

INVESTIGATION OF THE EFFECT OF SELECTED TOXICANTS
ON THE BEHAVIOR OF INTERMEDIATE GASES
IN ANAEROBIC DIGESTION

A Master's Project Presented

By

Eugenio Giraldo-Gomez

Submitted to the Department of Civil Engineering of the
University of Massachusetts in partial fulfillment
of the requirements of the degree of

MASTER OF SCIENCE IN CIVIL ENGINEERING

May 1989

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Department of Civil Engineering



UNIVERSITY OF MASSACHUSETTS
AT AMHERST

Department of Civil Engineering

Marston Hall
Amherst, MA 01003
(413) 545-2508

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Approved as to style and content by:

Dr. Michael S. Switzenbaum, chairperson

Dr. David A. Reckhow, member

Dr. James K. Edzwald, member

Dr. William H. Hightler, Department Head

To the memory of my parents

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As Ortega y Gasset said " I am myself and my circumstances", and it is to my circumstances that I want to thank. Alvaro Orozco that set the ball to run, the Organization of American States and the Commonwealth of Massachusetts that sponsored my studies, all the people in the EVE program, Dan, Kajsa and Juliana for their help in the lab, Bob Hickey for his permanent advice in the lab and his sharing of knowledge and literature, Barb for her moral support, the bugs that behaved so well during this time, and last but not least, Mike Switzenbaum for his advice through all this time, his patience and cheer and interest in me.

ABSTRACT

Development of an early warning system for process upsets in anaerobic digestion would be an important step in the improvement of the reliability of anaerobic processes. Conventional indicators present slow responses and supply limited information about the metabolic status of the microorganisms in the digester.

The objectives of this research were to examine the behavior of the trace gases hydrogen and carbon monoxide as indicators of toxic events in a suspended growth sucrose enrichment culture. A literature review of anaerobic process characterization was done. Hydrogen and carbon monoxide are trace gases present in anaerobic digestion and show potential for monitoring due to the short relaxation times, and relationship with the metabolic status of the ecosystem. Four different heavy metals and two organic substances were tested. A modification of the anaerobic toxicity assay, ATA, was used during the experimental part. A mathematical model was also developed, and a sensitivity analysis of the responses of methane, hydrogen and carbon monoxide to variation in the activity of the different populations of microorganisms in the ecosystem was done.

The mathematical model showed potential to describe the behavior of hydrogen and carbon monoxide, although calibration is required. Carbon monoxide seems to be, in fact, in thermodynamic equilibrium with hydrogen, methane and acetate concentrations. In addition, the carbon

monoxide source is most likely acetoclastic methanogens in this system. The toxicants acted differently in the different populations of the microbial ecosystem. Some populations are more affected than others at low applied dosages. This selective inhibition behavior raises a serious objection to the use of conventional indicators in toxic scenarios. Carbon monoxide showed to be a better indicator of low toxic effects than hydrogen. Statistically significant variations of carbon monoxide occur in the first 2.5 hours for toxic events of organic substances, and in the first 6 hours for heavy metals toxic events. This time response would be dependent on the volumetric load of the system.

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CHAPTER I
INTRODUCTION

The advantages and disadvantages of anaerobic digestion processes, when they are compared with aerobic processes for wastewater treatment, are well-known by environmental engineers. One of the principal obstacles for the wider use of anaerobic processes is its reputation for being an unstable process (i.e., difficult to control and operate). In fact, the nature of the microbial interactions between the different populations of microorganisms (Figure 1) in anaerobic digestion is complex and, still, not totally understood. The key of successful operation of an anaerobic process is maintenance of the delicate balance among the different trophic groups in the consortium, under the changing characteristics of their environment. The development of an early warning indicator for the potential imbalance situations would be an important contribution to the improvement of the reliability of anaerobic processes.

Studies on the principal causes of imbalances in anaerobic digesters for sludge stabilization, point to toxic events and organic overloads as the principal causes (Swanwick et al., 1969). When anaerobic digestion processes are applied to industrial wastes, temperature variations in the waste should be considered in addition (Iza, 1988).

In Figure 2 is presented an illustration of a typical monitoring/control scheme for an anaerobic digester. Controlable

