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Inhibition of anaerobic digestion by organic priority pollutants

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INHIBITION OF ANAEROBIC DIGESTION BY ORGANIC PRIORITY POLLUTANTS

Iowa State University PH.D. 1981

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Inhibition of anaerobic digestion by organic priority pollutants

by

Lyle D. Johnson

A Dissertation Submitted to the

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

^{o}C	degrees Celsius
cm	centimeter
cu ft/lb VS	cubic feet of gas produced per pound of volatile solids added
m^3	cubic meter
m^3/kg VS	cubic meters of gas produced per kilogram of volatile solids added
ft	feet
g/L	grams per liter
kg	kilogram
lb VS/cu ft/day	pounds of volatile solids added per cubic feet of reactor volume per day
mA	milliamp
mg/L	milligrams per liter
mL	milliliter
mm	millimeter
mV	millivolt
psig	pounds per square inch (gauge)
SRT	solids retention time
µL	microliter

INTRODUCTION

Anaerobic digestion is a biological process by which organic materials are converted into a product gas consisting of a high percentage of methane, the major component in natural gas. While this reaction involves the complex coexistence of different groups of bacteria in a single reactor system, the overall process of conversion of organic materials into product gas can be simplified into three steps. First, particulate and colloidal organic polymers are hydrolyzed and converted to soluble substrates by bacterial extracellular enzymes. The soluble substrates are then metabolized to form low molecular weight end products such as acetic acid, hydrogen and carbon dioxide. In a third step, acetic acid and hydrogen are converted biologically to a product gas containing methane and carbon dioxide.

Anaerobic digestion processes have been used widely for stabilization of municipal wastewater sludges and industrial wastes, and anaerobic digestion of municipal refuse recently has attracted attention as a possible renewable energy source. The performance of these processes is dependent upon maintenance of a delicate biochemical balance among the different groups of bacteria. Instability may occur as a result of a general change in the environment of the process as a whole or as a result of effects on specific cells or enzymes. The general environmental limits for anaerobic

treatment are well-defined and every attempt is made to maintain the proper environmental conditions in an anaerobic treatment process.

Instability often is caused by the presence of a toxic substance which inhibits the metabolism of the methane producing microorganisms. The specific effects of toxic organic chemicals on the biological reactions occurring during anaerobic digestion are not defined well, and application of various inhibition theories to an anaerobic waste treatment process is complicated by the presence of a mixed biological culture. The methane-forming bacteria are the key to the successful operation of any anaerobic process since these organisms are responsible for true waste stabilization. Inhibition of the methane-producing bacteria is generally characterized by a decrease in methane production and an increase in methane precursors such as acetic acid. In many cases, recovery from inhibition occurs with a return to stable operating conditions. The actual conditions which relate to the recovery are not defined well. The term "acclimation" has been loosely applied since microorganisms usually have the ability to adapt to some extent to inhibitory concentrations of most materials.

While a material may be inhibitory to the methane-forming bacteria within the treatment system, there may be other microorganisms present that are capable of degrading the compound and thus eliminating the inhibitory effects. Organic chemical inhibitors fall into this category and various chemical, physical and biological factors may

play a part in their inhibitory action.

One anaerobic treatment application expected to be subject to instability caused by the presence of inhibitory organic materials is the anaerobic digestion of municipal solid wastes. The technology exists to readily separate the organic fraction from the inert material and to prepare the organic material as a feedstock for an anaerobic digester. There is, however, little control of the actual composition of the waste materials, and the possibility exists that slug loads of toxic organic substances will be carried into the digestion process with the solid waste to be digested.

Because a better understanding of the inhibitory effects of organic toxins is needed to help improve the stability of anaerobic digestion processes, and because municipal solid waste digestion systems may be particularly prone to receive toxic organics, a research study was initiated to investigate the inhibitory effect of toxic organics on the anaerobic digestion of municipal solid waste.

OBJECTIVES AND SCOPE OF STUDY

The primary objectives of this research study were 1) to investigate the factors that affect inhibition of anaerobic digestion by organic chemicals and 2) to determine the conditions that relate to the recovery of methane production capacity in an inhibited system. It was of particular interest to evaluate the response to various inhibitors by the different groups of bacteria in an unacclimated culture.

To develop a useable set of data, initial screening for inhibition was performed with various organic chemicals to determine their inhibitory effect in anaerobic systems as measured by or affected by the following parameters:

1. Gas production,
2. Gas quality,
3. Individual volatile acids concentration,
4. Soluble inhibitor concentration, and
5. Inert solids concentration.

The laboratory study involved a series of tests in which mixed liquor from a laboratory-scale digester receiving a mixture of components of municipal solid waste was transferred to 250 mL serum bottles. Ethanol was added as a known biodegradable substrate and the serum bottles were then dosed differentially with organic test chemicals to determine their inhibitory effect. Suppression of gas

production was used as a measure of inhibition. Gas chromatography was used to measure organic acids, to provide a means of determining if the test chemicals were decomposed, and to identify decomposition products.

It was not the intent of this research project to make an in-depth study of the degradation of the inhibitory organic chemicals in anaerobic environments. However, pertinent data and experimental observations will be reported when the metabolism of various organic inhibitors was observed.

LITERATURE REVIEW

Biochemistry and Microbiology of Anaerobic Treatment

The process of anaerobic digestion involves the biological decomposition of complex organic matter by a wide variety of interacting species of bacteria to form methane and carbon dioxide. At least two large, physiologically different bacterial populations must be present to complete the two major phases of conversion of organic materials. The first phase involves a heterogeneous group of microorganisms that convert proteins, carbohydrates and lipids into primarily fatty acids by hydrolysis and fermentation. This phase has been called the acid fermentation phase because of the acidic end products of metabolism. The second, or methanogenic phase involves the formation of methane and carbon dioxide from the various end products of the first phase by a unique group of strict anaerobic bacteria. Bryant (1979) and other researchers (McInerney, et al., 1979; Zeikus, 1977; and Wolfe, 1979) reported the presence of an intermediate stage of conversion performed by a group of microorganisms called the acetogenic bacteria. These bacteria are involved in the conversion of high molecular weight organic acids and alcohols to acetate, molecular hydrogen and carbon dioxide. Because each phase plays an important role in the anaerobic treatment process and in the effect of toxicity of organic materials to the anaerobic reactions, the different stages of conversion will be considered separately in the following sections.

Acid formation phase

The first stage of anaerobic digestion is performed by a wide variety of bacteria. Some are facultative anaerobes while others are strict anaerobic species. Many of these bacteria excrete extracellular enzymes which cause the hydrolysis of complex organics such as polysaccharides, cellulose and proteins (Zeikus, 1980). This hydrolysis produces soluble substrates including hexoses, pentoses, amino acids and fatty acids that can be transported into the cell where metabolism occurs (Kaspar and Wuhrmann, 1979).

The fermentation of sugars (hexoses and pentoses) proceeds along pathways, such as the Embden-Meyerhof-Parnas (EMP) pathway, with the formation of pyruvate (Bryant, 1979; Kotze, et al., 1969). Pyruvate is then converted further to acetate, carbon dioxide and molecular hydrogen or to propionate, butyrate or ethanol (Figure 1).

Decomposition of proteins in anaerobic treatment processes is primarily the work of the proteolytic species of Clostridium (Gaudy and Gaudy, 1980). These organisms actively produce proteolytic enzymes which break down proteins into amino acids. The resulting amino acids are fermented in basically two ways. Some amino acids are fermented individually by pathways specific for each compound. The fermentation products are generally ammonia, carbon dioxide, hydrogen, acetic acid and butyric acid. Propionic acid and ethanol may also be produced, depending on the amino acid being metabolized. The second amino acid fermentation pathway is described by the Strickland reaction

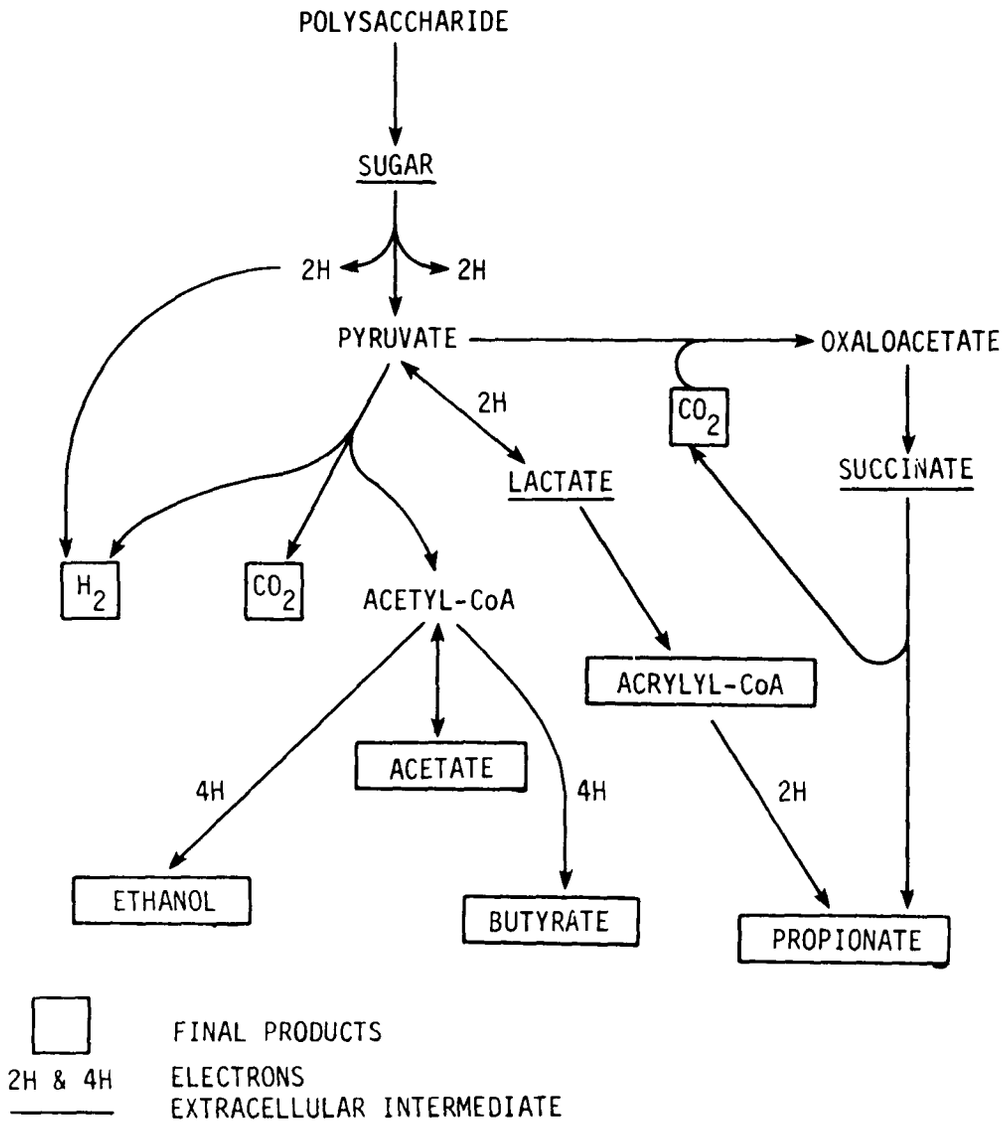

Figure 1. Pathways involved in carbohydrate metabolism by fermentative bacteria (from Bryant, 1979)

(Kotze, et al., 1969; Gaudy and Gaudy, 1980). In this reaction, the oxidation of one amino acid is coupled to the reduction of another amino acid. Figure 2 illustrates the oxidation of alanine and the reduction of glycine by the Strickland reaction.

The initial degradation of lipids is a hydrolytic process in which glycerol is liberated (Kotze, et al., 1969). The resulting fatty acids are then degraded by beta-oxidation to form butyric acid and acetic acid (McCarty, et al., 1963).

Microorganisms capable of fermentation require no external electron acceptor since metabolic products formed by the organism from the substrate are used as electron acceptors. The requirement for use of the products of the metabolism of the substrate as electron acceptors precludes the utilization of pathways that lead to complete oxidation of the substrate by fermentative bacteria (Gaudy and Gaudy, 1980). Electrons generated upon oxidation of the substrate are coupled to the reduction of a primary electron acceptor such as nicotinamide adenine dinucleotide (NAD) to form reduced NAD (NADH). NAD is a very common electron carrier molecule and acts as the primary electron acceptor in fermentative metabolism (Gottschalk, 1979). The amount of primary electron carriers is limited in bacteria and therefore the NAD is cycled continuously through a reaction in which NADH is reoxidized by reaction with a terminal electron acceptor. This reaction is illustrated in Figure 2 with the reoxidation of the NADH by the reduction of glycine to acetic acid. The acetic acid then becomes the terminal

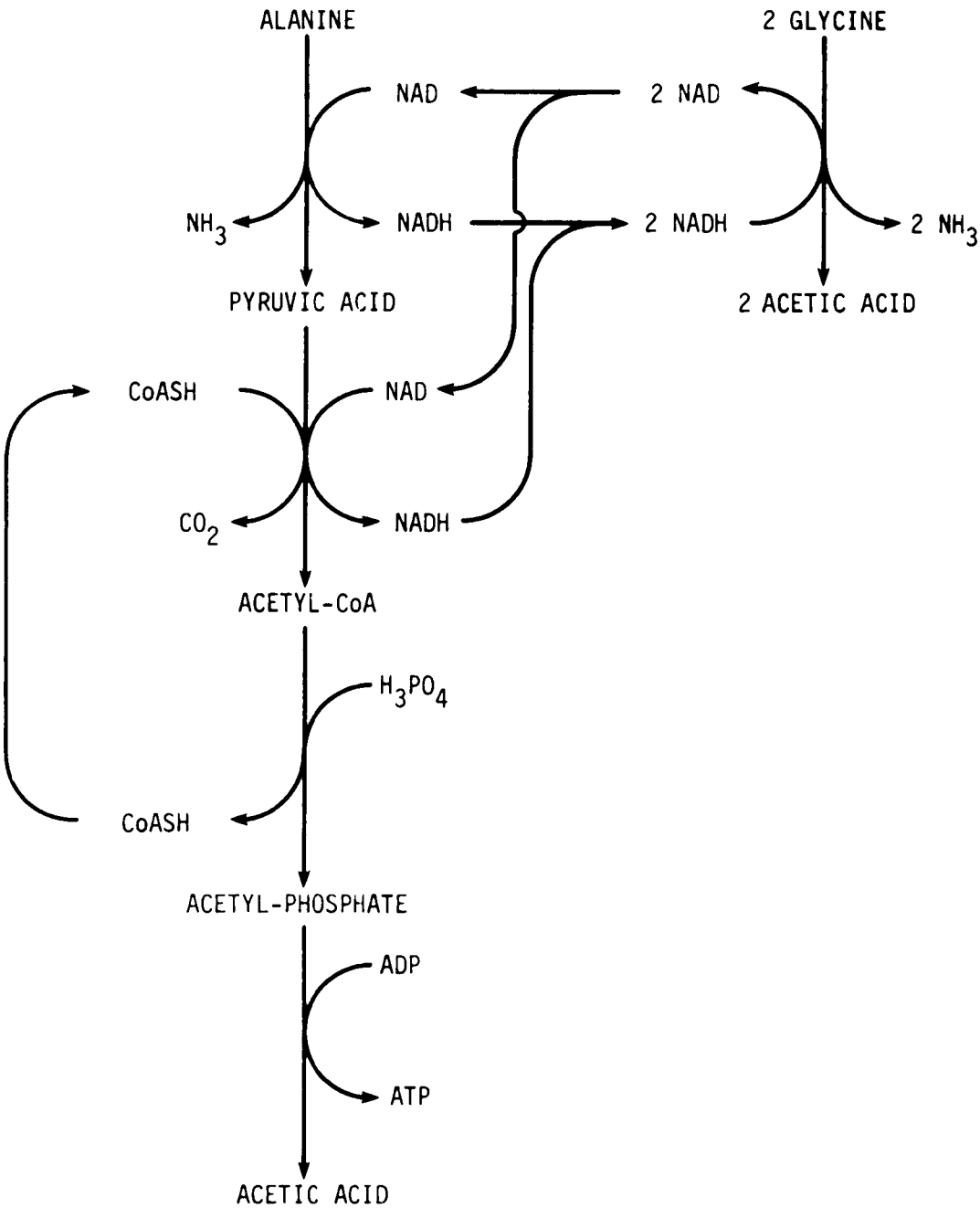

Figure 2. Strickland reaction for the fermentation of aniline and glycine amino acids by Clostridium

electron acceptor for this reaction.

Fermentative bacteria obtain energy for synthesis and cell maintenance by adenosine-5'-triphosphate (ATP) generation via substrate level phosphorylation which occurs in all bacteria that utilize organic compounds as the energy source (Gottschalk, 1979; Gaudy and Gaudy, 1980). This mechanism involves the transfer of a phosphate group from an organic intermediate to adenosine-5'-diphosphate (ADP). Substrate level phosphorylation is the only known mechanism for ATP formation by fermentative organisms, whereas this mechanism contributes only a minor fraction of the total ATP synthesized by aerobic organisms.

Aerobic organisms use an electron transport system in which oxygen is the terminal electron acceptor for reoxidation of primary electron carrier molecules. The organic substrate can be oxidized completely to carbon dioxide and water with the accompanying ATP generation by electron transport phosphorylation. This mechanism of ATP generation provides the aerobic organism with much more available energy than is available to fermentative bacteria. Gottschalk and Andreesen (1979) reported that as much as thirty-eight moles of ATP can be produced from the aerobic oxidation of one mole of glucose. By comparison, anaerobes that use organic compounds as the terminal electron acceptor can produce only four moles of ATP from one mole of glucose.

With some anaerobes, the reoxidation of NADH can occur by the enzyme system pyruvate:ferredoxin oxidoreductase and ferredoxin dehydrogenase (Gottschalk and Andreesen, 1979; Thauer, et al., 1977).

This reaction results in the formation of molecular hydrogen as follows (Bryant, 1979):

$$NADH + H^+ \rightleftharpoons H_2 + NAD^+ \quad (1)$$

The equilibrium of this reaction is strongly in the direction of NADH formation unless the partial pressure of molecular hydrogen is maintained at a very low level (Thauer, et al., 1977). Formation of molecular hydrogen by this mechanism allows for fermentation of the substrate to proceed to acetate and carbon dioxide. When the equilibrium of the reaction is directed toward NADH because of a high concentration of molecular hydrogen, there is a tendency for the electrons of NADH to be utilized for further conversion of pyruvate to propionate, butyrate, valerate and caproate (Bryant, 1979). In some extreme cases, lactate and ethanol may be formed.

Very little actual waste stabilization occurs in the acid formation phase of anaerobic treatment processes due to the incomplete oxidation of the substrate. Various facultative anaerobes can perform the complete oxidation of organic carbon to carbon dioxide when the appropriate terminal electron acceptors, such as nitrate, are available. In anaerobic treatment processes, the available nitrate will be used first as a terminal electron acceptor before anaerobic fermentation can take place.

Several anaerobic bacteria, for example, Desulfovibrio and Desulfotomaculum, can utilize sulfate as the terminal electron acceptor (Thauer, et al., 1977). These organisms are strict anaerobes and

convert some organic carbon to carbon dioxide but often do not carry out complete oxidation (Gaudy and Gaudy, 1980).

Cartwright and Cain (1959) reported on the use of the nitro group of para-nitrobenzoic acid as an electron acceptor. In experiments with Nocardia crythropolis and Pseudomonas fluorescens, para-nitrobenzoic acid was reduced to para-aminobenzoic acid as the result of the dehydrogenase activity of enzymes for which the nitro group was an electron acceptor.

Methanogenic phase

Methanogenic bacteria are responsible for converting the by-products of first-stage fermentation reactions to methane and carbon dioxide. Without the methanogens, organic acids having an energy content almost equivalent to the original organic matter would accumulate in the treatment system. Consequently, without the methanogens, little waste stabilization would occur.

The methanogenic bacteria represent a unique group of organisms. They are the only anaerobic organisms capable of effectively obtaining electrons from molecular hydrogen and metabolizing acetate anaerobically in the absence of light or exogenous electron acceptors such as nitrate or sulfate. These microorganisms are all strictly anaerobic, slow growing, sensitive to pH and organic and inorganic toxins (Zeikus, 1977). This sensitivity frequently is the cause of process instability.

Methanogenic bacteria contain three coenzymes which have not been found in other organisms (Bryant, 1979). Coenzyme 420 is an electron

transport coenzyme that replaces such electron carriers as ferredoxin found in other bacteria. This compound is strongly fluorescent and provides an easy way to identify methanogens in mixed cultures (Gottschalk, 1979). Coenzyme M (2-mercaptoethane-sulfonic acid) serves as the active carrier of methyl groups during methane formation. Factor B is a low molecular weight heat-stable coenzyme involved in the enzymatic formation of methane from methyl-Coenzyme M.

Barker (1956) reported that a number of substrates could be metabolized by methanogenic bacteria (Table 1). However, recent studies have shown that known species of the methanogenic bacteria cannot metabolize most of these compounds (Bryant, et al., 1967; Bryant, 1979) The only substrates known to be metabolized by methanogenic bacteria that only recently have been isolated in pure culture are acetate, formate, carbon dioxide, hydrogen, methanol and carbon monoxide (Zeikus, 1977; Wolfe, 1979). A possible explanation of this discrepancy concerns the extreme difficulty in isolating methanogenic bacteria in pure cultures (Zeikus, 1977). Many of the compounds reported by Barker (1956) were studied in enrichment cultures, that is, cultures in which the substrate and environmental conditions were chosen in such a way as to favor strongly the development of certain species of methanogenic bacteria without excluding a substantial and frequently significant number of other methanogenic and nonmethanogenic species.

Species of methanogenic bacteria isolated thus far are all able to oxidize molecular hydrogen coupled with the reduction of carbon

Table 1. Substrates metabolized by methanogenic bacteria according to Barker (1956)

Organic Acids	Alcohols
Formic	Methanol
Acetic	Ethanol
Propionic	n-Propanol
n-Butyric	Isopropanol
Isobutyric	n-Butanol
n-Valeric	Isobutanol
n-Caproic	1-Pentanol
n-Caprylic	
n-Capric	
Stearic	Gases
Oleic	Hydrogen
Benzoic	Carbon monoxide
Phenylacetic	Carbon dioxide
Hyrocinnamic	
Cinnamic	
Oxalic	Others
Succinic	Acetone
	2,3-Butylene glycol

dioxide to methane (Wolfe, 1979; Zeikus, 1977; Gottschalk, 1979; Bryant, 1979), as described by the following reaction:

$$4\ H_2 + CO_2 \longrightarrow CH_4 + 2\ H_2O \qquad (2)$$

Reduction of carbon dioxide to methane proceeds stepwise, but the intermediates remain firmly bound to carrier molecules that are yet

only partly known (Figure 3).

Many methanogenic species, for example, Methanobacterium formicum, Methanobacterium ruminantum, Methanobacterium mobile, Methanococcus vannieli and Methanospirillium hungatti, have the ability to metabolize formate (Zeikus, 1977). Formate is first converted to carbon dioxide and molecular hydrogen by formate dehydrogenase and is thus not a direct precursor of methane (Gottschalk, 1979). The hydrogen and carbon dioxide are then converted to methane as shown by the following reaction:

$$4\ HCOOH \longrightarrow 4\ CO_2 + 4\ H_2 \quad (3)$$

$$CO_2 + 4\ H_2 \longrightarrow CH_4 + 2\ H_2O \quad (4)$$

$$4\ HCOOH \longrightarrow CH_4 + 3\ CO_2 + H_2O \quad (5)$$

Methanol is converted to methane by only one known species of methanogenic bacteria, Methanosarcina barkeri (Bryant, 1979). This organism contains an enzyme system by which methanol and hydroidocobalamin are converted to methylcobalamin. Methane is then formed from methylcobalamin via methyl-Coenzyme M (Daniels and Ziekus, 1978; Gottschalk, 1979). This biochemical reduction is coupled to the oxidation of methanol to carbon dioxide:

$$\text{Oxidation:}\quad CH_3OH + H_2O \longrightarrow CO_2 + 6H \quad (6)$$

$$\text{Reduction:}\quad 3CH_3\ OH + 6H \longrightarrow 3CH_4 + 3H_2O \quad (7)$$

$$\text{Overall:}\quad 4CH_3OH + H_2O \longrightarrow 3CH_4 + CO_2 + 2H_2O \quad (8)$$

Methane produced in the reductive reaction is derived almost entirely from carbon contributed by the methanol (Barker, 1956).

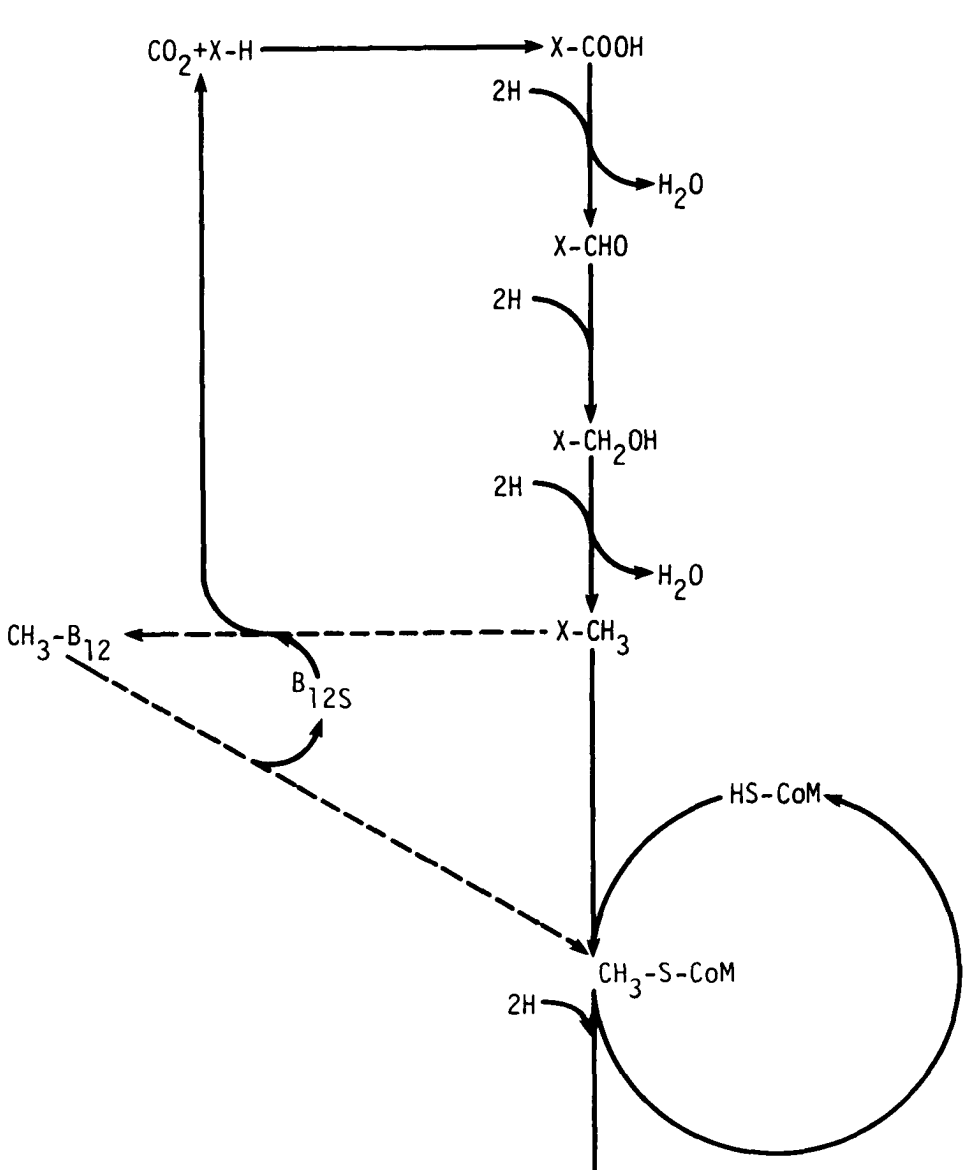

Figure 3. Scheme for the reduction of carbon dioxide to methane (from Gottschalk, 1979) X is an unknown carrier; B_{12S} is hydridocobalamin; HS-CoM is coenzyme M

Zeikus (1977) reported that cell suspensions of Methanosarcina barkeri and Methanobacterium formicicum converted carbon monoxide to carbon dioxide and methane. However, the use of carbon monoxide as an energy source for growth has not been documented.

Acetate accounts for approximately seventy percent of the total methane formed in anaerobic treatment processes (Jeris and McCarty, 1965; Mah, et al., 1978). Zeikus (1977) suggested that this mechanism would not allow for microbial growth with acetate as the sole electron donor. Microbial growth using acetate would be expected to occur only if the methyl carbon of acetate was oxidized to carbon dioxide to generate reducing equivalents for the conversion of acetate to cell material. If this reaction occurred, however, significant amounts of labeled carbon dioxide would be produced from the labeled methyl carbon of acetate, and this does not occur. Therefore, for acetate metabolism to be the major source of energy, other substrates would be needed to provide reducing equivalents or precursors for cell carbon synthesis (Zeikus, 1977).

The methanogenic species responsible for acetate metabolism have been extremely difficult to isolate and grow. In fact, only one species of acetate-metabolizing methanogen, Methanosarcina barkeri, has been isolated in pure culture (Bryant, 1979). Zeikus (1977) suggested that the repeated inability to isolate and grow methanogenic bacteria on acetate as the sole electron donor for growth and methanogenesis may be the result of improper cultivation conditions. Mah, et al., (1978) reported that pure cultures and long term enrichments of acetate-

utilizing methanogens exhibited nutrient-limited responses. This was confirmed by stimulation of methanogenesis upon addition of yeast extract to cultures. The actual mechanism of methane formation from acetate is not well understood (Gottschalk, 1979).

Various experiments have shown the importance of methanogenic bacteria to the metabolism performed by the fermentative bacteria. Zeikus (1977) reported that the end products of the rumen ecosystem in the absence of methanogens were predominantly molecular hydrogen, carbon dioxide, formate, acetate, succinate, lactate and ethanol. In the presence of methanogenic bacteria, the end products were mainly methane, acetate, and carbon dioxide. McCarty, et al. (1963) reported that during startup of anaerobic treatment processes, acetic and butyric acids were the major products, but significant concentrations of most other short-chain fatty acids also were present. After active gas production had begun, the major acids present were acetic and propionic. Similar results were reported by Pohland and Bloodgood (1963). The presence of fewer reduced products during methanogenesis could relate to the conversion of hydrogen to methane which would provide the fermentative bacteria with an alternate mechanism for reoxidation of some NADH (Gaudy and Gaudy, 1980). The low partial pressure of hydrogen created by methanogens permits reoxidation of NADH by causing electron flow to shift toward hydrogen production rather than production of organic electron sink products (see Equation 1).

According to Zeikus (1977), this altered electron flow or interspecies hydrogen transfer that occurs during coupled growth of methanogens and fermentative bacteria results in:

1. Increased substrate utilization,
2. Different proportions of reduced end products,
3. Increased ATP generation by nonmethanogenic bacteria,
4. Increased growth of both groups of organisms, and
5. Displacement of unfavorable reaction equilibria.

The narrow range of substrates utilizable by methanogenic bacteria has generated much interest in isolating intermediate species of bacteria that degrade fermentation products other than those used by methanogenic species. It is possible that methanogens may exist that are capable of using other compounds. The methods for isolation and study of these strict anaerobic organisms have been developed only recently. Several examples of the isolation of these intermediate acetate-forming, or acetogenic, bacteria have been reported and are discussed in the following section.

Acetogenic bacteria

Existence of acetogenic bacteria was first realized with the isolation of the S organism from strains of Methanobacillius omelianskii by Bryant, et al. (1967). M. omelianskii was one of the few methanogenic bacteria isolated in pure culture as early as 1936. Barker (1956) described this bacterium as a strict anaerobe that obtained its energy

for growth from the oxidation of ethanol to acetate. Electrons generated in the oxidative steps are in turn used to reduce carbon dioxide to methane. Bryant, et al. (1967) worked with strains of M. omelianskii supplied by Barker and determined that the culture was actually a mixture of two distinct species, the S organism and Methanobacterium MoH. The symbiotic association of both species was necessary for the metabolism of ethanol. Bryant and his co-workers determined that the S strains were anaerobes but not as strict as the methanogenic bacteria. The S organism used ethanol as an energy source and was inhibited by high partial pressures of molecular hydrogen. Consequently, hydrogen would build up to inhibitory levels unless it was converted to methane by the methanogenic bacteria. Gottschalk (1979) provided a scheme to illustrate the association of the two species (Figure 4).

Bryant, et al. (1967) suggested that fatty acids other than formate and acetate are not degraded by methanogenic bacteria but by another group of bacteria which, like the ethanol degraders, produce acetate and molecular hydrogen from fatty acids in association with hydrogen-utilizing methanogens.

Recently, McInerney, et al. (1979) reported on a species of anaerobic bacteria that degraded the even-numbered carbon fatty acids, butyrate, caproate and caprylate to form acetate and molecular hydrogen. The odd-numbered carbon fatty acids, valerate and heptanoate, were converted to acetate, propionate and molecular hydrogen. Metabolism of even-numbered carbon fatty acids did not proceed via beta-oxidation which

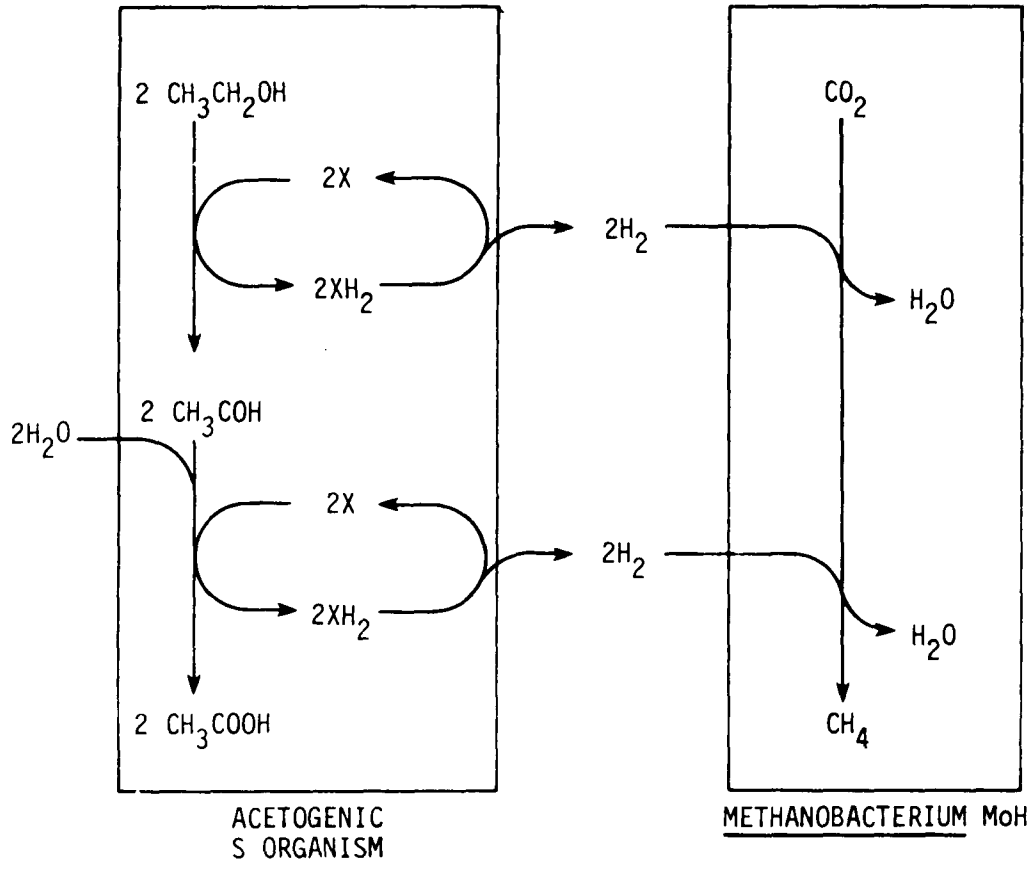

Figure 4. Conversion of ethanol by M. omelianskii culture (from Gottschalk, 1979)

conflicts with results reported by Jeris and McCarty (1965). However, valerate did appear to be degraded by beta-oxidation (McInerney, et al., 1979). This organism could be grown only in association with either a hydrogen-utilizing methanogen or hydrogen-utilizing desulfovibrio.

Bryant (1979) speculated on the existence of an acetogenic organism that ferments propionate to acetate. His indirect evidence was based on experiments with propionate enrichments which always contained hydrogen-utilizing methanogens as the most numerous species. These species do not metabolize propionate. Addition of molecular hydrogen to these enrichments caused an immediate blockage of propionate metabolism and resulted in the accumulation of propionate.

Wolfe (1979) discussed two other acetogenic bacteria, Acetobacterium woodii and Clostridium acetium, that oxidize hydrogen anaerobically with the reduction of carbon dioxide to acetate. C. acetium was isolated in 1936 by Wieringa (Gottschalk, 1979) and converted hydrogen and carbon dioxide according to the following equation:

$$4H_2 + 2\ CO_2 \longrightarrow CH_3COOH + 2\ H_2O \qquad (9)$$

Unfortunately, this culture was later lost and little is known about the details of its metabolism, Braun, et al., (1979) reported that the relative numbers of these acetate-forming bacteria were quite low in anaerobic digesters and therefore may contribute very little to the overall flow.

A general scheme of the relationships of the three steps involved in the breakdown of complex organics with the eventual formation of methane and carbon dioxide is shown in Figure 5. Fermentative bacteria initiate the conversion of complex organics into acetate and other organic acids, molecular hydrogen and carbon dioxide. Acetogenic bacteria convert organic acids (other than acetate) into acetate, molecular hydrogen and carbon dioxide. Finally, methanogenic bacteria utilize the products of the first two stages with the production of methane and carbon dioxide.

Inhibition of Anaerobic Reactions

Anaerobic treatment processes are subject to occasional operational instability due to disturbances of the interdependency of the mixed bacterial cultures present. The consequences of process failure are especially serious in the case of anaerobic digesters since the unit may be out of service for several weeks, and disposal of large quantities of incompletely treated and noxious sludge will be required. Process instability usually is attributed to inhibition of the metabolism of strict anaerobic methanogenic bacteria which are responsible for true waste stabilization. These bacteria are more sensitive to environmental stresses and chemical and physical factors, and have slower growth rates than other bacterial groups present in digesters. Thus, the methanogenic bacteria usually are the key to successful anaerobic treatment, and conditions agreeable to this group must be closely controlled.

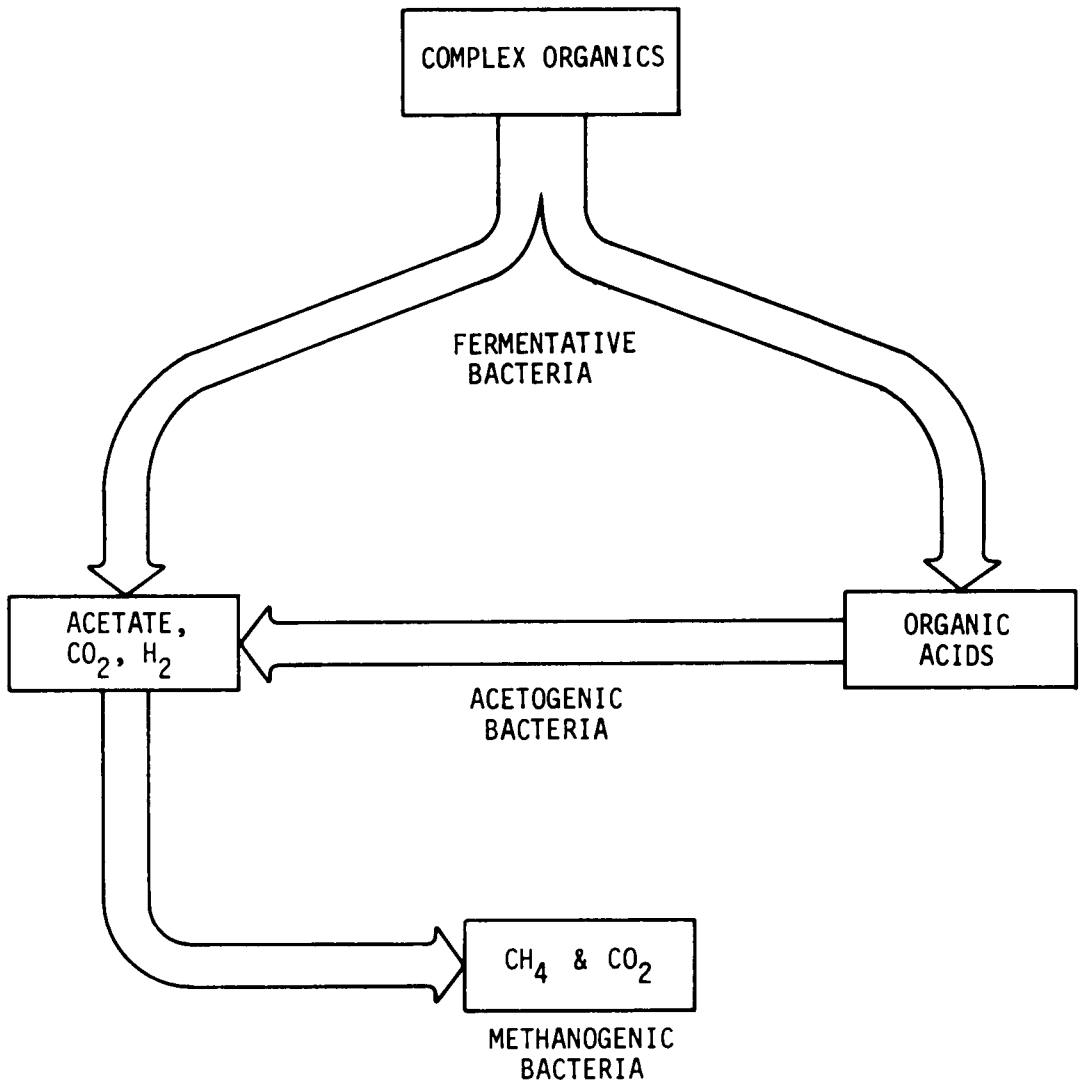

Figure 5. Substrate flow in the anaerobic degradation of complex organics

Environmental stresses

The reactions responsible for methane formation are relatively slow and therefore heating often is used to increase the rate of reaction. It has been observed that the rate of reaction for microorganisms increases with increasing temperature, doubling with about every 10^{o} C of rise in temperature, until some limiting temperature is reached. However, experience with sludge digestion has shown that the best performance is obtained in two specific temperature ranges associated with mesophilic (20^{o}-45^{o} C) or thermophilic (45^{o}-75^{o} C) microorganisms (Metcalf and Eddy, Inc., 1972; McCarty, 1964a). Mesophilic operation is most efficient at 35°-37°C while the optimum temperature for thermophilic digestion is about 55^{o} C. Once steady-state operation has been established, the process is sensitive to temperature changes of only one to three degrees Celsius, but the process is usually able to recover from such minor temperature fluctuations (Kotze, et al., 1969).

