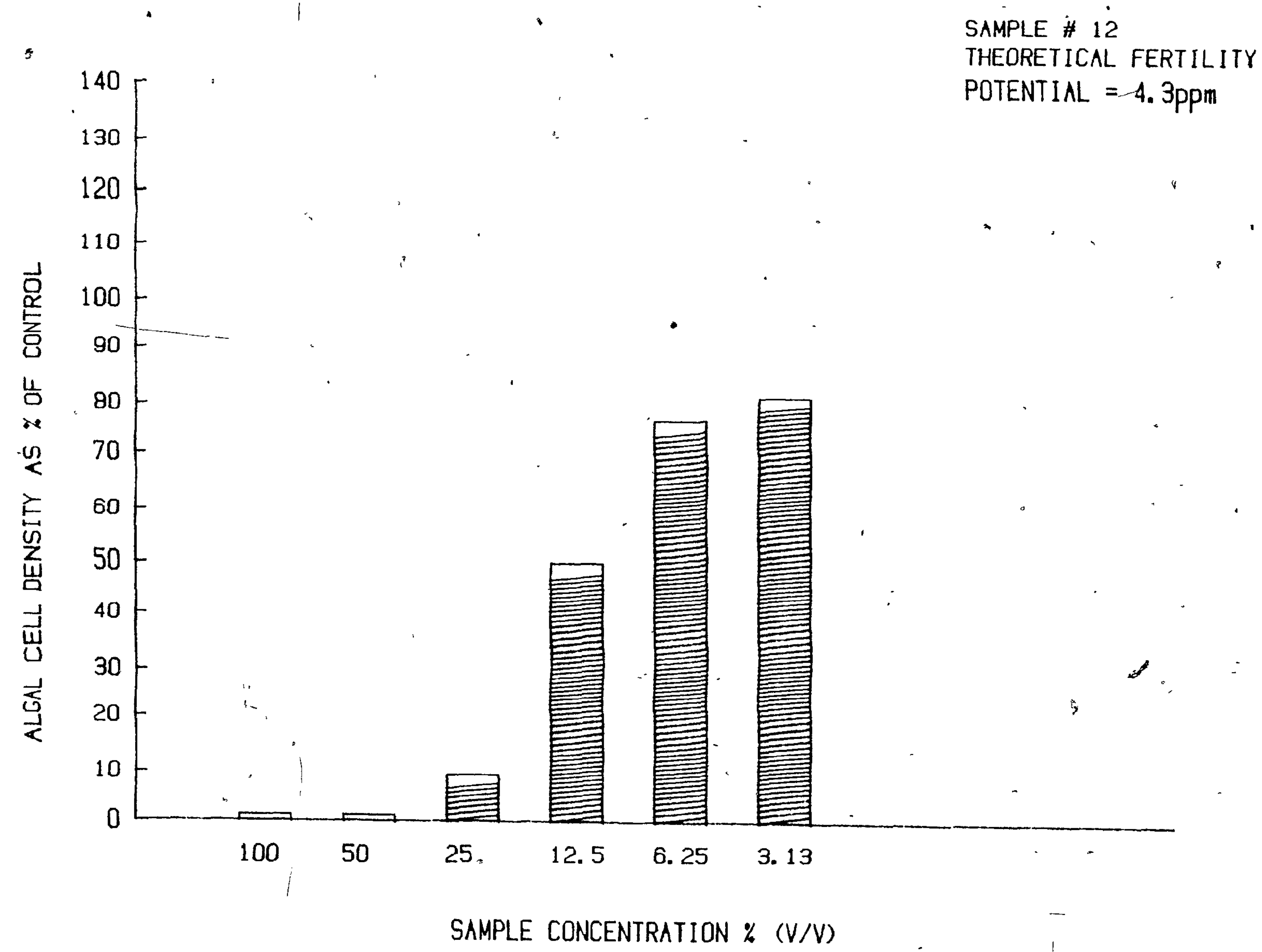


FIG. C.6 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. oapricoronutum*

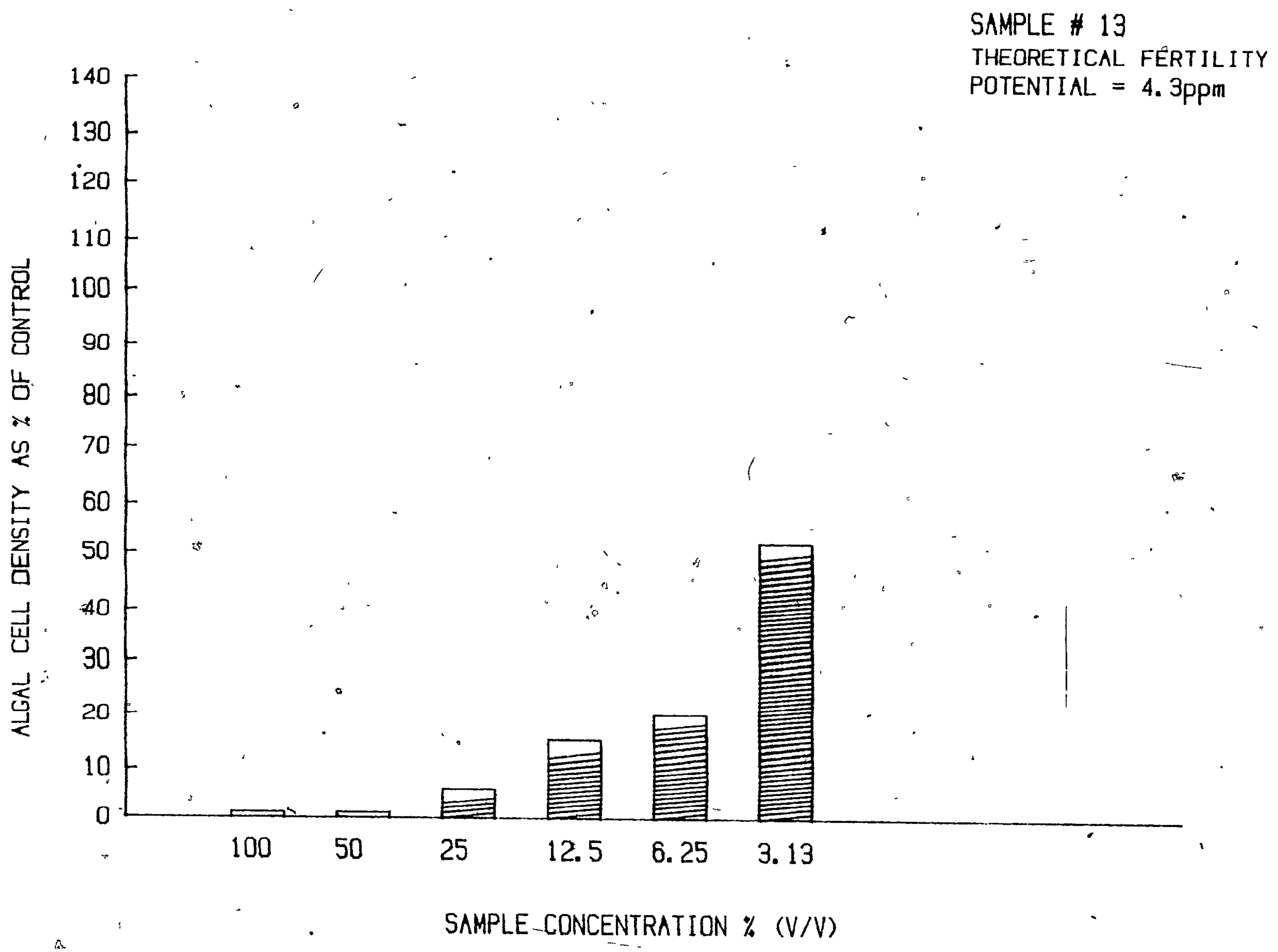


FIG. C.7 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

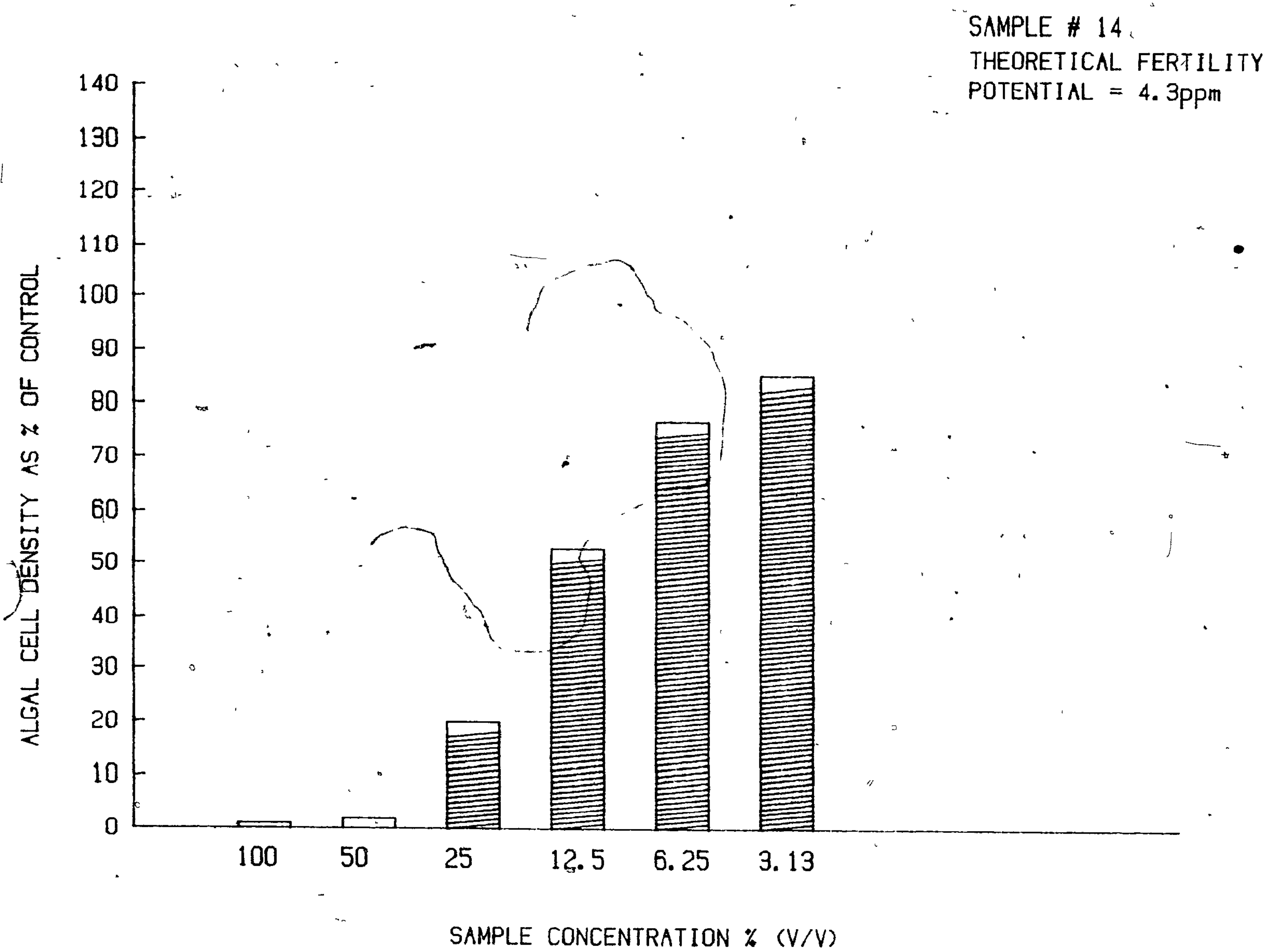


FIG. C.8 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

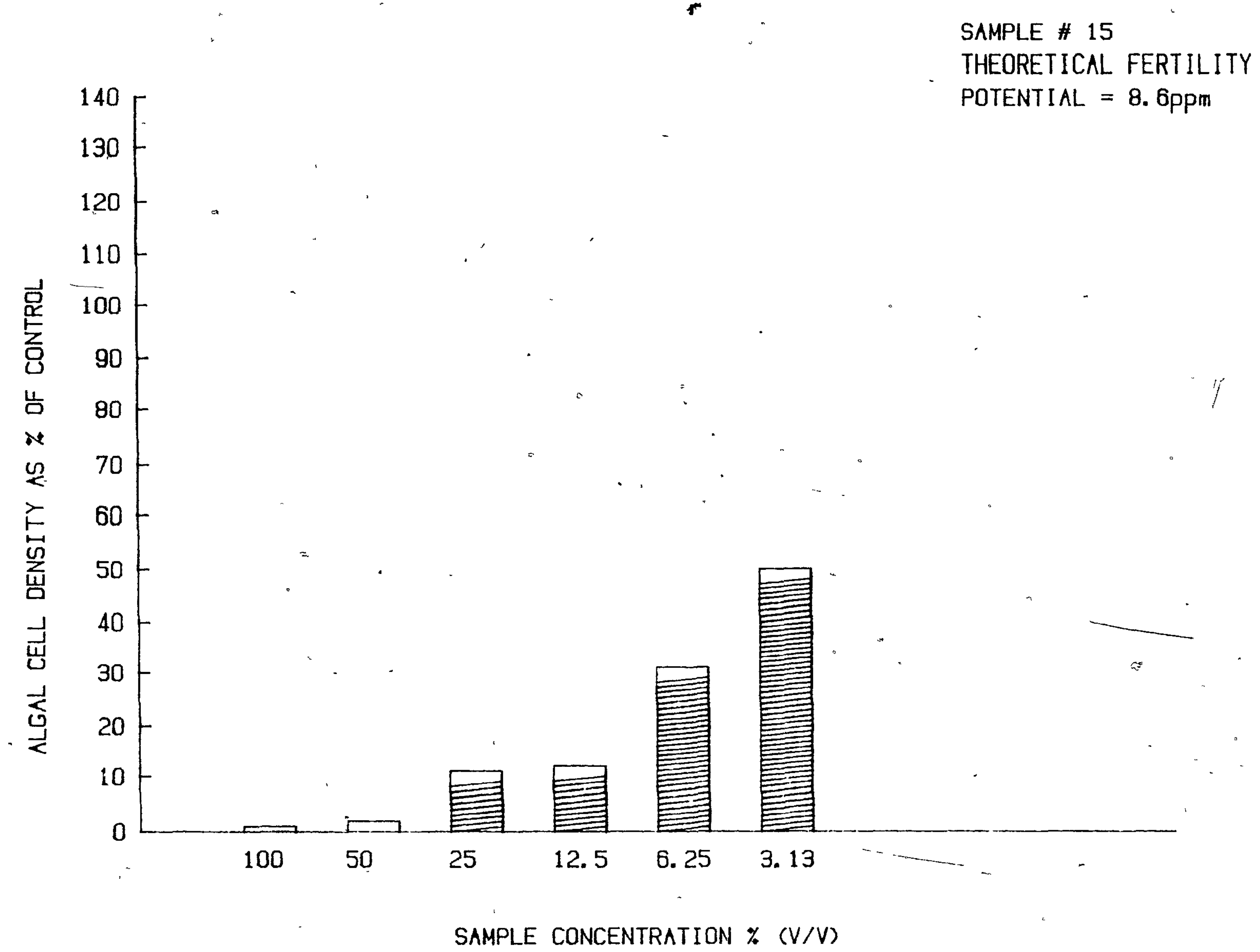


FIG. C.9 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

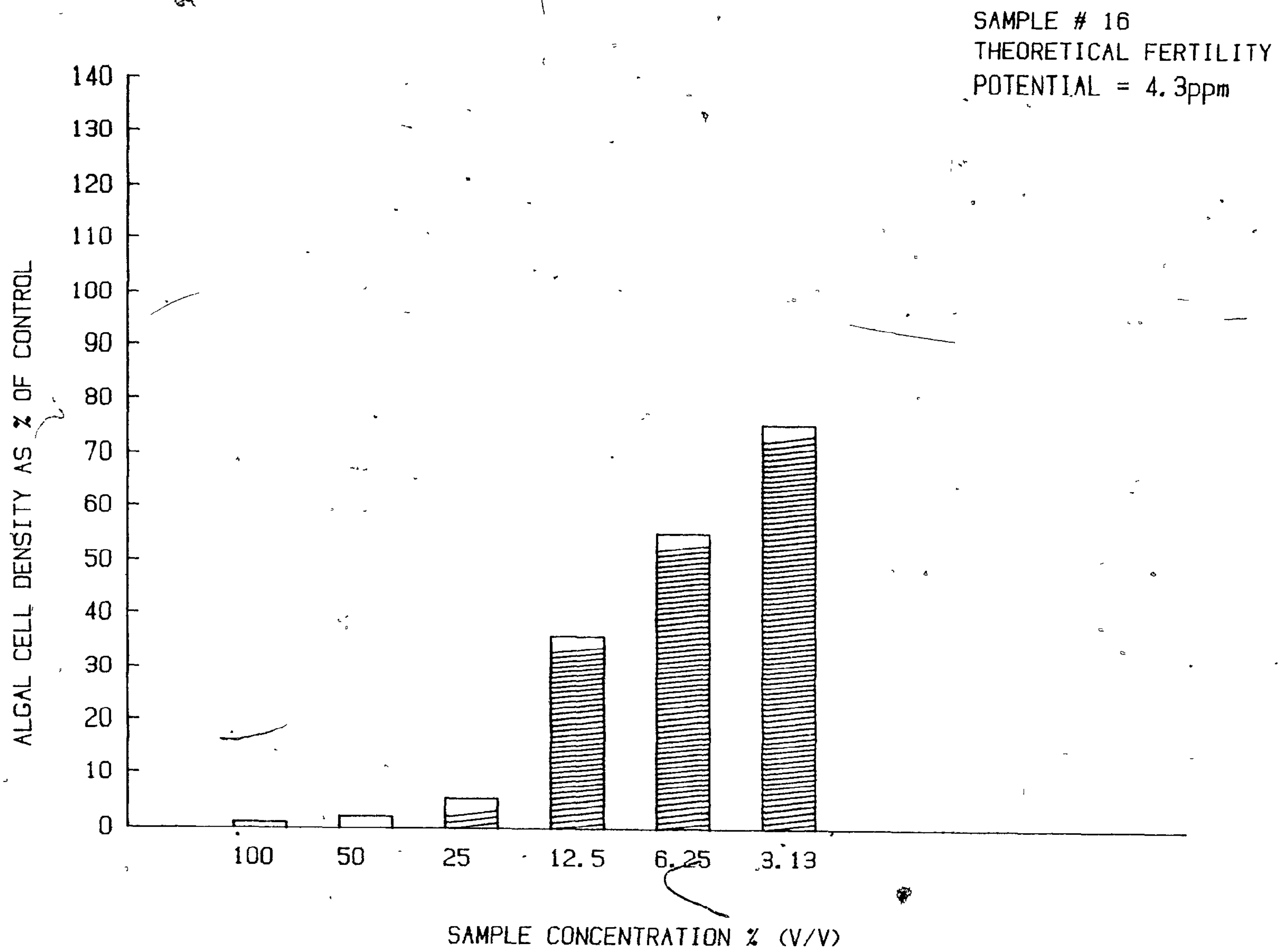


