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FORMIC ACID: DEVELOPMENT OF AN ANALYTICAL METHOD AND USE AS A PROCESS INDICATOR IN ANAEROBIC SYSTEMS

by
Sharon L. Perkins

In Partial Fulfillment
of the Requirements for the Degree
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A Special Research Problem Report

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APPROVED:

Dr. W. H. Cross, Advisor

Dr. J. P. Gould

Dr. F. M. Saunders

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ABSTRACT

Anaerobic degradation requires a diverse yet interrelated group of organisms. These organisms exist in a synergistic relationship that requires that a delicate balance be maintained for the system to function properly. Therefore, anaerobic treatment systems are frequently considered to be somewhat unreliable due to the sensitivity of methanogens to toxic substances. Considerable research has been done to determine reliable methods of predicting when this balance has been upset.

Formic acid is a common intermediate in anaerobic degradation and half of all methanogens can utilize it as a substrate. However, most of the research performed regarding its role in anaerobic systems has focused on natural rather then engineered environments. Further, many of the methods used to analyze for formic acid require extensive pretreatment to remove interfering substances and the use of unstable enzymatic solutions and are not amenable to use as a process monitoring method.

In this research an analytical method which could be routinely used for determining formic acid was developed. This procedure was utilized to examine the fluctuations of formic acid concentration in anaerobic batch reactors which were fed substrates containing various amounts of substances known to induce stress in these systems. The results were examined to determine possible correlation between these fluctuations and system performance;

however, the health or inhibition of the experimental systems was not conclusively established. Also several full scale and pilot systems were analyzed for the presence of formic acid.

Based on the results of this research it was concluded that formic acid concentrations in system that were not subjected to stressful substances are very low. There was correlation between system stress and formic acid accumulation in systems exposed to toxic levels of ammonia. There was an indication that formic acid increases were dependent on the type of toxicity induced. Finally, when the system was stressed the concentration of formic acid increased an order of magnitude over unstressed systems.

I. INTRODUCTION

GENERAL

Anaerobic processes occur in nature in a variety of environments. Anaerobic organisms have been found in such diverse habitats as lakes, marshes, cattle and human intestines (Winfrey *et al.*, 1977; Strayer and Tiedje, 1978; Phelps and Zeikus, 1985; Jones and Paynter, 1980; Beijer, 1952; Carroll and Hungate, 1955; Hungate *et al.*, 1970; Dakin *et al.*, 1913; Miller and Wolin, 1981). Anaerobic systems have been utilized to treat municipal and industrial wastewaters since the 1890's (McCarty 1985). Although anaerobic treatment was recognized as a waste treatment process long before aerobic treatment, it was not widely used. Some of the products of anaerobic degradation are offensive volatile sulphur compounds; such as hydrogen sulfide, sulfur dioxide, methyl mercaptan and dimethyl sulphide (Wheatland, 1981). These compounds have been found to be harmful to human health by hindering oxygen utilization at the cellular level (Kangus *et al.*, 1984). Additionally, their obnoxious odors caused anaerobic treatment to be considered undesirable (McKinney 1986).

Anaerobic degradation involves the conversion of complex organic matter to carbon dioxide (CO_2) and methane gas (CH_4). Methane fermentation is an important aspect of anaerobic processes because the degradation of complex organic matter to CO_2 and CH_4 results in a relatively low growth yield. Organic matter is

stabilized (McKinney 1986), while most of the energy provided by the substrate is retained as CH_4 (Bryant 1977).

Anaerobic degradation has typically been used to stabilize primary sludges (Grady and Lim, 1980); however, it has also been applied to biological sludges from aerobic processes and mixtures of sludges containing components of industrial wastes (Parkin and Owen, 1986), including meat packing, brewing, pharmaceutical, chemical, and food processing wastes (Grady and Lim, 1980). In addition, with the increasing concern regarding the deleterious effects of trihalomethanes in drinking water, researchers have examined the possibility of utilizing anaerobic treatment to degrade halogenated organic compounds. Bouwer *et al.* (1981) and Bouwer and McCarty (1983), in studies of 1- and 2-carbon organic compounds, found that, in low concentrations, trihalomethanes were anaerobically degradable, while no aerobic degradation occurred. Further, brominated halogens were found to be more readily degraded than chlorinated ones.

There are several reactor configurations used to facilitate anaerobic processes. Baffled, fixed bed and packed-bed reactors are just a few of the systems available. The type of system used depends upon the characteristics of the waste, the space available for the system, and the desired objective of the treatment.

The advantages of using anaerobic treatment over aerobic include (1) reduced electrical power requirement, (2) lower microbial cell production, and (3) the use of biogases produced as fuel. Since anaerobic processes by definition due not require O_2,

the electrical costs associated with generating and providing sufficient O_2 to an aerobic system are avoided. It has been recognized that anaerobic processes are most efficient at temperatures ranging from 30 °C to 55 °C; however, the power required to generate this heat can be obtained by using the biogas (CH_4 and CO_2) produced as a fuel, thereby offsetting this operating cost. In some instances the CH_4 and CO_2 produced are sold to local utilities. Since aerobic reactions yield more energy than anaerobic ones, more cell mass is produced (McKinney, 1986). The disposal costs of excess cells produced by aerobic processes far exceed that of anaerobic systems (Speece, 1983). The economic advantages which are most significant are the decreased costs for sludge disposal and electricity (Speece, 1983).

The disadvantages of anaerobic treatment include (1) an delicate ecosystem, (2) long hydraulic retention time (HRT), and (3) high capital costs. The interdependent relationships of anaerobic organisms are inherently unstable and require constant monitoring. Since anaerobic organisms have low growth rate, they take longer to respond to upsets, therefore, longer hydraulic retention times are required to allow for acclimation and recovery. Further, anaerobic systems are most efficient at temperatures of 30 to 55 °C, high capital costs may be encountered due to the requirement for heating the system. However, the operating costs may be deferred by using the biogas produced as a fuel to heat system. When considering the use of anaerobic treatment systems,

if the waste is high strength, then the advantages typically outweigh the disadvantages (Grady and Lim, 1980).

BIOCHEMISTRY

Anaerobic degradation requires a diverse yet interrelated group of organisms. As illustrated in figure 1.1, it is a three-stage operation; hydrolysis, acidogenesis, and methanogenesis (Grady and Lim, 1980). Organic wastes contain various kinds of lipids, carbohydrates, and proteins. First these organic constituents are hydrolyzed and liquified by extracellular enzymes into a soluble form that can pass through the bacterial cell wall. There the fermentation process yields short-chain volatile acids. Depending on the characteristic of the substrate, the overall rate of stabilization to CH_4 can be limited by this stage of the process.

Once soluble, acid-producing bacteria convert the material into a variety of end products depending on various factors. Acid-producers are composed of a very diverse group of organisms. The results of their fermentations depend upon the species present and the physical characteristics of the medium, such as pH and temperature. The fermentation products include long-chain fatty acids, short-chain volatile acids, amino acids, and sugars (McCarty 1986; Parkin and Owen, 1986; Grady and Lim, 1980). The resulting end products of this phase are due to the combination of activity by hydrogen-producing and acid-producing bacteria. When the electrons are transferred to hydrogen ions, hydrogen (H_2), CO_2, and

acetic and formic acids are the major end products (Grady and Lim, 1980). The last phase, methanogenesis, involves the formation of CH_4 and CO_2 (Chung and Neethling, 1990; Grady and Lim, 1980). Methanogenic bacteria can utilize H_2/CO_2, acetate, formate, and a few other substrates as sources of both carbon and energy, as indicated in table 1.1 (Daniels, 1984).

Acetic acid is the most common intermediate produced in the acid formation stage. In anaerobic digestors, it has been established that approximately 70% of the CH_4 produced comes from acetate (Baresi *et al.*, 1978; Mah *et al.*, 1978). Since acetic acid is an important intermediate, it has been the subject of numerous studies regarding anaerobic processes. Formic acid is also a common intermediate in anaerobic systems (Hungate *et al.*, 1970; Zeikus, 1977; Daniels *et al.*, 1984;), and as shown in table 1.2, there are methanogens that utilize it as a substrate. However, it is generally considered that the CH_4 derived from formate is minor, and there have been relatively few studies regarding its role in anaerobic systems. Thiele and Zeikus (1988) have examined the role of formic acid in H_2 transfer between hydrogen producers and users.

OBJECTIVES OF RESEARCH

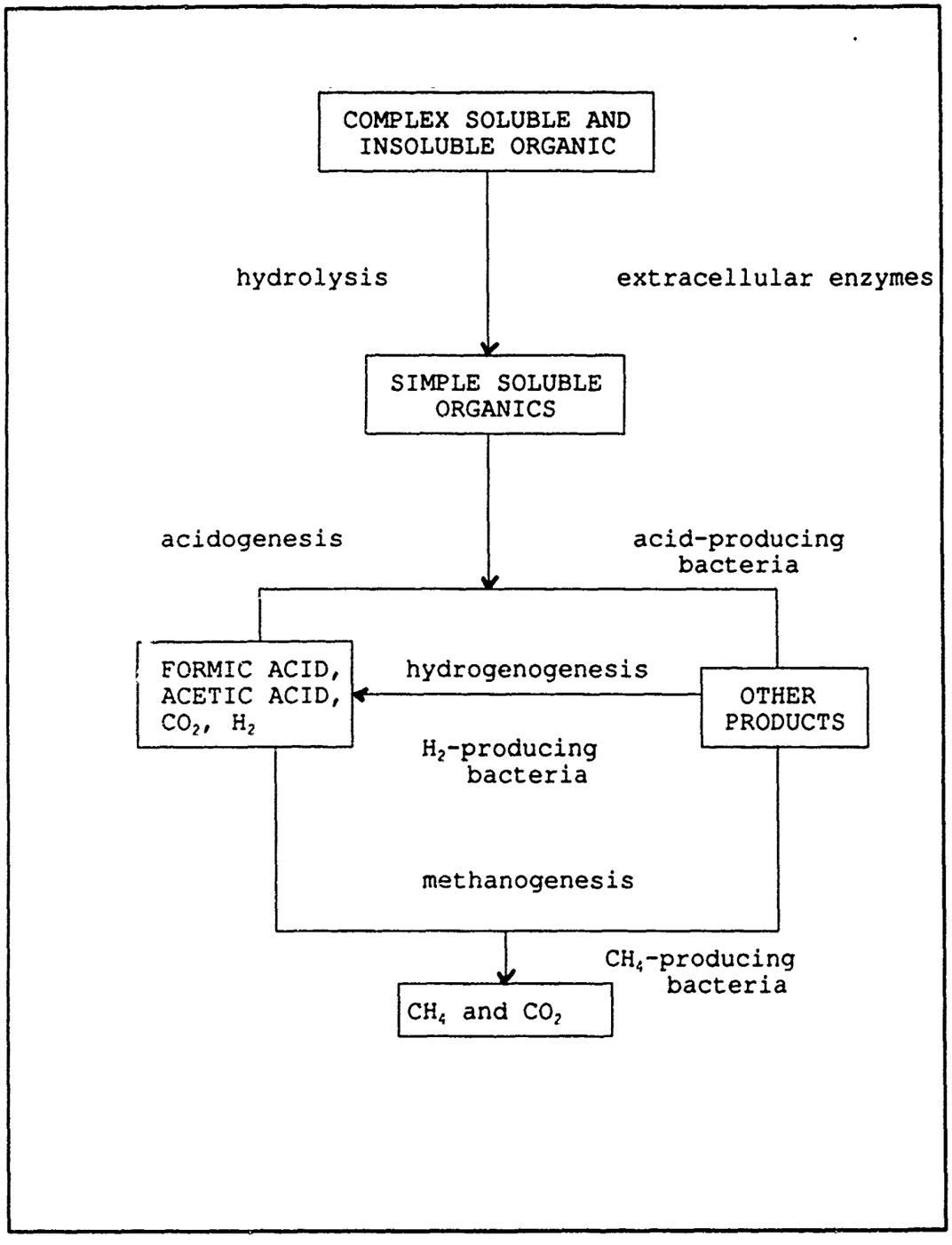
This research paper presents experimental data and results from a study designed to asses the performance of anaerobic batch reactors when subjected to shock or toxic loadings. The purpose of this study is to determine if there is a correlation between (1) an increase in formic acid concentration and system shock, and (2) the

health of an anaerobic system and formic acid concentration. In this research an analytical method was developed for determining formic acid that was simple and straight forward enough to use on a daily basis. This procedure was used to examine the fluctuations of formic acid concentration in anaerobic batch reactors subjected to stress, and examined the possible correlation between these fluctuations and system performance. Additionally, the method was used to determine the concentration of formic acid in some pilot scale and full scale anaerobic systems.

[a]Table 1.1 - Energy metabolism of methanogenic bacteria

Equation	$\Delta G°$ per reaction (KJ)	$\Delta G°$ per CH_4 (KJ)
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-138.8	-138.8
$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$	-119.5	-119.5
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	-310.5	-103.5
$CH_3COOH \rightarrow CH_4 + CO_2$	-27.6	-27.6
$4CO + 2H2O \rightarrow CH_4 + 3CO_2$	-185.6	-185.6
$4CH_3NH_3^+ + 2H_2O \rightarrow 3CH_4 + CO_2 + 4NH_4^+$	-225.7	-75.2

[a]Daniels *et al.* 1984



[a]Figure 1.1 - Multistage anaerobic process

[a]Grady and Lim, 1980

Table 1.2 - Sole electron source for methanogenesis and growth

Species	Substrate
Methanobacterium arbophilicium[β]	H_2
Methanobacterium formicium[β]	H_2, HCOOH
Methanobacterium mobile[β]	H_2, HCOOH
Methanobacterium thermoautotrophicum[α]	H_2
Methanococcus vannieli[α]	H_2, HCOOH
Methanosarcina barkeri[α]	H_2, CH_3OH, CH_3NH_2, CH_3COOH
Methanospirillum hungatei[α]	H_2, HCOOH
Methanobacterium bryantii[α]	H^2
Methanococcus thermolithotrophicus[α]	H_2, HCOOH

[α]Daniels *et al.* 1984 [β]Zeikus 1977

II. LITERATURE REVIEW

PROCESS INHIBITION

A discussion of anaerobic treatment processes typically includes dialogue regarding the difficulties involved with operation and control of these systems. Although there are advantages with using anaerobic processes to treat wastes, the sensitivity of the organisms that exist in this delicate ecosystem has limited its use.

Of the organisms required to carryout the interrelated anaerobic processes, methanogens are commonly regarded as the most sensitive to toxicity. Studies have demonstrated that waste constituents and environmental conditions can adversely affect the balance of these delicate systems. Physical parameters such as pH, temperature, organic and hydraulic loading increases, and the induction of toxic materials can adversely affect CH_4 production. A pH range of 6.5 to 7.6 is considered optimum for CH_4 production. While the optimum temperature range varies from 30 to 38 °C in the mesophilic range and from 50 to 60 °C in the thermophilic range (Parkin and Owen, 1986). There are many ways to induce stress in anaerobic systems; only a few will be discussed here.

Hickey and Switzenbaum (1991) studied a 10-day HRT reactor subjected to a 2.65 fold organic loading increase. They found that the total gas production increased 220% during the first day, stabilized over the next few days, then declined. The CH_4

concentration first increased then declined gradually over the testing period of 11 days. Also as a part of their research, they found that a hydraulic loading increase of two times the normal rate caused an upset condition. Additionally, when the rate was increased 4 fold, system performance declined.

The literature indicates that there is wide variation regarding the levels of ammonia, ammonium ion, and total ammonia that can be tolerated by anaerobic systems. While concentrations of ammonia ranging from 50 to 200 mg/L have been considered nutritionally beneficial (Bhattacharya and Parkin, 1989), ammonia may induce severe toxicity in two ways, as the un-ionized ammonia (NH_3) or the ammonium ion (NH_4^+). Most researchers have found that the un-ionized form is the most toxic (Parkin and Owen, 1986). The toxicity associated with the ammonium ion is the same as with any other cation. Values ranging from 40 to 100 mg/L of NH_3, and 200 to 7000 mg/L of total ammonia nitrogen have been reported to be the thresholds which marked the inhibition of CH_4 production (Heinrichs *et al.*, 1990; Bhattacharya and Parkin, 1989).

Bhattacharya and Parkin (1989) found that when ammonia was applied in the toxic threshold as a slug dose, the bacteria had minimal chance to acclimate. However, when ammonia concentration was gradually increased, acclimation was possible. They found that with a 15-day solids retention time (SRT), system failure occurred with a continuous dose of 33 mg/L NH_3. While a slug dose of 19 mg/L NH_3 was tolerated by the system. They also concluded, as did Heinrichs *et al.* (1990), that toxicity levels were species related.

That is, based on percentages of substrate removal, acetate-utilizing organisms were much more sensitive to ammonia toxicity then propionate users. Heinrichs *et al.* (1990) also determined that systems supplemented with sulfate could tolerate higher concentrations of un-ionized ammonia.

Depending on the SRT, formaldehyde concentrations of 200 to 400 mg/L is the threshold at which inhibition begins to occur in anaerobic systems (Bhattacharya and Parkin, 1989). Some have shown that slug dosages of 200 mg/L of chloroform could be tolerated in acclimated systems (Yang *et al.*, 1980). However, Hickey *et al.* (1987) found that chloroform concentrations of 1.0 mg/L completely inhibited CH_4 production.

Hickey *et al.* (1987) also demonstrated that bromoethanesulfonic acid dosages of 5 mM, and 150 mg/L of trichloroacetic acid completely inhibited CH_4 production. In a survey of biodegradability of organic chemicals, Battersby and Wilson (1989) found that at concentrations of 50 mg/L as carbon mono-, di-, tri-, and pentachlorophenol, mono-, and dinitrophenol, 4-nonylphenol, and 2-phenylphenol inhibited CH_4 production.

Trace levels of metals such as nickel, cobalt, and molybdenum are required nutrients for some methanogens (Mckinney, 1986); however, Hickey et al. (1989) demonstrated that copper dosages of 25 to 150 mg/L, 100 to 900 mg/L of zinc, and 25 to 100 mg/L of cadmium, caused inhibition of CH_4 production.

Methane inhibition negatively affects anaerobic process efficiency diminishing the extent of substrate removal. System

stress adversely affects anaerobic processes by altering the systems kinetics (Parkin and Owen, 1986). If toxic compounds are present in the waste treatment system using an anaerobic process, the system may become upset and fail.

PROCESS INDICATORS

Failure or inhibition of anaerobic systems is marked by decreasing pH, an increase in VOA concentrations, and a decrease in CH_4 production. Typical healthy systems have a pH around neutral, bicarbonate alkalinity from 1000 to 5000 mg/L as $CaCO_3$, volatile acid concentrations under 500 mg/L, and a biogas that is 55 to 75% CH_4 (Parkin and Owen, 1986). Monitoring of these parameters, is the conventional means by which reactor efficiency is evaluated. However, unless the problem develops slowly, by the time these indicators are noted, the system is well on its way to failure (Hickey and Switzenbaum, 1991), and may have to be restarted. Over a long period of time the system may recover if the toxicant is removed (Parkin and Owen, 1986).

Anaerobic processes are very sensitive and subject to rapid failure. A readily detectable, early indicator of system stress is needed in order to determine when a system is approaching failure. Many studies have been conducted towards this end, with several studies focusing on the possibility of utilizing H_2 concentration as a process indicator. The concentration of H_2 in the system controls the proportion of intermediates produced. It must be maintained at low concentration, partial pressure below 10^{-4}

atmospheres, to facilitate the production of H_2, CO_2 and acetate instead of other higher molecular weight intermediates such as ethanol, lactate, propionate, etc. (Speece, 1983; Wise, 1981; Bryant and Wolin, 1975). Hickey and Switzenbaum (1991) found that increased loading, either organic or hydraulic, caused the concentrations of H_2 and CO to increase earlier than conventional indicators. They noted, however, that monitoring H_2 did not indicate the stress induced on acetate-utilizing anaerobes; thereby limiting the usefulness of this indicator. Harper and Pohland (1986) concluded that certain treatment options could be employed to control H_2 and volatile acids, thereby avoiding process instability. However, in a study of carbohydrate wastewaters, Harper (1989) found that H_2 concentration had no effect on wastewater treatment efficiency. Mosey and Fernandes (1989) noted that with automatic continuous monitoring, H_2 concentration could be used to indicate the induction of a toxic substance. However, with the more standard method of daily sampling, the chances of missing the peak H_2 concentration triggered by a toxic substance would be greatly increased.

Others have centered their research on enzyme related indicators. This area of study focused on the fact that various enzymatic reactions are required to carry out the degradation of organic matter. Mackie and Bryant (1990) correlated the rate of protein synthesis with cell growth and substrate degradation. Their purpose was to monitor the growth of the complex anaerobic population. Chung and Neethling (1989) suggested that

dehydrogenase activity, which is very sensitive to adverse environmental conditions, could serve as an early warning mechanism. Likewise, Zhenglan *et al.* (1990) observed increased phosphatase activity in early stages of system failure. The measurement of these enzymatic parameters involves extensive laboratory procedures and are not suited for routine process analysis required to be performed in daily plant operations.

FORMIC ACID

Biochemistry. Formic acid (or formate) has been found in many of the various habitats of anaerobic bacteria. Approximately half of the genera of methanogens can utilize formate as both a carbon and energy source (Daniels *et al.*, 1984). Originally it was believed that CH_4 production from formate involved the cleavage of formate to H_2 and CO_2 which were then used for methanogenesis; however, it is now accepted that formate can be used directly (Daniels *et al.*, 1984). The enzymes that act on formic acid are formic hydrogenlyase and formic dehydrogenase; see table 2.1 for these reactions.

[α]Table 2.1 - Enzymes that act on formic acid

Enzyme	Reaction
formic dehydrogenase	$HCOOH + {}^{\beta}X \rightarrow XH_2 + CO_2$
formic hydrogenlyase	$HCOOH \rightarrow H_2 + CO_2$

[α]Stephenson and Strickland, 1932
[β]X represents an intracellular hydrogen carrier

Woods (1936) found that under the appropriate conditions, formic hydrogenlyase is reversible, acting to degrade as well as synthesize formic acid. Formic acid can be produced by the reduction of CO_2 with electrons donated in the reoxidation of NADH to NAD^+ or FADH to FAD^+ (Bryant and Wolin, 1975), or it can be formed as an intermediate in the degradation of pyruvate (Bailey, 1986).

Most studies regarding formic acid have observed its contributions to CH_4 production in natural environments. Several studies have found that the addition of formic acid/formate stimulated CH_4 production. In studies of lake sediments, Strayer and Tiedje (1978) found that addition of formate immediately stimulated methanogenesis, while Winfrey *et al.* (1977) observed that formate additions gave a more rapid increase in initial CH_4 production than H_2. Of the natural intermediates studied, Phelps and Zeikus (1985) found formate to have the highest rate of transformation to CH_4. Jones and Paynter (1980), in an investigation of marsh sediments, found that when adding CO_2,

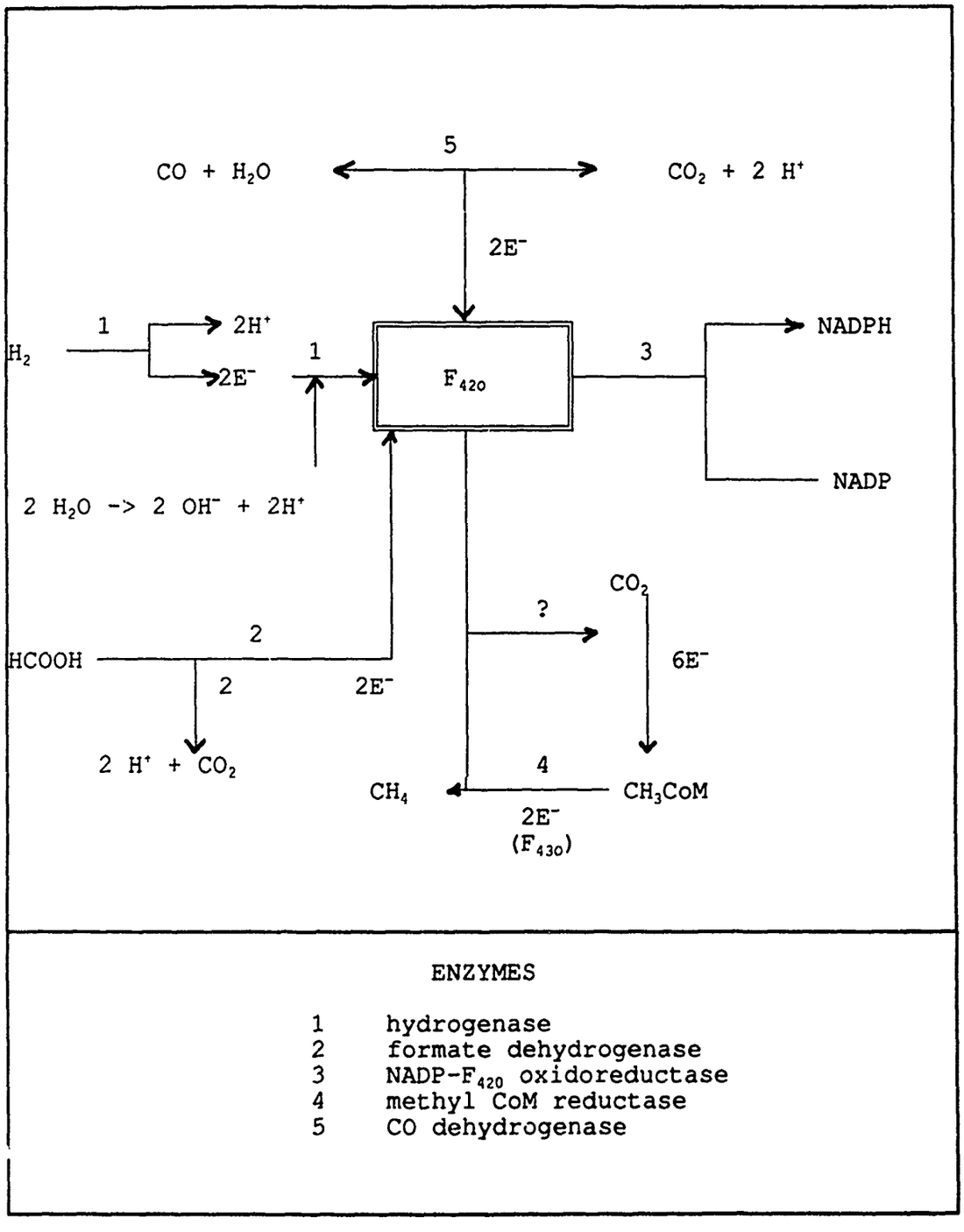
acetate, or formate, the greatest stimulation of CH_4 production occurred for formate.

Kinetic studies of formate metabolism indicated that when it was added to anaerobic systems, it was rapidly depleted; in most cases in a matter of minutes. In a study of whey waste, Chartrain and Zeikus (1986) found that more than 95% of the formate added to the system was removed within the first minute. The formate was converted to CO_2, with little attributing to CH_4 formation. Hungate *et al.*, (1970) found formate degradation occurring in the first eight seconds, and determined a Michaelis constant, K_m, of 30 nmoles/g. In a study by Strayer and Tiedje (1978) the rate of conversion was so rapid that they could not determine a K_m.

Hungate *et al.*, (1970) examined the probability that formate may be a significant source of H_2 to be used for methanogenesis. They found that in the bovine rumen of alfalfa-fed heifers, approximately 18% of the CH_4 produced came from H_2 supplied by formate. In another rumen oriented study, Beijer (1952) determined that 1.0 mmole of formic acid yielded 0.2 mmole CH_4.

CH_4 can be produced from formic acid in two ways. It can be cleaved to CO_2 and H_2 which are used by methanogens to produce CH_4, or it can enter the methanogenic pathway directly at the formate oxidation level (Daniels *et al.*, 1984). Figure 2.2 indicates the major electron flow in methanogens.

As noted earlier, H_2 concentration must be controlled to allow for anaerobic degradation to proceed to its final level. Interspecies H_2 transfer has been found to be the route by which



[a]Figure 2.1 - Major methanogenic electron transfer reactions

[a]Daniels *et al.* 1984

electrons flow between organisms in the synergistic relationship between hydrogen producers and users. Thiele and Zeikus (1988) concluded that interspecies formate transfer between acetogens and methanogens links electron flow to CO_2 reduction to CH_4.

Few studies have examined the role of formic acid in anaerobic treatment systems. One such study was performed using a baffled anaerobic reactor. Grobicki and Stuckey (1989) examined the fluctuations of formate concentration under hydraulic shock-loading conditions. Shock loadings were applied for the first 3 hours, then was reduced to the normal loading. At the fourth hour, formate levels reached maximum values of 2500 mg/L in the reactor effluent. Formate concentration decreased until none was detected after 11 hours. The system demonstrated a rapid recovery with 99% COD removal. They concluded that formate production was important to the stability of the anaerobic process.

Methods of detection. As the lowest molecular weight organic acid, formic acid is difficult to detect and measure when it is in a mixture of other organic compounds. Many of the procedures which are used to analyze for formic acid involve oxidizing it to CO_2 or reducing it to formaldehyde. Since the other higher molecular weight organic acids can also be oxidized to CO_2 and reduced to other possibly interfering substances, their presence could adversely affect the detection of formic acid. In most cases it is necessary to remove these higher molecular weight compounds, as well as any CO_2 or formaldehyde, present in the sample before analyzing for formic acid.

A study to distinguish between three different enzymes that act on formic acid or H_2, hydrogenase, formic dehydrogenase and formic hydrogenase (see table 2.1 for reactions) was performed by Stephenson and Strickland (1932). They found that placing bacteria possessing the enzyme formic hydrogenlyase in contact with formic acid, in the presence of O_2, gives the following reactions:

$$HCOOH \rightarrow H_2 + CO_2$$

$$HCOOH + \tfrac{1}{2}O_2 \rightarrow CO_2 + H_2O$$

$$H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O \ .$$

The CO_2 produced was measured using a manometer; the displacement of air observed was a measure of the formic acid present in the original sample. *Escherichia coli*, which produce formic hydrogenlyase in the presence of formate, were cultivated and placed in contact with known quantities of formate. The actual production of H_2 observed was found to be between 97 and 106% of theoretical values. While this method was not practical due to the unstable nature of formic hydrogenlyase (Pickett *et al.*, 1944).

Another of the earliest methods used to analyze for formic acid was based on Stephenson's and Strickland's work. It involved oxidizing a sample with mercuric chloride and measuring the precipitate, calomel (mercurous chloride - Hg_2Cl_2). This procedure required the extraction of formic acid from aqueous solutions. Dakin *et al.* (1913) developed the following method to analyze for formic acid in urine. First they used ether to extract formic acid; they found that after four hours of extraction most of the formic acid had been removed from solution. They then neutralized

the acid by adding carbonate; this solution was acidified with phosphoric acid then subjected to steam distillation. The dilute formic acid solution was neutralized with an excess of caustic soda. This solution was evaporated then neutralized with acetic acid. At this point the formic acid solution was oxidized with mercuric chloride. To assist this reaction, the mixture was heated for six hours. Once formed, the precipitate was dried and weighed; one gram calomel represented 0.0977 gram formic acid. This method was found to be about 99.8% accurate for samples containing a total quantity of formic acid around 47 mg. Due to the large amount of formic acid required for this method (Woods, 1936), and the fact that it was very time consuming, it was not appropriate for routine analysis.

Pickett *et al.*, (1944) developed another manometric method of determining formic acid. They utilized ceric sulfate in the presence of palladium to oxidize formic acid to CO_2,

$$2H_4Ce(SO_4)_4 + HCOOH \xrightarrow{Pd} Ce_2(SO_4)_3 + 5H_2SO_4 + CO_2$$

the oxidation was faster than with mercuric chloride, and the reagents were stated to be stable for six months. The procedure requires H_2SO_4, ceric sulfate and palladinized asbestos. The sample was first distilled to remove compounds such as cinnamic, glycolic and levulinic acids which can be oxidized to formic acid by ceric sulfate, thus interfering with the analysis. The distillate was then neutralized, redissolved, redistilled then oxidized with ceric

sulfate. The CO_2 produced in 20 to 30 minutes was measured using a manometer. Although the reagents were more stable then the formic hydrogenlyase, this method did not allow for direct analysis of the sample due to possible interfering substances.

Grant (1948) evaluated the use of colormetric analysis for formic acid reduced to formaldehyde. His work was based on findings by MacFadyen (1945) who use chromotrophic acid to measure concentrations of formaldehyde. MacFayden's work was fashioned after experiments by Boyd and Logan (1942). They oxidized amino acids to formaldehyde then colorimetrically analyzed the formaldehyde using chromotrophic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid). Grant's procedure required the use of chromotrophic acid and magnesium ribbon. A coil of magnesium ribbon was added to a solution containing formic acid that was then immersed in an ice bath. HCl was added periodically, the chromotrophic acid was added, then the mixture was heated for 30 minutes. After centrifugation, the supernatant was analyzed spectrometrically. A calibration curve was established correlating the colormetric response to known quantities of formic acid, from 0 to 15 μg. This method also required distillation of the sample to remove interfering organic substances, formaldehyde present in the sample may be removed by reaction with phenylhydrazine, and HCl may be required to acidify the sample to pH 2 if carbonates are present. With this method only 29% of the theoretical amount of formaldehyde was recovered; this low recovery was due to the lack

of complete conversion of formic acid to formaldehyde. Pickett *et al.*, (1954) also found the colormetric method to be unsatisfactory.

Perlin (1954) proposed the use of lead tetraacetate to oxidize formic acid. Formic acid concentration was then determined by titrating the reduced lead tetraacetate. The oxidizing reaction was allowed to proceed for 20 to 30 minutes, then the stopping solution, potassium iodide and sodium acetate, was added. Finally the solution was titrated with thiosulfate. This procedure could be modified to measure formic acid by manometrically determining the CO_2 produced. For this analysis, a calibration curve relating the quantity of formic acid with CO_2 produced must first be established. For samples containing 1.05 to 32.3 mg formic acid, recovery values ranged from 96.3 to 101% of theoretical values. The recovery attained by this analysis was satisfactory; however, the considerable laboratory methods required for this analysis make it undesirable for the purposes of this research.

Rabynowitz and Pricer (1957) developed an enzymatic method for analyzing for formic acid which entailed a spectrophotometric determination of N^5-N^{10}-imidazolinium (5-10-methenyl-tetrahydrofolic acid). Formic acid was catalyzed by tetrahydrofolic formylase as follows.

$$HCOOH + tetrahydrofolic\ acid + ATP \rightarrow N^{10}\text{-}formyl\ tetrahydrofolic\ acid + ATP + P_i$$

When treated with acid, N^{10}- formyltetrahydrofolic acid was converted to N^5-N^{10}-imidazolinium which was analyzed at a wavelength of 350 μm. This method demonstrated 94 - 98% recovery of formic

acid, and could be used to determine formic acid in biological samples containing 0.02 to 0.2 µmole/mL of sample (or 0.92 to 9.2 mg/L of formic acid). Not only is the sample preparation complex, but the range of detection would require dilution of most samples for wastewater treatment plants.

Asnis and Glick (1956) analyzed formic acid by developing an alternate enzymatic procedure. They used an *E. coli* enzymatic system in the presence of nitrate to oxidize formic acid as shown,

$$\textit{formic acid} + KNO_3 \xrightarrow[\textit{enzymesystem}]{\textit{particulate}} CO_2 + H_2O + KNO_2$$

CO_2 is measured manometrically as previously described. *E. coli* were cultivated, sonically treated, and centrifuged. The resultant supernatant was further centrifuged and the resulting cell-free sediment was resuspended, recentrifuged, and resuspended. This suspension, containing the enzyme system, was then used to oxidize formic acid. The procedure must be performed in an O_2 free environment, as O_2 is a preferential electron acceptor over NO_3^-. This method was applied to samples containing 2.5 to 20 µmole (0.12 to 0.92 mg) formate, with a recovery of 102.7%. The main difficulty associated with this method was the unstable nature of the enzyme system; its active life was less than 24 hours.

Gas chromatography, the technique typically used to analyze for organic acids, is not successful when analyzing formic acid using the standard flame ionization detector (FID). In 1976, Brown and Moore dealt with issue by converting formic acid to

dimethlyformamide which is readily detectable with an FID. The conversion of formic acid was accomplished by treating a sample with diazomethane and dimethylamine; these substances were found to be specific for formic acid. Bricknell and Finegold (1978) improved Moore and Brown's method by using a thermal conductivity detector. The purpose of their assay was to develop a means of detecting the presence of formic acid; this information is required for the identification and classification of certain microorganisms. Their procedure calls for methylation of the sample followed by extraction with chloroform. The sample was then chilled and centrifuged to ensure a homogenous mixture. A calibration curve was established for concentrations from 1.0 to 10.0 µmole/mL formic acid. This procedure proved to be 50 to 60% accurate.

Jorgensen (1981) developed a method of measuring formic acid by distilling a sample, stripping the mixed gases which are sorbed onto a porous medium coated with Ag_2O. Ag_2O acts as an oxidizing agent for formic acid. The formic acid was converted to CO_2 [and H_2] which was absorbed into a mixture of barium chloride and NaOH; $BaCO_3$ was formed and titrated. This method demonstrated a 90% recovery for concentrations from 0.513 to 5.125mM (23.6 to 235.8 mg/L). As described this method was time consuming with extensive sample pretreatment required.

Guerrant *et al.*, (1982) developed a method for analyzing short-chain acids using high-performance liquid chromatography. This method involved ether extraction of the sample. After a

single extraction with ether, the recovery of formic acid was only 51%. High pressure liquid chromatography is the most current method used to analyze for formic acid in bacterial cultures.

Ion chromatography can also be used to detect formic acid as the formate ion in a mixture of organic acids. However, there was no indication in the literature that is method was beino used to analyze formic acid in biological experiments.

These previously described methods were developed for various reasons. For some analyses, the mere detection of formic acid in a samples was sufficient. Due to the extensive pretreatment required or the low recovery obtained using these methods, they are not suited for routine daily analysis of formic acid for reactor monitoring purposes. For use as a routine process indicator at wastewater treatment facilities employing anaerobic processes, formic acid must be analyzed using a simple, relatively accurate process requiring minimal sample preparation.

III. ANALYTICAL METHODS AND MATERIALS

This research was conducted in two phases. In the first phase, an analytical method was developed to analyze for formic acid in effluent from anaerobic treatment systems using ion chromatography. During the second phase, anaerobic batch systems were subjected to toxic loadings then monitored over time periods ranging from twelve hours to several days. The effect these loadings had on various parameters, including formic acid concentration, was monitored.

DEVELOPMENT OF FORMIC ACID ANALYTICAL METHOD

As noted in the literature review, present methods for detecting formic acid are time consuming, involve preparation of unstable enzymatic solutions, extensive pretreatment procedures or provide inadequate recoveries. One of the objectives of this research was to develop a reliable and simple method of analyzing for formic acid.

An application note (AN 24) from the Dionex Corporation entitled "Determination of Formaldehyde as Formate Ion" indicated that Kim, Geraci, and Kupel (1978), of the National Institute for Occupational Safety and Health, were using ion chromatography to analyze for formaldehyde in ambient air. Appendix A provides a copy of this note. For detection of formate they used an AS4A separator column and an AG4A guard column. A 5mM $Na_2B_4O_7 \cdot 10H_2O$

(borax) solution was the eluent applied at a flowrate of 2.0 mL/min. From the note, at flow rates of 200 mL/min, recovery of formaldehyde ranged from 59 to 122% with an average of 99%.

For the purposes of this research, the ion chromatograph used consisted of a Dionex System 2000i/SP ion chromatograph equipped with a model CDM-1 conductivity detector. The range of the detector was 0.1 to 10,000 µs, and the size of the sample injection loop was 50 µL. The column configuration was an AG4A guard column followed by an AS4A separator column. A 5 mM $Na_2B_4O_7 \cdot 10H_2O$ (borax) solution was used as the eluant.

An investigation was conducted to determine what anions would interfere with formic acid analysis. The chemicals tested, Cl^-,

Table 3.1 – Data from chromatographic analysis of chemicals that have elution times similar to formic acid

COMPOUND	CONCENTRATION mg/L	RETENTION TIME chart movement mm	RETENTION TIME time min
NaF	10	6.0	1.2
NaCl	100	10.5	2.1
CH_3COOH	100	6.5	1.3
HCOOH	75	7.5	1.5

INSTRUMENT SETUP

Chart speed:	30cm/hr
Flowrate:	1.9mL/hr
Detector range:	100µs
	300µs for HCOOH

F^-, and acetic acid, were chosen based on fact that they have a similar elution time as formic acid. Analyses of individual and mixed solutions was accomplished. Table 3.1 and Figure 3.1 presents the results of the chromatographs of the mixed samples. To increase the separation between acetic and formic acids, the flowrate was changed to 1.23 mL/min. Table 3.2 and figure 3.2 indicate the results of this change.