One of the most important environmental requirements in anaerobic digesters is the maintenance of proper pH. The pH in a digester is a function of the volatile acids, alkalinity and carbon dioxide in the product gas. Low pH problems are the most common and usually occur when the buildup of volatile acid fermentation intermediates exceeds the buffering capacity provided by the alkalinity in the system. This condition can result from organic overloading of the system or inhibition of the methanogenic population. McCarty (1964a) reported that the optimum pH for methanogenic bacteria occurred in the range of 6.7 to

7.3. Clark and Speece (1970) determined that there was no inhibition in an anaerobic filter between pH 6 and 8 for an acetate enriched culture. These researchers reported that the inhibition at pH 5 and pH 9 was only temporary when the period of low and high pH was short (12 hours or less).

Nutrients

The input stream to an anaerobic treatment process must provide the essential nutrients for the growth of anaerobic bacteria. Macronutrients are nitrogen and phosphorus and micronutrients include sulfur, calcium, cobalt, copper, iron, magnesium, potassium, selenium and zinc. These nutrients essentially are the same as those required by aerobes. However, lower nutrient quantities are needed in anaerobic processes because of lower yields of anaerobic bacterial cells (McCarty, 1964a; Gottschalk, 1979). Domestic wastewater sludges usually will contain all the nutrients required for bacterial growth, but some industrial wastes and municipal refuse are deficient in nitrogen and phosphorus (McCarty, 1964a; Pfeffer, 1974a). Pfeffer (1974a) also reported that improved digestion of municipal refuse was observed after addition of sewage solids even when adequate nitrogen and phosphorous were present. This effect was attributed to the trace nutrients in the wastewater solids. McCarty and Vath (1962) reported similar results after the addition of supernatant liquor solids from a sewage sludge digester to laboratory digesters.

Toxic substances

A critical requirement for successful anaerobic treatment is that the waste input stream be free of toxic materials. While the above environmental requirements are relatively easy to monitor and control, detection and exclusion of toxins or inhibitory levels of certain other materials is much more difficult.

Molecular oxygen is toxic to strict anaerobic bacteria and its exclusion is elementary to anaerobic process control. The toxicity of molecular oxygen originates in its reaction with reduced electron carriers or reduced iron-sulfur proteins (Gottschalk, 1979; Gaudy and Gaudy, 1980). The reaction, which occurs in the presence of various oxidases that are present in all organisms, produces hydrogen peroxide and the superoxide anion (O_2^-). Aerobic organisms contain the enzymes catalase and superoxide dismutase which are responsible for the conversion of hydrogen peroxide to molecular oxygen and water and the superoxide anion to hydrogen peroxide and oxygen, respectively. Facultative anaerobes form superoxide dismutase but are devoid of catalase. Strict anaerobes lack both enzymes. It is believed that the toxicity of oxygen to strict anaerobes is caused by the superoxide anion (Gottschalk, 1979).

Process inhibition from high concentrations of ammonia has in particular been experienced in the treatment of wastes that are high in protein, such as meat packing wastes. The amount of free ammonia

(NH_3) in aqueous solution is related to the ammonium ion (NH_4^+) and pH according to the following equilibrium (Butler, 1964):

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad , \quad pk_B = 4.75 \qquad (10)$$

McCarty and McKinney (1961a) and Kugelman and McCarty (1965) determined that the toxicity of ammonia is related to the concentration of free ammonia in solution. Ammonia nitrogen concentrations between 1500 and 3000 mg/L are inhibitory but this inhibition can be temporarily relieved by lowering the pH of the digester liquor (McCarty, 1964 b). Ammonia nitrogen concentrations greater than 3000 mg/L are highly toxic regardless of pH.

There have been many contradictory reports concerning the character of inhibition from volatile acids in anaerobic digestion. This disagreement is whether the inhibitor is the acid anion, the corresponding cations or the unionized acid. Inhibition from the hydrogen ion, as expressed by a low pH, has already been discussed but a substitute cation may also cause inhibition. McCarty and McKinney (1961a). researched this problem and concluded that cations caused the inhibition. Sodium and ammonium ions were found to be the most toxic of the commonly present cations. It was also reported that volatile acid concentrations up to 10,000 mg/L could be neutralized without inhibition

by the use of calcium or magnesium hydroxide, but not sodium, ammonium or potassium hydroxide (McCarty and McKinney, 1961a).

McCarty (1964b) reported that high levels of alkali and alkaline-earth cations are inhibitory to anaerobic treatment systems. As can be seen from Table 2, low concentrations of these cations are stimulatory while the cations only exhibit inhibition at relatively high concentrations. These cations would normally be no problem in the digestion of wastewater sludges, but could be a problem in the treatment of some industrial wastes.

McCarty (1964 b) also reported that soluble sulfides in excess of 200 mg/L are toxic to anaerobic treatment systems. Sulfides can result from introduction with the input waste stream and/or biological production in the digester from reduction of sulfates and other sulfur-containing inorganic compounds, as well as from anaerobic protein degradation. Sulfides in anaerobic systems exist in three forms; hydrogen sulfide (H_2S) and the ions HS^- and $S^=$. These forms are related by the following equilibrium (Butler, 1964). At neutral pH conditions, as are present

$$H_2S \rightleftharpoons H^+ + HS^- \qquad pka_1 = 7.00 \qquad (11)$$

$$HS^- \rightleftharpoons H^+ + S^= \qquad pka_2 = 12.92 \qquad (12)$$

At neutral pH conditions, as are present in well-operated systems,

Table 2. Stimulatory and inhibitory concentrations of alkali and alkaline-earth cations (from McCarty, 1964)

	Concentrations in mg/L		
Cation	Stimulatory	Moderately inhibitory	Strongly inhibitory
Sodium	100-200	3500-5500	8,000
Potassium	200-400	2500-4500	12,000
Calcium	100-200	2500-4500	8,000
Magnesium	75-150	1000-1500	3,000

much of the sulfide exists as hydrogen sulfide gas which escapes from solution with the other gases produced during treatment.

The concentration of soluble sulfides is also influenced by the presence of heavy metals. Sulfides of heavy metals are very insoluble and therefore their precipitation as heavy metal salts can occur in anaerobic digesters. Lawrence and McCarty (1965) reported that this precipitation is an important mechanism in the reduction of inhibition from heavy metals.

Heavy metal toxicity is, next to overloading, probably the most common cause of digester failure. Copper, zinc, and nickel in solution are associated with most of the problems of heavy metal toxicity

(McCarty, 1964b). Cadmium, lead and mercury are also toxic to the methanogenic bacteria. These heavy metals combine with sulfides to form insoluble sulfide salts which do not adversely affect the microorganisms. Sufficient sulfides must be available to precipitate the heavy metals from solution. If sufficient sulfide is not formed during waste treatment, additional sulfides in the form of sodium sulfide, sodium sulfite or a sulfate salt may be added to control heavy metal inhibition (Lawrence and McCarty, 1965).

Organic chemical inhibition

Many inhibitory organics have been identified in various streams (Table 3). Evaluation of petroleum-chemical wastes streams has provided a significant amount of data on the inhibitory effect of these organic chemicals. In a study of inhibitory petrochemical pollutants, Hovious, et al., (1973) introduced the activity concept. Activity was described as the ratio of gas production in spiked samples to that of the respective control. Activity was calculated according to Equation 13:

$$A = \frac{R}{R_c} \tag{13}$$

where A = activity ratio
R = gas production rate with test chemical
R_c = gas production rate in control

Since its introduction, the use of activity has been a common means of expressing quantitative inhibition data. Concentrations reported in

Table 3. Organic chemicals inhibitory to anaerobic cultures

Chemical	Concentration mg/L[a]	Reference
Acetaldehyde	440	Chou, et al., 1978
Acrolein	20-50	Hovious, et al., 1973
	11.2	Chou, et al., 1978
Acrylic acid	864	Chou, et al., 1978
Acrylonitrile	100	Hovious, et al., 1973
	212	Chou, et al., 1978
Aniline	2418	Chou, et al., 1978
Carbon tetrachloride	2.2	Thiel, 1969
Catechol	2640	Chou, et al., 1978
Chloroform	20	Stickley, 1970
	0.96	Thiel, 1969
3-Chloro-1,2-propandiol	662.7	Chou, et al., 1978
1-Chloropropane	149.1	Chou, et al., 1978
1-Chloropropene	7.6	Chou, et al., 1978
2-Chloropropionic acid	867.6	Chou, et al., 1978
Crotonaldehyde	455	Chou, et al., 1978
Diethylamine	300-1000	Hovious, et al., 1973
Ethylacetate	968	Chou, et al., 1978
Ethylacrylate	300-600	Hovious, et al., 1973
Ethylbenzene	339.2	Chou, et al., 1978
Ethylene dichloride	150-500	Hovious, et al., 1973
	2.5-7.5	Stuckey, et al., 1978
2-Ethyl-1-hexanol	500-1000	Hovious, et al., 1973
Formaldehyde	50-100	Hovious, et al., 1973
	72	Chou, et al., 1978
	200	Pearson, et al., 1980
Lauric acid	592.8	Chou, et al., 1978

[a]The reported concentration represents the level which produced 50% reduction in activity.

Table 3. continued

Chemical	Concentration mg/L	Reference
Methylene chloride	100	Thiel, 1969
	1.8-2.16	Stuckey, et al., 1978
2-Methyl-5-ethylpyridine	100	Hovious, et al., 1973
Methyl isobutyl ketone	100-300	Hovious, et al., 1973
Nitrobenzene	12.3	Chou, et al., 1978
Phenol	2444	Chou, et al., 1978
	300-1000	Hovious, et al., 1973
	500	Pearson, et al., 1980
Propanol	5220	Chou, et al., 1978
Resorcinol	3190	Chou, et al., 1978
Vinyl acetate	592	Chou, et al., 1978
	200-400	Stuckey, et al., 1978
Vinyl chloride	5-10	Stuckey, et al., 1978

Table 3 represent the levels responsible for a fifty percent reduction in activity.

Chou, et al., (1978) studied the effect of petrochemical structure on inhibition of methane formation in anaerobic cultures. On the basis of the results by these researchers, the following conclusions were reported:

1. Aldehydes, chloro functional groups, double bonds and benzene ring compounds exhibit toxicity to unacclimated methane cultures,

2. Amino, hydroxyl and carboxyl substitutions do not increase the toxicity to unacclimated methane cultures, and addition of hydroxyl groups was shown to decrease toxicity, and

3. An increase in the carbon chain length decreased the toxicity of aldehydes and compounds with double bonds.

Several investigators have reported the accumulation of molecular hydrogen during inhibition of methanogenesis. Thiel (1969) reported that chloroform, carbon tetrachloride and methylene chloride caused inhibition of methanogenic bacteria and subsequent production of molecular hydrogen. Thiel also reported that uninhibited controls produced molecular hydrogen when the pH was depressed from 7.0 to 6.0. Toerien, et al., (1970) also reported a decrease in pH and production of molecular hydrogen during inhibition by chloroform. Sykes and Kirsch (1972) reported that the primary source of molecular hydrogen was insoluble polysaccharides in the feed sludge when anaerobic cultures were inhibited by carbon tetrachloride. These researchers also reported a reduction in pH in both controls and inhibited cultures, but the accumulation of hydrogen

in the controls was not noted. Chynoweth and Mah (1971) reported the absence of molecular hydrogen in anaerobic cultures inhibited by chloroform and carbon tetrachloride. The pH of the culture medium was not reported.

Organic chemicals that inhibit methanogenesis have been reported to also inhibit acid forming bacteria. Chynoweth and Mah (1971) observed that carbon tetrachloride inhibited acetate production in anaerobic cultures. Chloroform did not produce the same effect. Sykes and Kirsch (1972) reported that the inhibition of methanogenesis by carbon tetrachloride also inhibited the formation of propionate but stimulated the formation of valerate and caproate.

Swanwick and Foulkes (1971) observed a high variation in quantitative inhibition data while studying the suppression of gas production in anaerobic cultures by chlorinated hydrocarbons. It was determined that the solids content of the anaerobic culture was an important factor influencing inhibitory effects. These researchers reported that increased solids in anaerobic digester reduced the inhibition caused by chloroform, 1,1,1-trichloroethane, chlorobenzene, orthodichlorobenzene and paradichlorobenzene. Adsorption of these materials on the solids with the subsequent reduction of concentration in solution was determined to be the mechanism responsible for the reduced inhibition.

Reversible inhibition has been observed by several investigators. Roberton and Wolfe (1969) reported that inhibition of cultures of

Methanobacterium strain MoH by 1.68 mg 2,4-dinitrophenol/L was reversed within three hours. These researchers believed that the recovery was due to biological reduction of this compound, but no supporting data was presented. Sykes and Kirsch (1972) reported that anaerobic cultures inhibited by 16 mg carbon tetrachloride/L achieved partial recovery in twelve days. At this time, the disappearance of molecular hydrogen and appearance of methane was observed. No explanation of the partial recovery was given and the fate of chloroform was not discussed. Stuckey, et al., (1978) observed that anaerobic cultures inhibited by 400 and 100 mg vinyl acetate/L recovered in three to four days. These researchers reported that acclimation was probably responsible for recovery, but gas production data indicated that anaerobic decomposition of vinyl acetate had occurred and therefore the vinyl acetate was removed from solution. Parkin, et al., (1980) observed that inhibition by shock loads of chloroform and formaldehyde was reversed more quickly in anaerobic filters than in complete-mix reactors. Since the flow pattern of anaerobic filters is characteristic of plug flow, the toxicant slug would not be readily diluted and would therefore pass through the system quickly. Complete-mix systems would rapidly dilute the toxicant slug but the toxicant would remain in the system longer. This information would indicate that the period of inhibition was related to the time of contact between inhibitor and microorganisms.

Measurement of Inhibition in Anaerobic Processes

The occasional process instability of anaerobic treatment systems has generated much research effort in the area of inhibition. Many different bioassay techniques have been used to measure inhibition, each with its own advantages and disadvantages.

Earlier in this review it was mentioned that the methanogenic bacteria are the key to successful operation of anaerobic treatment processes. The measurement of methanogenic metabolism is made easily since methane and carbon dioxide are the end products. Consequently, the techniques that have been used are based on the measurement of gas production.

Apparatus

Continuous or semi-continuous bioassays have been described widely in the literature. These systems usually involve a mixed reactor that receives incremental or continuous feeding from a stock waste storage container. Gas often is recirculated to provide mixing, and excess gas is passed through a meter for measurement (Figure 6). For toxicity or biodegradability studies, the feed material is spiked with the test chemical which is added continuously or on a draw-and-fill schedule. The gas production is then measured by various methods to determine inhibition. These laboratory systems closely approximate full-scale anaerobic treatment operation. Both shock loading and continuous gradual feeding of inhibitors can be simulated using continuous

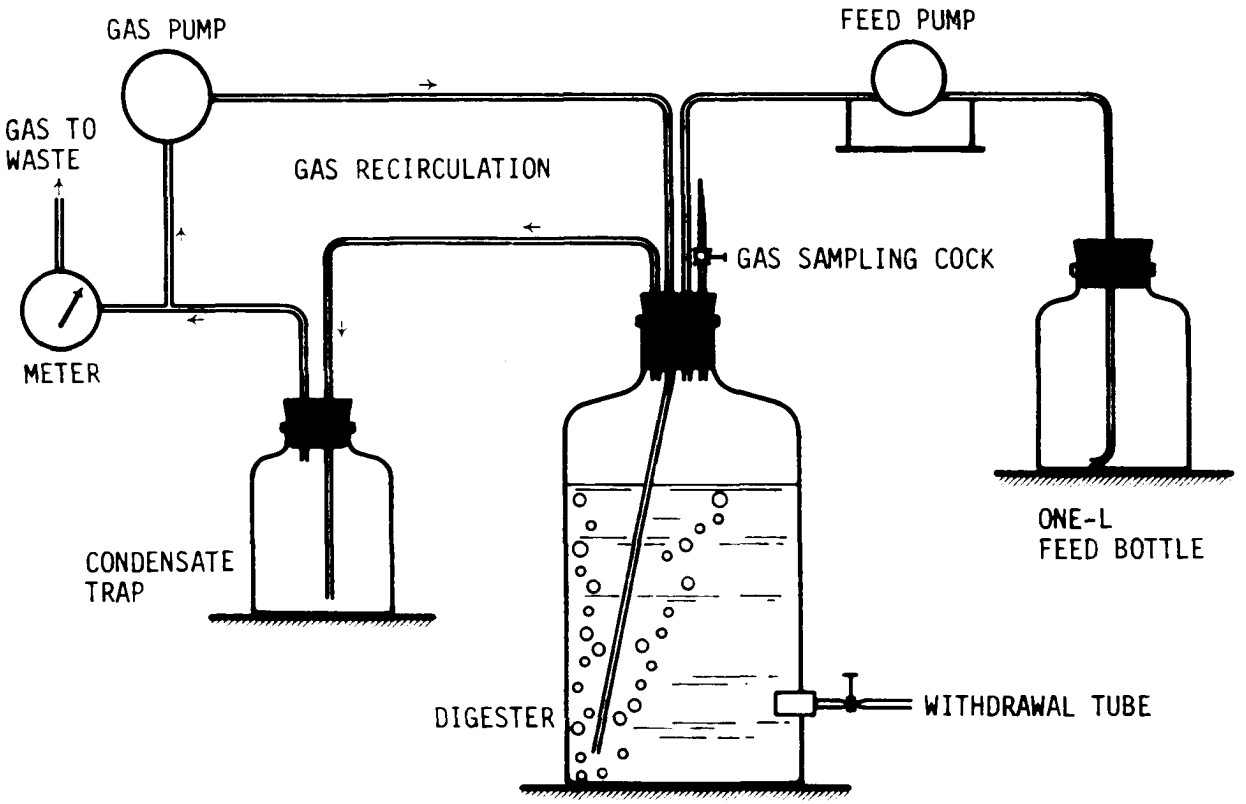

Figure 6. Schematic layout of continuous feed bioassay apparatus (from Kugelman and McCarty, 1965)

bioassays. However, they are costly in terms of facilities, equipment, time and personnel.

Batch bioassays involve the addition of anaerobic seed, nutrients, buffer, substrate, and test chemical to a closed, constant-volume container. Controls are set up in the same fashion but without the addition of the test chemical. The gas production can then be monitored by following the change in pressure of the system. No fluid is removed from the container until the termination of the experiment. Batch bioassay techniques can be used to evaluate the effects of shock loading, but do not simulate the true conditions of anaerobic treatment since anaerobic systems are not operated as batch reactors. The batch bioassay technique is a relatively simple device adaptable to small-scale laboratory setups and it needs very little auxiliary equipment. The information gained from batch bioassays can lead to the design of efficient continuous experiments.

The Warburg respirometer is possibly the most widely used apparatus for conducting batch bioassays. The procedure involves the addition of anaerobic seed to a Warburg flask containing nutrients and a buffer. The flask is then connected to a manometer which is used to measure gas production. Specially-designed Warburg flasks are available for transfer of the seed to the flask (Figures 7 and 8). However, Warburg respirometers have several limitations (Owen, et al., 1979);

1. The system is relatively costly and requires skilled operation,
2. Only a fixed number of assays can be tested on a given instrument at the same time,

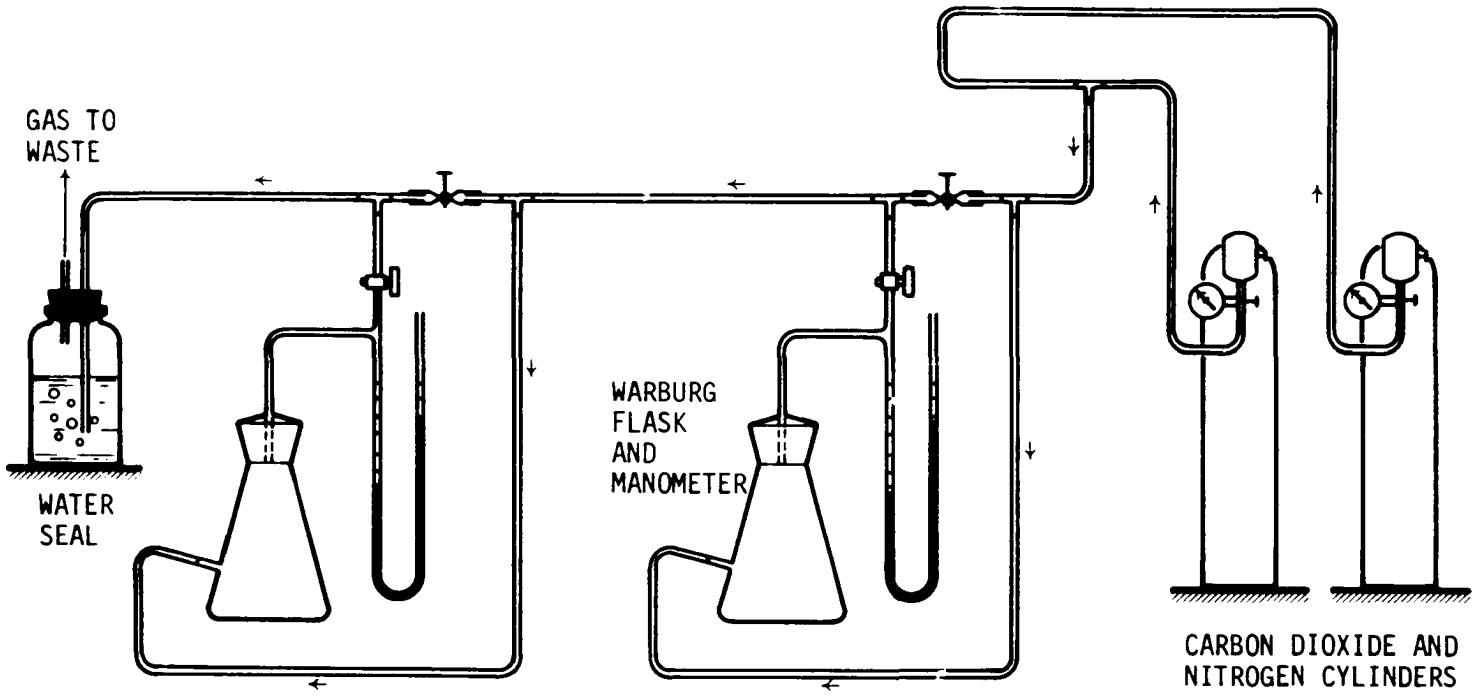

Figure 7. Schematic diagram of gas flushing apparatus Warburg flasks (from McCarty, et al., 1963)

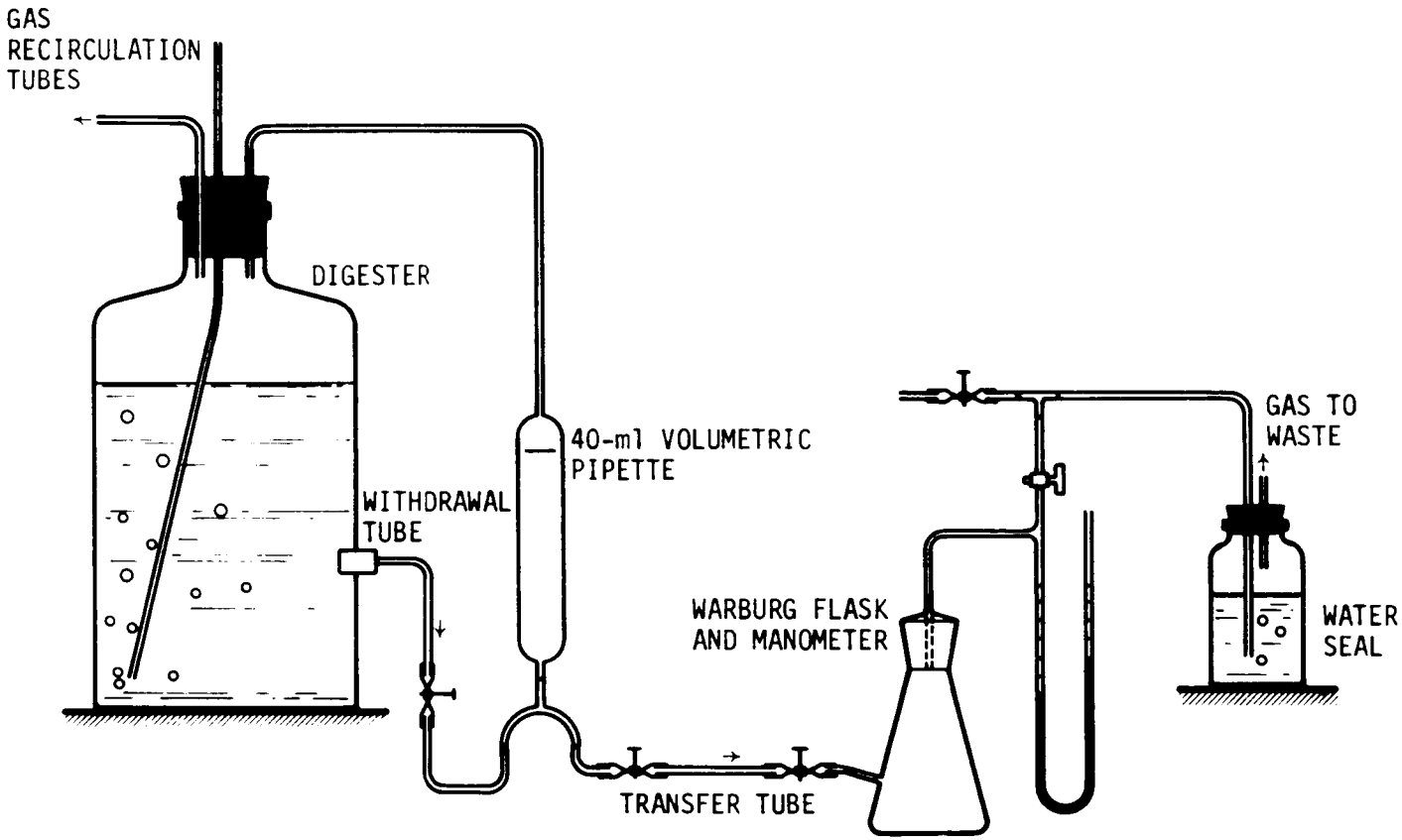

Figure 8. Schematic diagram showing anaerobic transfer of seed to Warburg flasks (from McCarty, et al., 1963)

3. Sample size is limited to the specially-designed Warburg flask,
4. Gas and liquid sampling are difficult during the test period, and
5. Extended incubation times are impractical and lead to inconsistent results.

Based on these deficiencies, Owen, et al., (1979), Speece, (1978) and Stuckey, et al., (1978) described a batch bioassay technique developed as a modification of the Hungate serum bottle procedure (Miller and Wolin, 1974). This procedure involves the addition of anaerobic seed, nutrient media and buffer solution to a serum bottle which is sealed with a rubber flanged septum. Gas production is then monitored by the use of a hypodermic syringe. This technique is not limited by the various deficiencies of the Warburg respirometer. Stuckey, et al., (1978) compared this batch bioassay procedure to semi-continuous bioassays and reported that the batch bioassay gave a conservative figure compared to the semi-continuous bioassay procedure. Except in cases where disappearance of the test chemical occurred due to volatilization or biodegradation, the results from both bioassays were comparable.

One problem with batch bioassays concerns the length of time that should be used in evaluating inhibitory effects. Hovious, et al., (1973) based their results on the gas production rate during a three and one-half hour period following the initial two hours of the test. Chou, et al., (1978) reported that activities were based on measurements of gas production over a period of time after a steady rate of gas production was attained both initially and after injection of the test

compound. No time was reported for the length of the test runs. Stuckey, et al., (1978) collected inhibition data over a ten day period and reported the recovery of anaerobic cultures inhibited by vinyl acetate after three to four days. Short run lengths allow for a larger number of chemicals to be tested but might not provide complete information. It is possible for a lag period to occur and a short run length might not show this important information.

Seed culture

As mentioned earlier in this literature review, the microbial populations involved in the complete decomposition of complex organic matter to methane and carbon dioxide have a large physiological variation. Facultative and strict anaerobes break down the organic matter into fatty acids, some of which are further degraded to acetate and molecular hydrogen by the acetogenic bacteria. Finally, the strict anaerobic methanogens form methane and carbon dioxide from the acetate and molecular hydrogen. However, toxicity evaluations often are performed using anaerobic cultures that are not representative of the heterogeneous cultures present in anaerobic treatment systems.

Chou, et al., (1978) used acetate enrichment cultures in the study of inhibitory petrochemical pollutants. Parkin, et al., (1980) and Speece et al., (1980) used similar acetate enrichments in inhibition studies. Use of acetate enrichments has been promoted since it is generally accepted that the conversion of acetate to methane is the rate-limiting step in the anaerobic decomposition of complex organics and

that approximately 70 percent of the methane produced from such wastes results from acetate conversion. Acetate enrichments provide only a selective group of microorganisms.

McCarty, et al., (1963) reported on the ability of enrichment cultures to utilize various fatty acids. Acetate enrichments were observed to utilize only acetic and formic acids. Cultures enriched with propionate could utilize propionic, acetic, butyric, valeric and caproic acids. Butyrate enrichments were capable of utilizing valeric, butyric and acetic acids. Propionic acid was not degraded by this culture. Cultures maintained on primary municipal sludge were capable of utilizing all of the volatile fatty acids.

Inclusion of acid forming bacteria in test cultures can provide a means of degradation of inhibitory organic compounds. Healy and Young (1978) reported that anaerobic cultures obtained from a municipal digester degraded phenol and catechol to methane and carbon dioxide when added as the sole carbon source. Jagnow, et al., (1977) reported that species of Clostridium, Bacillaceae and Enterobacteriaceae actively degraded gamma-hexachlorocyclohexane under anaerobic conditions. The mechanism of degradation of this chemical was dechlorination which was completed in four to six days. Anaerobic degradation of DDT, DDD, aldrin, dieldrin, lindane, endrin, heptachlor and heptachlor epoxide by municipal digester cultures was reported by Hill and McCarty (1967). Evans (1977) reviewed the biochemistry of the anaerobic degradation of

aromatic compounds and reported that the decomposition of aromatics in mixed anaerobic cultures was the result of reduction and cleavage of the benzene nucleus by facultative organisms.

Summary of Literature Review

A survey of the literature provided a base of knowledge for this research project. This survey indicated that a number of questions concerning inhibition of anaerobic reactions by organic chemicals remained unanswered. A first question concerned the adequacy of the length of time for batch bioassay tests which varied from a few hours to several days in length. In some cases, the length of time was not specified. The importance of the length of inhibition evaluations by batch bioassays needs examination.

Several investigators reported the reversal of inhibition after varying periods of time. Possible explanations were given for this occurrence, but no data were presented to support the theories. The presence, concentration and condition of the inhibitory chemical also were not evaluated. Questions then remain as to whether the recovery of methane production was due to acclimation, degradation or removal of the inhibitory chemical from solution by physical means.

Swanwick and Foulkes (1971) reported that increased solids levels in anaerobic digestion systems reduced the degree of inhibition by organic chemicals. Since many organic chemicals are only slightly soluble in aqueous solution, their adsorption onto solids in anaerobic

treatment systems may provide a mechanism for removal of the inhibitor from solution. The effect of this mechanism on the recovery of an inhibited system is not known.

A better understanding of inhibition in anaerobic treatment systems could lead to improved control methods. In addition, knowledge of the degradation of synthetic organic chemicals could provide information on possible applications of anaerobic treatment. While the anaerobic cultures in a digester receiving solid wastes may not be much different from municipal sludge cultures, the response of these cultures to inhibitory organic chemicals has not been established in the literature.

Therefore, there seemed to be a definite need to investigate further the topic of inhibition of anaerobic digestion reactions by organic chemicals.

EXPERIMENTAL STUDY

Test Program

This research was divided into three phases based on the objectives of each phase. Phase I involved screening various organic chemicals for inhibitory effects. Inhibitory organics from Phase I were used in the later two phases. Phase II was designed to examine the conditions during inhibition and recovery. The third phase involved a study of the effects of various concentrations of inert solids on inhibition by toxic organic compounds.

Laboratory digesters maintained on a complex substrate at 37^{o} C were used to provide heterogeneous anaerobic cultures for anaerobic batch bioassays. This technique involved the addition of anaerobic seed, nutrients and buffer to 250 mL serum bottles. Ethanol was added as a known biodegradable substrate. The serum bottles were then dosed differentially with organic test chemicals and suppression of gas production was used as a measure of inhibition.

During Phase II, additional serum bottles were used to provide liquid samples for measurement of organic acids and to examine the behavior of the inhibitory test chemicals. Methane and carbon dioxide were monitored in the product gas.

The third phase involved the addition of various amounts of sterilized solids to serum bottles containing active seed plus the

test chemicals used in Phase II. Gas production was measured to determine the effect of the solids on inhibition and recovery.

The following sections describe the apparatus and procedures that were used in this study.

Apparatus

Anaerobic reactor

The anaerobic reactors used for this study consisted of two plexiglass columns, 183 cm long with an inside diameter of 14 cm (outside diameter of 15.2 cm). These columns were connected to a base 7.6 cm long and the completed unit was mounted on concrete blocks (Figure 9). Flanged connections were used on the reactors and O-rings in the flanges provided a positive seal. Feeding and sludge wasting were made possible by the use of 2.5 cm ball valves[1] located at the top and bottom of the reactors.

A small compressor[2] was used on each reactor to provide gas recirculation for mixing. A 0.95 cm plastic tube mounted in the base of each reactor provided the outlet for the compressed gas in the reactors. All gas lines were 0.95 cm I.D. Tygon tubing. The gas collected at the top of the reactor was passed through

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[1] "Compact" Type 500, GF Plastic Systems, Inc., Santa Ana, California.

[2] Speedaire Model 4Z026, Dayton Electric Manufacturing Co., Chicago, Illinois.

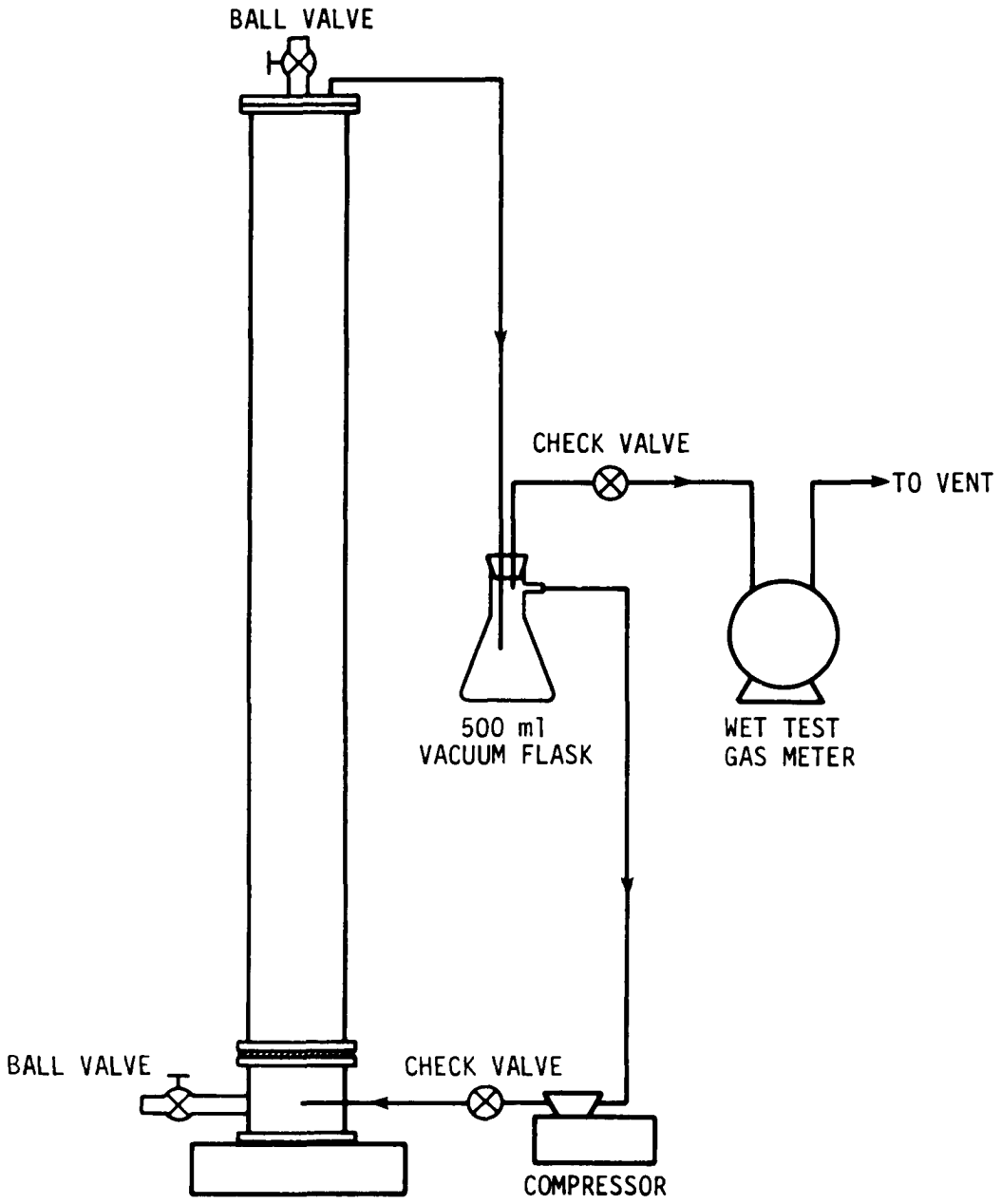

Figure 9. Schematic diagram of laboratory digesters used in this study

a 500 mL vacuum flask to collect condensate. Excess gas was diverted to a wet test meter[1] for measurement of gas production. After measurement, the gas was vented to a fume hood. Check valves[2] were placed in the gas lines between the compressor and the reactor and between the vacuum flask and the gas meter.

Both anaerobic reactors were set up in a constant temperature room maintained at 37°C. The temperature was controlled to within ± 1°C except in cases when the high pressure steam to the building was interrupted for maintenance. Thermostatically-controlled electric heaters[3] were added to provide a more dependable temperature control during periods of steam interruption.

Artificial substrate

An artificial substrate was developed as a substitute for municipal refuse. The reasoning behind this was twofold:

1. Sampling of municipal solid waste is relatively difficult and the acquisition and storage of a truly representative sample is almost impossible, and
2. Use of an artificial substrate would prevent undesirable contamination with toxic materials that might be present in municipal solid waste and would provide better control of the feed composition.

Pfeffer (1974b) reported that typical municipal refuse contained about 78 percent combustible material (Table 4). Resource Planning

[1]No. 63110, Precision Scientific Co., Chicago, Illinois.

[2]Nalge 6120, Fisher Scientific Co., Itasca, Illinois.

[3]Titan T760 B, W.W. Grainger, Inc., Des Moines, Iowa.

Table 4. Composition of a typical domestic refuse (from Pfeffer, 1974b)

Component	% Total by weight Wet wt.	Dry wt.
Paper	48.0	35.0
Leaves	9.0	5.0
Wood	2.0	1.5
Synthetics	2.0	2.0
Cloths	1.0	0.5
Garbage	16.0	8.0
Combustibles	78.0	52.0
Glass	6.0	6.0
Metal	8.0	8.0
Ashes, stone, etc.	8.0	6.0
Total moisture content	———	28.0

Table 5. Average solids content of substitute substrates

Component	% Total solids	% Volatile solids
Kraft paper	93.3	94.6
Newsprint	94.0	97.9
Dog food	92.0	80.4
Hay	90.0	80.0

Associates (1976) reported similar compositions, as did Tchobanoglous, et al., (1977) and Golueke and McGauhey (1967). Klein (1972) reported that the paper fraction of municipal organic waste consists of approximately 40 percent Kraft paper and 33 percent newsprint. Kraft paper, the brown paper used in grocery bags and wrapping paper, is composed of less lignin-bound cellulose than newsprint. The garbage fraction of municipal refuse is primarily kitchen and food waste. Garden wastes and lawn trimmings are included in the leaves and wood fractions listed in Table 4 and therefore are quite variable, depending on the geographical location and the season of the year.

The major digestible fraction of organic municipal refuse is represented by the paper, leaves and garbage materials. Wood contains lignin-bound carbohydrates and is therefore fairly resistant to biodegradation (Zeikus, 1980). Synthetics, such as plastic and synthetic cloth fibers, are also resistant to biodegradation, but these two categories represent only a small fraction of the total organic refuse. Tchobanoglous, et al., (1977) found the textile or cloth fraction represented only 0.2 percent of the total weight of municipal refuse. Based on this information, an artificial refuse was formulated that contained the following materials:

40 % Kraft paper,

35 % newsprint,

12 % lawn and garden wastes, and

13 % garbage.

The amount of each material was determined by refactoring the composition based on these materials only.

The selection of appropriate substitutes for these materials was necessary to provide a controlled substrate. The Kraft paper was obtained in a 27.3 kg roll of wrapping paper. The newsprint was obtained as end rolls from a local newspaper office. The newsprint was unprinted and therefore contained no ink. A bale of grass hay was chosen as a substitute for the lawn and garden fraction since it was similar in texture and character and the bale would provide an adequate supply for the duration of the project. Dog food[1] was used as a substitute for the garbage since it contains similar components such as proteins, fats, and carbohydrates.

These materials were reduced in size by grinding in a Wiley mill containing a 0.32 cm screen. The ground materials were stored in separate plastic garbage cans. Five random samples of each material were taken to determine moisture and volatile solids content. These data are presented in Table 5.

Pfeffer (1974 a) reported an improvement in the anaerobic digestion of municipal refuse when sewage solids were added. Therefore, primary municipal wastewater solids were added to the substrate for the anaerobic seed reactors. Twenty gallons of primary sludge were collected at the Ames, Iowa wastewater treatment

[1]Purina Dog Chow, Ralston Purina Company.

plant, divided into one gallon portions, and then frozen for later use. Analysis of this sludge indicated a total solids concentration of 4.3 percent and a volatility of 74.4 percent.

The five components of the artificial substrate were analyzed for cellulose, lignin, total Kjeldahl nitrogen and total phosphorus (Table 6).

Table 6. Composition of the components of the artificial substrate

	Component				
	Sludge	Newsprint	Kraft paper	Hay	Dog food
Total cellulose	16.3 g/L	77.2 %	84.1 %	31.2 %	7.92 %
Total lignin	3.65 g/L	20.9 %	6.7 %	11.1 %	0.45 %
Total Kjeldahl nitrogen	980 mg/L	0.09 %	0.02 %	3.71 %	4.70 %
Total phosphorus	857 mg/L	0.001 %	0.003 %	0.38 %	1.17 %

The recipe for the artificial substrate is listed in Table 7 . This substrate yielded a mixture containing 4 percent municipal sludge and 96 percent organic refuse based on total volatile solids. Nutrients and alkalinity (K_2HPO_4, NH_4Cl, and $NaHCO_3$) were added since Pfeffer (1974 b) reported a deficiency in these materials

Table 7. Components of artificial substrate used in this study

Component	Amount
Kraft paper	26.8 grams
Newsprint	23.2 grams
Dog food	10.2 grams
Hay	8.3 grams
Dipotassium hydrogen phosphate	0.2 grams
Ammonium chloride	0.4 grams
Sodium bicarbonate	3.5 grams
Municipal primary sludge	80 mL
Ethyl alcohol (after first 6 months)	10 mL
Tap water	740 mL
Total volume	833 mL

when the amount of organic refuse exceed 60 percent of the total volatile solids. This substrate was fed to each reactor at a loading rate of 2.4 grams of volatile solids/liter/day (0.15 lb VS/cu ft/day). Based on a reactor volume of 25 liters, the solids retention time (SRT) was 30 days. These are fairly conservative operating parameters based on the information available on the anaerobic digestion of organic municipal refuse. Golueke (1977) reported the range in loadings for organic municipal refuse from

1.05 to 4.86 grams VS/liter/day (0.08 to 0.30 lb VS/cu ft/day). Pfeffer (1974 a & b) worked with municipal refuse digesters operating with SRTs of 4 to 30 days and reported that a 10 day SRT was adequate when operating at 35°C.