FIG. C.10 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

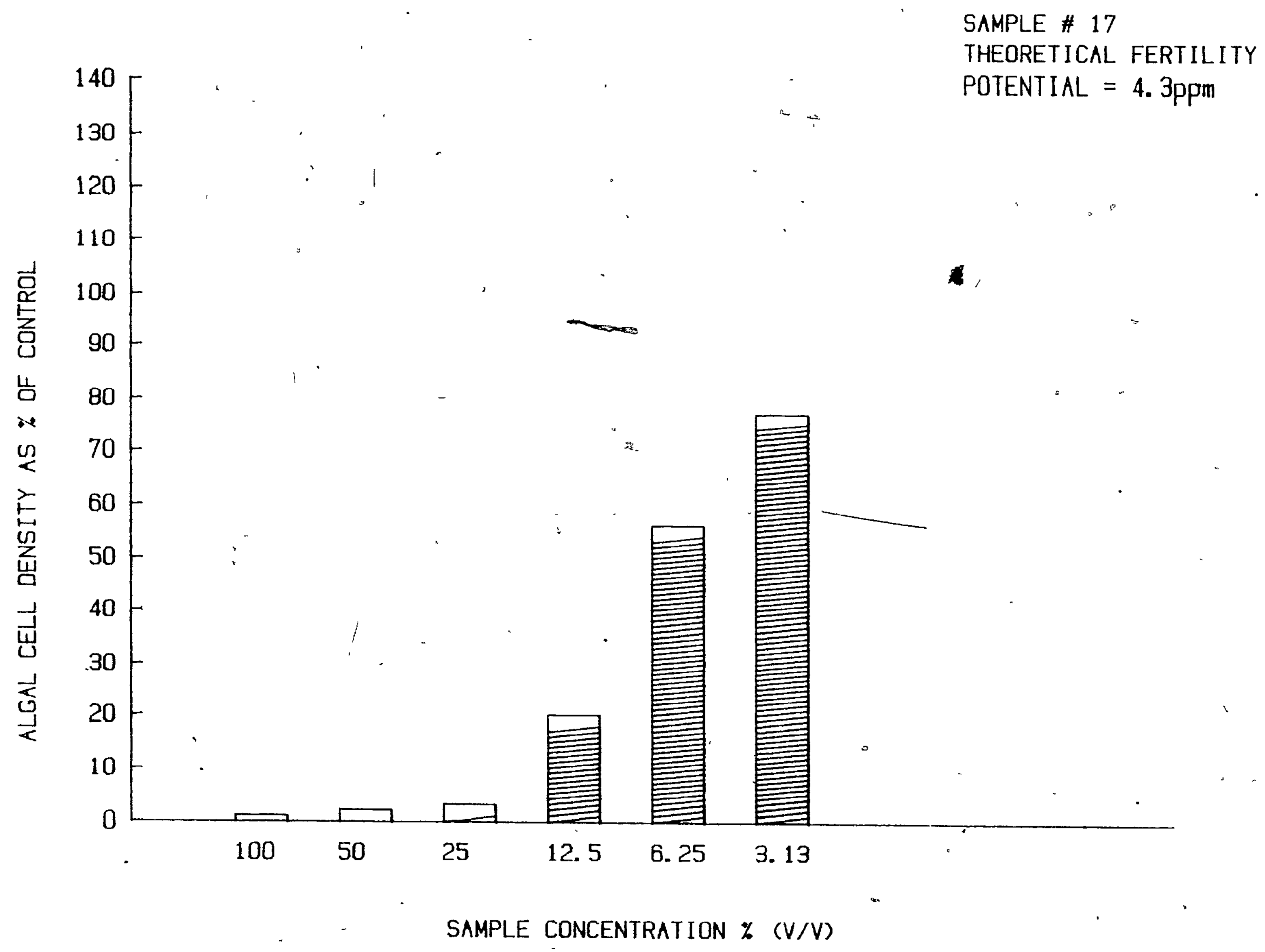


FIG. C.11 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

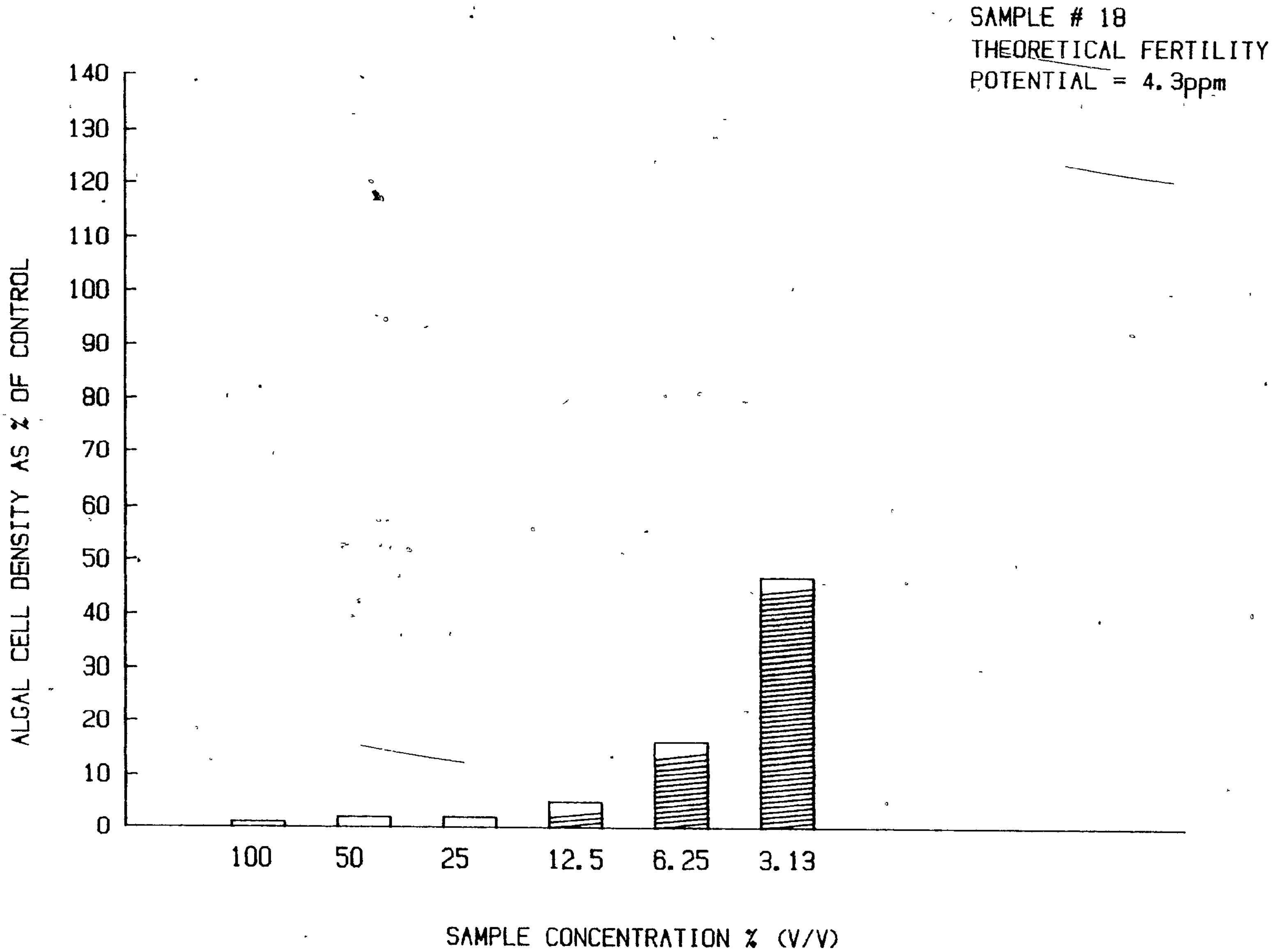


FIG. C.12 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

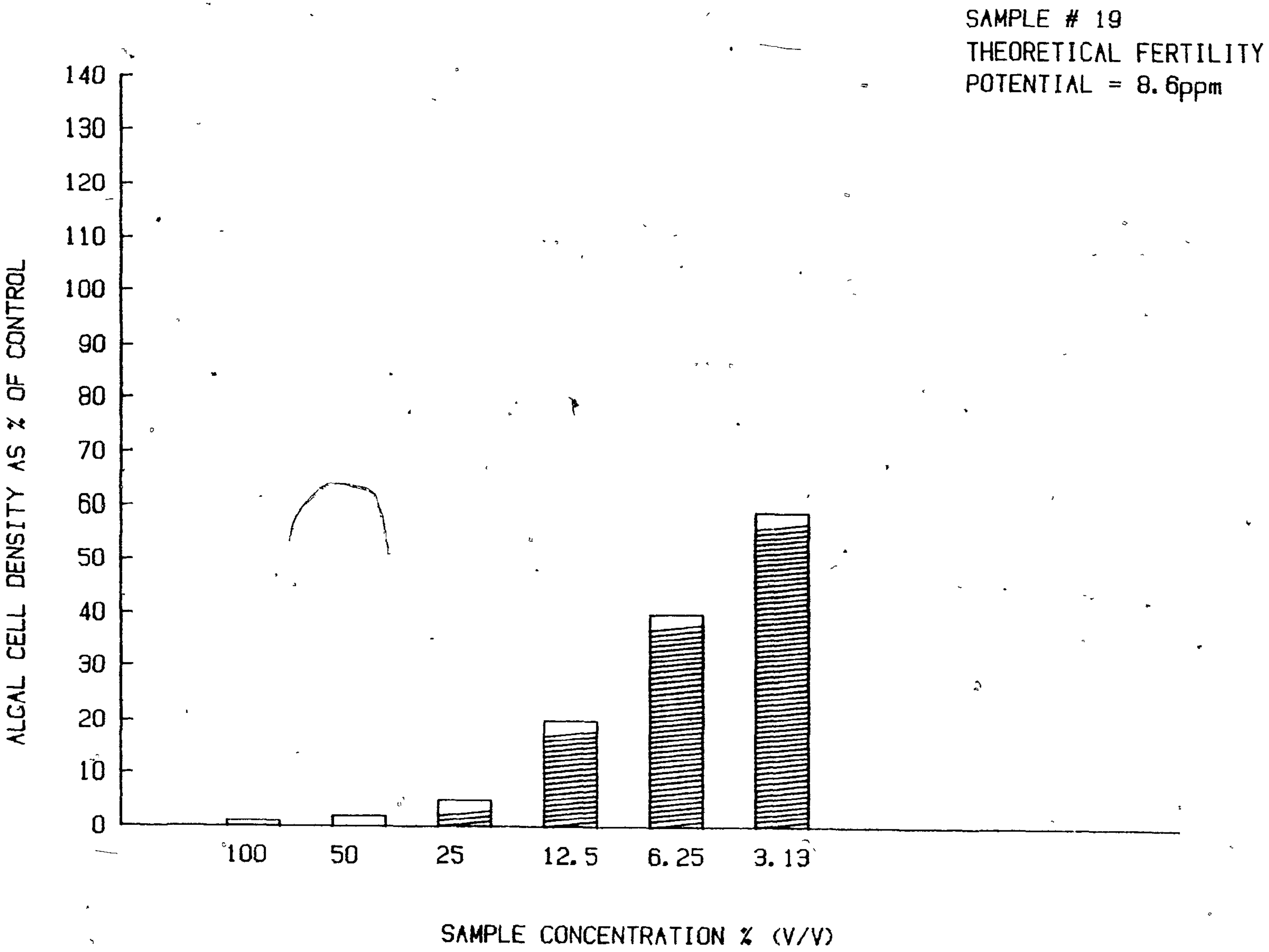


FIG. C.13 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

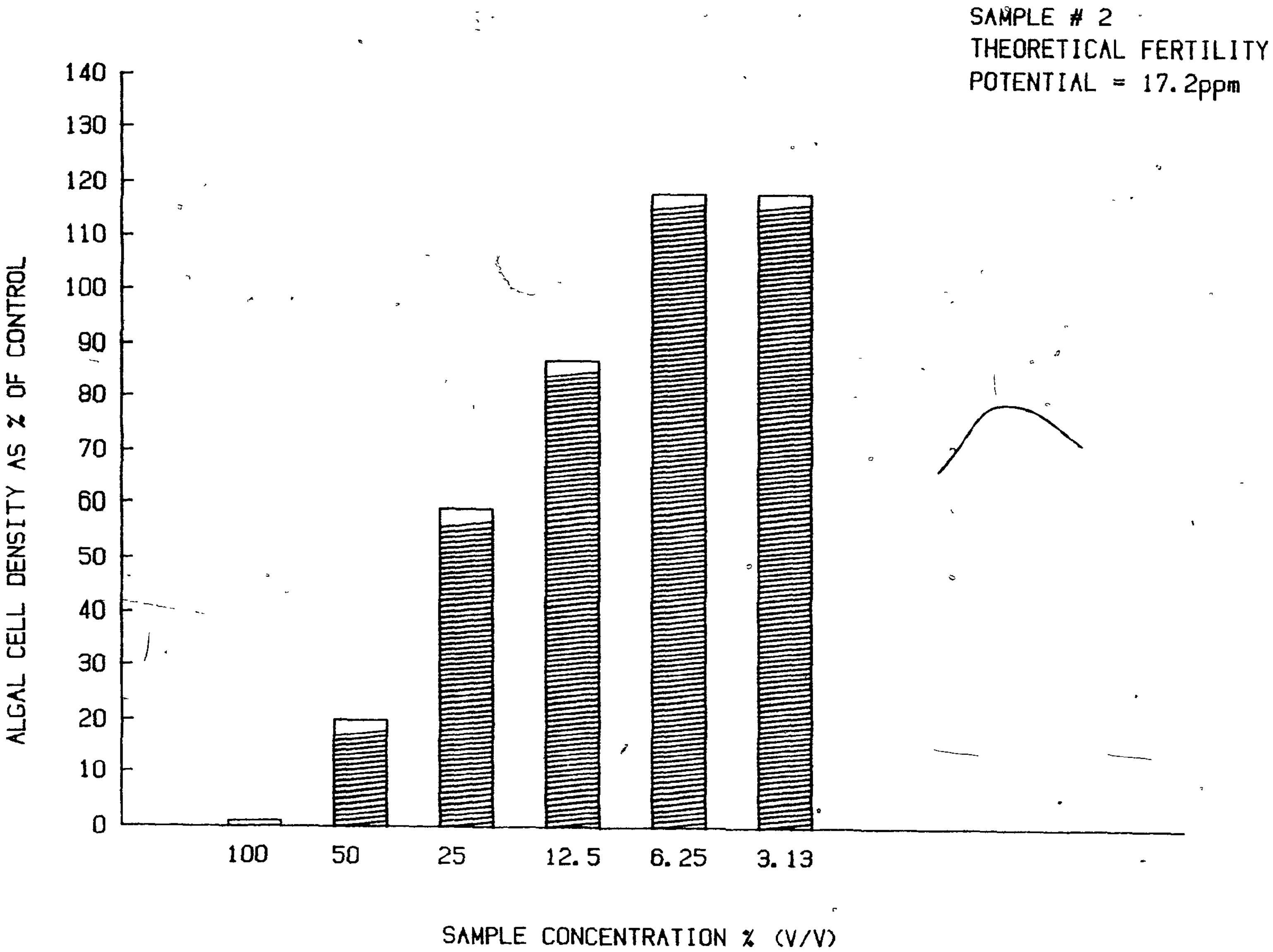


FIG. C.14 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