Table 3.2 - Data from chromatographic analysis of acetic and formic acids

COMPOUND	CONCENTRATION mg/L	RETENTION TIME chart movement mm	RETENTION TIME time min
CH_3COOH	100	9.5	1.9
HCOOH	75	11.5	2.2

INSTRUMENT SETUP

Chart speed:	30 cm/hr
Flowrate:	1.23 mL/min
Detector range:	100 µs

Next standards were prepared using 88% formic acid. A calibration curve of instrument response, peak height, versus formic acid concentration was established for concentrations ranging from 5 to 100 mg/L; figure 3.3 presents this information. As can be seen, the curve is linear up to approximately 8 mg/L. However, a plot of the natural log of the concentration versus the natural log of the peak height gave a straight line. This relationship is shown in figure 3.4. From this graph it is clear

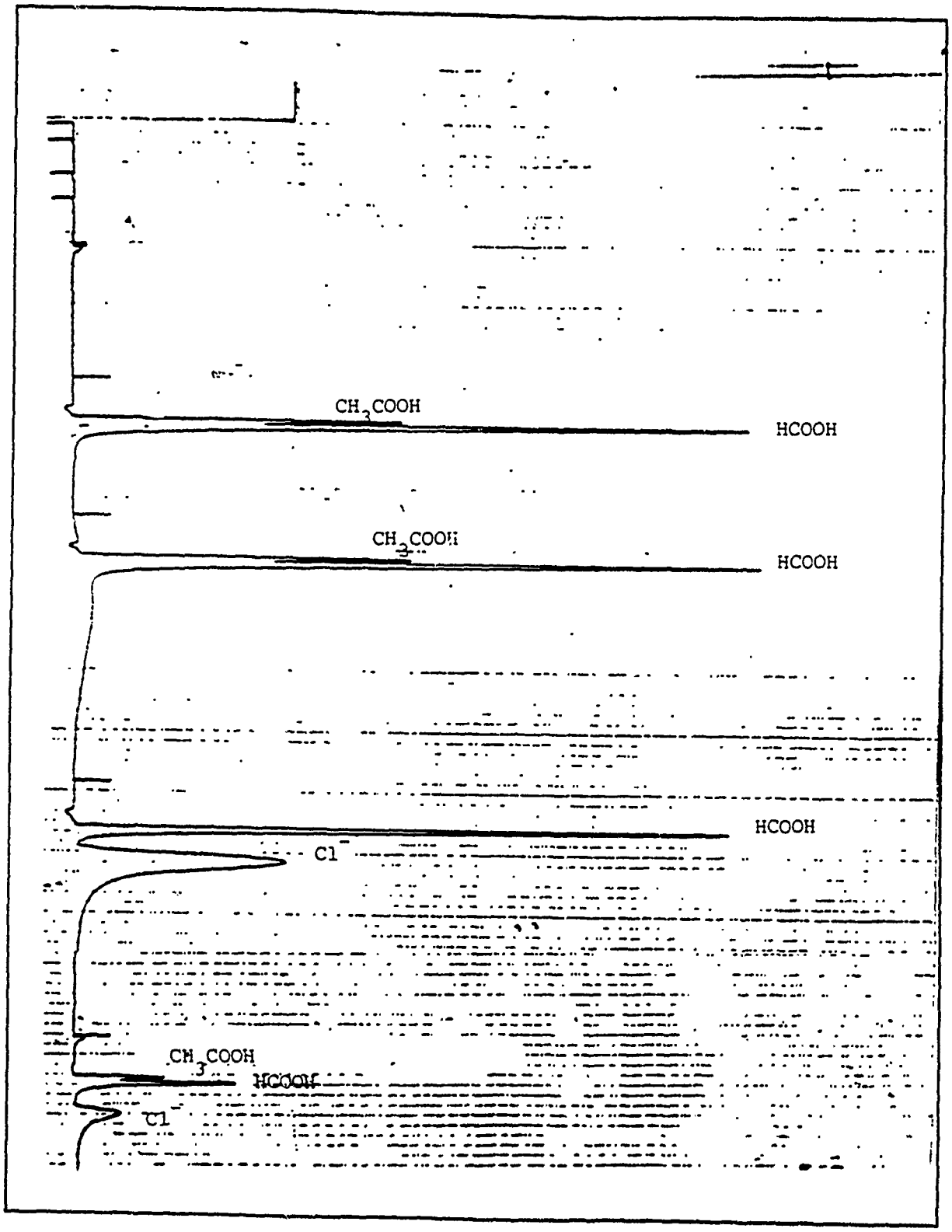


Figure 3.1 - Ion chromatographic elution peak separation of chemicals that might interfere with analysis of formic acid

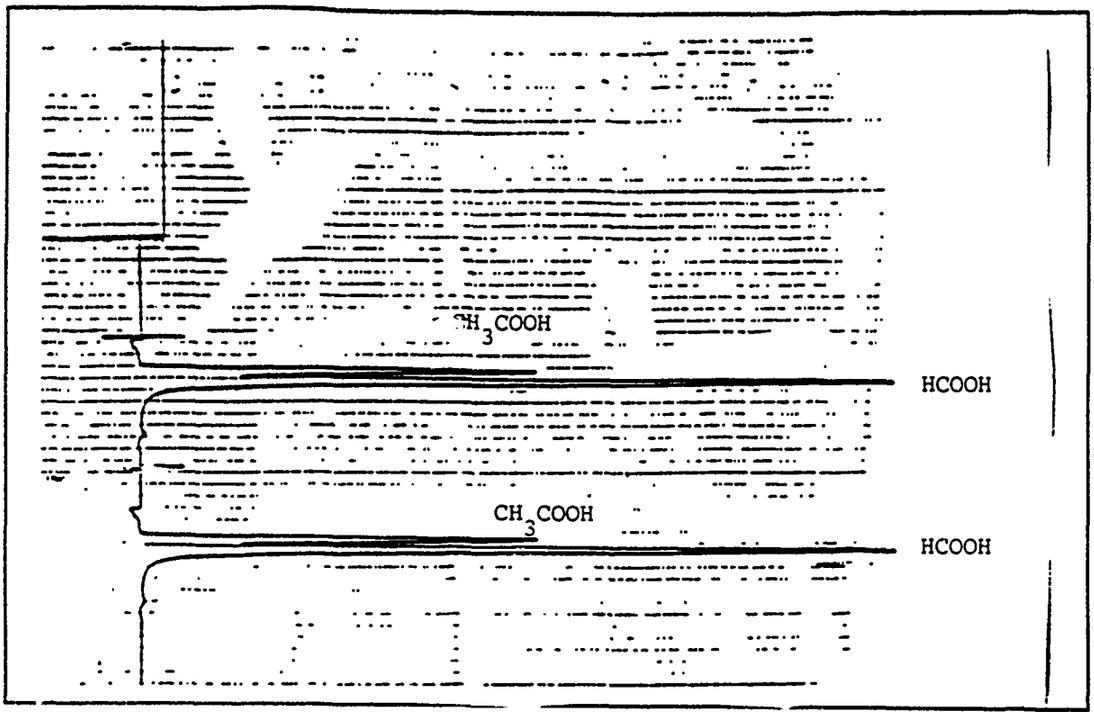


Figure 3.2 - Acetic and formic acid elution peak separation at an eluent flow of 1.23mL/min

that the useful range is from 5 to 75 mg/L.

Recovery analysis was performed using reagent grade formic acid and leachate from two established experimental landfill columns, identified as columns 1 and 2. As shown in figure 3.5, chromatographic analysis of unspiked anaerobic leachate samples produced large peaks in a range that would interfere with formic acid determination. However, due to the stability of the landfill system producing the leachate it was unlikely that formic acid was present. Formic acid in spiked samples was undetectable until the formic acid exceed a concentration of 75 mg/L. Known leachate constituents which could have caused the interferences were thought to be either low molecular weight acids or high concentrations of

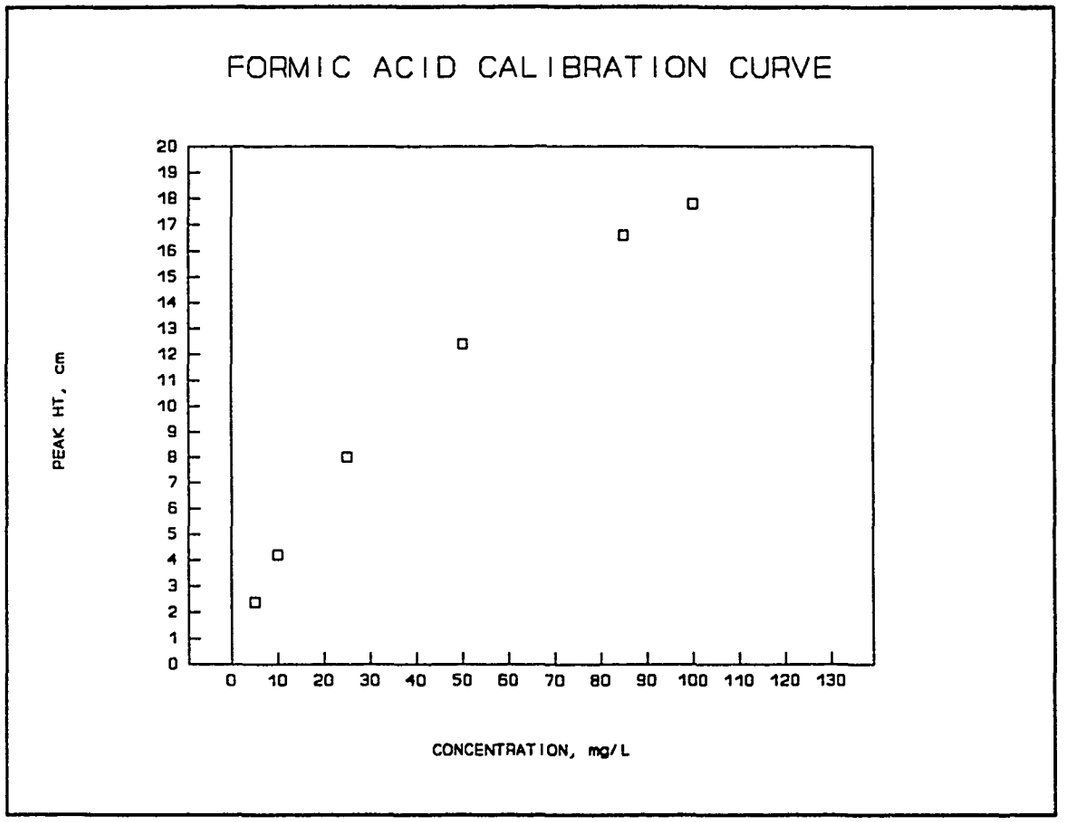


Figure 3.3 - Formic acid calibration curve

chloride ion. In an attempt to alleviate these interferences, leachate samples were pretreated with Onguard pretreatment cartridges, manufactured by the Dionex Corporation.

The first cartridge used was the "RP" cartridge. It contained macroporous, divinylbenzene, reversed-phase packing and was recommended for the removal of hydrophobic compounds including some carboxylic acids. It contained no anion or cation exchange sites. Use of this cartridge alone did not remove the interferences. Next an "AG" cartridge was utilized to attempt to remove the interfering substances. This cartridge contained a silver cation exchange resin and was recommended for removal of Cl^-, Br^-, I^-, CrO_4^{2-} as well

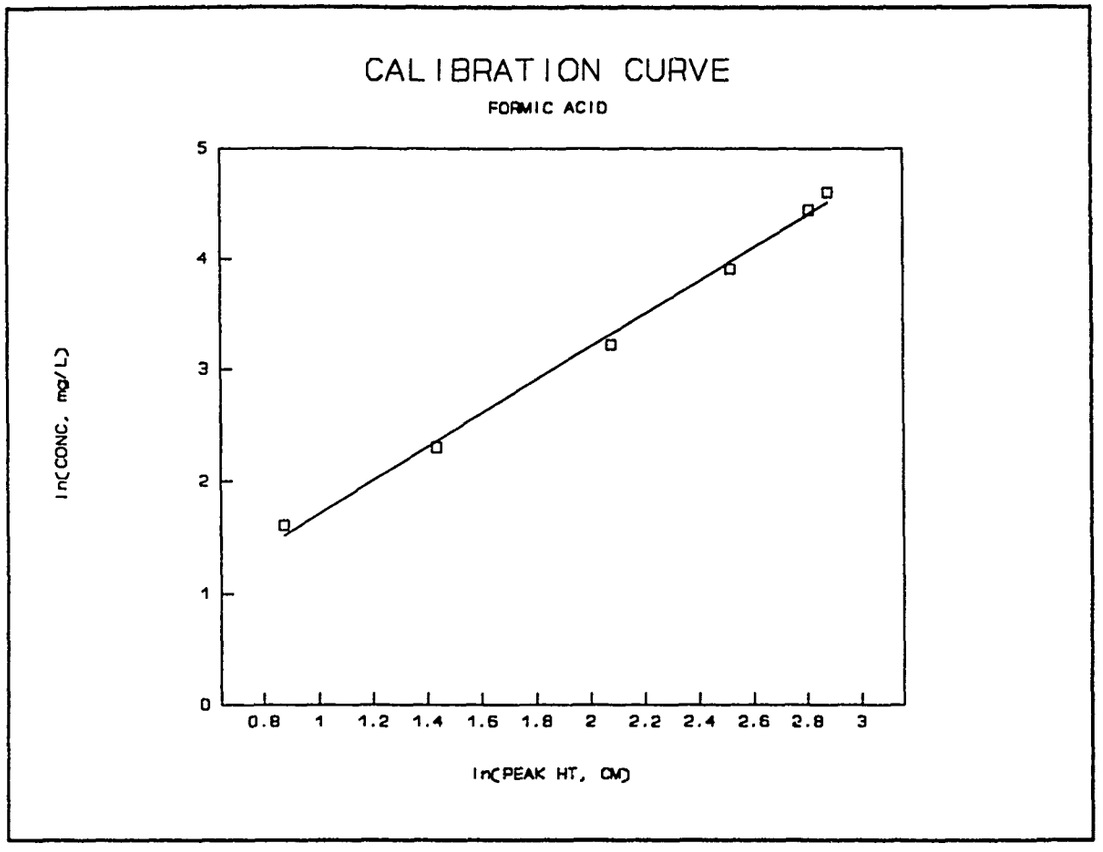


FIGURE 3.4 - Linearized formic acid calibration curve

as a number of other anions. Its capacity was stated to be 1.8 to 2.0 meq/cartridge. Use of this pretreatment cartridge removed enough of the interfering materials to allow the detection of 50 mg/L and 10 mg/L formic acid in spiked leachate samples of column 1 and 2, respectively. Greater removal of the interfering substances was obtained by pretreating the samples with both the RP and AG cartridges. In each case, the cartridges were prepared as noted in the Onguard user information sheet. The procedure was as follows:

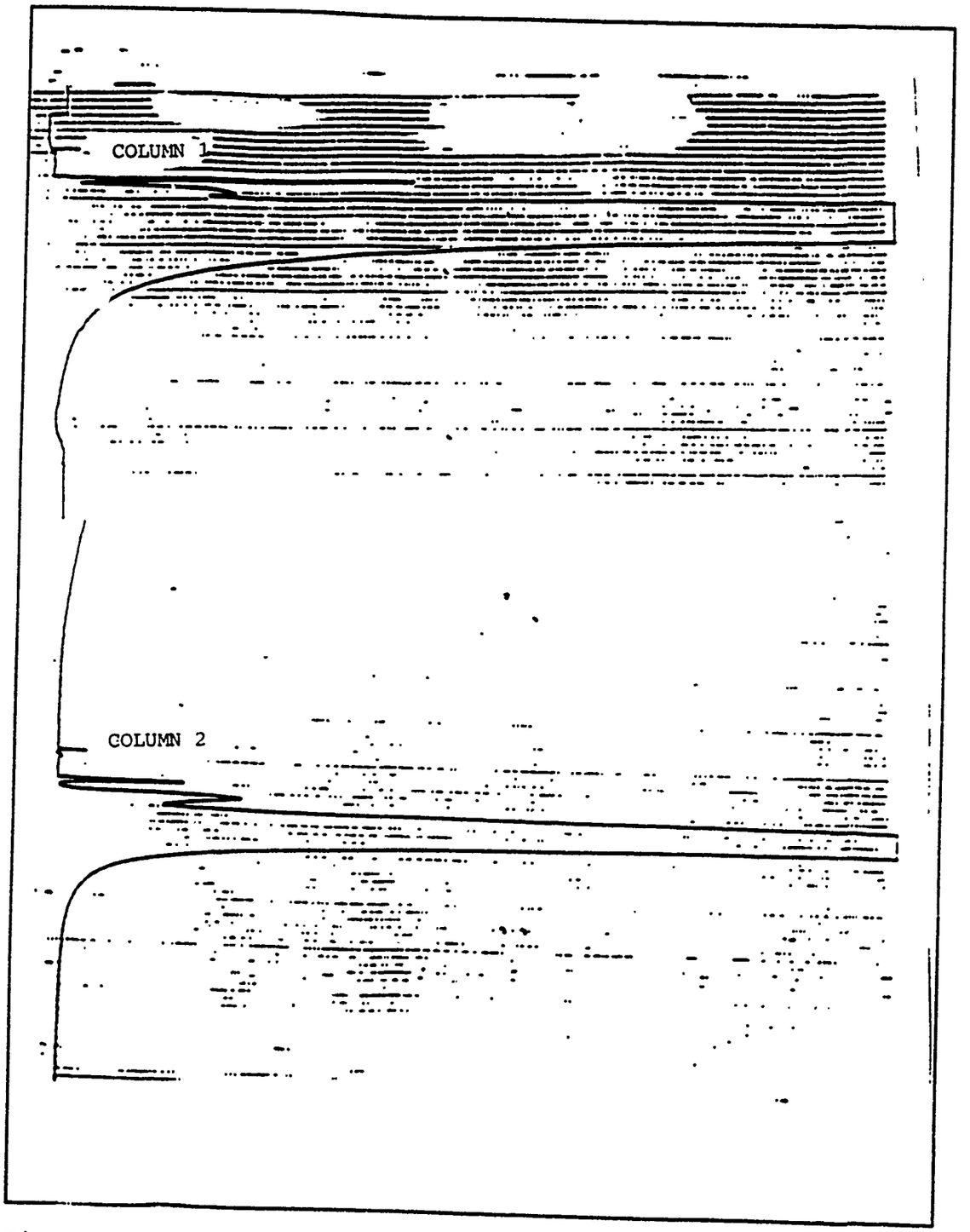


Figure 3.5 - Analysis of leachate from experimental landfill columns 1 and 2

1) Filter 30 mL of leachate through a 0.45 µm membrane filter. If spiked sample was to be analyzed, the sample was spiked after filtration.
2) Pass 12 mL of filtered leachate through a RP cartridge, allowing the first 3 mL to pass to waste.
3) Pass the remaining 9 mL through an AG cartridge, again wasting the first 3 mL.

This protocol was used to run reproducibility analysis on leachate samples from both columns spiked with 10 mg/L formic acid. The results of this analysis indicated recovery of formic acid ranging from 109 to 157%, with an average of 132%. The average concentration, based on instrument response and the calibration curve generated previously, was 13.2 mg/L, with a standard deviation of 1.2, less than a 10% error. Table 3.3 and figure 3.6 present these data. The reason the recovery was much greater than 100% was attributed to the fact that the calibration curve was established using peak height values produced from samples of deionized water spiked with known concentrations of formic acid which were not subjected to pretreatment with the RP and AG cartridges. The calibration curve was reestablished using standards that were subjected to the same pretreatment process as the samples.

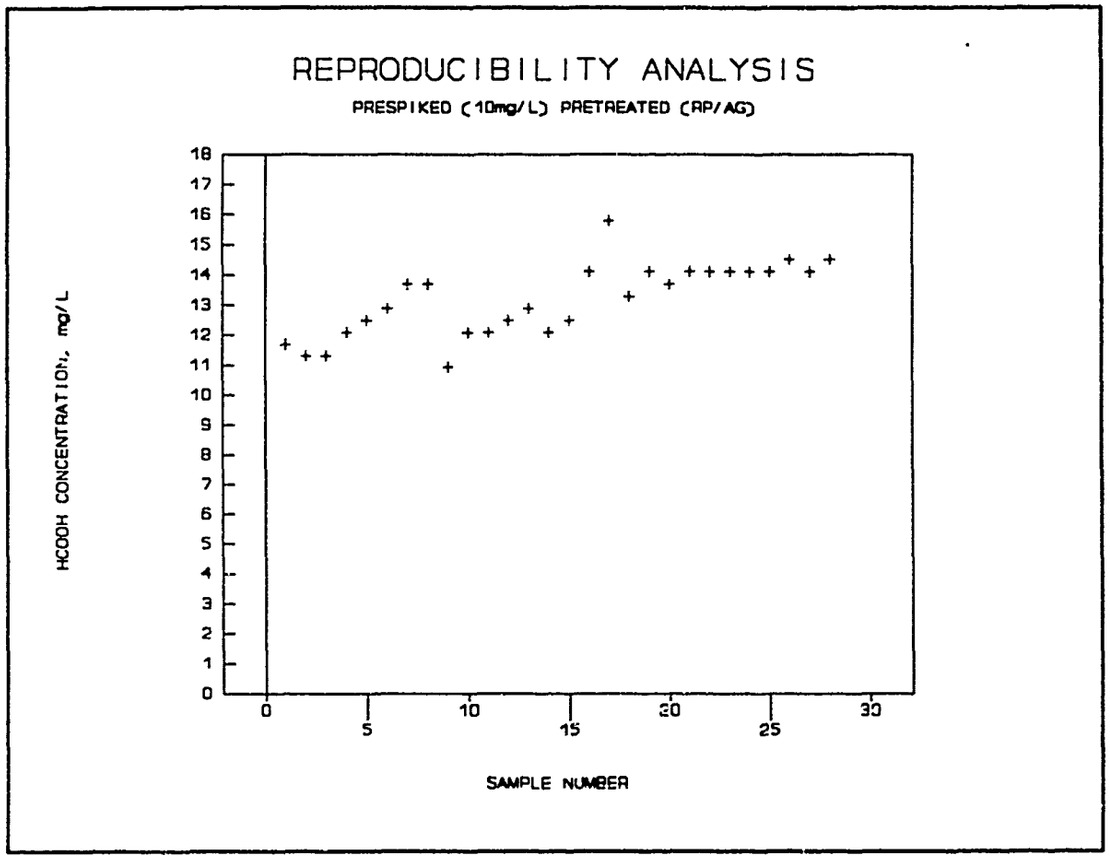


Figure 3.6 - Ion chromatographic reproducibility analysis of leachate from experimental landfill column 1, spiked with 10 mg/L formic acid and pretreated with RP and AG cartridges

Chromatographs were run on samples of anaerobic leachate from both columns, pretreated and untreated, unspiked and spiked with formic acid. Samples of deionized water using the pretreatment cartridges, unspiked and spiked with formic acid were also analyzed to determine the baseline due to pretreatment. Individual chromatographs of deionized water samples spiked with formic acid that were untreated and pretreated prior to analysis were run. The data indicated that the untreated sample gave a response of 4.3 cm, the sample pretreated with both cartridges provided a peak height

Table 3.3 - Data from ion chromatographic reproducibility analysis of leachate from experimental landfill column 1, spiked with 10 mg/L formic acid and pretreated with RP and AG cartridges

COLUMN 1 SAMPLE #	RUN #	PK HT cm	ln(PK HT)	CALC ln(CONC)	CONC mg/L	% RECOVERY
1	1	4.5	1.50	2.46	11.7	117
	2	4.4	1.48	2.43	11.3	113
	3	4.4	1.48	2.43	11.3	113
	4	4.6	1.53	2.49	12.1	121
	5	4.7	1.55	2.52	12.5	125
2	6	4.8	1.57	2.56	12.9	129
	7	5.0	1.61	2.62	13.7	137
	8	5.0	1.61	2.62	13.7	137
	9	4.3	1.46	2.39	10.9	109
	10	4.6	1.53	2.49	12.1	121
*4	11	4.6	1.53	2.49	12.1	121
	12	4.7	1.55	2.52	12.5	125
	13	4.8	1.57	2.56	12.9	129
	14	4.6	1.53	2.49	12.1	121
	15	4.7	1.55	2.52	12.5	125
5	16	5.1	1.63	2.65	14.1	141
	17	5.5	1.70	2.76	15.8	158
	18	4.9	1.59	2.59	13.3	133
COLUMN 2						
1	19	5.1	1.63	2.65	14.1	141
	20	5.0	1.61	2.62	13.7	137
	21	5.1	1.63	2.65	14.1	141
	22	5.1	1.63	2.65	14.1	141
	23	5.1	1.63	2.65	14.1	141
2	24	5.1	1.63	2.65	14.1	141
	25	5.1	1.63	2.65	14.1	141
	26	5.2	1.65	2.68	14.5	145
	27	5.1	1.63	2.65	14.1	141
	28	5.2	1.65	2.68	14.5	145
			STANDARD DEVIATION		1.2	
				AVERAGE	13.2	132

*Sample 3 turned purple; unusable due to reduction and precipitation of silver in cartridge.

of 5.5 cm, while the samples pretreated with only an RP or AG cartridge gave responses of 5.2 and 3.5, respectively. It was clear from these data that the RP cartridge increased instrument

response; however, the cause was undetermined. These chromatographs are presented in Appendix B. The results of these analyses lead to the conclusion that using the AG cartridge alone was the best method of pretreatment.

The next step of this phase involved determining the lowest concentration that could be detected using the method developed. A calibration curve for concentrations from 10 mg/L to 0.1 mg/L was developed. Figure 3.7 displays this information.

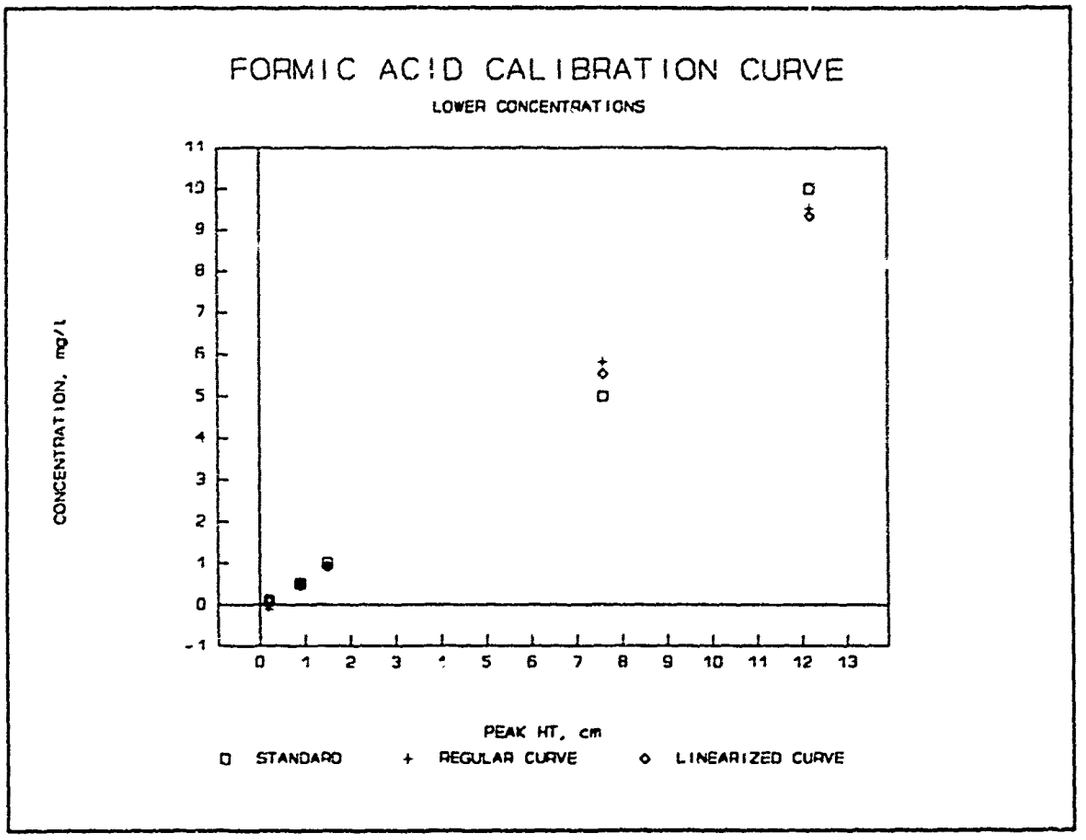


Figure 3.7 - Calibration curve for low concentrations of formic acid

Since the equation generated from the linearized and regular calibration curves are very similar, the non linearized calibration

curve was used for this analysis. Since leachate from column 1 contained more interfering substances than that from column 2, column 1 leachate was spiked with various amounts of formic acid for the detectibility analysis. The lowest detectible limit in leachate was determined to be 5 mg/L. This information is presented in table 3.4. This concentration is considered to be

Table 3.4 - Data from ion chromatographic analysis to determine the lowest concentration of formic acid detectable

LOWEST DETECTIBLE CONCENTRATION

SPIKE mg/L	PEAK HT cm	CONC mg/L	RECOVERY %
10.0	10.3	7.98	80
5.0	4.4	3.26	65
1.0	ND	ND	ND
0.5	ND	ND	ND
0.1	ND	ND	ND

ND - not detectible

very conservative since anaerobic leachate was the substance spiked. Analysis of effluent from bench scale and full scale anaerobic systems indicated that they contained fewer interfering substances than the leachate. In reactor effluent it is probable that the detection limit could be as low as 1 mg/L.

To see if formic acid could be detected in anaerobic systems, samples were obtained from anaerobic digester effluents from the Georgia Tech Research Institute (GTRI) test reactors and the Utoy

Table 3.5 - Formic acid analysis of effluent from various anaerobic reactors

SOURCE	PEAK HT cm	CONC mg/L
GTRI #4	2.0	16
Utoy	2.0	16
Entrenchment Creek	2.2	19

and Entrenchment Creek wastewater treatment facilities. The effluent samples obtained from GTRI were taken from four reactors. Reactor 1 was a packed column used to treat blood wastes; reactor 4 was an empty bed column also treating blood wastes; reactors 10 and 11 were treating egg wastes and a mixture of wastes respectively. The first chromatographic analyses were inconclusive; there were peaks noted that could have been formic or acetic acid. When samples were spiked with formic acid, the peaks in question increased. However, since it was highly unlikely that these samples contained formic acid and not acetic acid, it was determined that the concentration of acetic acid in the samples produced a peak that masked a possible formic acid peak. By varying the recorder speed and the eluent flow, as demonstrated in figure 3.8, reasonable separation of acetic and formic acids was obtained. Using the amended instrument setup, formic acid was detected in a sample of effluent from GTRI's laboratory anaerobic reactor 4, as well as in the samples obtained from the Utoy and Entrenchment Creek anaerobic digester effluents. As indicated in table 3.5, the concentration of formic acid was below 20 mg/L for each of these reactors.

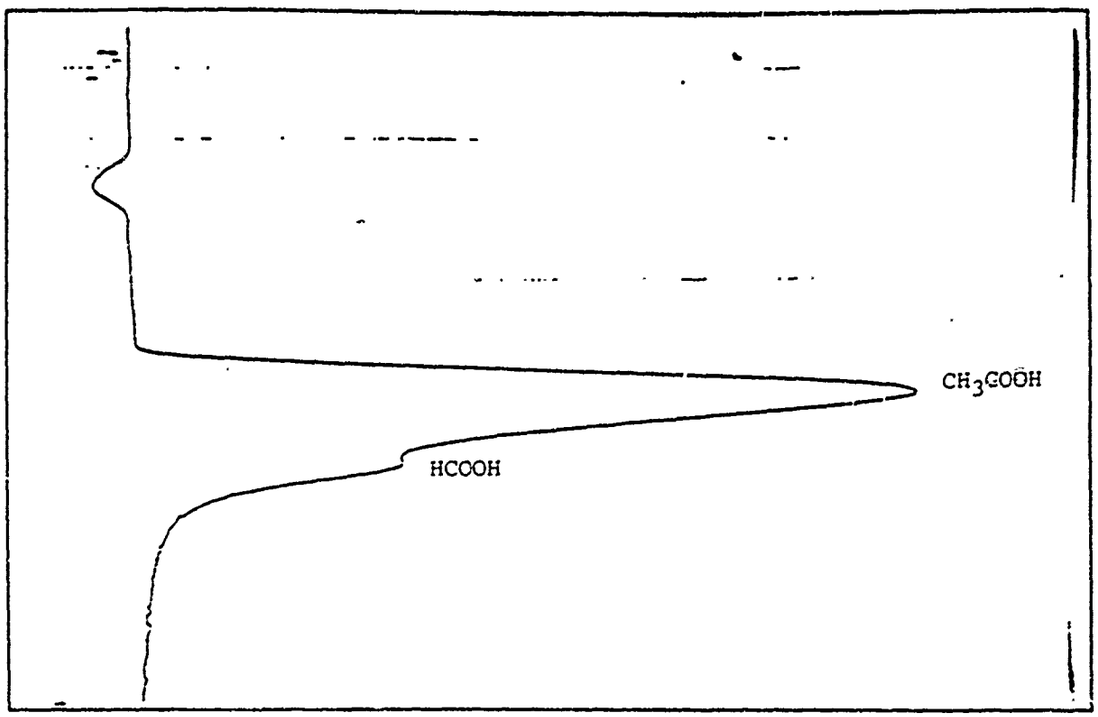


Figure 3.8 - Acetic and formic acid elution peak separation at an eluent flow of 0.22mL/min

The analytical procedure developed for the analysis of formic acid in anaerobic effluents was as follows:

(1) Filter sample with 0.45 µm membrane filter

(2) Pass 15 mL of sample through a prepared AG Onguard cartridge, wasting the first 3 mL.

(3) Inject 1 mL of sample into the sample port of the ion chromatograph set up as follows:

Detector range: 100 µs

Recorder speed: 30 cm/min

Flow: 0.22 mL/min (setting 1.0)

In the second phase of this research, the procedure established above was used to analyze for formic acid in the effluent samples

of the batch reactors under study; minor adjustments were made as necessary. Another calibration curve was generated with the standard prepared using the method noted above; the results were very similar to those of the first curve generated by using standards of deionized water and formic acid without pretreatment. Figure 3.9 displays these data. It is presumed that reproducibility analysis would be more favorable with the AG cartridge alone The calibration curve generated with the pretreated standards was used in the analysis of formic acid in the second phase of research.

The advantages associated with this procedure were: (1) the pretreatment may not be required depending on the constituents present in the sample; (2) if required, the pretreatment was uncomplicated and not very time consuming, the only preparation required of the AG cartridge is filtering 5 mL of deionized water through it prior to passing the sample through; (3) after injecting the sample into the chromatograph, data were automatically registered on a strip chart recorder; and (4) the recovery analysis produced less than 10% error, which compares favorably with the other methods described previously.

There were a few disadvantages noted with using this method of analysis; (1) if the capacity of the pretreatment cartridge was exceeded, the pretreated sample would not be useful for analysis; (2) With the decrease in eluent flow from 1.3 mL/min to 0.22 mL/min, the time to elute the formic acid peak increased from 2.5 min to 14 min; (3) if a large peak was encountered that eluted

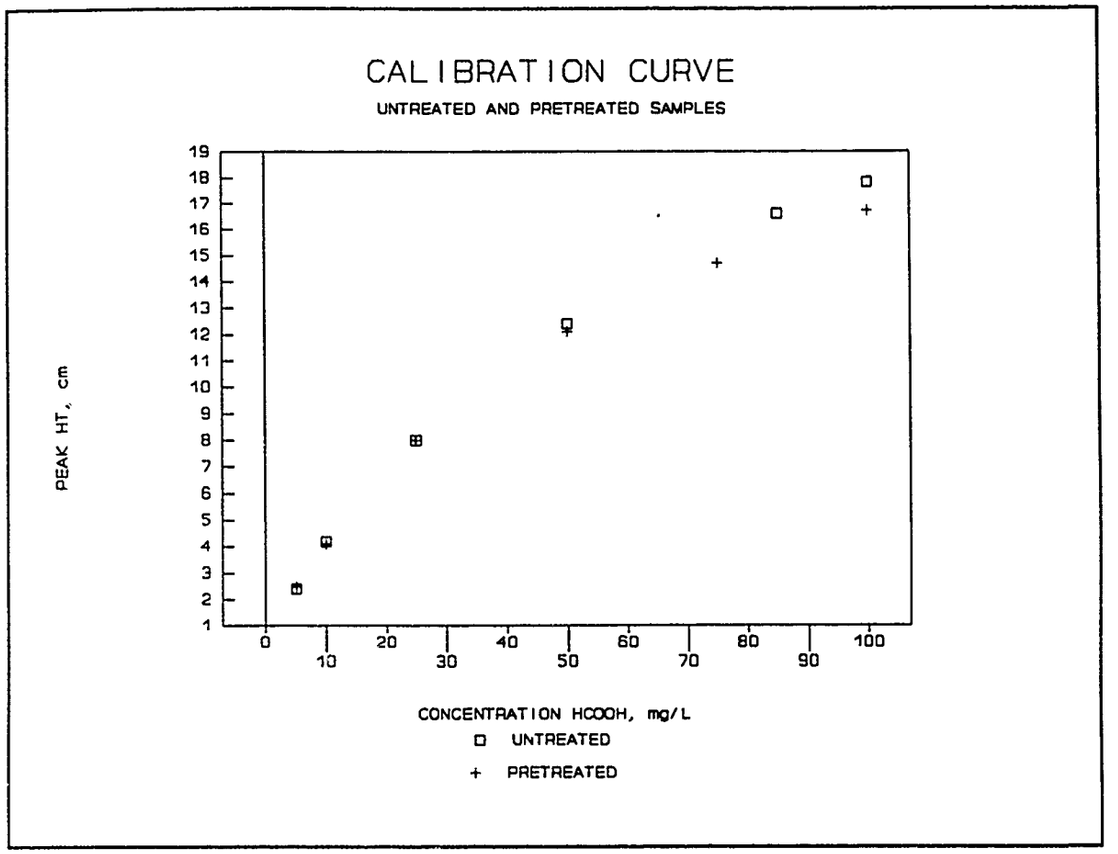


Figure 3.9 - Comparison of formic acid calibration curves generated from untreated and pretreated standards

after formic acid, say phosphate, the time for the system to return to baseline was extremely long at a flow of 0.22 mL/min. A dual system that allows for switching from one flow and eluent to another, or a gradient pumping system, would have made the analysis less time consuming. These types of ion chromatographs are available. Finally, (4) if the concentration of acetic acid was much greater than formic acid, on the order of 100:1, determining the quantity of formic acid was not as precise as when the concentration of acetic acid was not that much greater than formic acid, on the order of 5:1.

ANAEROBIC BATCH REACTOR SETUP AND OTHER ANALYSES

An 8 L anaerobic batch reactor was established to provide seed for smaller (1 L) test reactors. The test reactors were exposed to toxic loadings and their effluents were analyzed for various process indicators. The 8 L reactor was seeded from a wastewater treatment plant's anaerobic digester. The reactor was started June 12, 1991 with 4 L of supernatant from the plant digester and 1 L of substrate. See Table 3.6 for the contents of the seed reactor substrate. The reactor was fed daily and gradually brought up to a final working volume of 8 L on June 17, 1991. At this time the substrate was increased to a dextrose concentration of 4000 mg/L to allow for more gas production. The 8 L reactor was configured as shown in figure 3.10. The temperature in the reactor was maintained at 35 ± 2 °C by using a heated water bath connected to copper coils wrapped around the reactor, enclosed in insulation. The reactor was fed once a day using the fill and draw method; 1 L of effluent was drawn off, then one 1 L of substrate was fed to the system. The reactor had an 8-day hydraulic retention time (HRT).

The 8 L reactor was fed and monitored for more than 40 days, when the first test batch reactors (1 L) were started. All parameters were measured using a sample of the effluent from the 8 L or 1 L reactors. As a minimum, every HRT, a sample of the effluent from the 8 L reactor was analyzed for the following: pH, chemical oxygen demand (COD), volatile organic acid (VOA) concentration, and formic acid. Gas composition analysis was not performed due to the fact that to test for gas leakage the reactor

Table 3.6 - Seed reactor substrate

Substrate

Constituent	Concentration
dextrose	4 g/L
NH_4Cl	0.5 g/L
KH_2PO_4	4.25 g/L
Na_2HPO_4	17.72 g/L
trace salt solution	2 mL/L

Trace Salt Solution

Compound	Quantity, grams*
Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	3.27
$ZnCl_2$	4.74
$Na_2B4O_7 \cdot 10H_2O$	1.15
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	2.08
$FeCl_3 \cdot 6H_2O$	34.38
$CaCl_2 \cdot 2H_2O$	2.04
$CoCl_2 \cdot 6H_2O$	2.85

*compound were dissolved in 1L of deionized water

head space was filled with natural gas to test air tightness. This procedure was performed several times throughout this phase of the research. Under these circumstance, gas composition analysis would have been misleading. Formic acid was determined by the procedure established in the first phase of this research, and pH was measured using a pH meter.