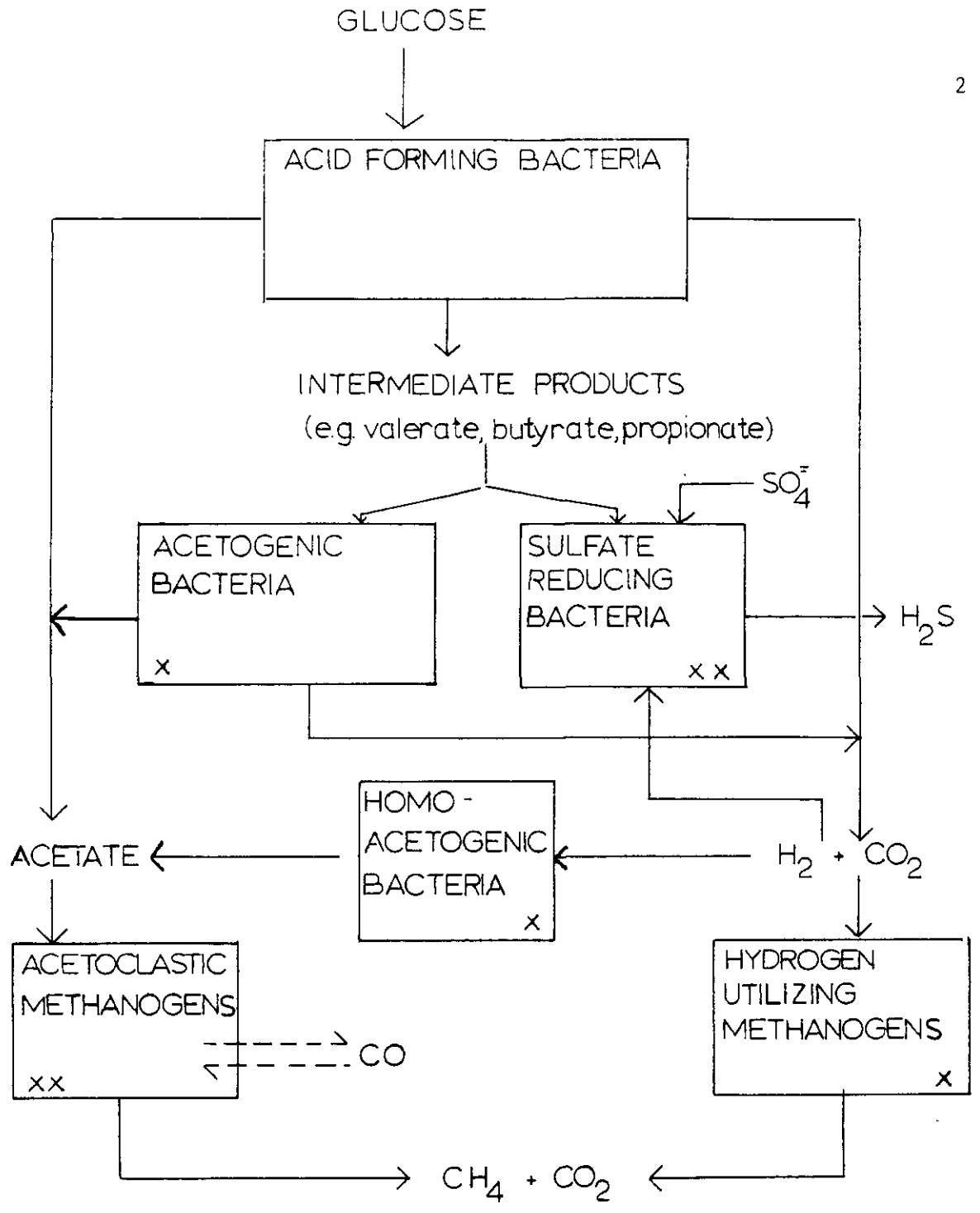


Figure 1. Different microbial populations in an anaerobic digester. Bacterial species marked with x use Acetyl-CoA pathway in an anabolic way. Those marked with xx use the pathway in a catabolic way.