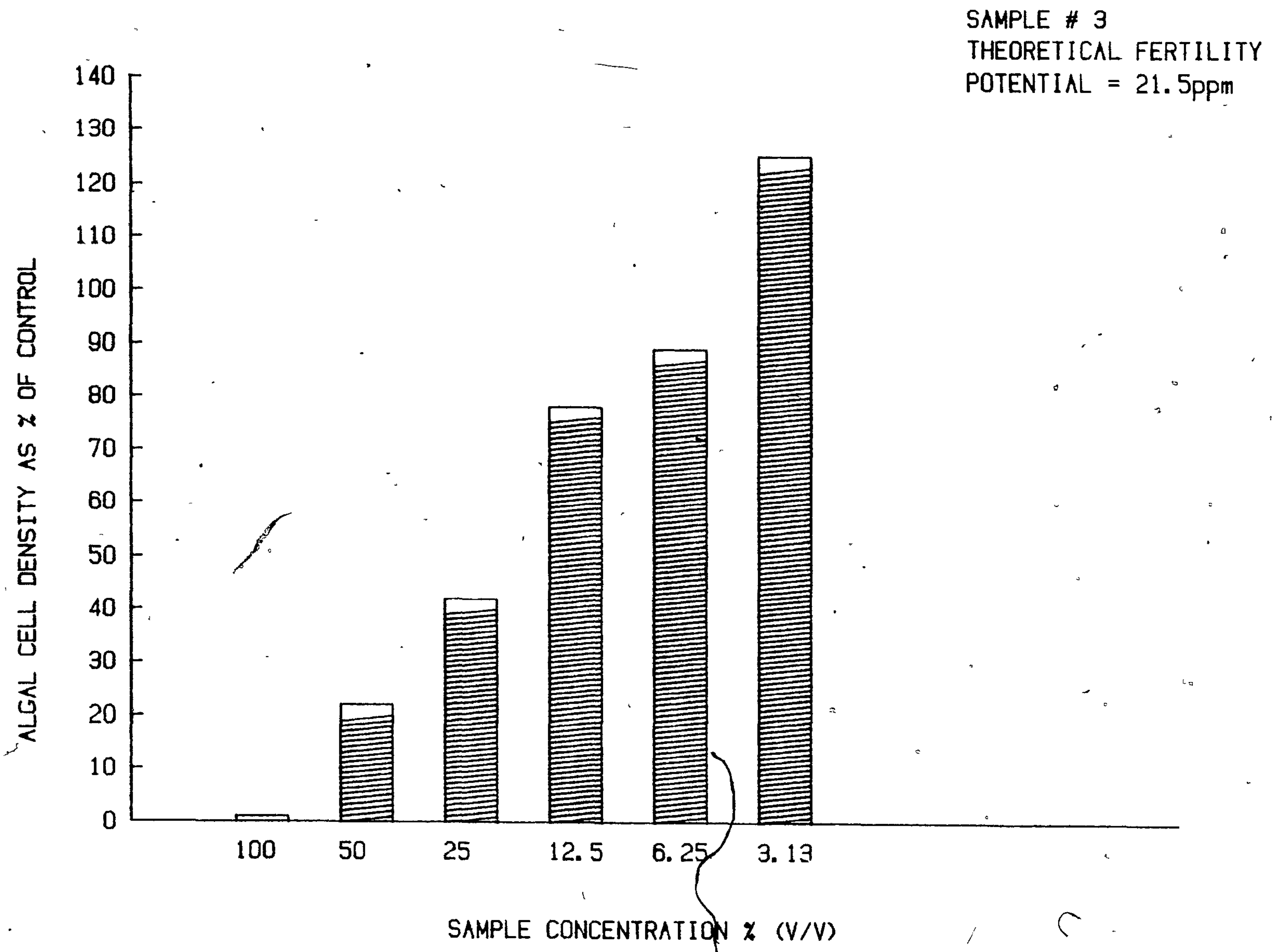


FIG. C.15 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

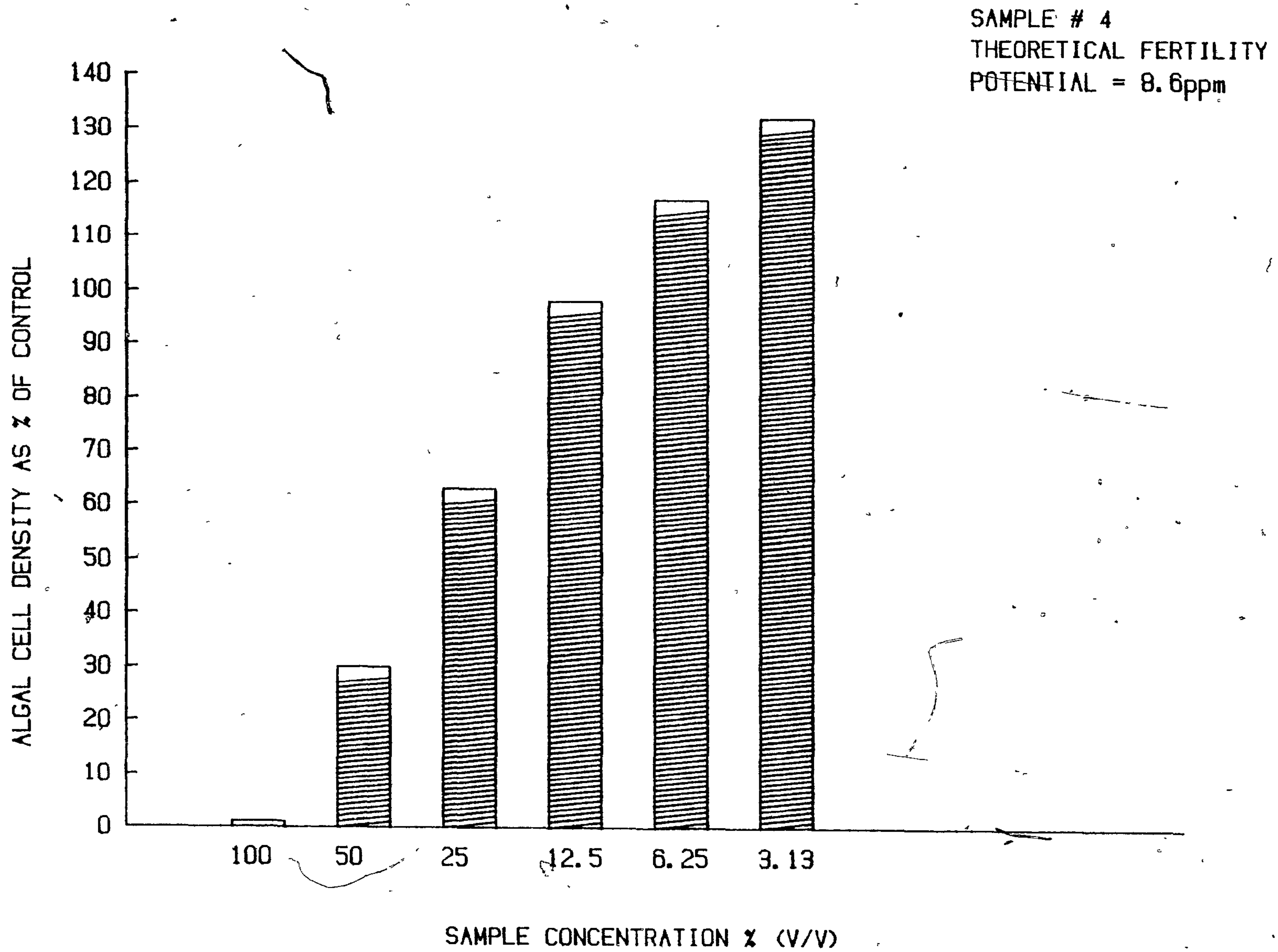


FIG. C.16 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

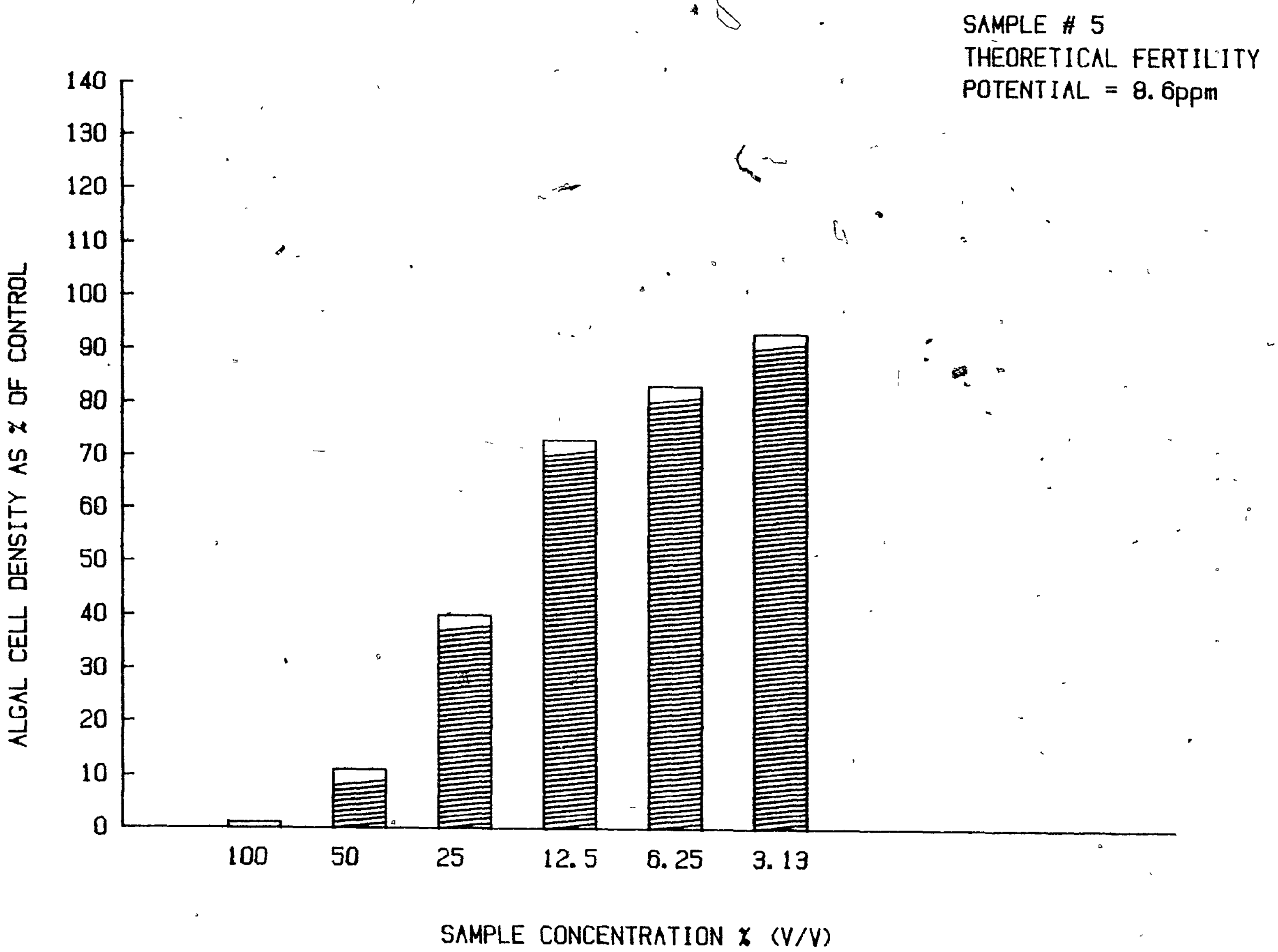


FIG. C.17 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. *capricoronutum*

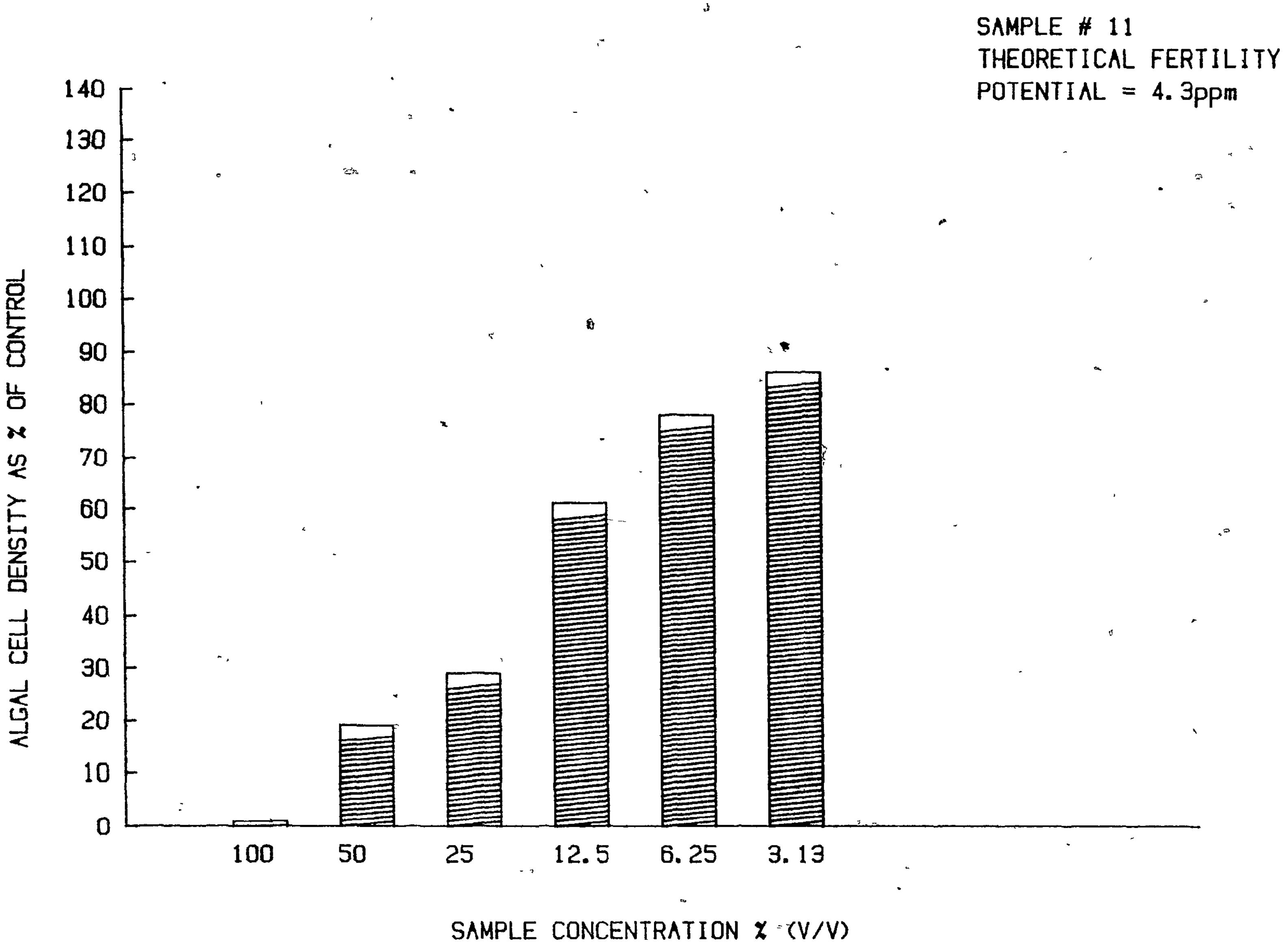


FIG. C.18 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. *capricoronutum*