Soluble COD was determined using manufacturer-prepared vials. A calibration curve was generated using KPH standards from 180 to 900 mg/L. This curve was used to correlate absorbance at 600 nm

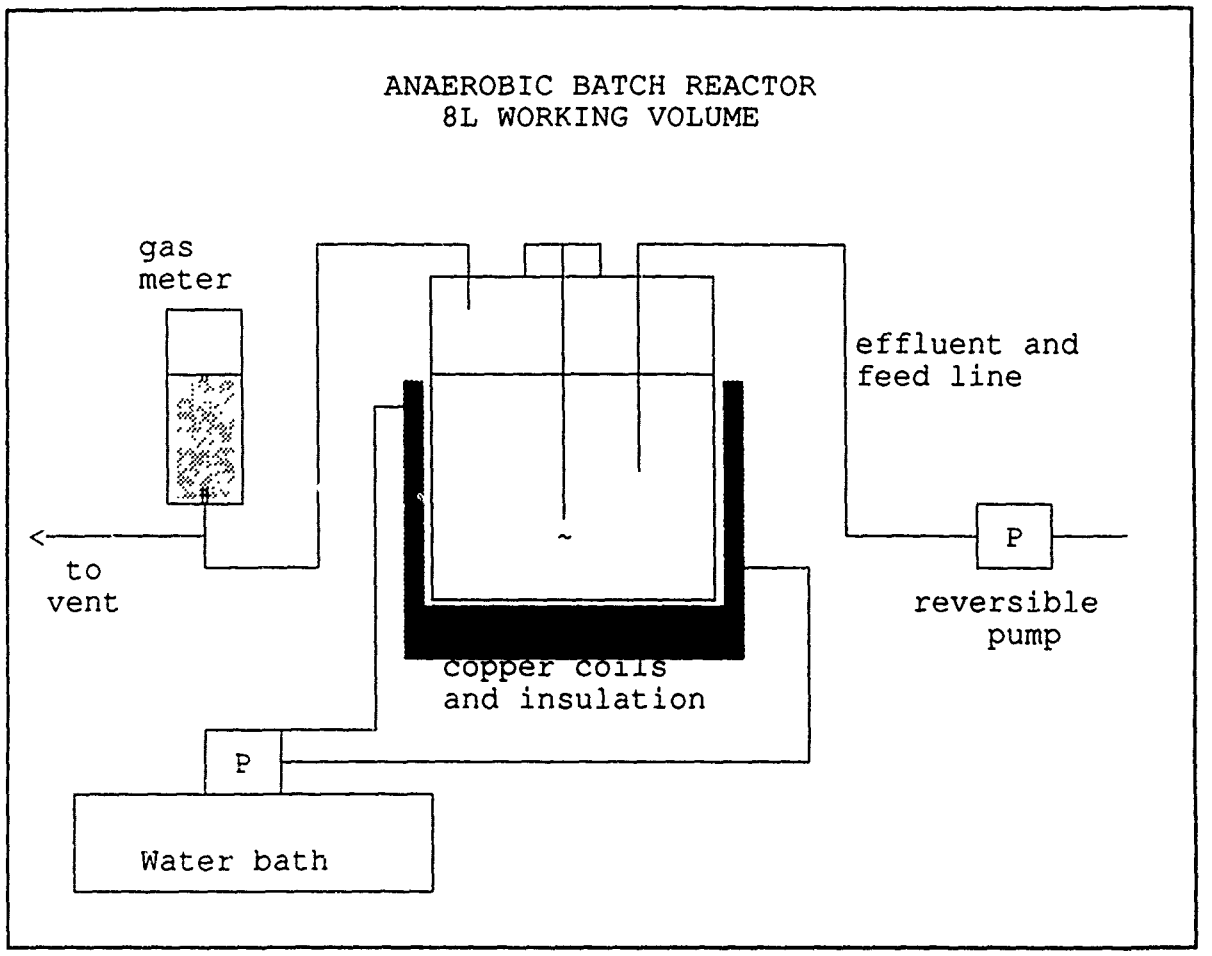


Figure 3.10 - Experimental setup for seed batch reactor

with COD. Two milliliters of sample, filtered with a 0.45 µm membrane filter and diluted if necessary, was placed in a vial. The vial was thoroughly shaken then heated for 2 hours. After cooling, the absorbance at 600 nm was observed.

Volatile acids were determined using a Hewlett-Packard model 5830A gas chromatograph equipped with a flame ionization detector. To prepare the standards, the following acids, in the approximate amounts shown, were dissolve in 100 mL of methanol:

8.5g	acetic acid	1.0g	i-valeric acid
4.0g	propionic acid	3.0g	valeric acid
1.0g	i-butyric acid	8.0g	hexanoic acid
10.0g	butyric acid		

For analysis of the standard, 100 µL of the stock solution was diluted to 10ml with organic free water; 2 µL of benzyl alcohol, an internal standard, was added. Five drops of H_3PO_4 (concentrated) were then added to the standard to ensure the pH was less then 4. Samples were also prepared in 10 mL volumetric flasks with the internal standard and H_3PO_4 added. The standards and samples were place into 5 mL sealed vials, ensuring no head space was present. Due to the small volume of sample available for analysis, no internal standard was used during testing. For analysis, 2 µL of the sample was injected into the gas chromatograph. The instrument parameters were as follows:

Injection temperature - 250 °C

Detector temperature - 300 °C

Initial oven temperature - 110 °C

Initial time - 2 min

Rate - 2 °C/min to 125 °C

Final oven temperature - 125 °C

Final time - 5 min

Carrier gas - helium

Flow - 10 mL/min

The concentration of each acid was determined as follows:

$$RF = \frac{\text{area acid in std}}{\text{area IS (std)}} \times \frac{\text{conc IS}}{\text{conc acid in std}},$$

where RF was the response factor, and IS was the internal standard.

$$\text{conc acid in sample} = \frac{\text{area acid in sample}}{\text{area IS (sample)}} \times \frac{\text{conc IS}}{\text{RF acid}}.$$

Without the internal standard,

$$\text{conc acid in sample} = \frac{\text{area acid in sample}}{\text{area acid in standard}} \times \text{conc acid in std}$$

The samples were analyzed immediately for pH. For the other analyses, the samples were stored at 25°F until analyzed; if VOA analysis was to be performed, the samples were stored with no head space in the bottle. Appendix C provides data for the process indicators monitored during the start-up phase of the research.

One-liter test reactors were used to analyze the effect of toxic loadings. They were seeded with 0.5 L or 1 L of effluent from the 8 L reactor at the time of feeding. To factor out the possible stress induced during transfer of effluent drawn from the seed reactor to the test reactors, a control reactor was started and monitored along with the reactors subject to toxic loadings.

In the first test, the test reactors were subjected to increased organic loadings of dextrose 2, 3 and 5 times that of the seed and control reactors. In the second test, chloroform was added to the substrate of the test reactors. In the first test reactor a concentration of 4 mg/L chloroform did not exhibit an increase in formic acid production; therefore, the third and fourth reactors were fed 20 and 100 mg/L chloroform, respectively. The

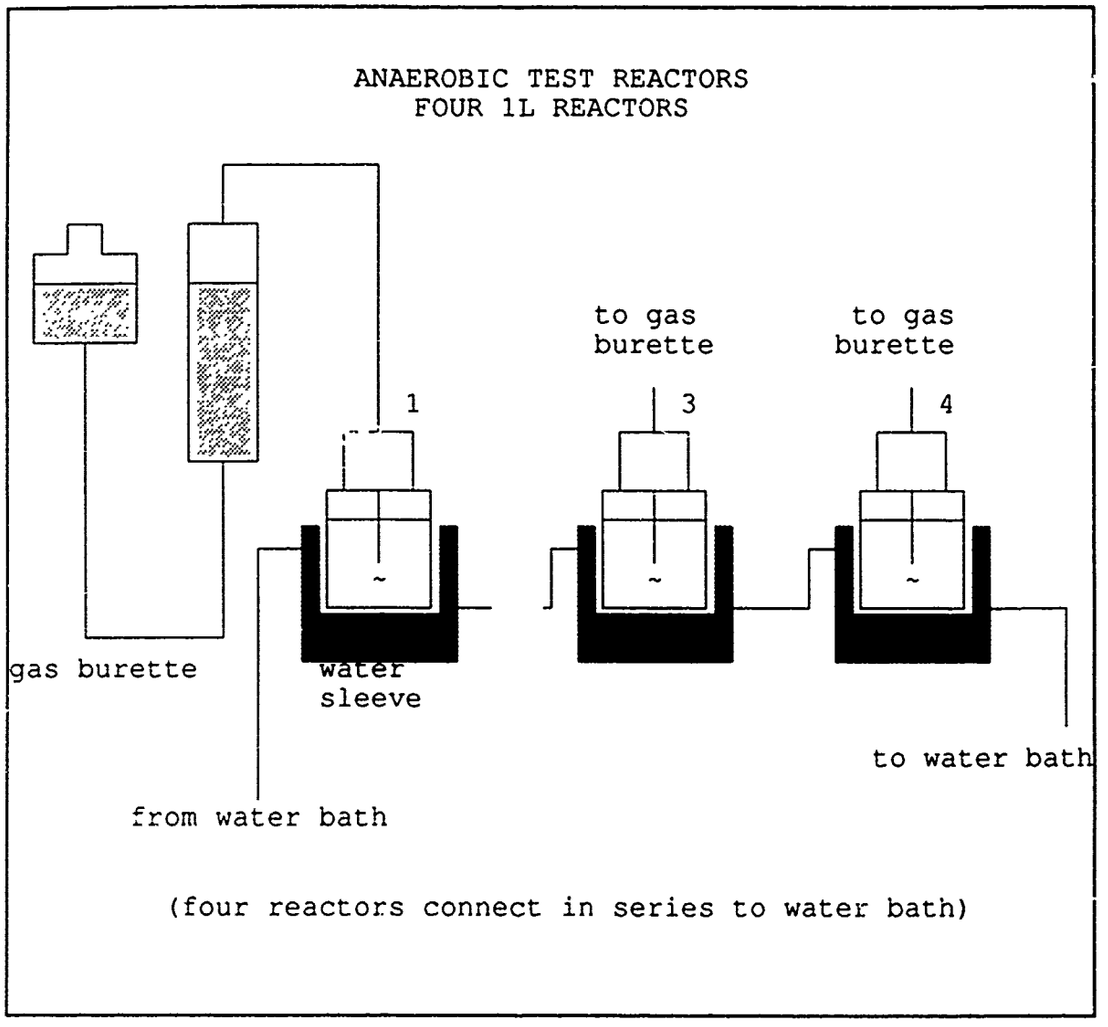


Figure 3.11 - Test reactor configuration

third test involved ammonia toxicity. A HRT of 8 days is relatively short for anaerobic systems; therefore, 50 mg/L NH_3 was selected because it was at the lower end of the toxicity threshold range of 40 to 100 mg/L. At a pH of 7, a concentration of 77.52 g/L $(NH_4)_2SO_4$ would provide 50 mg/L NH3 in solution. Due to the relatively short timeframe of the experiment, it was presumed that the amount of $NH_{3(g)}$ stripped out of solution was negligible. The

control reactor, at a pH of 7, contained less than 0.1 mg/L NH_3. The set up of the 1 L reactors is shown in figure 3.11.

The control reactor was fed the same substrate as the 8 L reactor for all analyses. The test reactors were fed the same

Table 3.7 - Test reactor substrates

ORGANIC LOADING

Test reactor	Dextrose
1	8 g/L
2	12 g/L
3	20 g/L

CHLOROFORM TOXICITY

Test reactor	Chloroform
1	4 mg/L
2	20 mg/L
3	100 mg/L

AMMONIA TOXICITY

Test reactor	NH_3
1	50 mg/L

substrate along with various toxic substances as noted in table 3.7. The testing was performed as indicated in table 3.8. To obtain samples, 20 mL of reactor contents was withdrawn then replaced with 20 mL of deionized water. The dilution factor was

not accounted for in the analysis of the reactor samples, nor is it reflected in the results presented in chapter 4. Assuming complete mixing, the dilution after the completion of twelve samplings left approximately 64% of the original sample in the reactor. Appendix D contains a table that estimates the approximate percentage of reactor volume not composed of deionized water, the percentage of original, undiluted sample. This refilling of the reactor volume was an attempt to maintain a constant liquid volume so the gas burettes could be used to determine gas production. However, the head space in the reactors was much greater than the reactor contents, and due to fluctuations in the temperature of the reactor and the laboratory, the gas burette readings were unreliable. For ammonia analysis test number 4 the samples removed were not replaced with deionized water in an attempt to obtain more accurate COD data. The test reactors were analyzed for pH, soluble COD, VOA's, formic acid and gas composition. The gas composition was determined on a Fisher gas analyzer. Since the samples from the 1 L reactors indicated no CH_4 production, samples were analyzed on VOA GC setup to determine if CH_4 was present since this instrument was more sensitive to CH_4.

The results of analyses for all parameters are presented in chapter 4. The raw data are contained in appendix D.

Table 3.8 - Test conditions

TEST CONDITIONS

TEST #	TOXIN	DURATION	SAMPLING
1	organic loading	12hrs	every hr
2	chloroform	12hrs	every hr
3	ammonia	12hrs	every hr
4	ammonia	12hrs	every hr
5	ammonia	24hrs	every 4hrs
6	ammonia	120hrs	every 12hrs
7	unknown	120hrs	every 12hrs

IV. RESULTS

The data from the results of the toxicity analyses are provided for pH, COD, VOA, and formic acid. The data for all these parameters are presented based on the substances added to produce the shock or toxicity.

TEST 1 - SUBSTRATE CONCENTRATION INCREASE

As indicated earlier, to study the affect of increased substrate concentration on anaerobic batch reactors, test reactors were subjected to substrate concentrations (as dextrose) 2, 3, and 5 times that of the control and seed reactors. The pH data are presented in table 4.1. The buffer capacity of the system was sufficient to maintain the pH around neutral for all tests. In the substrate concentration test, the pH ranged from 6.93 to 7.11.

Table 4.1 - pH analysis of effluent from reactors fed various concentrations of substrate

SUB CONC	SEED	TIME, HRS 1	2	3	4	5	6	7	8	9	10	11	12
CONTROL	7.38	NA	NA	7.09	7.09	7.09	7.06	7.07	7.09	7.09	7.05	7.09	7.05
8 g/L	7.38	NA	NA	7.08	7.07	7.11	7.10	7.05	7.09	7.08	7.06	7.09	7.08
12 g/L	6.93	7.05	7.02	7.00	7.02	6.96	7.02	7.02	6.96	6.97	6.99	6.98	7.01
20 g/L	6.93	7.05	7.01	6.99	6.98	7.02	7.02	7.01	6.97	6.97	6.98	7.00	7.01

During this test, the change in COD was sporadic; however, the overall trend indicated a slight decrease, as indicated in figure

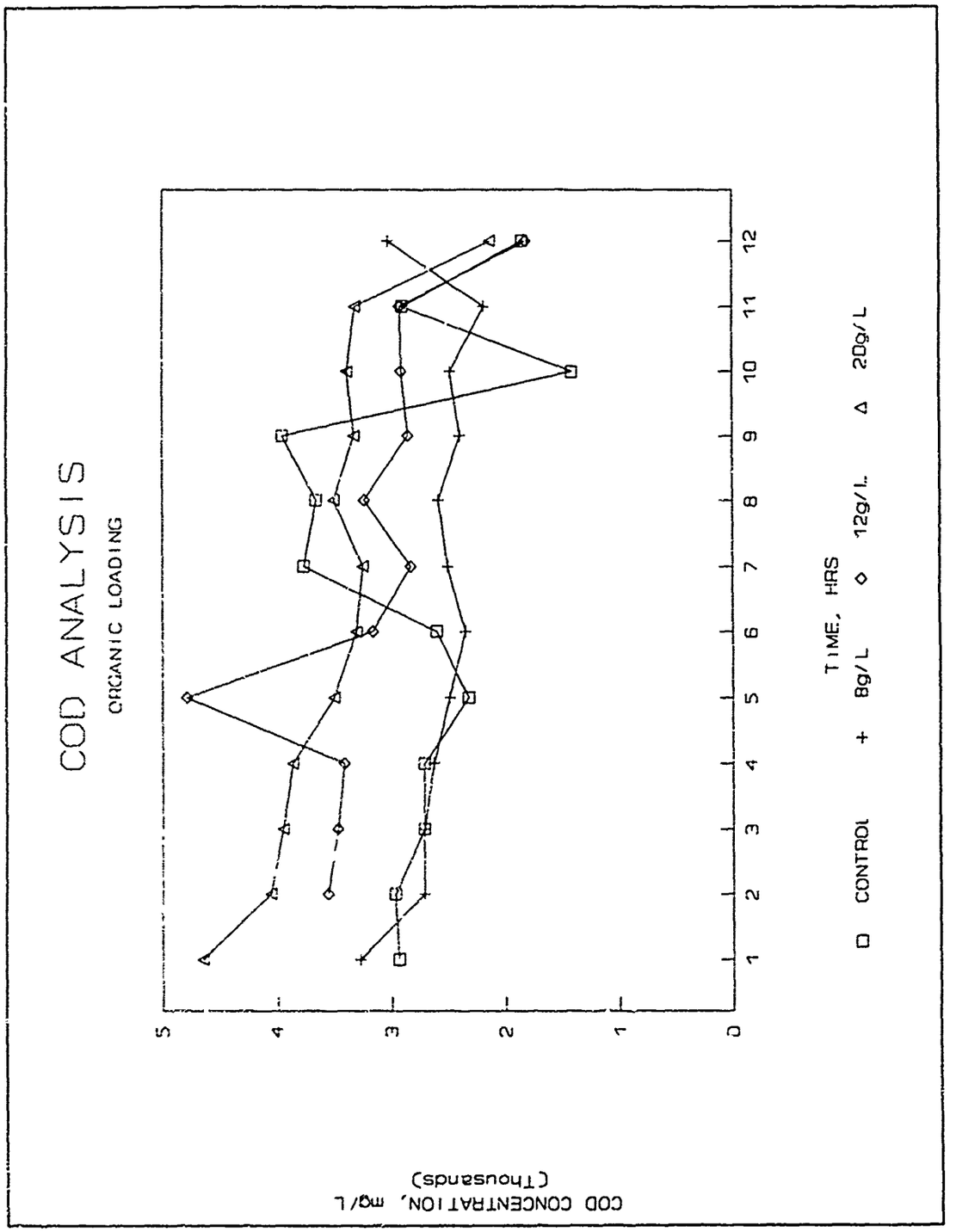


Figure 4.1 - COD analysis of effluent from reactors fed various substrate concentrations

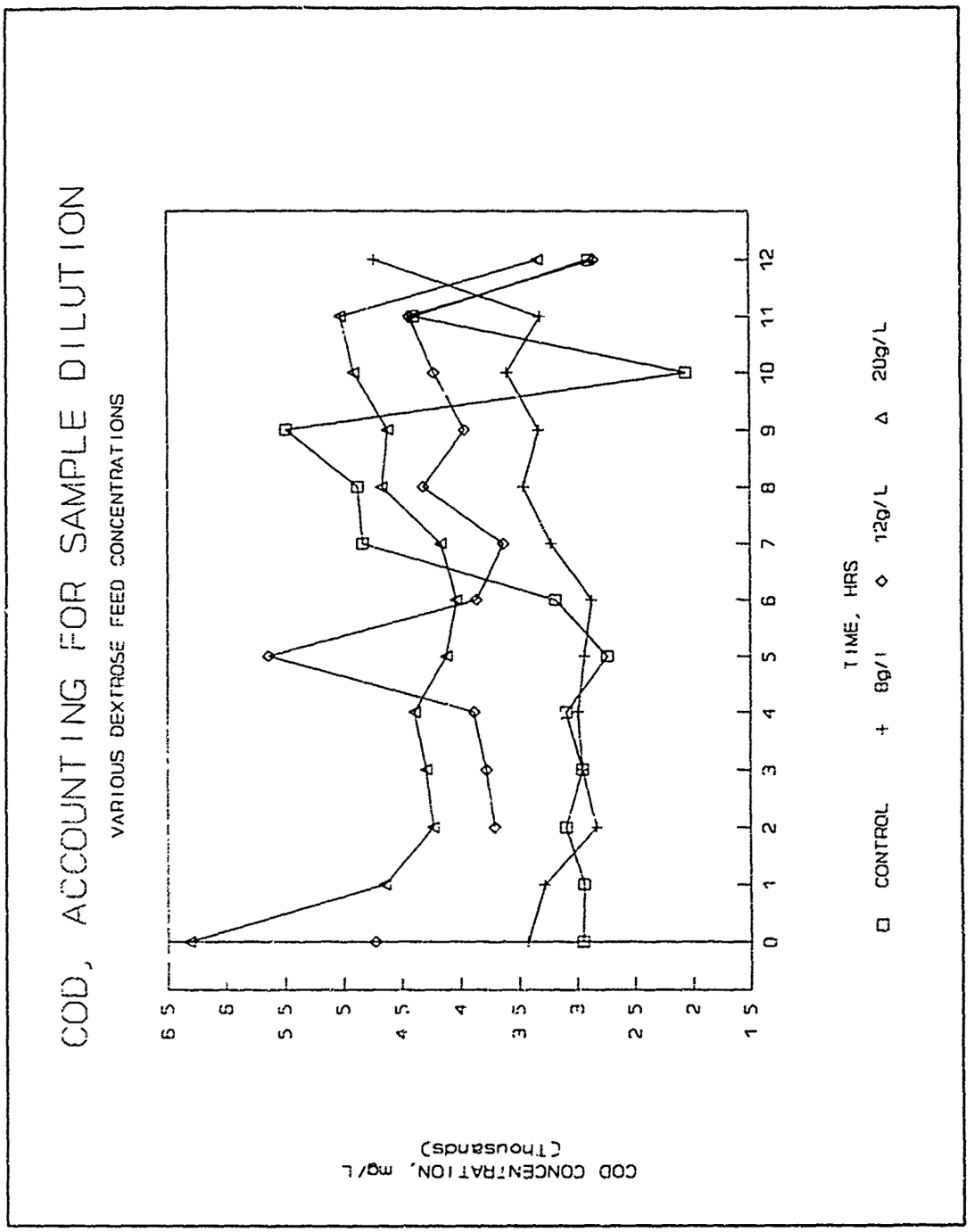


Figure 4.2 - COD concentration corrected for the dilution of the samples of effluent from reactors fed various substrate concentrations

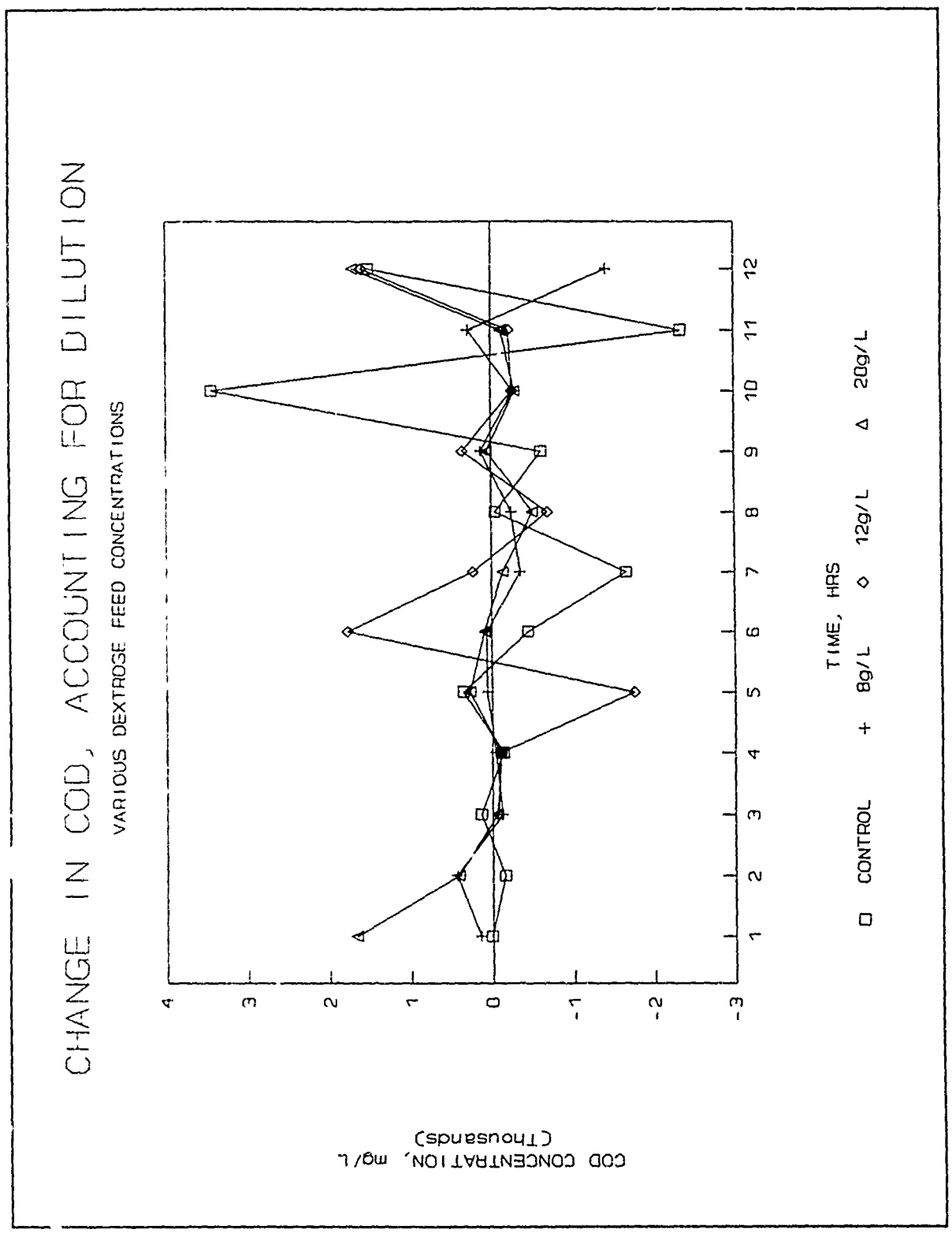


Figure 4.3 - Change in COD concentration versus time for effluent from reactors fed various substrate concentrations

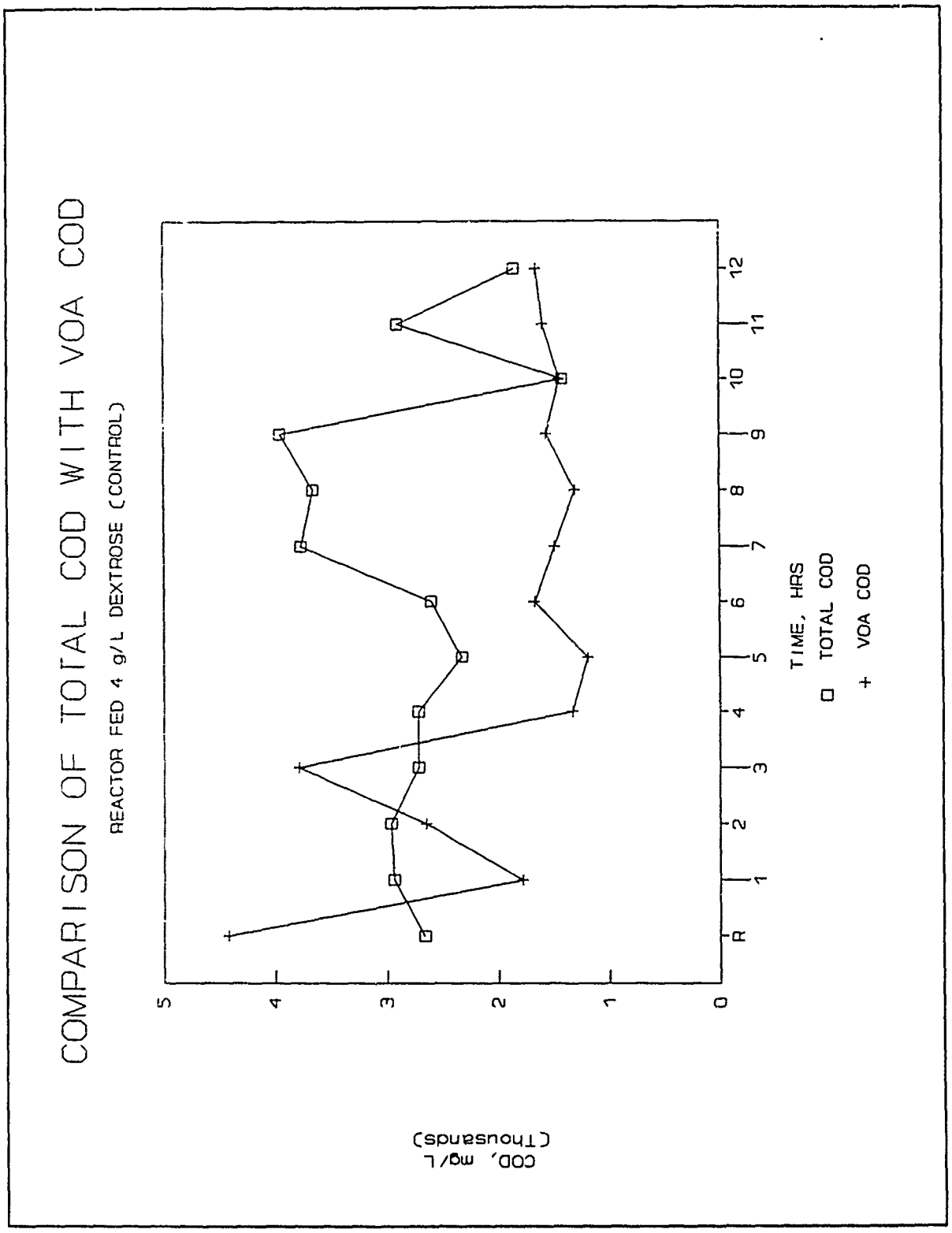


Figure 4.4 - Comparison of total COD with COD due to VOA, reactor fed substrate containing 4 g/L dextrose (control)

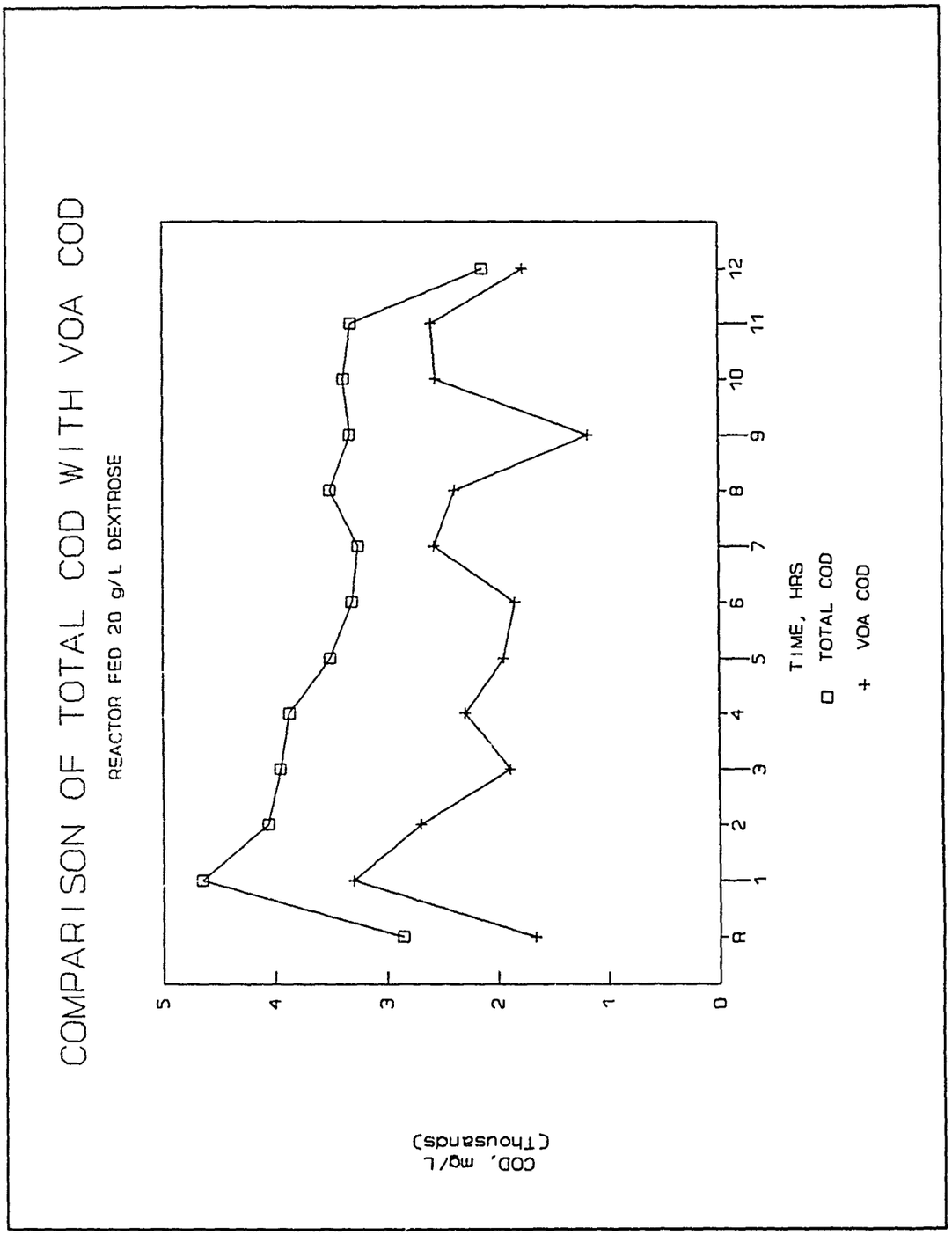


Figure 4.5 - Comparison of total COD with COD due to VOA, reactor fed substrate containing 20 g/L dextrose

4.1. The change in COD noted was due primarily to the dilution of the reactor contents with deionized water during sampling. A plot of COD concentration which has been corrected for dilution of the sample is provided in figure 4.2. As shown, the trends are similar to that of the uncorrected graph. A plot of the change in COD versus time is shown in figure 4.3. The results were erratic, but indicate that the overall change in COD was minimal in all reactors. There was no significant change in COD versus time in this test, and the remaining tests indicated only minor changes in COD. Therefore, a plot of the change in COD versus time is not provided for the remaining tests. Figures 4.4 and 4.5 are graphs comparing COD due to volatile acids with the total COD for the control reactor and the reactor fed substrate containing 20 g/L dextrose. As shown the average percentage of COD due to VOA's was 65 to 75%. The remaining tests proved that the percentage of COD from VOA's ranged from 54% to 94%, averaging around 80%. This data is not presented for the remaining tests. Gas analyzer data showed no indication of CH_4 production. However, analysis of gas phase samples on the VOA GC setup indicated CH_4 was present in the control reactor at the end of the test period of 12 hours. This same analysis of the test reactor did not indicate the presence of CH_4. It was concluded that there was some CH_4 production in the control reactor; however, it was very low. Therefore, the change in COD was also very low. As noted in chapter 3, the gas volume data was not useful.

Appendix D provides the VOA concentrations, while figure 4.6 indicates the total VOA's for the control and test reactors. The concentration of total organic acids was dynamic for all reactors, but generally the control reactor maintained the lowest concentrations.

The formic acid analysis indicated the greatest concentration increase for the test reactor containing 8 g/L of dextrose, but the other test reactors contained considerably less formic acid then the control reactor. The results of this analysis are shown in figure 4.7. The separation between acetic and formic acid on the ion chromatograph was good for the control reactor and the reactor feed 8 g/L of dextrose. For the test reactors with 12 and 20 g/L the separation between the acetic and formic peaks was not a distinct. Figures 4.8 through 4.11 graphically display the comparison between the concentrations of formic acid, acetic acid and total VOA's in the control and test reactors. For the 12 and 20 mg/L reactors, the acetic acid concentrations are slightly higher than the control reactor, while the formic acid concentrations were lower.