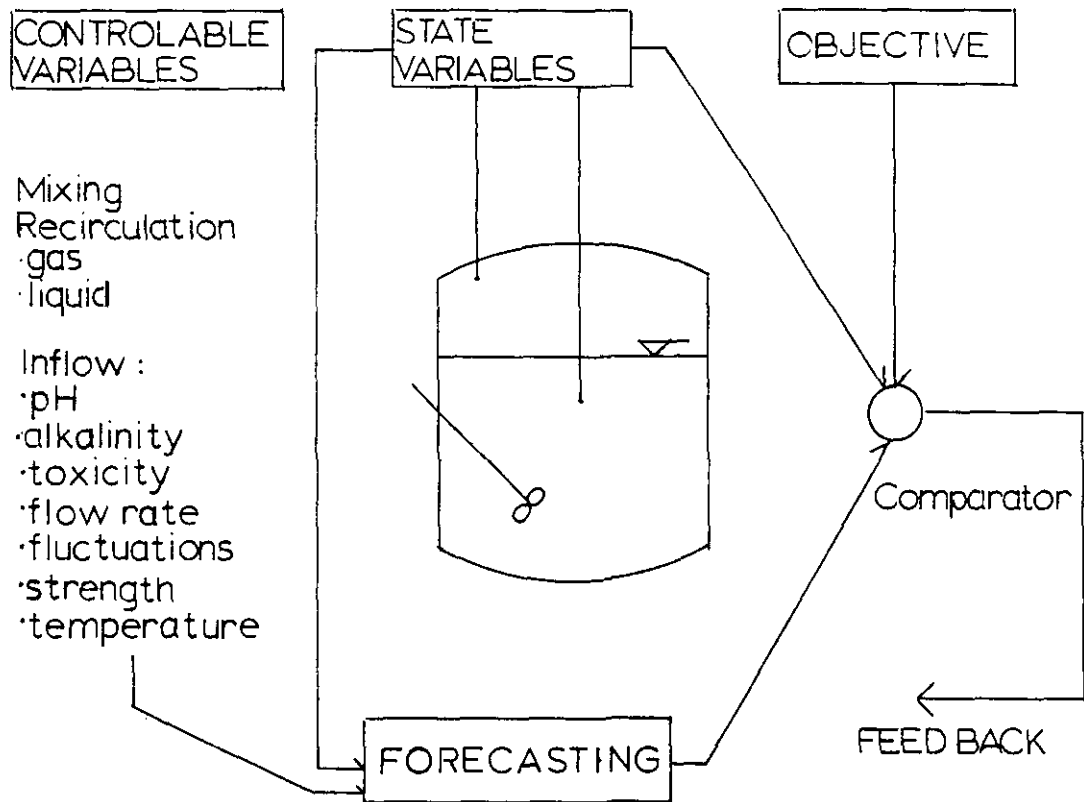


Figure 2. Schematic of a control system for an anaerobic digester.

variables include the parts of the system that can be manipulated (e.g. characteristics of the inflow stream, digester mixing, recirculation). State variables include the parameters that we actually measure in the reactor to characterize its chemical, physical and biological status. This variables will be discussed in chapter 2.

In order to achieve the objective it is necessary to be able to predict the behavior of the system when a defined input occurs. This prediction usually requires the definition of the system in term of the state variables. Most of the work reported in the literature focuses on the monitoring of the liquid phase and solid phase of the digester as a means of determining the current status of the process. However, monitoring of the process in the liquid phase presents several disadvantages. Measurement of the parameters in the liquid phase usually involves laboratory techniques not suitable for on-line data analysis. The environmental conditions inside the liquid phase of the digester rapidly generate chemical and physical fouling of the measurement probe. Mixing problems in the liquid phase are common; thus, several samples are necessary to obtain a composite characteristic of the digester. On the other hand, monitoring of the gas phase of anaerobic digesters allows a real-time on-line estimation. The gas phase gives a composite picture of the system, and the environmental conditions are less severe than those in the liquid phase.

The objective of this research effort is to investigate the behavior and usefulness of the trace gases, hydrogen and carbon monoxide, as early warning indicators of imbalances in a suspended

growth sucrose enrichment culture subject to toxic concentrations of heavy metals and selected organic toxicants. This investigation is part of an ongoing research effort on the monitoring of anaerobic processes in the Department of Civil Engineering of the University of Massachusetts at Amherst. This project is a direct continuation of the doctoral work of Robert Hickey (1987). In his dissertation, the importance of carbon monoxide as an indicator of the activity of acetoclastic methanogens in mixed cultures which naturally occur in anaerobic digesters treating waste activated sludge was first observed and proposed (Hickey, 1987).

CHAPTER II

BACKGROUND

2.1. CHARACTERIZATION OF ANAEROBIC DIGESTION

The anaerobic digestion process as applied in current engineering practice can be pictured as a three-phase process (solid-liquid-gas) (Figure 3). Each phase is closely related to the other two, and sometimes information drawn from one phase can be directly related to the status of the others. The objective of a good monitoring strategy is to fully characterize the current status of the anaerobic digestion process. Achievement of this objective allows us to control the process in order to produce the desired results in a reliable way. Several parameters have been proposed to characterize the anaerobic digestion process, and they will be discussed in the following sections.

2.1.1. Solid Phase Characterization.

Solid phase, as discussed in this section, is the combination of non-soluble materials immersed in the liquid phase. This mixture is comprised of organic and inorganic solids, the former of which can be divided into inert organic solids and cells (Figure 3). Measurement of the active cells and their current metabolic status are very important parameters in defining the control strategies that can be undertaken