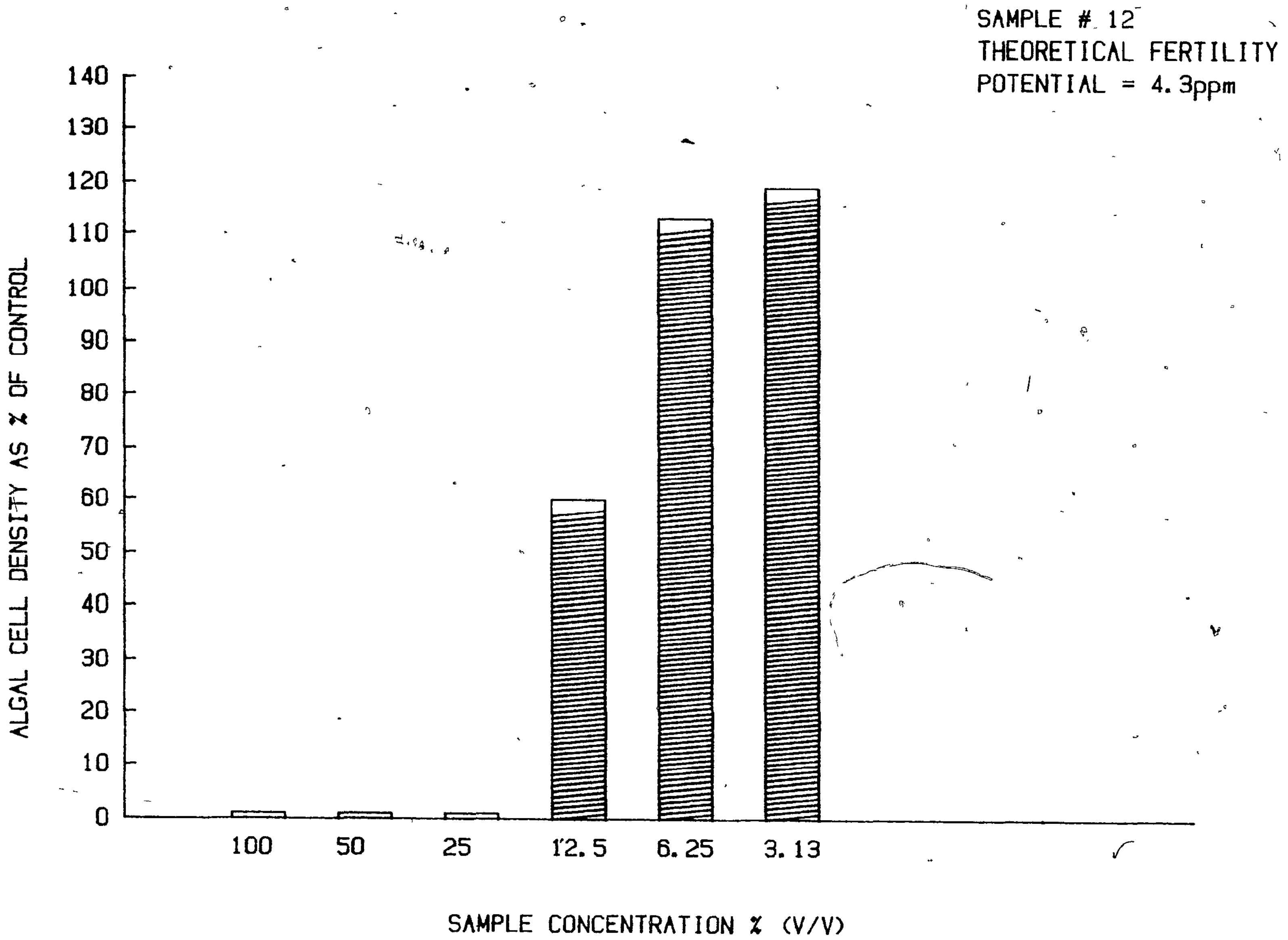


FIG. C.19 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

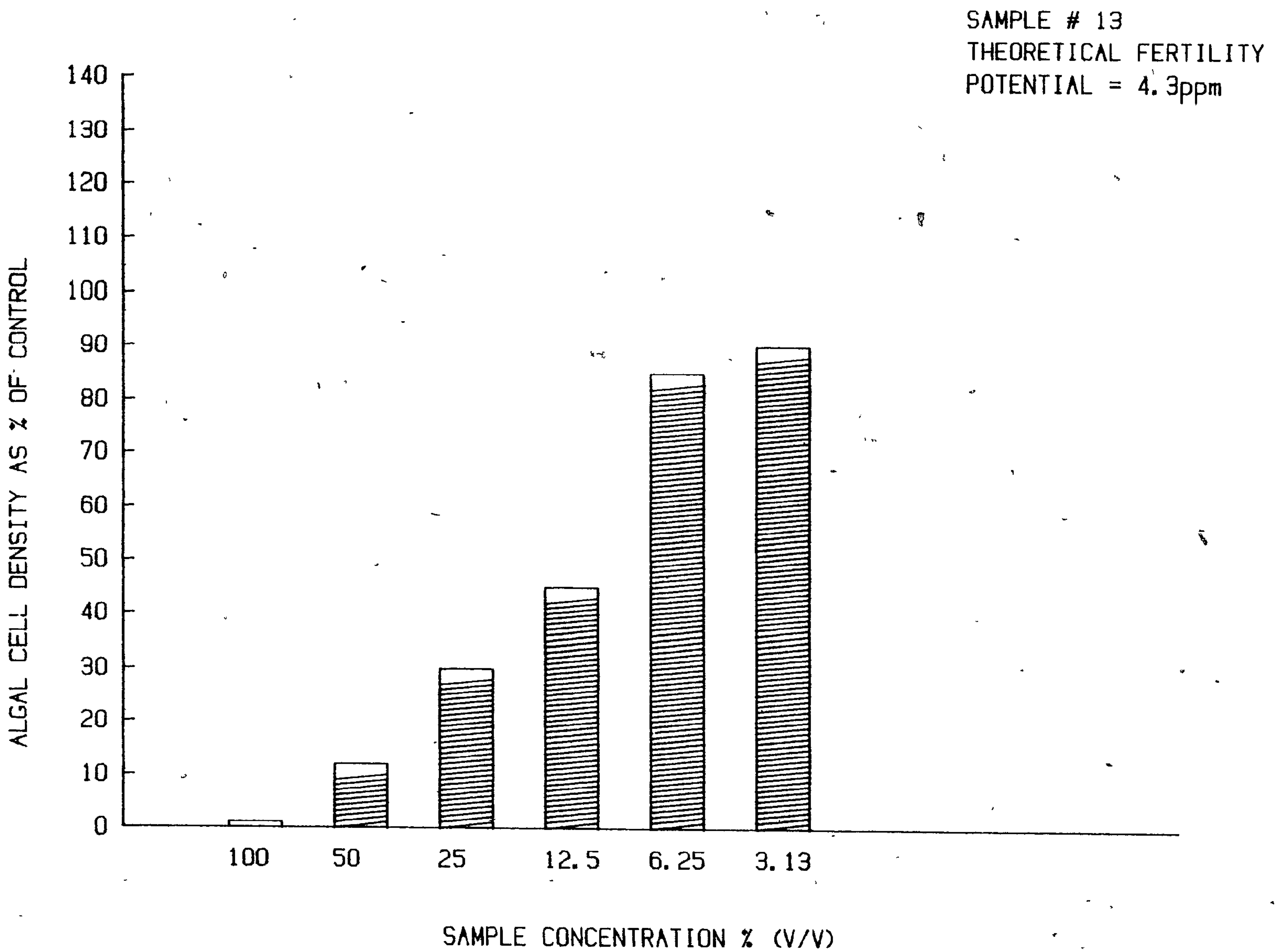


FIG. C.20 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

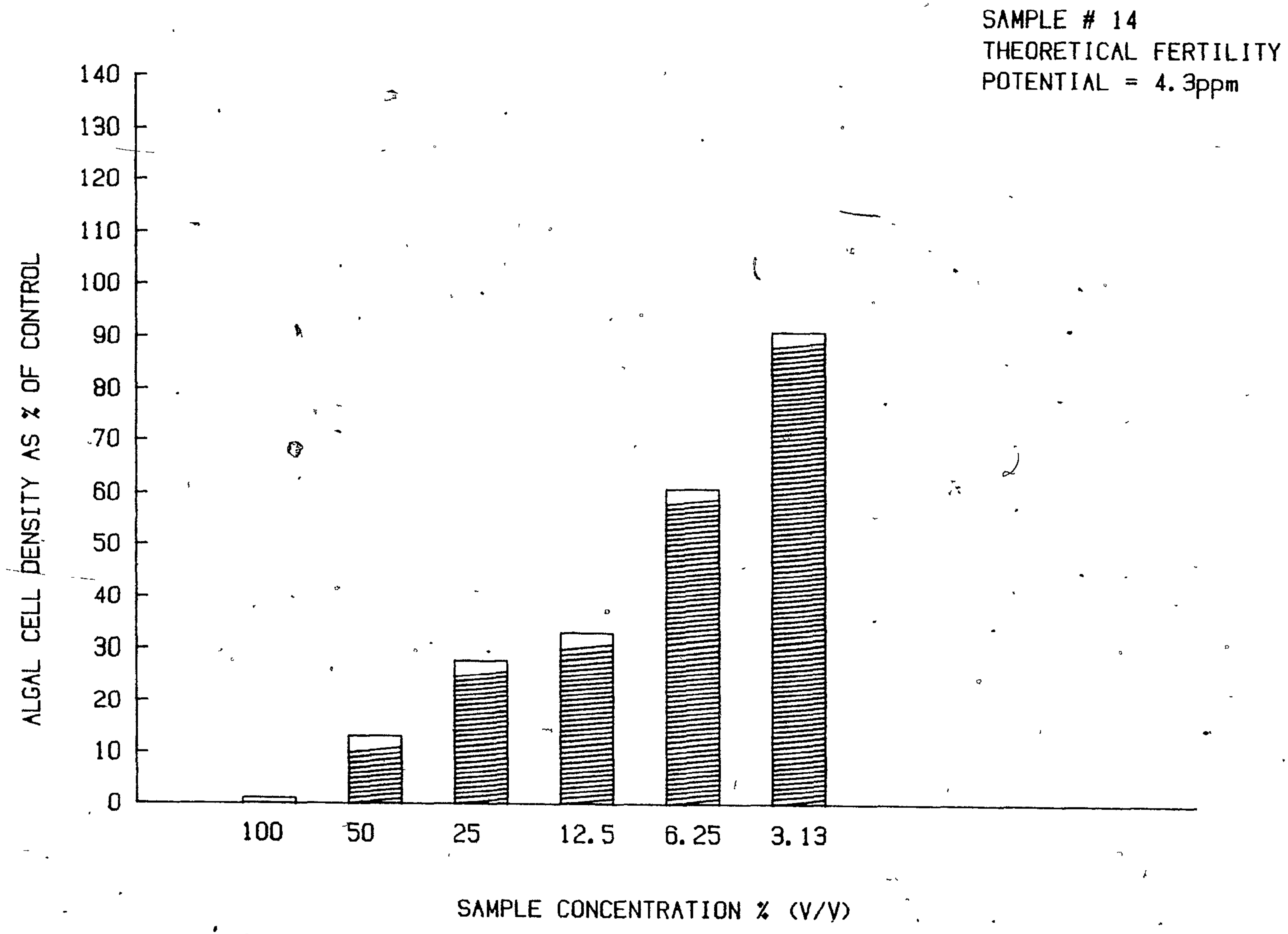


FIG. C.21 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

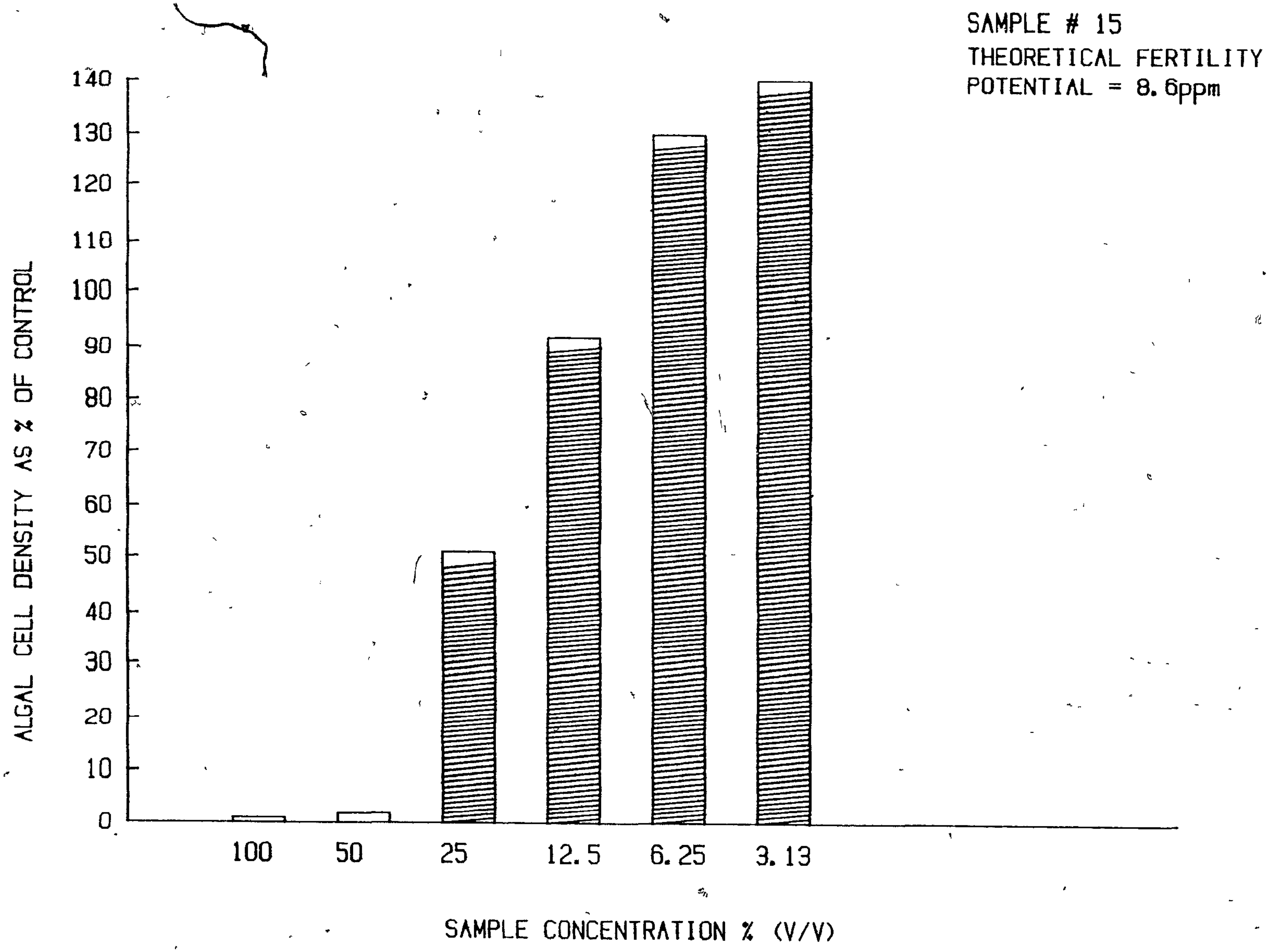


FIG. C.22 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

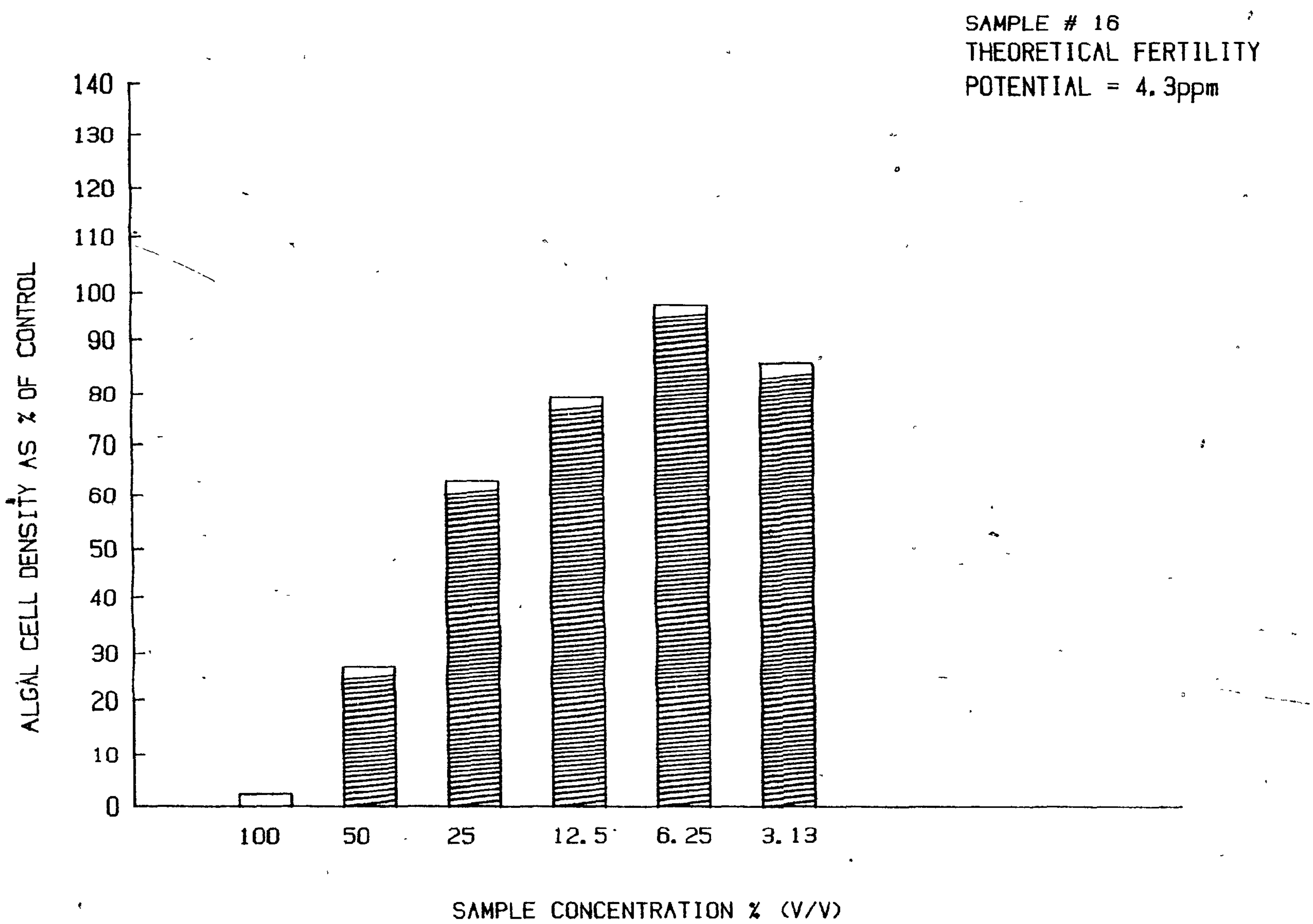


FIG. C.23 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF *S. capricoronutum*