TEST 2 - CHLOROFORM TOXICITY

As indicated in table 3.7, the test reactors contained 4, 20 and 100 mg/L $CHCl_3$. During this test pH range was very narrow, from 6.89 to 7.10, as shown in table 4.2. The soluble COD indicated a downward trend, as presented in figure 4.12. The gas chromatographic analysis indicated some CH_4 was produced in all of

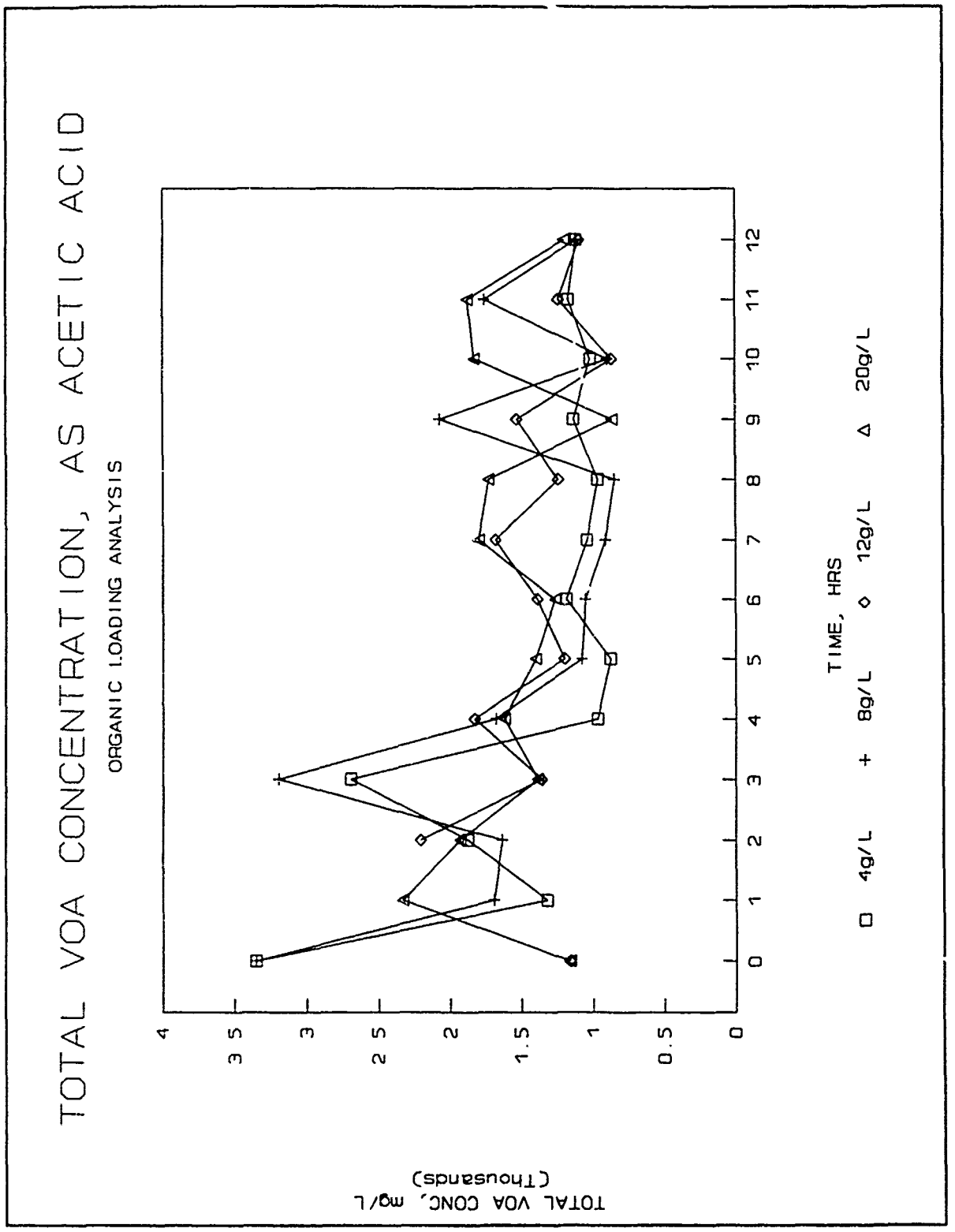


Figure 4.6 - VOA analysis of effluent from reactors fed various substrate concentrations

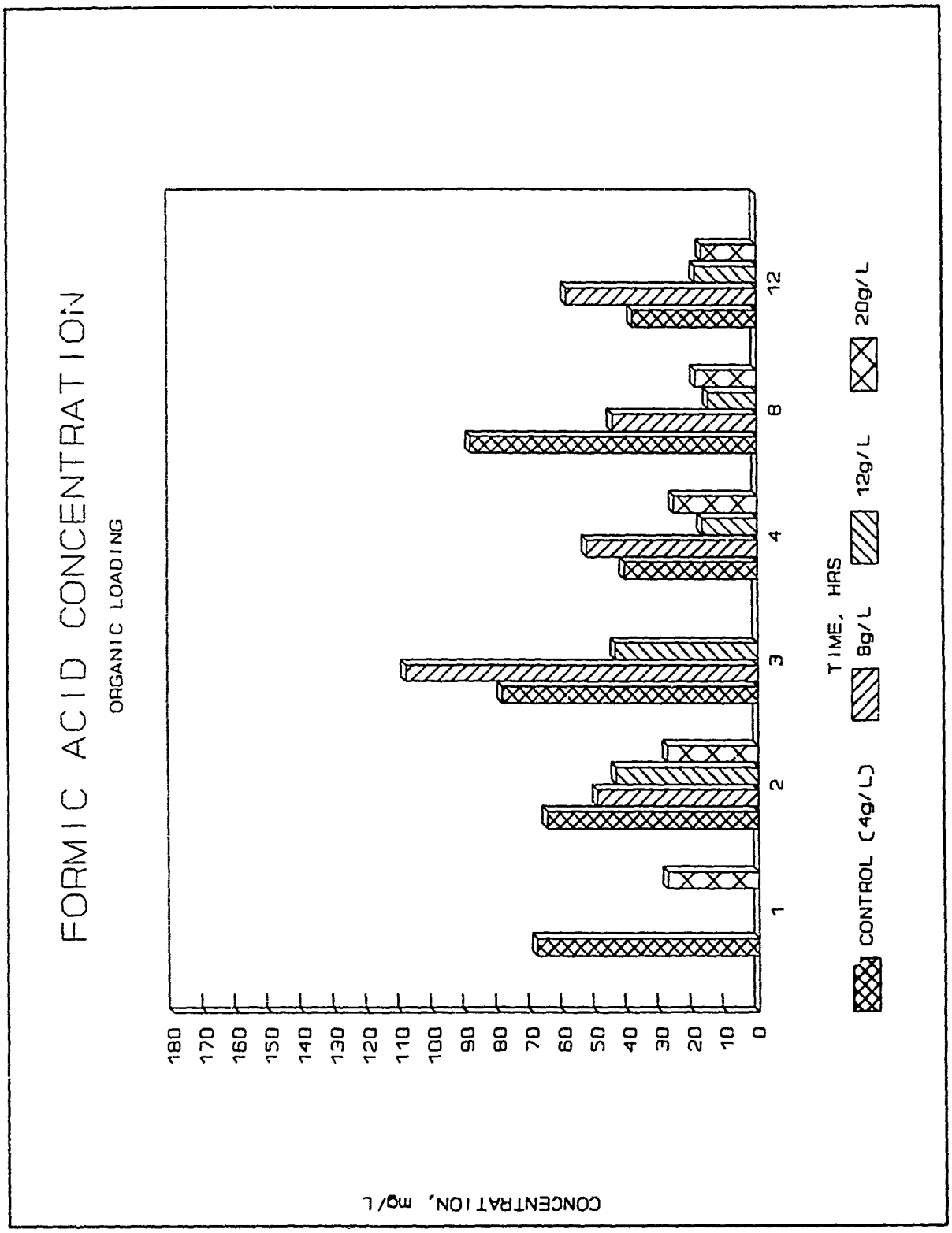


Figure 4.7 - Formic acid analysis of effluent from reactors fed various substrate concentrations

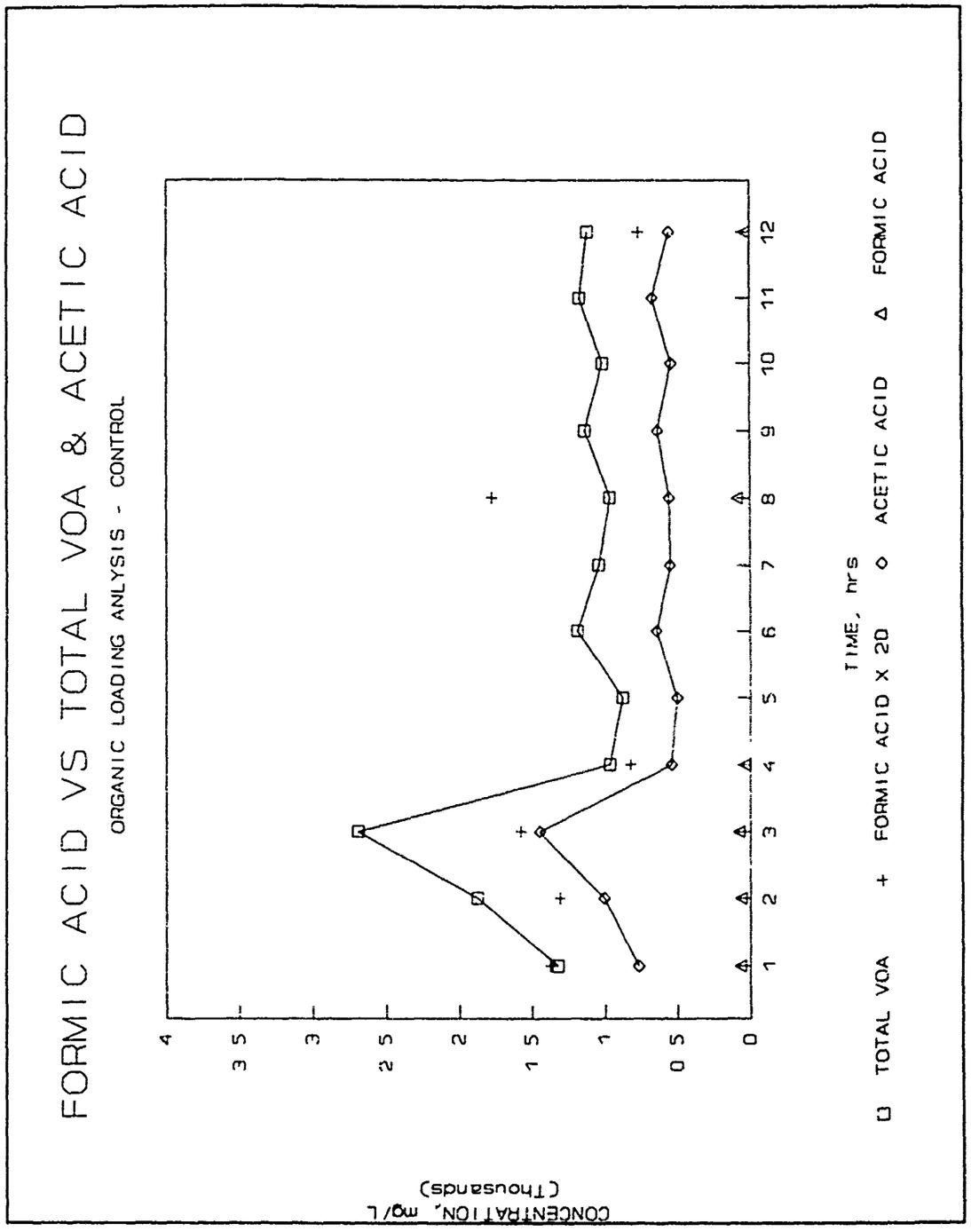


Figure 4.8 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate concentration of 4 g/L dextrose

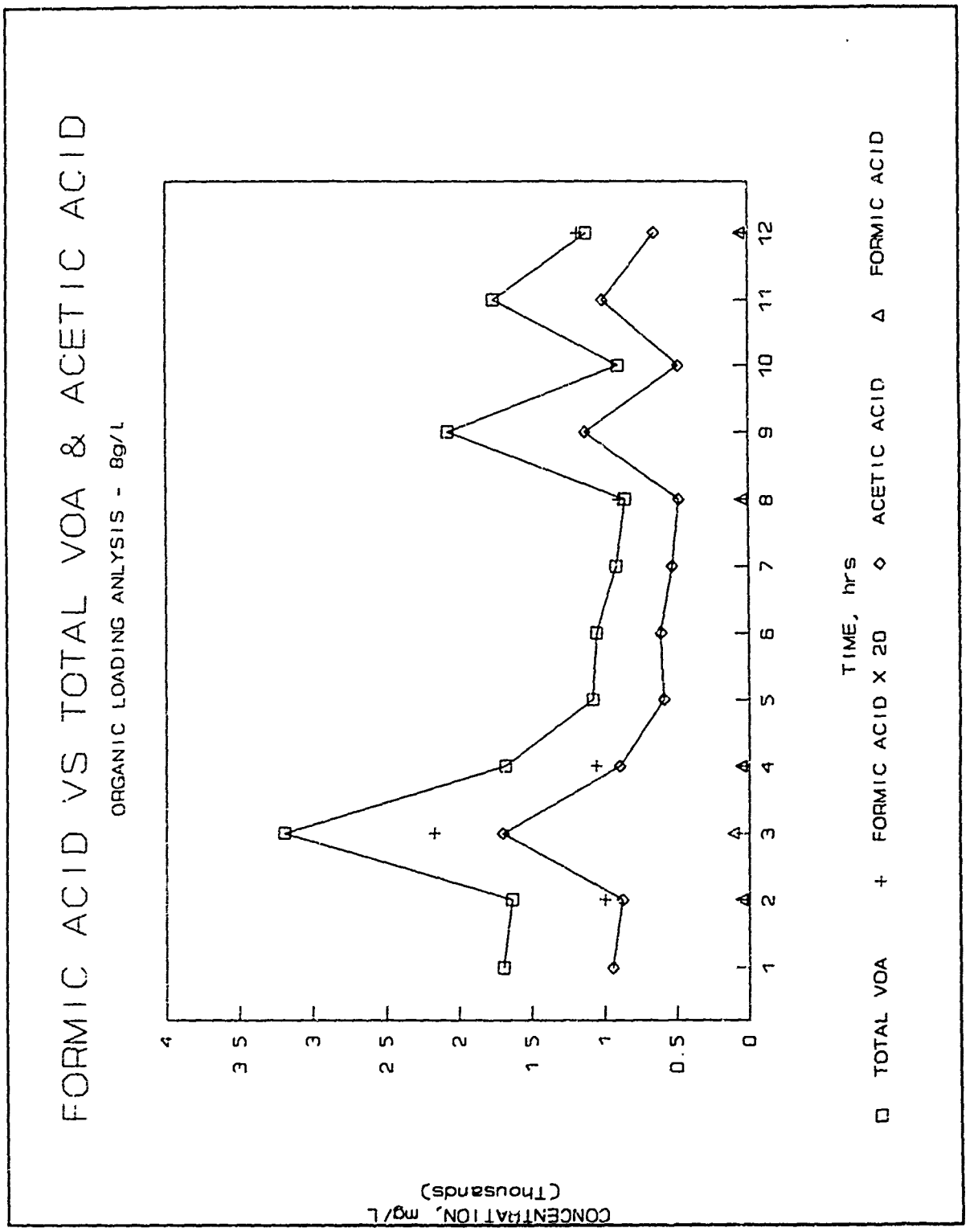


Figure 4.9 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate concentration of 8 g/L dextrose

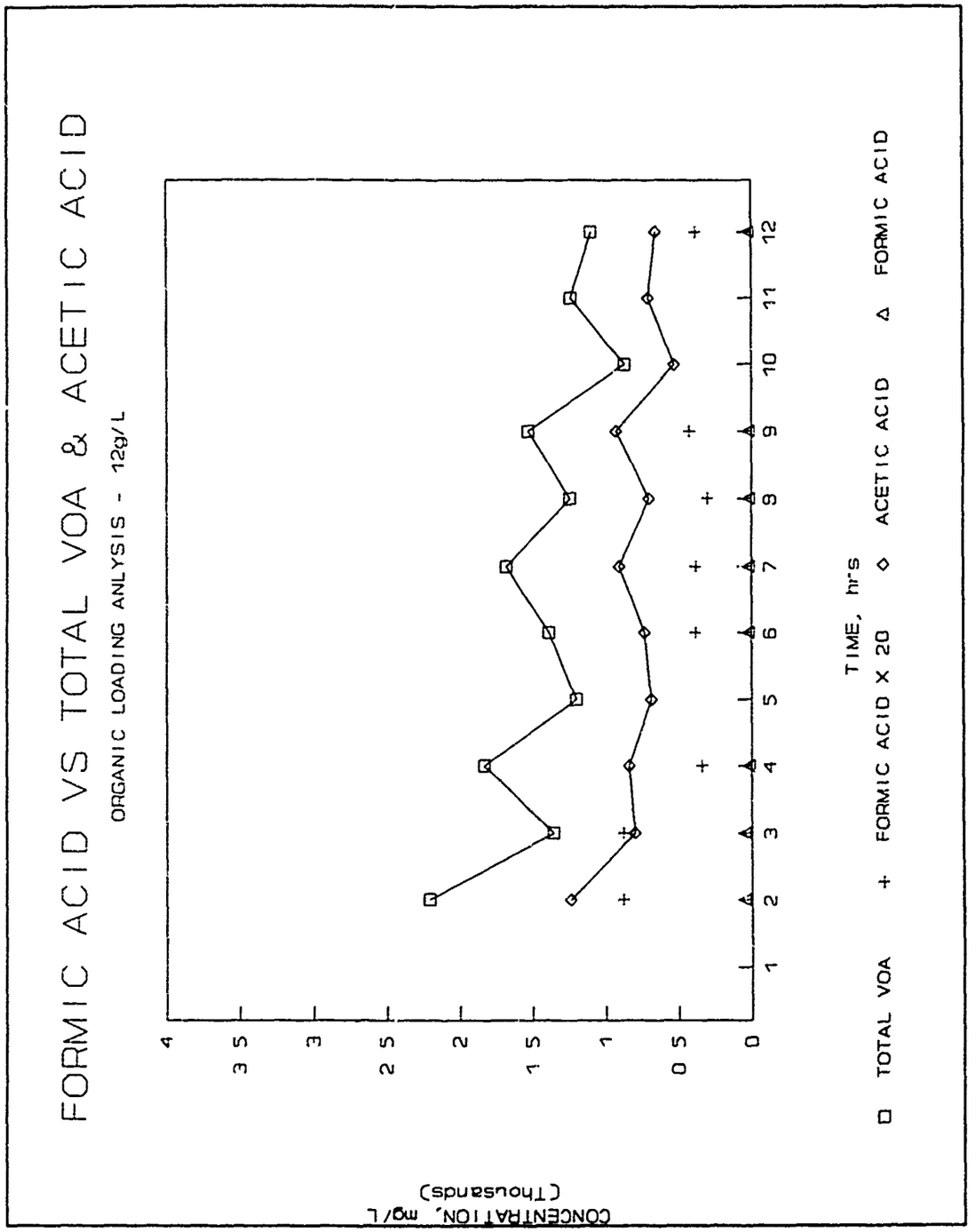


Figure 4.10 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate concentration of 12 g/L dextrose

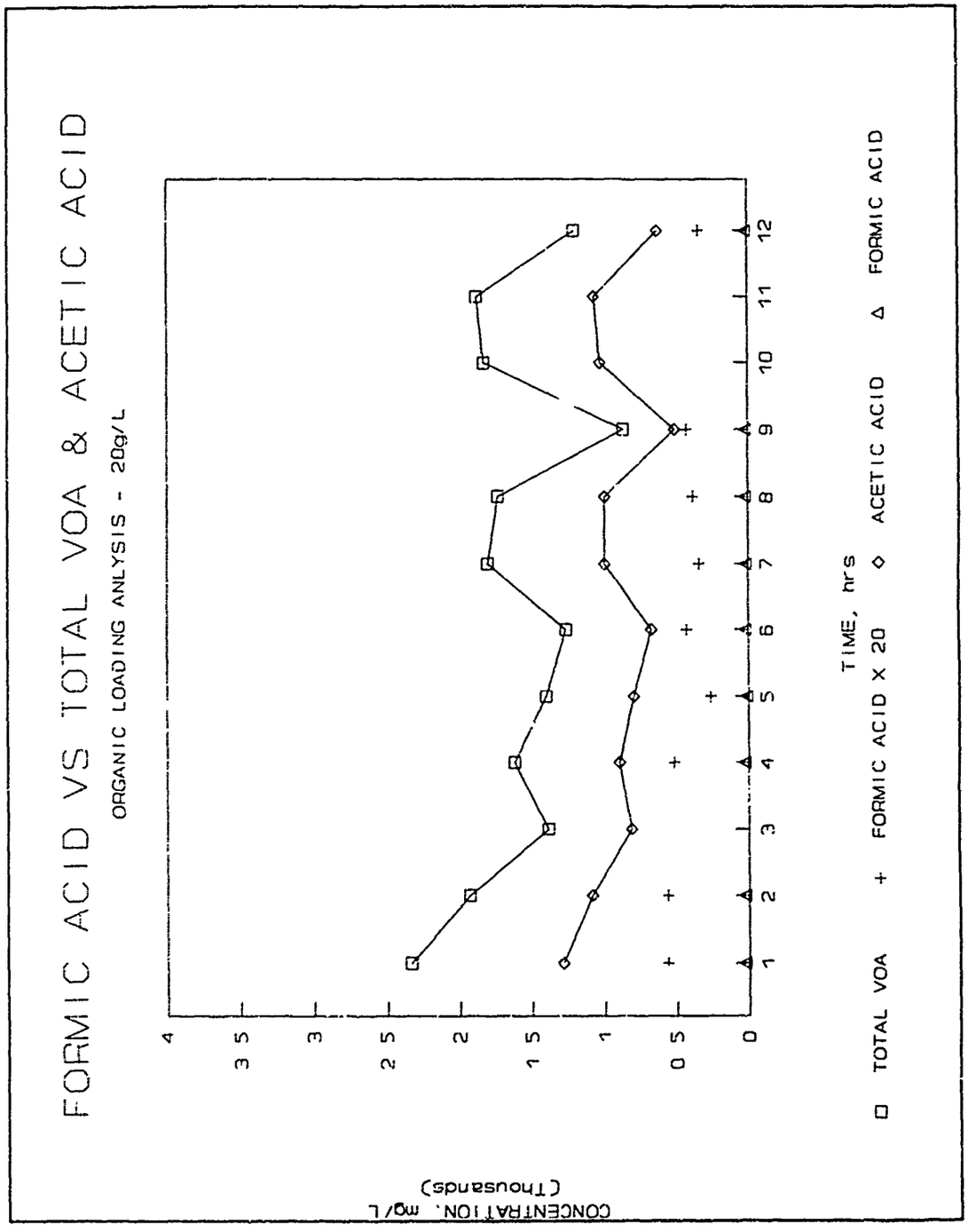


Figure 4.11 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate concentration of 20 g/L dextrose

Table 4.2 - pH analysis of effluent from reactors fed substrate containing various concentrations of chloroform

CH_3Cl CONC	TIME, HRS											
	SEED	1	2	3	4	5	6	7	8	9	10	11	12
CONTROL	6.89	6.99	7.03	7.02	7.02	7.01	7.02	7.06	7.10	7.07	7.05	7.07	7.05
4 mg/L	6 89	6.98	6.99	6.99	7.00	6.98	6.98	7.04	7.10	7.07	7.02	7.06	7.05
20 mg/L	6.89	6.96	7.03	7.02	7.04	7.04	7.04	7.05	7.00	NA	7.05	7.05	7.06
100 mg/L	6.89	6.93	6.95	7.03	7.02	7.00	7.00	7.03	6.98	NA	7.04	7.03	7.05

the reactors. As noted in chapter 3, no gas production or CH_4 concentration data was obtained.

The VOA analyses indicated a slightly higher concentration in the control than the test reactors; figure 4.13 presents these data. The results from the formic acid analysis indicate a more rapid decrease in formic acid concentration in the control reactor than in the test reactors and a considerable increase in formic acid in all reactors at the end of the test period, especially in the reactor fed 100 mg/L $CHCl_3$. The large increase in formic acid concentration at hour 12 followed the same pattern as the other VOA's in the control and 20 mg/L reactors. Figure 4.14 presents the formic acid data, and figures 4.15 through 4.18 indicate the relationship between formic and acetic acids and total VOA's.

TEST 3 - AMMONIA TOXICITY NUMBER 1

Four tests were run on reactors exposed to toxic dosages of ammonia as indicated in table 3.11. All test reactors were feed 50 mg/L NH_3 as indicated in chapter 3. In test number 1, the substrate was fed at the time the seed effluent was added to the 1 L reactors. In tests number 2, 3 and 4 the 1 L reactors were

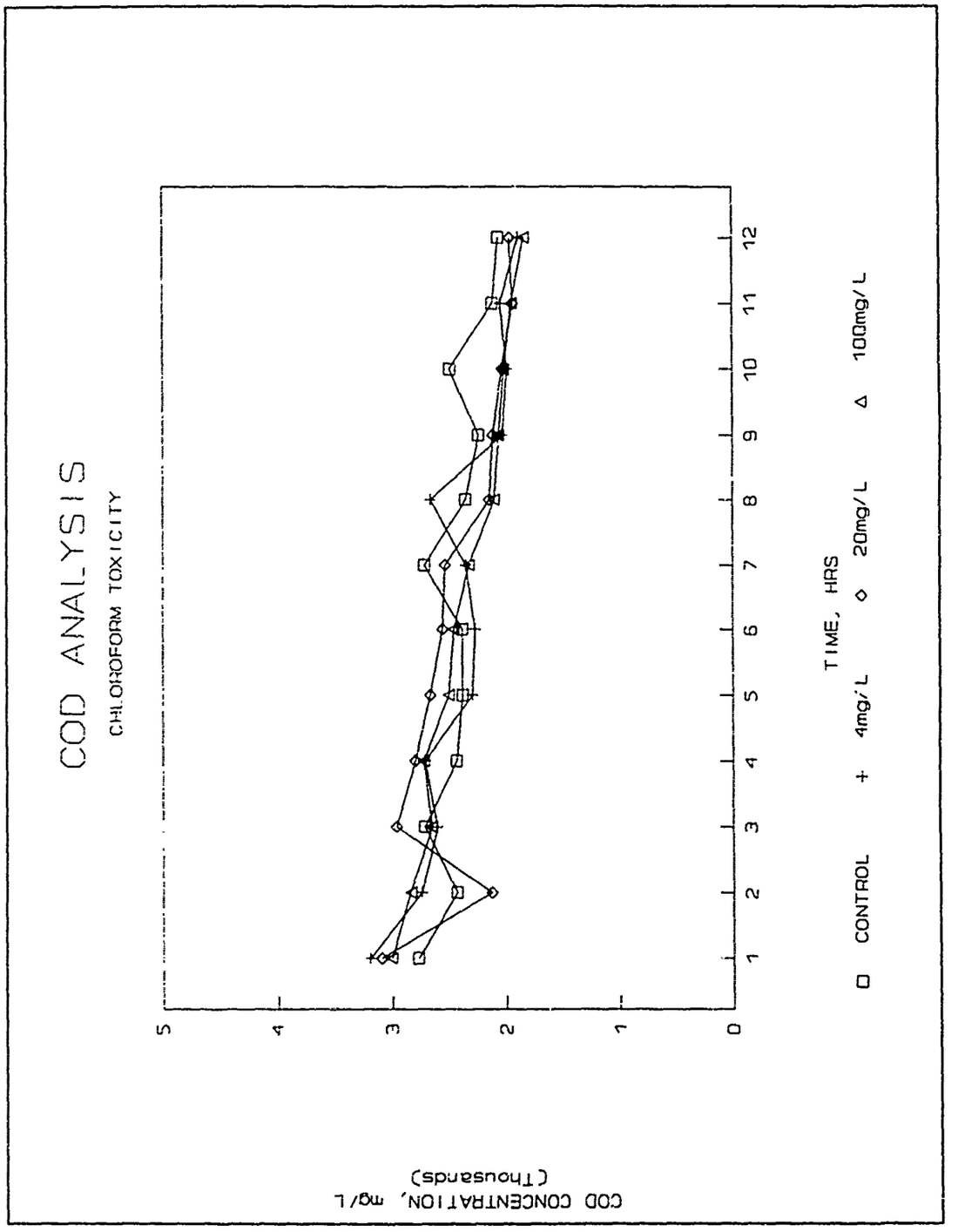


Figure 4.12 - COD analysis of effluent from reactors fed substrates containing various concentrations of chloroform

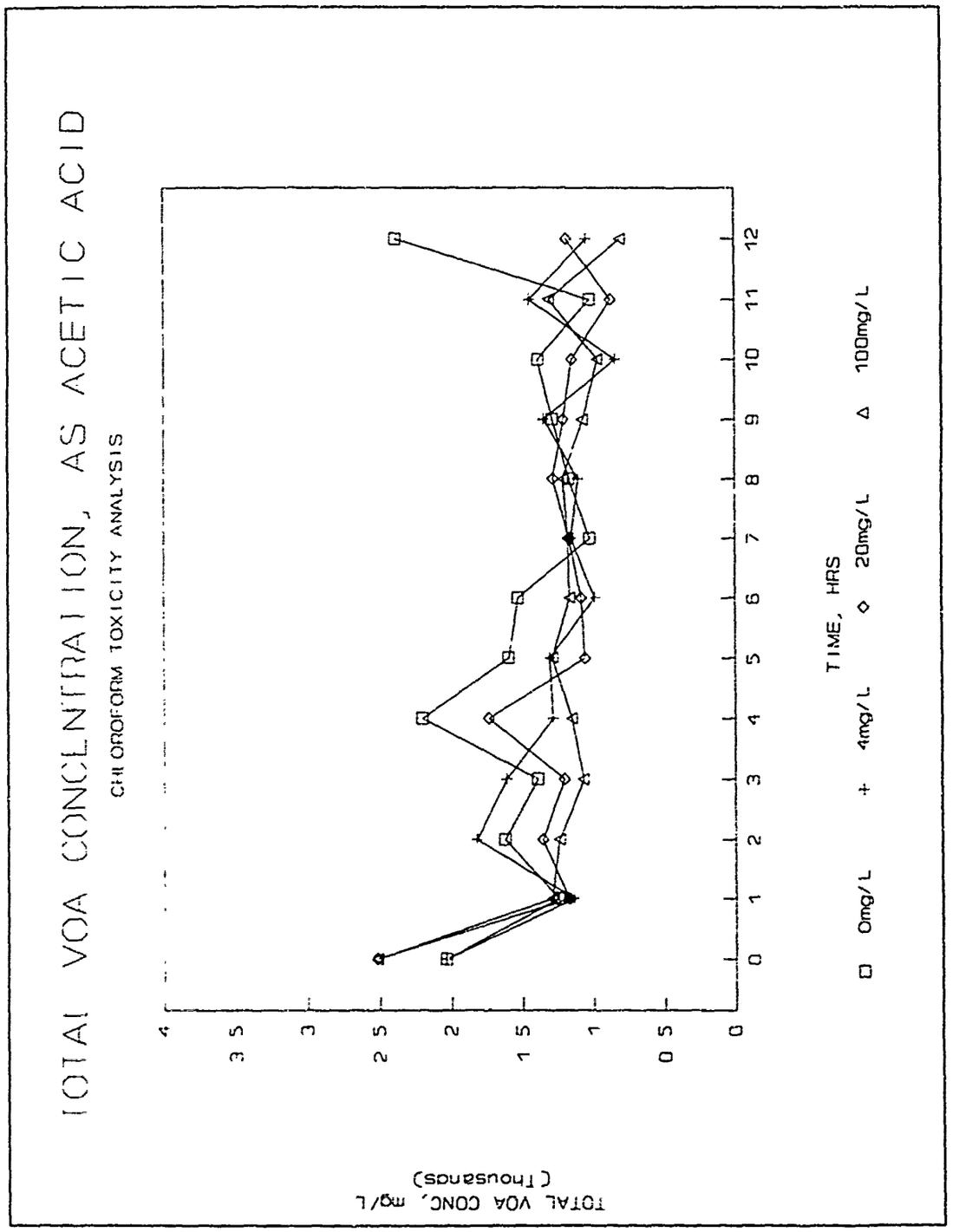


Figure 4.13 - VOA analysis of effluent from reactors fed substrates containing various concentrations of chloroform

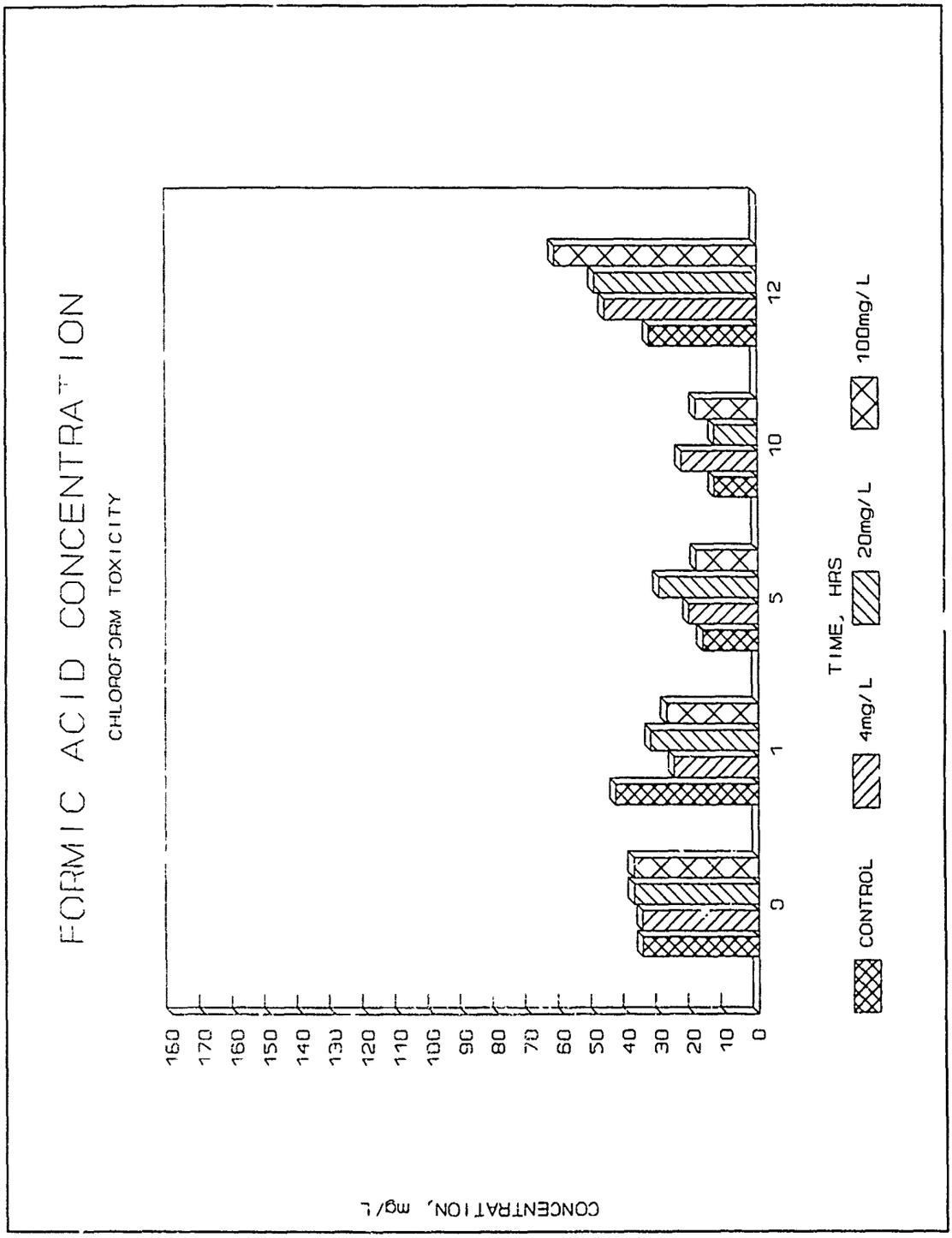


Figure 4.14 - Formic acid analysis of effluent from reactors fed substrates containing various concentrations of chloroform

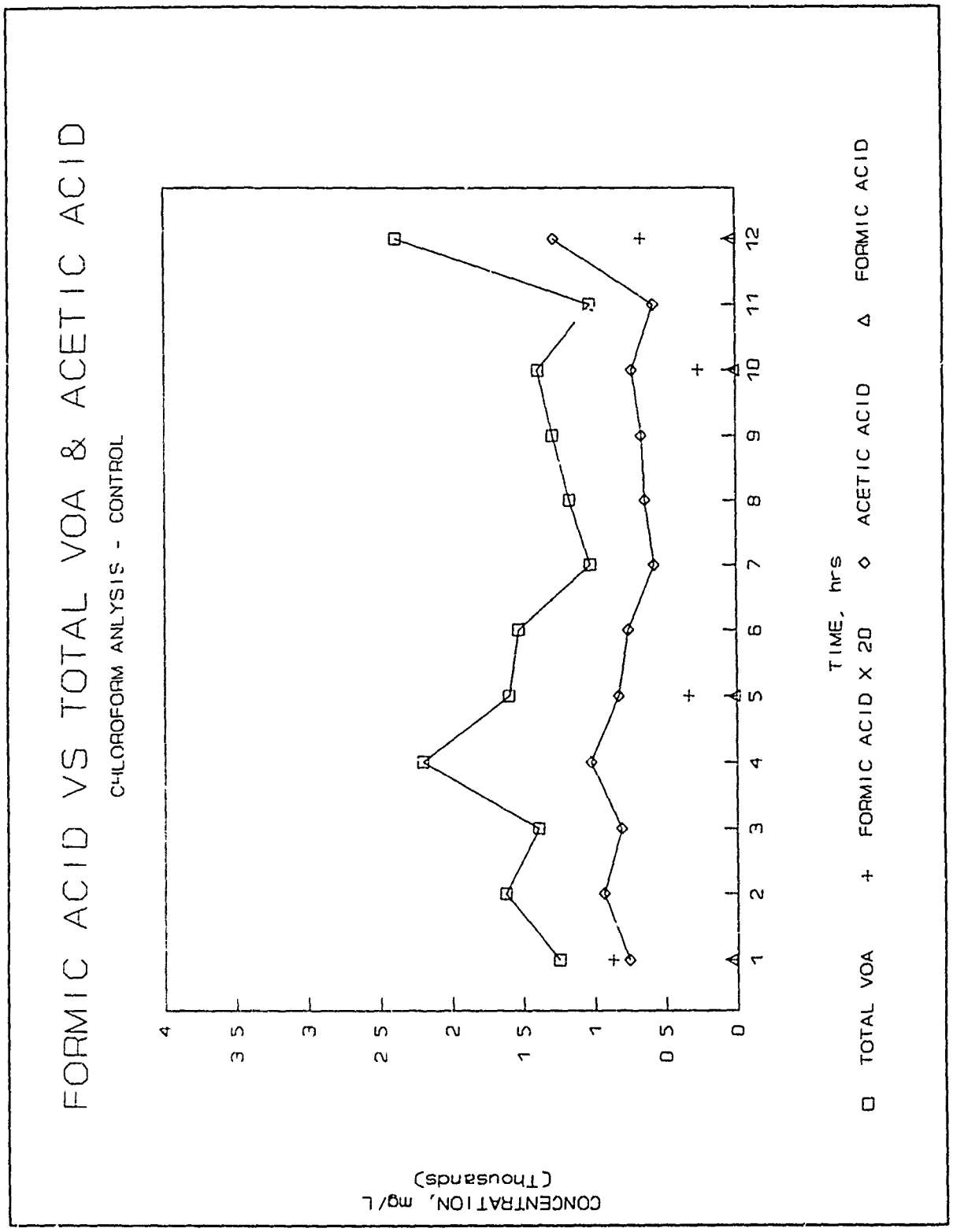


Figure 4.15 - Comparison of HCOOH with VOA concentrations of effluent from control reactor in chloroform toxicity analysis

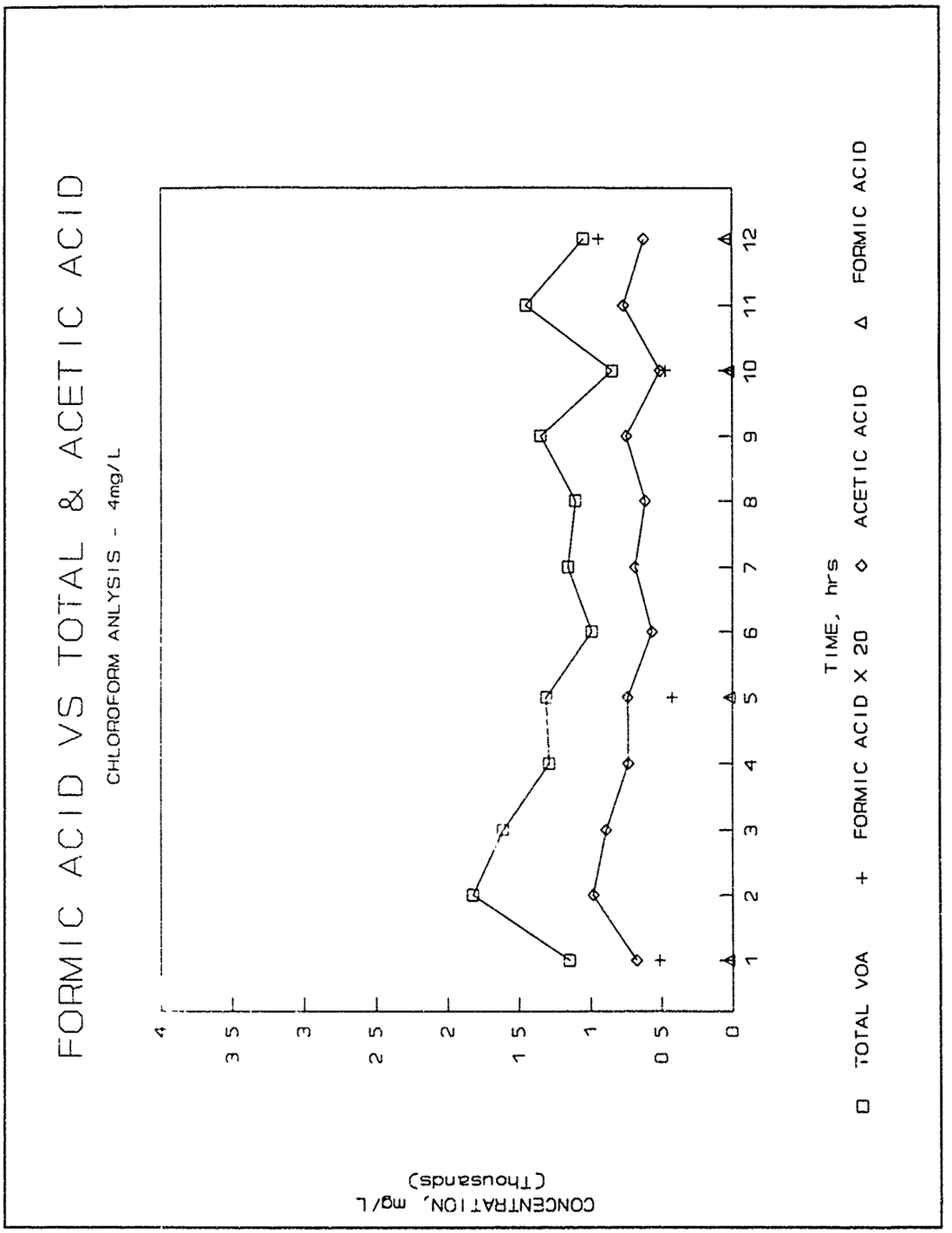


Figure 4.16 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate containing 4 mg/L chloroform