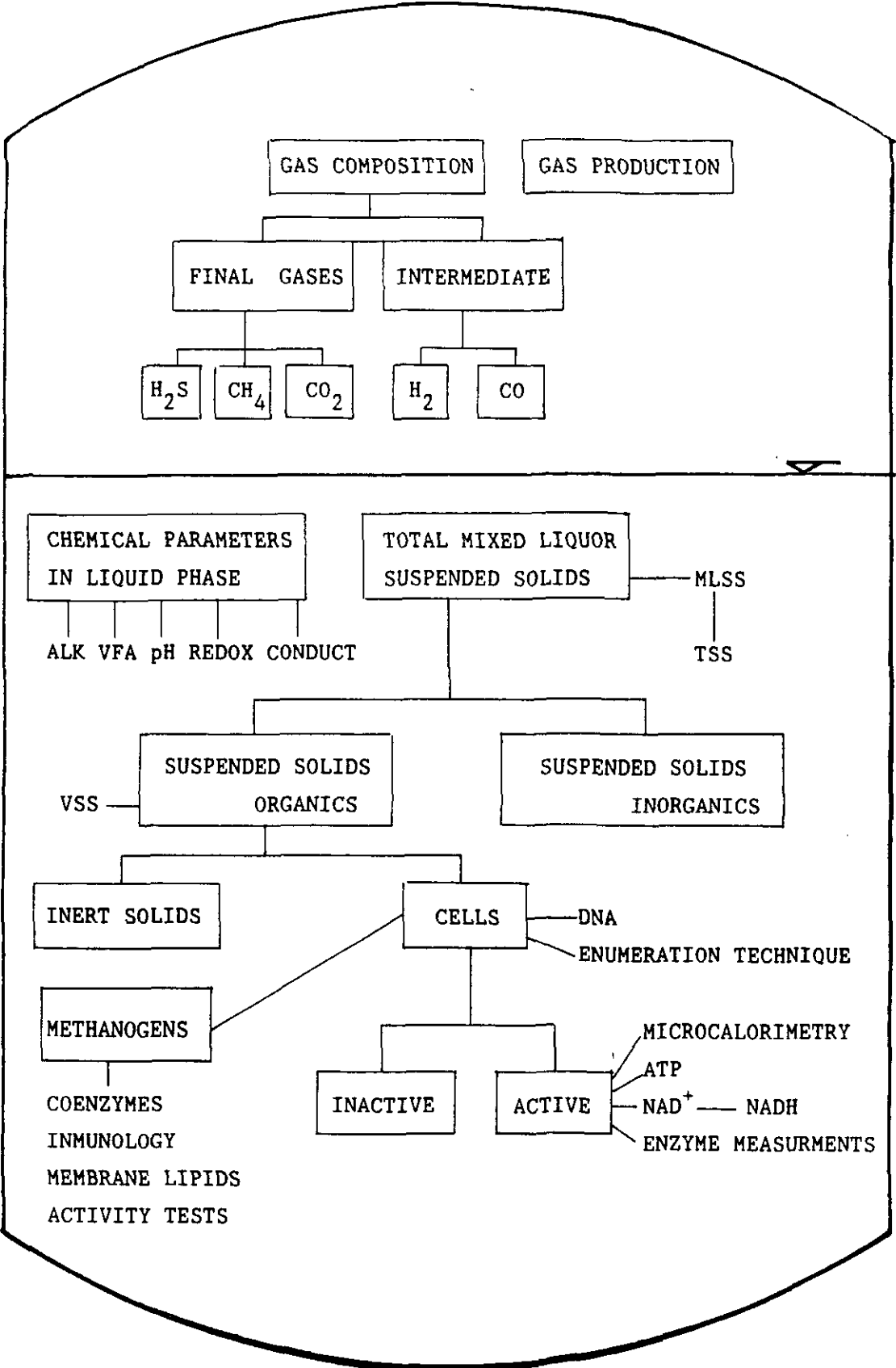


Figure 3. Characterization tests for anaerobic digesters.

(e.g. determination of maximum potential substrate utilization rate). Chemical parameters in the liquid phase provide little, if any, information about the metabolic status of the microorganisms. Thiel et al. (1968) found high variation in the microbiological and biochemical characteristics of anaerobic digesters when the liquid phase parameters reflected steady state conditions. A brief discussion of the different proposed parameters for the characterization of the microbiological status is now presented.

a. Solids.

In general, solids are not a good estimation of the active microorganism population when the substrate entering the reactor has suspended solids itself (Figure 3). In spite of these drawbacks solid measurements are the most common parameter of microbial biomass characterization used in practice. Solids are usually measured gravimetrically; however, Grune (1965) found a good correlation between solids content in a digester and conductivity measurements, proposing this last parameter as a way to control the degree of stabilization of the sludge in the digester.

b. Cell Enumeration.

Several researchers have tried to use direct cell enumeration techniques to study the microbial ecosystem in an anaerobic digester (Heukelekian and Heineman, 1939; Smith, 1965; Siebert and Hattingh, 1967; Siebert et al. 1968). The procedures are elaborate and time-consuming,

and are therefore not suitable for regular use in engineering applications.

c. Deoxyribonucleic Acid (DNA).