Startup and operation of anaerobic reactors

The anaerobic reactors were initially seeded with active digested sludge from the Ames, Iowa wastewater treatment plant. Approximately 20 liters of digested sludge were added to each reactor. Warm (37°C) tap water was added to give a total volume of 25 liters in each reactor. After closing the feed valves, the compressors were started. Output from the compressors was much more than was required for mixing and pinch clamps were used on the compressor influent lines to control the gas recirculation rate. Reactor and gas line connections were tested for leaks using a liquid-type leak detector[1]. Gas meters were reset to zero and the contents of each reactor were allowed to mix for two days. At that time gas production was observed.

The reactors were fed at approximately the same time each day, using the following procedure:

1. The date and time were logged in and the temperature in the room was recorded,

[1]Snoop, Nupro Company, Willoughby, Ohio.

2. Gas meters were read and the gas production was recorded,

3. Dry substrate was added to a 2 liter Nalgene beaker and 80 mL of primary sludge were added. Tap water was added to give a total volume of 833 mL,

4. The solution was mixed by hand,

5. The compressor to the reactor which was to be fed was shut off and the gas line between the reactor and compressor was closed with a pinch clamp,

6. The feed valve at the top of the reactor was opened to vent the reactor and approximately one liter of mixed liquor from the bottom of the reactor was added to provide a thinner mixture for addition to the reactor,

7. A 2 liter Nalgene funnel was placed in the feed valve and the diluted feed material was added,

8. The funnel was removed and the level of mixed liquor in the reactor was lowered to give a volume of 25 liters by wasting sludge from the bottom valve. (This procedure of withdrawing waste sludge after adding the feed material was deemed satisfactory because little settling of the mixed liquor occurred and there was no mixing of the digester contents when waste sludge withdrawals were made.)

9. Both valves were closed, the pinch clamp was removed from the gas line to the reactor, and the compressor was started.

10. The waste mixed liquor was bottled and stored at 4^{o} C for further analysis with a portion used for immediate pH measurement.

After approximately six months of operation, one of the reactors was converted to a different substrate to conserve the artificial refuse material. The new substrate consisted of 25 mL of absolute ethanol and the nutrients listed in Table 8. The amount of each nutrient was based on the nutrient requirements reported by Speece and McCarty (1964). Primary sludge supernatant was obtained by centrifuging municipal sludge for one hour at G = 8200. This was

added to provide micronutrients. Daily feeding of 500 mL of this substrate produced an SRT of 50 days which would provide an active culture of methanogenic bacteria in case of failure of the unit receiving solid waste.

At this time, 10 mL of absolute ethanol were added to the substrate for the solid waste digester. The addition of ethanol allowed the anaerobic bacteria in the solid waste digester to become acclimated to the ethanol which was to be used during the inhibition study.

Table 8. Ethanol substrate components

Component	Amount
Absolute ethanol	25 mL
Primary sludge supernatant	25 mL
$FeCL_3$	60 mg/L
$CoCl_2 \cdot 6\ H_2O$	8 mg/L
K_2SO_4	10 mg/L
NH_4CL	147 mg/L
$(NH_4)_2\ HPO_4$	22 mg/L
Bicarbonate buffer (as $CaCO_3$)	3000 mg/L
Thiamine hydrochloride	2 mg/L
Tap water	450 mL

Both reactors were operated for approximately eighteen months prior to the inhibition study and then for another year during the inhibition study. Throughout this period, process instability occurred with both reactors several times. Once, during initial operation, the substrate was added after three days at no feeding. This resulted in decreased gas production, increased volatile acids concentration and a subsequent lowering of pH. The pH was then adjusted to 7.2 and feeding was discontinued until gas production resumed to normal levels. The feeding was then renewed, but at a lower loading with a gradual return to normal loading.

Another cause of instability occurred when air was introduced into the digesters. This problem occurred with both reactors at various times during the research project and was discovered when the weekly gas analysis showed high concentrations of nitrogen. During introduction of air, the pH of the mixed liquor went from the normal of 7.2 to 8.3. The most common cause of air leaks was loose connections between the compressor and gas lines. Once again, corrective action involved pH adjustment and termination of feeding. Several days were required to return the system to a normal loading rate.

The last cause of process instability was major temperature fluctuations resulting from interruption of steam and ventilation to the temperature controlled room. Two electric heaters subsequently were placed in the room to reduce temperature variations. During

periods of temperature control problems, the feeding of each reactor was discontinued until the temperature returned to normal. Usually the reactors recovered quickly when this procedure was followed.

During steady-state operation, the solid waste digester produced an average of 0.038 m^3 of gas/day (1.327 cu ft/day) which consisted of 55.7 % methane and 44.3 % carbon dioxide. This gas production represented 0.566 cu m/kg VS (9.1 cu ft/lb VS) added to the reactor. The characteristics of the contents of the solid waste digester are listed in Table 9. The reactor fed the ethanol substrate produced an average 0.021 m^3 of gas/day (0.75 cu ft/day) with a composition of 79.2 % methane and 20.8 % carbon dioxide.

Anaerobic toxicity assay

Design of the anaerobic toxicity assay (ATA) apparatus was based on the work of Speece (1978), Stuckey, et al. (1978), and Owen, et al. (1979). The only deviations from the apparatus described by these researchers were the use of larger serum bottles and manometers rather than syringes for measurement of gas production.

The arrangement of the ATA apparatus is illustrated in Figure 10. The manometers were 140 cm long and constructed of 2 mm I.D. glass chromatography tubing (6.4 mm O.D.). Three manometers were mounted on plywood and metric tapes were located between the arms of each manometer. Each manometer was half-filled with Merium Manometer

Table 9. Characteristics of mixed liquor in solid waste digester

Parameter	Concentration Total	Soluble
COD	55490 mg/L	8040 mg/L
Total solids	45041 mg/L	————
Volatile solids	39213 mg/L	————
Total dissolved solids	————	9867 mg/L
Volatile dissolved solids	————	4606 mg/L
Total volatile acids	————	35 mg/L[a]
Cellulose	19.26 g/L	————
Lignin	10.79 g/L	————
Sodium	1250 mg/L	1280 mg/L
Potassium	469 mg/L	459 mg/L
Calcium	364 mg/L	135 mg/L
Magnesium	97.7 mg/L	63.3 mg/L
Sulfate	————	12.2 mg/L
Chloride	————	619.9 mg/L
Phosphate	659 mg/L	175 mg/L
Ammonia-nitrogen	————	320 mg/L
Total Kjeldahl Nitrogen	1250 mg/L	————
Nitrate-nitrite nitrogen	————	LT[b] 0.5 mg/L
Barium	3.47 mg/L	0.8 mg/L

[a] As acetic.
[b] LT = less than.

Table 9. continued

Parameter	Concentration Total	Soluble
Silver	0.17 mg/L	0.02 mg/L
Selenium	0.5 µg/L	0.3 µg/L
Mercury	110 µg/L	10 µg/L
Lead	1.7 mg/L	0.4 mg/L
Arsenic	3.9 µg/L	0.9 µg/L
Chromium	3.23 mg/L	0.88 mg/L
Zinc	3.91 mg/L	0.91 mg/L
Copper	1.6 mg/L	0.6 mg/L
Nickel	3.0 mg/L	1.3 mg/L
Manganese	5.67 mg/L	2.3 mg/L
Iron	14.1 mg/L	6.44 mg/L

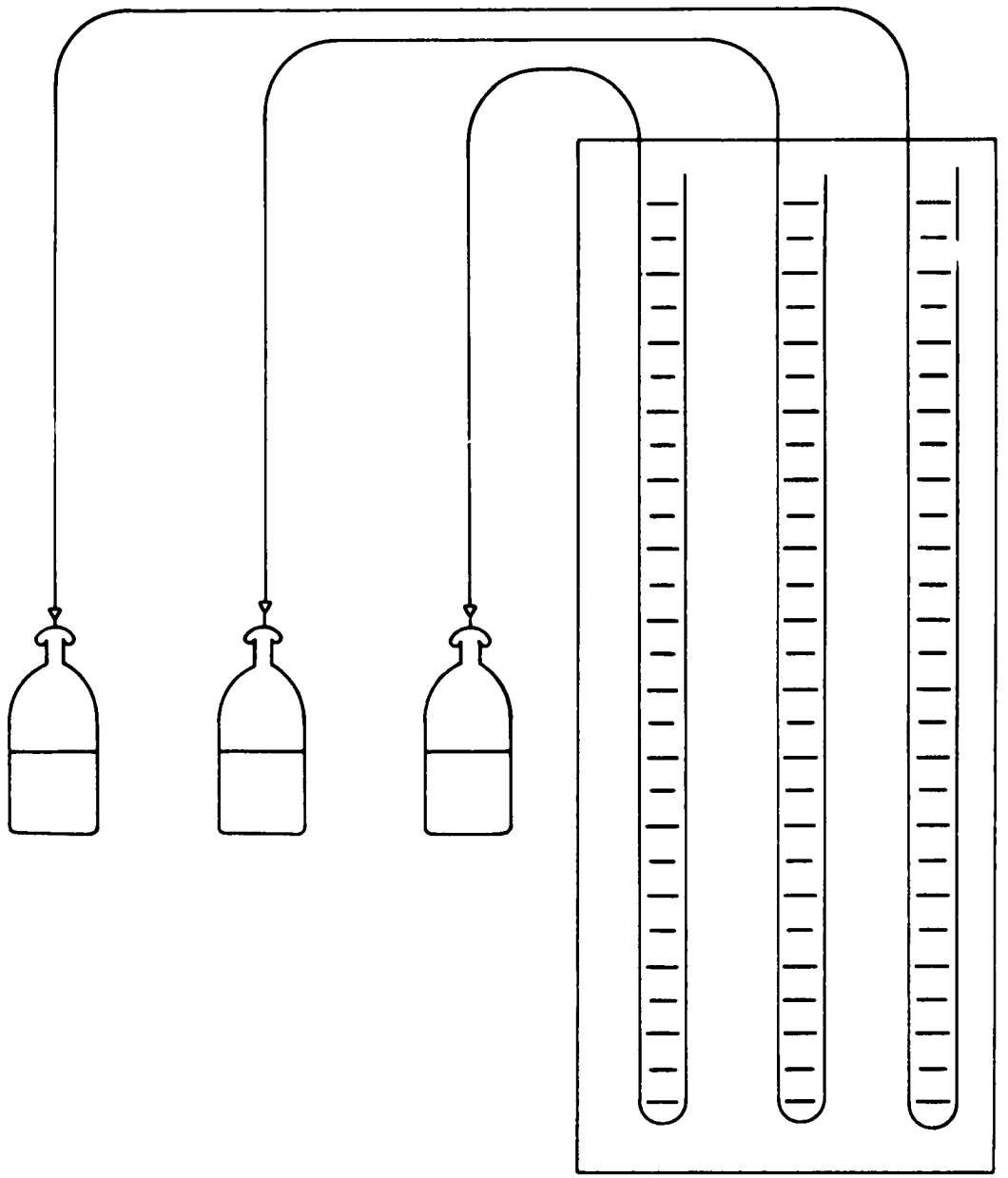

Figure 10. Schematic diagram of anaerobic toxicity assay apparatus used in this study

Fluid No. 3[1], which is pure acetylene tetrabromide with a red dye added. This fluid has a specific gravity of 2.95.

One arm of each manometer was approximately 5 cm longer than the other arm so that it extended above the mounting board. This allowed for the attachment of 0.48 cm I.D. rubber tubing. The tubing was 80 cm long with one end attached to the manometer and the other end connected to a 22 gauge stainless steel syringe needle.

Anaerobic cultures for toxicity testing were transferred from the large reactors to 250 mL serum bottles[2]. Red rubber flanged stoppers[3] were used to seal the serum bottles. The stoppers were easily pierced by the syringe needles to provide measurement of gas production and sampling of the gas.

Measurement of inhibition by this apparatus was based on the same theory of operation that applies to the anaerobic Warburg procedure. The biological production of gas in a constant volume/ constant temperature system causes an increase in pressure. This increased pressure produces a displacement of the fluid in the manometers. The amount of displacement, adjusted to constant atmospheric conditions, corresponds to a specific volume of gas. This was basically the theory of operation used in this study, with some modifications.

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[1]Merium Company, Cincinnati, Ohio.

[2]No. 223950, Wheaton Industries, Millville, New Jersey.

[3]No. 224127, Wheaton Industries, Millville, New Jersey.

Manometer measurements used to determine gas production requires knowledge of the exact volume of each serum bottle and the volume inside the tubing attached to the manometers. Due to variations in serum bottle stoppers and tubing, the determination of these volumes would be an extremely difficult task. Therefore, another procedure, as described in the following section, was devised that provided gas production measurements without concern for these volumes.

Stuckey, et al. (1978) and Owen, et al. (1979) used glass syringes lubricated with glycerine to measure gas production. These syringes are fairly expensive and there was a possibility that the syringe plunger would stick causing an incorrect volume to be recorded. The procedure in this study required the use of inexpensive plastic syringes[1]. The manometers were connected to the serum bottles and the gas production was measured by withdrawing it into the plastic syringe until the manometer pressure was the same as for a serum bottle containing only 100 mL of tap water. The tap water blank provided a thermo-barometric control to compensate for changes in temperature and barometric pressure. The manometer levels for the assay bottles were always reset to the same level as that for the thermo-barometric control.

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[1]Plastipak Disposable Syringes, 10 mL, 20 mL, 30 mL, and 50 mL, Becton-Dickinson, Rutherford, New Jersey.

Anaerobic toxicity assay procedure

After the ATA apparatus was constructed, the system was checked for leaks by connecting the manometers to stoppered serum bottles and pressurizing the serum bottles to approximately 10 cm of manometer fluid displacement. All three manometers were allowed to set under these conditions for at least 24 hours. A reduction in pressure indicated a leak in the system and this procedure was used each time needles were changed.

The serum bottles were cleaned by first rinsing them with tap water, then soaking them in potassium dichromate-sulfuric acid cleaning solution to remove organic chemical residues and followed by several rinses with tap water and distilled water. Preparation of the serum bottles for test purposes involved first adding to each 60 mL of nutrient-buffer solution containing 5.7 g/L sodium bicarbonate and 0.37 g/L ferrous chloride (Stuckey, et al. 1978). These bottles were then purged with a mixture of 30 percent carbon dioxide and 70 percent nitrogen[1]. Stoppers were placed immediately in each serum bottle after purging. An adequate number of serum bottles was set up to provide for duplicate analysis. This included two seed controls, two fed controls, and two bottles for each dosage of the test chemical.

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[1]Linde specialty gas, Union Carbide Corporation, New York, New York.

Prepared serum bottles were stored overnight at 37° C to allow for temperature equilibration. The next day, 40 mL of anaerobic seed from the refuse digester were added to each bottle. This operation was performed in the temperature controlled room. These bottles were then allowed to set undisturbed for two hours so that the cultures could recover from possible oxygen exposure.

After the two hour equilibration period, the pressure in each serum bottle was reduced to atmospheric pressure by removing excess gas with a syringe. This was then considered the start of each test run, and substrate and test chemicals were added immediately to each serum bottle. In the case of the fed controls, 0.100 mL of absolute ethanol was added with a 0.250 mL syringe[1]. The seed control did not receive additional substrate and therefore provided an indicator of background gas production. Chemicals for toxicity evaluation were added in the desired concentrations to the ethanol prior to its addition to the respective test serum bottles.

Selection of test chemicals

A major objective of this research project was to study the inhibitory effects of synthetic organic chemicals on anaerobic digestion systems. However, the number of organic chemicals available for testing is almost undefinable. Therefore, the semivolatile organic Priority Pollutants were chosen for use as a test base

[1]Series 5000, Unimetrics Corporation, Anaheim, California.

because these chemicals reflect an overall environmental concern due to toxicity, carcinogenicity or a general environmental hazard. Basically, the Priority Pollutant list originated as the result of a court settlement between several environmentally concerned plaintiffs and the Environmental Protection Agency (Keith and Telliard, 1979). The suit concerned Part 307 of the Federal Water Pollution Control Act (Public Law 92-500) which required EPA to establish effluent limitations for toxic materials. At first, a total of 65 compounds and classes of compounds appeared on the Toxic Pollutant List. However, the nonspecific nature of this list could have encompassed thousands of compounds if all compounds in each class were included.

Therefore, EPA developed a more specific list which contained 113 organic compounds, 13 metals and 3 miscellaneous categories including total cyanides, total phenols and asbestos (Keith and Telliard, 1979). The organic compounds were divided into two general classes based on the analytical technique required for quantitation. Compounds in each class were further arranged according to carbon skeleton and functional groups.

The first class of compounds is the volatile organics, so named because the analytical technique involves gas stripping from solution (U.S. Environmental Protection Agency, 1979). The volatile organics class includes 34 compounds. These compounds were not selected for evaluation in the immediate study because of their volatility.

The second class of organic Priority Pollutants is the semi-volatile organics. These organic pollutants require the use of solvent extraction for concentration and analysis. They all are, however, volatile enough to be analyzed by gas chromatography techniques. There are nine groups of chemical compounds in this class (Table 10). The numbers in parentheses in Table 10 represent the number of industrial discharges in which the compound was found when a total of 32 industrial discharge categories were sampled (Keith and Telliard, 1979).

Twenty-four compounds were selected for testing from the nine groups of semivolatile organic Priority Pollutants (Table 10). Each group produced at least one representative compound, except the benzidine group due to the commercial unavailability of these two compounds. Only two PCB's (Arochlors) were selected from the chlorinated hydrocarbon pesticide group because of the extensive research that has been performed on these compounds.

Laboratory Analysis

Chemical analyses were performed by the Analytical Services Laboratory (ASL) of the Engineering Research Institute and by research personnel in the Iowa State University Sanitary Engineering Laboratory (SEL) working in conjunction with this project. The analysis of the refuse digester contents was quite involved, as shown in Table 9, to provide adequate background information concerning the anaerobic

Table 10. List of semivolatile organic priority pollutants

1. Organochlorine pesticides and PCB's	
aldrin	(5)[a]
alpha-BHC	(4)
beta-BHC	(6)
delta-BHC	(4)
gamma-BHC	(3)
chlordane	(4)
4,4'-DDD	(2)
4,4'-DDE	(1)
4,4'-DDT	(2)
dieldrin	(3)
endosulfan I	(3)
endosulfan II	(4)
endosulfan sulfate	(2)
endrin	(3)
endrin aldehyde	(2)
heptachlor	(3)
heptachlor epoxide	(1)
toxaphene	(2)
Arochlor 1016	(2)
Arochlor 1221	(1)
Arochlor 1232	(2)
Arochlor 1242[b]	(3)
Arochlor 1248	(2)
Arochlor 1254[b]	(3)
Arochlor 1260	(1)
2,3,7,8-tetrachlorodibenzo-p-dioxin	
2. Benzidines	
benzidine	(4)
3,3'-dichlorobenzidine	(0)

[a]Number of industrial effluent categories in which the chemical was found.

[b]Test chemicals selected for Phase I screening.

Table 10. continued

3. Chlorinated hydrocarbons	
hexachlorocyclopentadiene*	(1)
hexachlorobenzene	(7)
hexachlorobutadiene*	(1)
hexachloroethane*	(5)
2-chloronaphthalene	(9)
1,3-dichlorobenzene*	(9)
1,4-dichlorobenzene*	(9)
1,2-dichlorobenzene	(9)
4. Nitrosamines	
n-nitrosodimethylamine*	(1)
n-nitrosodiphenylamine	(5)
n-nitrosodi-n-propylamine	(2)
5. Haloethers	
bis(2-chloroethyl)ether*	(4)
bis(2-chloroethoxy)ether	(3)
bis(2-chloroisopropyl)ether	(6)
4-bromophenylphenyl ether*	(1)
4-chlorophenylphenyl ether	(2)
6. Phthalate esters	
benzyl butyl phthalate	(13)
bis(2-ethlhexyl)phthalate	(29)
di-n-butyl phthalate*	(23)
di-n-octyl phthalate	(12)
diethyl phthalate*	(20)
dimethyl phthalate*	(15)

Table 10. continued

7. Polynuclear aromatic hydrocarbons	
acenaphthene	(14)
acenaphthylene	(12)
anthracene	(16)
benzo(a)anthracene	(6)
benzo(a)pyrene	(8)
benzo(b)fluoranthene	(6)
benzo(ghi)perylene	(7)
benzo(k)fluoranthene	(6)
chrysene	(9)
dibenzo(a,h)anthracene	(4)
fluoranthene	(12)
fluorene	(11)
indeno(1,2,3-cd)pyrene	(4)
naphthalene*	(18)
phenanthrene	(16)
pyrene*	(14)
8. Phenols	
4-chloro-3-methylphenol*	(8)
2-chlorophenol*	(10)
2,4-dichlorophenol*	(12)
2,4-dimethylphenol*	(15)
2,4-dinitrophenol	(6)
2-methyl-4,6-dinitrophenol	(6)
2-nitrophenol*	(11)
4-nitrophenol*	(9)
pentachlorophenol	(18)
phenol*	(25)
2,4,6-trichlorophenol*	(12)

Table 10. continued

9. Nitroaromatics and isophorone	
isophorone	(13)
nitrobenzene*	(9)
2,4-dinitrotoluene	(3)
2,6-dinitrotoluene	(9)

seed. This was not a routine analysis and was performed on a 14 day composite sample of the waste mixed liquor from the refuse digester The analytical procedures involved in this chemical analysis are not included in the body of this report but can be found in Appendix I.

Routine analyses performed during this study included pH, total and volatile suspended solids, gas composition, individual volatile acids, soluble concentration of the test chemical and the presence of degradation products of the test chemical. Suspended solids and pH were measured according to Standard Methods (1976) and will be discussed in the following section. The other analyses were performed using gas chromatography procedures. These procedures were performed without the aid of documented methods and therefore will be discussed in a later section.

pH

The hydrogen ion concentration, pH, is an important quality parameter in the control of biological treatment systems. The acceptable pHs for anaerobic bacteria typically range from 6 to 8 with an optimum of 6.7 to 7.3. The pH of the waste mixed liquor from both laboratory digesters was checked each day following feeding using a Beckman Zeromatic pH meter. This instrument was calibrated at pH 7.0 using a certified buffer solution[1] prior to each measurement.

[1]Fisher Scientific Company, Itasca, Illinois.

Suspended solids

The anaerobic seed from the refuse digester was withdrawn into a 2-liter Nalgene beaker. A 100 mL sample was taken from this beaker after thorough mixing by hand. This sample was stored at 4^{o} C until analysis on the same day.

Due to the large amount of disposed solids in the sample, only a limited amount of sample could be filtered. A 5 mL graduated pipette with an enlarged tip was used to transfer the sample to the filter pad. Normally 2.0 mL of sample were filtered.

Three filter discs were used for each suspended solids analysis. The average of the three determinations was used in calculating the suspended solids concentration.

Gas chromatographic analyses

Gas chromatography was used to analyze all gaseous and semi-volatile organic compounds of importance to the immediate study. This section describes the sampling procedures and the methods employing gas chromatography that were used. A description of how gas chromatography works and the necessary calculation procedures are presented in Appendix II.

Gas analysis Gas analysis included the determination of carbon dioxide, methane and nitrogen in the serum bottles. The gas chromatographic conditions are listed in Table 11. A typical chromatogram of the gas produced by the refuse digester is presented

in Figure 11.

The general method of gas sampling was as follows:

1. Gaseous samples were collected using a 1.0 mL gas tight syringe[1] equipped with a Mininert syringe valve,
2. The syringe valve was opened and the syringe plunger was completely depressed,
3. The syringe needle (28 gauge) was introduced into the serum bottle through the stopper,
4. 0.5 mL of gas was drawn into the syringe,
5. After approximately 5 seconds, the syringe valve was closed and the needle withdrawn from the serum bottle, and,
6. The syringe was immediately transferred to the instrument room for analysis.

Volatile acids A review of the literature concerning the chromatography of acetic, propionic, iso-butyric, normal butyric, iso-valeric, and normal valeric acids was first performed. Nikelly (1964) obtained very good resolution using a column packed with 0.25 % Carbowax and 0.47 % isophthalic acid on 200-micron glass microbeads. This packing material was not commerically available, however. Hauser and Zabransky (1975) reported a method developed specifically for analysis of organic acids produced by anaerobic

[1] Pressure-Lok Series C syringe, Precision Sampling, Baton Rouge, Louisiana.

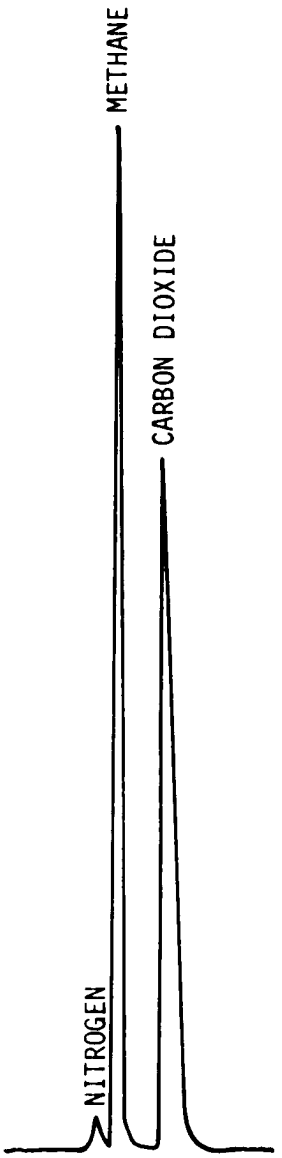

Figure 11. Typical gas chromatogram of laboratory digester gas

Table 11. Operating conditions for gas analysis

Gas chromatograph	Packard Model 7411S
Column	10 ft X 4 mm glass
Packing	Porapak Q, 80/100 mesh
Temperature	95° C
Carrier gas	Helium
Flowrate	30 mL/min
Column head pressure	29 psig
Detector	Thermoconductivity
Temperature	110° C
Bridge current	250 mA
Sensitivity	10 mV
Injector block temperature	105° C
Sample size	0.5 mL

bacteria. This method, however, employed an ether extraction procedure described by Dowell and Hawkins (1974). Attempts were made in the Sanitary Engineering Laboratory to use this column (15 % SP-1220/1 % H_3PO_4 on 100/120 Chromosorb W AW) with direct aqueous injection but failed to totally resolve the acetic acid. The method described by Ottenstein and Bartley (1971 a & b) was used with good success, and the operating conditions for this analysis are given in Table 12. A typical volatile acid standard chromatogram is shown in Figure 12 .

There are some important points which should be discussed concerning the analysis of volatile acids. First, the use of glass columns is mandatory. Only silanized glass is inert enough to eliminate adsorption of sample components. Secondly, glass wool treated with phosphoric acid must be used in the columns to retain the packing since the use of silane-treated glass wool causes tailing of the component peaks. Lastly, a glass injection port liner is necessary.

The procedure for analysis of individual volatile acids was as follows:

1. Liquid samples from the serum bottles were first centrifuged at G = 39100 for 30 minutes,
2. 5 mL of centrate was transferred by pipette to 7 mL specimen bottles,
3. The centrate in the specimen bottles was acidified to pH ~ 2 using concentrated sulfuric acid, and
4. The acidified samples were stored at 4^{o} C until the termination of the test run at which time the samples were analyzed.

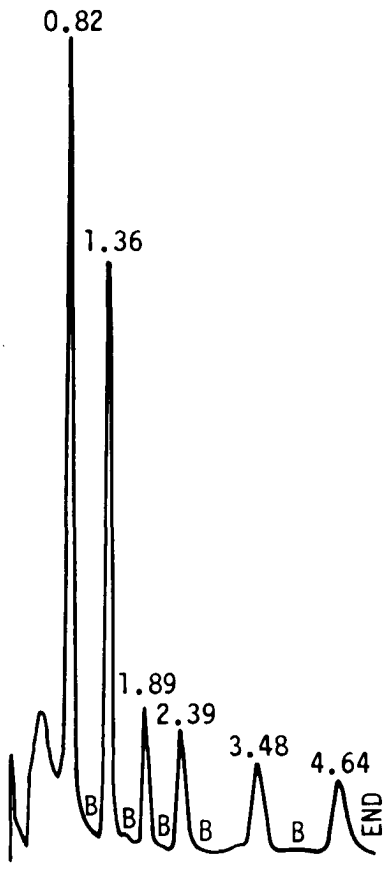

Figure 12. Typical gas chromatogram of volatile acids standard

Table 12. Operating conditions for volatile acids analysis

Gas chromatograph	Perkin-Elmer Sigma I
Column	6 ft X 2 mm ID glass
Packing	10 % SP-1200/1 % H_3PO_4 on 80/100 Chromosorb W AW
Temperature	115° C
Carrier gas	Nitrogen
Flowrate	35 mL/min
Detector	Flame ionization
Hydrogen flowrate	44 mL/min
Temperature	280° C
Injection port temperature	225° C
Sample size	1.0 μL

Organic chemical analysis Concentrations of various test chemicals were measured in solution and in the product gas during the inhibition study. Direct aqueous injection was used with the liquid samples to eliminate recovery problems with solvent extraction procedures. This required modification of procedures suggested by Supelco (1978) and the U.S. Environmental Protection Agency (1979). The same method for each compound was used in the analysis of gas and liquid samples, except larger sample volumes were injected in the gas analysis. Specific methods for each chemical are listed in

Appendix II.

The general method of liquid sampling was as follows:

1. Samples from the serum bottles were centrifuged at G = 39100 for 30 minutes to remove the solids,

2. 5 mL of centrate were transferred by pipette to a 7 mL specimen bottle,

3. The centrate was acidified to pH $\leq$ 2 using concentrated sulfuric, and

4. The samples were stored at 4^{o} C until analysis.

TESTING AND RESULTS

The experimental investigation was divided into three phases. Organic Priority Pollutants were screened for inhibitory effects during the first phase. Chemicals found to be inhibitory during Phase I were used in the second phase to study the conditions during inhibition and to examine the various factors that relate to the recovery of an inhibited system. Phase III was a study of the effect of the inert solids concentration on inhibition and recovery.

Phase I

The objective of Phase I testing was to identify the compounds among the 24 Priority Pollutants selected for testing that produced inhibitory effects in anaerobic digestion systems. This was determined by performing a batch bioassay on each individual chemical.

Testing

Stock solutions of each test chemical were prepared by dissolving each chemical in absolute ethanol. Since the dilution factor in the serum bottles was 1000 to 1 (0.100 mL of ethanol in 100 mL of culture solution), stock solutions were made at the 100 gram per liter level. Other concentrations were made by dilution of the 100 g/L stock solution. The only exceptions to this procedure were the solutions of naphthalene and pyrene. These two chemicals are only slightly soluble in ethanol and since they are not liquids, the maximum test concentration was

limited without the use of an alternate substrate. Naphthalene was put into solution at a maximum concentration of 50 g/L and pyrene had a maximum concentration of 10 g/L in ethanol. After preparation, the chemical solutions were stored in the dark at 4^{o} C until use.

Chemical dosages used in the ATA procedure were 1, 5, 10, 50 and 100 mg/L for all the test chemicals except for naphthalene (1, 5, 10 and 50 mg/L) and pyrene (1, 5, and 10 mg/L). This low range of test concentrations was selected for two reasons:

1. Many of these organic compounds have a low water solubility, and,
2. A realistic concentration range which would represent a slug load of the chemicals to an anaerobic digester was desired.

Table 13 lists the solubilities of the test chemicals in aqueous solution for which data were available.

During this phase of the study, only gas production in the bioassays was monitored. Gas measurements were taken at least three times during the first day and then once a day for the next three days. Comparison of gas production in the spiked serum bottles to the control provided evidence of inhibition.

A total of seven separate test runs were made during Phase I. Four were made using anaerobic seed from the reactor fed with ethanol. An air leak in the unit receiving solid waste caused unstable operation and the period of time for recovery would have caused an unnecessary delay in the project. Therefore, only the last three test runs were made using anaerobic seed from the refuse digester.

Table 13. Solubilities of organic test chemicals in water

Compound	Solubility, mg/L	Reference
Dimethylphthalate	5,000 @ 20° C	Verschueren, 1978
Diethylphthalate	7,000 @ 25° C	Handbook of Chem. and Phys., 1963
N-nitrosodimethylamine	Miscible	Dobbs & Cohen, 1980
Di-n-butylphthalate	400 @ 25° C	Handbook of Chem. and Phys., 1963
4-Bromodiphenyl ether	38 @ 20° C	Dobbs & Cohen, 1980
1,3-Dichlorobenzene	123 @ 25° C	Verschueren, 1978
1,2-Dichlorobenzene	145 @ 25° C	Verschueren, 1978
2-Chloroethyl ether	11,000 @ 20° C	Dobbs & Cohen, 1980
Phenol	67,000 @ 16° C	Handbook of Chem. and Phys., 1963
2,4,6-Trichlorophenol	800 @ 25° C	Handbook of Chem. and Phys., 1963
4-Chloro-3-methylphenol	3,846 @ 20° C	Merck Index, 1976
2,4-Dimethylphenol	17,000 @ 160° C	Dobbs & Cohen, 1980
Arochlor 1242	0.24	Dobbs & Cohen, 1980
2-Chlorophenol	28,500 @ 25° C	Handbook of Chem. and Phys., 1963
Arochlor 1254	0.012	Dobbs & Cohen, 1980
2-Nitrophenol	2,100 @ 25° C	Handbook of Chem. and Phys., 1963
4-Nitrophenol	16,000 @ 25° C	Handbook of Chem. and Phys., 1963
2,4-Dichlorophenol	4,600 @ 20° C	Handbook of Chem. and Phys., 1963
Hexachlorocyclopentadiene	0.805	Dobbs & Cohen, 1980
Hexachloro-1,3-butadiene	2 @ 20° C	Dobbs & Cohen, 1980

Table 13. continued

Compound	Solubility, mg/L	Reference
Naphthalene	30 @ 20° C	Verschueren, 1978
Nitrobenzene	1,900 @ 20° C	Handbook of Chem. and Phys., 1963
Pyrene	0.175 @ 20° C	Dobbs & Cohen, 1980
Hexachloroethane	50 @ 22° C	Lange's Handbook of Chem., 1970

Results of Phase I

The order of test runs is listed in Table 14. An additional supply of serum bottles received after the fourth test run allowed for the testing of several chemicals during subsequent runs.

Table 14. Chronological order and test chemicals for each screening test

Run # 1	
4-Nitrophenol	1,3-Dichlorobenzene
	Phenol
Run # 2	2,4,6-Trichlorophenol
2-Nitrophenol	1,2-Dichlorobenzene
	4-Chloro-3-methylphenol
Run # 3	
2,4-Dichlorophenol	Run # 6
	Naphthalene
Run # 4	Hexachloro-1,3-butadiene
2,4-Dimethylphenol	2-Chloroethylether
	Nitrobenzene
Run # 5	2-Chlorophenol
Hexachloroethane	Pyrene
N-Nitrosodimethylamine	
Diethylphthalate	Run # 7
Dimethylphthalate	Arochlor 1254
4-Bromodiphenylether	Arochlor 1242
Di-n-butylphthalate	

Total gas production in the controls during each test run was different. Table 15 lists the average gas production for the controls at the termination of each run. Data listed are based on the average of duplicate analysis. The relatively low gas production through the first four runs can be attributed to the low background gas production in the seed cultures from the ethanol reactor.

Table 15. Summary of gas production data for the controls during each test run

Test run	Total gas production, mL
1	86.2
2	125
3	84.8
4	84.3
5	227
6	217
7	206

In order to compare the data from all of the test runs, relative activity was established as a comparison basis. Relative activity is the ratio of gas production in the spiked culture to that of the respective control. For the initial determination of inhibition, the procedure of Hovious, et al., (1973) was used. This procedure consisted of using the gas production data over the initial five hour period of the test run. The gas production data for all the test chemicals are presented in this manner in Figures 13-16. Controls are represented by the horizontal line at 100% relative activity.

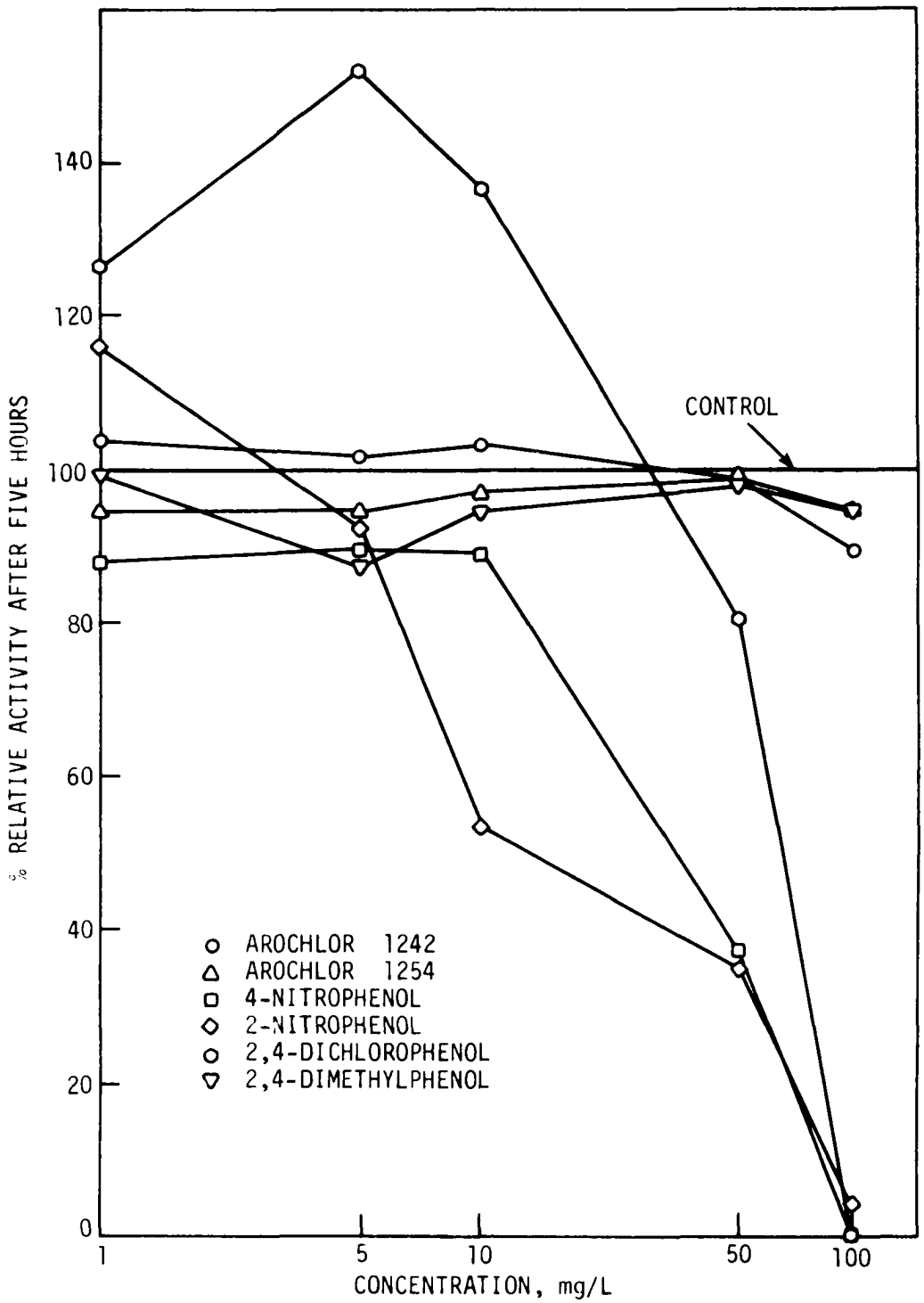

Figure 13. Relative activity versus concentration for test runs 1, 2, 3, 4 and 7 during Phase I testing

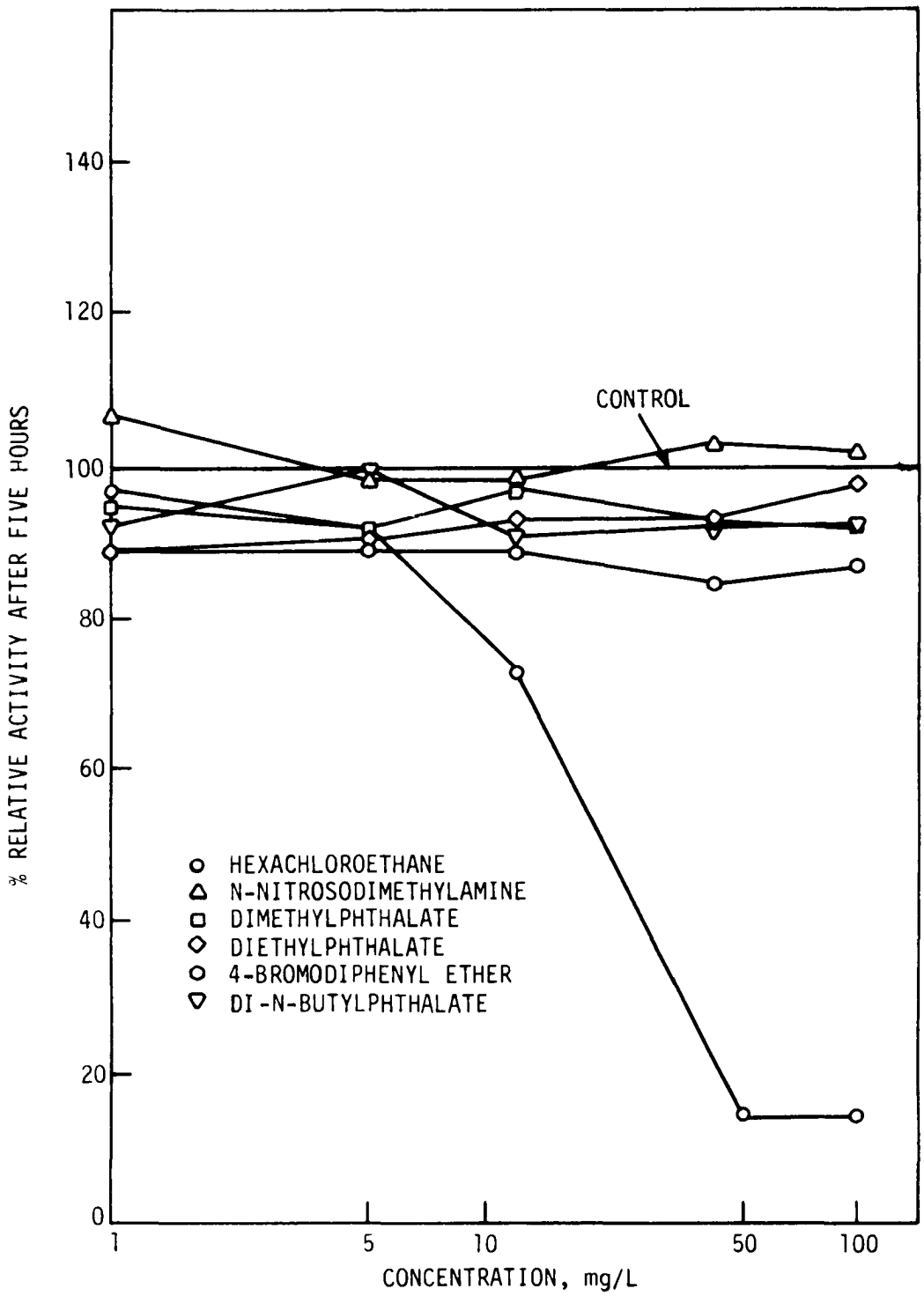

Figure 14. Relative activity versus concentration for test run 5 during Phase I testing

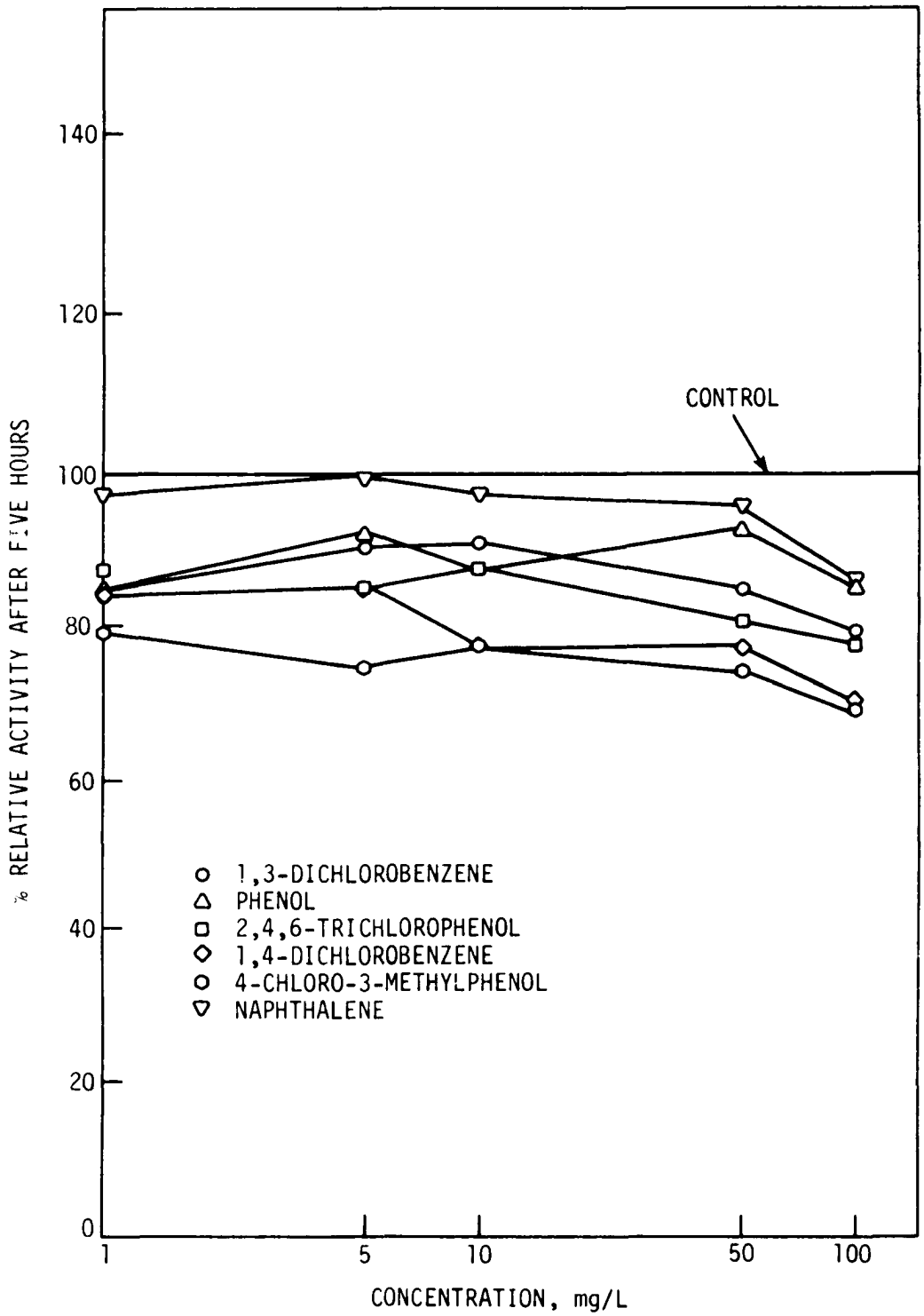

Figure 15. Relative activity versus concentration for test runs 5 and 6 during Phase I testing.