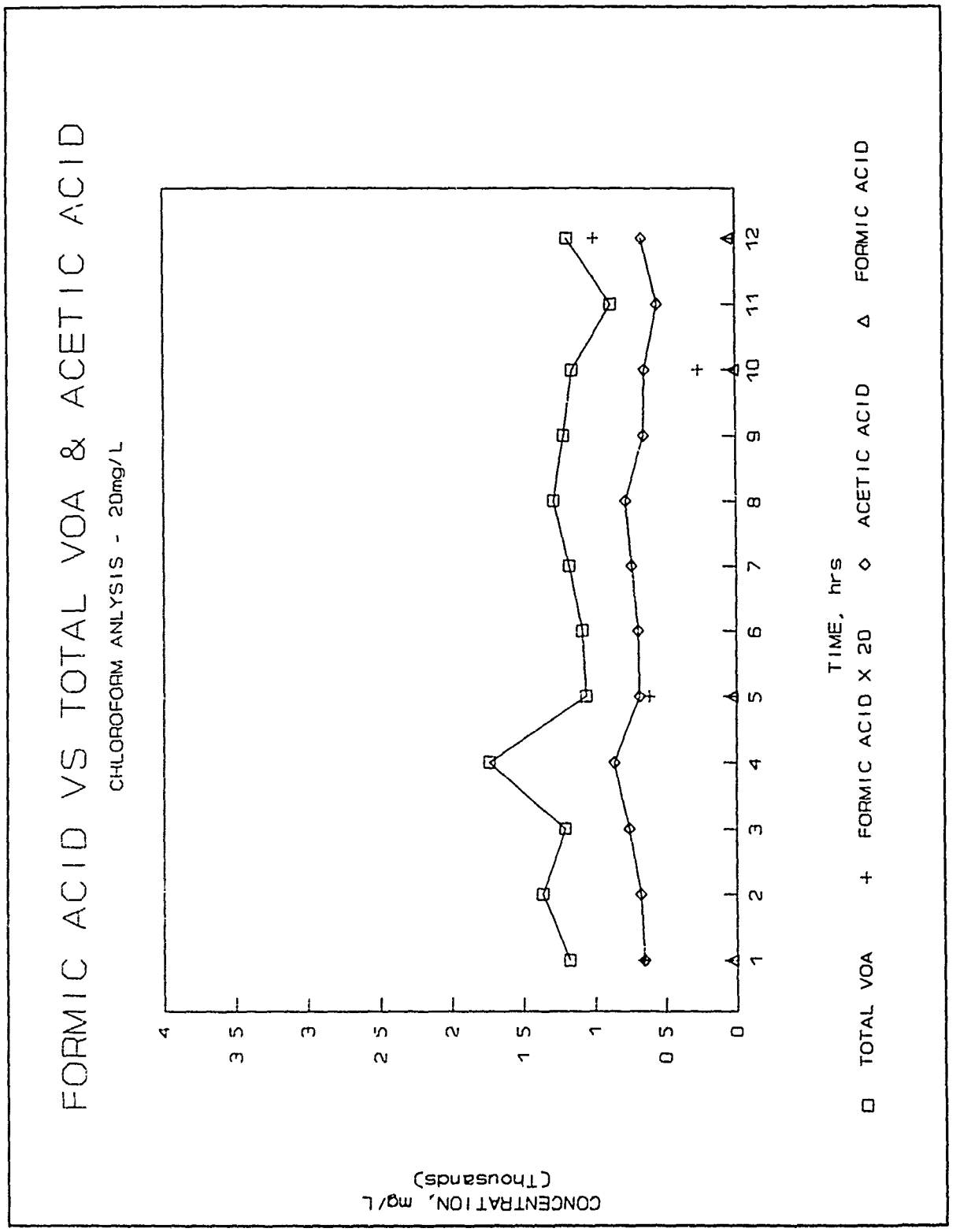


Figure 4.17 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate containing 20 mg/L chloroform

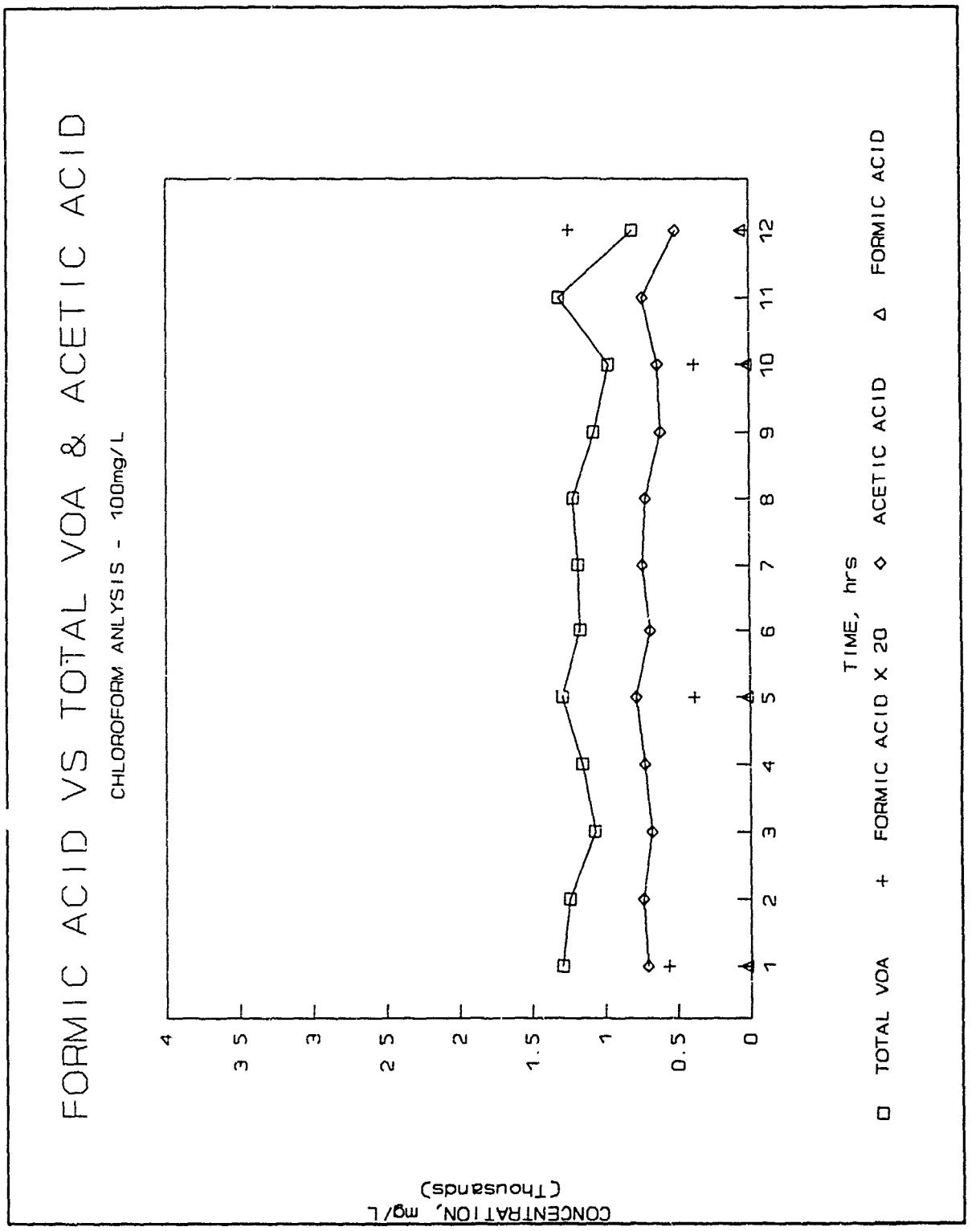


Figure 4.18 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate containing 100 mg/L chloroform

seeded, one hour later, at time zero, samples were obtained, then the reactors were fed the appropriate substrate. As presented in table 4.3, in the first analysis the pH for both the control and test reactor ranged from 6.83 to 6.96. In figure 4.19 the COD demonstrated a general downward trend.

The VOA concentration showed similar trends for both the test

Table 4.3 - pH analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #1

NH_3 CONC	SEED	TIME, HRS 1	2	3	4	5	6	7	8	9	10	11	12
CONTROL	6.88	6.95	6.94	6.92	6.92	6.95	6.95	6.96	NA	6.96	6.96	6.96	6.96
50 mg/L	6.88	6.86	6.83	6.86	6.84	6.86	6.84	6.87	NA	6.87	6.89	6.89	6.91

and control reactors, as shown in figure 4.20. However, the formic acid concentration was much greater in the test reactor. Again, there was good separation between the formic and acetic acid peaks. Figures 4.21, 4.22 and 4.23 indicate the formic acid concentration alone as compared with acetic in the control and test reactors. The volatile acid concentrations in the control reactor showed more activity then the test reactor. The VOA and formic acid analysis indicated high concentrations of each from the seed reactor that were quickly reduced. The formic acid concentration followed the same general trend as that in the test reactor, only at lower levels.

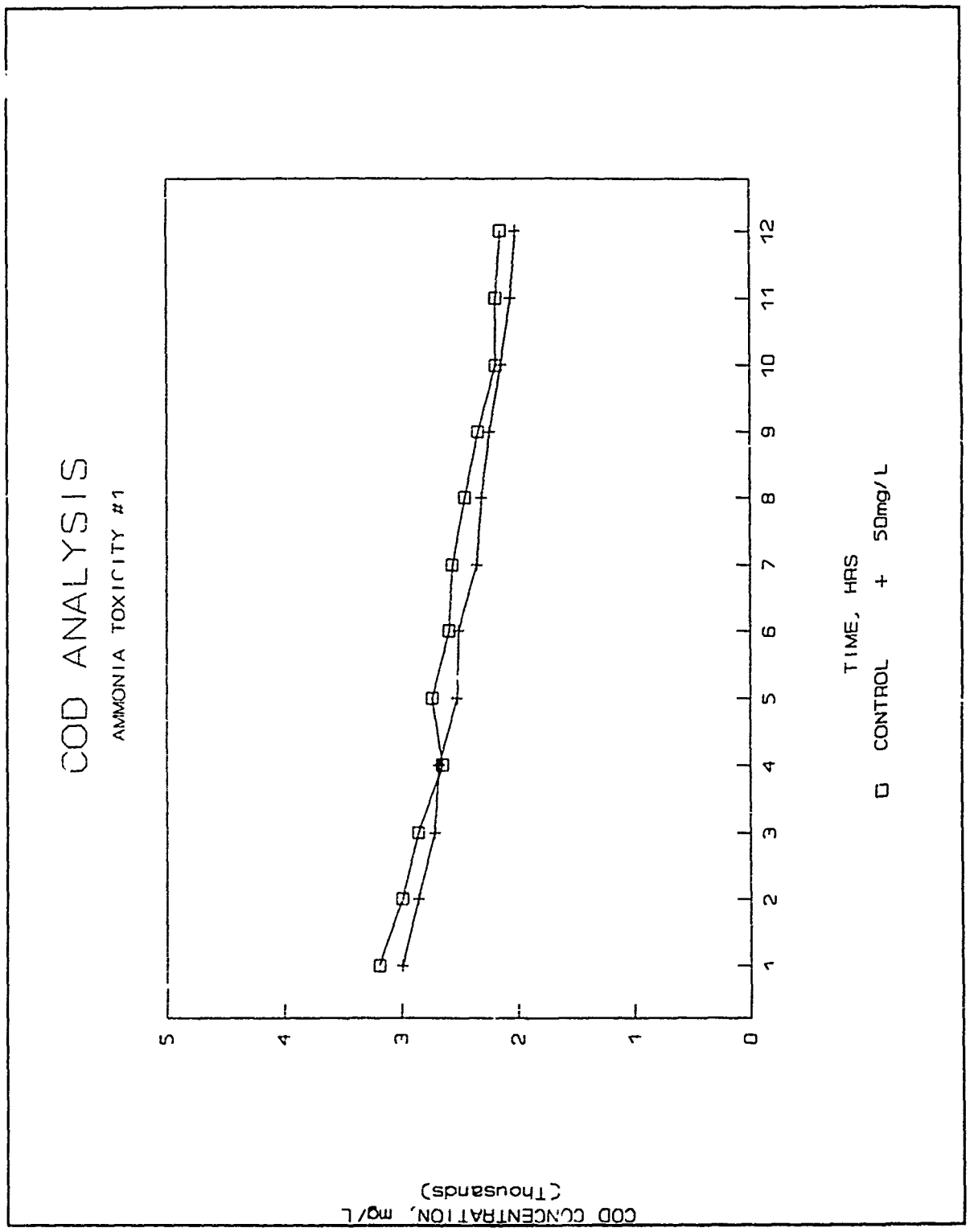


Figure 4.19 - COD analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #1

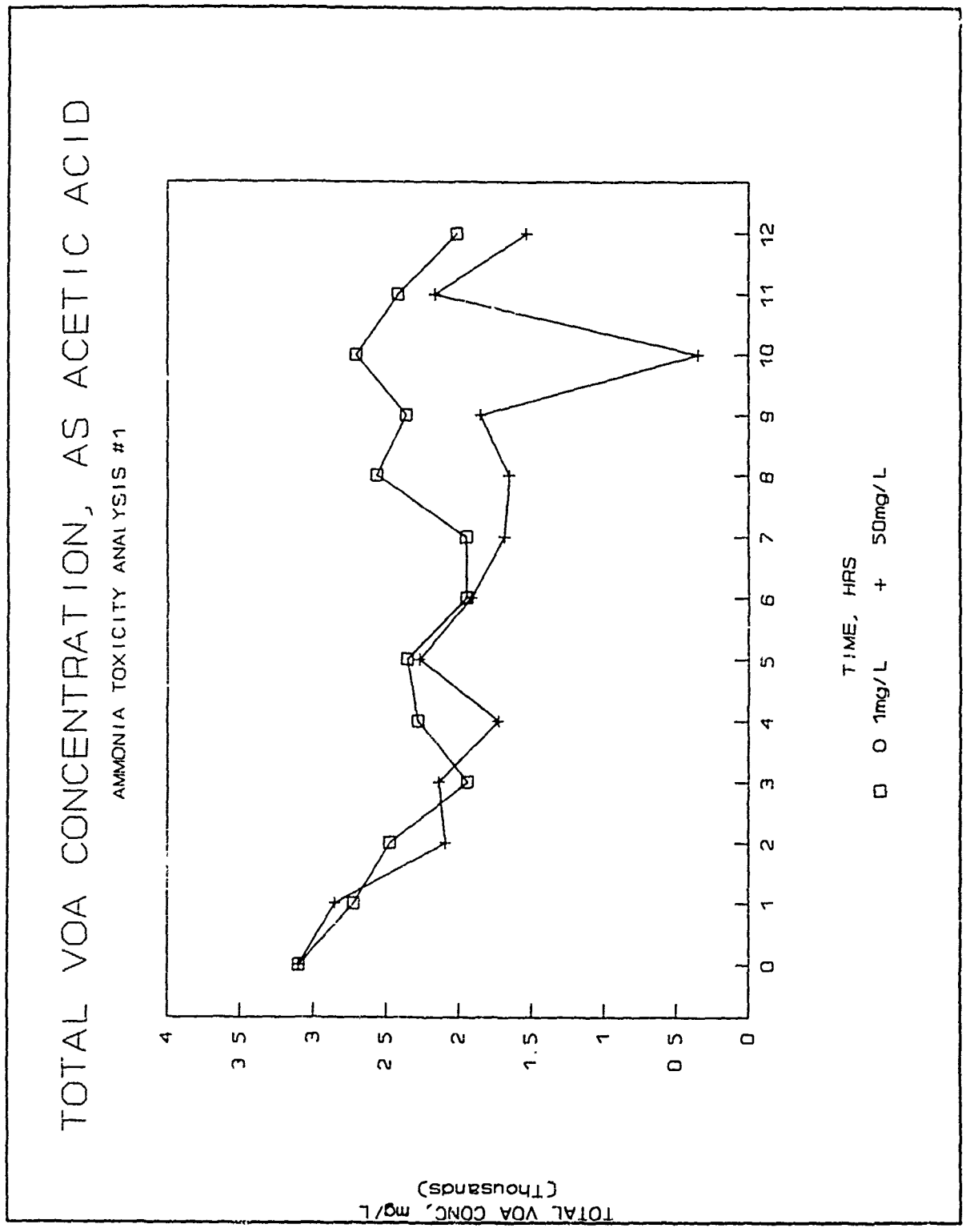


Figure 4.20 - VOA analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #1

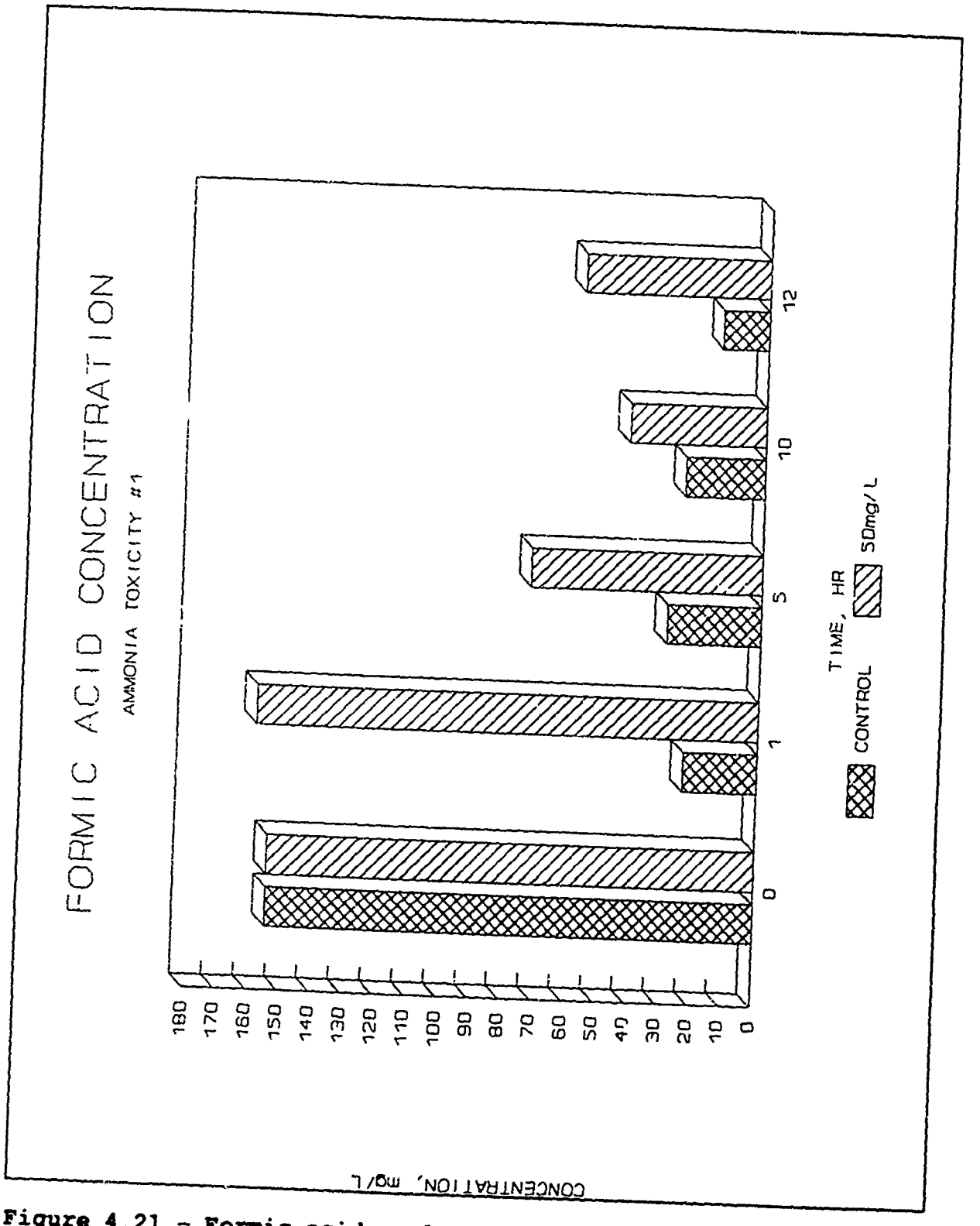


Figure 4.21 - Formic acid analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #1

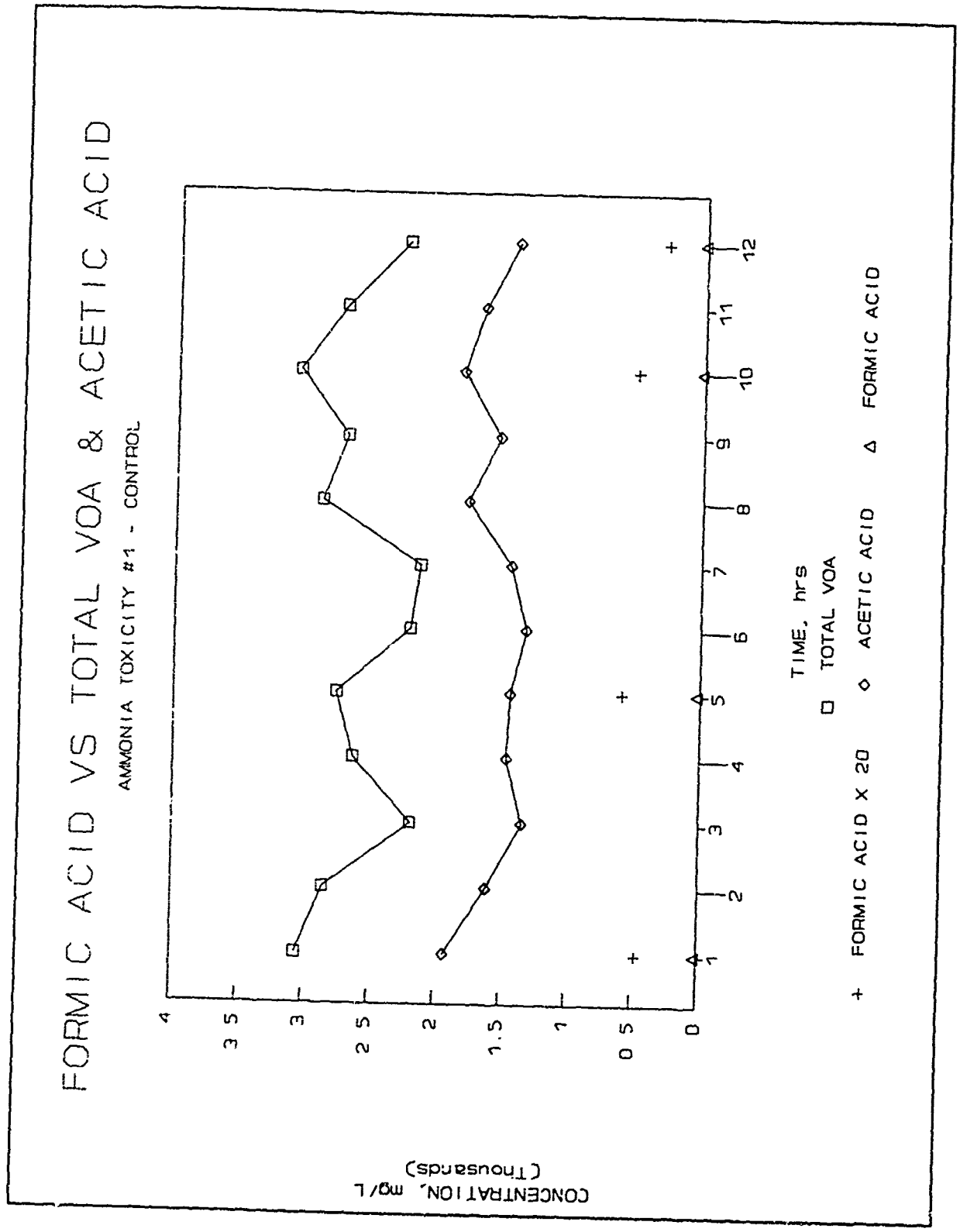


Figure 4.22 - Comparison of HCOOH with VOA concentrations of effluent from control reactor in ammonia toxicity analysis #1

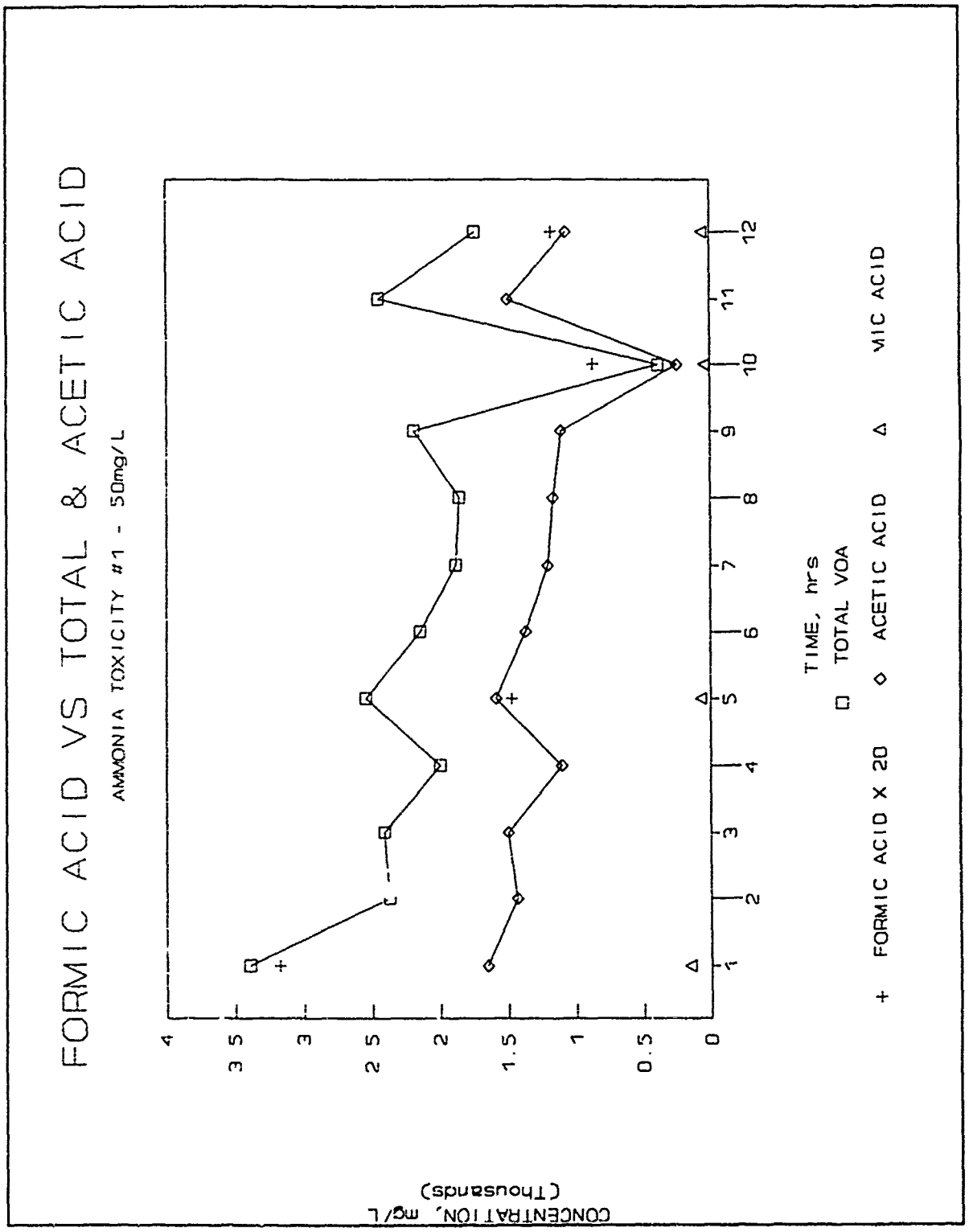


Figure 4.23 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate containing 50 mg/L NH_3, ammonia toxicity analysis #1

TEST 4 - AMMONIA TOXICITY NUMBER 2

Analysis #2 for ammonia toxicity was a repetition of the first ammonia toxicity test. This test was repeated since it appeared to give the greatest difference in formic acid concentration between the control and test reactors. The samples obtain were analyzed for pH and formic acid only. These results are indicated in table 4.4 and figure 4.24. Although the concentration of formic acid was not as great was with ammonia test #1, the results of test number two followed the same pattern.

TEST 5 - AMMONIA TOXICITY NUMBER 3

Analysis #3 for ammonia toxicity involved the analysis of a test reactor only over a 24-hour period as compared to the previous

Table 4.4 - pH analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #2

SUB CONC	SEED	TIME, HRS 1	2	3	4	5	6	7	8	9	10	11	12
CONTROL	6.90	6.89	6.85	6.87	6.88	6.87	6.83	6.90	6.90	6.87	6.88	6.89	6.90
50 mg/L	6.90	6.79	6.80	6.83	6.81	6.81	6.72	6.78	6.80	6.78	6.80	6.83	6.77

12-hour periods. The samples were analyzed for all the parameters. The pH varied from 6.79 to 6.96, as indicated in table 4.5, while the COD was sporadic, as demonstrated in figure 4.25. There was an indication of some CH_4 production.

The VOA analysis indicated very little change, as demonstrated in figure 4.26. The separation between the acetic and formic acid

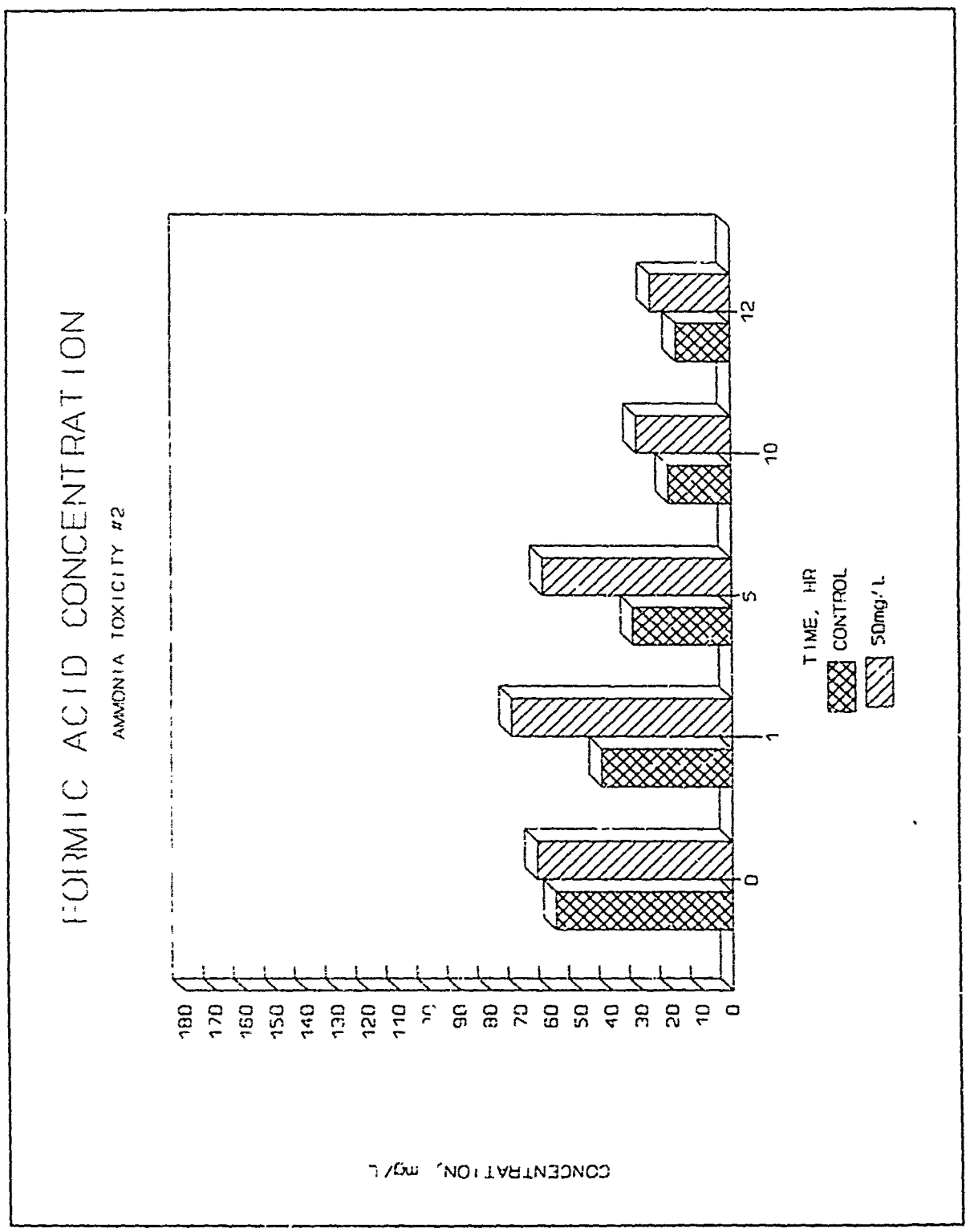


Figure 4.24 – Formic acid analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #2

Table 4.5 - pH analysis of effluent from reactor fed substrate containing 50 mg/L NH_3, ammonia toxicity analysis #3

NH_3 CONC	TIME, HRS 0	1	4	8	12	16	20	24
50 mg/L	6.79	6.81	6.84	6.96	6.80	6.83	6.84	6.87

peaks was very good. Figures 4.27 and 4.28 indicate the concentration of formic acid, and compare it to acetic acid. It is apparent that the formic acid concentration is cyclic.

TEST 6 - AMMONIA TOXICITY NUMBER 4

In ammonia toxicity analysis #4 all parameters were again measured. Both the control and test reactors were filled with 1 L of effluent from the seed reactor and monitored for a 120-hour period. As with all the other tests, the pH remained relatively unchanged; see table 4.6.

The COD data with the exception of 2 or 3 data points were relatively constant over the 120-hour period; see figure 4.29. As

Table 4.6 - pH analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #4

NH_3 CONC	TIME, HRS 0	1	12	24	36	48	60	72	84	96
CONTROL	6.81	6.78	6.80	6.83	6.88	6.87	6.86	6.87	6.85	[illegible]
50 mg/L	6.81	6.72	6.72	6.74	6.79	6.78	6.79	6.79	6.[illegible]	[illegible]

indicated in chapter 3, gas composition [illegible]

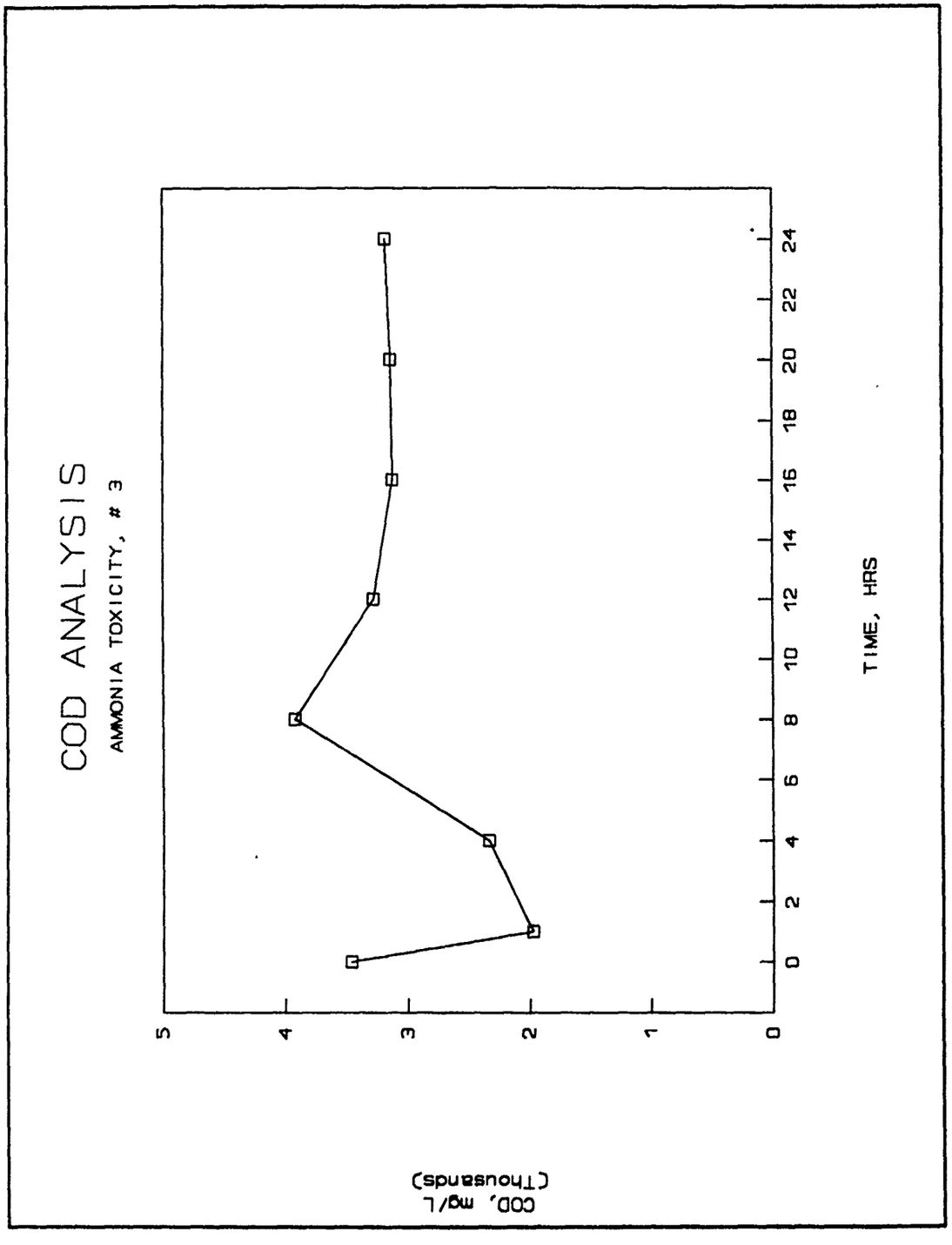


Figure 4.25 - COD analysis of effluent from reactor fed substrate containing 50 mg/L NH_3, ammonia toxicity analysis #3

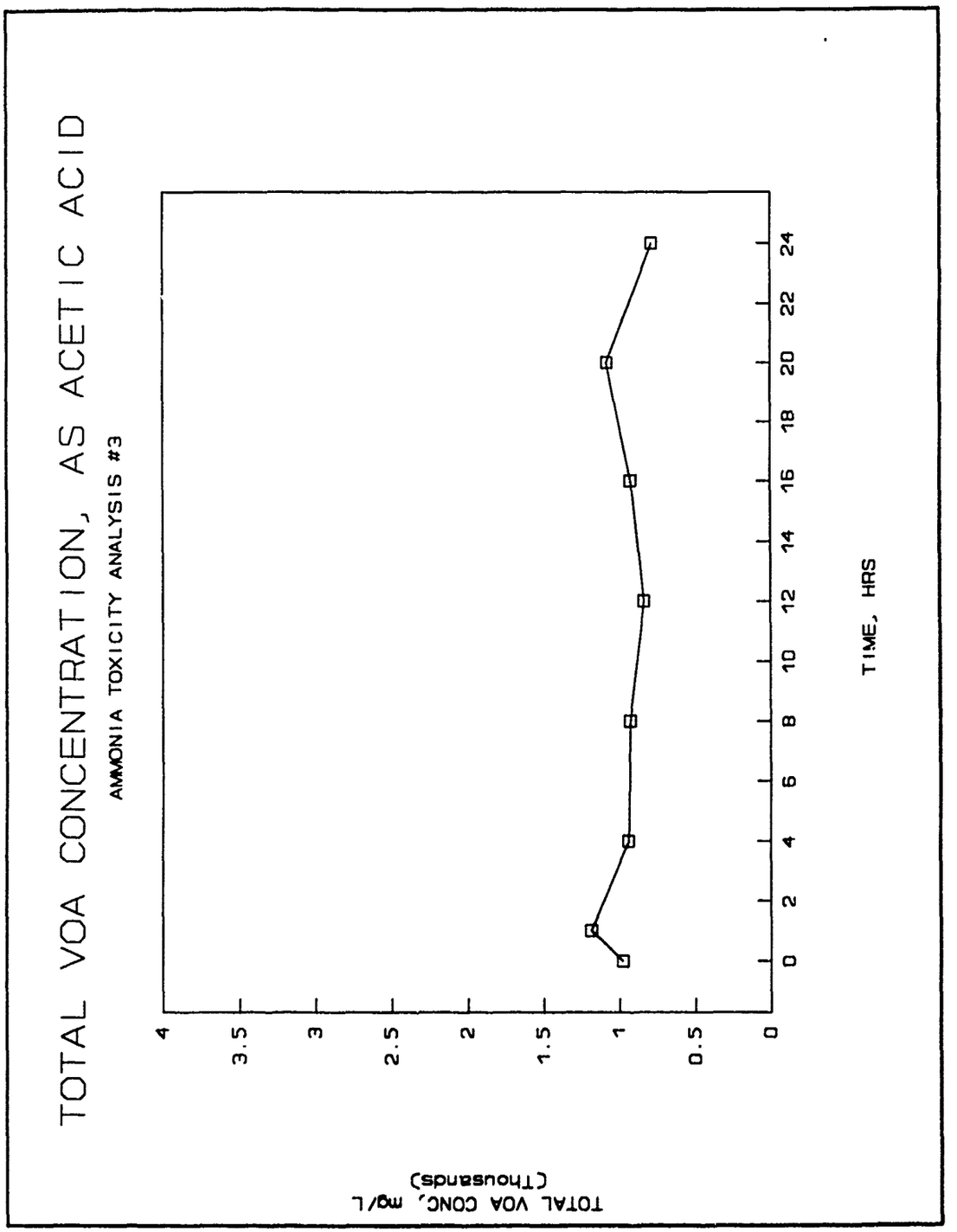


Figure 4.26 - VOA analysis of effluent from reactor fed substrate containing 50 mg/L NH_3, ammonia toxicity analysis #3