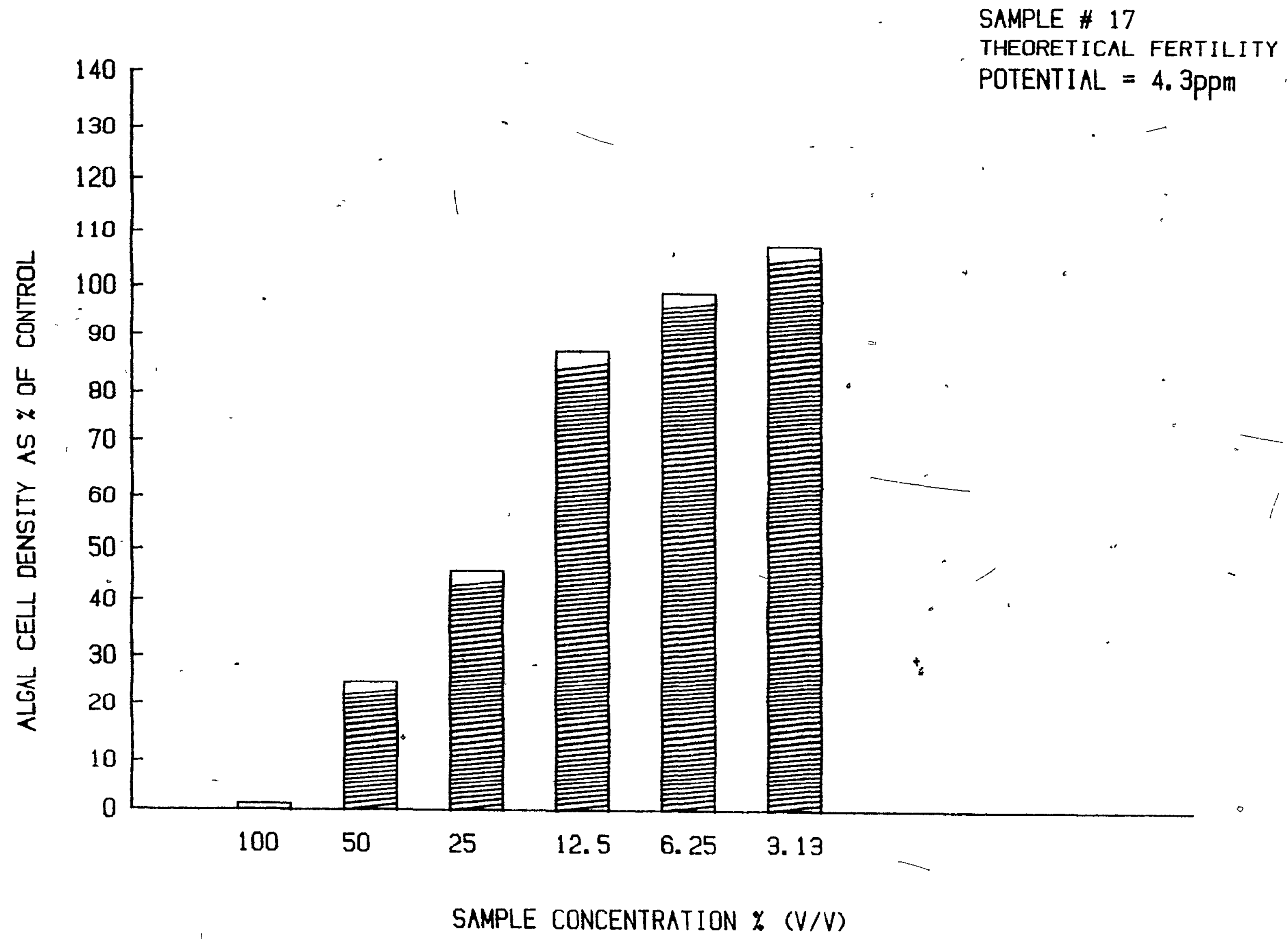


FIG. C.24 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

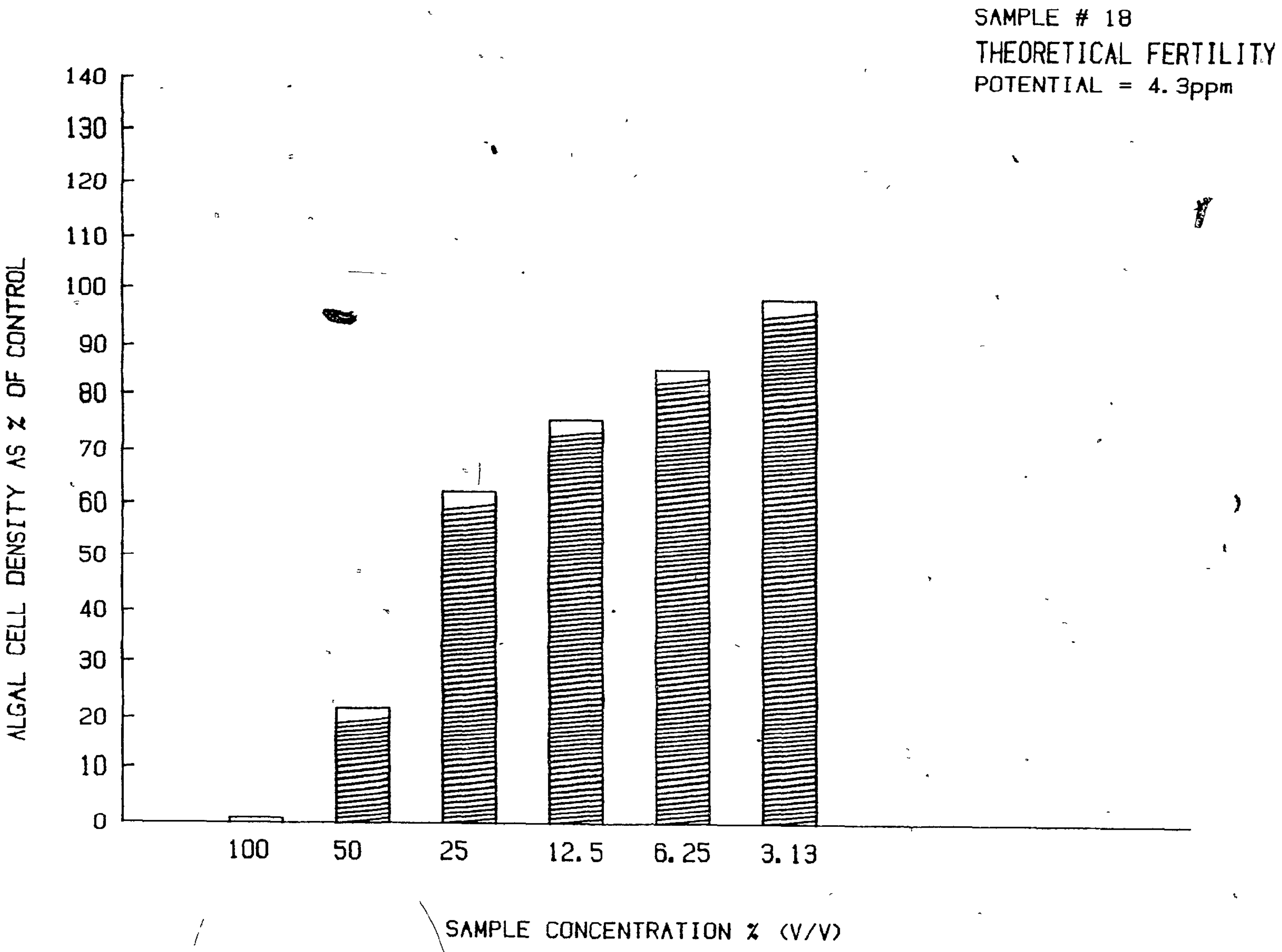


FIG. C.25 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. oapricoronutum

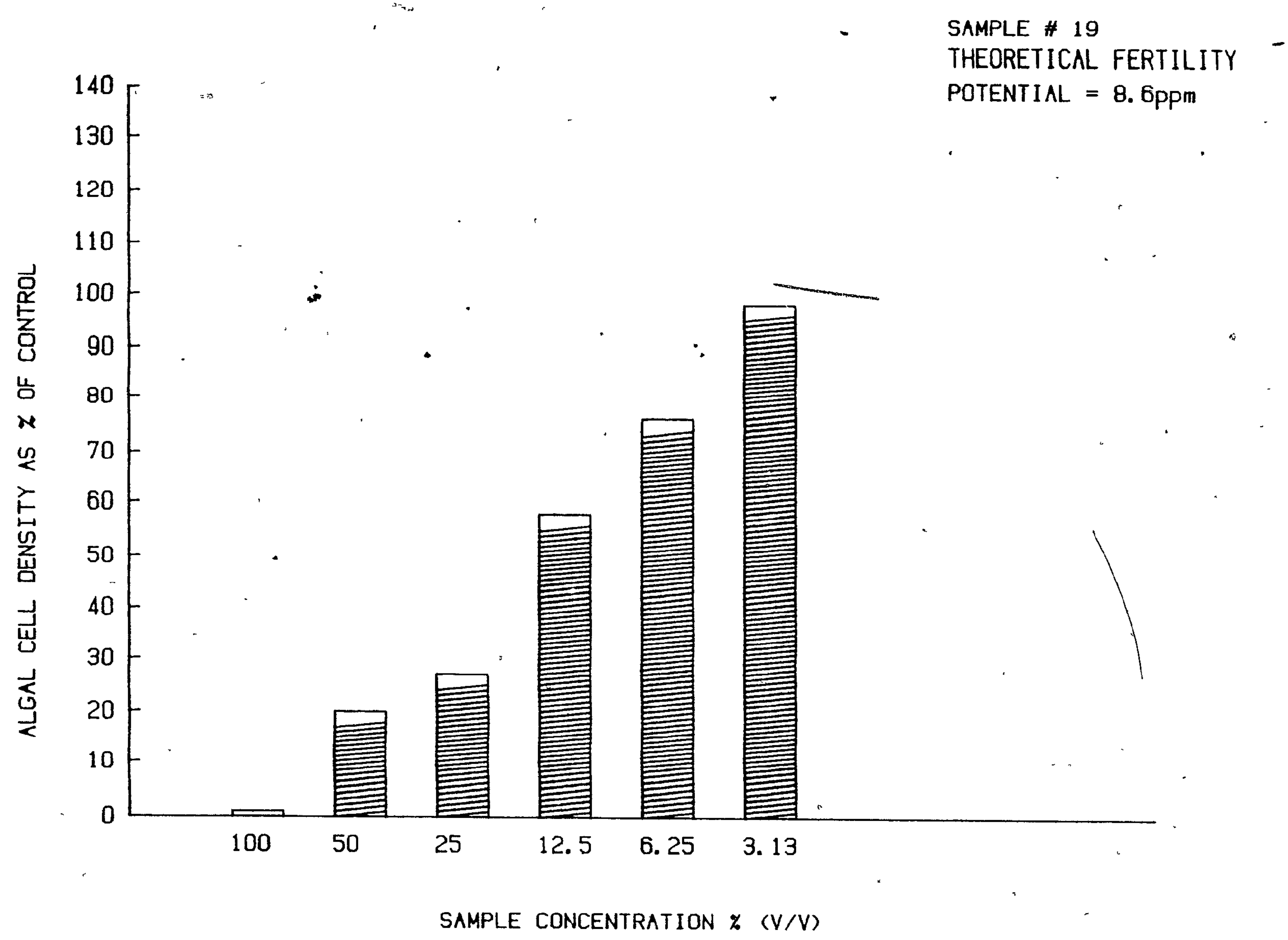


FIG. C.26 THE EFFECT OF SAMPLE CONCENTRATION ON THE GROWTH OF S. capricoronutum

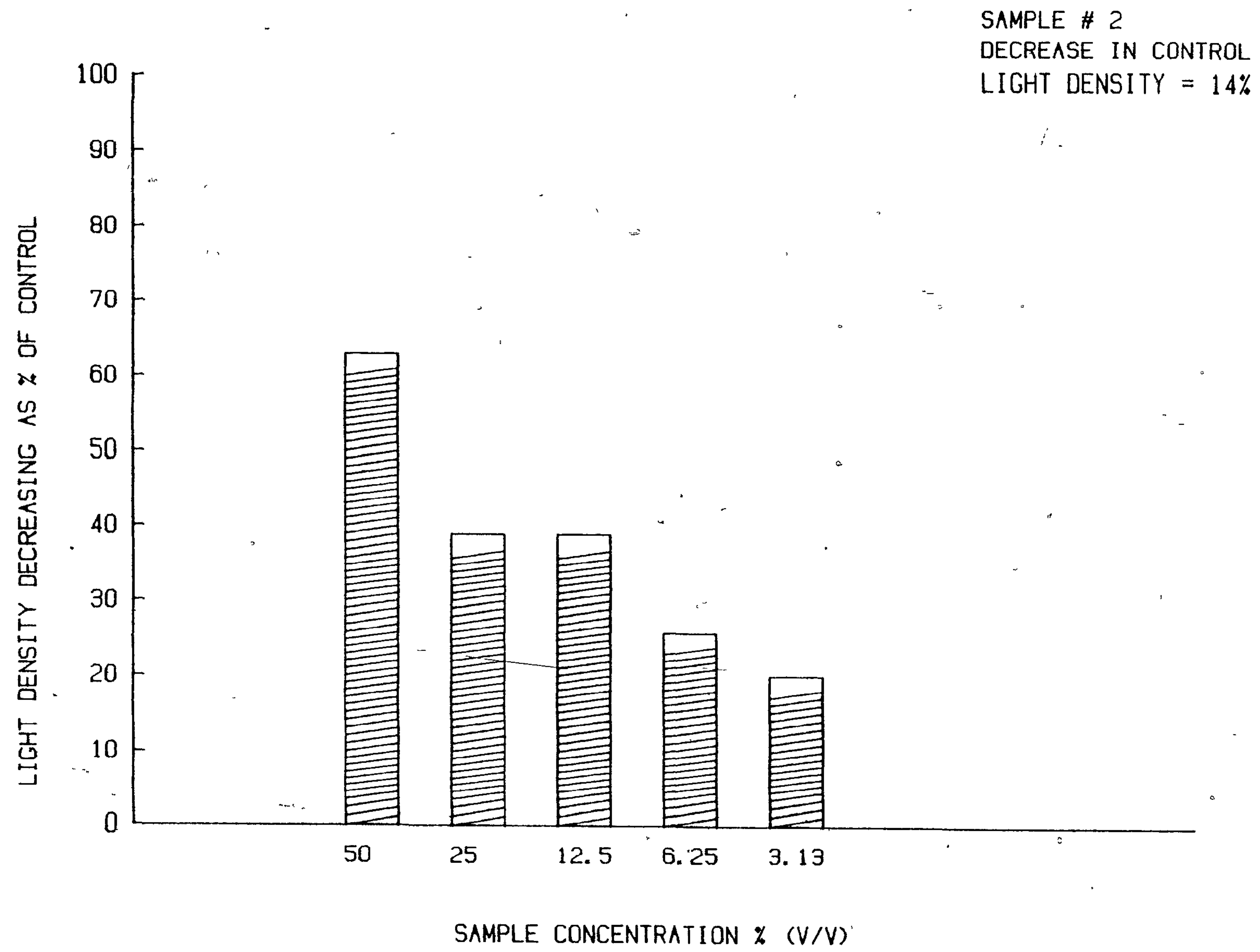


FIG. C.27 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