Determination of DNA gives an estimate of the microorganisms present in the anaerobic sludge since the amount of DNA per cell is fairly constant and this substance is synthesized only by the living material present. DNA, like volatile suspended solids measurements, is an aggregated measurement that does not distinguish between the different trophic groups in the anaerobic ecosystem. (Agardy and Shepherd, 1965; Hattingh and Siebert, 1967; Hattingh et al., 1967). These researchers also found good correlations between the DNA content of the sludge and suspended solids. Determination of DNA probably did not become a more popular measurement due to this finding and the ease of suspended solids analyses. Furthermore, no information about the metabolic status of the microorganisms is supplied by DNA determination.

d. Protein Content.

Hattingh et al. (1967a) and Hattingh et al. (1967b) report the determination of protein content of sludges as an indirect way of measuring the microbial biomass. Good correlation was found between the protein content and DNA content of the sludges. They also concluded that the protein content cannot be estimated by information on the organic-N of the sludges. Similarly, the determination of protein content provides no information about the metabolic status of the microorganisms.

e. Bacterial Lipids.

Henson et al. (1985) measured the bacterial lipid content of an anaerobic thermophilic digester. Phospholipids are found in the membrane of the cells in a relatively constant proportion, and have a relatively rapid turnover in dead and living cells. Additionally, certain phospholipids can act as fingerprints of different types of bacteria (e.g. Archaeobacteria have different lipids in the outer layer). These researchers propose that bacterial lipids analysis would provide relevant information about microorganism numbers, ecosystem composition and nutritional status. More recently Henson's group reported the characteristic phospholipid fatty acid composition of the syntrophic anaerobic bacterium Syntrophomonas wolfei (Henson et al., 1988). The methods are destructive, however, and are not suitable for on-line measurements .

f. Adenosine Triphosphate (ATP).

Chung and Neethling (1988) have recently proposed the use of ATP as an indicator of activity in anaerobic digesters. Their results show that ATP reflects changes in activity in the digester as well as toxic inhibition. Adebowale and Kiff (1988) used ATP measurements to investigate the development of granulation in an UASB reactor. No difference in ATP concentrations was detected upon granulation. ATP, though, correlated satisfactorily with reactor COD removal and gas production. The determination is not suitable to on-line monitoring and

no distinctions can be made between the different populations of bacteria in the digester.

g. Enzyme Activities.

Enzymes are responsible for all biological changes brought about by microorganisms. Activities of well-selected enzymes will give an estimate of the substrate flow in the microbial ecosystem. Agardy et al. (1963) developed a method to measure protease activity in anaerobic digesters. Protease activity was shown to be a good indicator of digester stress during an organic overload. Thiel and Hattingh (1967) and Thiel et al. (1968) measured four hydrolytic enzymes. During normal digestion, enzymes reached stable activities and presented significant variations approaching failure of the reactor.

Ashley and Hurst (1981) measured phosphatase activity in a laboratory sludge digester. Phosphatase activity measurements were found to reflect toxic events and organic overload before accumulation of volatile acids occurred. Lenhard (1968) proposed a method for measuring dehydrogenase activity in anaerobic digesters. Florent (1983) reported that dehydrogenase could provide an estimation of the active biomass in anaerobic digesters. In aerobic ecosystems, an analogous procedure is used for toxicity screening and process control. More recently, hydrogenase activity of methanogenic bacteria in pure and mixed culture was determined by Liwen et al. (1988). These researchers found good correlation between the enzyme activity and the methanogenic activity of the sludge tested.

Although these proposed measurements can potentially help to characterize more precisely the metabolic status of the biological phase, they are not suitable for on-line monitoring.

h. Methanogenic Activity Measurements.

A number of activity assays for evaluating the anaerobic digestion process have been proposed (Speece, 1988; Iza, 1988; Dolfing and Bloemen, 1985; Valcke and Verstraete, 1983; Reynolds et al., 1987a,b). The purpose of these tests is to evaluate the maximum potential utilization rate(MPUR) of intermediate products in the anaerobic digestion process. Comparison of these data with microbiological data from pure cultures (e.g. maximum specific growth rate, yield coefficient, substrate affinity constant) allows an estimation of the size of the different populations in the consortium and provides a diagnosis of the status of the microorganisms. Currently, these assays have not been implemented on-line, and require a long time (hours) to be performed.

Another diagnostic assay recently proposed is bioavailability of iron, cobalt and nickel (Speece, 1988). In this assay the stimulatory effect of these nutrient metals on the methanogens is evaluated .

i. Microcalorimetry.