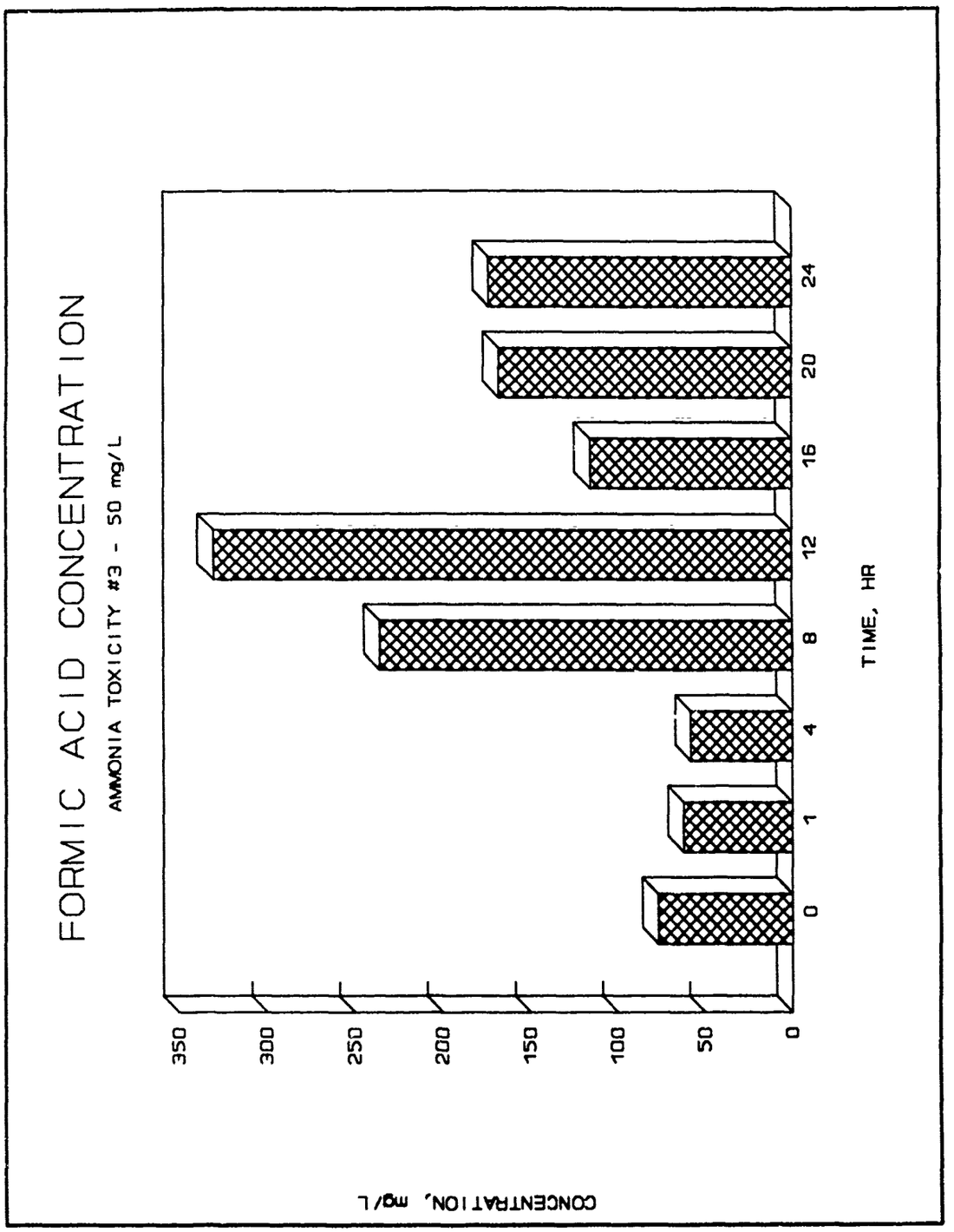


Figure 4.27 - Formic acid analysis of effluent from reactor fed substrate containing 50 mg/L NH_3, ammonia toxicity analysis #3

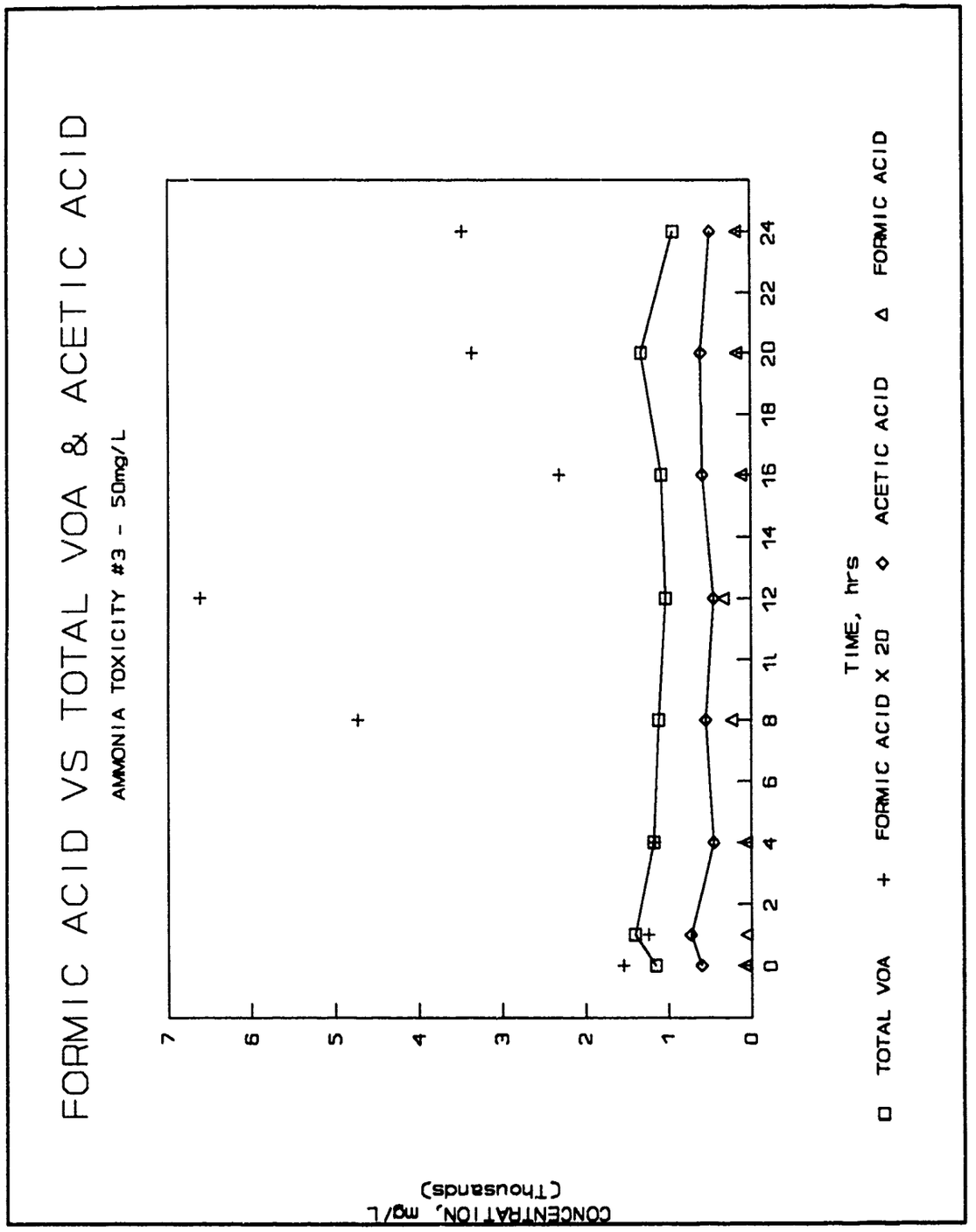


Figure 4.28 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate containing 50 mg/L NH_3, ammonia toxicity analysis #3

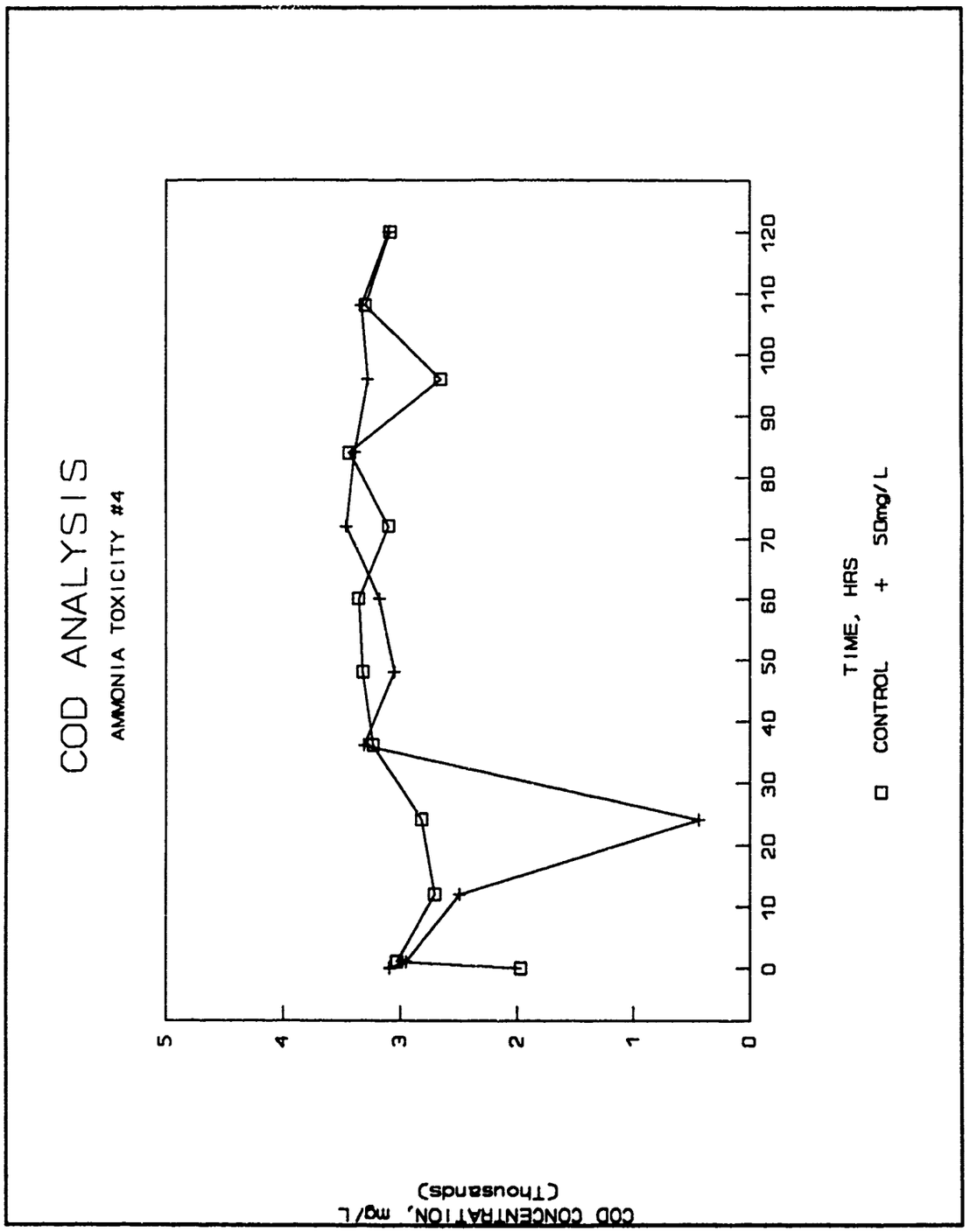


Figure 4.29 - COD analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #4

reliable, and was not taken during the course of this test. The VOA analysis indicated a constant slightly decreasing trend, shown in figure 4.30. The formic acid concentration again appeared to be cyclic. Figures 4.31 through 4.33 indicate the trends of formic and acetic acids. The concentration of formic acid remained higher in the samples from the test reactor than those from the control reactor, indicating that the seed reactor was stressed, causing the concentration of formic acid to be higher in both reactors then in previous tests.

TEST 7 - SEED REACTOR DURING UPSET

During the last ammonia test, it was noted that the seed reactor's formic acid concentration had drastically increased. A gas composition analysis indicated the headspace contained approximately 30% N_2, a sign of possible O_2 inhibition due to air in the reactor. Since the seed reactor was stressed it was monitored for a period of 6 days. During the upset test period, the pH ranged from 6.6 to 6.72, as shown in table 4.7. The COD analysis showed a decrease in COD towards the end of the test

Table 4.7 - pH analysis of effluent from seed reactor during upset period

SAMPLE NUMBER											
1	2	3	4	5	6	7	8	9	10	11	12
6.71	6.72	6.72	6.70	NA	6.67	6.70	6.67	6.68	6.66	6.69	6.67

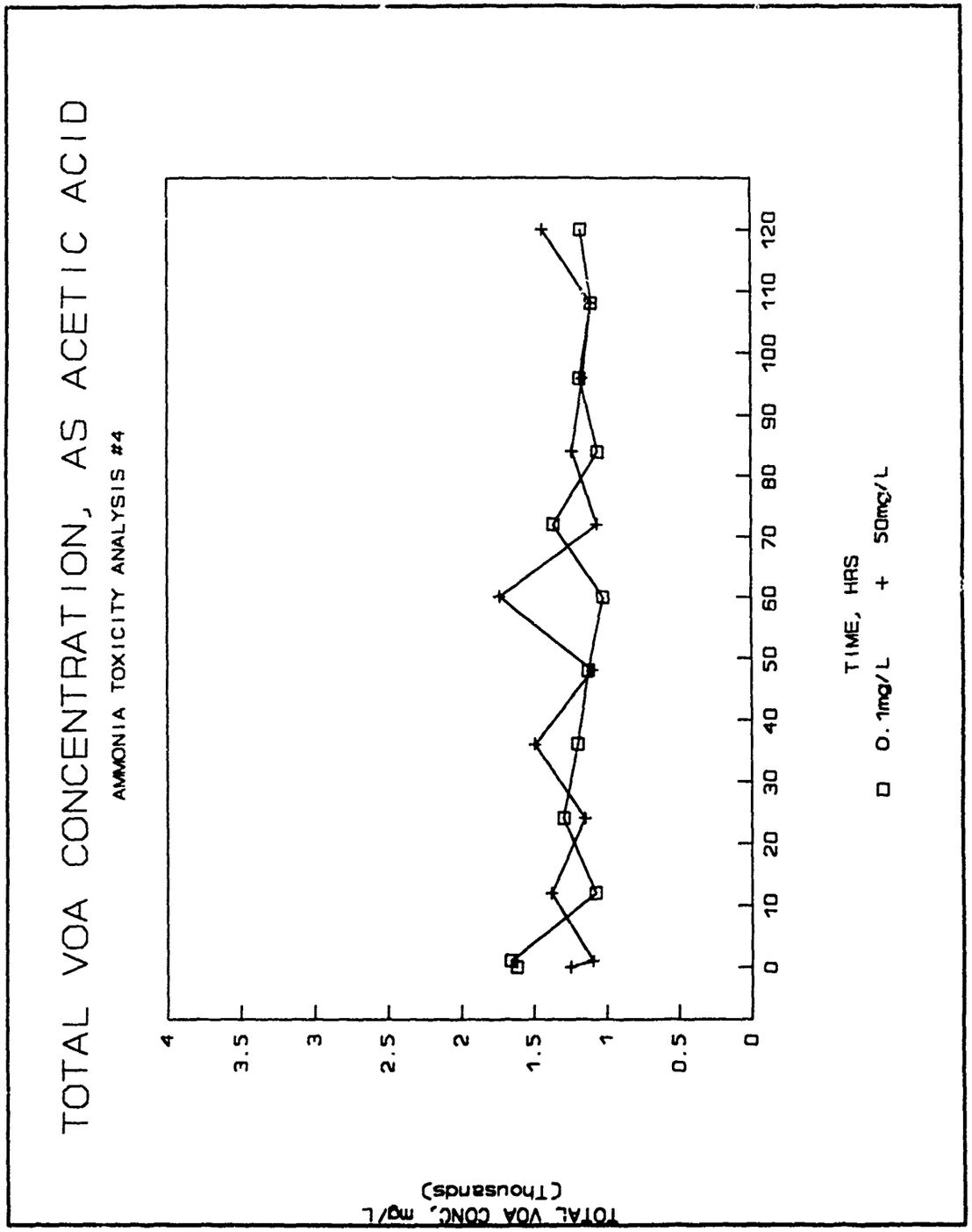


Figure 4.30 - VOA analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #4

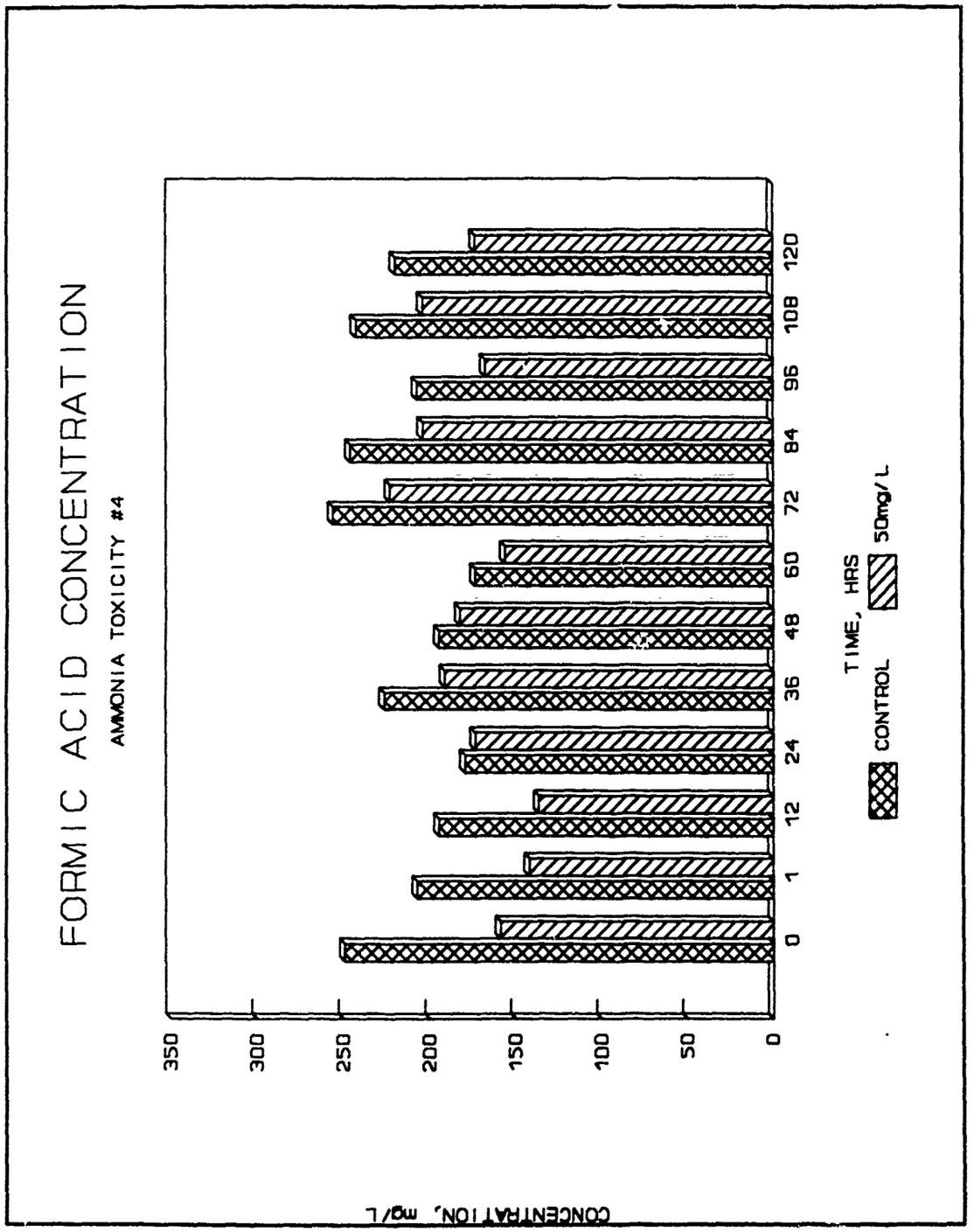


Figure 4.31 - Formic acid analysis of effluent from reactors fed substrate containing various concentrations of NH_3, ammonia toxicity analysis #4

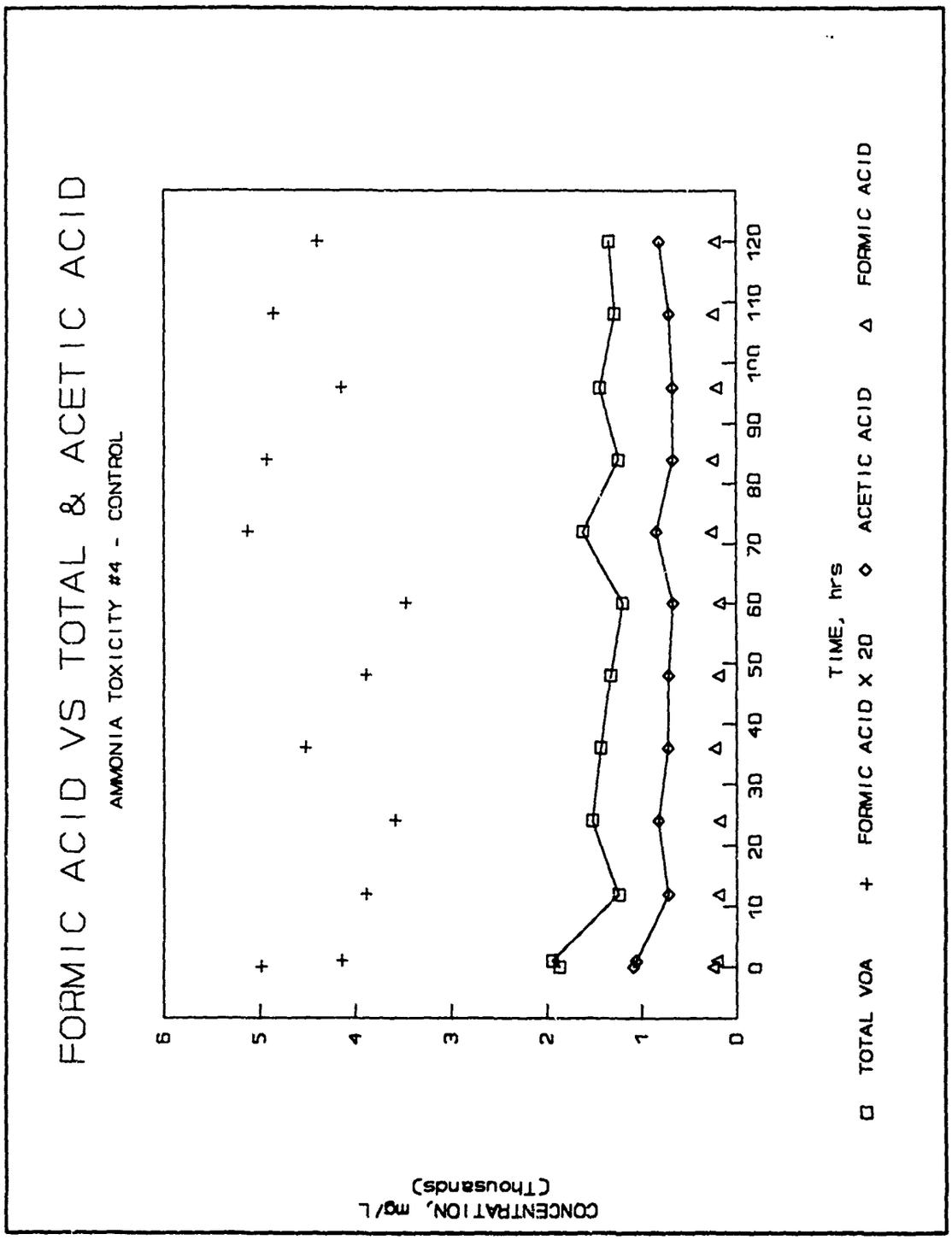


Figure 4.32 - Comparison of HCOOH with VOA concentrations of effluent from control reactor in ammonia toxicity analysis #4

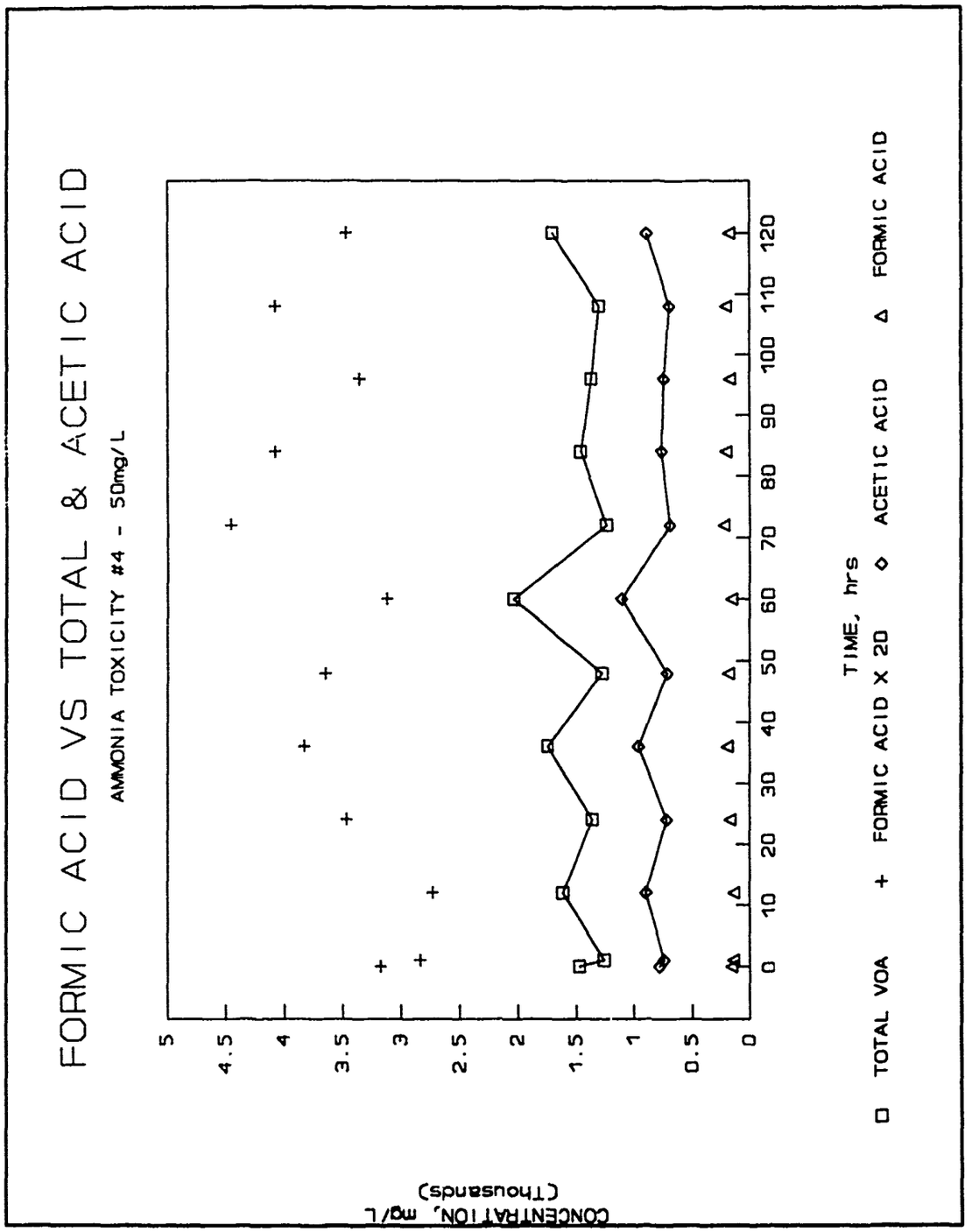


Figure 4.33 - Comparison of HCOOH with VOA concentrations of effluent from reactor fed substrate containing 50 mg/L NH_3, ammonia toxicity analysis #4

period, indicating the reactor was becoming more stable. This findings are shown in figure 4.34.

Figure 4.35 indicates the total VOA trend, while figures 4.36 and 4.37 present the results of the formic acid analysis and compare it to the total VOA and acetic acid concentrations. The formic acid concentration, although cyclic, was decreasing, another indication that the system was recovering.

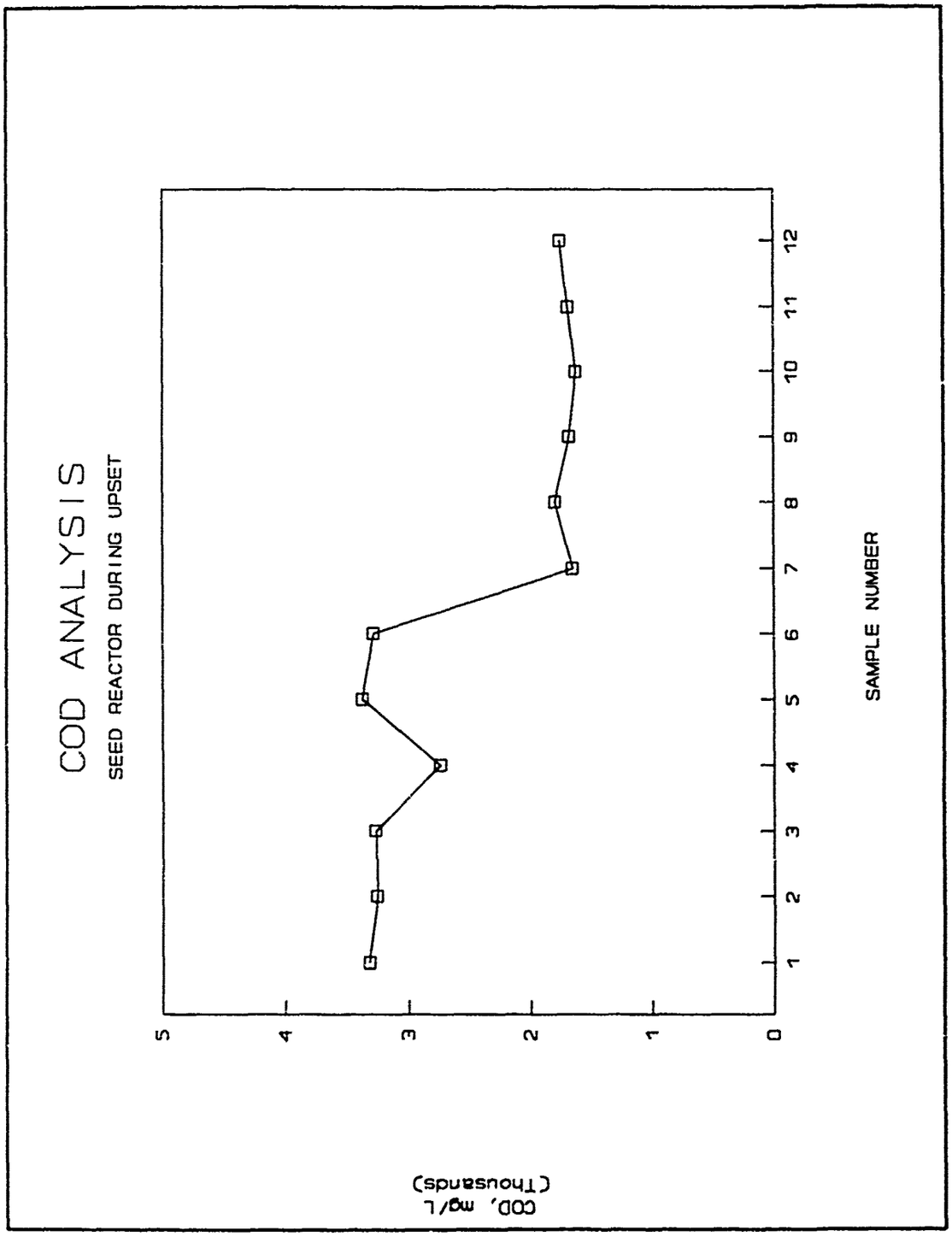


Figure 4.34 - COD analysis of effluent from seed reactor during upset period

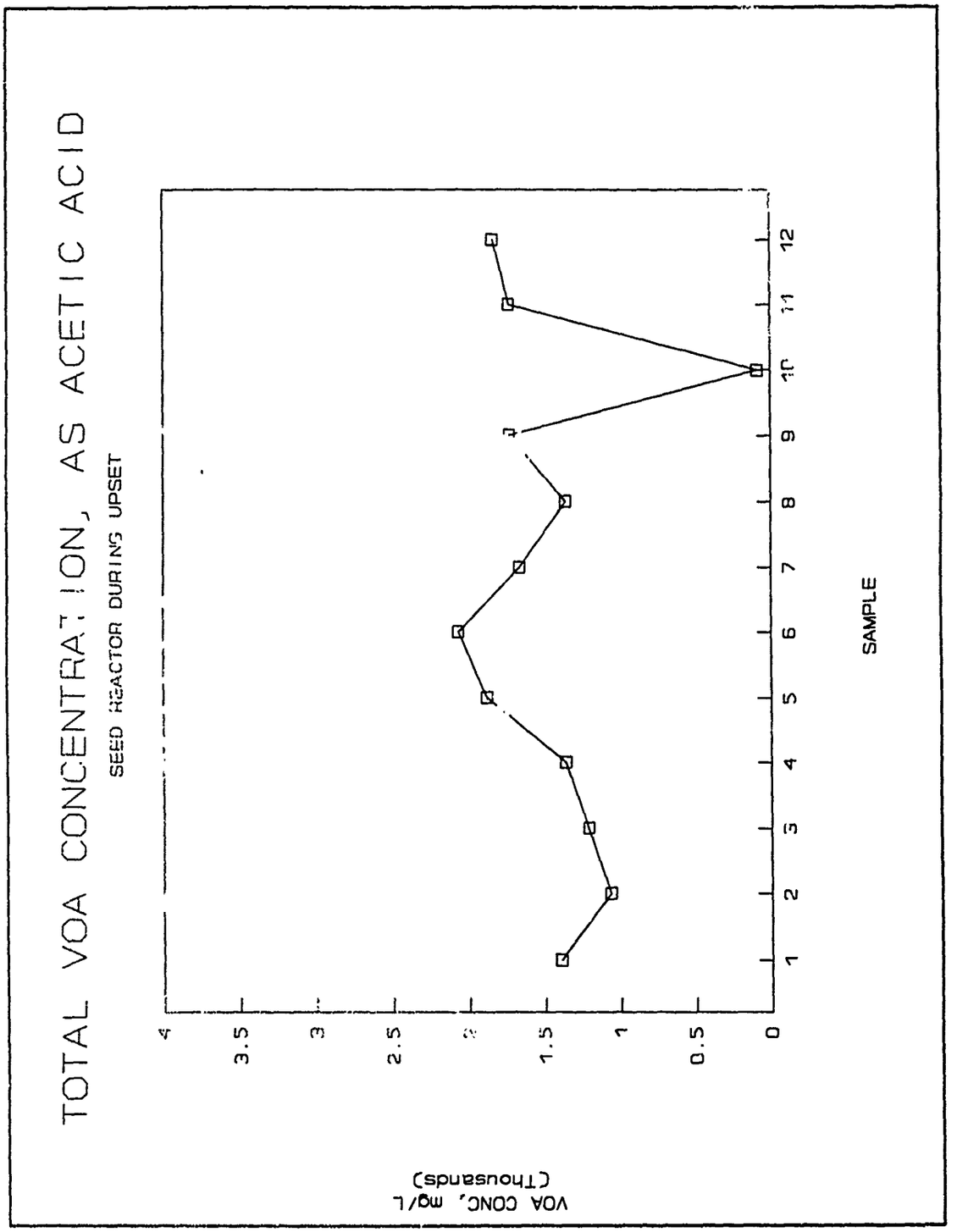


Figure 4.35 - VOA analysis of effluent from seed reactor during upset period

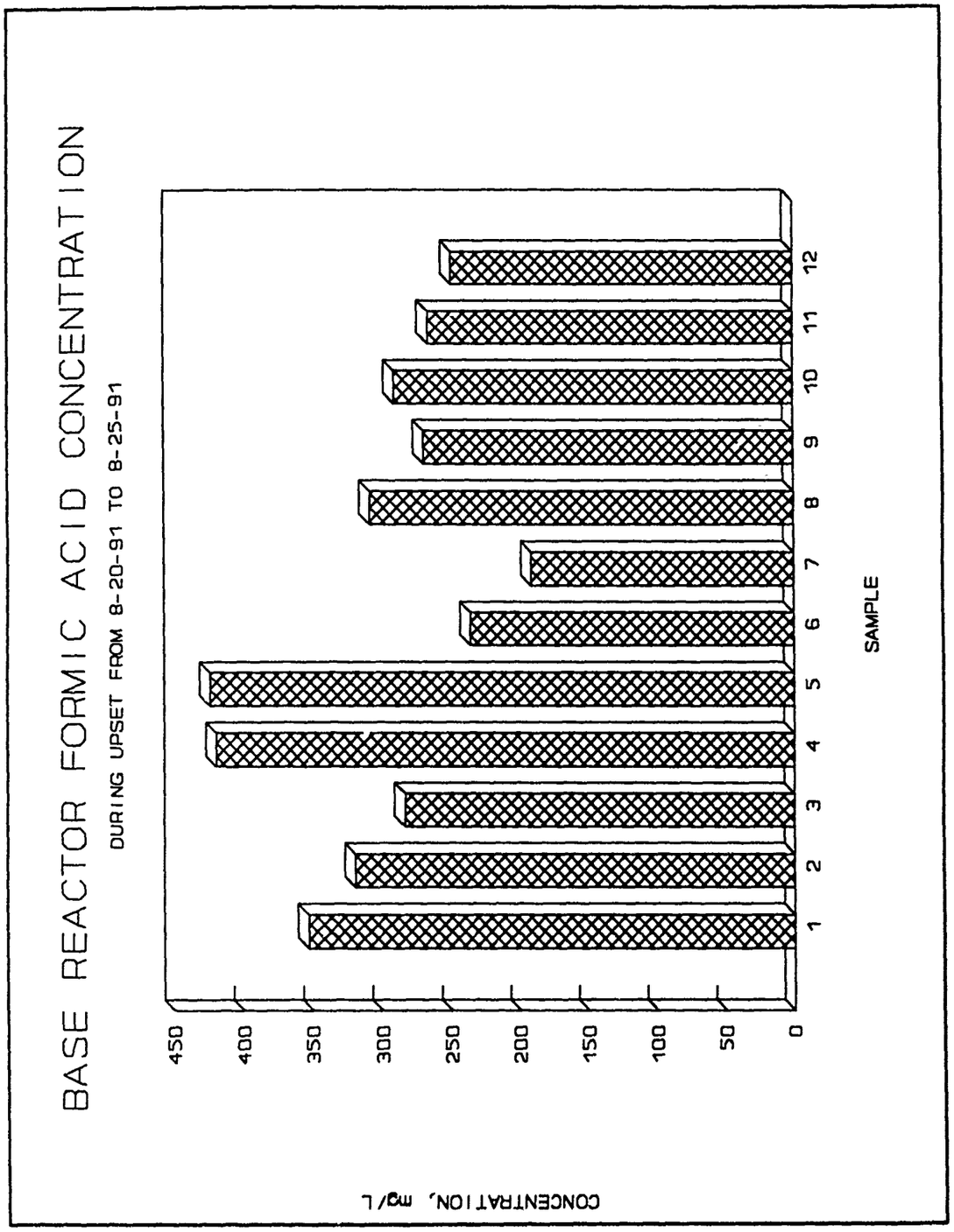


Figure 4.36 - Formic acid analysis of effluent from seed reactor during upset period

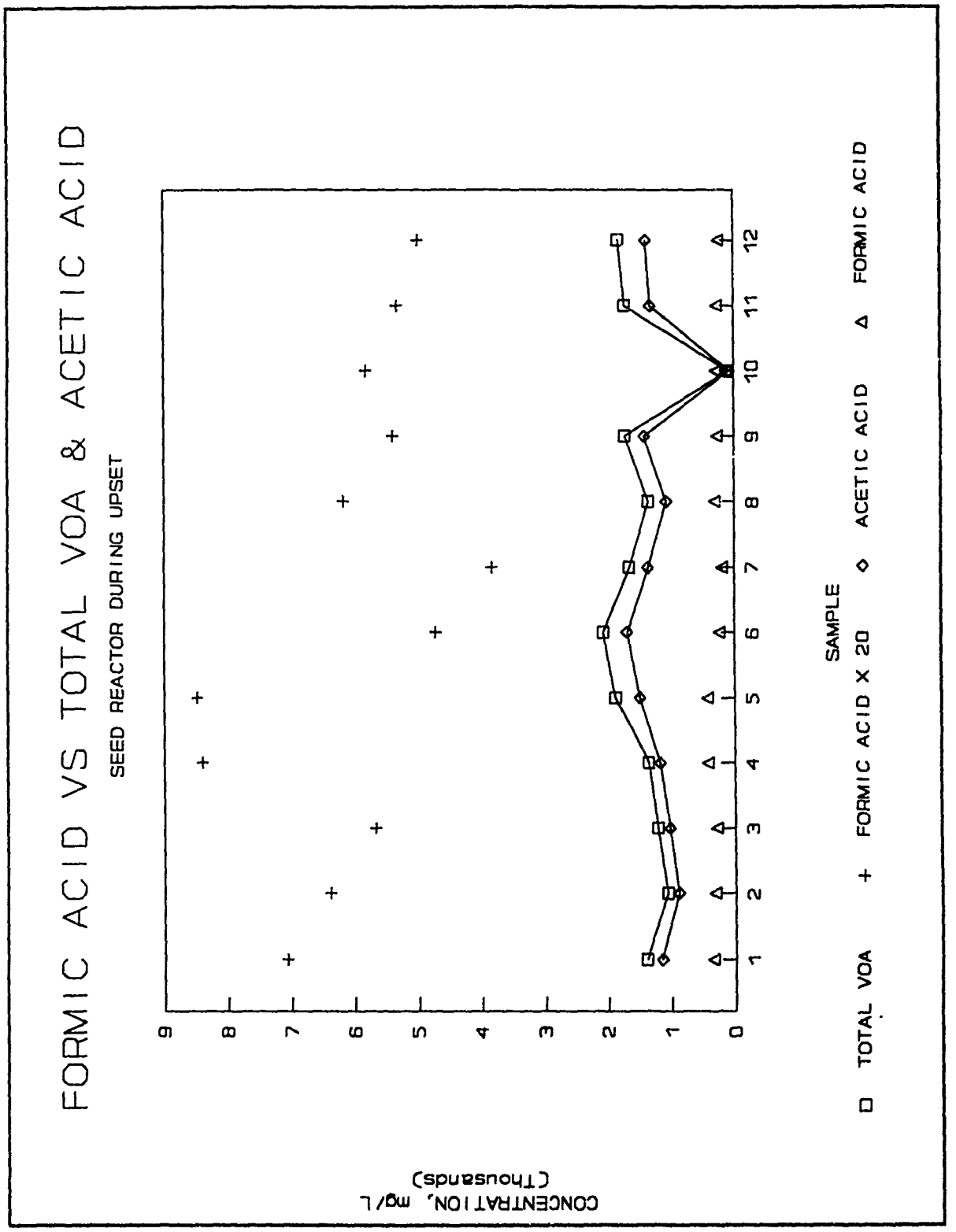


Figure 4.37 - Comparison of HCOOH with VOA concentrations of effluent from seed reactor during upset period

V. CONCLUSIONS

FINDINGS

(1) Based on the results presented in chapters 3 and 4 it is concluded that formic acid can be determined using the method developed in this research with very little pretreatment.