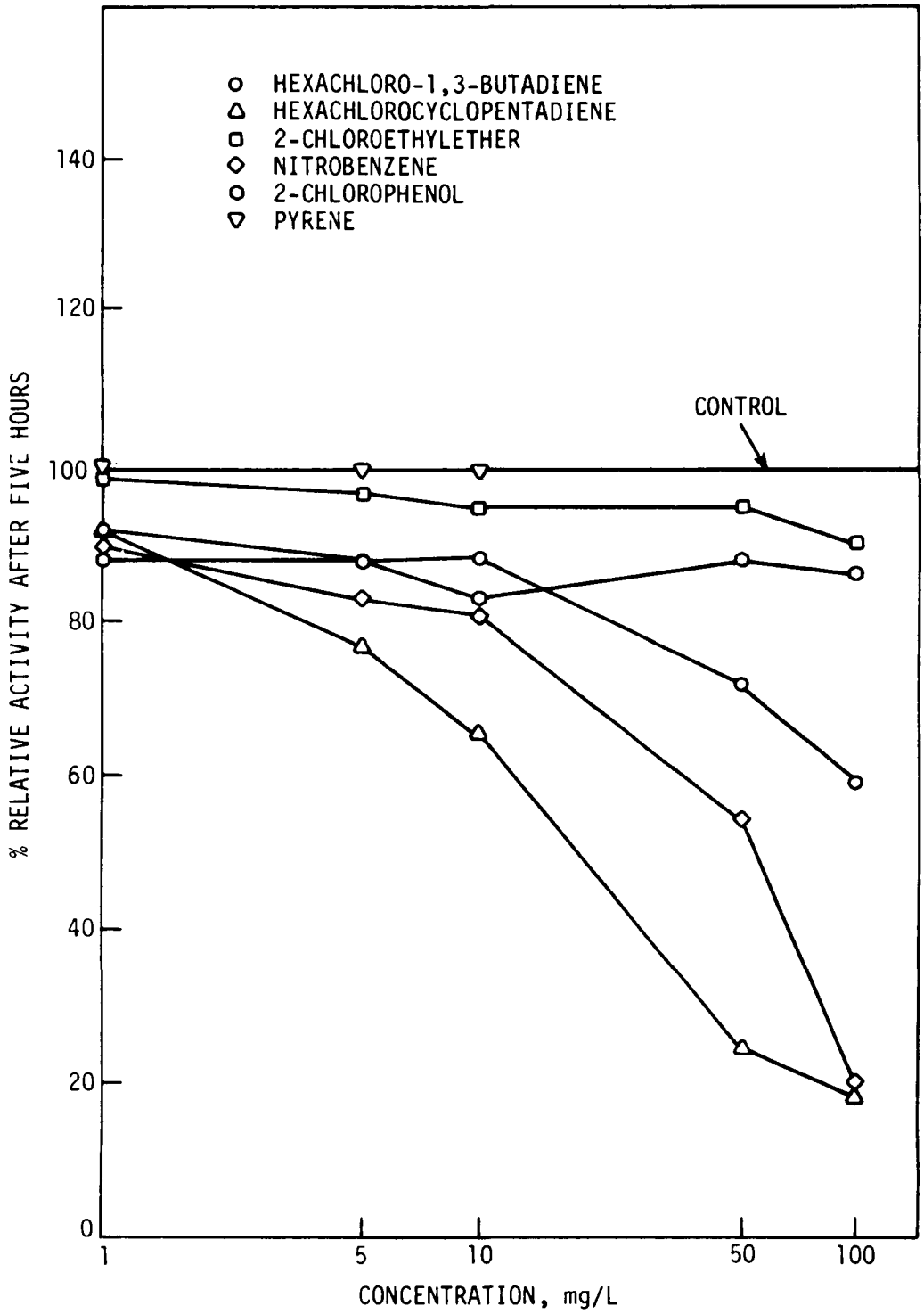

Figure 16. Relative activity versus concentration for test run 6 during Phase I testing

As shown by these figures, the test chemicals producing significant inhibition (> 50% relative activity) were 4-nitrophenol, 2-nitrophenol, 2,4-dichlorophenol, hexachloroethane, hexachlorocyclopentadiene and nitrobenzene. All of these chemicals produced a decrease in relative activity with increasing concentrations. Samples dosed with hexachloro-1,3-butadiene exhibited a trend toward decreasing activity with increasing concentration, but this chemical only produced a forty percent reduction in relative activity during the initial five hours.

Anaerobic cultures exposed to 2,4-dichlorophenol responded in such a manner as to suggest stimulation at low concentrations. McCarty (1964b) and Kugelman and Chin (1971) reported that low concentrations of many materials can produce this same effect.

Gas production in cultures dosed with phenol, 4-bromodiphenyl ether, 4-chloro-3-methyl phenol, 2,4,6-trichlorophenol and 1,3-dichloro-benzene was fairly erratic with little consistency between replicates. These inconsistent measurements produced lower relative activities (Figures 14 and 15). All of these compounds were tested during the same run but the cause of the inconsistent gas production measurements was unknown.

There appeared to be significant variation in the relative activities produced by the test chemicals over the entire run. It is important to note that the data in Figures 13-16 were based on the initial five hours of gas production while the run lengths varied between 72 and 285 hours. Table 16 lists the relative activities produced by the

Table 16. Relative activities of samples dosed with the test chemicals

Chemical	Run hrs.	Relative activity, percent of control Dosage, mg/L 1	5	10	50	100
4-Nitrophenol	72	88[a] 93[b]	90 96	89 99	37 91	0 72
2-Nitrophenol	100	116 102	92 104	54 98	35 103	5 103
2,4-Dichlorophenol	96	130 116	154 108	137 107	80 99	0 76
2,4-Dimethylphenol	96	99 103	87 100	95 100	98 98	95 92
Hexachloroethane	193	97 97	92 96	73 96	14 91	14 90
N-Nitrosodimethylamine	193	107 97	98 96	98 95	103 96	102 93
Dimethylphthalate	193	95 93	92 93	97 95	93 92	92 91
Diethylphthalate	193	89 92	90 93	93 94	93 94	98 95
4-Bromodiphenylether	193	89 91	89 91	89 91	85 90	87 91
Di-n-butylphthalate	193	92 93	100 95	90 92	92 93	92 92
1,3-Dichlorobenzene	193	85 91	90 94	90 91	85 91	79 86
1,2-Dichlorobenzene	193	84 90	85 91	77 88	77 87	70 83
Phenol	193	89 91	92 92	87 91	92 92	85 90
2,4,6-Trichlorophenol	193	87 91	85 91	87 89	80 86	77 79

[a] Relative activity after 5 hours.

[b] Relative activity at the termination of the run.

Table 16. continued

Relative activity, percent of control						
Chemical	Run hrs.	Dosage, mg/L 1	5	10	50	100
4-Chloro-3-methylphenol	193	79 90	74 87	77 87	74 85	69 84
Naphthalene	285	97 99	99 95	97 95	95 98	— —
Hexachlorocyclopentadiene	285	92 105	77 104	65 101	24 90	18 91
Hexachloro-1,3-butadiene	285	92 99	88 102	88 100	72 68	59 41
Bis(2-chloroethyl)ether	285	99 98	97 97	95 98	95 98	90 97
Nitrobenzene	285	90 101	83 100	81 103	54 101	20 97
2-Chlorophenol	285	88 102	88 94	83 99	88 102	86 93
Pyrene	285	100 101	100 100	100 100	— —	— —
Arochlor 1242	167	104 102	102 101	103 103	99 100	89 90
Arochlor 1254	167	95 104	95 105	97 105	99 105	95 102

test chemicals after the initial five hours and at the termination of the test run.

The most significant variation was experienced in the cultures dosed with nitrobenzene, 2-nitrophenol, 4-nitrophenol, 2,4-dichlorophenol, hexachloroethane and hexachlorocyclopentadiene. All of these chemicals produced a higher relative activity at the termination of the run than during the initial five hours of the test. Cultures dosed with 100 mg/L of hexachloro-1,3-butadiene exhibited a reduction in relative activity over the length of the test run. This was the only chemical producing significant inhibition that displayed this pattern. The variation of relative activities with respect to time for these seven chemicals are displayed in Figures 17a and b. It is interesting to note that the initial five-hour period produced the maximum inhibition from all of these chemicals except nitrobenzene and hexachloro-1,3-butadiene.

As shown by Figures 17a and b, the length of the inhibition evaluation using batch bioassays is an important consideration. The variation of methanogenic activity in this study was quite significant. In the cases of all seven inhibitory Priority Pollutants, the degree of inhibition was dependent on the time over which the measurements were made. A short period of evaluation, such as less than ten hours, would not give a true representation of the response of the anaerobic culture. However, a short run would be appropriate for screening organic chemicals for inhibitory effects since the chemicals producing no inhibition could be eliminated from further testing.

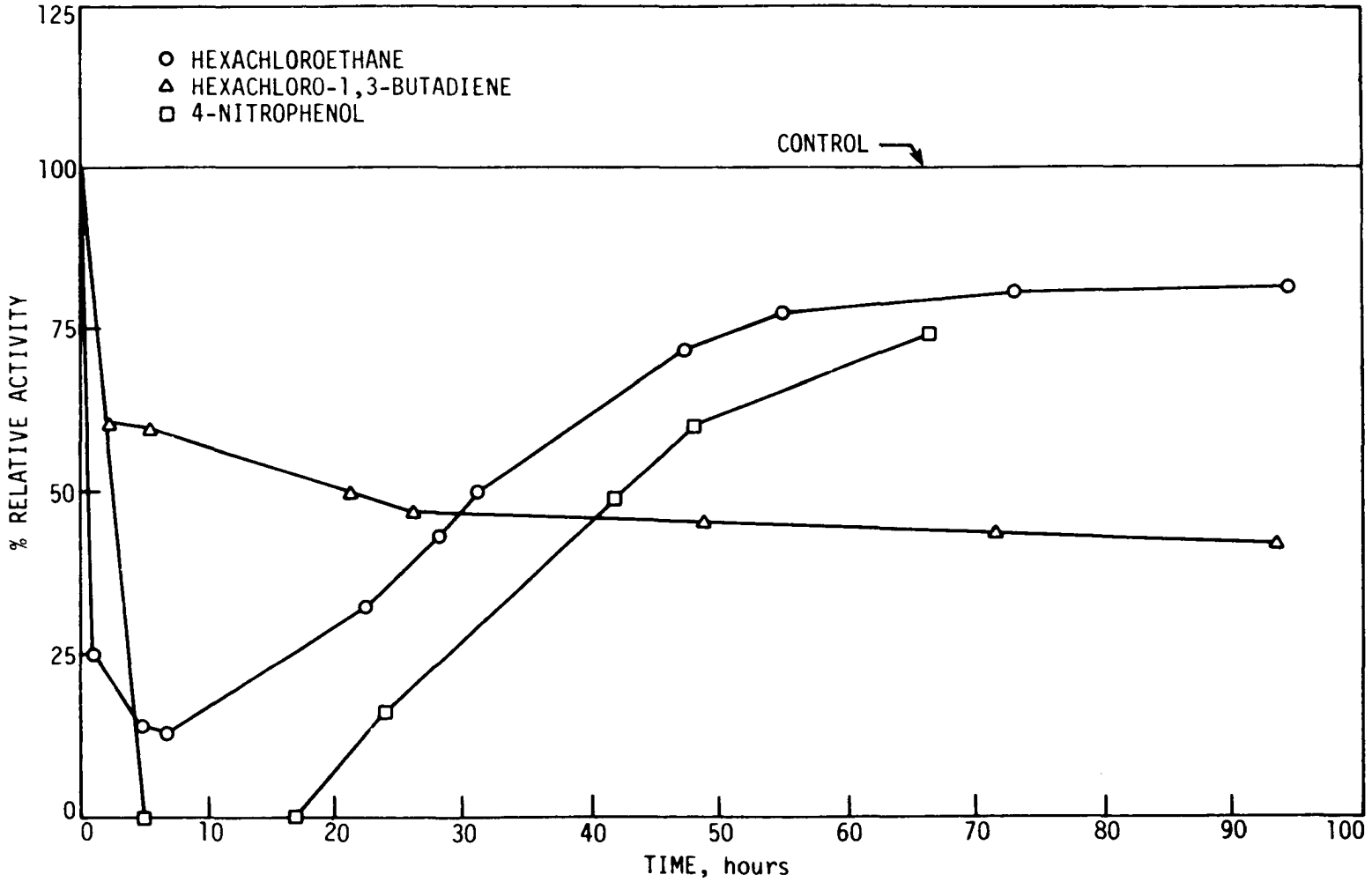

Figure 17a. Variation of activity with time for 100 mg/L dosages of hexachloroethane, hexachloro-1,3-butadiene and 4-nitrophenol

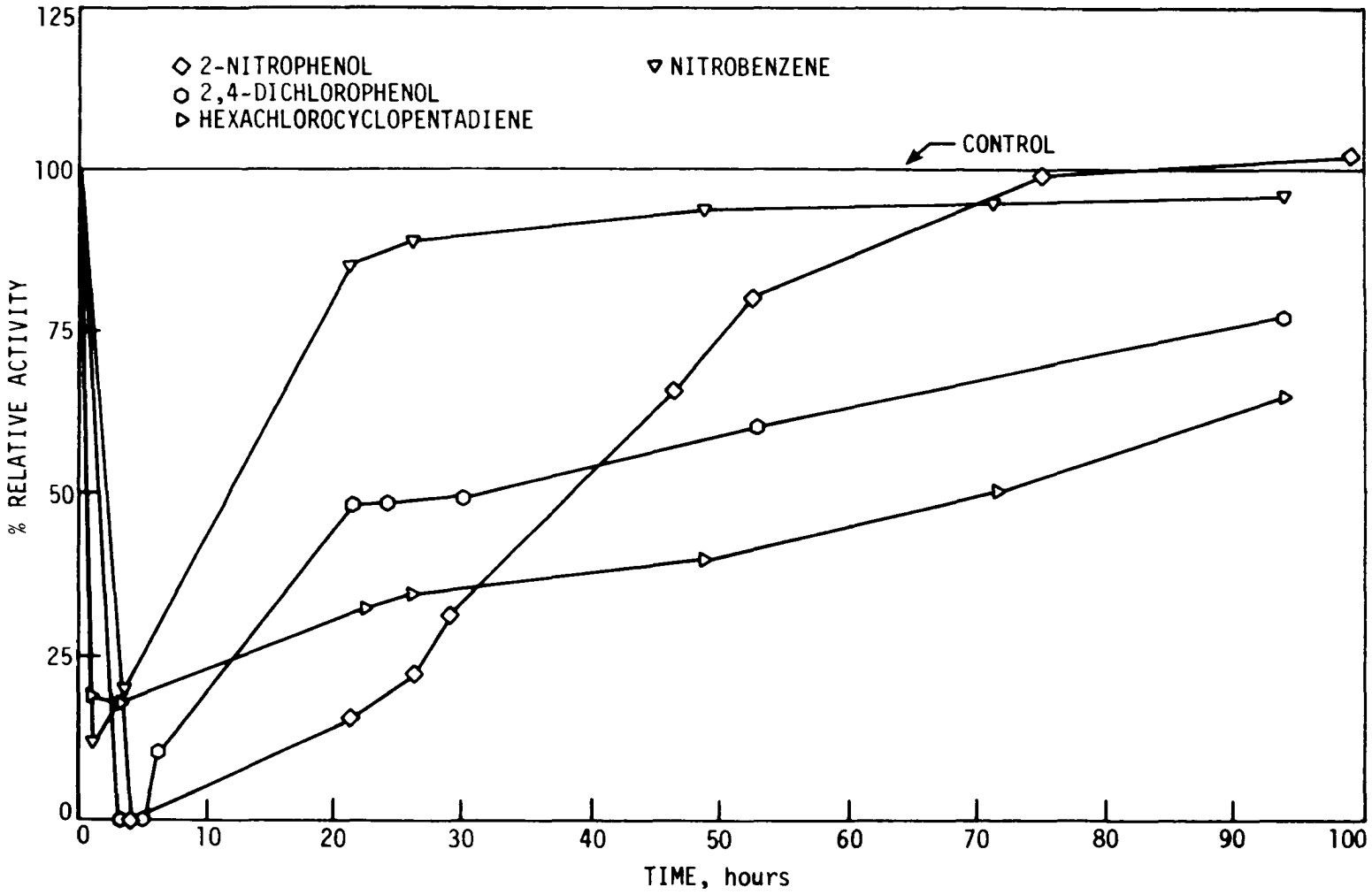

Figure 17b. Variation of activity with time for 100 mg/L dosages of 2-nitrophenol, 2,4-dichlorophenol, hexachlorocyclopentadiene and nitrobenzene

Nitrobenzene, 2-nitrophenol, 4-nitrophenol, 2,4-dichlorophenol, hexachloroethane, hexachlorocyclopentadiene and hexachloro-1,3-butadiene were selected for testing in Phase II because they all produced at least fifty percent reduction in relative activity at some point during the screening tests. Chemical structures of these seven compounds are shown in Figure 18. The common element of all of these chemicals is the presence of a nitro or chloro functional group.

Inhibition of methane formation of anaerobic cultures by chlorinated organic compounds has been well-established (Thiel, 1969; Hovious, et al., 1973; Stickley, 1970; Mosey and Hughes, 1975; Chou, et al., 1978). Some organic compounds containing the nitro group, such as nitrobenzene and 2,4-dinitrophenol, have been identified as being inhibitory to methanogenic bacteria but the nitro group in general has not been linked to inhibition problems. Chou, et al., (1978) reported that 12.3 mg/L of nitrobenzene produced a fifty percent reduction in gas production in acetate-enriched anaerobic cultures. These researchers did not report any variation in the degree of inhibition during testing. Roberton and Wolfe (1970) reported recovery of methane production in pure cultures of Methanobacterium MoH that had been inhibited by 3.7 mg/L of 2,4-dinitrophenol. The recovery occurred in three hours.

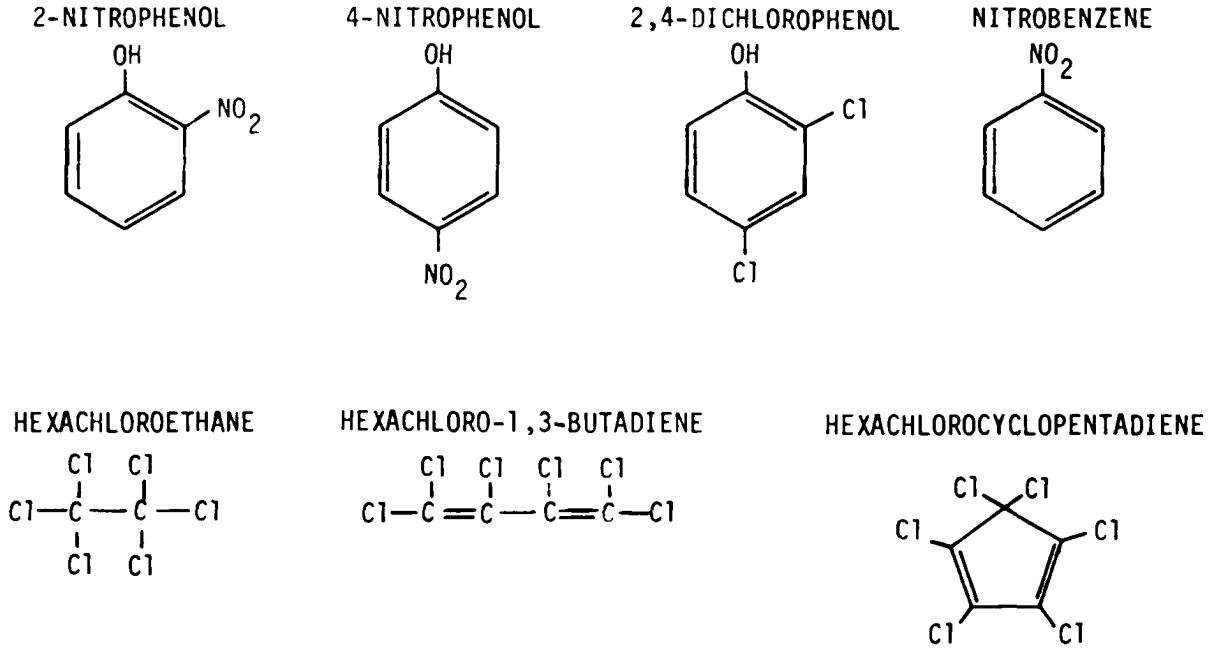

Figure 18. Chemical structures of organic priority pollutants inhibitory to anaerobic digestion

Phase II

Hexachloroethane, hexachlorocyclopentadiene, hexachloro-1,3-butadiene, 4-nitrophenol, 2-nitrophenol, nitrobenzene and 2,4-dichlorophenol were found to produce significant inhibition of anaerobic cultures during Phase I. These chemicals were used in Phase II to explore further the causes of this inhibition.

Testing

The anaerobic toxicity assay (ATA) procedure used in Phase II was slightly different from that used in Phase I to allow for the collection of samples for chemical analysis. As before, the ATAs were set up in duplicate for measurement of gas production at each concentration of the test chemicals. Additional replicate serum bottles containing the test chemicals were set up to provide samples for chemical analysis. Using this procedure, no liquid was removed from the two serum bottles that were used for gas production measurements.

Run lengths during this phase were not set rigidly since the goal was to allow all inhibited samples to recover before terminating the test. As in Phase I, gas production was measured at least three times during the first day and then once a day for the duration of the test run. In addition, an analysis of each serum bottle for gas quality (methane, carbon dioxide and nitrogen) was performed periodically throughout the run. Samples for chemical analysis were collected after 4, 8 and 24 hours and then periodically over the duration of the test.

These samples were analyzed for individual volatile acids, soluble concentration of the test chemical and the presence of any detectable degradation products of the test chemical. Gas chromatography conditions and procedures used are explained in Appendix II. A sample of the seed was taken before each test run for analysis of total suspended solids, volatile suspended solids, and individual volatile acids. Anaerobic seed from the refuse digester was used exclusively in Phases II and III.

A supplemental study during Phase II was performed to examine the behavior of the test chemicals in the absence of biological activity. The objectives of this study were to determine if the observations noted in Phase II were due to microbial action. Seed cultures were added to serum bottles which were then sterilized at 120° C for one hour to kill all living organisms. Sterilized cultures closed with 100 mg/L of each test chemical and samples were taken after 2, 4, 8, 24 and 48 hours for for analysis of the soluble test chemical concentration and the presence of degradation products.

Results of Phase II

Individual test runs were set up for each test chemical and these are discussed separately in the following sections.

Hexachlorocyclopentadiene Seed cultures in this test run were dosed at concentrations of 50, 100 and 500 mg/L of hexachlorocyclopentadiene. Only the cultures dosed at 50 and 100 mg/L were analyzed in depth. The dosage of 500 mg/l was an attempt to induce irreversible inhibition.

Total gas production and methane production are shown in Figure 19. At 50 mg/L, cultures were inhibited initially. While the total gas production in cultures at this concentration was less than that of the controls, the methane production in the spiked cultures exceeded that of the control after 70 hours. This might suggest that early methane production in these spiked cultures resulted from utilization of molecular hydrogen and carbon dioxide since this mechanism would result in the formation of methane only in the product gas.

Cultures dosed with 100 mg/L were also inhibited but continued to produce gas throughout the test period. Early gas production resulted in very little methane formation but after 122 hours the methane production in these cultures equalled that of the control. At 500 mg/L, cultures were inhibited throughout the test run.

It was hoped to determine the cause of recovery by monitoring the soluble concentration of hexachlorocyclopentadiene. At no time during the test period was hexachlorocyclopentadiene detected in the liquid samples. This is especially remarkable since the electron capture detector is capable of detecting nanogram quantities of this compound (based on the detector conditions and injection volume). In addition, no peaks were noted that would represent degradation products of this compound. However, the selectivity of this detector is an important consideration and dechlorination of the compound could account for the negative detection.

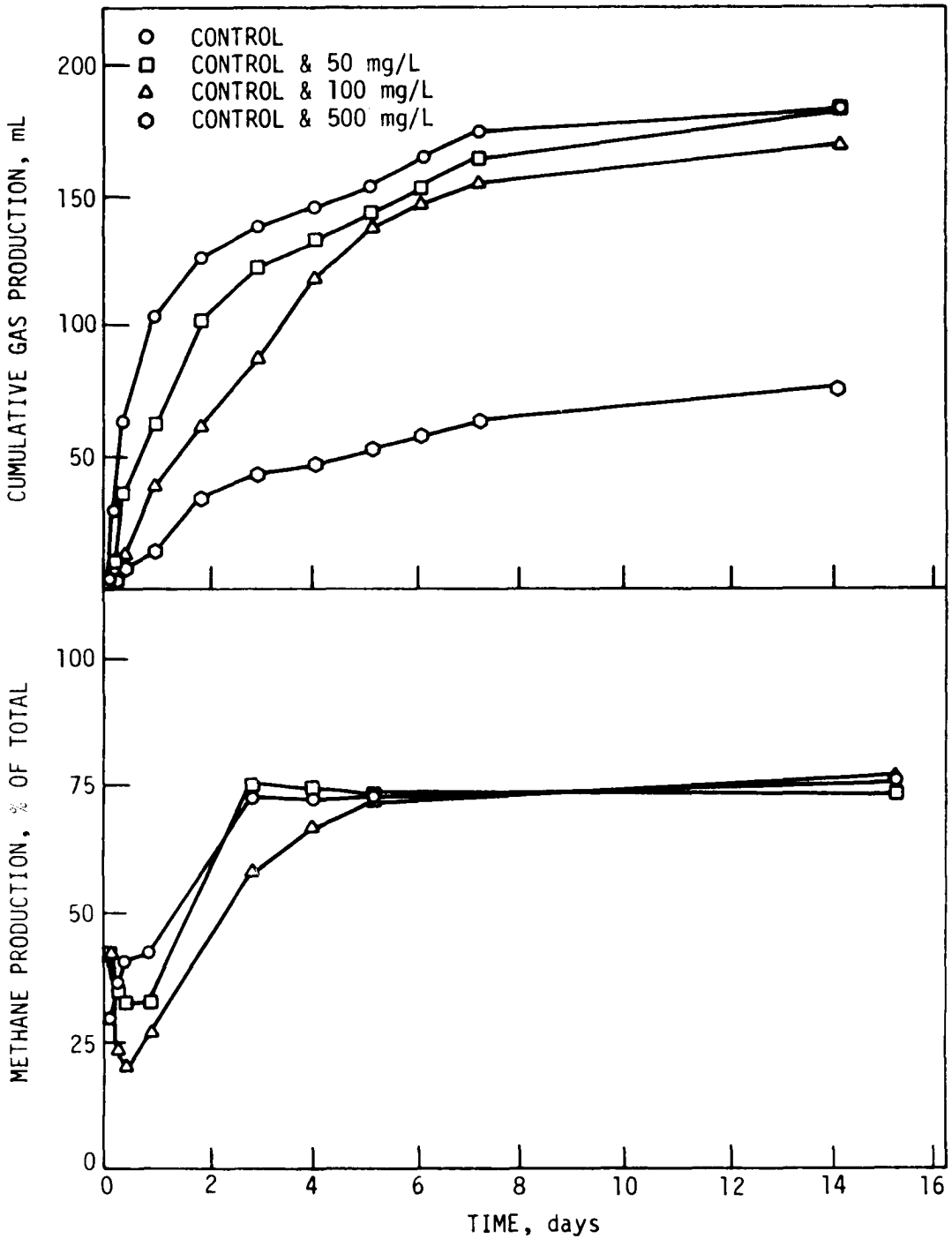

Figure 19. Total gas production and methane production versus time for hexachlorocyclopentadiene during Phase II testing

Samples of the gas yielded a maximum hexachlorocyclopentadiene concentration of 4×10^{-4} ppm at the beginning of the test which dropped to 5×10^{-5} ppm at the termination of the run. These data were collected from the cultures dosed with 100 mg/L. The volatility of this compound was therefore not great enough to cause significant removal under the conditions of this study.

Concentrations of the volatile acids are plotted against time in Figures 20a and b. The concentration of normal valeric acid was less that 1 mg/L in all of the samples and therefore is not shown. Acetic, propionic and normal butyric acids followed similar patterns with an initial accumulation due to inhibition of methane production followed by a gradual disappearance with the return of active gas production. The rate of disappearance was less in the cultures dosed with 100 mg/L, especially that of propionic acid. This would indicate that the microorganisms responsible for propionate utilization were sensitive to this concentration of hexachlorocyclopentadiene. The sensitivity of these microorganisms to foreign materials has been observed by other researchers (Sykes and Kirsch, 1972; McCarty and McKinney, 1961b).

Iso-butyric and iso-valeric acids in cultures dosed with 50 mg/L followed the patterns of the other volatile acids at this concentration of hexachlorocyclopentadiene. Accumulation of iso-butyric and iso-valeric acids in cultures dosed with 100 mg/L showed a maximum concentration at approximately 70 hours. These accumulations occurred during a period when gas production was gradually increasing and the other

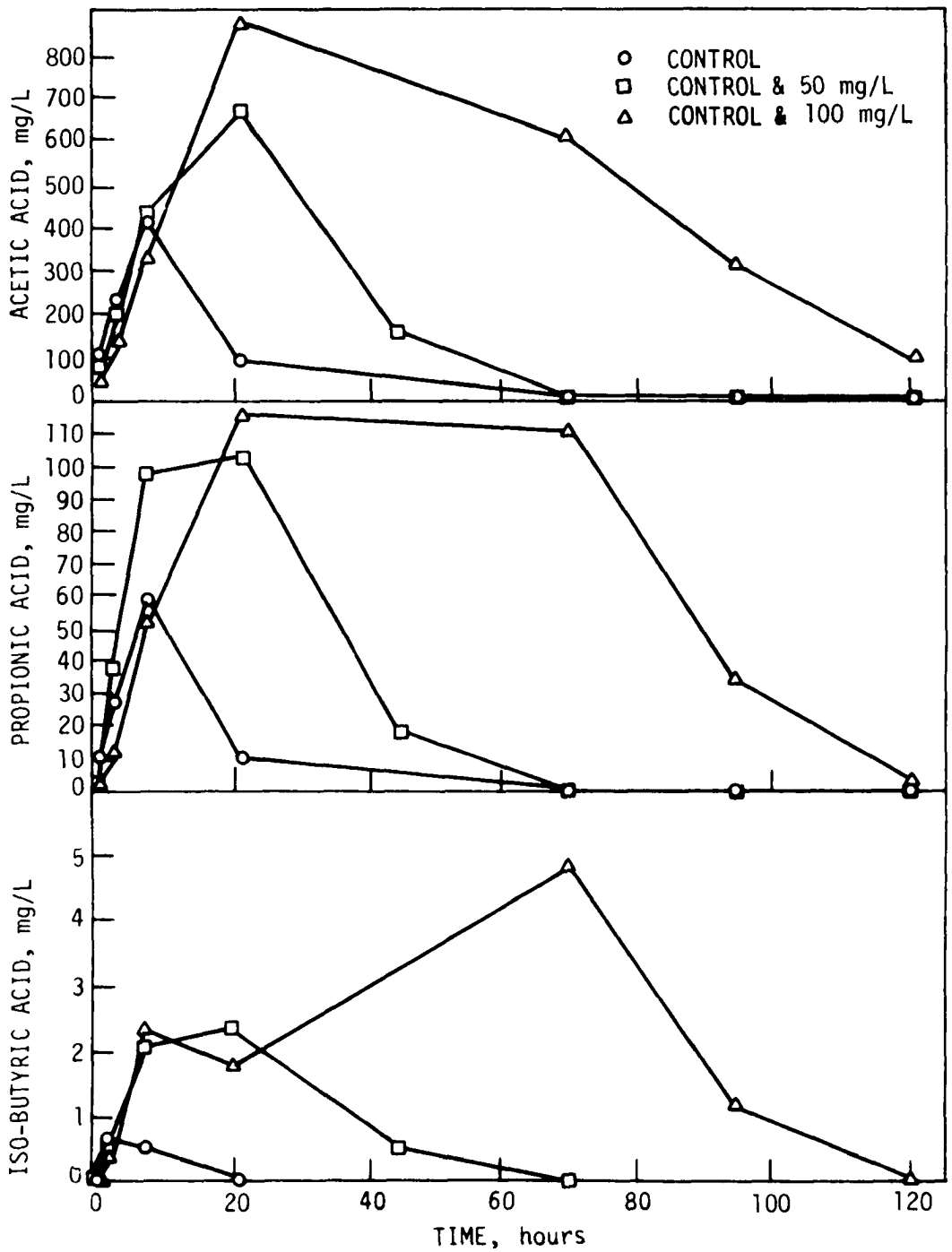

Figure 20a. Concentrations of acetic acid, propionic acid and iso-butyric acid versus time for hexachlorocyclopentadiene during Phase II testing

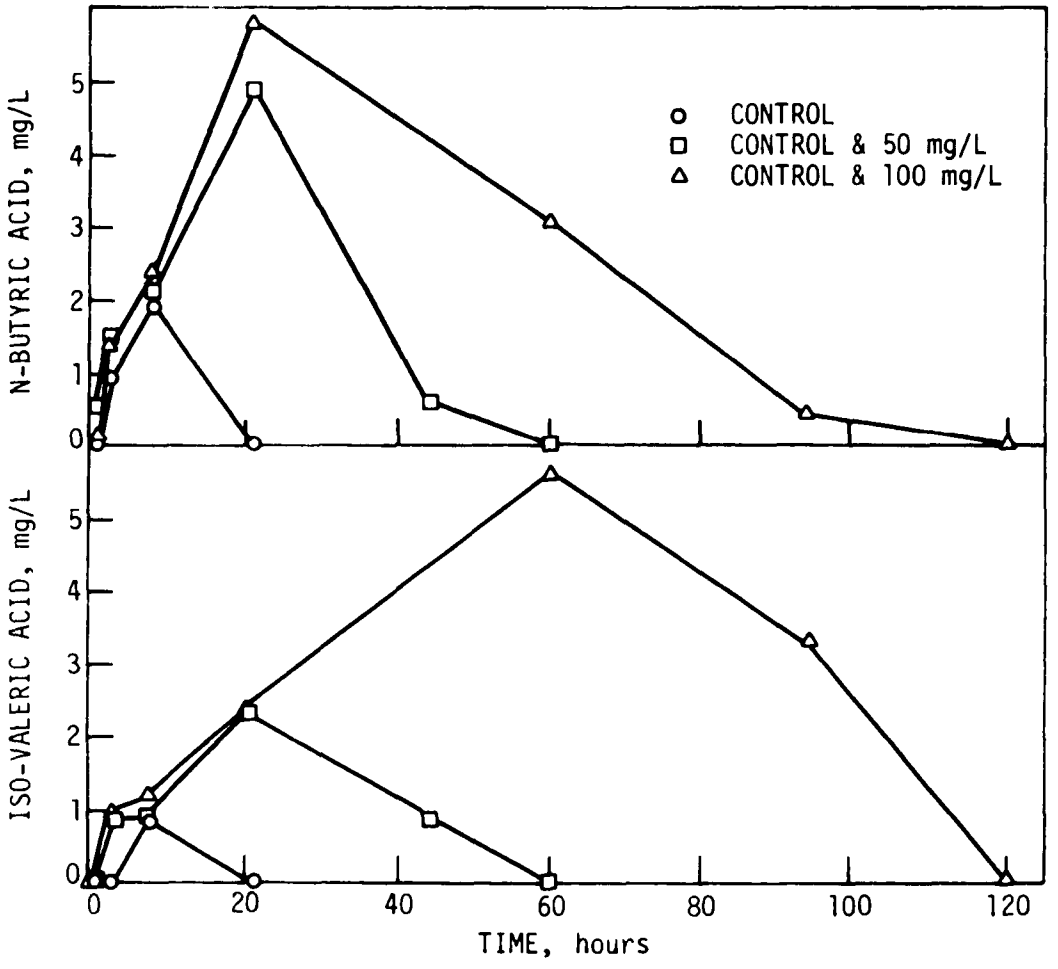

Figure 20b. Concentrations of n-butyric acid and iso-valeric acid versus time for hexachlorocyclopentadiene during Phase II testing

volatile acids were gradually disappearing. Occurrence of the delayed peak concentrations for these two acids could suggest that the formation of iso-butyric and iso-valeric acids was triggered by high concentrations of acetic and propionic acids. Sykes and Kirsch (1972) reported the formation of valeric acid from acetate and propionate in anaerobic cultures inhibited by carbon tetrachloride which would support the observations in this study. Bryant (1979) also reported that increased concentrations of the higher acids occurred during inhibition of methanogenesis, but little data are available specifically on the formation of the isomeric forms of butyric acid and valeric acids.

4-Nitrophenol Evaluation of inhibition from 4-nitrophenol was conducted at concentrations of 50, 100 and 500 mg/L. Total gas production and methane production data are presented in Figure 21. As in Phase I, initial inhibition was evident followed by a period of recovery. A significant lag in gas production was present in cultures dosed with 500 mg/L. This lag period lasted slightly more than one day followed by a period of active gas production. The rate of gas production following the lag period at this concentration was much lower than that of the control.

Methane production followed a pattern similar to that for total gas production. Very little methane production occurred initially at either the 100 or 500 mg/L dosages.