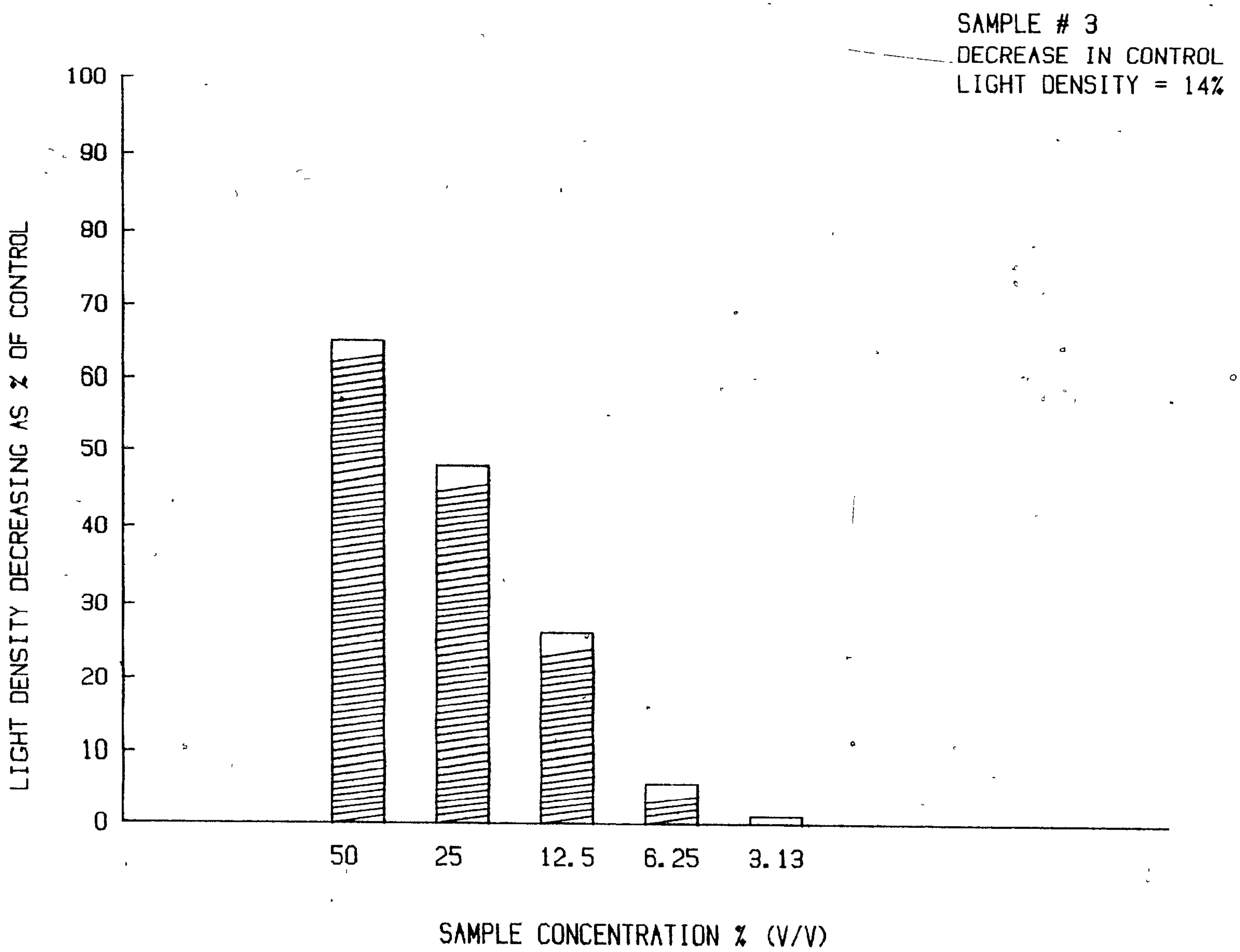


FIG. C.28 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

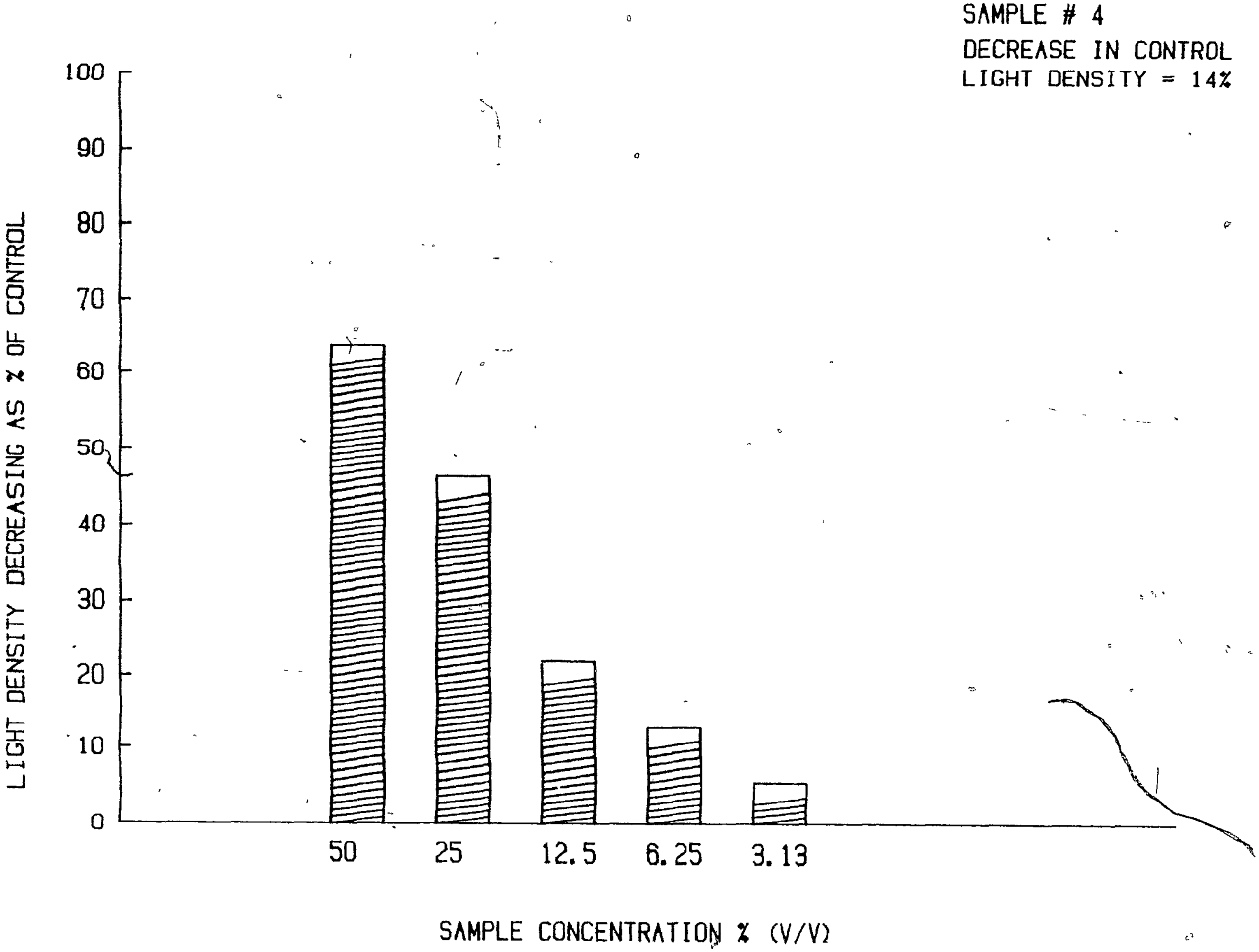


FIG. C.29 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

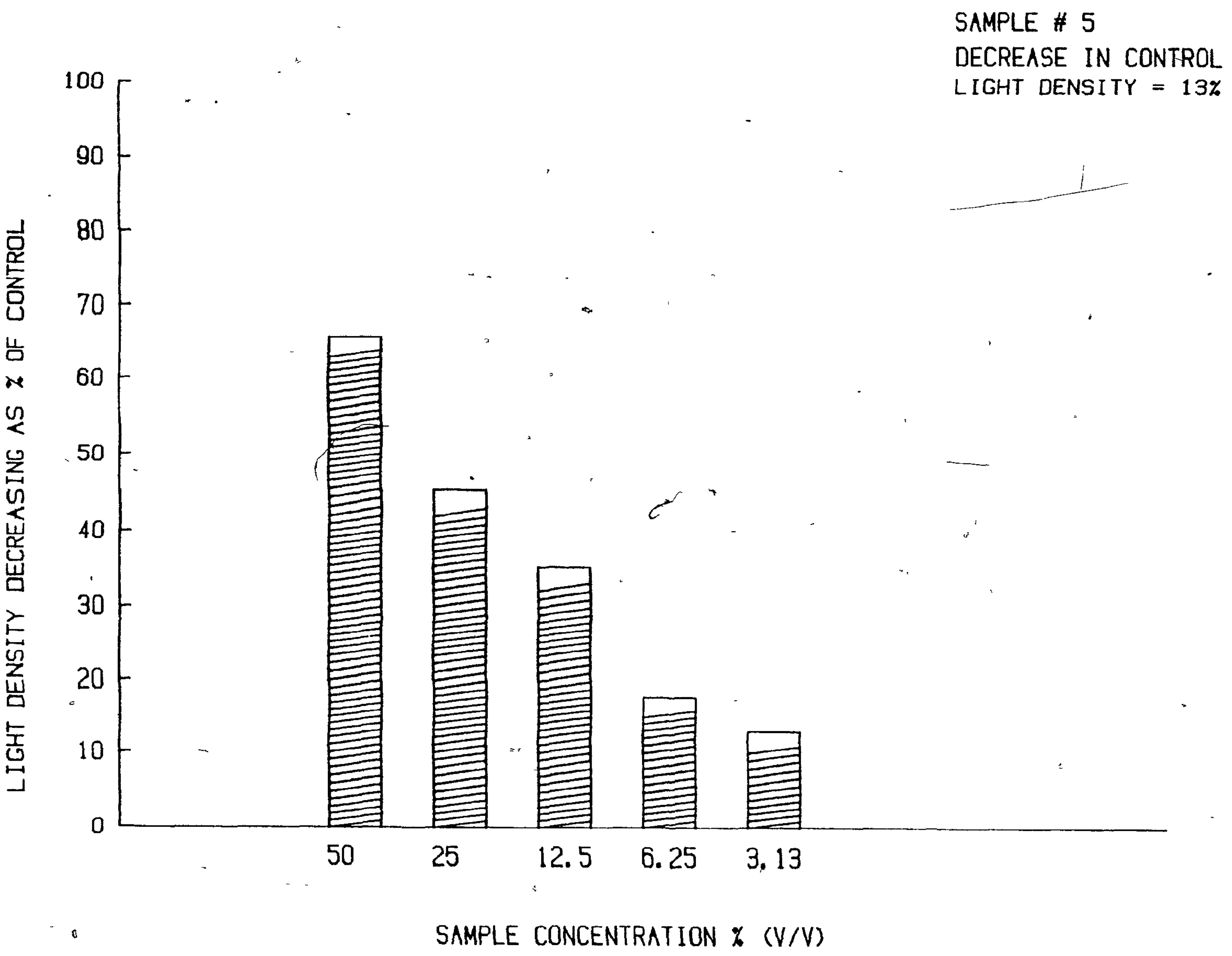


FIG. C.30 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

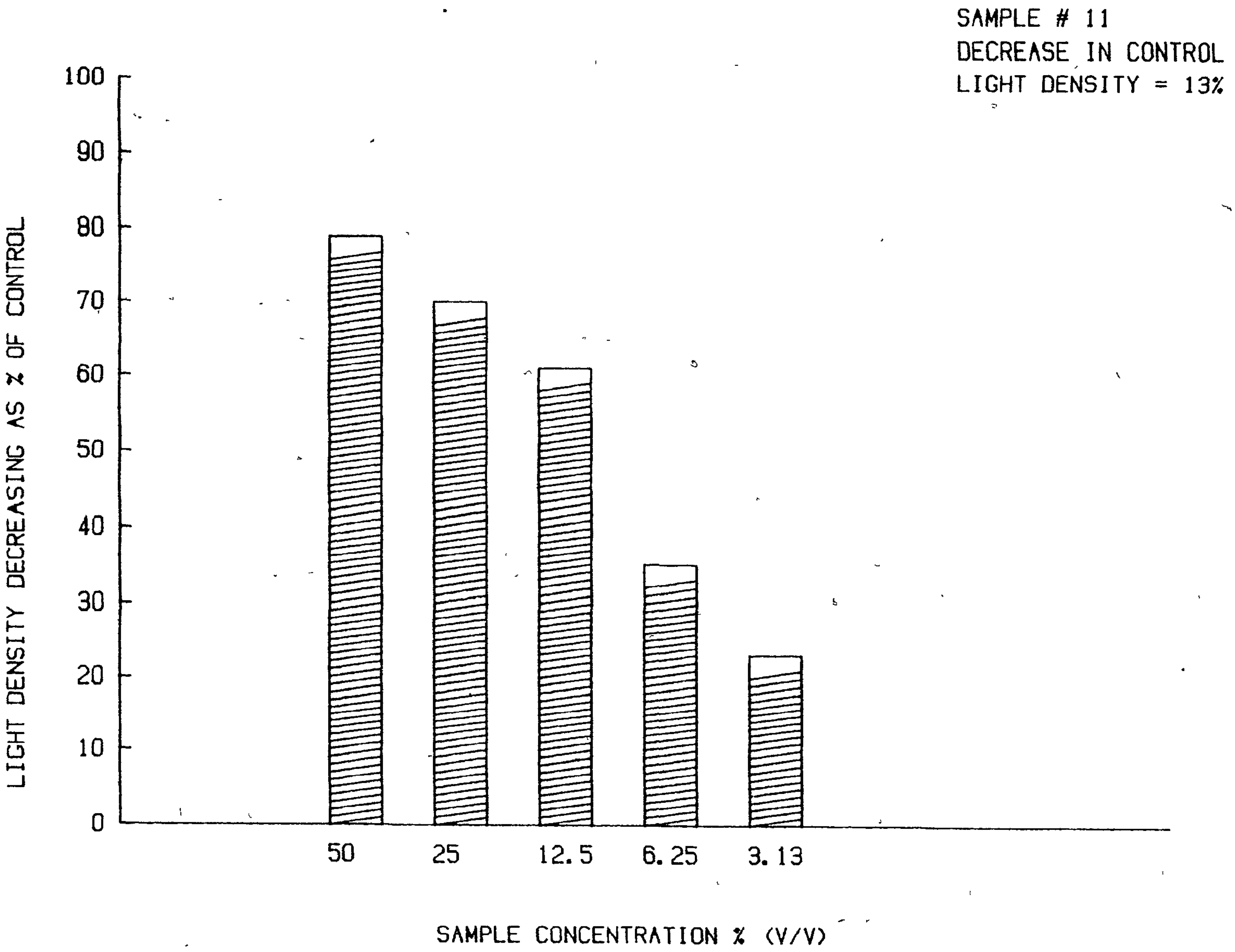


FIG. C.31 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