Heat released during the metabolic activity of the microorganisms can be theoretically related to the size of the microbial population(s), the metabolic status and the metabolic activity of the microorganisms

involved. Heijnen et al. (1986), Pauss et al. (1988) and Samson et al. (1988) have used microcalorimeters to evaluate the activity of the anaerobic digestion ecosystem. In this method, continuous heat flow from an auxiliary reactor is measured. Toxic effects generate a drop in the heat signal, allowing rapid identification of toxicity problems. Samson et al. (1988) found that 95% of the heat flow from a combined ecosystem was generated by the acidogenic microorganisms. This assay is suitable for on-line monitoring of the process; but its application to full-scale systems remains to be proven.

j. Co-enzymes and C1-carriers of Methanogens.

Methanogens possess a unique set of structural and functional molecules that permit the development of several tests to qualify and quantify their presence (Taylor, 1982). One of the tests that has received much attention by researchers is the quantification of coenzyme factor F-420 (Gorris et al., 1988; Reynolds and Colleran, 1987; Dolfig and Mulder, 1985; Zeeuw de, 1984; Pause and Switzenbaum, 1983; Delafontaine et al., 1979). Coenzyme factor F-420 is a low molecular weight compound that fluoresces and has been shown to be present in anaerobic environments only in methanogenic bacteria (Taylor, 1982; Cheeseman et al., 1972). Attempts have been made to relate the activity of the methanogens in an anaerobic ecosystem and the F-420 content of the ecosystem biomass. Results show that the methanogenic activity cannot be unambiguously characterized by F-420 (Pause and Switzenbaum, 1983; Switzenbaum and Eimstad, 1987; Dolfig and Mulder, 1985).

More recently, Gorris et al. (1988) used High Performance Liquid Chromatography (HPLC) to distinguish structurally different types of coenzymes F-420 and Methanopterin (MPT, a C1 carrier specific for methanogens) present in the hydrogenotrophic and acetotrophic groups in an anaerobic digester. Using this technique they were able to develop good correlations between the different coenzymes measured and the hydrogenotrophic and acetotrophic methanogenic potential of granular sludge. Shulze et al. (1988) used acetone extraction with reverse phase HPLC to separate F-420 from an UASB sludge, finding a satisfactory correlation between the methanogenic activity and the cofactor. Although these techniques look promising to use in evaluating the capacity of the anaerobic consortia, expensive equipment and sophisticated laboratory protocols are necessary. In addition, it is not clear if the changes in F-420 are rapid enough to reflect a change in the metabolism of methanogens in a time frame adequate for an oportune correction action.

Samson et al. (1988), used a miniaturized fluorescence detector to monitor the level of F-420 on-line, without an extraction procedure. Although the probe was useful in pure bacterial cultures, when applied to anaerobic sludge did not produce satisfactory results due to the dark color of the sludge.

k. Immunology of Methanogens.

Use of immunological reactions for the study of microbial ecology is a developed tool (Schmidt et al., 1968). Strayer and Tiedje (1978) and Ward and Frea (1980) used Fluorescent Antibody technique to study methanogenic populations in lake sediments and anaerobic digesters. Robinson and Erdos (1985) used immuno-electron microscopy to identify Methanosarcina spp. in anaerobic digester fluid. Conway de Macario et al. (1981) used polyclonal antibody probes to determine the antigenic map ("fingerprint") of 17 methanogens. The same researchers (Macario and Conway de Macario, 1983) used monoclonal antibodies to identify methanogens in microbial ecosystems resembling anaerobic digesters.

Two different types of assays have been developed for this purpose - Slide Immunoenzymatic Assay (SIA) and Indirect Immunofluorescence (IIF). Both of them are run on glass slides and provide a relatively quick procedure for identifying methanogens in mixed cultures (Macario and Conway de Macario, 1985). Archer (1984) and Kemp et al. (1988) have applied the Enzyme-linked Immunoabsorbent Assay (ELISA) to detect and quantify methanogens in complex systems.

These new developments seem promising for the ecological study of digester populations, but do not allow for on-line monitoring.

2.1.2. Liquid Phase Characterization.

Parameters used to characterize the chemical status of the liquid phase are the most commonly used (Zickefoose and Hayes, 1976).

Nevertheless, variation in liquid phase characteristics are the result of the imbalances that are to be prevented. On-line monitoring of these parameters can be implemented, but calibration and maintenance problems make the long term performance difficult.

a. pH.

pH is an important parameter in the characterization and control of anaerobic digestion due to the inhibitory effects of low pH on the activity of anaerobic digestion bacteria (Clark and Speece, 1970). Additionally, pH can produce several side effects; it controls the fraction of undissociated fatty acids that are thought to freely permeate the cellular membrane of microorganisms, internally dissociate which lowers the cytoplasmic pH, and in this way affect the bacterial metabolism (Zoetemayer et al., 1982). This subject has been a matter of several decades of discussion among researchers (Anderson et al., 1982; McCarty and McKinney, 1961). Nevertheless, the occurrence of low pH is the result of a well-developed imbalance and as such is not useful as an early warning indicator.

b. Volatile Fatty Acids (VFA).