Data on the soluble concentration of 4-nitrophenol are shown in Figure 22. Complete disappearance of 4-nitrophenol in solution took

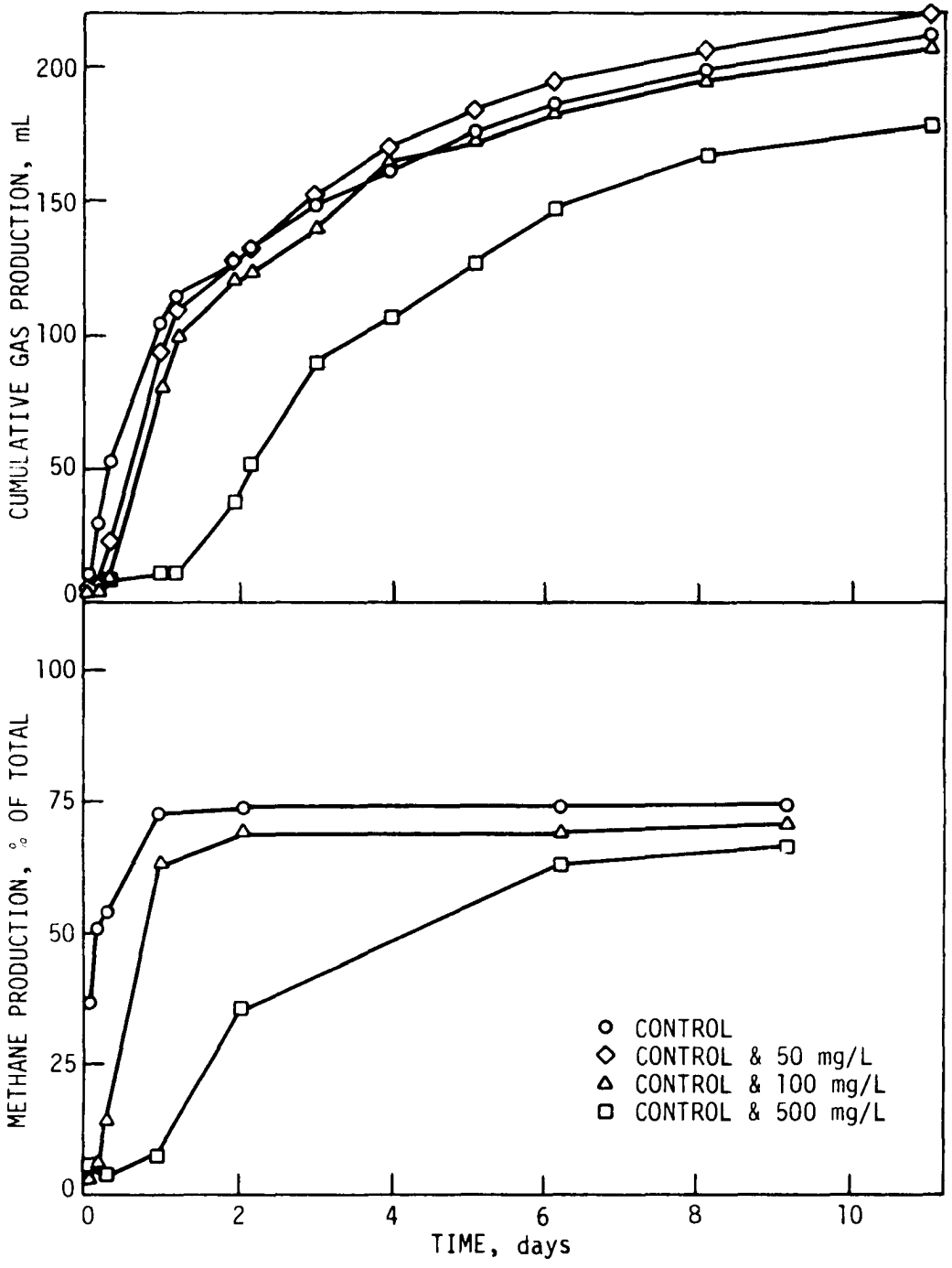

Figure 21. Total gas production and methane production for 4-nitrophenol during Phase II testing

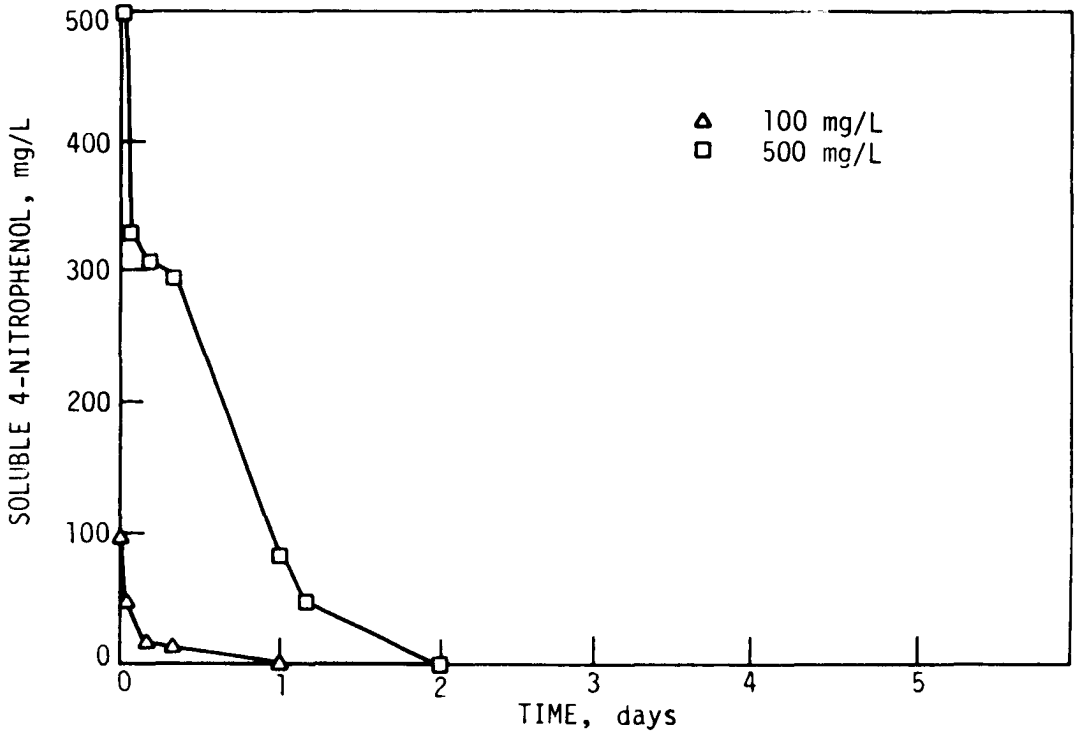

Figure 22. Soluble concentration of 4-nitrophenol versus time during Phase II testing

approximately 24 hours at a dosage of 100 mg/l and 48 hours at 500 mg/l. After these times, no 4-nitrophenol was detected in any of the samples.

Based on the results of Cartwright and Cain (1959), it was likely that reduction of the nitro group of 4-nitrophenol to an amine group could have occurred. Volatile acid data substantiate this removal mechanism because maximum concentrations of acetic and propionic acids occurred later than in the controls (Figure 23). Since the biological reduction of the nitro group would proceed without the production of volatile acids it was

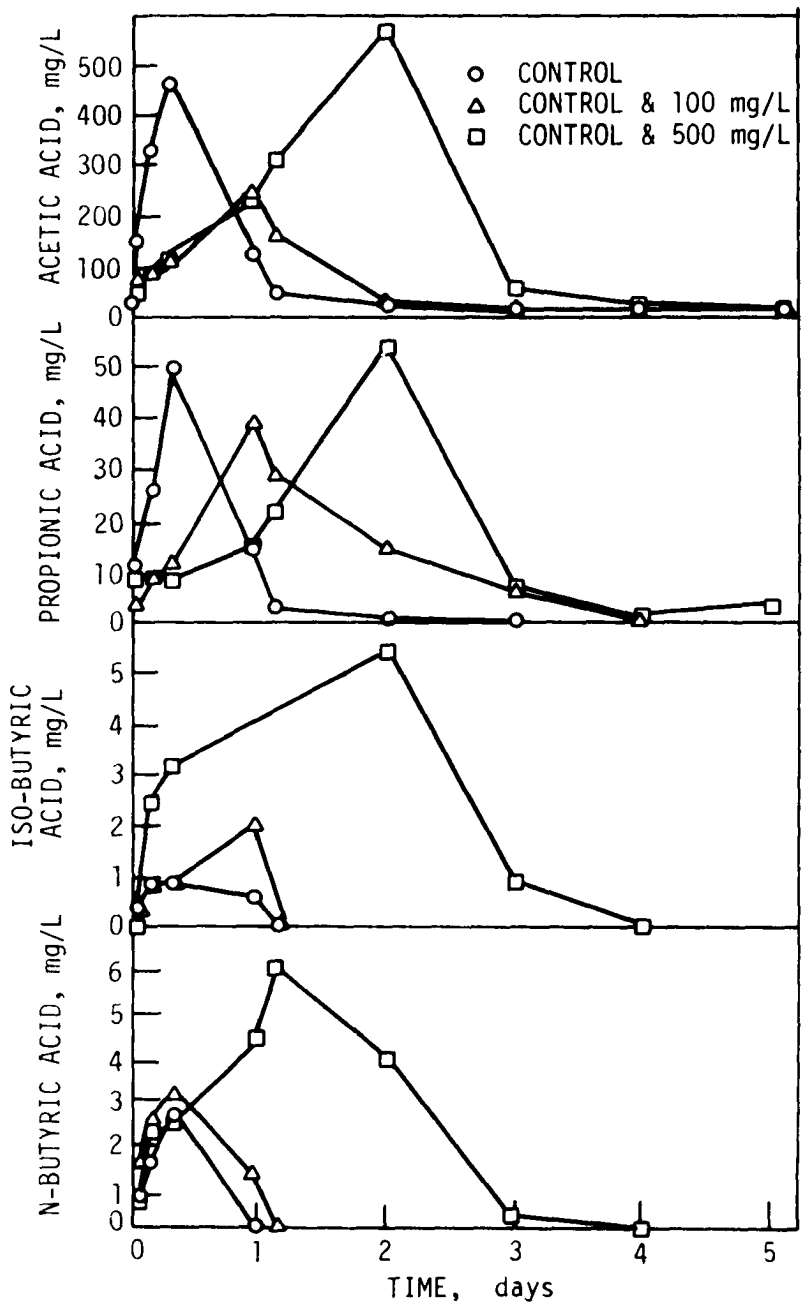

Figure 23. Concentrations of acetic, propionic, iso-butyric and n-butyric acids versus time for Phase II testing of 4-nitrophenol

likely that the use of the nitro group of 4-nitrophenol as an alternate electron acceptor allowed the utilization of the ethanol substrate without the need for fermentation reactions.

Active gas production in the spiked cultures corresponded to the disappearance of 4-nitrophenol. The lag period represented the time for removal of this compound. The slow rate of recovery experienced in the cultures dosed with 500 mg/L of 4-nitrophenol could have been due to a buildup of a slightly inhibitory degradation product.

Nitrobenzene Analysis of inhibition by nitrobenzene was performed at concentrations of 100, 500 and 1000 mg/L. Complete analyses were performed on the samples dosed with 100 and 500 mg/L. The dosage of 1000 mg/L was used in an attempt to induce irreversible inhibition. However, gas production data presented in Figure 24 show that recovery occurred in all spiked cultures. The lag period in cultures dosed with 500 and 1000 mg/L had lag periods of approximately one and two days, respectively. Problems with one of the gas chromatographs prevented the determination of product gas quality.

Analysis of the liquid samples provided evidence of a degradation product of nitrobenzene. The concentration of nitrobenzene steadily decreased with time. Disappearance of the nitrobenzene peak during gas chromatographic analysis coincided with the appearance of an earlier eluting peak. The compound causing this peak was identified as aniline by injecting various solutions of organic compounds into the gas chromatograph and matching retention times.

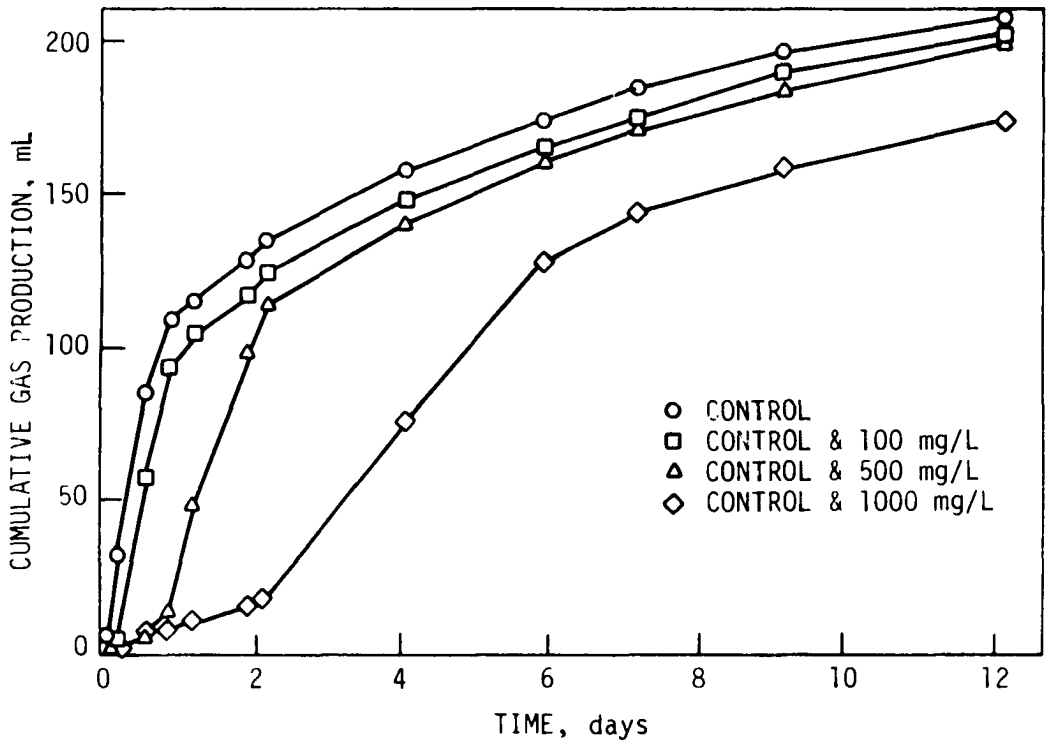

Figure 24. Total gas production versus time during Phase II testing of nitrobenzene

Nitrobenzene and aniline concentrations are shown with the gas production data in Figures 25 and 26. It should be noted that the gas production in the spiked cultures began actively only when the soluble concentration of nitrobenzene was significantly reduced. The rate of gas production in the spiked culture closely paralleled that of the control after active gas production had begun. This indicated that the concentration of aniline present in the spiked cultures was not significantly inhibitory. Chou, et al., (1978) reported that aniline was significantly less toxic than nitrobenzene.

If it is assumed that one mole of nitrobenzene would produce one mole of aniline, 100 mg/L of nitrobenzene should have resulted in 75.6 mg/L of aniline and 500 mg/L of nitrobenzene should have produced 378 mg/L of aniline. The maximum concentrations of aniline detected were 65 and 366 mg/L, respectively. This is approximately a stoichiometric conversion based on a mole-to-mole conversion ratio, considering that some of the aniline may have been adsorbed onto culture solids. A mole-to-mole conversion for para-nitrobenzoic acid to para-aminobenzoic acid was reported by Cartwright and Cain (1959).

Volatile acid data seem to support the conversion of nitrobenzene to aniline (Figure 27). Reduction of the nitro group would not result from fermentation processes. Therefore, this reaction would not result in the formation of volatile acids. The rate of formation of acetic and propionic acids in the cultures dosed with 500 mg/L of nitrobenzene was initially much slower than that of the controls. Active production

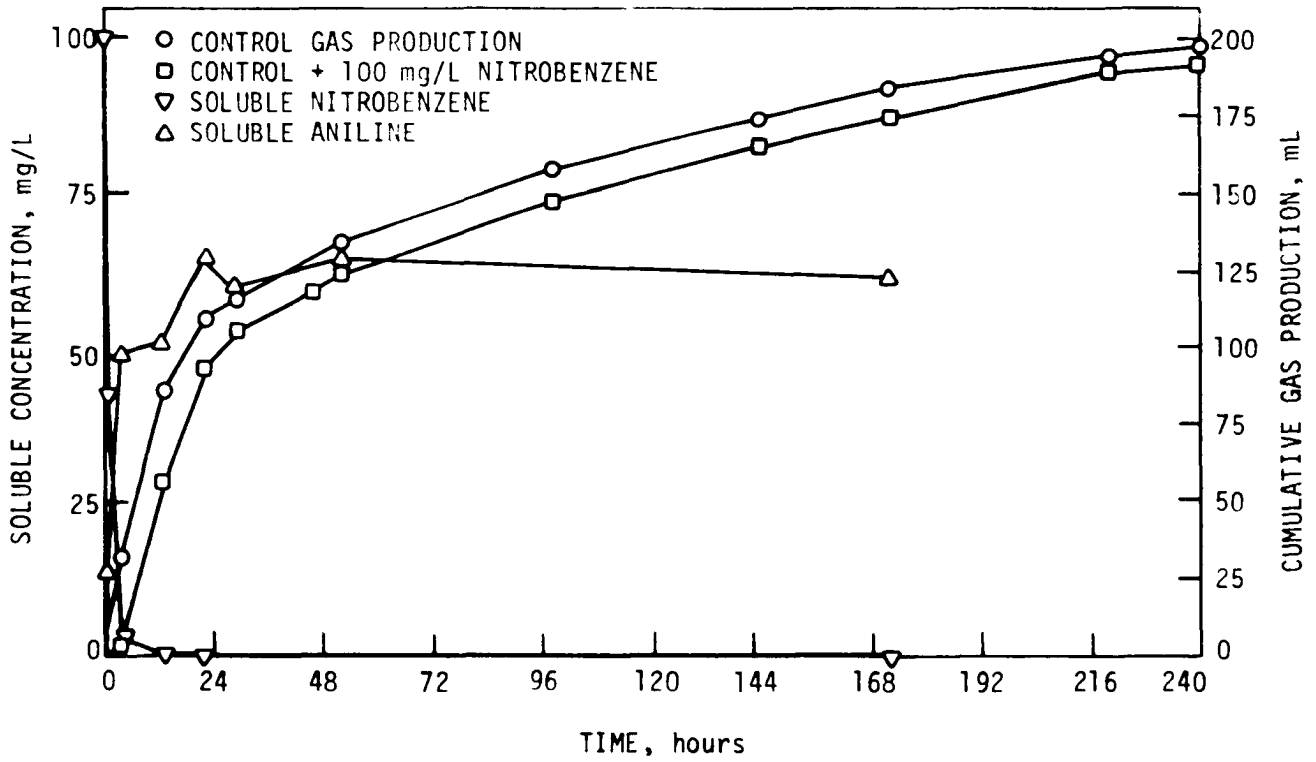

Figure 25. Total gas production and soluble concentrations of nitrobenzene and aniline versus time for 100 mg/L of nitrobenzene

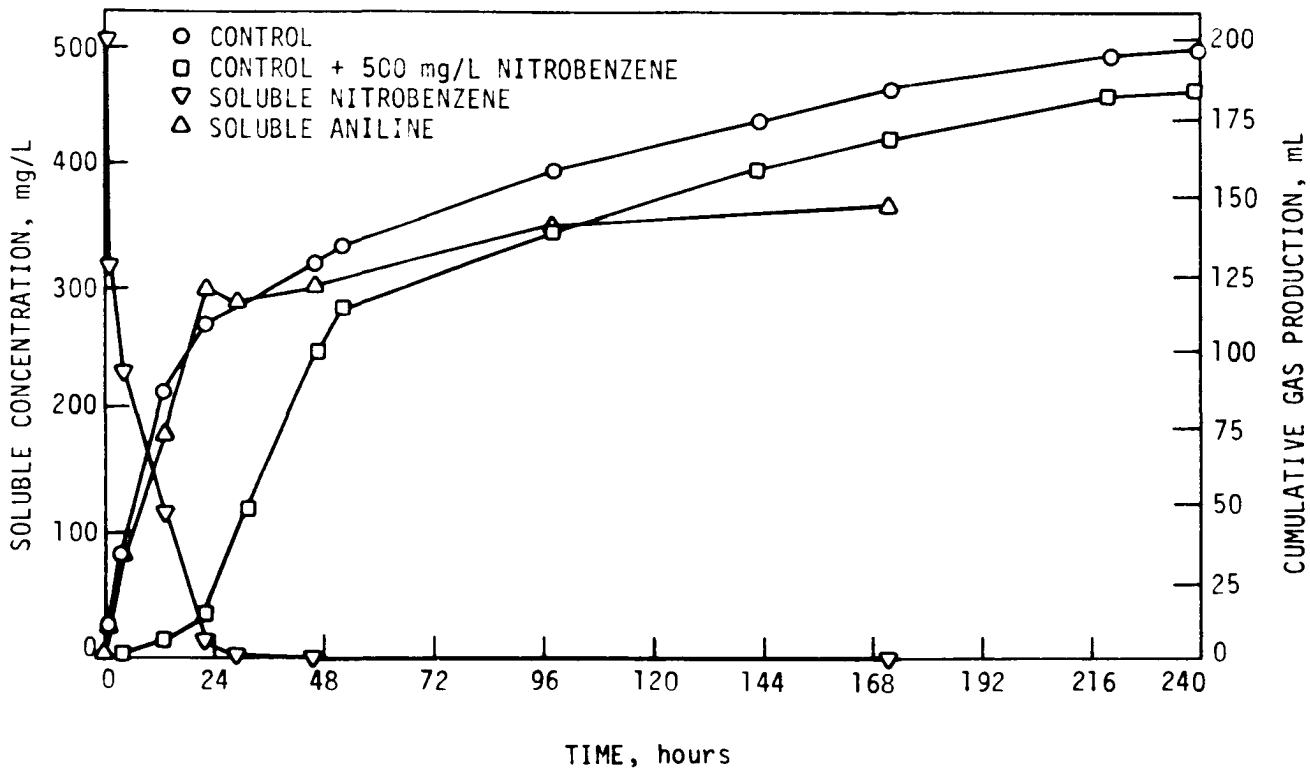

Figure 26. Total gas production and soluble concentrations of nitrobenzene and aniline versus time for nitrobenzene dosage of 500 mg/L

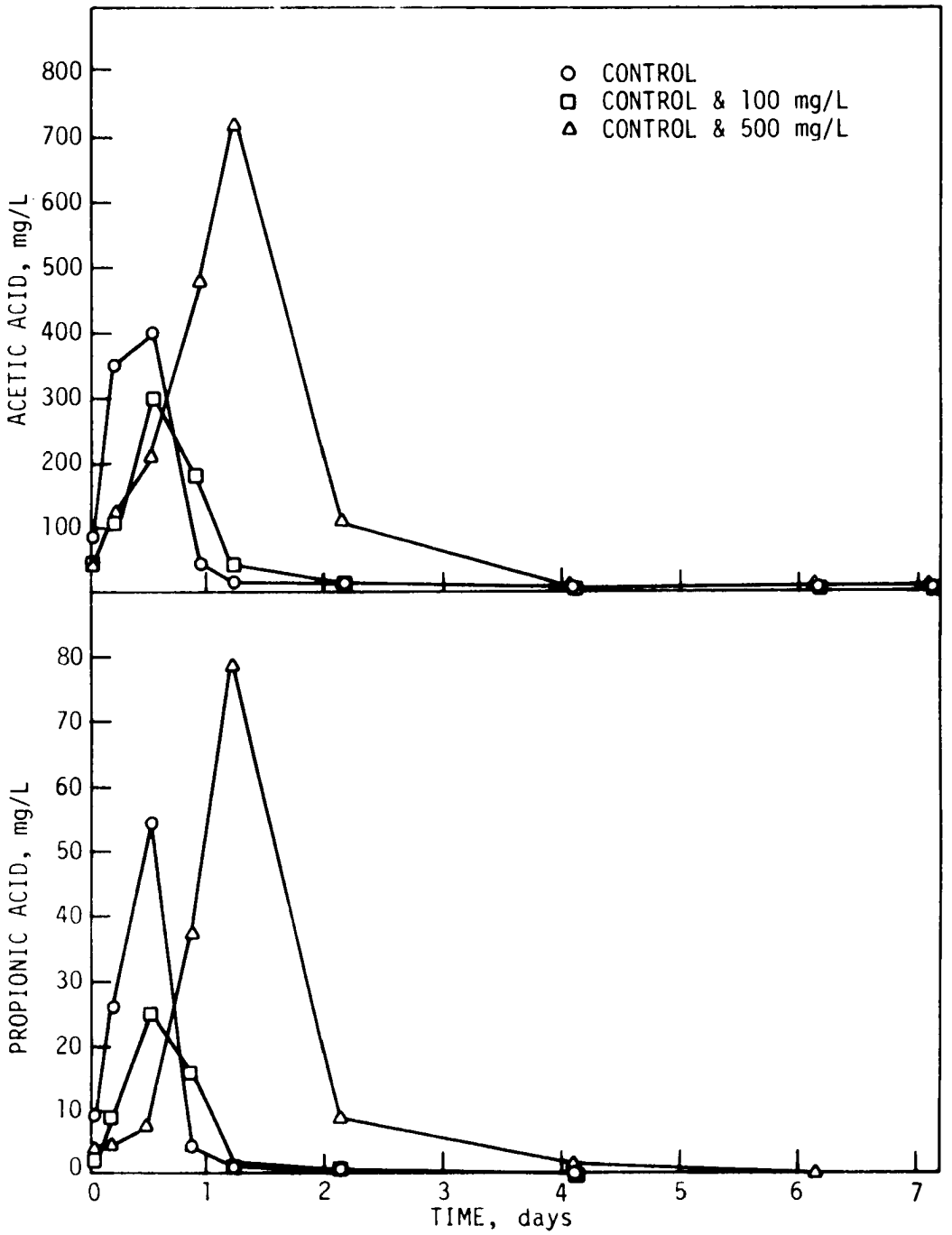

Figure 27. Concentrations of acetic and propionic acids during Phase II testing of nitrobenzene

corresponded to the disappearance of nitrobenzene, i.e., the reduction of the nitro group. This occurrence was also noted in the cultures dosed with 100 mg/L of nitrobenzene. While there was essentially no gas production during the initial four hours in these spiked cultures, the volatile acid levels were less than those in the control. The absence of gas production should have resulted in the rapid accumulation of acetic and propionic acids.

2,4-Dichlorophenol Analysis of inhibition from 2,4-dichlorophenol (2,4-DCP) was conducted at concentrations of 100 and 500 mg/L. Gas production in cultures dosed with 100 mg/L of 2,4-DCP initially was very close to that of the control (Figure 28). However as time progressed, the difference in gas production between the control and the spiked cultures increased. This difference is not apparent in the methane production data (Figure 29). Methane production equalled that of the control after 43 hours and remained at that level throughout the test run. The difference in gas production was probably due to accumulation of propionic acid in the spiked cultures (Figure 30a). Initial formation of propionic acid in the controls and spiked culture was essentially the same. However, propionic acid gradually accumulated in the cultures dosed with 100 mg/L of 2,4-dichlorophenol. Inhibition of the organisms responsible for propionic acid utilization was evident.

Gas production in the cultures dosed with 500 mg/L of 2,4-dichlorophenol indicated very little activity (Figure 31). After 71 hours, there was essentially no gas production. Before that time, there

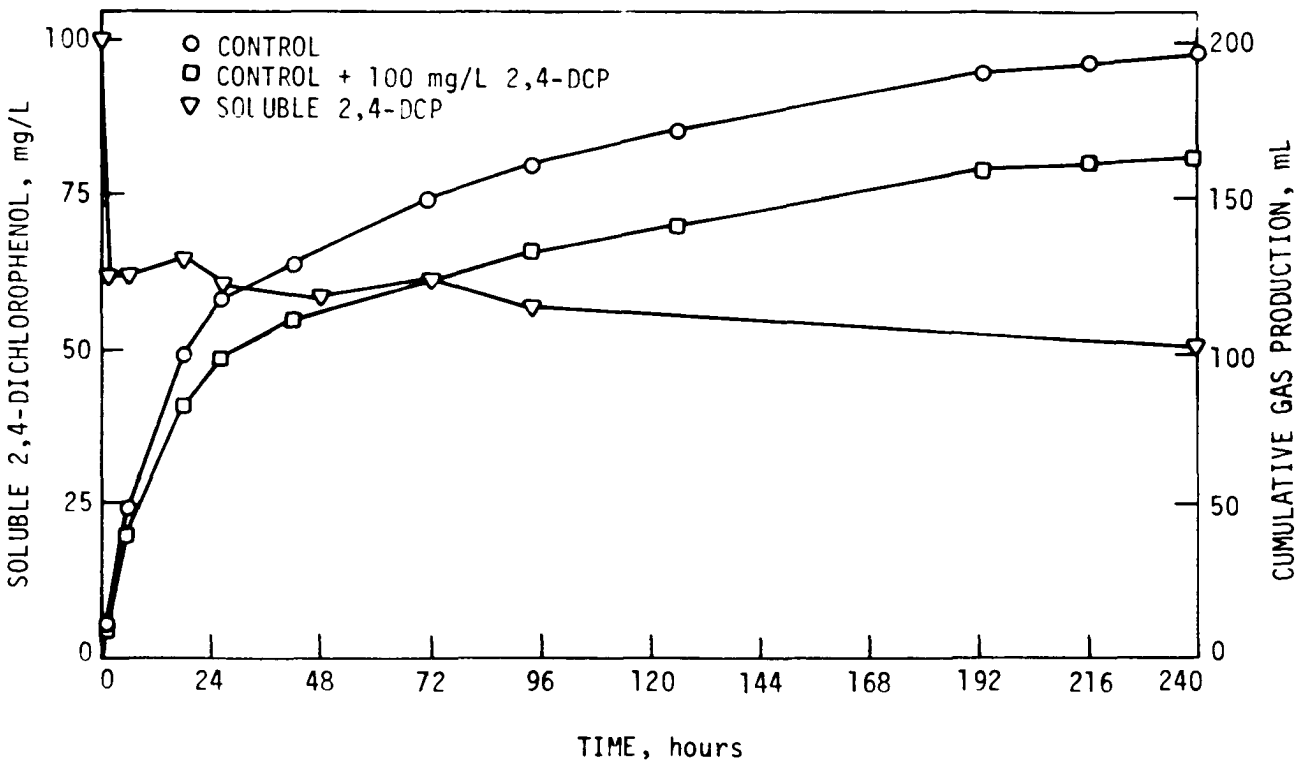

Figure 28. Total gas production and soluble concentration of 2,4-dichlorophenol (2,4 DCP) versus time for Phase II testing of 2,4-dichlorophenol at a dosage of 100 mg/L

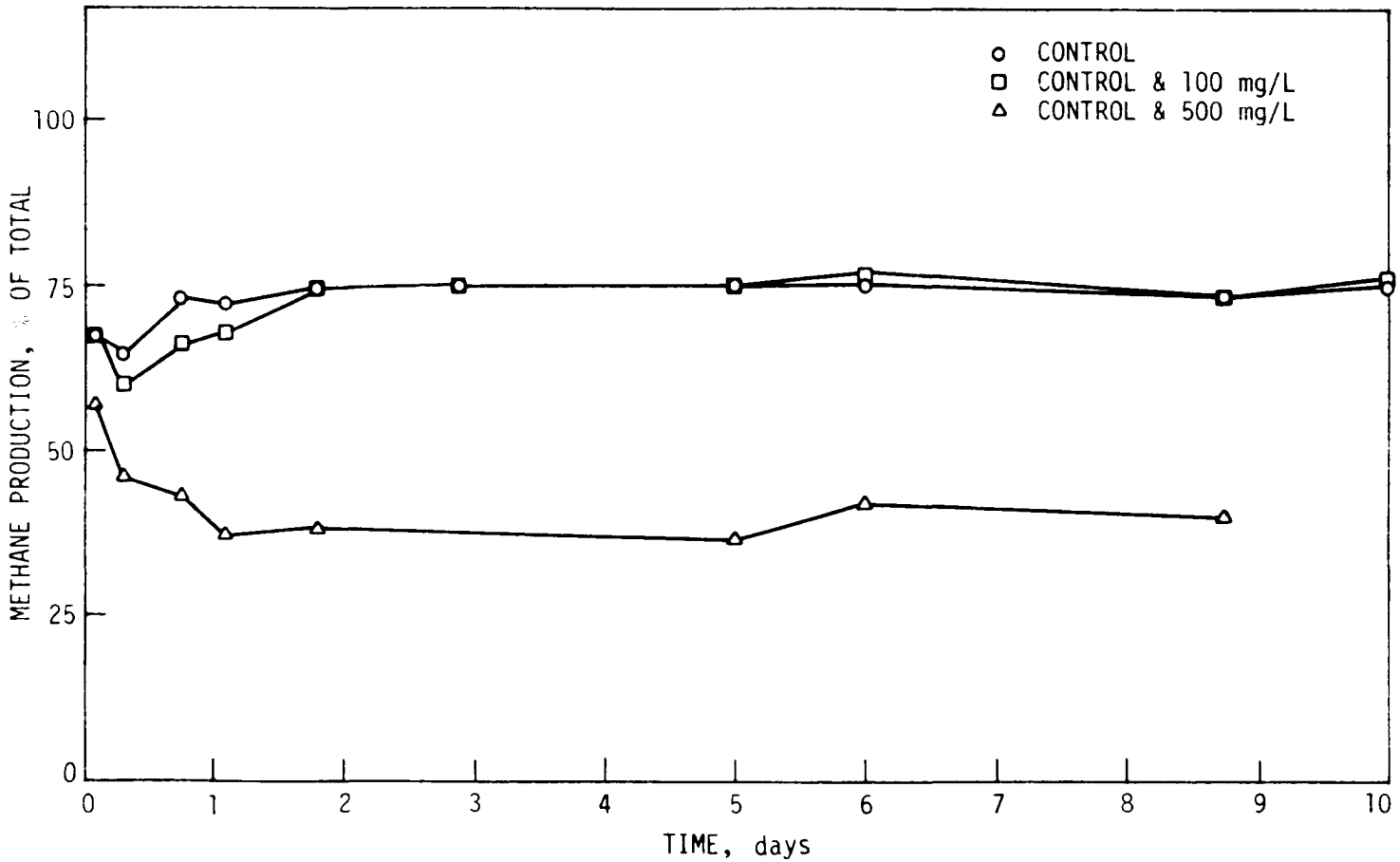

Figure 29. Methane production versus time for Phase II testing of 2,4-dichlorophenol

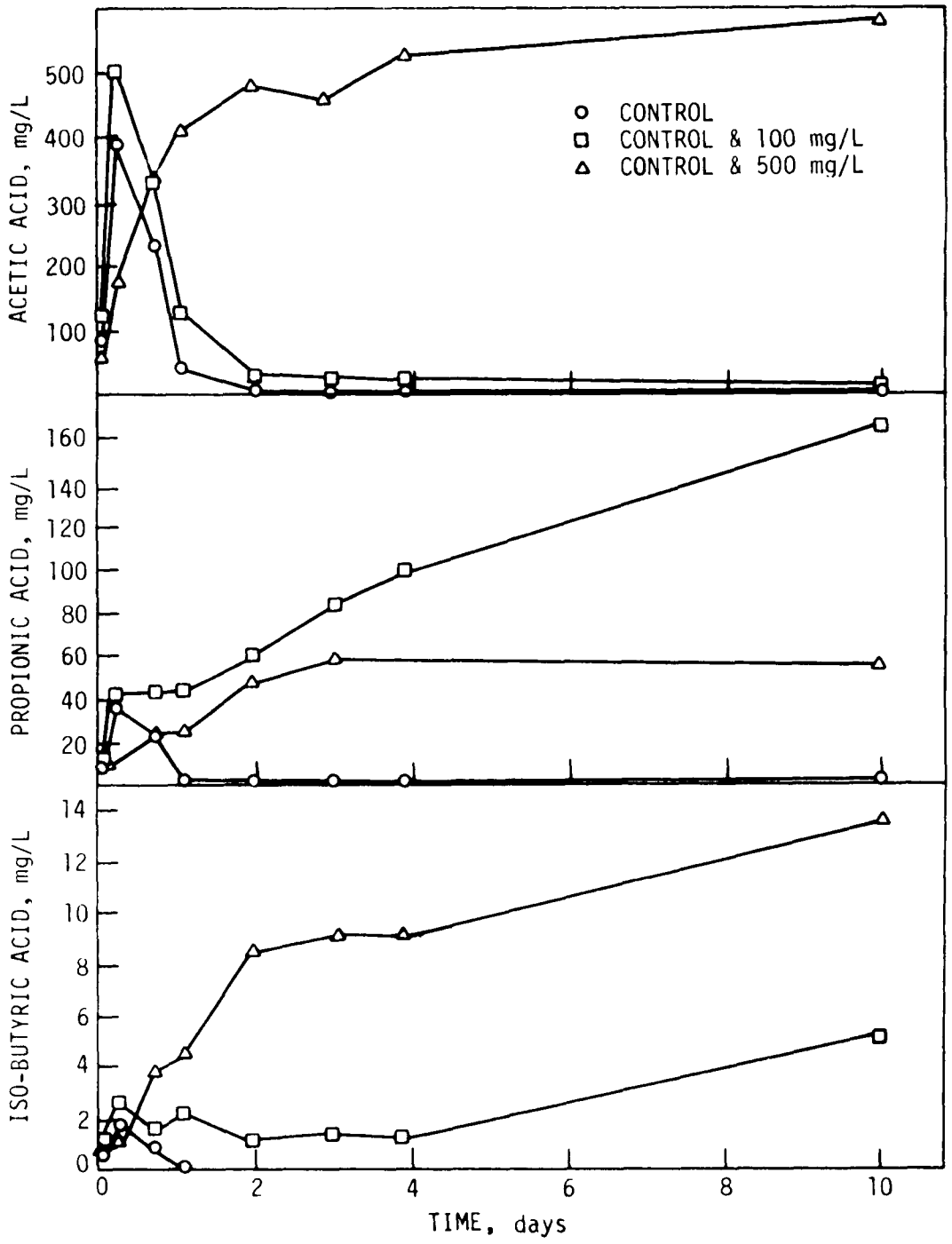

Figure 30a. Concentrations of acetic, propionic and iso-butyric acids versus time during Phase II testing of 2,4-dichlorophenol

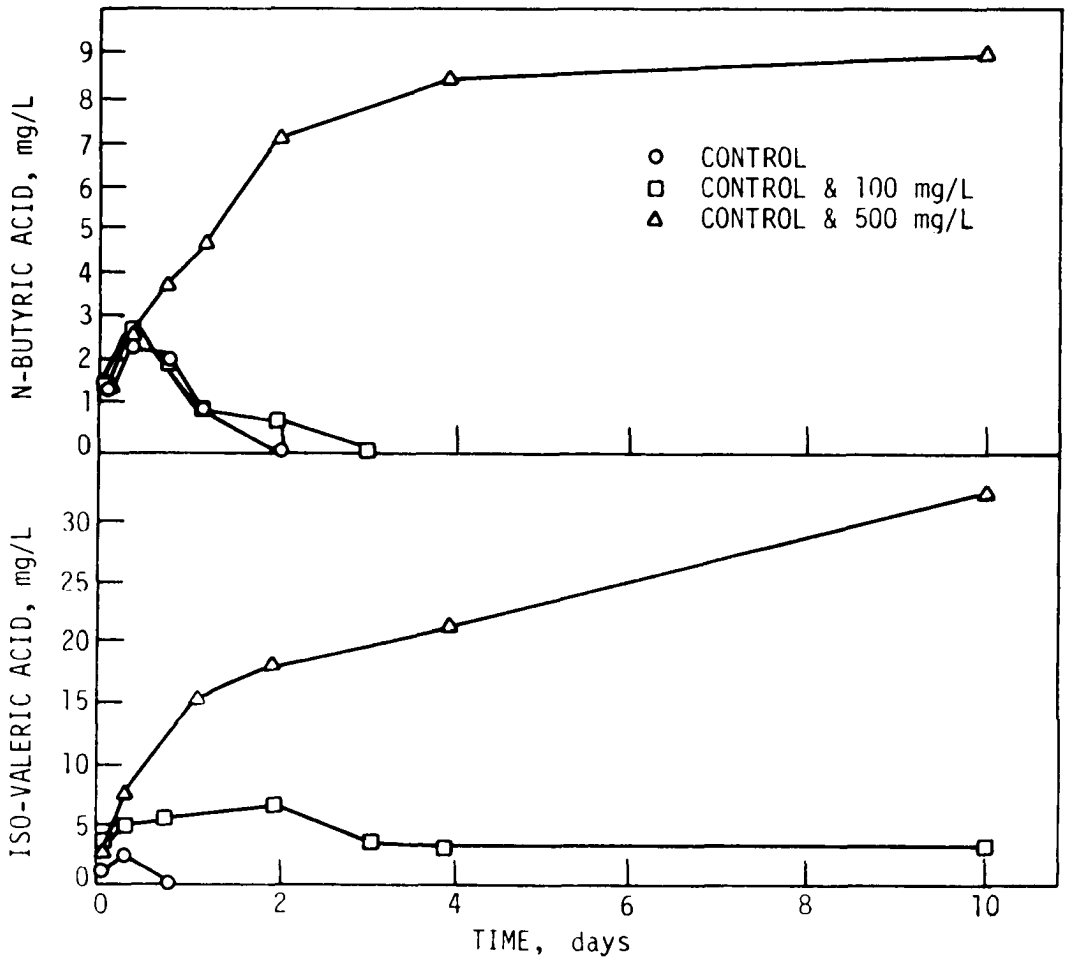

Figure 30b. Concentrations of n-butyric and iso-valeric acids versus time during Phase II testing of 2,4-dichlorophenol

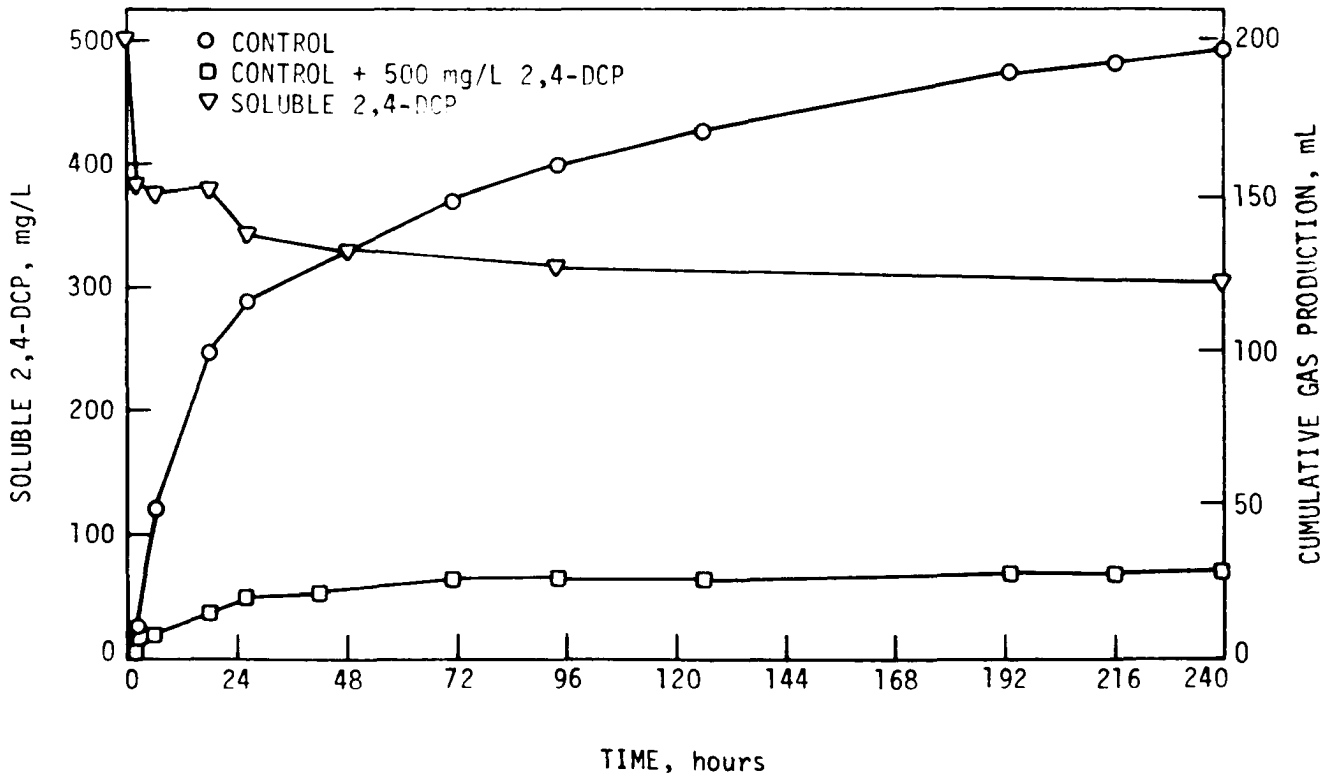

Figure 31. Total gas production and soluble concentration of 2,4-dichlorophenol versus time for a dosage of 500 mg/L of 2,4-dichlorophenol

evidently was some methane production since methane represented approximately 40% of the total gas production. Volatile acids data indicated that formation of acetic and propionic acids was inhibited (Figures 30a and b). Sykes and Kirsch (1972) and Chynoweth and Mah (1971) reported a similar occurrence during inhibition of anaerobic cultures by carbon tetrachloride.