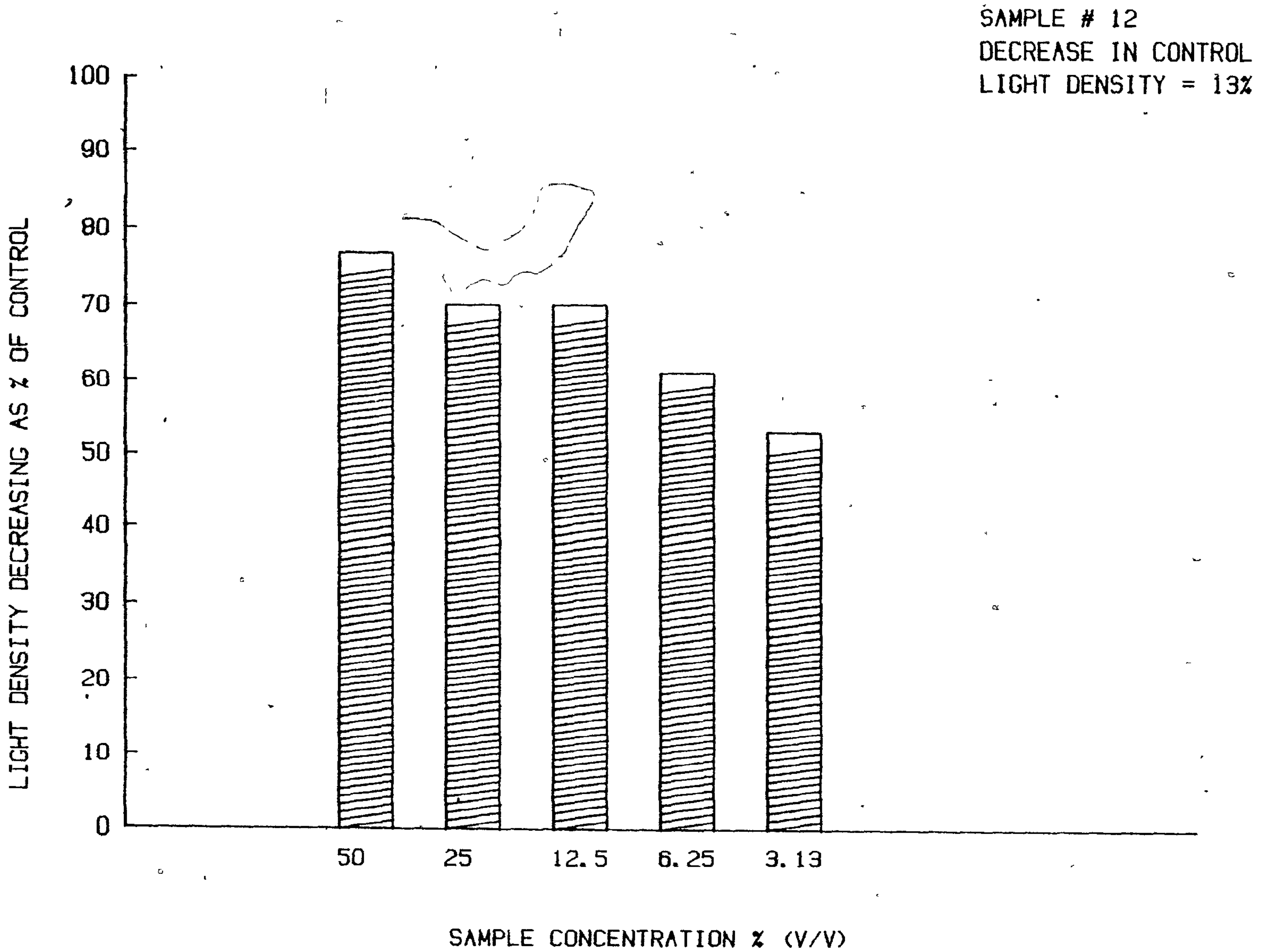


FIG. C.32 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

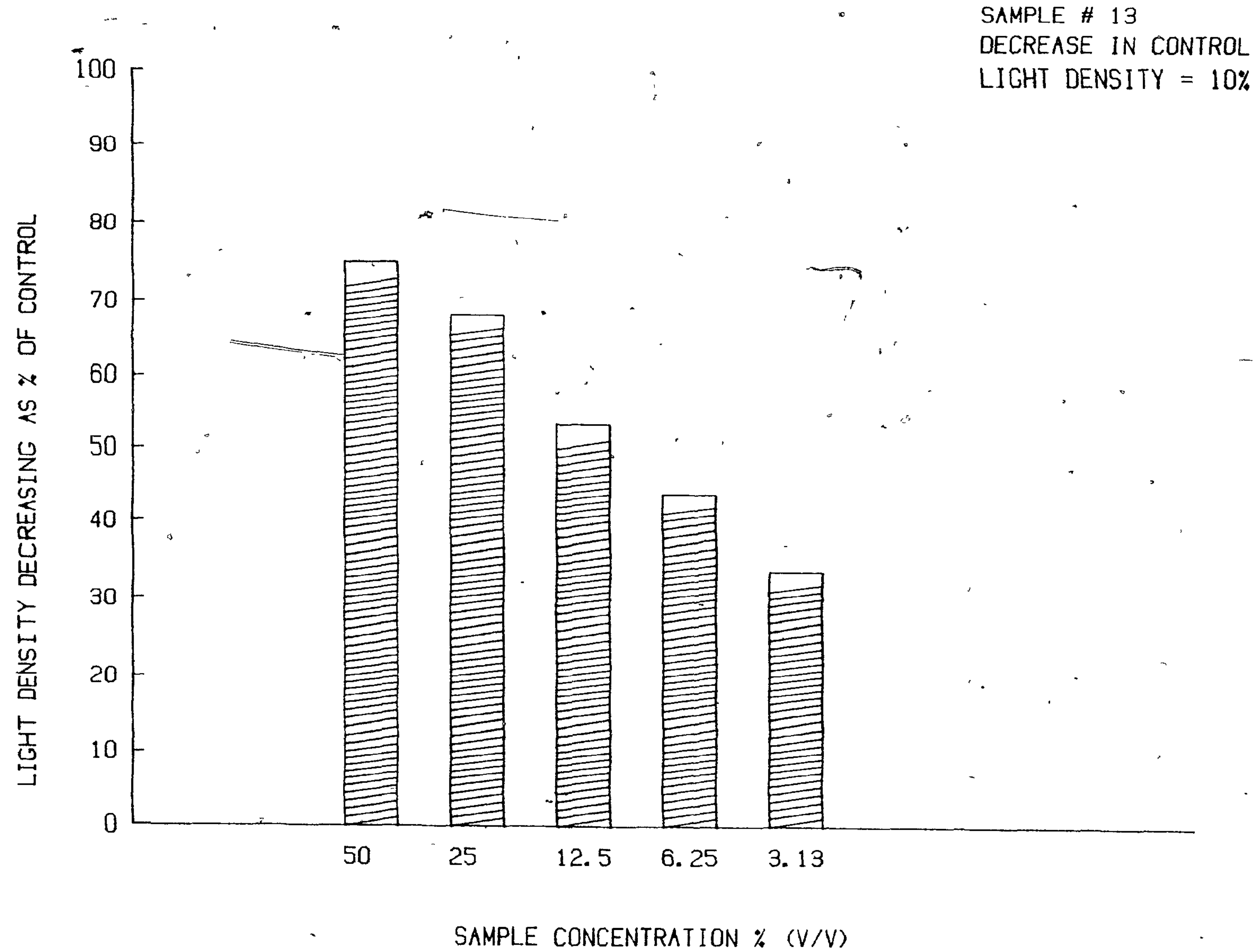


FIG. C.33 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

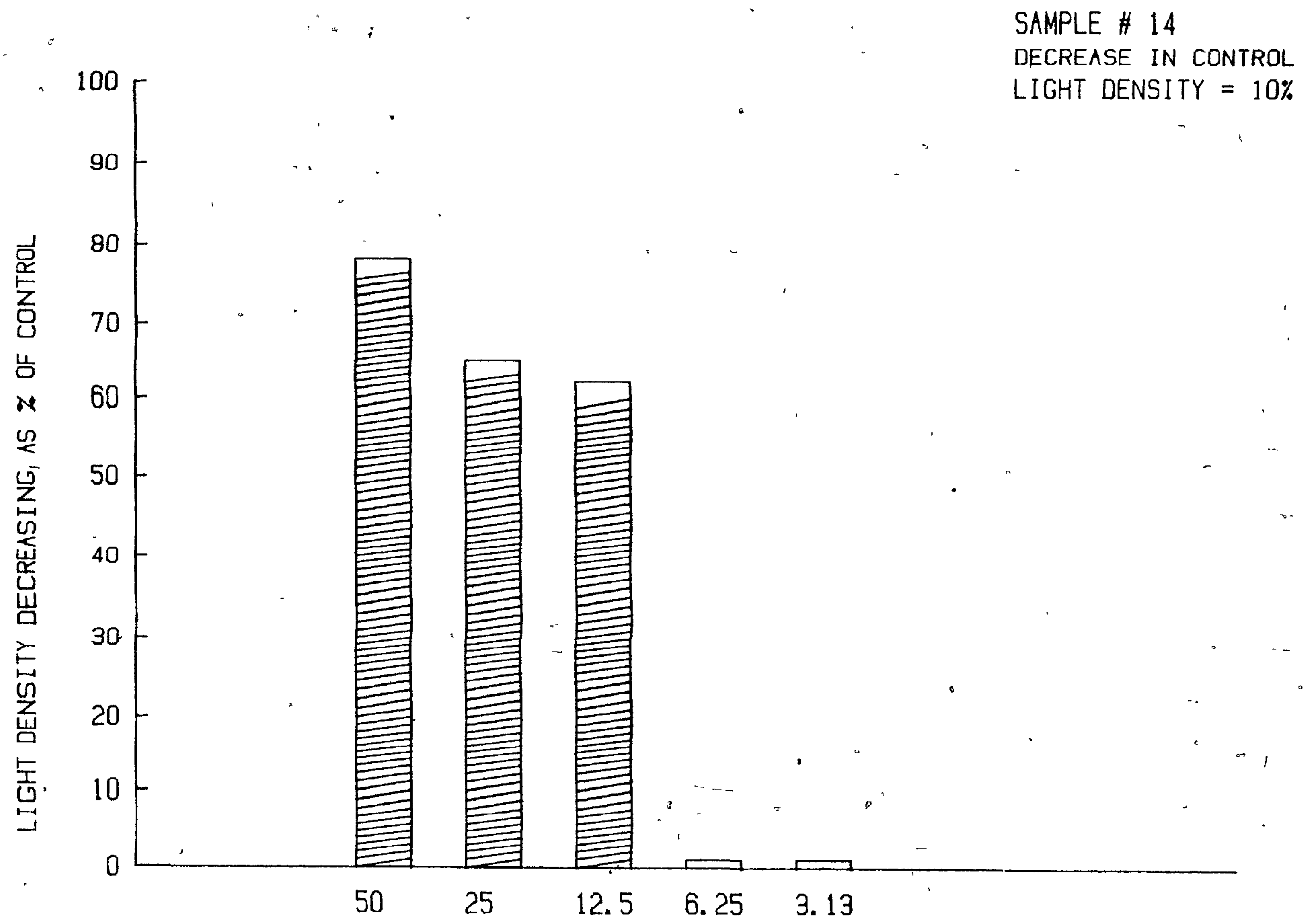


FIG. C.34 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

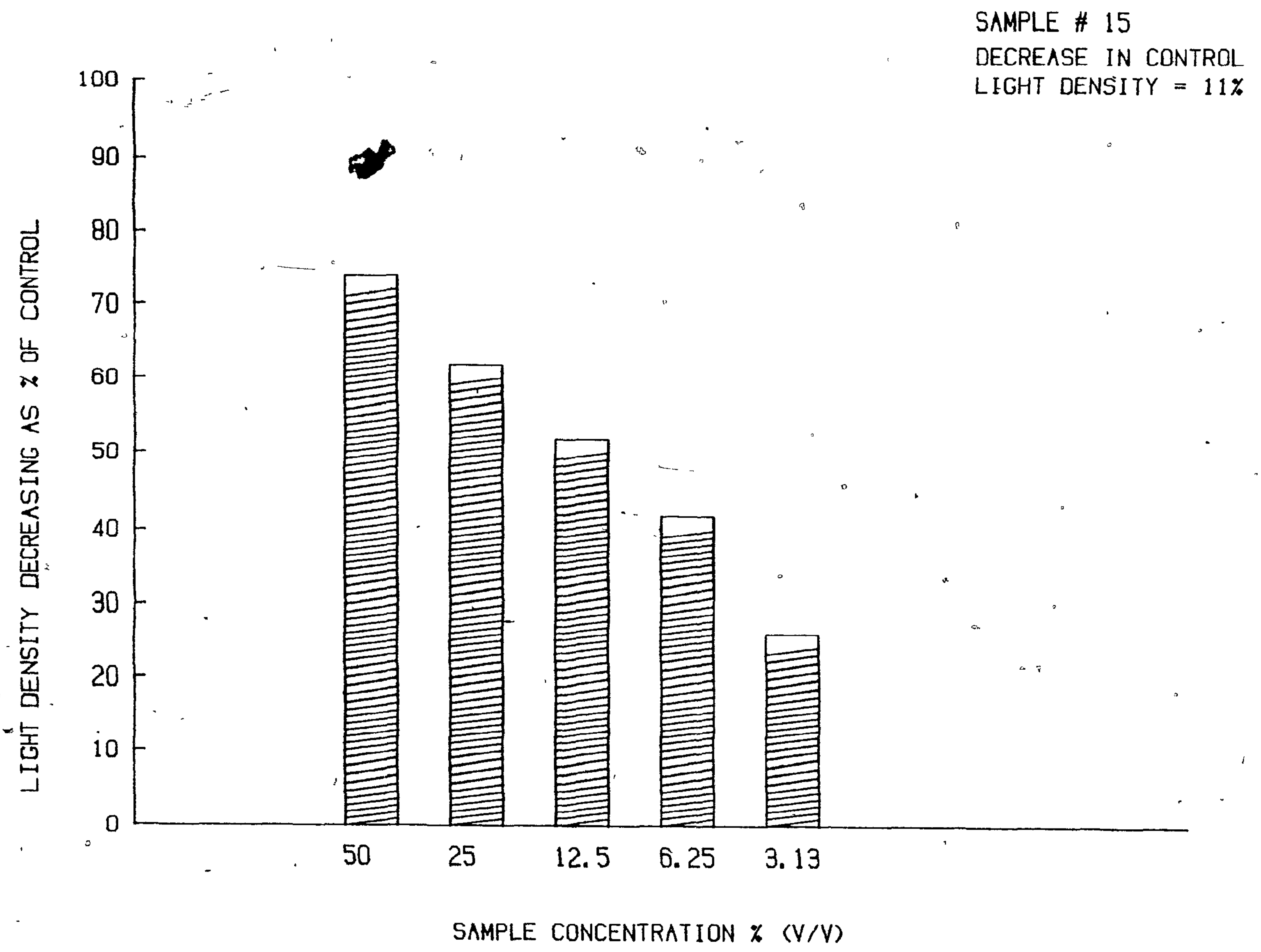


FIG. C.35 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