(2) As noted in the data from the seed reactor and other reactors, in systems which are not operating under the stress of the toxins addressed in this research, formic acid, when present will be at low levels, less than 50 mg/L.

(3) The concentration of formic acid increased when the systems were subjected to toxic levels of ammonia, and possibly under conditions of O_2 toxicity.

(4) Formic acid concentration did not in general increase when subjected to chloroform or increased substrate concentrations. This factor appears to indicate that formic acid increases are subject to the type of toxicity induced.

(5) In stressed anaerobic batch systems, within the timeframe of the sampling formic acid concentrations increased to over 400 mg/L, an order of magnitude higher than the unstressed systems.

AREAS OF FUTURE RESEARCH

While there appeared to be a correlation between formic acid and VOA concentration, there were insufficient data collected in this research to determine if it could be detected sooner than

other indicators. Increasing the number of samples analyzed by decreasing the time between samples for formic acid will allow for a more definitive determination.

Since landfills employ anaerobic degradation, analyzing leachate could provide more insight into the stability or state of stabilization of these systems. It has been determined that formic acid could be detected when spiked in landfill leachate, but no determination was made as to whether formic acid would be a significant intermediate in these systems.

ENGINEERING SIGNIFICANCE

Once established by further research that formic acid concentration can be used to predict pending system upset, daily monitoring for formic acid could be accomplished as easily as any other parameter measured. A baseline concentration of formic acid in the system would first have to be established then monitored daily thereafter.

As with H_2 concentration, the concentration of formic acid appears to be cyclic. The pattern would have to be established for a specific treatment system then samples taken at relatively the same "time" in the cycle for comparison.

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Zhenglan *etal* 1990

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APPENDIX A
DIONEX APPLICATION NOTE (AN 24)

DIONEX

Application

AN 24

Determination of Formaldehyde as Formate Ion

INTRODUCTION

An analytical method combining formaldehyde collection on oxidizer impregnated charcoal with subsequent IC analysis of the formaldehyde as formate ion has been developed.

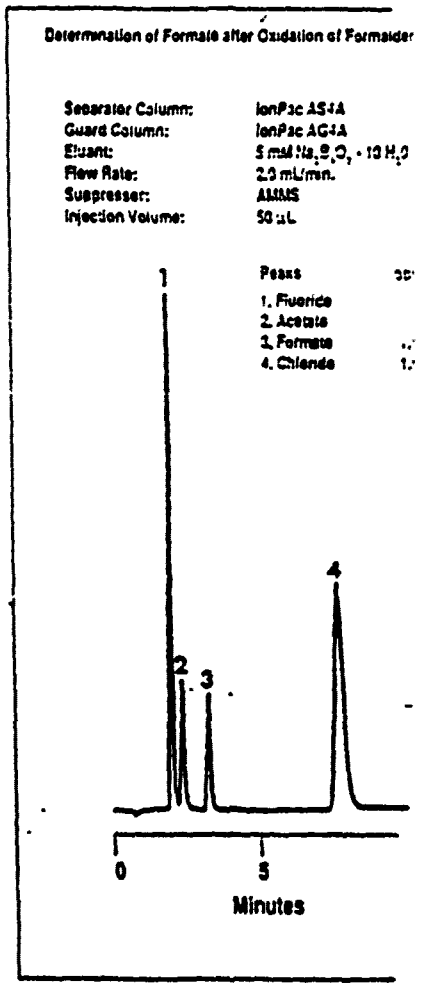
Formaldehyde is a sensory irritant and can also form a suspected carcinogen, bis-chloromethyl ether, when reacted with HCl in humid air. Because formaldehyde is common to chemical industry work environments, OSHA has set exposure standards to ensure the safety of workers.

Previously, a reliable method for formaldehyde monitoring was not available. Problems were encountered (1) in the sampling procedure as devices used were made of glass and were susceptible to breakage and (2) in the formaldehyde determination because of a lack of sensitivity with former methods (e.g., gas polarography).

The following method to determine formaldehyde in ambient air was adapted from the method developed at the National Institute for Occupational Safety and Health.[1]

PROCEDURE

Air samples were passed through a solid sorbent tube (4 mm i.d. × 7 cm long) containing 150 mg of impregnated charcoal (Barnebey-Cheney Co., Columbus, OH, No. 580-20, M-2820) at 50–200 cc/min. for 3–5 hours. The charcoal was removed to a tube containing 15.0 mL of 0.1% H_2O_2 and shaken for one hour, then sonicated for 20 minutes. The solution was filtered through a 0.45 µ membrane filter, then injected into an Ion Chromatograph.



LPN 032028-01 10/88

CONDITIONS

Eluant:	5 mM $Na_2B_4O_7 \cdot 10H_2O$ (Borax)
Flow Rate:	2.0 mL/min.
Guard Column:	IonPac® AG4A
Separator:	IonPac® AS4A
Suppressor:	AMMS
Injection Volume:	50 µL

DISCUSSION

The table below lists the recovery of standards collected at 200 cc/min. Average recovery was 99% with 8% RSD. A typical chromatogram is shown on the previous page. Generated samples were stored up to nine days without refrigeration or protection from light without loss of formaldehyde.

Recovery Data for Formaldehyde Samples Collected at 200 cc/min.

Generated µg HCHO	Minutes Sampled	Found µg HCHO	% Recovery
16	80	16	100
16	77	16	100
16	78	16	100
16	65	19	119
16	80	17	106
80	80	74	93
80	154	81	101

CONCLUSION

In addition to formate ion determination in collected air samples, IC can also determine formaldehyde after this irritant is oxidized to formate. A method for formaldehyde collectic and oxidation has been developed in NIOSH laboratories.

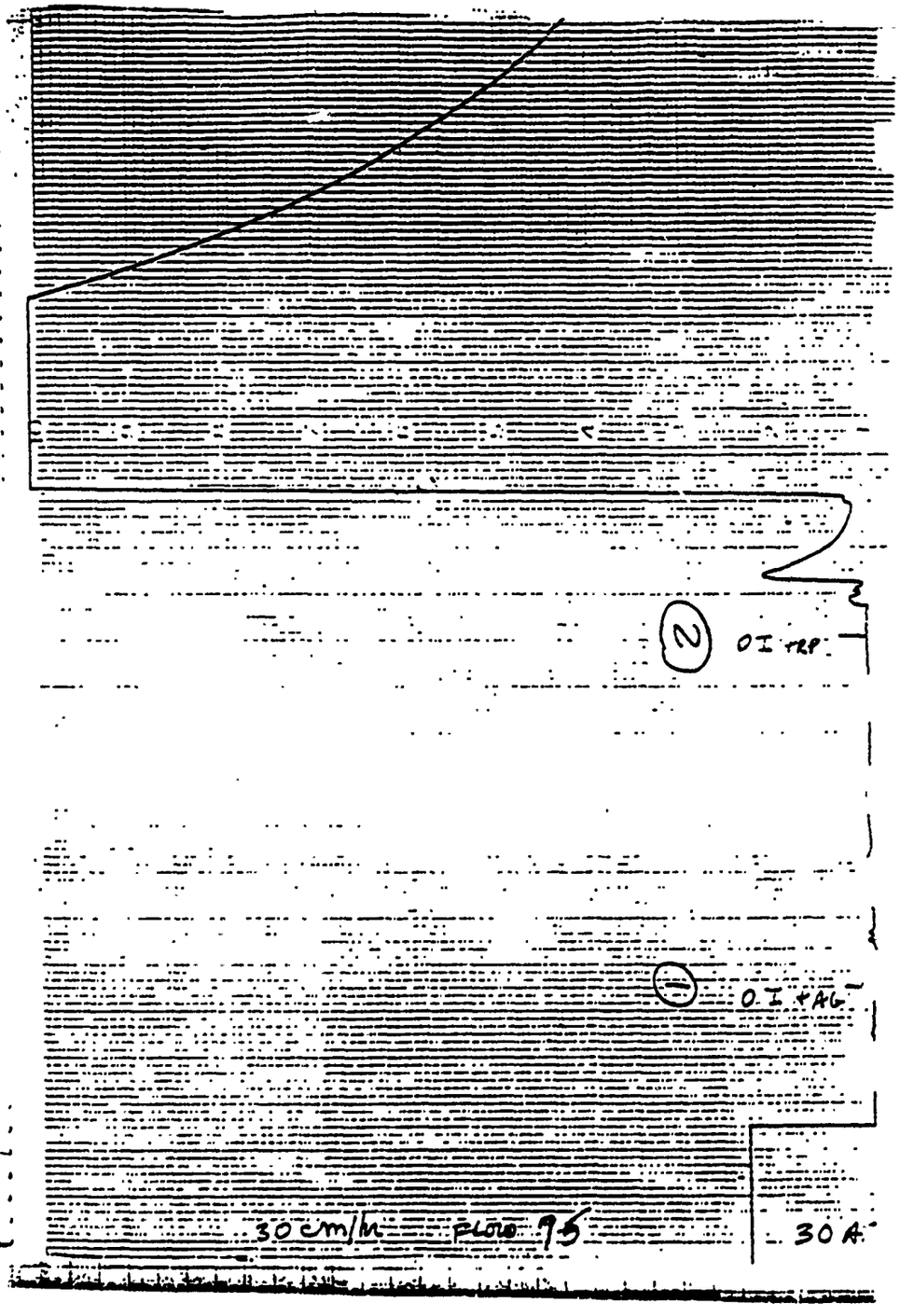
REFERENCE

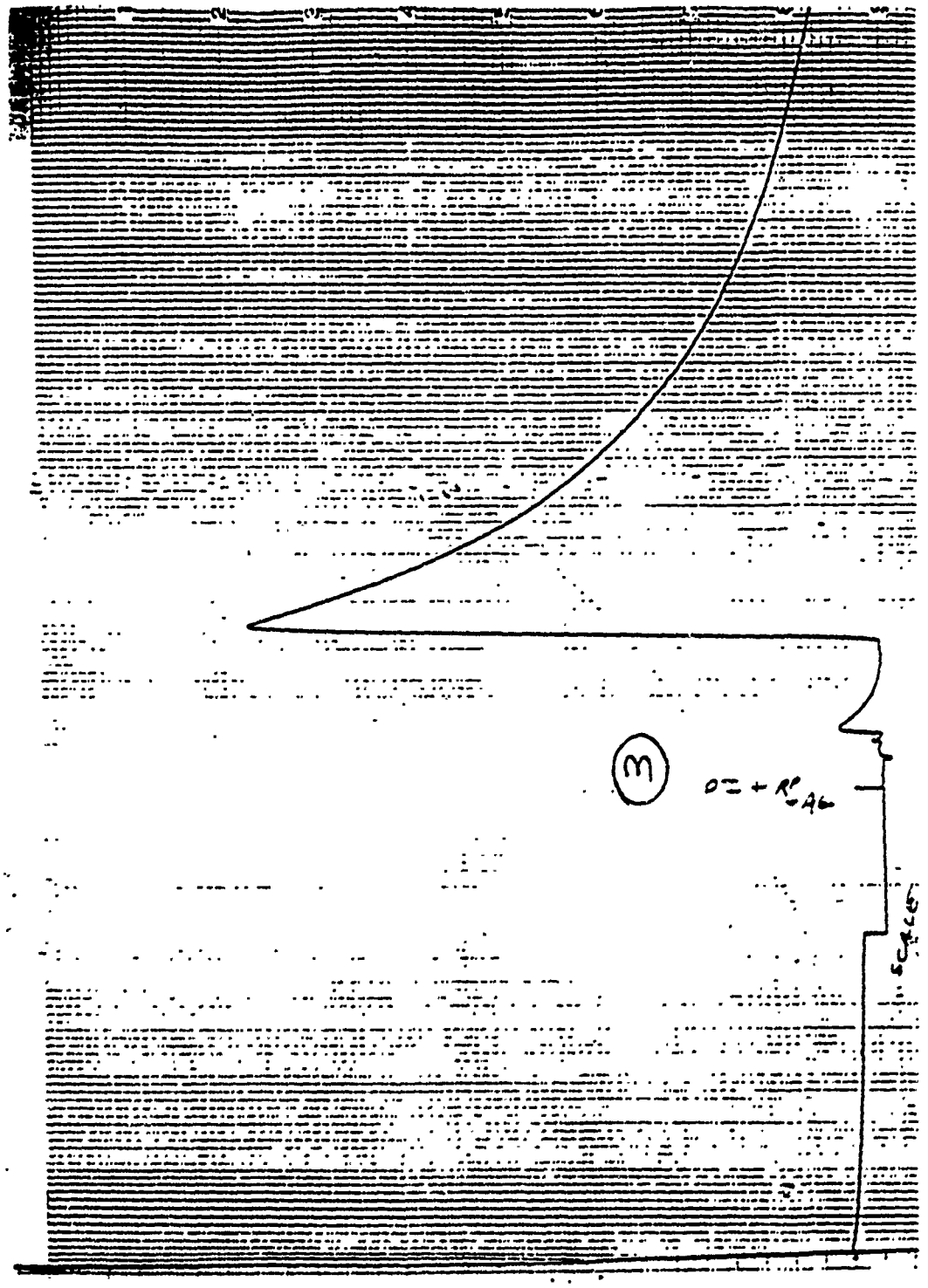
[1]Kim, W.S., Geraci, C. L. Jr. and Kupel, R. E. "Sampling and Analysis of Formaldehyde in : Industrial Atmosphere." U.S. Dept. of Health Education and Welfare, NIOSH, Cincinnati, C 45226, Oct. 1973.

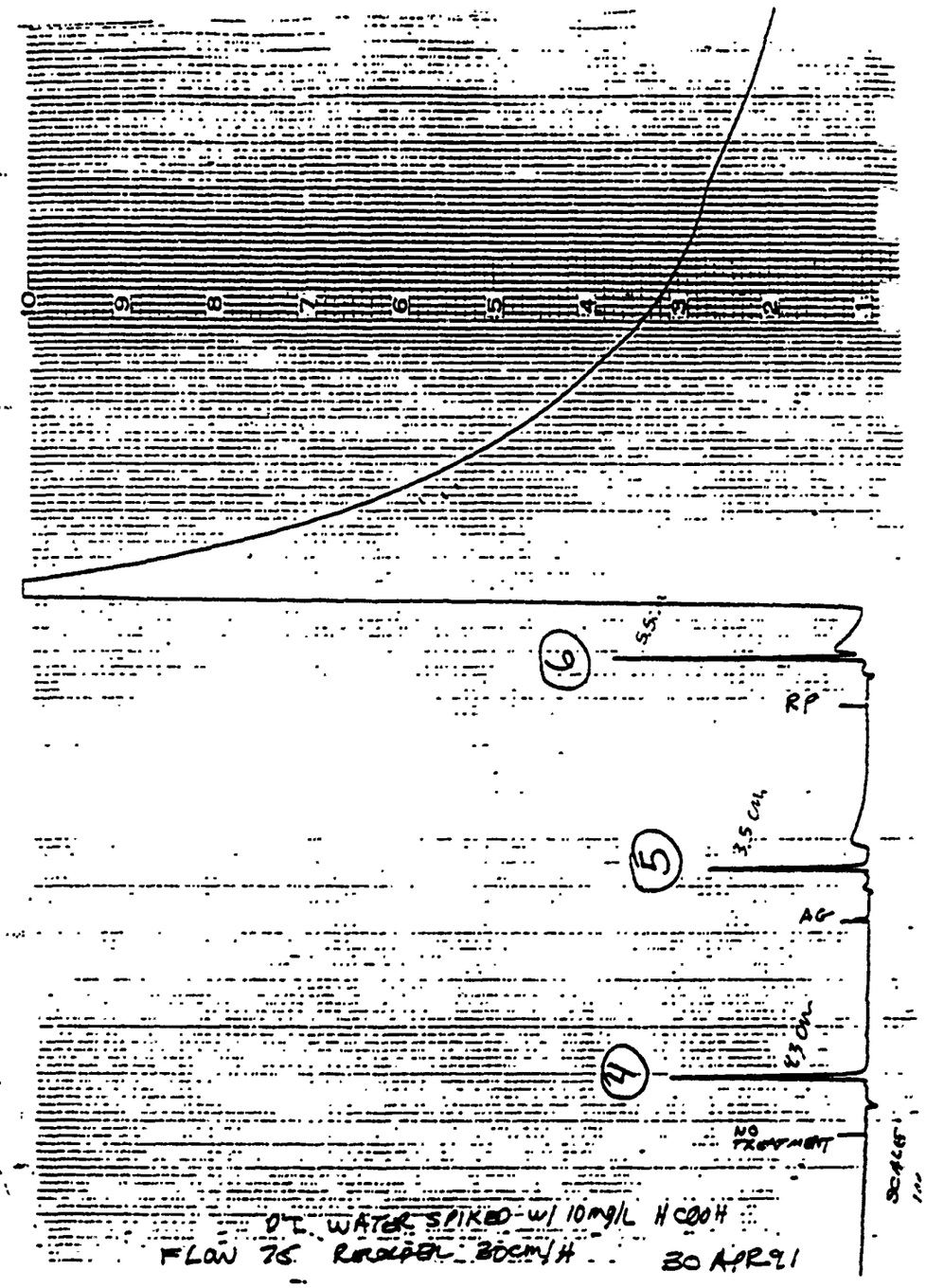
APPENDIX B
INDIVIDUAL CHROMATOGRAPHS

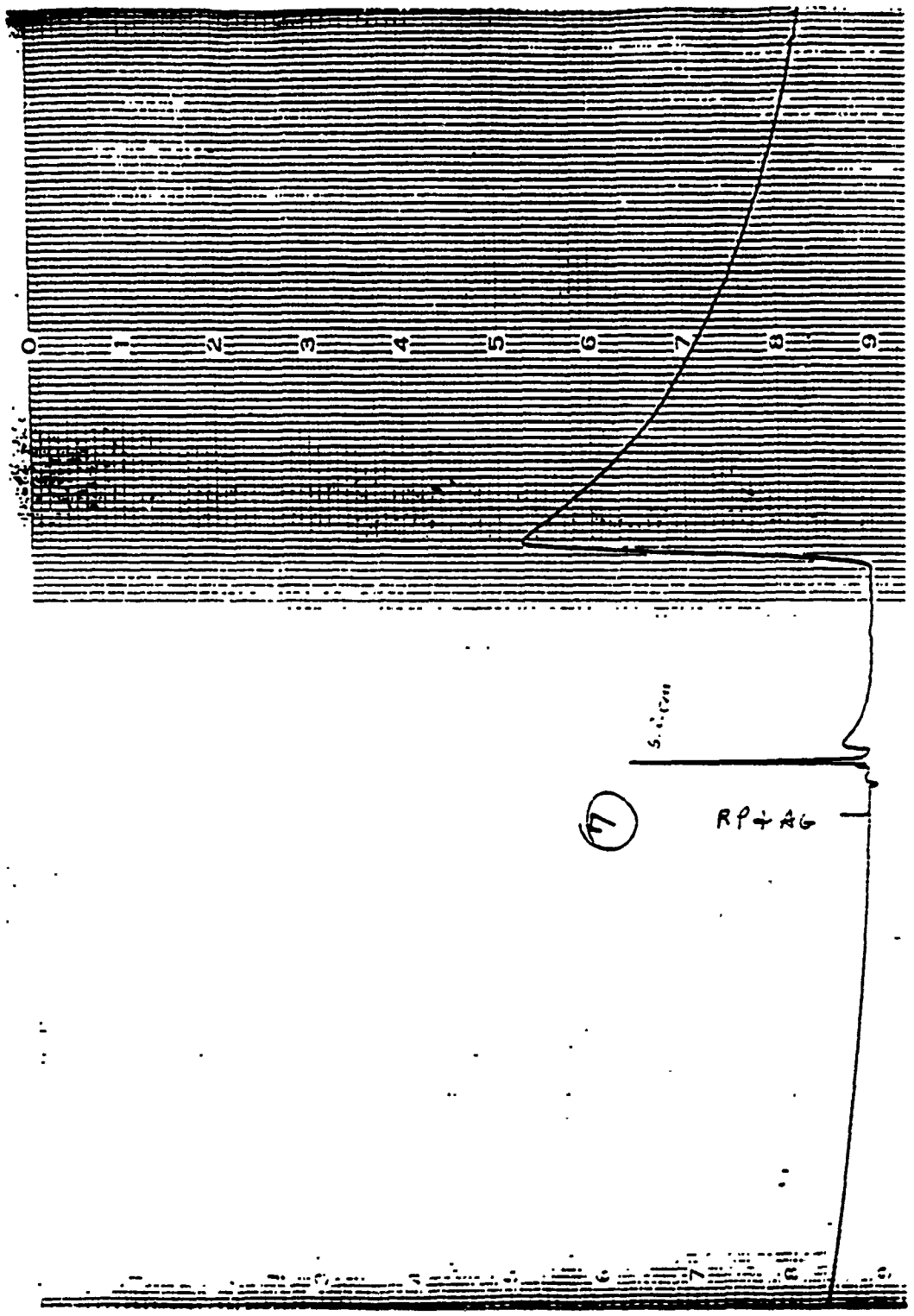
INDIVIDUAL CHROMATOGRAPHIC ANALYSES

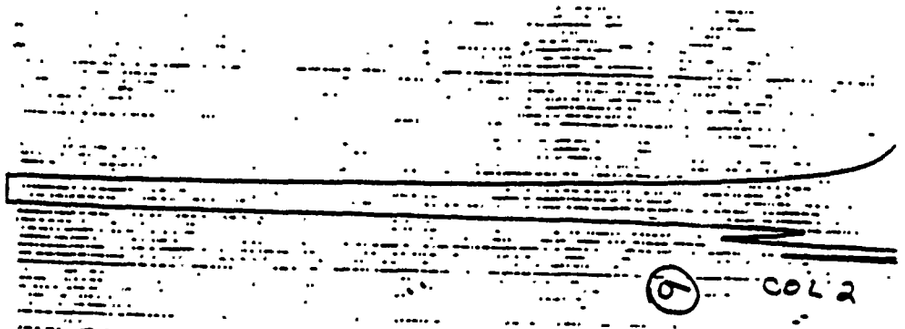
1 Deionized water treated with AG cartridge
2 Deionized water treated with RP cartridge
3 Deionized water treated with RP and AG cartridges
4 Deionized water prespiked with 10mg/L HCOOH
5 Deionized water prespiked with 10mg/L HCOOH AG cartridge
6 Deionized water prespiked with 10mg/L HCOOH RP cartridge
7 Deionized water prespiked with 10mg/L HCOOH RP and AG cartridges
8 Column 1 leachate no pretreatment or spiking
9 Column 2 leachate no pretreatment or spiking
10 Column 1 leachate treated with AG cartridge
11 Column 1 leachate treated with RP cartridge
12 Column 1 leachate treated with RP and AG cartridges
13 Column 2 leachate treated with AG cartridge
14 Column 2 leachate treated with RP cartridge
15 Column 2 leachate treated with RP and AG cartridges
16 Column 1 leachate treated with 10mg/L HCOOH
17 Column 1 leachate treated with 10mg/L HCOOH and RP cartridge
18 Column 1 leachate treated with 10mg/L HCOOH and AG cartridge
19 Column 1 leachate treated with 10mg/L HCOOH and RP and AG cartridges

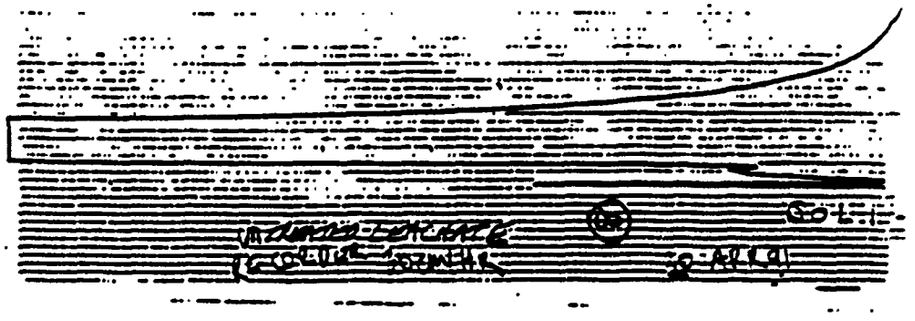

2
OI rep
1
OI +AG
30 cm/h
FLOW 95
30 A

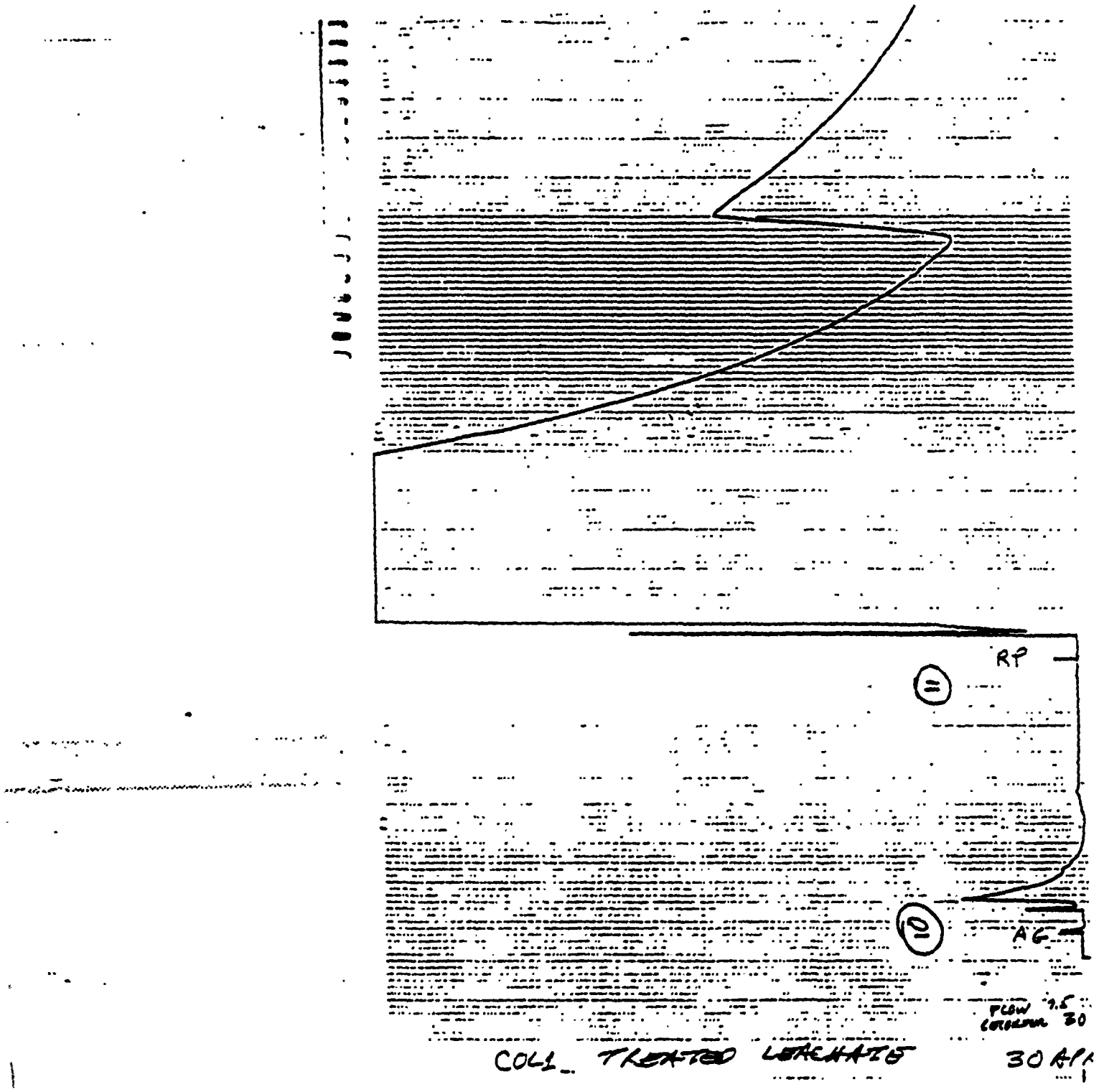

(3)
SCALE

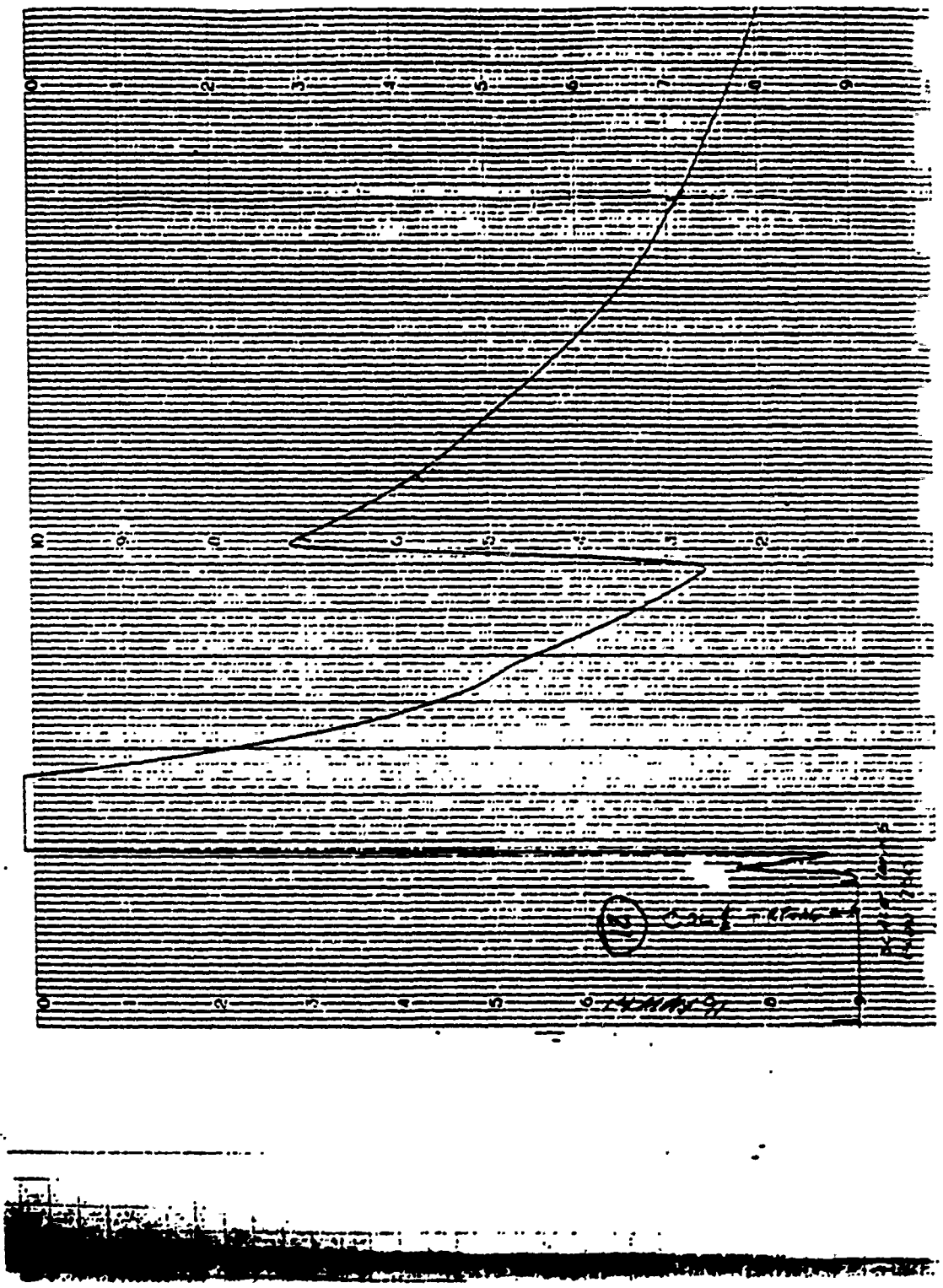

10
9
8
7
6
5
4
3
2
1
6
SS
RP
5
3.5 CAL
AC
4
#3 CAL
NO TREATMENT
SCALE: 1"=1'
0.2 WATER SPIKED w/ 10 mg/L HCOOH
FLOW 75 RECIRC. 20cm/H
30 APR 91