Analysis of the soluble concentrations of 2,4-dichlorophenol indicated a rapid reduction in concentration followed by very little additional removal at both dosages (Figures 28 and 31). This suggests that there was rapid adsorption of this compound and subsequent equilibrium established between the liquid and solids.

2-Nitrophenol Evaluation of the inhibition from 2-nitrophenol was conducted at concentrations of 100 and 500 mg/L. Gas production in spiked cultures at both concentrations was initially suppressed but active gas production occurred within the first day (Figure 32). Methane production in cultures spiked with 100 mg/L of 2-nitrophenol was almost equal to that of the controls throughout the test period. Cultures dosed with 500 mg/L showed much less methane production initially, but methane production approached that of the control toward the end of the test period.

Analysis of 2-nitrophenol in the samples showed a rapid disappearance of this compound at both concentrations (Figures 33 and 34). No decomposition peaks were apparent during gas chromatography analysis. However, the acidified samples had a reddish color while 2-nitrophenol

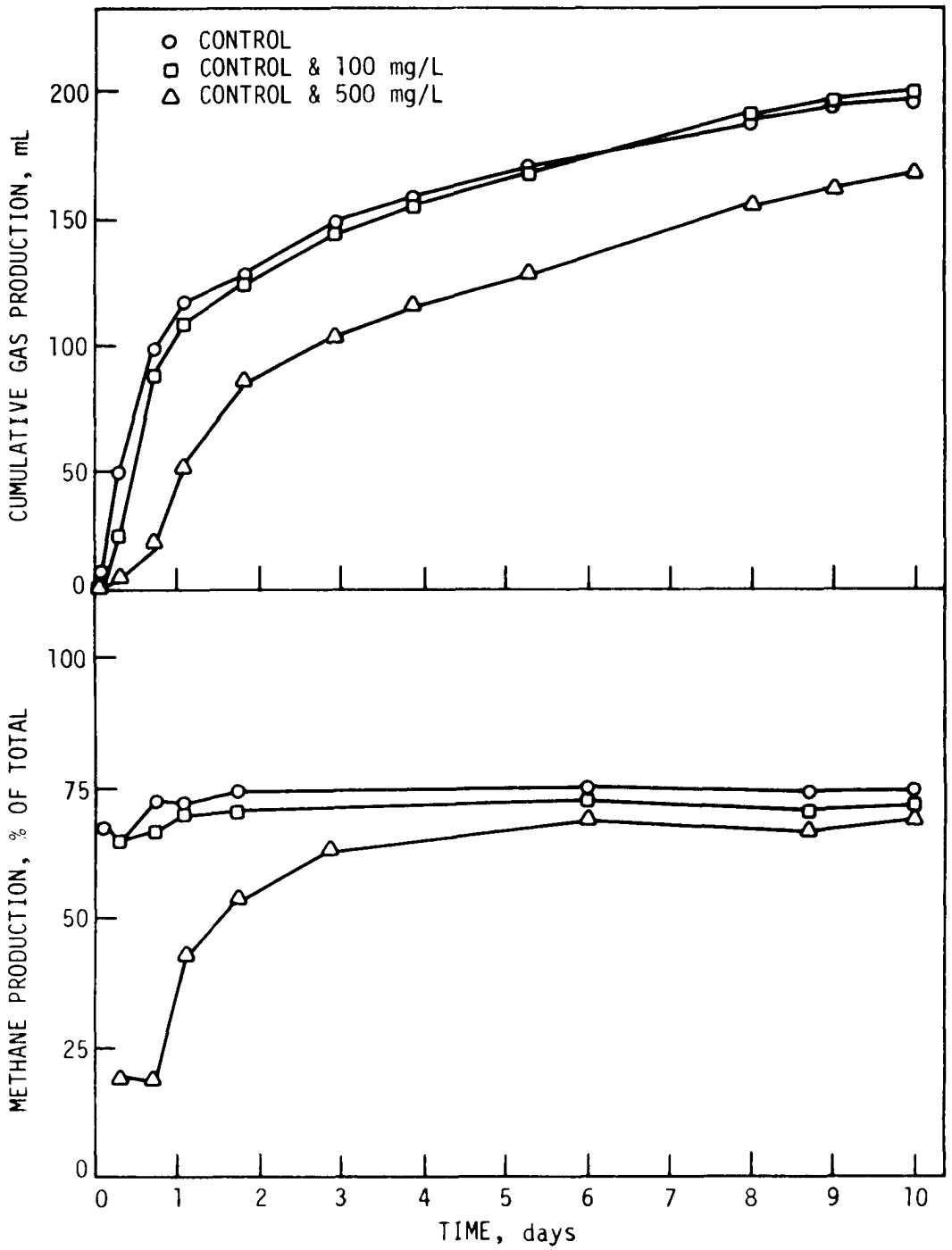

Figure 32. Total gas production and methane production versus time during Phase II testing of 2-nitrophenol

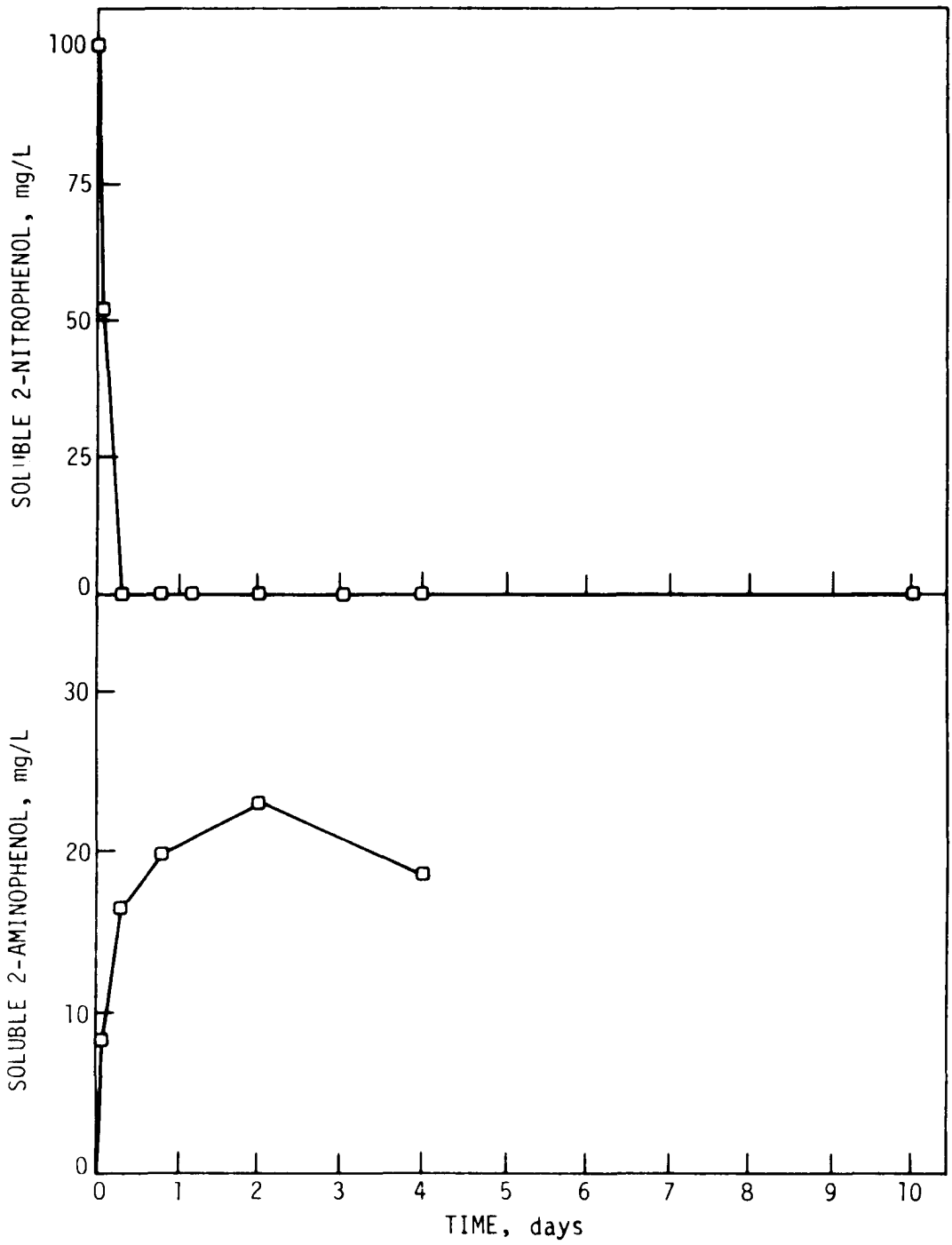

Figure 33. Soluble concentrations of 2-aminophenol and 2-nitrophenol versus time at a dosage of 100 mg/L of 2-nitrophenol

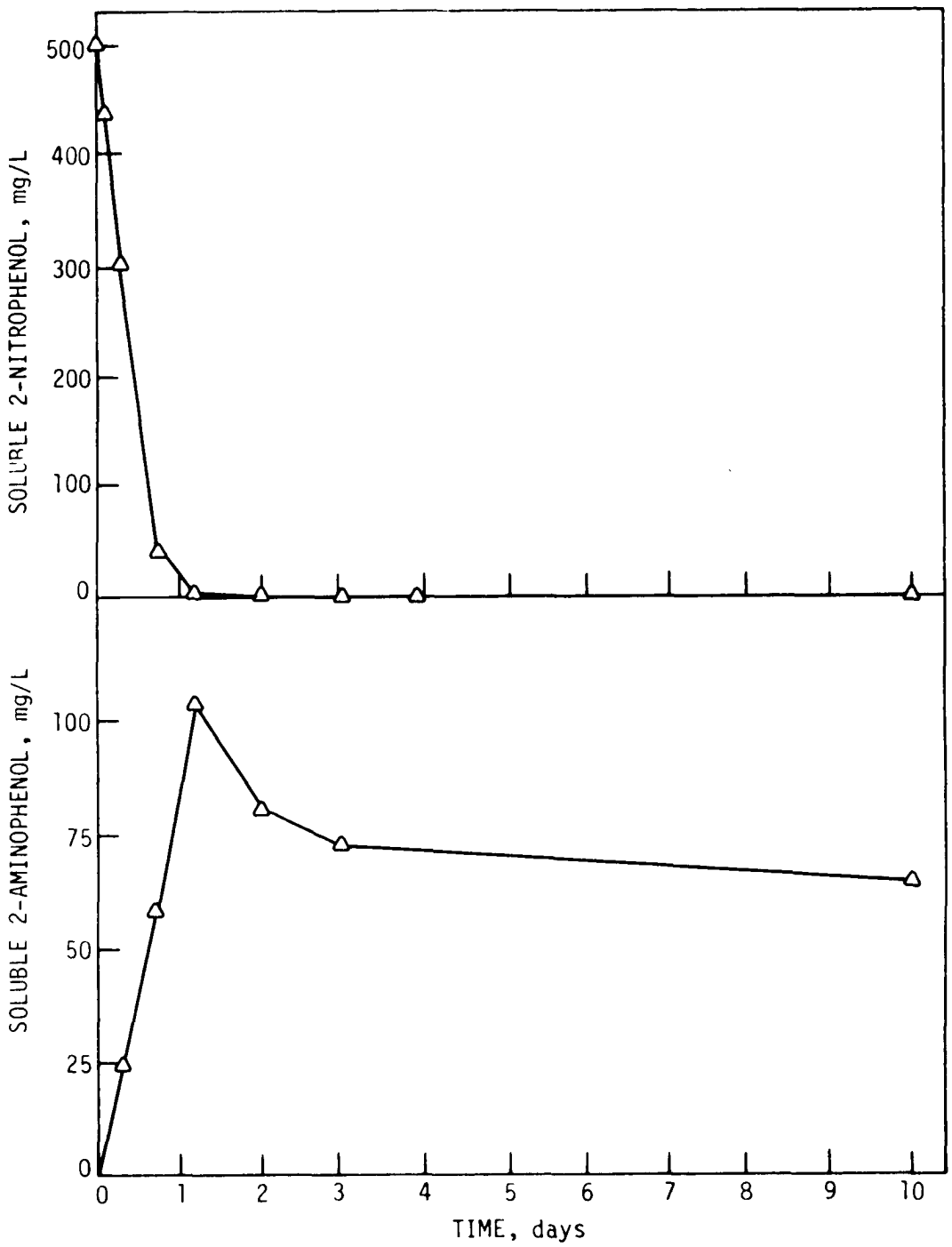

Figure 34. Soluble concentrations of 2-nitrophenol and 2-aminophenol versus time at a dosage of 500 mg/L of 2-nitrophenol

is colorless at a pH $\leq$ 2. Based on the results with nitrobenzene, it was possible that the reduction of the nitro group of 2-nitrophenol could have occurred. Standards of 2-aminophenol were prepared and upon acidification the same reddish color occurred. A similar occurrence was noted with 4-nitrophenol.

Approximate concentrations of 2-aminophenol in the samples were determined using spectrophotometric analysis. Samples and standards were analyzed at a wavelength of 535 nm. Resulting data are shown in Figures 33 and 34. The acidified samples were stored at 4^{o} C for several weeks prior to the analysis so it is difficult to determine the reliability of the results. However, formation of 2-aminophenol followed the disappearance of 2-nitrophenol at both concentrations. The observed concentrations of 2-aminophenol were much less than would be expected based on a mole-to-mole conversion of 2-nitrophenol. Apparently, 2-aminophenol was degraded to products other than methane precursors since the gas production was not significantly greater than that of the control. Alexander and Lustigman (1966) reported that 2-aminophenol was rapidly degraded by soil microorganisms. No degradation products were reported.

Only acetic and propionic acids were present at significant concentrations. The patterns of formation and utilization of these acids (Figure 35) in the spiked cultures is similar to the patterns observed in the cultures dosed with nitrobenzene and 4-nitrophenol. However, due to the rapid decomposition of 2-nitrophenol, the time lag of volatile acids formation was much less pronounced.

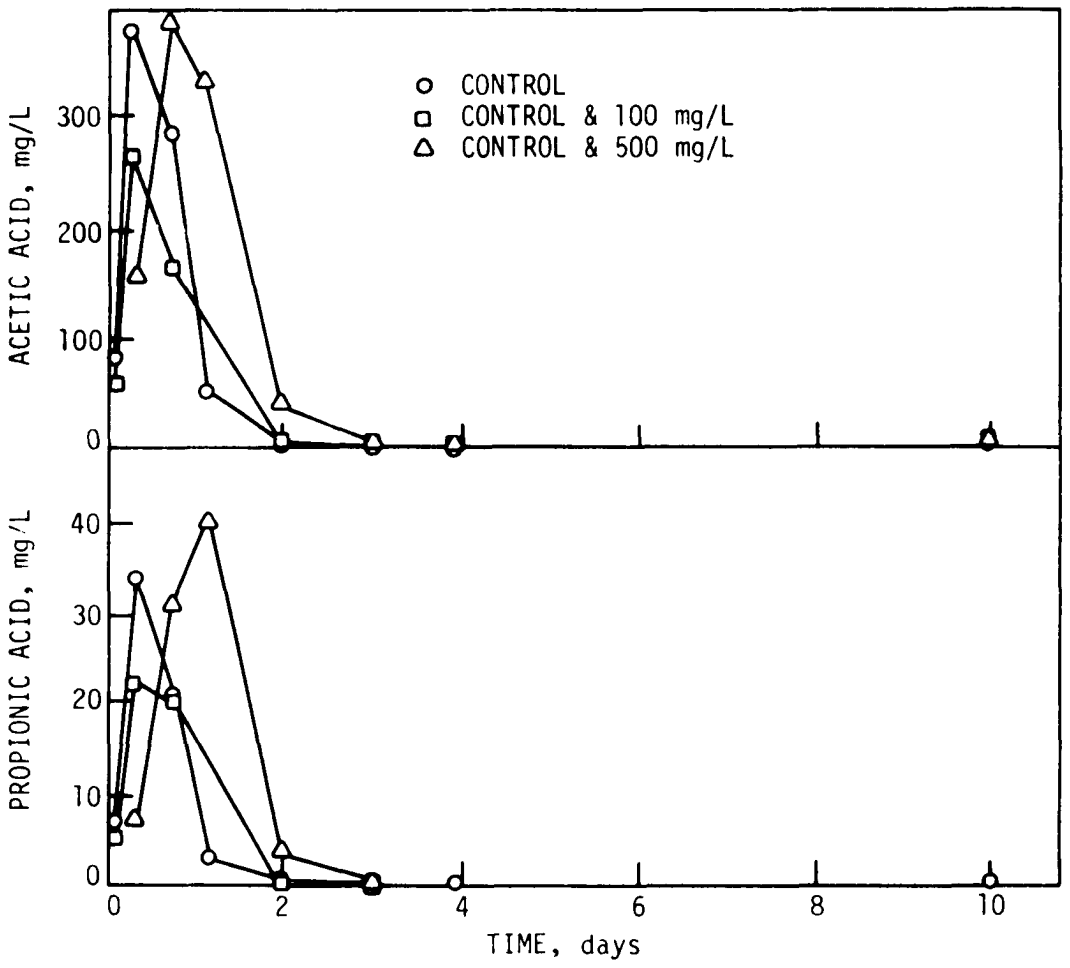

Figure 35. Concentrations of acetic acid and propionic acid versus time during Phase II testing of 2-nitrophenol

Hexachloro-1,3-butadiene The first evaluation of inhibition from hexachloro-1,3-butadiene was performed at concentrations of 50, 100, 500 and 988 mg/L. However, due to a loss of samples, only total gas production and methane production were available. These data are shown in Figure 36.

In all cases, the gas production of the spiked samples initially followed the gas production of the controls. However, the gas production rate in spiked cultures quickly fell off. This is also shown by the methane production for all of the spiked cultures (Figure 36). It is interesting to note that there was very little difference in gas production in the cultures dosed with 500 and 988 mg/L. The increased concentrations above 500 mg/L had little effect on the cultures.

Tests with this chemical were rerun to collect samples for chemical analysis. Only one concentration (100 mg/L) was used during this evaluation. Analysis of the samples for hexachloro-1,3-butadiene indicated a rapid reduction in concentration within the first four hours (Figure 37). After this time, the concentration remained relatively constant at approximately 1 mg/L. Even at this low concentration, the spiked cultures continued to exhibit inhibitory responses. Gas chromatographic analysis did not indicate any decomposition products but again the selectivity of the electron capture detector must be considered.

Volatile acids data are shown in Figures 38a and b. The decrease in acetic and propionic acids at approximately four days was not accompanied by an increase in gas production. However, the higher

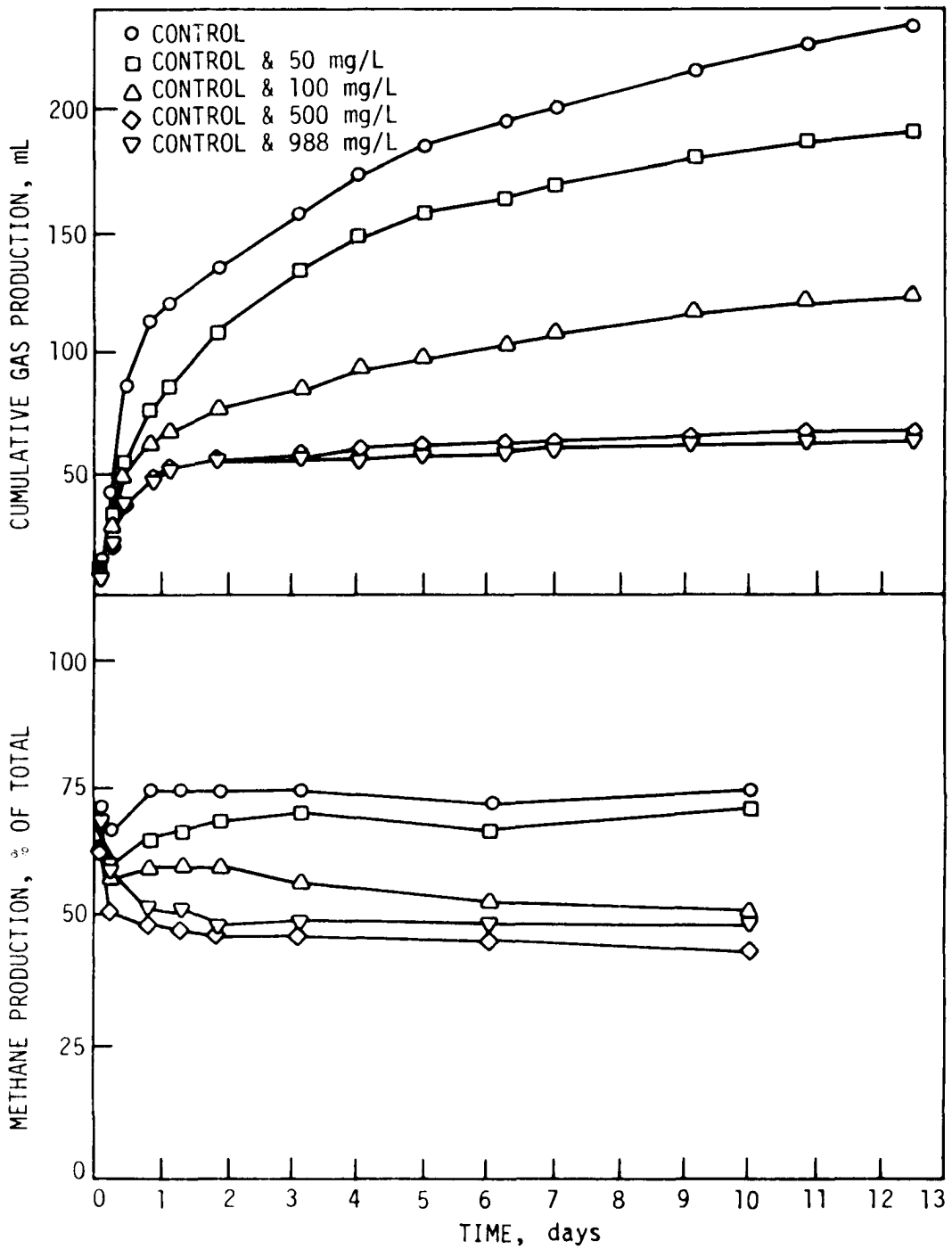

Figure 36. Total gas production and methane production versus time for hexachloro-1,3-butadiene during Phase II testing

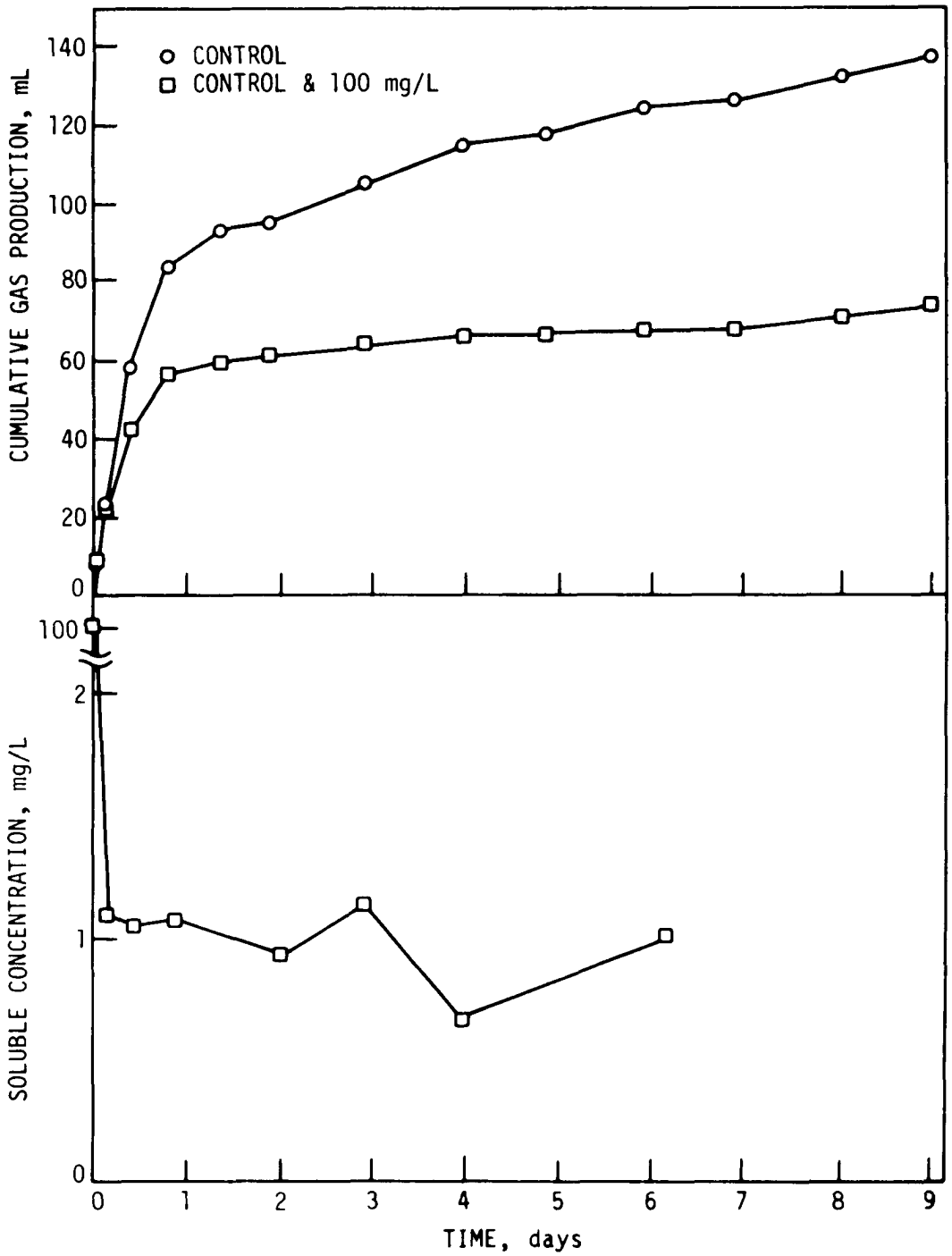

Figure 37. Total gas production and soluble concentration of hexachloro-1,3-butadiene versus time at a dosage of 100 mg/L of hexachloro-1,3-butadiene

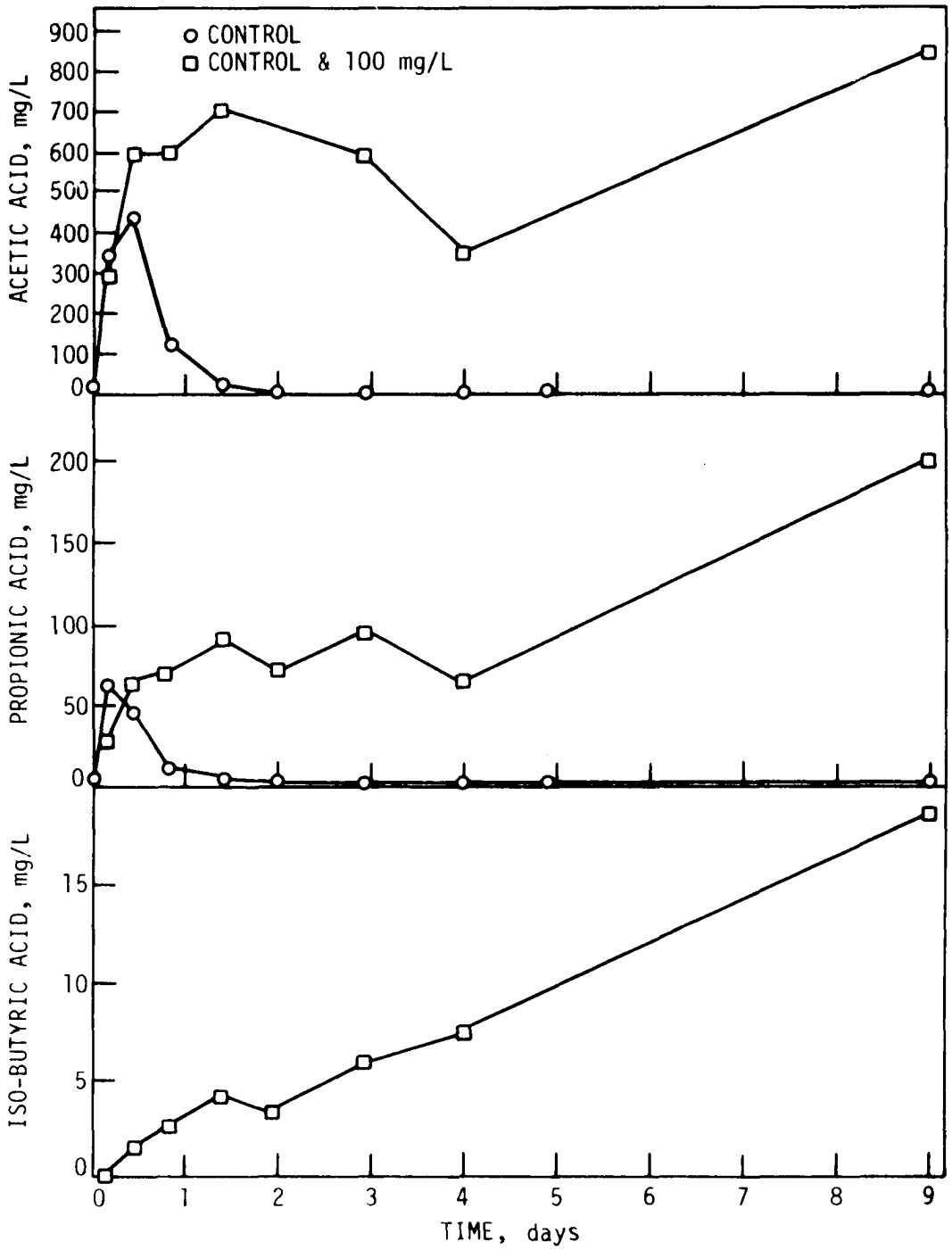

Figure 38a. Concentrations of acetic, propionic and iso-butyric acids versus time at a dosage of 100 mg/L of hexachloro-1, 3-butadiene

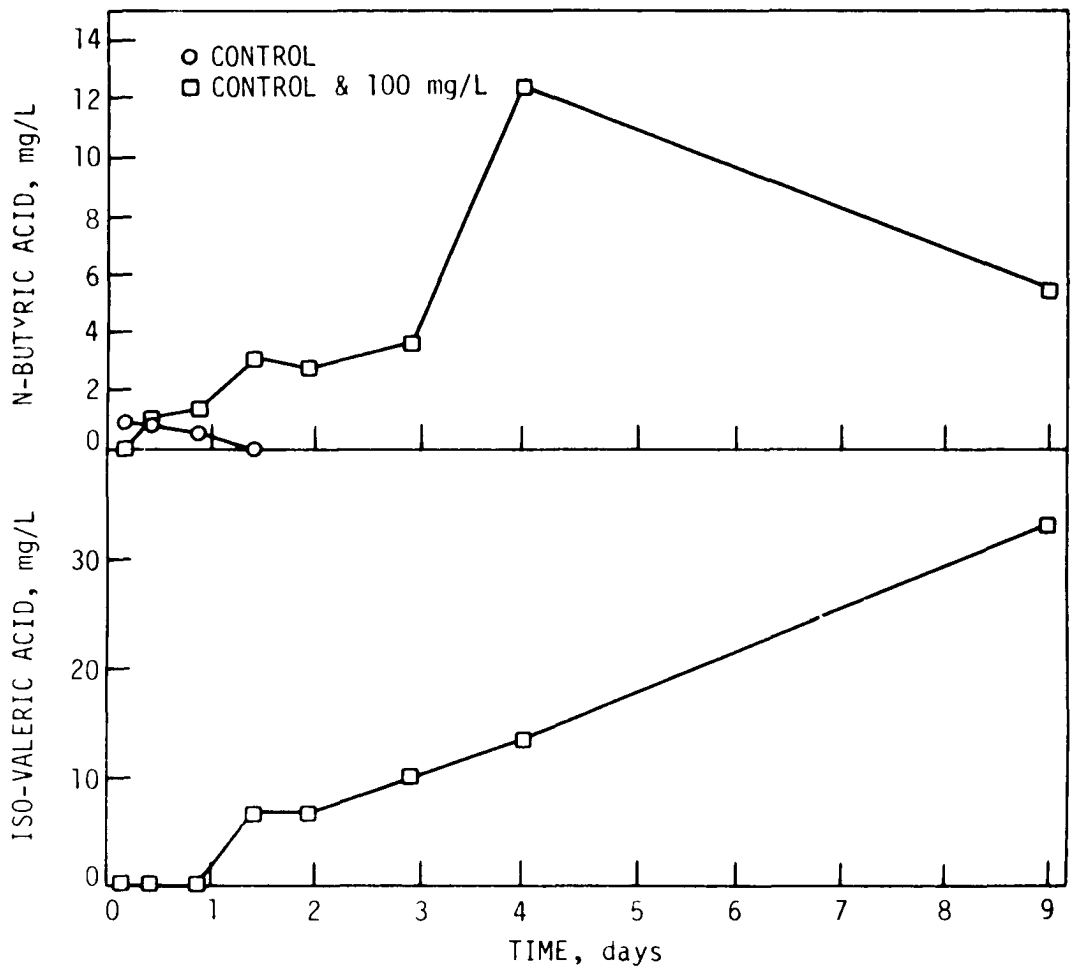

Figure 36b. Concentrations of n-butyric and iso-valeric acids versus time at a dosage of 100 mg/L of hexachloro-1,3-butadiene

acids were increasing during the decrease in concentrations of acetic and propionic acids. The slight increase in concentrations of n-butyric, iso-butyric and iso-valeric acids could not be solely responsible for the large decrease in acetic acid. These data were consistent among replicate samples and therefore the erratic patterns were not associated with experimental error.

Hexachloroethane Hexachloroethane was tested at concentrations of 50, 100 and 164 mg/L. Total gas production and methane production data are shown in Figure 39. Gas production in cultures spiked with 50 mg/L of hexachloroethane was initially delayed but eventually reached that of the control at the termination of the run.

Cultures dosed with 100 mg/L showed a unique gas production pattern unlike any observed in this study. The rate of gas production was much lower than that of the controls but the total amount of gas produced during the test period was very close to that of the controls. Gas production in cultures at this concentration showed a steady increase after five days with a higher percentage of methane than the controls at the termination of the run.

Cultures dosed with 164 mg/L of hexachloroethane showed very little activity until the later period of the test (Figure 39). After approximately twelve days, the gas production showed an upward trend. Methane production at this concentration exhibited the same trend. It appears that if sufficient time had been allowed, these cultures would also have shown a recovery from inhibition.

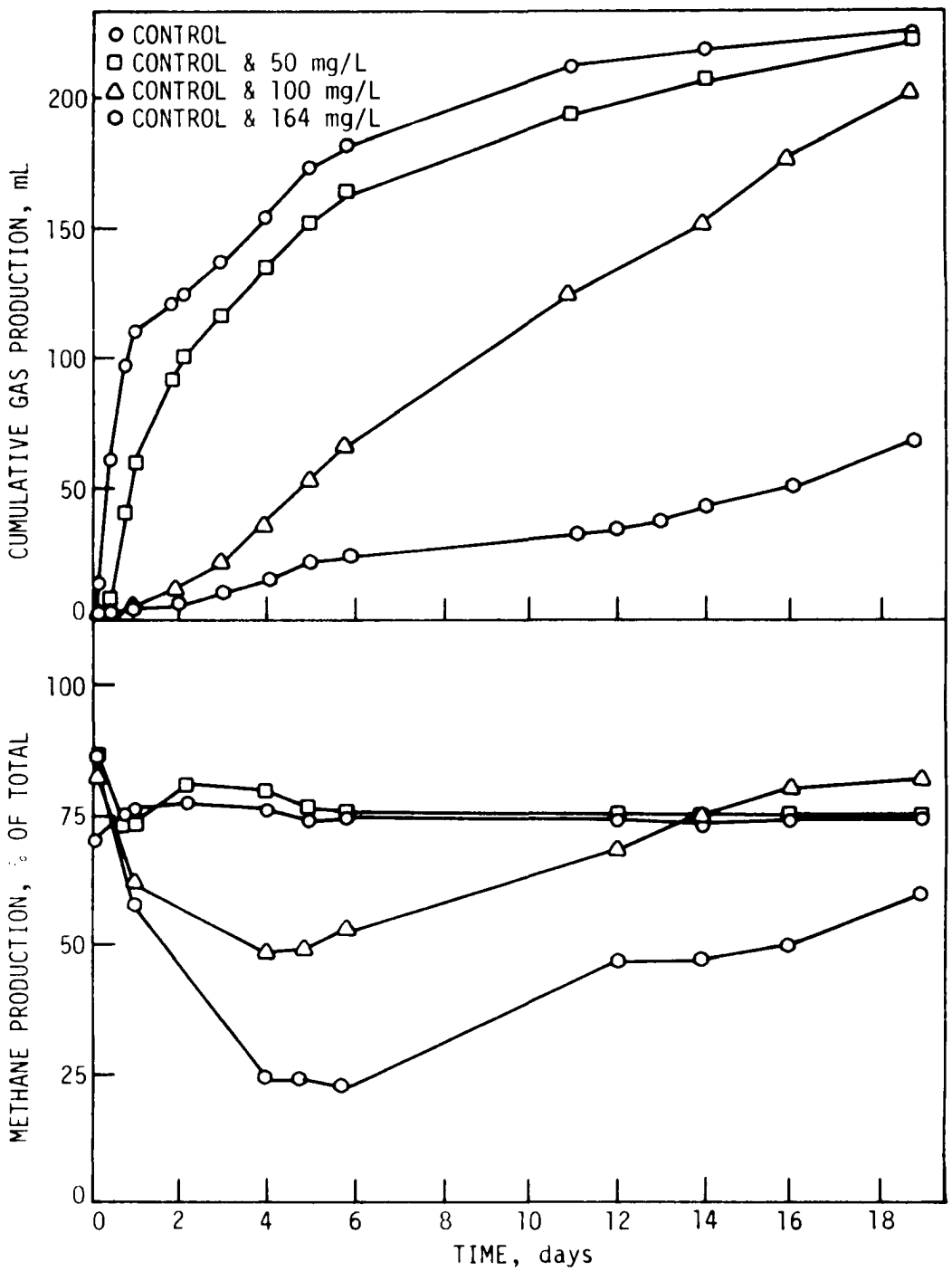

Figure 39. Total gas production and methane production during Phase II testing of hexachloroethane

Analysis of the soluble concentration of hexachloroethane showed a very rapid removal at all concentrations. However, the removal stabilized quickly and hexachloroethane was present at low concentrations in all liquid samples throughout the test period. This suggests that there was rapid adsorption and subsequent equilibrium established between the liquid and the solids (Figure 40).

In cultures dosed with 50 mg/L of hexachloroethane, the formation of acetic acid was initially inhibited (Figure 41a). The concentration of this acid peaked at approximately one day which was later than that of the control. Utilization of acetic, propionic and iso-butyric acids occurred at a much slower rate than that of the controls. Both acid forming and methanogenic bacteria were inhibited by this concentration of hexachloroethane (Figure 41a and b).

The same effect, but more pronounced, was observed in cultures dosed with 100 mg/L of hexachloroethane. At this concentration, inhibition of the propionic acid-utilizing microorganisms was the most severe. Gradual utilization of all volatile acids indicated that the organisms responsible for their degradation gradually became acclimated to the low concentration of hexachloroethane remaining in solution.

At 164 mg/L of hexachloroethane, gas production in the spiked cultures was inhibited throughout most of the test which allowed an accumulation of all of the volatile acids. Formation of iso-butyric and iso-valeric acids in the spiked cultures resulted in significantly higher concentrations than were observed in the controls.