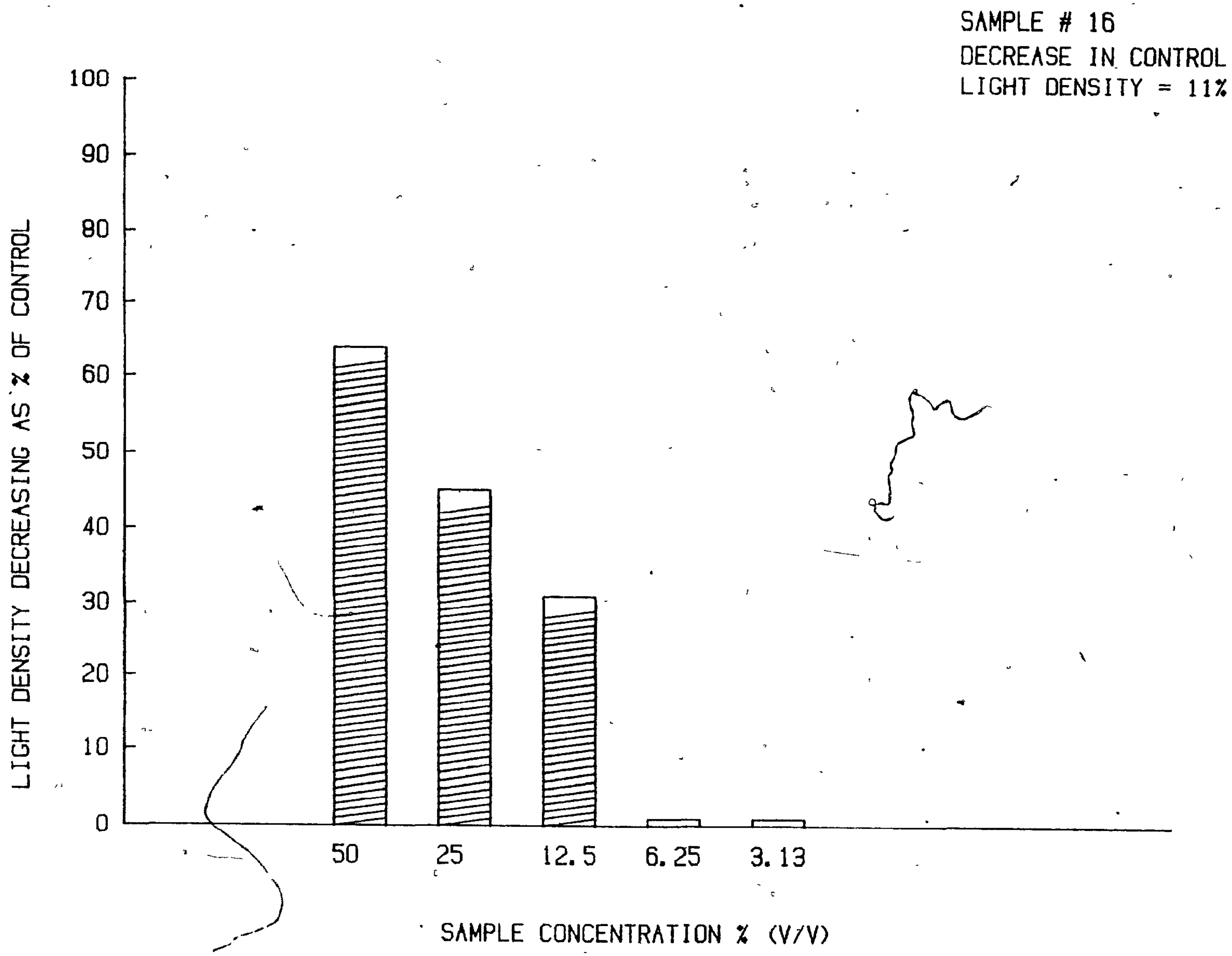


FIG. C.36 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

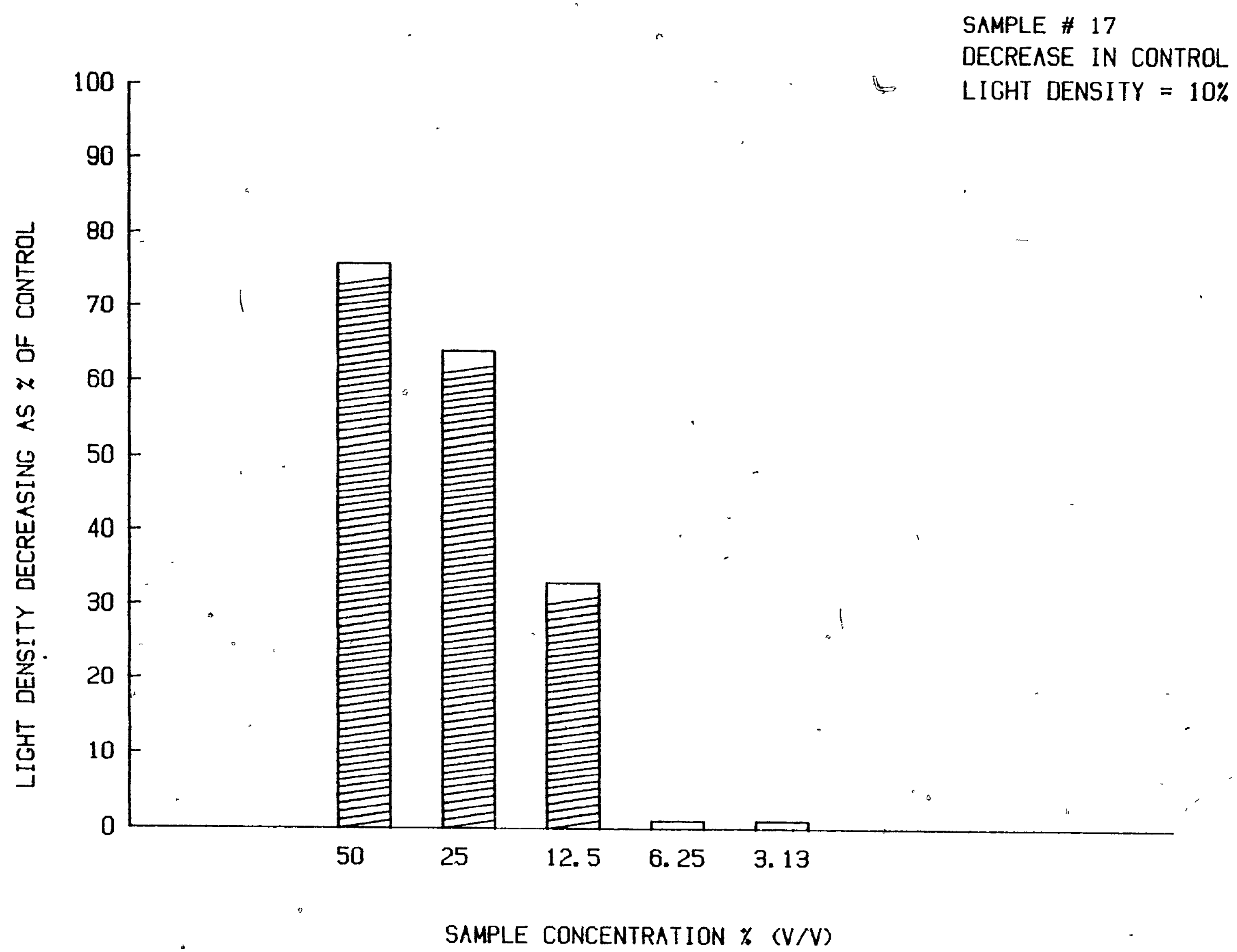


FIG. C.37 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

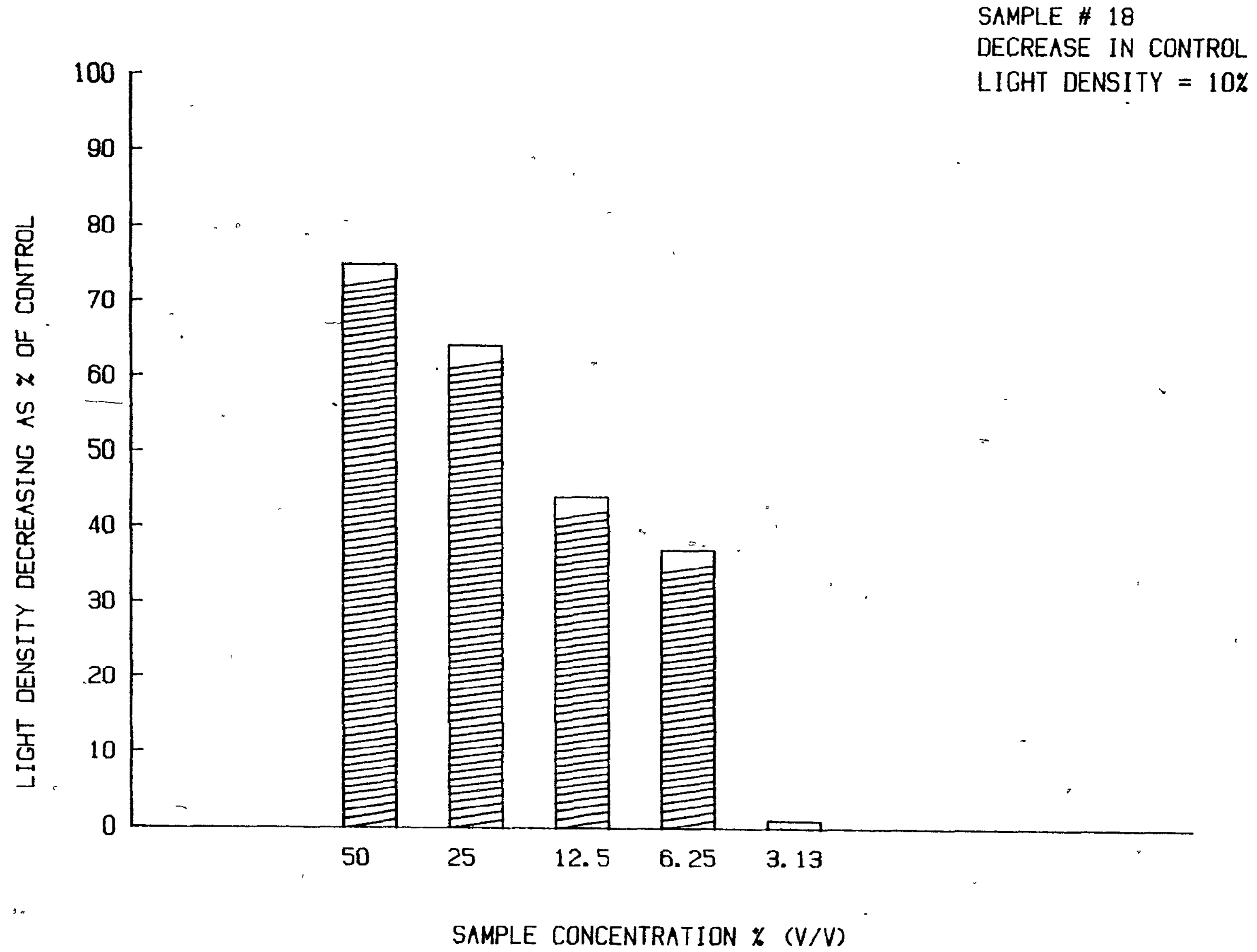


FIG. C.38 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA

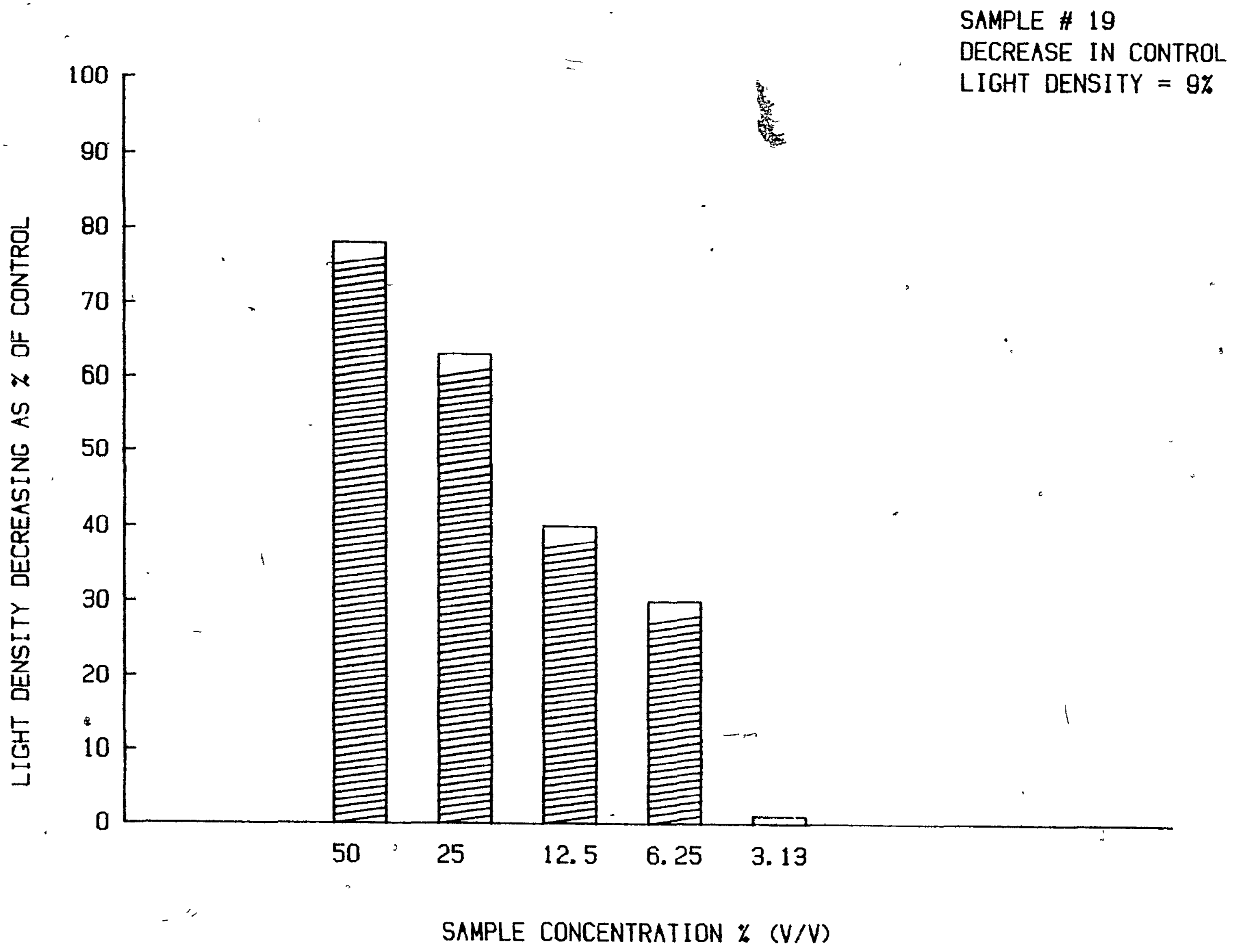


FIG. C.39 THE EFFECT OF SAMPLE CONC. ON THE LIGHT DENSITY EMITTED BY MARIN BACTERIA