The most popular parameter for sludge digestion monitoring is the determination of volatile fatty acid (VFA) concentration. Volatile fatty acid accumulation reflects a kinetic uncoupling between acid producers and consumers and is typical of stress situations (Zickenfoose and Hayes, 1976; Dague, 1968; Asinari di San Marzano et al., 1981;

Pohland and Engstrom, 1963). Accumulation of VFA can also be a cause of subsequent problems if the system lacks enough buffering capacity to avoid a drop in the pH. Along with other parameters such as alkalinity and pH, determination of VFA levels allows the control of slowly developing failures caused by organic overloads. Its utility as operation control parameter in anaerobic processes functioning under toxic stress scenarios is not clear. Several laboratory procedures exist for the determination of VFA's.

c. Alkalinity.

A direct relationship exist between alkalinity variations and VFA accumulation in anaerobic digestion. Jenkins et al. (1983) and Ripley et al. (1986) have proposed the control of anaerobic digestion based on alkalinity titration. Automatic control of anaerobic digestion based on alkalimetric measurements was proposed by Rozzi (1983) and Colin (1984). A more sensitive parameter for monitoring digesters is the ratio VFA/Alkalinity (Zickenfoose and Hayes, 1976). These researchers recommend a ratio between 0.1 and 0.35 for a healthy digester. Similarly to VFA measurements, the utility of alkalinity as a control parameter in anaerobic digesters under toxic stress scenarios has not been established.

d. Redox Potential.

In theory, the measurement of redox potential would provide a good means of accurately describing variations in the intermediate product

composition in an anaerobic digester (Dirasian et al., 1963; Molof et al., 1963; Grune, 1965). These researchers measured redox potential in anaerobic digesters subject to overloading, and found a good correlation between the electrode potential measurements and the volatile fatty acids accumulation. Electrolytic pH control was practiced with apparent success. Nonetheless, according to Stumm (1966) the application of redox measurements to multi-redox component systems not in equilibrium or metastable equilibrium is meaningless.

e. Specific Sulfide Electrode.

Mosey et al. (1971; 1975) proposed the use of sulfide ion activity monitoring as a way to detect inhibitory concentrations of heavy metals in anaerobic digestion. The rationality of the proposal stands on the low solubility of heavy metals sulfide salts. Detection of a decrease in the activity measurements of the sulfide ion via a silver/silver specific sulfide electrode would indicate an inhibitory concentration of heavy metals in the digester. pS values over 14 were proposed as inhibitory. Although the reported experiences with laboratory digesters were promising, no further reports indicating the use of the electrode in practice are known by the author of this review.

2.1.3. Gas Phase Characterization.

a. Gas Production.

Rates of gas production, and more specifically the methane yield, can potentially be a good indicator of the metabolic status of the digester (Dague, 1968). Lowering of methane production rates, when compared to the influent rate of organic matter, gives warning of the accumulation of soluble acid products in the liquid phase. Unfortunately, this is again the result of an imbalance rather than a warning of it.

b. Gas Composition.

The principal gases in the gas phase of an anaerobic digester are CO_2 and CH_4 . Variations in the relative proportions of both gases are typical of digester imbalances (Zickefoose and Hayes, 1976). Unfortunately the variations are significant only after the imbalance is well developed.

Better understanding of the microbiology of the anaerobic digestion during the recent past has indicated that some other gases are present in trace amounts in the digester atmosphere. These gases could potentially be good indicators of the complex process occurring in the other phases of the digester. A more detailed discussion of H_2 and CO as trace gas indicators will be presented in Sections 2.2 and 2.3.

2.1.4. Summary.

Substantial amounts of research have been done in order to identify the parameter or combination of parameters that characterize the

physical, chemical and metabolic status of an anaerobic digester. Microbial ecosystems developed in a particular anaerobic reactor are unique to that particular system with the common characteristic of producing methane and carbon dioxide as final products.

Characterization of the metabolic status of the anaerobic digestion microbial ecosystem has been the least successful effort. Much work is needed to develop simple tests that allow the recognition of the relative importance of the different populations present in an anaerobic digester as well as their metabolic status. Ideally, these tests should quickly reflect changes in the metabolism of the population affected permitting an opportune remedial action. Physical-chemical parameters measured in the liquid phase are the most commonly used parameters. Nonetheless, variations of physical-chemical parameters in the liquid phase are slow, see Table 1, and their determination usually involves aqueous laboratory techniques not suitable for real-time data acquisition.