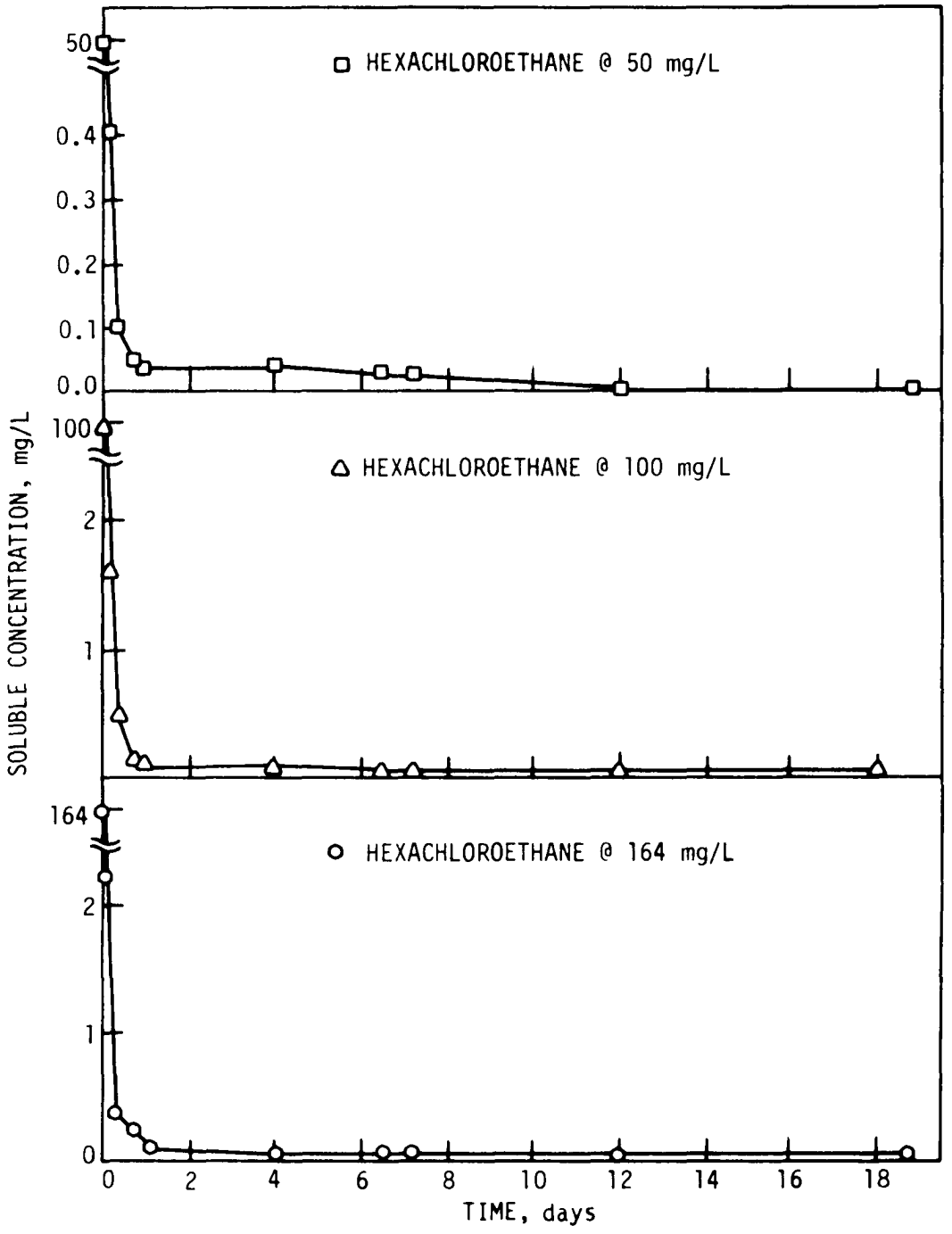

Figure 40. Soluble concentrations of hexachloroethane during Phase II testing

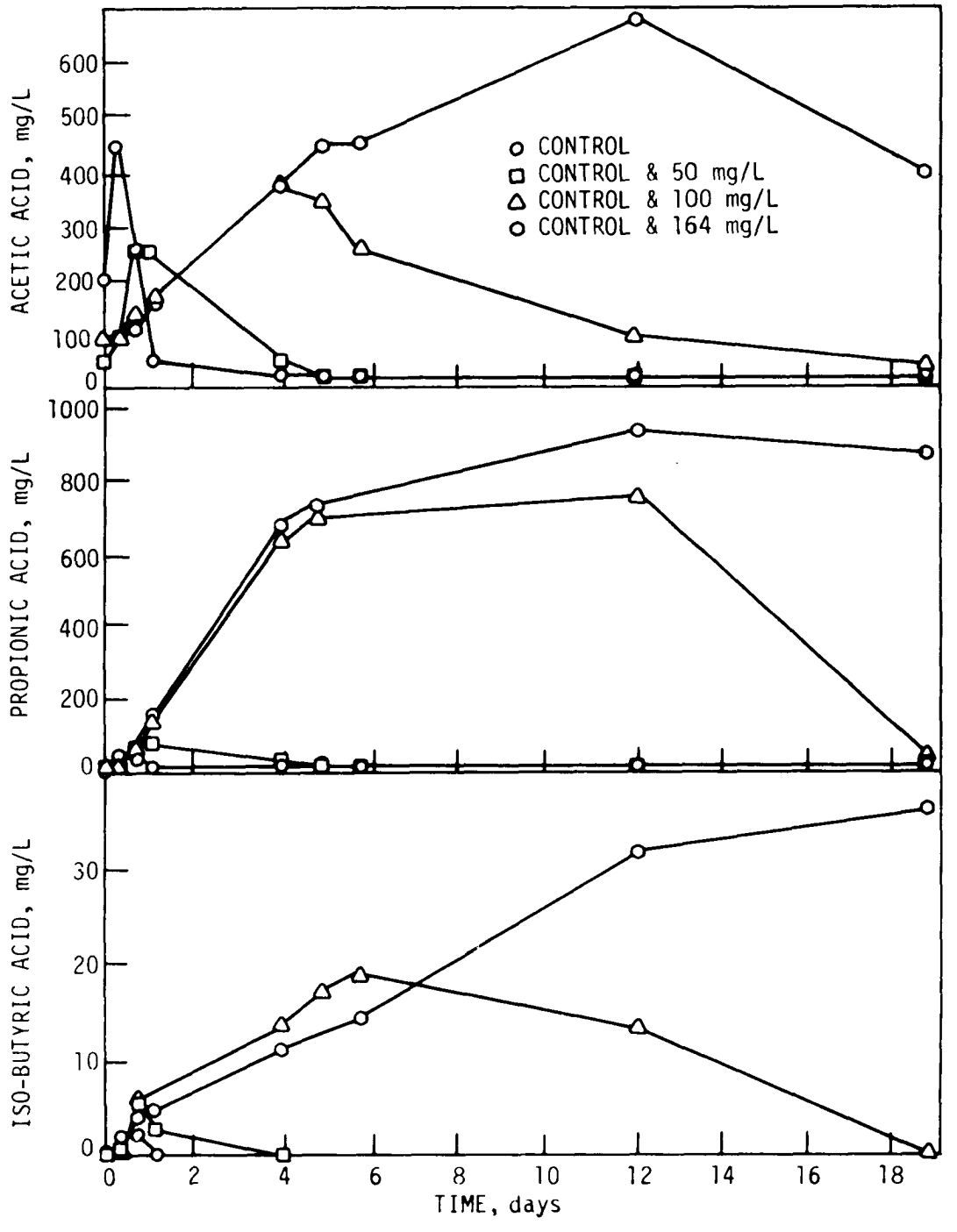

Figure 41a. Concentrations of acetic, propionic and iso-butyric acids versus time during Phase II testing of hexachloroethane

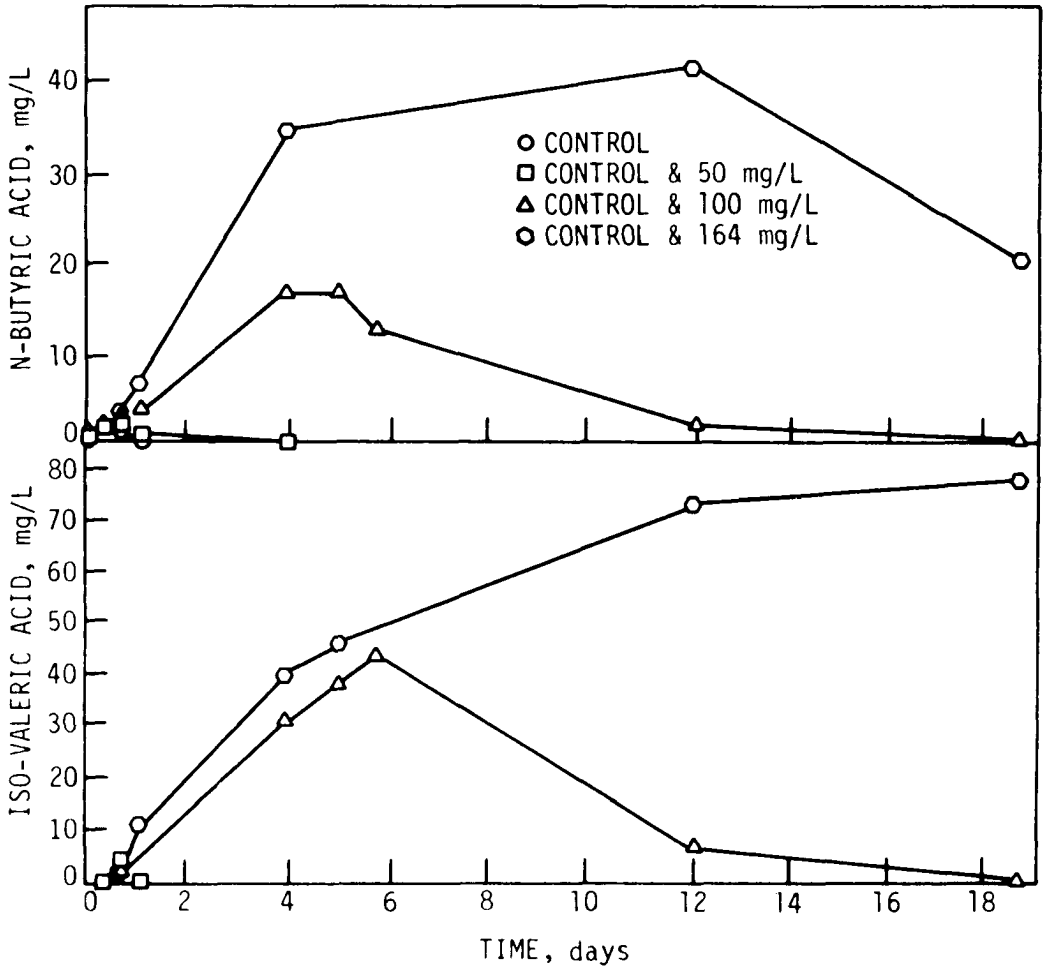

Figure 41b. Concentrations of n-butyric and iso-valeric acids versus time during Phase II testing of hexachloroethane

These acids could have been formed from acetic acid and propionic acid which had accumulated to relatively high concentrations.

Phase II supplemental study results The last stage of Phase II was conducted in the absence of biological activity to determine if the results observed were actually due to microbial activity. Individual sterilized cultures were inoculated with 100 mg/L of each test chemical and samples were taken to determine the fate of the inhibitors. Results of this study are shown in Figures 42a, b and c.

Removal by adsorption was observed for all the test chemicals. Volatility was not a major removal mechanism for any of the test compounds since none were detected in significant concentrations in the gas phase of the serum bottles. Removals were related to solubility (Table 17) which would be expected based on removal by adsorption.

Complete removal of the nitro organics was not observed in the sterilized cultures as occurred with the active cultures. In addition, aniline was not detected in any samples from the sterilized cultures dosed with nitrobenzene.

Removal of 2,4-dichlorophenol in the sterilized cultures was similar to that observed in the active cultures, which would indicate that adsorption was the major removal mechanism in the active cultures and the active microorganisms had little effect on this compound.

High removals of the chlorinated hydrocarbons was due to the very low solubility. The patterns of removal for hexachloroethane and

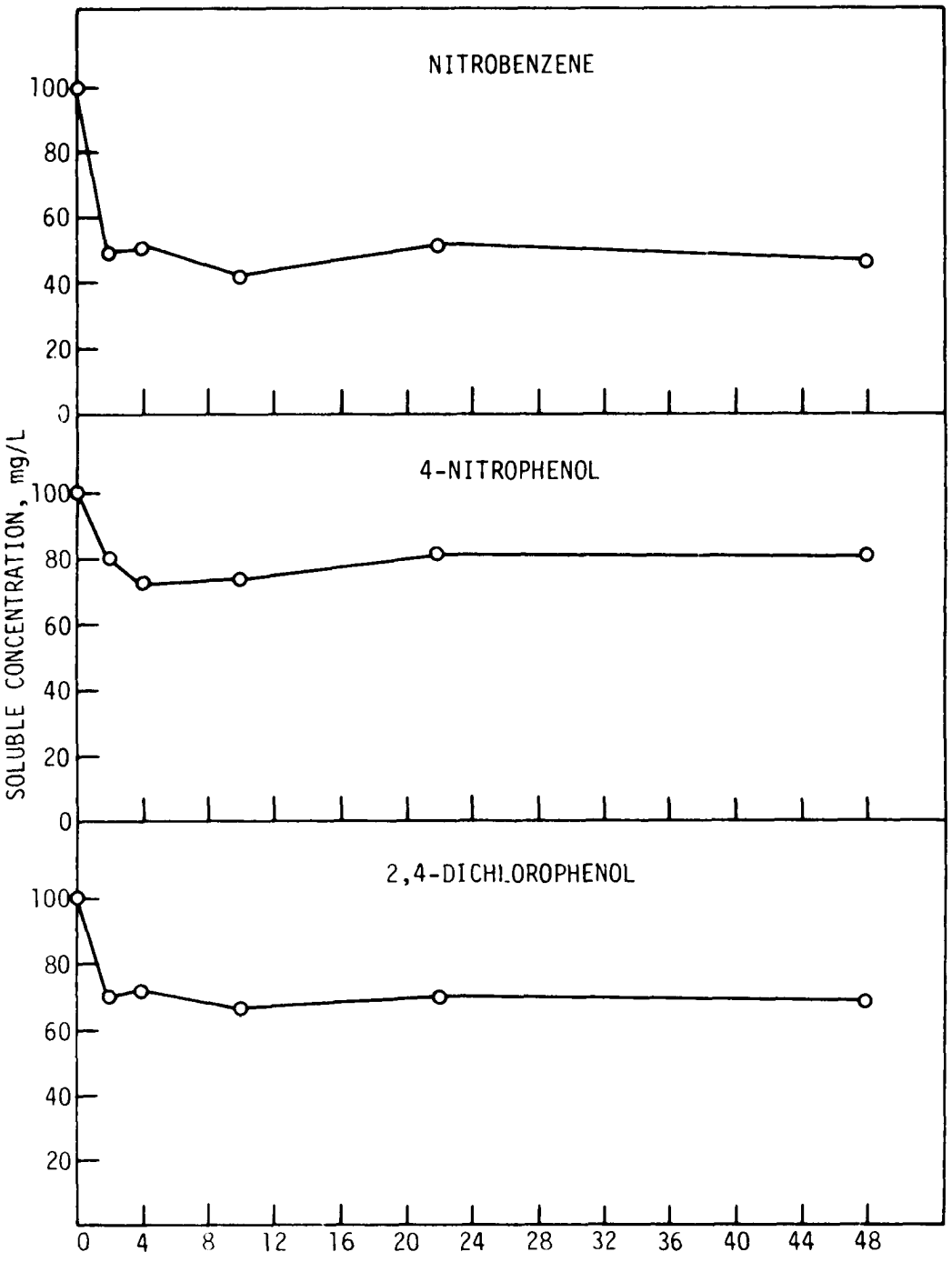

Figure 42a. Soluble concentrations of nitrobenzene, 4-nitrophenol and 2,4-dichlorophenol in the absence of biological activity

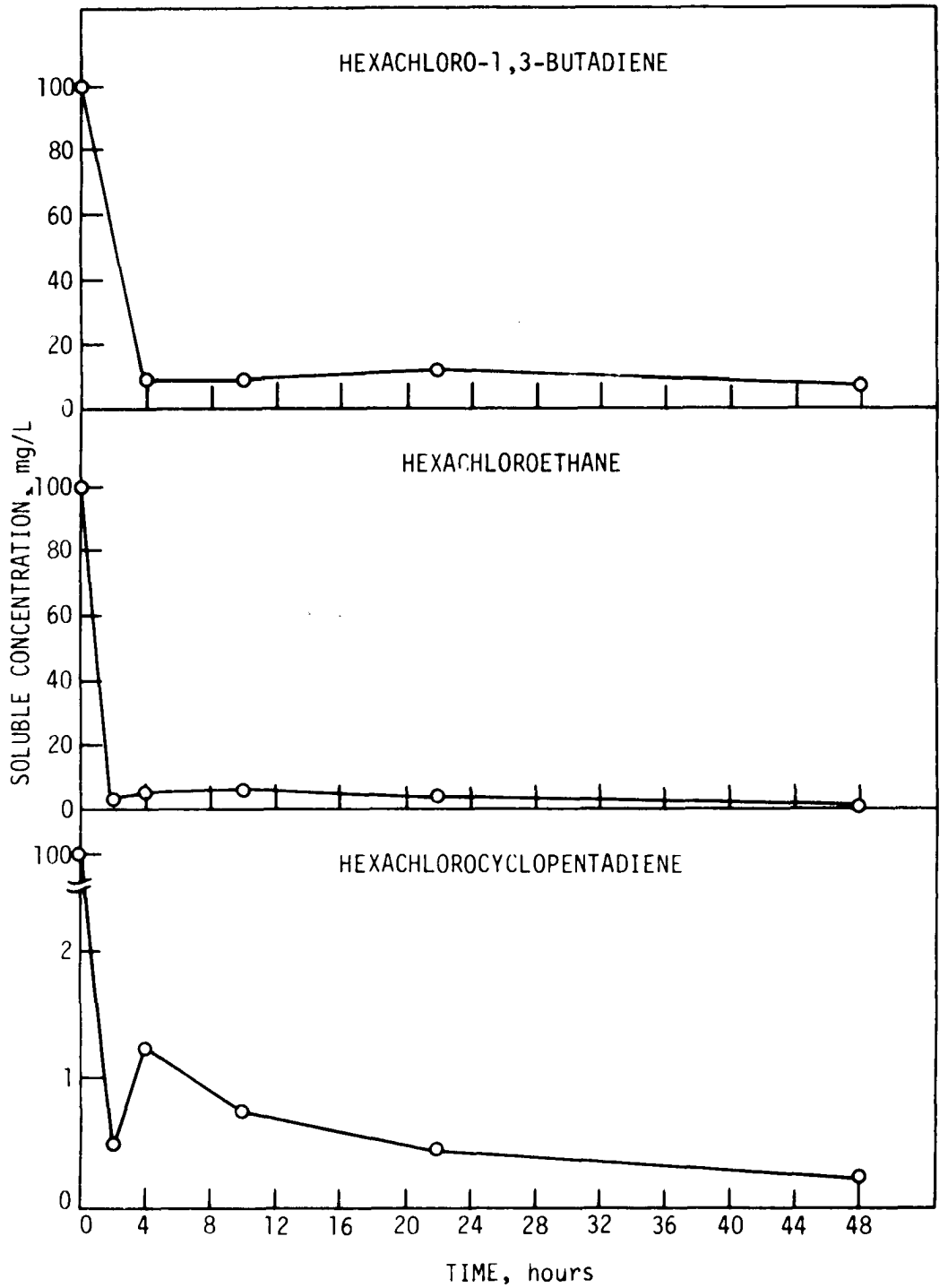

Figure 42b. Soluble concentrations of hexachloro-1,3-butadiene, hexachloroethane and hexachlorocyclopentadiene versus time in the absence of biological activity

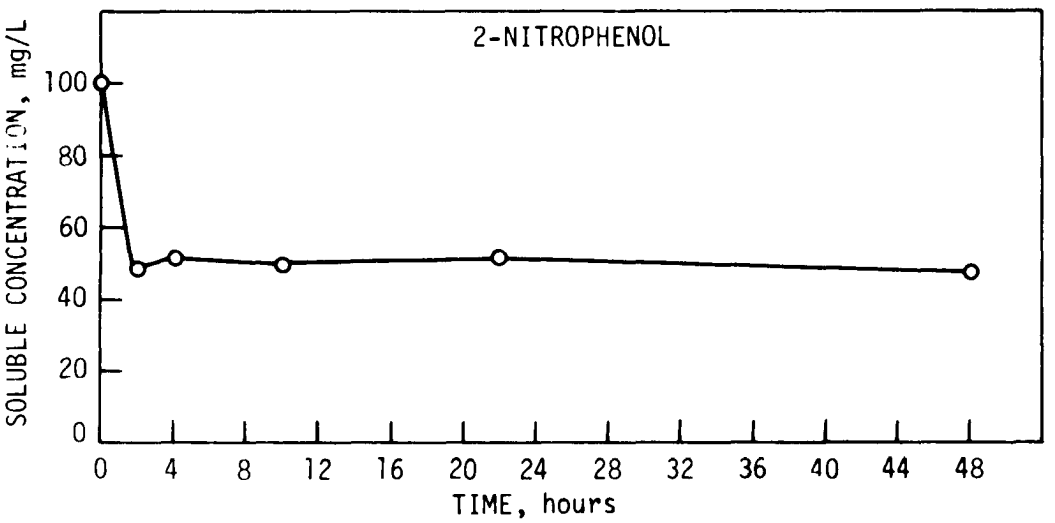

Figure 42c. Soluble concentration of 2-nitrophenol versus time in the absence of biological activity

Table 17. Test chemical solubilities and removals during Phase II testing

Chemical	Solubility, mg/L[a]	Removal, %[b]	
		Active cultures	Sterilized cultures
Nitrobenzene	1,900	100	52
4-Nitrophenol	16,000	100	19
2-Nitrophenol	2,100	100	51
2,4-Dichlorophenol	4,600	40	31
Hexachlorocyclopentadiene	0.085	100	99
Hexachloroethane	50	99	99
Hexachloro-1,3-butadiene	2	99	96

[a]See Table 13 for references.

[b]Removal based on soluble concentration after 48 hours of contact.

hexachloro-1,3-butadiene were almost identical to that observed during testing with active microorganisms (Figures 39 and 37, respectively). These patterns were similar to removal patterns reported by Hill and McCarty (1967) for chlorinated hydrocarbon pesticides. However, these researchers reported that the high initial removal occurred only in active cultures. It should be noted that hexachlorocyclopentadiene was not detected in any of the samples during test with active microorganisms but it was present in all of the samples from sterilized cultures. Removal of hexachlorocyclopentadiene in active cultures was therefore the result of both microbial activity and adsorption.

Phase III

This part of the research study was designed to investigate the effect of inactive solids on the inhibition and recovery of anaerobic cultures. Inert solids used in this phase were obtained by centrifuging mixed liquor from the refuse digester at 6,000 rpm (G=8200) for one hour. The supernatant was discarded and the solids were collected and dried at 103^{o} C. After sufficient solids had been collected, they were reground in the same Wiley mill used for grinding the substrate for the refuse digester. The ground solids were used as processed with no screening performed to separate the solids into size fractions.

Testing

The procedure for this phase was to add different levels of inactive solids to the serum bottles containing nutrient-buffer solution and anaerobic seed. Six serum bottles were set up for each concentration of test chemical: two bottles without additional solids, two bottles with 1.0 gram of additional inactive solids, and two with 5.0 gram of additional inactive solids. The chemical concentration used for each test was one which was known to cause rather severe inhibition. The run length once again was extended to allow for adequate time for recovery to occur. Only gas production and gas quality were monitored during the test run, but at the end of the run the assays were analyzed for individual volatile acids, soluble concentration of the test chemical

and presence of any degradation products. In addition to the assays set up for the test chemicals, six serum bottles were used for a seed control and six bottles for fed controls. The total and volatile solids were analyzed at each level of added solids prior to the test run.

Results of Phase III

Gas production in the controls was significantly different due to the biodegradation of the added solids (Table 18). Relative activities were used to provide a means of comparing all of the data.

Table 18. Gas production in controls during Phase III testing

Control	Gas production, mL
Seed	136.5
Seed + 1 gram solids	183.3
Seed + 5 grams solids	330

At a dosage of 500 mg/L of 4-nitrophenol, there initially was very little difference between the three levels of solids but after 120 hours the relative activity of the samples containing the highest solids concentration exceeded the relative activity of the control (Figure 43). No 4-nitrophenol was detected in any of the samples at the termination of the run. Gas production in both samples at the highest concentration of solids were similar and therefore the increased gas production in

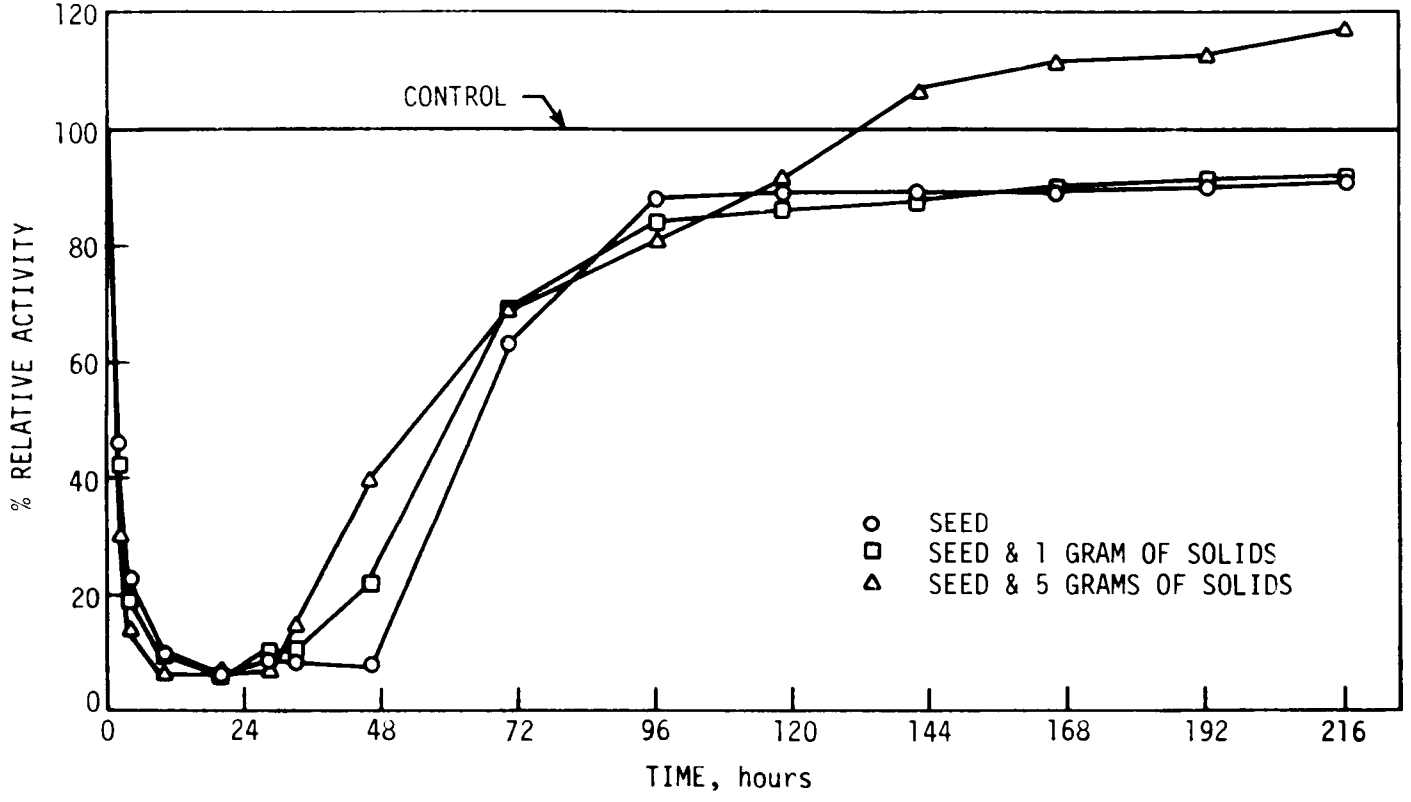

Figure 43. Relative activity versus time for various levels of inactive solids at 500 mg/L of 4-nitrophenol

these samples was not due to experimental error. There was no obvious explanation for this occurrence.

Data for cultures dosed with 500 mg/L of 2-nitrophenol revealed little evidence of any effect of the higher concentrations of inactive solids (Figure 44). No 2-nitrophenol was detected in any of the samples at the termination of the run.

The effect of adding solids on the inhibition from 100 mg/L of hexachloro-1,3-butadiene is shown in Figure 45. The relative activities at all levels of solids were essentially the same. Analysis of hexachloro-1,3-butadiene in the samples at the termination of the test run showed that the highest concentration of solids produced the highest removal of this chemical (Table 19). This low level of hexachloro-1,3-butadiene apparently had little effect on the response of the anaerobic seed culture since there was no difference in relative activities of the three solids levels.

Data for hexachlorocyclopentadiene at a dosage of 100 mg/L are shown in Figure 46. Inhibition was less severe at the highest concentration of solids. Recovery was also faster. Hexachlorocyclopentadiene was not detected in any of the samples at the termination of the test period.

Inhibition from 250 mg/L of 2,4-dichlorophenol was less severe at the highest level of solids (Figure 47). This also is reflected in the soluble concentrations of 2,4-dichlorophenol shown in Table 19. The concentration of 70 mg/L in the samples containing the highest level of

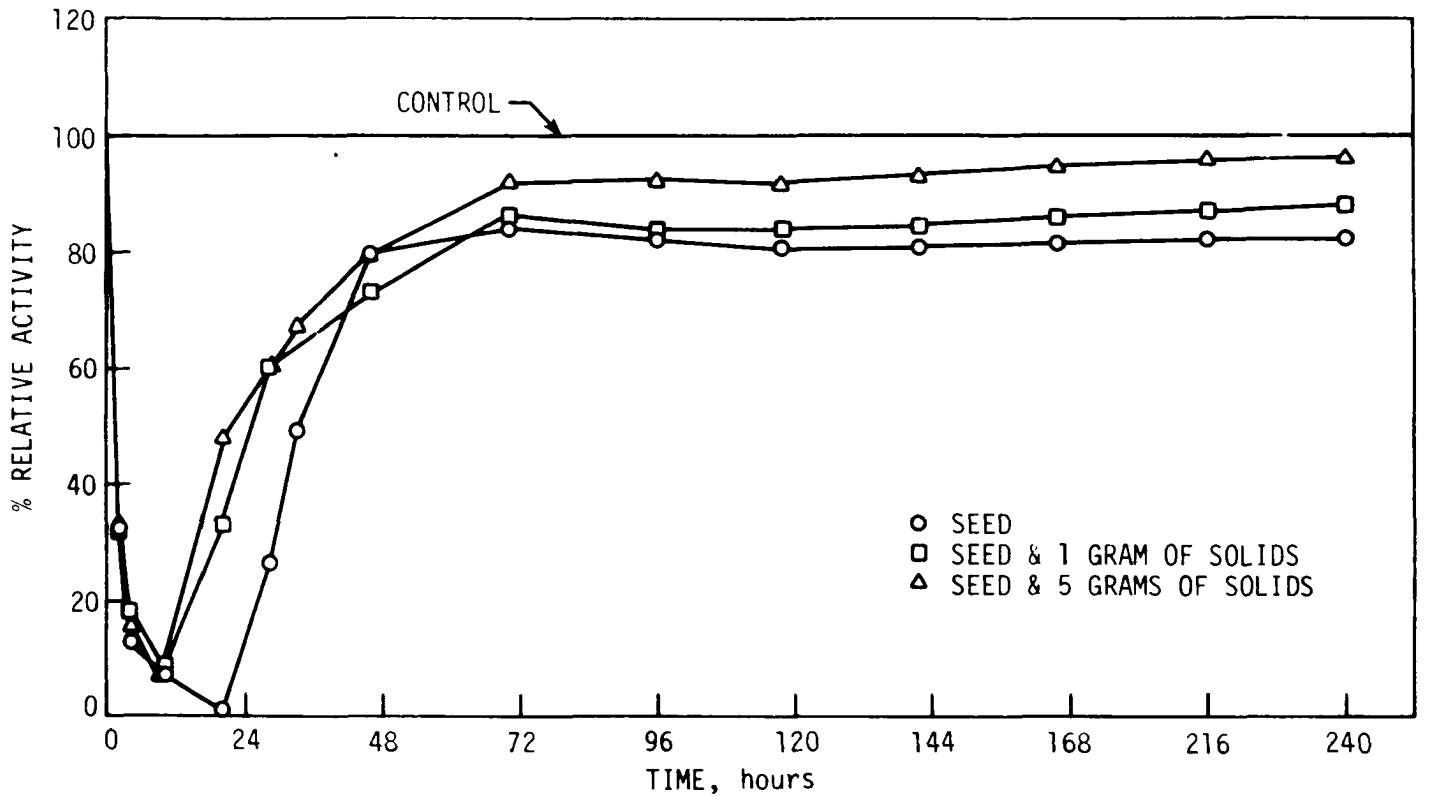

Figure 44. Relative activity versus time for various levels of inactive solids at 500 mg/L of 2-nitrophenol

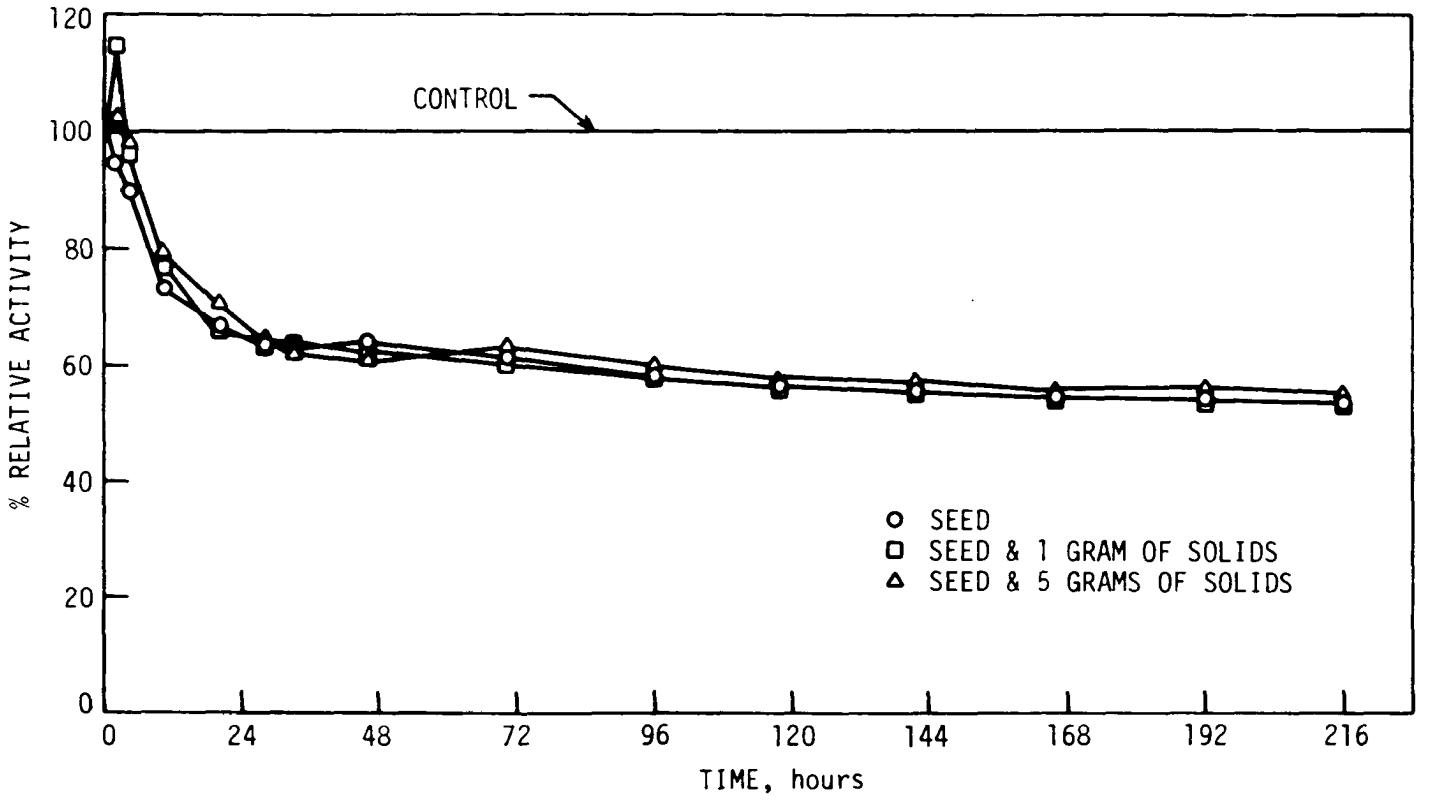

Figure 45. Relative activity versus time for various levels of inactive solids at 100 mg/L of hexachloro-1,3-butadiene

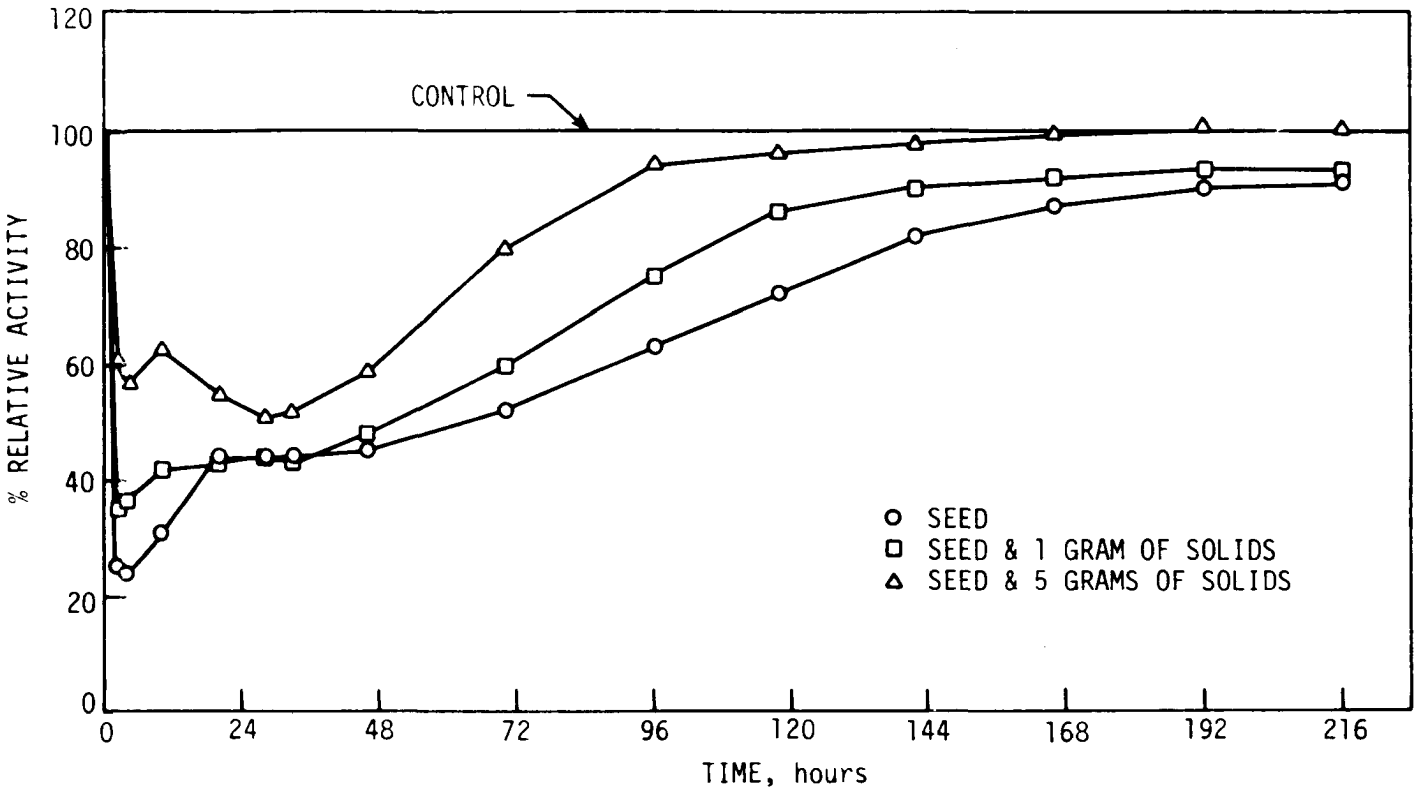

Figure 46. Relative activity versus time for various levels of inactive solids at 100 mg/l of hexachlorocyclopentadiene

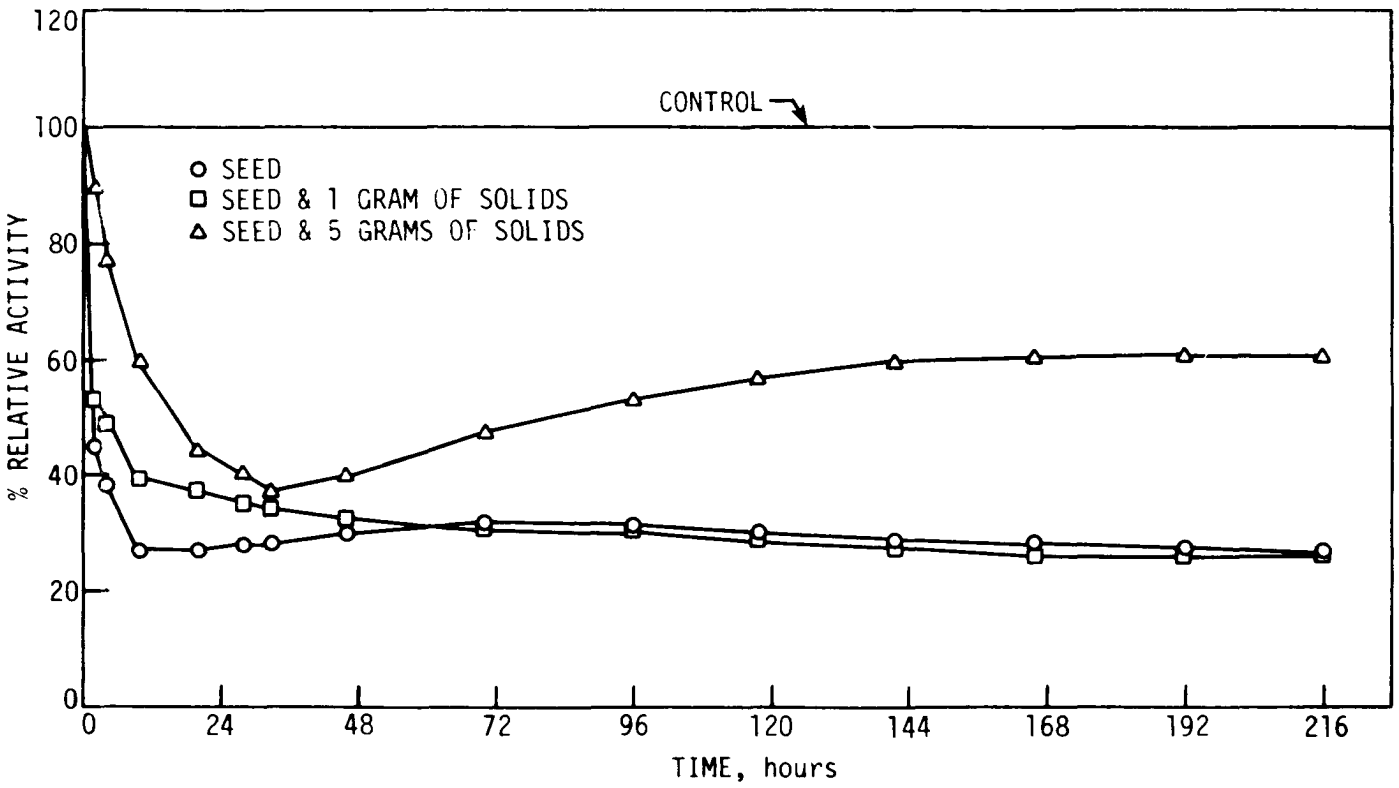

Figure 47. Relative activity versus time for various levels of inactive solids at 250 mg/L of 2,4-dichlorophenol

Table 19. Soluble concentrations of test chemicals at the termination of Phase III testing

Compound	Concentration, mg/L		
	Seed	Seed +1 gram solids	Seed +5 grams solids
Hexachloro-1,3-butadiene	1.3	0.9	0.6
2,4-Dichlorophenol	227	161	70
Hexachloroethane	0.2	0.08	0.04
4-Nitrophenol	ND[a]	ND	ND
Hexachlorocyclopentadiene	ND	ND	ND
2-Nitrophenol	ND	ND	ND
Nitrobenzene	ND	ND	ND
Aniline	314	287	213

[a]ND: none detected.

solids was similar to that observed during Phase II testing (60 mg/L). This concentration was apparently above the tolerable limit of the microorganisms responsible for propionic acid utilization since a buildup of this acid was observed in the samples collected at the termination of Phase III testing. The same occurrence was noted during Phase II.