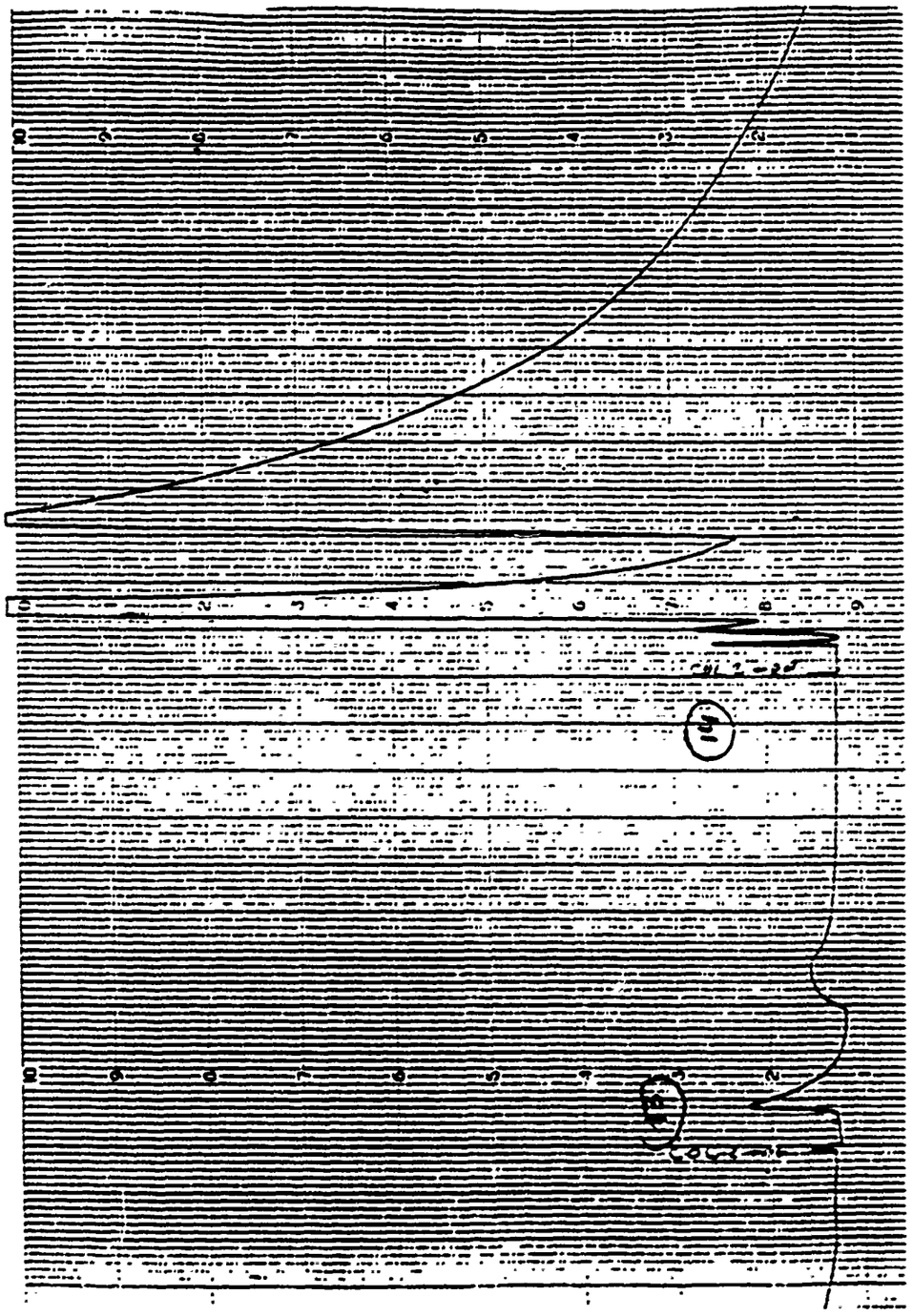

0
1
2
3
4
5
6
7
8
9
RP+AG

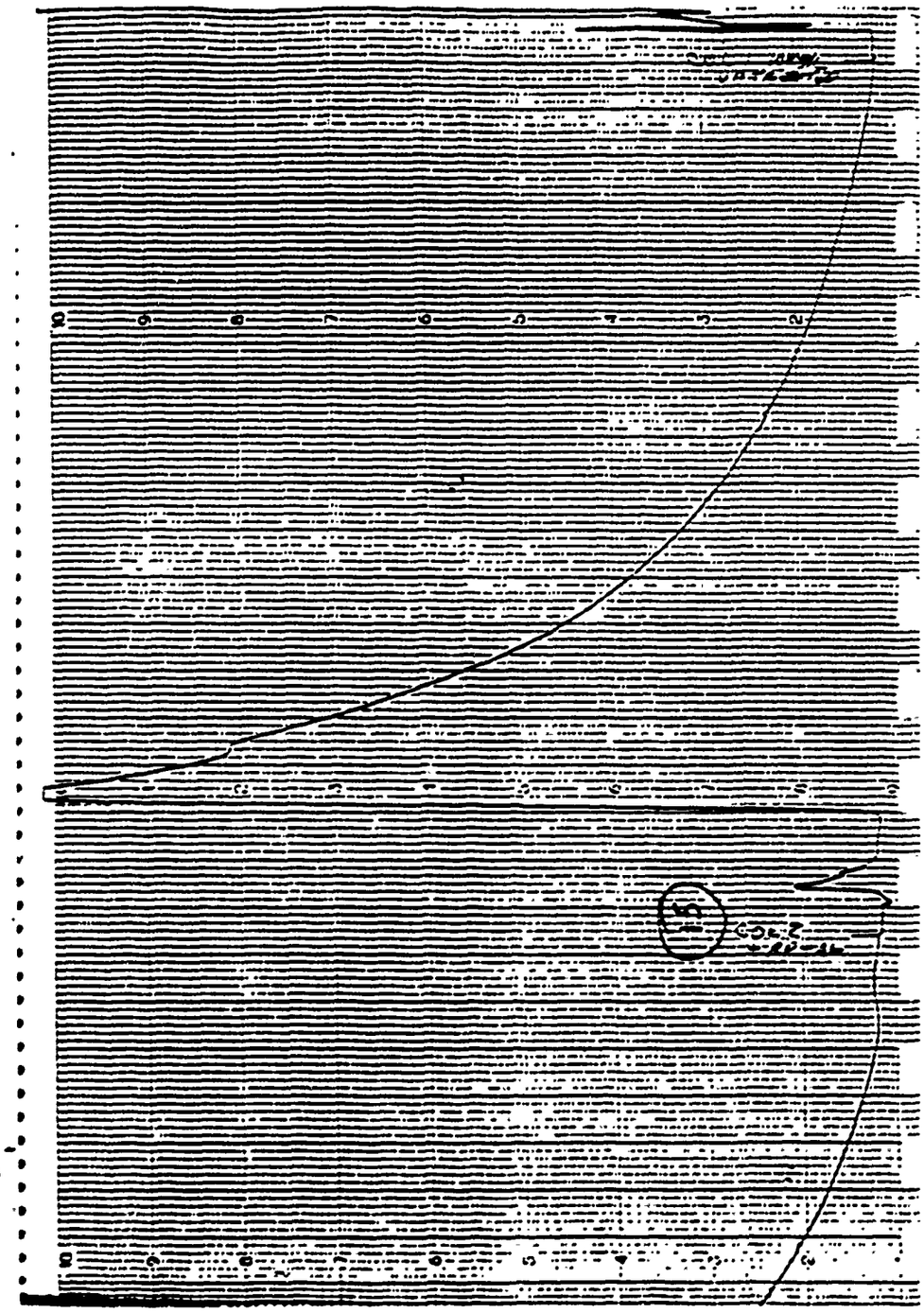

COL 2

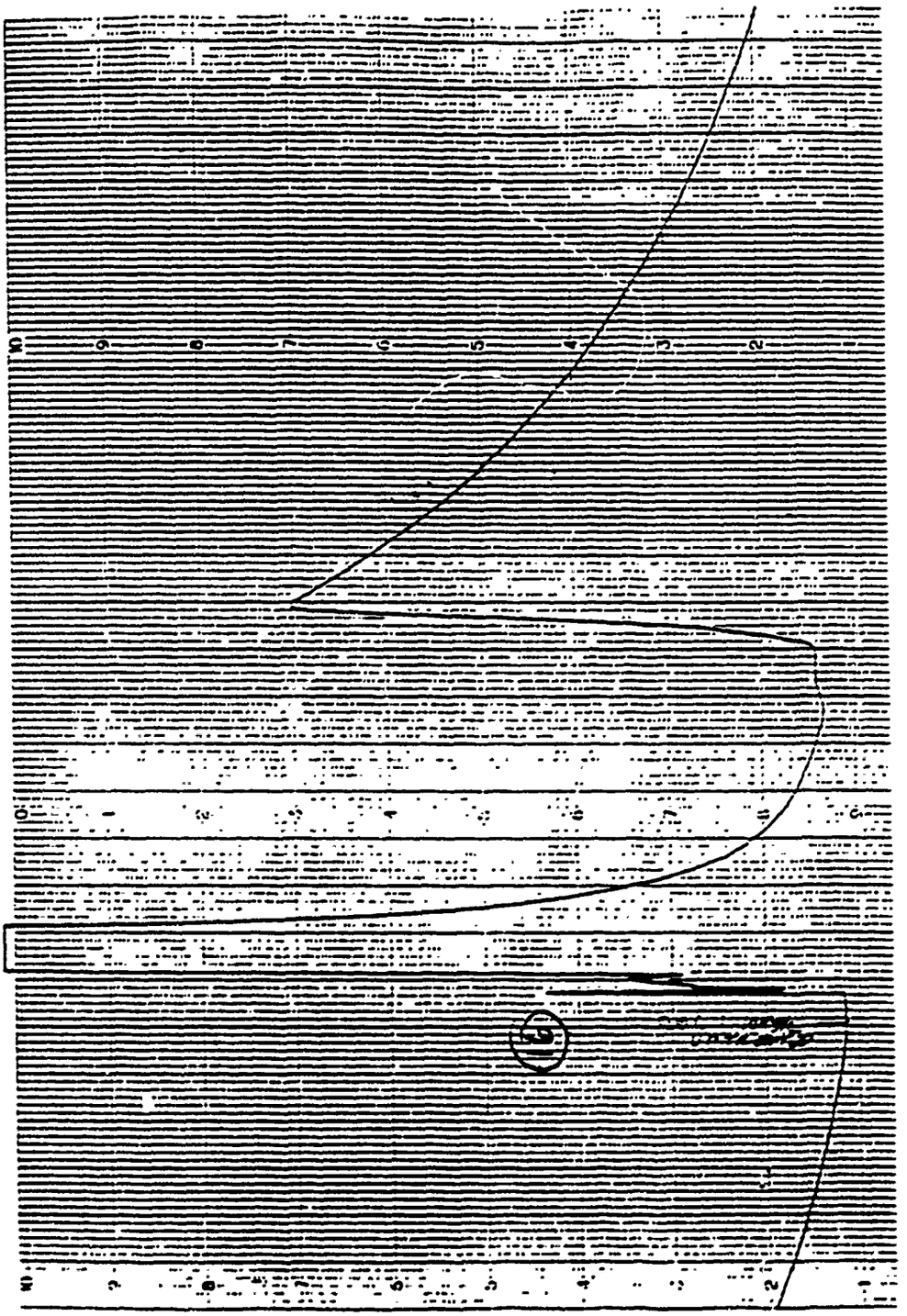


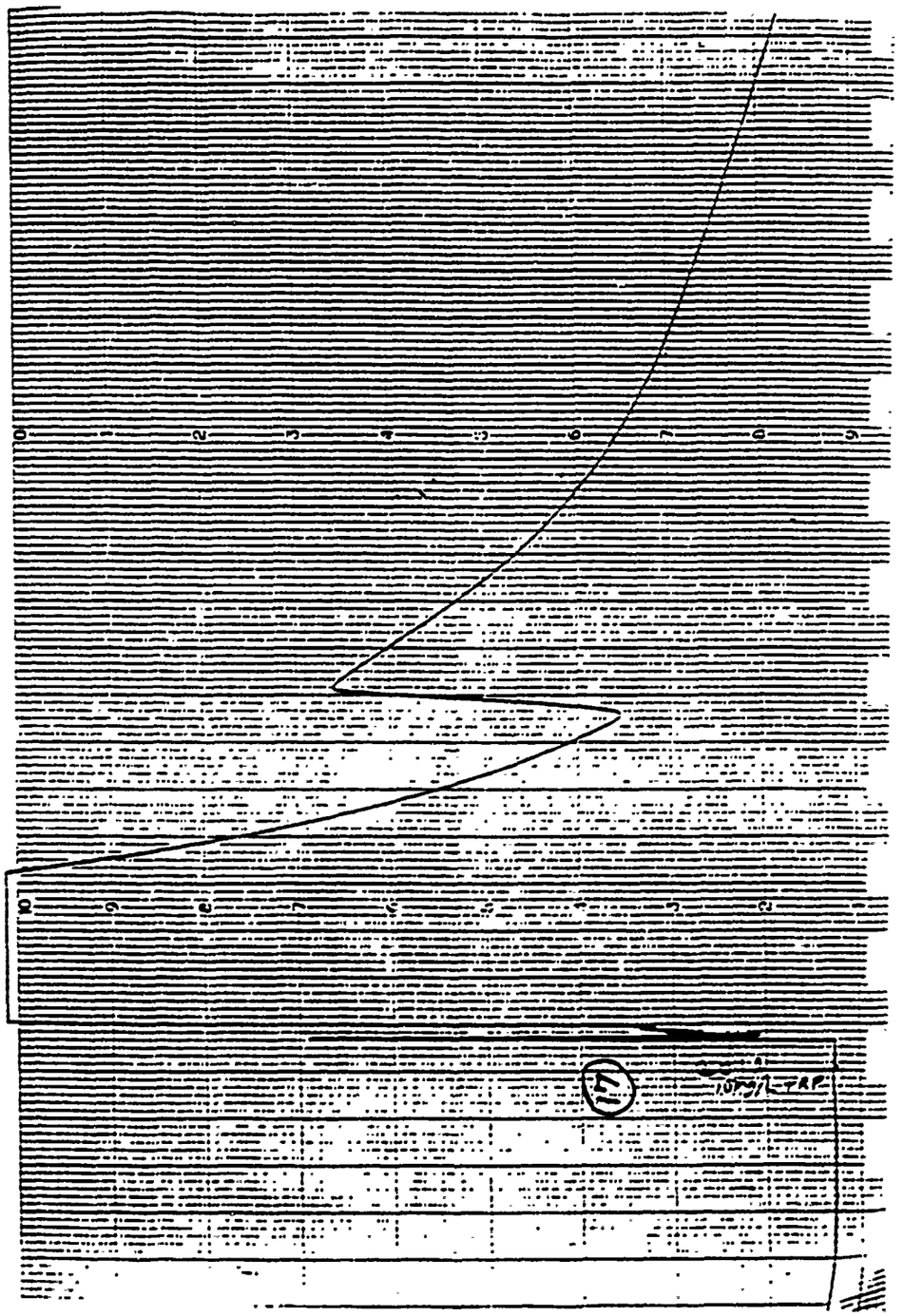

RP
AG
FLOW 1.5
COLLECTOR 30
COL_ TREATED LEACHATE
30 AP

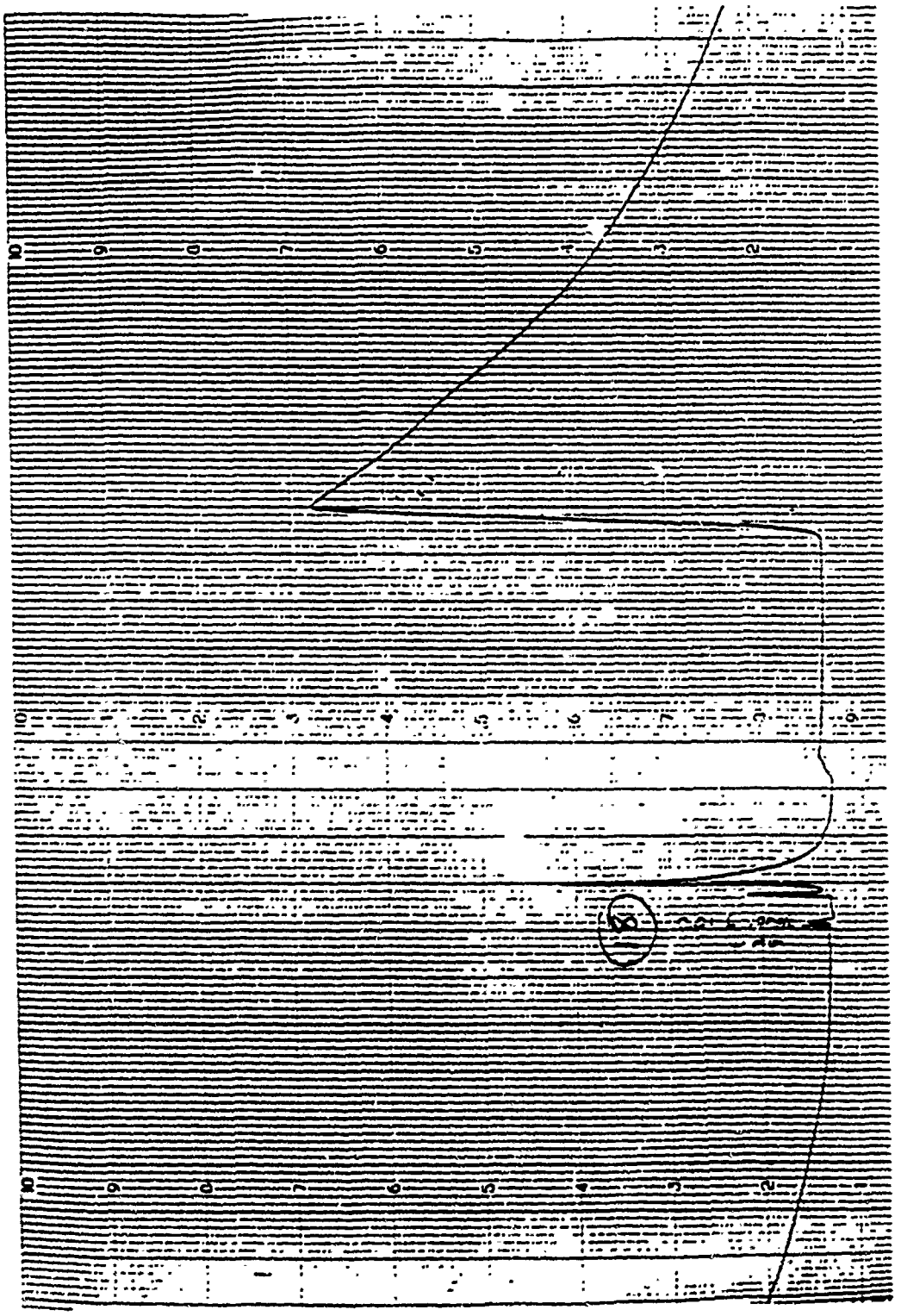


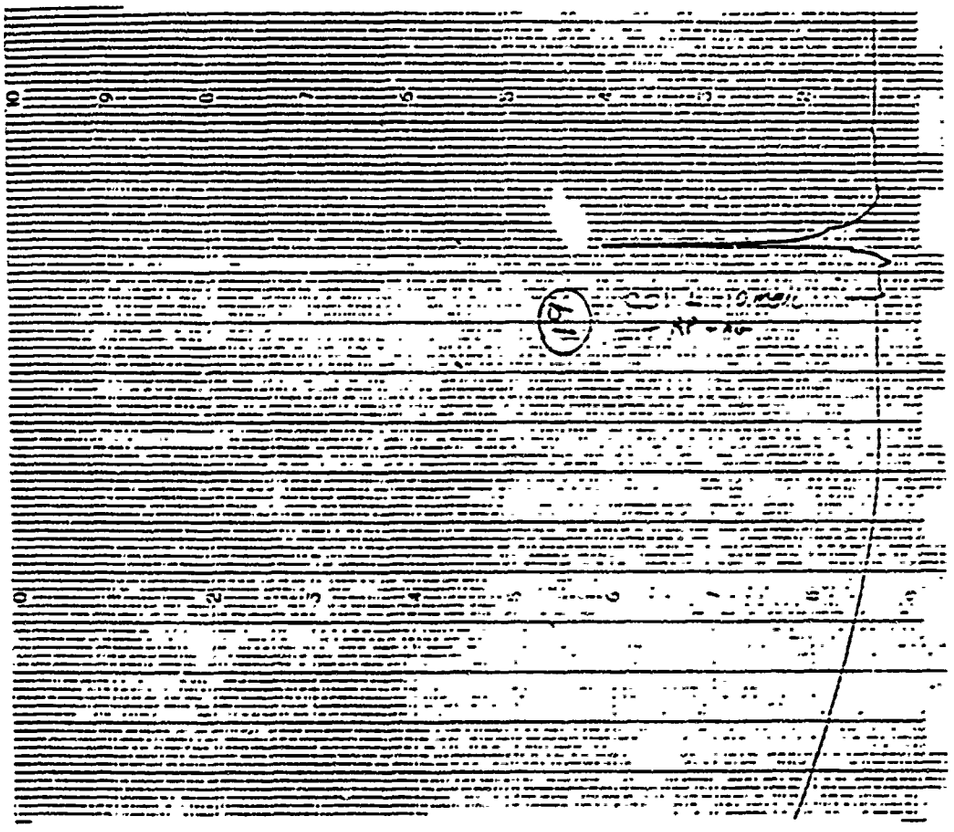












APPENDIX C

SEED REACTOR DATA

SEED REACTOR DATA

COD, mg/L

DATE	6-24-91	07-01-91	07-08-91	07-11-91	07-17-91	07-19-91	07-21-91
COD	3529.10	4481.30	2618.76	2898.84	2912.84	3388.96	3122.90

VOA ANALYSIS

DATE	6-24-91	07-01-91	07-08-91	07-11-91	07-17-91	07-19-91	07-21-91
ACID							
ACETIC	0	321.1	416.3	516.1	1532.6	1532.67	844.29
PROPIONIC	583.7	475.9	138.7	166.1	1461.94	1828.76	996.22
I-BUTYRIC	0	0	0	7.2	13.04	7.97	11.58
BUTYRIC	177.9	165.9	386.5	494.1	3364.82	233.63	237.43
I-VALERIC	0.00	0.00	0.00	5.80	7.96	6.42	9.03
VALERIC	184.30	386.20	475.70	492.40	382.21	236.54	246.74
HEXANOIC	0.00	0.00	35.40	62.80	49.84	27.02	33.34
TOTAL AS CH3COOH	702.98	1047.25	1090.42	1318.11	5276.33	3337.07	1989.51

FORMIC ACID CONCENTRATION

DATE	6-24-91	7-1-91	7-8-91	7-11-91	7-26-91	7-27-91	7-28-91	8-2-91	8-7-91	8-14-91	8-16-91
CONC, mg/l	21.08	24.12	26.77	22.08	28.12	35.71	38.38	27.89	21.96	77.22	249.30

GAS PRODUCTION
SEED REACTOR

DATE	06/18/91	06/19/91	06/20/91	06/21/91	06/22/91	06/23/91	06/24/91
METER	7771	7774	7779	7782	7783	7786	7787
GAS, L		2.43	4.05	2.43	0.81	2.43	0.81

DATE	06/25/91	06/26/91	06/27/91	06/28/91	06/29/91	06/30/91	07/01/91
METER	7788	7788	7789	7790	7791	7792	7794
GAS, L	0.81	0	0.81	0.81	0.81	0.81	1.62

DATE	07/02/91	07/03/91	07/04/91	07/05/91	07/06/91	07/07/91	07/08/91
METER	7795	7795	7796	7796	7797	7798	7799
GAS, L	0.81	0	0.81	0	0.81	0.81	0.81

DATE	07/09/91	07/10/91	07/11/91	07/12/91	07/13/91	07/14/91	07/15/91
METER	7799.5	7800	7801	7801	7802	7803	7803.5
GAS, L	0.405	0.405	0.81	0	0.81	0.81	0.405

DATE	07/16/91	07/17/91	07/18/91	07/19/91	07/20/91	07/21/91	07/22/91
METER	7804	7804	7805	7807	7807	7807	7809
GAS, L	0.405	0	0.81	1.62	0	0	0

DATE	07/23/91	07/24/91
METER	7810	7810
GAS, L	0.81	0

APPENDIX D
TEST REACTOR DATA

PERCENT REACTOR CONTENTS REMAINING

Sample #	% Remaining
1	100
2	96
3	92
4	88
5	85
6	82
7	78
8	75
9	72
10	69
11	66
12	64

ORGANIC LOADING

REACTOR	SEED	FEED	1	2	3	4	5	6	7	8	9	10
CONTROL	2660.88	4173.26	2940.95	2968.95	2716.89	2716.89	2324.79	2604.76	3767.06	3655.03	3949.10	1414.46
8g/L	2660.88	6609.88	3277.03	2716.89	2716.89	2632.87	2492.03	2352.80	2506.74	2590.76	2394.71	2478.73
12g/L	2856.93	12533.68	NA	3557.10	3473.08	3417.07	4789.42	3165.00	2828.92	3235.95	2852.37	2911.38
20g/L	2856.93	20725.76	4649.38	4061.23	3949.20	3865.18	3501.09	3305.04	3249.02	3501.50	3324.46	3383.47

CHLOROFORM TOXICITY

REACTOR	SEED	FEED	1	2	3	4	5	6	7	8	9	10
CONTROL	2492.83	3277.03	2772.90	2436.82	2716.89	2436.82	2380.80	2380.80	2716.89	2352.80	2240.67	2492.73
4mg/L	2492.83	3221.02	3193.01	2744.90	2604.86	2716.89	2296.78	2268.78	2352.80	2660.88	2030.62	1988.60
20mg/L	2842.82	3529.10	3094.12	2128.70	2962.47	2797.91	2666.26	2556.55	2534.61	2142.64	2114.64	2030.62
100mg/L	2842.82	3622.30	3006.35	2841.79	2655.29	2721.11	2501.70	2457.82	2326.17	2100.63	2058.62	2016.61

AMMONIA TOXICITY #1

REACTOR	SEED	FEED	1	2	3	4	5	6	7	8	9	10
CONTROL	0.211	0.150	0.117	0.217	0.207	0.192	0.198	0.188	0.186	0.178	0.170	0.159
50mg/L	0.211	0.144	0.217	0.207	0.197	0.194	0.183	0.182	0.171	0.168	0.163	0.156

AMMONIA TOXICITY #3

TIME	0	1	4	8	12	16	20	24
COD, mg/L	3465.51	1972.83	2334.22	3921.17	3276.96	3119.84	3135.55	3182.69

AMMONIA TOXICITY #4

TIME	0	1	12	24	36	48	60	72	84	96	108	120
CONTROL	1970.58	3031.50	2707.64	2819.32	3232.52	3321.86	3355.36	3098.51	3433.54	2651.80	3299.52	3087.34
TEST	3087.34	2953.33	2495.46	440.62	3310.69	3053.84	3176.68	3455.87	3388.87	3277.19	3333.03	3098.51

SEED REACTOR COD ANALYSIS DURING 8-20-91 TO 8-25-91

DATE	8-20-91	8-20-91	8-21-91	8-21-91	8-22-91	8-22-91	8-23-91	8-23-91	8-24-91	8-24-91	8-25-91	8-25-91
TIME	0800	1928	0720	1915	0715	1945	0720	1955	0800	1700	0700	1930
SAMPLE #	1	2	3	4	5	6	7	8	9	10	11	12
CONC,mg/L	3322.90	3256.50	3269.78	2738.53	3378.03	3283.06	1649.47	1795.57	1676.04	1622.91	1689.32	1755.72

VOLATILE ACID ANALYSIS
ORGANIC LOADING TESTS

CONTROL REACTOR
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	2060.35	767.77	1004.38	1447.02	542.95	506.01	644.53	549.32	556.33	636.25	541.68	672.56	556.82
PROPIONIC	1291.58	558.20	866.16	1250.57	425.22	373.26	540.48	476.78	415.06	497.08	467.02	499.07	531.93
I-BUTYRIC	10.93	3.50	8.30	7.58	4.22	0.00	0.00	5.39	3.35	4.37	5.68	0.00	7.38
BUTYRIC	197.51	82.93	136.14	190.46	65.51	59.02	87.35	79.06	59.85	77.54	76.98	76.02	100.02
I-VALERIC	12.39	4.17	0.00	12.62	0.00	3.70	4.51	9.84	0.00	0.00	0.00	5.90	6.88
VALERIC	157.35	66.50	111.92	151.30	54.38	45.69	72.68	69.26	48.06	64.26	65.05	63.60	86.65
HEXANOIC	13.96	0.00	8.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL AS CH3COOH	3356.75	1320.86	1875.31	2692.44	967.26	877.95	1187.73	1040.00	964.22	1132.94	1014.97	1169.93	1116.36

TEST REACTOR (T - 8g/L)
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	2060.35	943.87	874.13	1698.91	892.29	588.17	607.28	528.94	478.94	1126.66	479.90	1003.42	648.56
PROPIONIC	1291.58	754.48	751.50	1458.00	753.49	486.73	449.11	387.99	374.06	955.15	409.09	755.28	481.21
I-BUTYRIC	10.93	5.24	8.89	9.61	10.20	4.22	0.00	0.00	0.00	0.00	4.22	6.70	0.00
BUTYRIC	197.51	111.26	124.25	278.64	137.38	76.57	67.03	57.36	58.33	144.02	68.14	113.61	69.55
I-VALERIC	12.39	4.17	8.34	12.97	9.96	0.00	3.59	0.00	0.00	9.61	0.00	5.33	0.00
VALERIC	157.35	86.51	97.70	173.15	110.34	63.86	54.65	47.67	51.35	113.37	60.31	91.78	52.51
HEXANOIC	13.96	10.02	0.00	16.23	6.80	4.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL AS CH3COOH	3356.75	1693.58	1636.61	3195.48	1678.13	1077.88	1051.38	910.67	852.21	2071.64	896.41	1754.96	1117.04

VOLATILE ACID ANALYSIS
ORGANIC LOADING TESTS

TEST REACTOR (1A - 12g/L)
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	634.89	NA	1240.91	803.78	839.02	687.74	731.79	906.15	703.23	927.72	527.96	707.18	657.67
PROPIONIC	479.50	NA	904.86	527.76	838.18	417.38	572.97	682.40	493.94	563.28	321.82	492.04	409.02
I-BUTYRIC	5.87	NA	5.74	4.10	15.03	105.34	9.15	11.07	5.60	6.01	0.00	5.19	0.00
BUTYRIC	133.99	NA	233.33	128.46	278.62	103.93	175.64	207.31	136.68	140.86	81.55	129.81	106.22
I-VALERIC	0.00	NA	8.33	2.60	16.25	0.00	9.38	11.88	0.00	4.27	0.00	0.00	4.27
VALERIC	70.27	NA	110.24	60.21	159.16	50.78	98.82	114.96	72.75	70.14	41.09	66.79	55.12
HEXANOIC	6.45	NA	0.00	0.00	15.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL AS CH3COOH	1163.70	NA	2207.33	1359.02	1829.90	1198.70	1386.00	1682.95	1243.53	1528.35	868.67	1237.47	1096.66

TEST REACTOR (1B - 20g/L)
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	634.89	1287.39	1085.38	813.81	893.09	794.67	672.25	991.51	992.12	507.00	1023.41	1062.90	625.16
PROPIONIC	479.50	969.45	787.83	539.15	665.49	562.71	514.08	716.59	692.66	329.80	739.20	750.98	501.73
I-BUTYRIC	5.87	6.69	7.24	4.24	7.38	6.01	7.51	10.52	5.33	3.42	7.92	9.02	8.61
BUTYRIC	133.99	253.95	204.08	135.06	181.43	146.79	154.61	208.53	175.77	86.94	196.66	195.32	153.80
I-VALERIC	0.00	8.96	7.81	4.48	7.08	4.38	8.13	10.73	6.15	3.33	8.65	4.90	8.96
VALERIC	70.27	122.04	99.32	65.92	92.24	73.62	85.79	113.22	84.79	44.20	98.08	96.21	86.90
HEXANOIC	6.45	9.12	0.00	0.00	0.00	0.00	8.34	7.12	0.00	0.00	0.00	0.00	0.00
TOTAL AS CH3COOH	1163.70	2332.92	1931.26	1387.35	1619.84	1400.98	1259.16	1798.48	1730.70	863.97	1825.03	1870.60	1199.09

CONTROL REACTOR
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	1073.47	759.23	940.09	812.53	1024.25	830.59	760.69	573.43	639.25	662.84	730.11	578.09	1277.04
PROPIONIC	805.33	434.10	593.72	501.01	922.42	623.14	614.07	374.05	441.76	500.21	538.50	376.06	900.05
I-BUTYRIC	12.98	0.00	6.75	0.00	18.18	10.73	12.12	5.19	5.37	9.18	9.00	0.00	7.27
BUTYRIC	314.28	152.01	213.67	185.13	415.83	259.87	267.29	147.44	179.61	216.04	223.45	148.55	360.48
I-VALERIC	13.65	0.00	5.77	7.60	22.52	10.70	12.67	4.22	0.00	11.12	9.99	0.00	15.90
VALERIC	135.11	62.09	88.66	76.97	192.65	116.59	122.66	66.19	79.09	99.28	98.53	65.73	194.62
HEXANOIC	0.00	0.00	0.00	0.00	16.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL AS CH3COOH	2037.08	1251.38	1627.31	1394.73	2203.12	1595.22	1528.70	1022.20	1170.08	1286.91	1389.05	1022.95	2381.39

TEST REACTOR (T - 4mg/L)
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	1073.47	677.40	978.82	889.71	732.15	737.68	566.44	684.97	615.37	745.26	506.74	766.22	621.48
PROPIONIC	805.33	413.75	701.94	606.41	473.60	486.90	361.75	410.93	412.94	498.79	295.25	563.89	368.60
I-BUTYRIC	12.98	0.00	10.91	9.35	0.00	0.00	0.00	0.00	4.85	6.41	0.00	0.00	0.00
BUTYRIC	314.28	145.23	277.85	233.54	178.35	184.81	137.51	148.23	158.64	198.22	104.71	229.28	131.99
I-VALERIC	13.65	0.00	11.82	9.85	5.21	0.00	5.07	0.00	4.36	7.04	0.00	10.13	0.00
VALERIC	135.11	57.84	122.06	99.74	77.58	78.79	58.90	61.33	66.95	88.05	44.94	103.84	54.80
HEXANOIC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL AS CH3COOH	2037.08	1145.92	1823.59	1611.46	1286.45	1304.83	991.14	1155.30	1103.60	1345.13	843.95	1446.80	1042.58

TEST REACTOR (1A - 20mg/L)
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	1520.02	653.65	677.16	758.77	861.67	684.36	692.38	737.47	778.96	649.22	637.88	551.03	660.01
PROPIONIC	714.87	348.99	421.95	322.34	524.00	270.62	281.94	318.16	355.43	349.86	324.26	230.57	343.41
I-BUTYRIC	10.51	5.39	8.76	3.50	11.73	0.00	3.37	0.00	3.50	6.20	4.31	0.00	6.07
BUTYRIC	362.04	199.84	270.06	162.71	346.53	133.79	140.18	156.85	185.90	218.73	199.84	118.16	202.19
I-VALERIC	10.25	4.80	10.69	0.00	13.85	0.00	0.00	0.00	0.00	7.20	6.11	0.00	0.00
VALERIC	196.72	117.67	172.51	90.19	225.29	74.57	77.96	84.01	103.75	137.16	123.12	67.07	122.63
HEXANOIC	87.93	57.38	90.78	40.95	121.55	36.79	36.57	38.87	47.63	73.80	64.17	30.99	62.52
TOTAL AS CH3COOH	2520.88	1178.26	1364.11	1207.69	1734.34	1057.90	1083.63	1171.91	1281.95	1209.35	1149.20	874.02	1184.93

TEST REACTOR (1B - 100mg/L)
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	1520.02	709.80	739.13	676.89	726.12	784.21	683.53	738.57	713.40	610.77	630.14	733.59	510.09
PROPIONIC	714.87	376.50	345.85	286.29	307.89	351.25	324.96	316.77	337.49	301.27	247.81	376.85	212.11
I-BUTYRIC	10.51	7.14	4.99	0.00	0.00	3.91	4.58	3.64	4.58	0.00	0.00	4.72	0.00
BUTYRIC	362.04	225.25	187.99	142.00	154.25	184.60	180.43	160.37	185.38	177.57	121.55	220.43	108.13
I-VALERIC	10.25	7.31	3.82	0.00	3.82	6.54	0.00	0.00	4.25	4.80	0.00	4.80	0.00
VALERIC	196.72	138.25	108.35	78.45	84.50	106.05	109.07	85.10	109.92	109.20	66.10	135.83	60.65
HEXANOIC	87.93	70.85	53.44	34.49	38.87	52.12	55.74	41.50	53.87	54.97	30.55	70.52	28.14
TOTAL AS CH3COOH	2520.88	1295.79	1244.74	1069.82	1152.99	1290.73	1166.14	1178.76	1211.59	1071.60	968.62	1311.85	806.02

AMMONIA TOXICY TEST #1

CONTROL REACTOR
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	1906.88	1928.56	1612.25	1345.66	1469.43	1446.69	1327.14	1448.27	1782.57	1548.78	1833.88	1673.08	1421.83
PROPIONIC	515.57	404.99	394.57	283.63	359.75	382.73	293.33	266.40	390.27	380.93	417.55	374.83	305.18
I-BUTYRIC	12.82	0.00	8.88	0.00	9.37	11.84	0.00	0.00	0.00	12.58	0.00	0.00	0.00
BUTYRIC	763.65	489.60	542.99	375.00	515.20	581.27	387.19	320.87	484.23	502.52	553.72	463.51	364.52
I-VALERIC	10.05	6.44	8.53	0.00	0.00	0.00	0.00	0.00	0.00	6.82	0.00	0.00	0.00
VALERIC	277.31	152.14	189.21	126.74	185.17	216.86	133.93	104.72	156.63	175.51	180.23	147.19	113.71
HEXANOIC	150.70	75.16	94.33	63.53	98.59	124.36	69.93	0.00	76.32	80.39	87.17	68.18	54.24
TOTAL AS CH3COOH	3101.30	2722.90	2473.56	1938.73	2278.70	2353.28	1943.92	1944.65	2560.77	2357.67	2701.08	2414.87	2012.74

TEST REACTOR (T - 50mg/L)
SAMPLE CONCENTRATION

ACID	SEED	1	2	3	4	5	6	7	8	9	10	11	12
ACETIC	1906.88	1654.57	1433.46	1499.05	1102.34	1558.45	1368.40	1202.31	1165.81	1109.22	245.96	1497.47	1067.96
PROPIONIC	515.57	490.80	316.67	313.79	277.53	360.47	277.89	242.70	238.40	286.87	50.26	333.54	203.57
I-BUTYRIC	12.82	15.29	0.00	6.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BUTYRIC	763.65	772.19	415.47	394.99	399.87	446.93	339.40	301.37	302.10	515.44	69.25	415.96	309.90
I-VALERIC	10.05	16.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VALERIC	277.31	286.97	139.33	131.24	142.70	81.35	108.09	93.71	104.50	184.27	20.00	134.61	102.02
HEXANOIC	150.70	161.35	71.09	65.28	78.26	67.02	49.01	42.03	44.74	93.75	0.00	62.95	55.01
TOTAL AS CH3COOH	3101.30	2851.28	2092.22	2138.46	1724.42	2267.96	1914.06	1681.44	1649.69	1850.13	345.70	2163.26	1532.78

VOLATILE ACID ANALYSIS
AMMONIA TOXICY TEST # 3

TEST REACTOR

ACID	0	1	4	8	12	16	20	24
ACETIC	608.34	728.17	451.14	549.65	451.14	588.25	600.41	484.62
PROPIONIC	120.42	152.21	148.55	116.30	107.84	116.99	142.04	98.69
I-BUTYRIC	5.11	6.85	0.00	7.14	7.14	5.02	7.53	3.86
BUTYRIC	283.37	353.00	381.50	294.46	292.44	258.91	366.93	225.93
I-VALERIC	3.09	0.00	4.18	3.46	5.55	0.00	6.27	2.46
VALERIC	40.12	51.18	56.30	41.86	47.30	29.14	56.06	32.69
HEXANOIC	93.45	115.64	137.36	101.13	121.42	80.29	137.67	79.10
TOTAL AS CH3COOH	976.42	1186.86	938.33	928.55	836.74	921.73	1078.75	782.91

VOLATILE ACID ANALYSIS
AMMONIA TOXICY TEST # 4

CONTROL REACTOR
SAMPLE CONCENTRATION

ACID	0	1	12	24	36	48	60	72	84	96	108	120
ACETIC	1090.93	1056.64	720.79	826.88	721.22	716.29	662.71	842.31	671.28	668.28	706.64	809.73
PROPIONIC	188.02	212.08	122.97	155.43	146.61	131.22	117.09	161.16	123.25	147.87	128.85	130.94
I-BUTYRIC	4.95	0.00	6.24	6.13	5.70	3.98	0.00	6.02	4.84	8.28	4.95	4.95
BUTYRIC	421.55	471.18	279.20	372.45	373.10	324.86	286.83	428.21	308.42	410.80	316.16	290.59
I-VALERIC	0.00	0.00	0.00	4.00	8.29	0.00	3.14	0.00	3.05	7.33	3.62	0.00
VALERIC	49.72	58.99	33.95	46.56	48.70	41.65	36.27	52.03	38.77	56.58	39.14	32.83
HEXANOIC	109.10	142.05	77.80	106.95	123.59	103.50	85.56	119.97	90.65	131.18	82.28	66.24
TOTAL AS CH3COOH	1619.85	1658.03	1075.32	1296.09	1195.81	1124.92	1020.65	1361.71	1056.28	1179.36	1097.76	1170.98

TEST REACTOR (T - 50mg/L)
SAMPLE CONCENTRATION

ACID	0	1	12	24	36	48	60	72	84	96	108	120
ACETIC	785.30	740.08	897.40	718.43	961.48	715.43	1107.22	690.14	761.51	740.94	692.50	884.96
PROPIONIC	139.48	120.03	154.45	129.68	171.93	120.87	196.14	116.95	142.97	132.76	127.31	170.25
I-BUTYRIC	5.16	0.00	4.84	5.05	6.02	3.66	7.63	3.44	5.91	4.84	5.27	7.20
BUTYRIC	376.43	292.63	392.22	342.48	423.48	303.48	486.97	304.02	379.97	336.03	330.23	436.91
I-VALERIC	0.00	0.00	3.62	3.62	3.14	0.00	31.91	2.57	0.00	4.48	0.00	5.14
VALERIC	48.60	35.43	47.30	44.89	52.13	38.31	62.89	35.90	49.16	43.41	42.20	57.78
HEXANOIC	115.74	75.38	113.50	115.40	124.63	95.39	138.43	86.42	117.90	100.13	102.89	136.53
TOTAL AS CH3COOH	1247.02	1096.76	1382.01	1148.76	1490.70	1094.72	1730.84	1061.93	1230.44	1160.95	1102.51	1433.45

VOLATILE ACID ANALYSIS
SEED REACTOR DURING UPSET

DATE	8-20-91	8-20-91	8-21-91	8-21-91	8-22-91	8-22-91	8-23-91	8-23-91	8-24-91	8-24-91	8-25-91	8-25-91
TIME	0800	1928	0720	1915	0715	1945	0720	1955	0800	1700	0700	1930
SAMPLE #	1	2	3	4	5	6	7	8	9	10	11	12
ACID												
ACETIC	1151.28	893.99	1028.42	1169.75	1502.73	1698.79	1357.71	1073.38	1412.05	79.26	1314.17	1380.53
PROPIONIC	92.53	66.49	72.40	86.93	175.76	204.42	162.88	159.07	216.21	7.98	313.25	369.84
I-BUTYRIC	0.00	1.83	2.00	0.00	3.17	0.00	0.00	0.00	0.00	0.00	4.31	0.00
BUTYRIC	182.61	126.55	136.93	135.27	260.91	230.93	194.36	172.39	163.35	3.23	172.26	165.41
I-VALERIC	8.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.89	4.12
VALERIC	23.00	16.15	15.93	16.08	31.33	26.95	21.45	20.66	18.08	0.00	24.93	20.77
HEXANOIC	51.21	34.59	34.73	35.66	66.68	55.67	48.69	44.56	36.94	0.00	44.14	46.04
TOTAL AS CH3COOH	1395.80	1062.83	1209.19	1360.36	1878.21	2066.63	1660.09	1355.10	1728.47	87.94	1728.34	1831.64

ORGANIC LOADING

SAMPLE	1	2	3	4	8	12
CONTROL	68.62	65.32	78.85	41.12	88.58	38.38
8g/L	NA	49.73	108.49	52.73	44.78	58.91
12g/L	NA	43.93	43.93	16.98	15.00	19.05
20g/L	28.12	28.12	NA	25.74	19.05	16.98

CHLOROFORM TOXICITY

SAMPLE	0	1	5	10	12
0mg/L	35.71	43.93	16.98	13.11	33.11
4mg/L	35.71	25.74	21.20	23.43	46.80
20mg/L	38.38	33.11	30.58	13.11	49.73
100mg/L	38.38	28.12	19.05	19.05	62.09

AMMONIA TOXICITY TEST #1

SAMPLE	0	1	5	10	12
CONTROL	154.44	23.43	29.83	25.25	14.47
50mg/L	154.44	158.88	73.46	43.37	58.70

AMMONIA TOXICITY TEST #2

SAMPLE	0	1	5	10	12
CONTROL	58.21	42.97	32.66	20.56	17.86
50mg/L	64.33	72.83	62.27	31.04	26.36

AMMONIA TOXICITY TEST #3

SAMPLE	0	1	4	8	12	16	20	24
CONC,mg/L	77.22	62.27	58.21	235.99	330.74	115.47	167.55	173.43

AMMONIA TOXICITY TEST #4

SAMPLE	0	1	12	24	36	48	60	72	84	96	108	120
CONTROL	249.30	206.99	194.54	179.37	226.17	194.54	173.43	256.05	245.95	206.99	242.61	219.71
50mg/L	158.88	142.03	136.56	173.43	191.47	182.37	156.02	222.93	203.85	167.55	203.85	173.43

BASE REACTOR DATA DURING UPSET FROM 8-20-91 TO 8-25-91

SAMPLE #	1	2	3	4	5	6	7	8	9	10	11	12
DATE	8-20-91	8-20-91	8-21-91	8-21-91	8-22-91	8-22-91	8-23-91	8-23-91	8-24-91	8-24-91	8-25-91	8-25-91
TIME	0800	1928	0720	1915	0715	1945	0720	1955	0800	1700	0700	1930
PEAK HT	14.4	13.5	12.5	16.1	16.2	11.1	9.7	13.2	12.1	12.7	12	11.5
CONC,mg/L	353.30	319.66	283.70	420.03	424.08	235.99	191.47	308.71	269.75	290.77	266.30	249.30