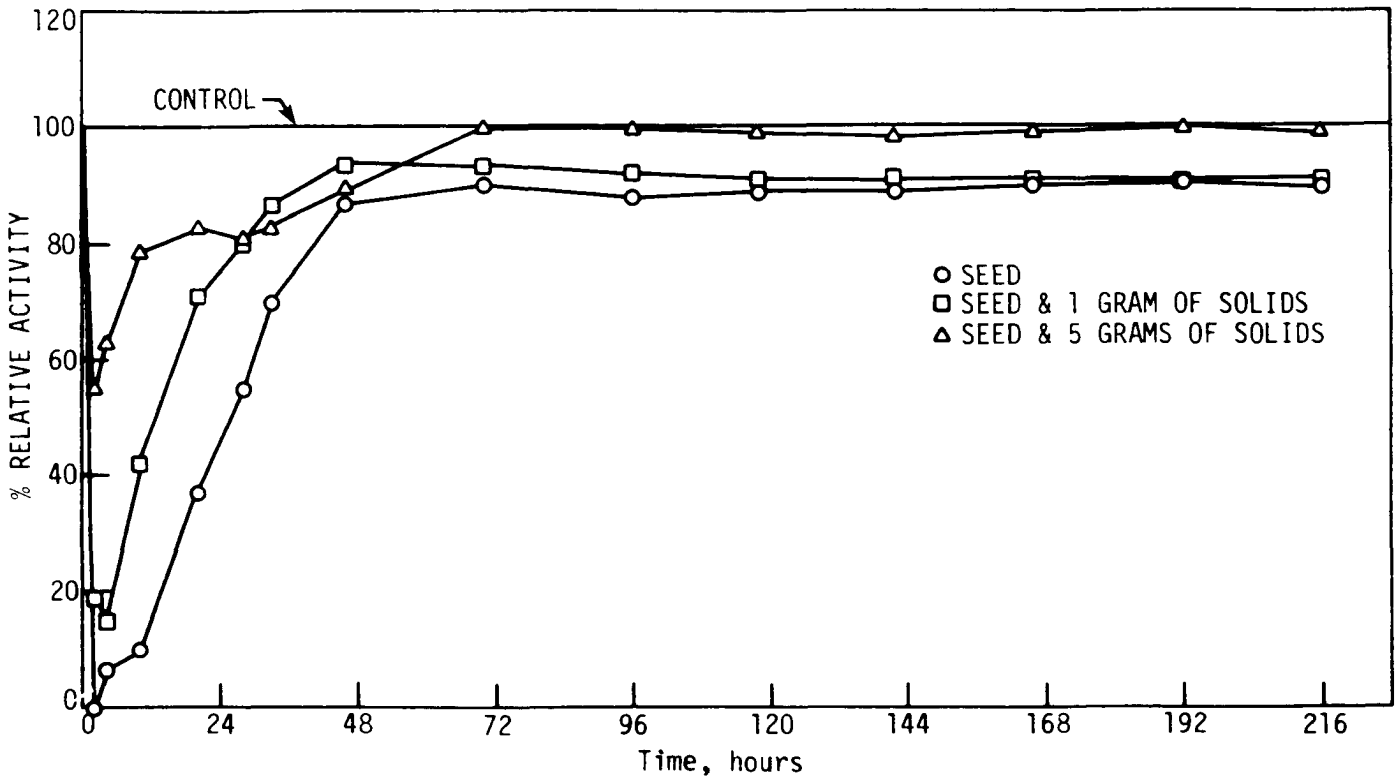

Figure 48. Relative activity versus time for various levels of inactive solids at 50 mg/L of hexachloroethane

Inhibition from 50 mg/L of hexachloroethane was less severe at the higher levels of solids (Figure 48). The highest concentration of solids produced the lowest reduction in relative activity. Very low soluble concentrations were observed at the termination of the test run with the lowest concentrations in the sample having the highest level of solids (Table 19).

Added solids had no effect on the inhibition caused by 500 mg/L of nitrobenzene (Figure 49). Nitrobenzene was not detected in any of the samples but the concentration of aniline was observed to decrease as the concentration of solids was increased (Table 19).

The overall effects of the added solids were difficult to analyze since the solids were indeed not inert and provided additional background gas production. Background gas production clouded the results since it is impossible to determine when the biodegradation of the solid material occurred. In addition, the increased level of microbial activity due to decomposition of the added solids could have had an affect on the results. This observation was reported by Swanwick and Foulkes (1971) and they noted that increased microbial activity reduced the inhibition from chlorinated hydrocarbons. The seed cultures contained approximately 32,000 mg/L of total suspended solids so the addition of five grams of solids created a slurry that was difficult to mix. The increased levels of solids did show increased removals of the test chemicals and appeared to reduce the toxicity of hexachloroethane, hexachlorocyclopentadiene and 2,4-dichlorophenol.

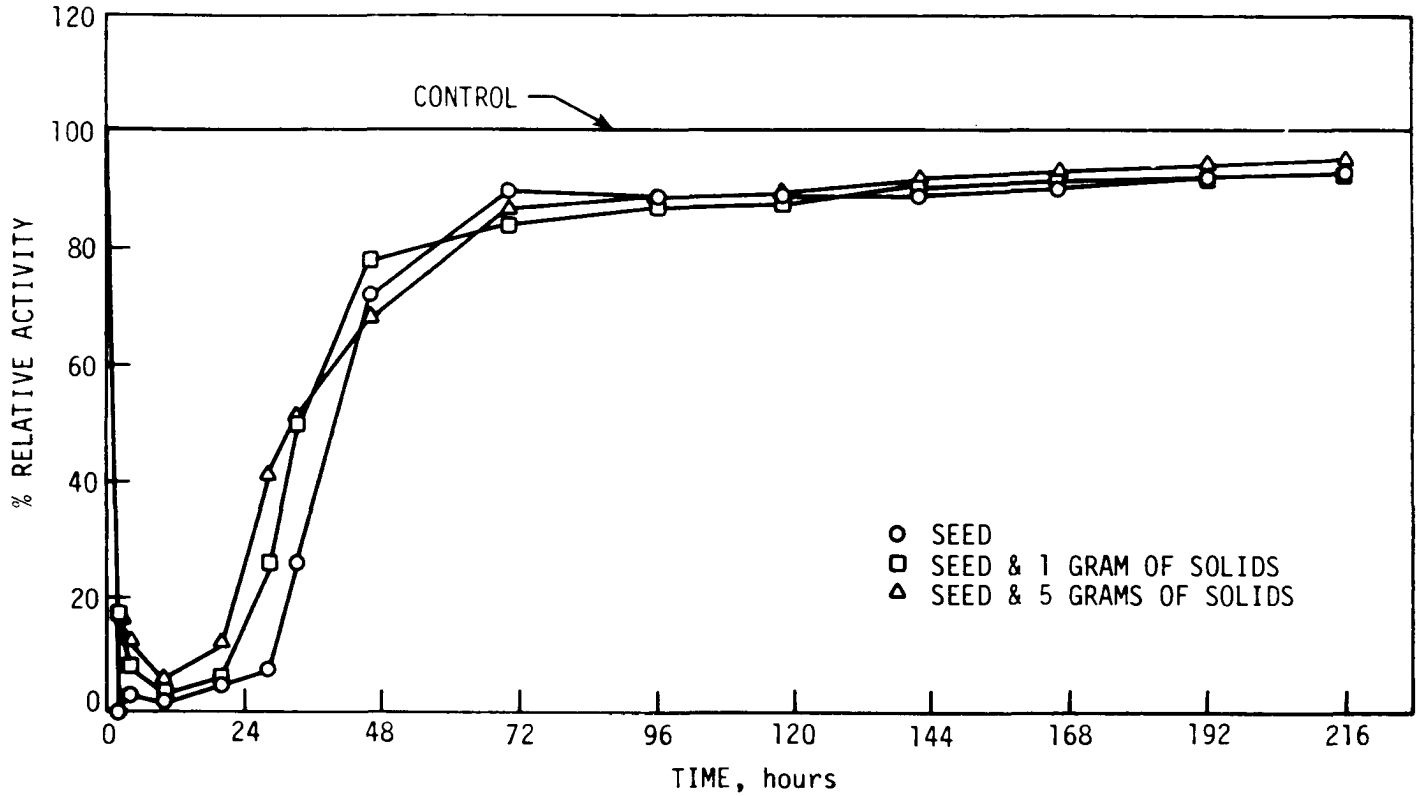

Figure 49. Relative activity versus time for various levels of inactive solids at 500 mg/L of nitrobenzene

Supplemental Study

Behavior of the chlorinated hydrocarbons in this research project prompted an analysis for the possible dechlorination of these compounds. The complete absence of hexachlorocyclopentadiene and the low levels of hexachloroethane and hexachloro-1,3-butadiene observed during test with active cultures indicated that this mechanism of removal could have occurred.

Since dechlorination would result in the formation of chloride ions in solution, an analysis of the chloride concentrations in samples from previous Phase II testing was performed. Chloride analysis was based on the potentiometric titration method recommended by Standard Methods (1976) and performed by the ERI Analytical Services Laboratory.

Results of these analyses are presented in Table 20. The samples were acidified supernatant samples collected during Phase II. Since each chemical was tested in separate runs, a control is included from each run for comparison purposes. These samples had been stored at 4^{o} C in the dark for several weeks so it is difficult to determine the reliability of these data. Only samples dosed with hexachlorocyclopentadiene showed a significant increase in chloride concentration.

Complete dechlorination of 500 mg/L of hexachlorocyclopentadiene should have resulted in a chloride concentration increase of 390 mg/L. The observed increase of 258 mg/L is approximately equivalent to the loss of four chlorine atoms from each molecule of hexachlorocyclopentadiene.

This observation is similar to that of Jagnow, et al., (1977) who reported that the dechlorination of hexachlorocyclohexane isomers resulted in the formation of tetrachloro isomers and therefore the loss of two chlorines from each molecule of hexachlorocyclohexane. These results indicate that hexachlorocyclopentadiene was biologically degraded by a dechlorination reaction.

Table 20. Chloride analysis of chlorinated hydrocarbon samples

Chlorinated Hydrocarbon	Test Concentration, mg/L	Chloride, mg/L	
		Control	Sample
Hexachloro-1,3-butadiene	988	286	288
Hexachlorocyclopentadiene	500	300	558
Hexachloroethane	164	296	291

DISCUSSION OF RESULTS

Anaerobic treatment systems are complex biological processes and the microbial groups responsible for waste conversion and stabilization have the remarkable ability to adapt to inhibitory organics. Evaluation of inhibition using batch bioassays represents a rather severe situation since the concentration of the toxic material is raised in one step to the level to be studied. In addition, the microorganisms in the seed culture are constantly exposed to the test chemical over the duration of the testing period.

Actual anaerobic treatment systems are continuous flow processes and whether they are designed as complete-mix or plug-flow reactors, they offer some protection against shock loads of toxic materials. Complete-mix reactors would provide dilution of the toxic material thereby exposing the microorganisms to a lower concentration of the toxin. Plug-flow systems would not provide dilution but would offer only a short contact time between the microorganisms and the slug load of toxin. It has been shown that in most cases only temporary inhibition of anaerobic activity occurs with a return to normal activity when the concentration of the inhibitor is reduced below some inhibitory level or removed from the system.

In the past, it has been common operating procedure to dump the contents of a "sour" digester and reseed with a new culture. However, this may not be the best solution when the inhibition is caused by

organic materials. The time required to start active digestion after reseeding usually takes a month or more and within that period of time acclimation to or removal of the toxic material may occur. This is assuming, of course, that proper environmental conditions are maintained during the period of inhibition.

If inhibition of a digestion system is known to be induced by a toxic organic or a combination of organic materials, the first action would involve close monitoring of environmental conditions. For recovery to occur the proper pH and temperature must be maintained. Based on the results of this study, it appears that approximately the initial 48 hours of exposure are the most critical. Suspension of feeding would eliminate further introduction of toxic materials. Increased gas production would indicate that recovery was in progress and an analysis of the propionic acid concentration would provide information on the degree of inhibition or recovery. Elimination of toxic organics from digester feed streams is still the best protection against digester upsets but anaerobic microorganisms can recover from exposure to toxic organics.

Solid waste digestion systems would probably be operated at a fairly high level of solids since water must be added to the solids before introduction to the digester. Adsorption of toxic organics on the solids would offer some degree of protection against slug loads of toxins.

Two phase anaerobic treatment systems may prove to be a viable alternative in cases where occasional slug loads of toxic organics

are experienced. Two phase anaerobic treatment involves the separation of the acid formation and methane production phases into separate reactors. While some of the organics examined during this study were found to inhibit acid formation, degradation of the organic toxins was determined to be an important removal mechanism. Series configuration of reactors in a two phase system would also provide an additional buffer to protect the methanogens against exposure to slug loads of inhibitory organic materials. In addition, it may be possible to adapt a two phase system to the treatment of organic chemical waste streams.

Batch bioassays are valuable tools in toxicity evaluations but the results must be interpreted properly. Short term assays, while allowing for many chemicals to be tested, may provide misleading results. For simple screening of organic chemicals for inhibitory effects, the short term assay would be appropriate since the nontoxic materials could be easily identified and eliminated from further testing. To properly evaluate the effects of toxic organics, however, longer assays are needed. Future toxicity evaluations should incorporate the results of this study to provide better information concerning the effects of inhibitory organics on anaerobic cultures.

SUMMARY AND CONCLUSIONS

Inhibition of complex anaerobic cultures by organic Priority Pollutants was studied. The study was conducted using anaerobic seed from a laboratory digester maintained on a synthetic substrate resembling municipal refuse. A batch bioassay technique using serum bottles was used to provide inhibition data at 37° C.

Based on the results of this study, the following conclusions can be drawn:

1. Hexachlorocyclopentadiene, hexachloroethane, hexachloro-1,3-butadiene, 4-nitrophenol, 2-nitrophenol, 2,4-dichlorophenol and nitrobenzene inhibited anaerobic cultures at 37° C,

2. Short term batch bioassays are adequate for screening of organic compounds for inhibitory effects but longer bioassays are needed for evaluation of inhibition of anaerobic cultures by toxic organics,

3. Inhibition from 4-nitrophenol, 2-nitrophenol, nitrobenzene, hexachloroethane and hexachlorocyclopentadiene was reversible under the conditions used in this study,

4. Recovery of methane production in anaerobic cultures inhibited by 4-nitrophenol, 2-nitrophenol and nitrobenzene was not caused by acclimation but by removal of the toxic substance from solution via biological reduction of the nitro group to an amine group,

5. Acclimation of microorganisms to low concentrations of hexachloroethane produced recovery of methane production in anaerobic cultures inhibited by this chemical,

6. Degradation of hexachlorocyclopentadiene at low concentrations by anaerobic dechlorination allowed recovery of methane production in inhibited cultures, and

7. Adsorption of low solubility organic inhibitors onto solids can be an important removal mechanism affecting the inhibition of organic chemicals.

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APPENDIX I. ANALYTICAL METHODS

Parameter	Method	Reference
Total solids	Evaporation and weighing	SMEWW[a], p. 91
Suspended solids	Glass fiber filter; nonfilterable residue	SMEWW, p. 94
Chemical oxygen demand	Dichromate reflux	SMEWW, p. 500
pH	Potentiometric	SMEWW, p. 460
Total nitrogen	Sum of all nitrogen forms	-----
Organic nitrogen	Kjeldahi, phenate method, automated	MCAWW[b], p. 351.2
Ammonia nitrogen	Phenate method, automated	MCAWW, p. 350.1
Nitrite-nitrate nitrogen	Cadium reduction method, automated	MCAWW, p. 353.2
Total phosphorus	Colorimetric, semi-automated block digester, AAII	MCAWW, p. 365.4
Heavy metals (except arsenic and mercury)	Atomic adsorption spectrophotometry	SMEWW, p. 144 & 147; AMAAS[c]
Arsenic	Atomic adsorption spectrophotometry, hydride generation	MCAWW, p. 206.5 MHS-10
Mercury	Manual cold vapor technique	MCAWW, 245.1

[a] Standard Methods for the Examination of Water and Wastewater, 1976.

[b] Methods for Chemical Analysis of Water and Wastes, 1979.

[c] Analytical Methods for Atomic Adsorption Spectrophotometry, 1973.

Parameter	Method	Reference
Sulfate	Methyl thymol blue, automated	SMEWW, p. 628
Sodium	Flame photometric method	SMEWW, p. 250; AMAAS
Potassium	Flame photometric method	SMEWW, p. 234; AMAAS
Chloride	Potentiometric titration	SMEWW, p. 408
Cullulose	Gravimetric	Dennis and Winfield (1977)
Lignin	Gravimetric	AOAC[a], 3.097

[a]Association of Official Analytical Chemists, 1970.

APPENDIX II. PACKED COLUMN GAS CHROMATOGRAPHY

Tracing the fate of the organic test chemicals over a period of time required the use of gas chromatography equipment. Since the analytical techniques employed were rather specific, no standard methods were available. This required the development of innovative methods. Therefore, to understand the procedures used in this study, a discussion of gas chromatography is presented.

Gas chromatography is a process by which a sample mixture can be separated into its individual volatile components. The modern gas chromatograph is a multi-component system. The basic components of a gas chromatograph are the injector, carrier gas, column, detector, amplifier and recorder. A schematic diagram of a typical gas chromatography is shown in Figure II-1.

Individual Components of a Gas Chromatography System

Each component of a gas chromatography has its own specific requirements for analysis of sample mixtures. Therefore, each component will be considered separately.

Carrier gas

The carrier gas transports the sample from the injection port, through the column and into the detector. Separation of the

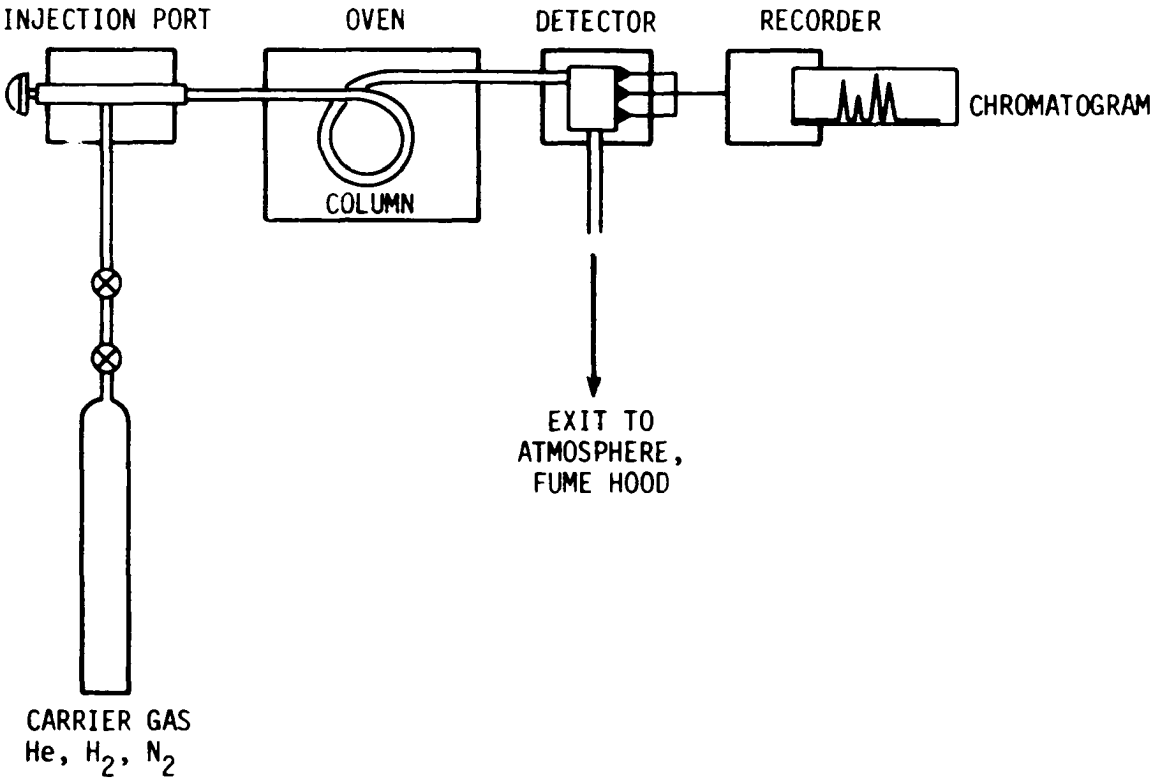

Figure II-1. Schematic diagram of gas chromatograph

constituents in the sample occurs because of the distribution of these compounds between the carrier gas and column packing material. The requirements of a carrier gas are (McNair and Bonelli, 1969):

1. Inert to avoid interation with the sample or packing material,
2. Able to minimize gaseous diffusion,
3. Readily available in pure form,
4. Inexpensive, and
5. Suitable for the detector used.

The most commonly used carrier gases are hydrogen, helium, and nitrogen. Specific carrier gases, such as argon/methane, are used to increase the sensitivity of electron capture and other detectors. The flow of carrier gas through the gas chromatograph must be controlled closely to provide reproducible analyses. Flow controllers and pressure regulators are used to meter the flow accurately.

Injection port

The injection port provides a means of quantitatively introducing the sample into the carrier gas stream. Samples are injected through a septum into the injection port by a syringe. The injection port is heated to provide flash-evaporation of the sample and the gaseous sample is transported through the column by the carrier gas. The temperature of the injection port will vary according to the type of analysis, and should be hot enough to cause flash-evaporation of the sample but not hot enough to cause thermal decomposition of

the sample components. The normal range of injection port temperature is 100°C to 300°C. Many injection ports contain glass liners. These liners are especially important when working with dirty samples since contaminants will be deposited on the glass liner and not carried to the column. The liner can then be removed for cleaning or replacement. The liners are also important since many sample components, such as organic acids, will interact with the metal of the injection port. This interaction causes incomplete resolution by the column.

Column

The column is the heart of a chromatography system since this is the component responsible for the actual separation of the sample mixture. Gas chromatography columns are generally glass, stainless steel, or aluminum tubes varying in length and diameter. Columns are usually coiled or U-shaped so that long lengths can be fitted into the column oven. The column contains a packing material which performs the actual separation of the sample mixture. The packing material selectively retards the sample components until they form separate bands in the carrier gas. The number of packing materials and the uses of these packings are too numerous to discuss in this dissertation. An excellent discussion of this subject is provided by Supina (1974).

There are basically two types of packing materials. The first type is solid adsorbents which are used for the separation of low molecular weight gases and liquids. When these packings, such as carbon or silica gel, are used the process is called gas-solid chromatography. Porous polymers, such as the Porapak[1] and Chromosorb[2] series, have extended the use of solid adsorbents into the separation of alcohols, organic acids, amines and other higher molecular weight compounds. The second type of packing material consists of a non-volatile liquid film or stationary phase on a solid support. The liquid film is responsible for the separation while the support simply acts to distribute the liquid film. The raw material for most supports is diatomaceous earth.

Separation of a sample mixture on a given column is a function of the linear velocity of the carrier gas, the uniformity and particle size of the packing material, the amount of the liquid film, the diffusivity of the sample components in the liquid film and the length of the column. A detailed discussion of these factors is given by McNair and Bonelli (1969).

Packings are usually classified according to polarity. The choice of the proper column packing depends on the composition of the sample. In general, a column packing should be similar in

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[1]Waters Associates, Inc., Milford, Massachusetts.

[2]Johns-Manville, Denver, Colorado.

chemical structure to the components of the mixture. Therefore, hydrocarbons should be separated on a non-polar column and organic acids on a polar column.

The temperature at which the column is operated is very important. Resolution can usually be improved by lowering the temperature of the column. This, however, results in a longer analysis time. A compromise can be reached by the use of temperature programming. Temperature programming is the process of beginning at a low temperature and gradually increasing the temperature to some practical maximum. In this way, complex mixtures can be separated with no loss in resolution or analysis time.

Detectors

The detector senses the presence of the gaseous components as they are eluted from the column. The most common detectors are the thermoconductivity, flame ionization and electron capture. Each of these detector types was used in this study.

The themoconductivity detector is based on the principle that a hot body will lose heat at a rate corresponding to the composition of the surrounding gas (McNair and Bonelli, 1969). This detector consists of an electrical filament supported inside a metal block. Changes in gas composition caused by compound eluting from the column change the detector filament temperature. This temperature change causes a change in electrical resistance in the filament which is

measured by a Wheatstone bridge circuit.

The flame ionization and electron capture detectors operate on the prinicple that the electrical conductivity of a gas is directly proportional to the concentration of charged particles within the gas. An ionization source ionizes specific molecules in the column effluent gas and these charged particles create a current in a collector electrode. The resulting signal is amplified by an electrometer and fed into the recorder. In the case of the flame ionization detector, the ionization source is a hydrogen flame. Electron capture detectors employ a radioactive foil of nickel^{63} or tritium (H^3) which emits beta radiation to ionize the sample components.

Each type of detector has specific uses and capabilities. For example, the electron capture detector responds to electron adsorbing compounds such as halogens, nitrates and conjugated carbonyls. The flame ionization detector responds to almost all compounds except those listed in Table II-1.

Table II-1. Compounds giving little or no response in the flame ionization detector (from McNair and Bonelli, 1969)

Helium	Argon	Krypton
Neon	Xenon	Oxygen
Nitrogen	Carbon disulfide	Hydrogen sulfide
Nitrogen oxides	Ammonia	Carbon monoxide
Carbon dioxide	Water	Sulfur dioxide

The thermoconductivity detector responds to all compounds but is not very sensitive. The electron capture detector, although limited in selectivity, is very sensitive and can detect some pesticides at the sub-picogram level (McNair and Bonelli, 1969).

The linear range of a detector may be defined as the ratio of the largest to the smallest concentration within which the detector is linear. Table II-2 lists the linear range of the above three detectors The high linear range plus good sensitivity make the flame ionization the most widely used detector for trace analysis.

Detectors are heated to prevent condensation of sample components in the detector. In general, the detector temperature should be at least 10^{o} C higher than the maximum column temperature.

Table II-2. Linear range of common gas chromatography detectors (McNair and Bonelli, 1969)

Detector	Linear range
Thermoconductivity	10^4
Flame ionization	10^7
Electron capture	5×10^2

Recorder

The recorder provides a visual display of the detector's response to each component of the sample mixture. The recorder output is called a chromatogram. Figure II-2 illustrates a typical chromatogram. Each component of the sample is represented by a peak which occupies a specific position along the time axis.

Methods of Analysis

Qualitative analysis

Each component of the sample mixture occupies a specific position along the time axis. This is referred to as the retention time. Depending on the complexity of the sample mixture, each component will have a different retention time. Tentative component identification is based on its particular retention time obtained from a standard analysis.

In many cases, the sample is too complex for component identification based on retention time. Packings specific to certain homologous series can be used in this case to aid in component identification. Retention time is still used for identification but the selectivity of these columns improves the resolution.

The retention time is measured from the leading edge of the solvent front. This eliminates any errors incurred in the sample injection technique. All gas chromatography condition, such as

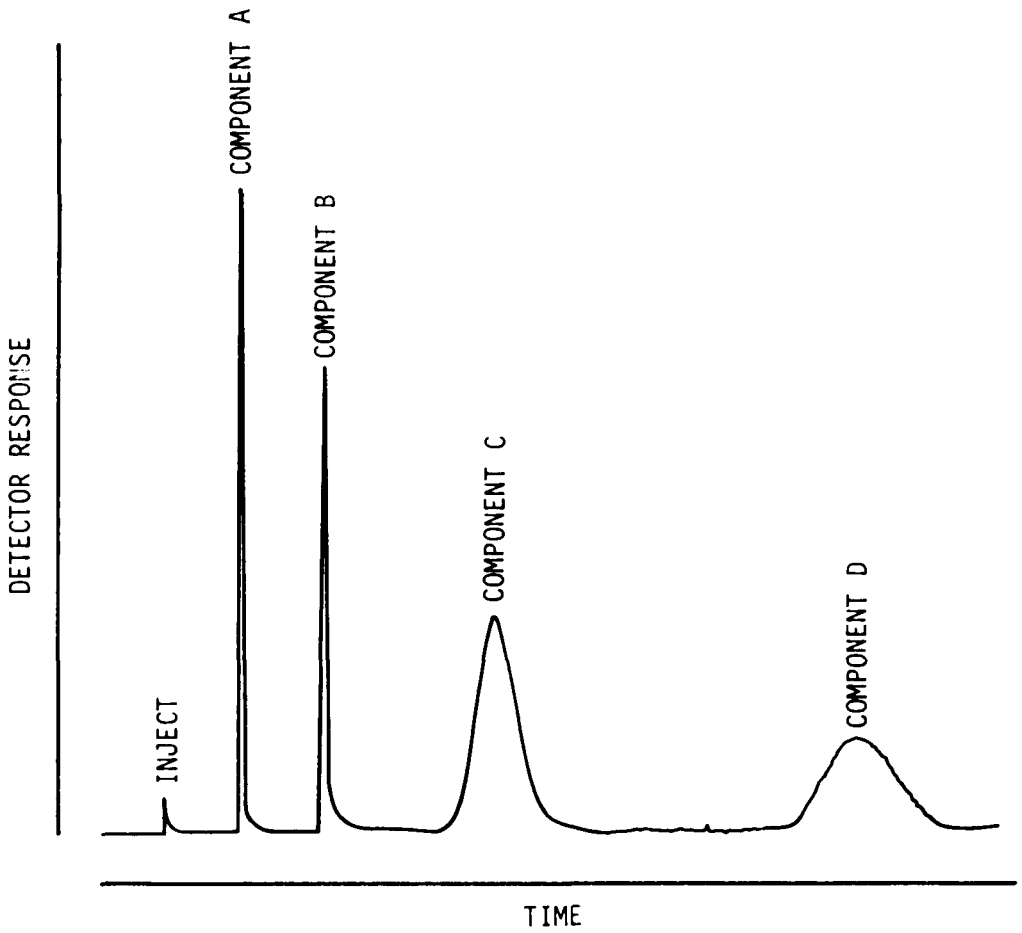

Figure II-2. Typical chromatogram from gas chromatography analysis

carrier gas flowrate, column temperature, etc., must remain the same so that retention time data does not vary appreciable. A more reliable method of identification is based on relative retention times. In this procedure, the retention time of an unknown relative to another component present in all samples is calculated. In general, the relative retention time is dependent only on column temperature and the type of packing material.

Confirmation of peak identity can be accomplished using specific detectors or a mass spectrometer. The electron capture detector is used to confirm the presence of halogenated compounds. Nitrogen-phosphorous or flame photometric detectors can confirm the identity of phosphorous-containing compounds. Dual detector operation is occasionally used for this purpose.

Quantitative analysis

Peak areas on the chromatogram are proportional to the concentration of their respective chemical components. In reference to Figure II-2, components displayed by peaks with very little baseline width can be quantified using peak height (component A for example). Peak height measurements are more rapid than the determination of peak area but the use of peak height results in a narrow linear range of calibration.

There are many different methods for the determination of peak area. Development of electronic integration systems has provided extremely rapid and accurate peak area determinations. A complete

discussion of the other methods of peak area evaluation is provided by McNair and Bonelli (1969).

Production of peaks on the chromatogram is the result of the detector response to compounds eluting from the column. However, different compounds have different detector responses. Peak areas are converted to concentrations through the use of response factors which are unique for each compound. The response factor for each compound is evaluated by the analysis of a known standard or by the use of response factor tables (Dietz, 1967). Analysis of standard solutions at various concentrations can be used to construct a calibration curve (Figure II-3). The slope of the calibration line is equal to the detector response factor for that particular compound.

There are several methods available for determination of component concentration based on peak areas. The first method, absolute calibration, requires the construction of a calibration curve for each component. New calibration curves must be determined each time any gas chromatographic conditions are changed. This method requires that the exact amount of sample injected must be known. After the calibration curve has been constructed, the concentration of the same component in the sample is determined by its peak area and the corresponding concentration from the calibration curve.

The second method is similar to the calibration curve except response factors are used. Based on the analysis of several injections of the same concentration of standard solution, the response factor

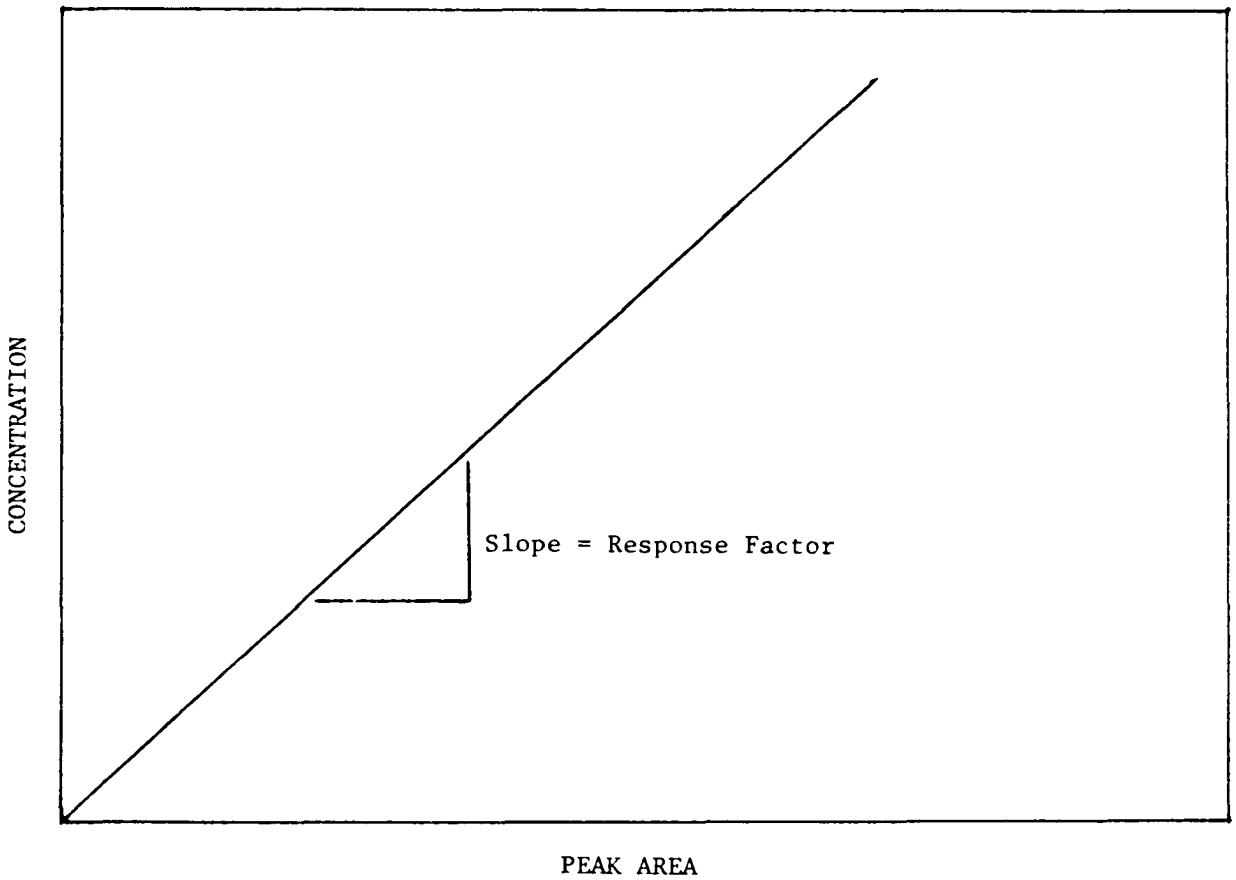

Figure II-3. Detector calibration curve

can be determined by the following equation:

$$\text{Response Factor (RF)} = \frac{\text{Standard Concentration}}{\text{Average Peak Area}} \qquad \text{(II-1)}$$

The concentration is then the product of the peak area and the calculated response factor. This method can be used only if it has been established that the calibration curve is linear within the analysis range.

Another method of calculating concentrations is called the area-normalization method. Using this method, the amount of one component is measured as a percentage of the total detector response for all the components of the sample. Referring to Figure II-3, the amount of component A could be expressed as follows:

$$\% \text{ A} = \frac{\text{Area A}}{\text{Total Area}} \times 100 \qquad \text{(II-2)}$$

This equation assumes that the detector response factors for all components is equal. The above equation requires modification to include the detector response factor for each component:

$$\% \text{ A} = \frac{(\text{Area A})(RF_A)}{(\text{Area A})(RF_A)+(\text{Area B})(RF_B)+(\text{Area C})(RF_C)+(\text{Area D})(RF_D)} \qquad \text{(II-3)}$$

A convenient method of expressing response factors is to make all response factors relative to the response factor of one of the sample components. Results from the area normalization method can be expressed as percent by weight or volume. This method is especially convenient since the exact injection volume need not be known.

Specific Methods of Analysis Used in this Study

Gas analysis

The gas chromatography conditions used to determine the amounts of nitrogen, methane and carbon dioxide in the product gas are shown in Table 11. Area normalization was used to determine the percent of each component in the gas. Response factors were expressed relative to nitrogen and were determined using standard gas mixtures[1]. The following relative response factors were used throughout this study:

1.000 - Nitrogen
1.174 - Methane
0.864 - Carbon dioxide

The gas composition was determined according to Equation II-3.

Further calculations were required to express the methane production as a percent of the total gas production which was used in Phase II. Each serum bottle contained a gas volume of approximately 150 mL. Samples for gas analysis were taken from this volume. However, gas production that occurred during the test must be taken into account. The total gas production is used to determine the percent methane production by the following equation:

$$\text{Methane production} = \frac{(150 + V) \times \% CH_4}{V} \qquad \text{(II-4)}$$

[1]Alltech calibration gases, 10% methane in nitrogen and 10% carbon dioxide in nitrogen, Alltech Associates, Deerfield, Illinois.

where:

150 = gas volume in serum bottles

V = total gas production prior to sample collection

% CH_4 = percentage of methane determined by analysis of the gas sample

Methane production = percent of total gas production

Volatile acids analysis

The concentrations of individual volatile acids were determined according to the gas chromatographic conditions listed in Table 12. Absolute response factors were used to determine concentrations. These response factors varied from day to day due to varying detector conditions and therefore are not presented here.

A volatile acid standard solution was prepared with the resulting concentrations shown in Table II-3. The standard solution was divided among twenty 2 mL serum bottles which were stored at $4^{o}C$, until needed. A new standard was used for each day's analysis. Normally, five injections of the standard solution were made to determine the response factor for each acid. This operation proceeded the analysis of samples. After analysis of the samples, several injections of the standard solution were made to check the calibration of the instrument. The results of seven injections of this type are also included in Table II-3. As shown by these results, the calibration of the instrument remained fairly consistent.

Table II-3. Volatile acid standard solution and accuracy and precision of analysis based on seven analyses

Component	Concentration in Standard, mg/L	Retention time, min.	Analysis of Standard. mg/L	Standard deviation, mg/L
Acetic acid	101.5	0.82	101.4	± 4.42
Propionic acid	49.9	1.36	48.7	± 1.59
Iso-butyric acid	10.1	1.89	9.67	± 0.43
N-butyric acid	10.0	2.39	9.87	± 0.35
Iso-valeric acid	9.8	3.48	9.25	± 0.79
N-valeric acid	10.5	4.64	10.1	± 0.47

Analysis of 2-nitrophenol

The gas chromatographic conditions used for the analysis of 2-nitrophenol are listed in Table II-4. Concentrations were determined

Table II-4. Operating conditions for the analysis of 2-nitrophenol

Gas chromatograph	Hewlett-Packard Model 5700
Column	6 ft X 2mm ID glass
Packing	Tenax GC, 80/100 mesh
Temperature	200° C
Carrier gas	Nitrogen
Flowrate	15 mL/min
Detector	Flame ionization
Temperature	250° C
Hydrogen pressure	18 psig
Injection port temperature	250° C
Sample size	
Liquid	2.0 μL
Gas	0.5 mL

using the absolute response factor method. The standard solution contained 100 mg/L of 2-nitrophenol in distilled water at pH < 2.

Analysis of nitrobenzene

The gas chromatographic conditions used for the analysis of nitrobenzene are listed in Table II-5. The standard solution contained 110 mg/L of nitrobenzene in distilled water. The absolute response factor method was used to determine concentrations.

Table II-5. Operating conditions for the analysis of nitrobenzene

Gas chromatograph	Perkin-Elmer Sigma I
Column	6 ft X 2mm ID glass
Packing	1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport
Temperature	100° C
Carrier gas	Nitrogen
Flowrate	30 mL/min
Detector	Flame ionization
Temperature	250° C
Hydrogen flowrate	44 mL/min
Injector temperature	225° C
Sample size	
Liquid	2.0 µL
Gas	0.5 mL

Analysis of 4-nitrophenol

The gas chromatographic conditions used for the analysis of 4-nitrophenol are listed in Table II-6. The standard solution contained

100 mg/L of 4-nitrophenol in distilled water at pH < 2 and absolute response factors were used to determine concentrations.

Table II-6. Operating conditions for the analysis of 4-nitrophenol

Gas chromatograph	Hewlett-Packard Model 5700
Column	6 ft X 2mm ID glass
Packing	Tenax GC, 80/100 mesh
Temperature	230° C
Carrier gas	Nitrogen
Flowrate	20 mL/min
Detector	Flame ionization
Temperature	300° C
Hydrogen pressure	18 psig
Injection port temperature	250° C
Sample size	
Liquid	2.0 μL
Gas	0.5 mL

Analysis of 2,4-dichlorophenol

The gas chromatographic conditions used for analysis of 2,4-dichlorophenol are listed in Table II-7. Concentrations were determined using the absolute response factor method. The standard solution contained 100 mg/L of 2,4-dichlorophenol in distilled water at pH < 2.

Table II-7. Operating conditions for the analysis of 2,4-dichlorophenol

Gas chromatograph	Hewlett-Packard Model 5700
Column	6 ft X 2mm ID glass
Packing	Tenax GC, 80/100 mesh
Temperature	215° C
Carrier gas	Nitrogen
Flowrate	20 mL/min
Detector	Flame ionization
Temperature	250° C
Hydrogen pressure	18 psig
Injection port temperature	250° C
Sample size	
Liquid	2.0 μL
Gas	0.5 mL

Analysis of chlorinated hydrocarbons

The same gas chromatographic conditions were used for the analysis of hexachloroethane, hexachloro-1,3-butadiene and hexachlorocyclopentadiene and are listed in Table II-8. Using this method, the following retention times were produced:

hexachloroethane,	1.23 minutes
hexachloro-1,3-butadiene,	2.33 minutes
hexachlorocyclopentadiene,	4.18 minutes

The standard solution contained 1 mg/L of each chlorinated hydrocarbon.

Table II-8. Operating conditions for the analysis of chlorinated hydrocarbons

Gas chromatograph	Perkin-Elmer Sigma I
Column	6 ft X 2mm ID glass
Packing	3% OV-1 on 100/200 Supelcoport
Temperature	100° C
Carrier gas	Nitrogen
Flowrate	30 mL/min
Detector	Electron capture
Temperature	300° C
Make-up gas	Nitrogen
Flowrate	30 mL/min
Cell current	0.25 nA
Injector temperature	225° C
Sample size	
Liquid	1.0 μL
Gas	0.5 mL