As a result, physical-chemical characterization in the liquid phase usually reflects a posteriori a stress situation in the biological phase. This situation is acceptable when the characteristic time frame of digester operation is of several days as is the case in sludge digestion, and there is not a substantial risk of toxic events. With the recent development of anaerobic digesters with short hydraulic residence time for domestic and industrial wastewater treatment, where the objective is low concentrations of BOD in the effluent, it is necessary to develop characterization strategies that permit rapid identification

TABLE 1. THEORETICAL RELAXATION TIMES FOR DIFFERENT SUBSTANCES AND MICROBIAL POPULATIONS IN ANAEROBIC DIGESTION (THIS STUDY).

<u>SUBSTANCE</u>	<u>RELAXATION TIME</u>
H ₂	15 sec
Glucose	3 min
CO ₂	1 hr
Acetate	2 hr
Propionate	4 hr
Methane	2 days
CO	?*
<u>MICROBIAL POPULATION</u>	
Acidogens	30 min
Propionate Consumers	5 days
Acetoclastic Methanogens	10 days
Hydrogenotrophic Methanogens	25 days

*Most likely controlled by H₂ relaxation time.

of stress situations of the biological population in the digester while they are occurring and remedial action can be taken.

2.2. HYDROGEN MONITORING IN ANAEROBIC DIGESTION.

The presence of hydrogen in man-made anaerobic digesters has long been recognized (Imhoff, 1923; Hatfield, 1928). However, its importance in the regulation of the carbon and electron flow between different species in anaerobic ecosystems was not fully recognized until recently (Wolin, 1982).

One of the principal characteristics of anaerobic fermentations is the smaller free energy change during the microbial-catalyzed reactions relative to the aerobic counterpart (Thauer et al., 1977). Therefore, variations in the concentration (activity) of products and/or reactants of the fermentation reaction make a significant difference in the amount of free energy available in the reaction (Thauer et al., 1977). Molecular hydrogen is usually produced in high stoichiometric amounts during these reactions, and consequently it is an important factor in the determination of the free energy change. Anaerobic bacteria are sensitive to free energy change variations and adjust their biochemical machinery to the energetic conditions of the environment (Gottschalk, 1986).

Several effects of hydrogen on anaerobic digestion have been identified:

- Shifts in the fermentation products of the acidogenic step (Scheifinger et al., 1975; Chung, 1976; Winter and Wolfe, 1980; Braun and Gottschalk, 1981; Rode et al., 1981; Laube and Martin, 1983; Marty, 1985; Van Andel et al., 1985; Cohen, 1982).

- Inhibition of the Beta-oxidation of long chain fatty acids generated during lipid degradation (Stieb and Schink, 1985; McInerney and Bryant, 1979).

- Inhibition of the degradation of alcohols and volatile fatty acids (Ahring and Westermann, 1988; Smith, 1987; Kaspar and Wuhrman, 1978; Boone, 1984).

- Modification of the rate of degradation of butyrate by syntrophic associations in mesophilic and thermophilic conditions, and the rate of benzoate degradation (Ahring and Westermann, 1988; Dwyer et al., 1988; Dolfing and Tiedje, 1988).

- Reversal of the Beta-oxidation reactions of fatty acids degradation (Smith, 1987)

- Shift in the degradation products of propionic acid and ethanol (Smith, 1987).

- Inhibition of the methanogenesis from acetate in Methanosarcina strains grown in pure culture. (Baresi et al., 1978).

These metabolically diverse phenomena are usually explained based on the energetic interactions of products, reactants and intracellular

electron carriers (e.g. NADH) of the microorganism catalyzing the reaction. Few of these studies have been designed to determine energetic-kinetic interactions in the reactions studied. Anaerobic digestion ecosystems as they occur in engineering practice are dynamic systems subject to changing environmental conditions. Therefore, the direct extension of these results and rational explanations to engineering systems may prove to be insufficient to explain the complex phenomena usually seen, as suggested by the results of Smith (1987). Additional research is needed in this area.

2.2.1. Sources and Sinks of Hydrogen in Anaerobic Digestion.

As has been pointed out before, microbial populations, the sequence of reactions giving rise to the end-products, and the prevailing environmental conditions are characteristic for each anaerobic reactor and will be dependent on several factors. These factors include: composition of the substrate, number of species of microorganisms present in the inoculum, temperature, loading rate, hydraulic residence time, sludge residence time, mixing characteristics, and the type of reactor. As a result, hydrogen can be transported from the influent organic matter to methane by different intermediate products characteristic of a particular reactor.

Molecular hydrogen can be produced at several points in its conversion to methane (see Figure 1).

a. Carbohydrate Fermentation.

The fermentation of carbohydrates has been extensively studied for industrial purposes. Different fermentation products can be produced, and the principal metabolic routes have been studied and indentified (Wood, 1961). Metabolic pathways such as glycolysis, the tricarboxylic acid cycle, the Entner-Douduroff pathway and the pentose phosphate cycle (shunt) for the degradation of carbohydrates have been found operative in anaerobic digesters (Kotze et al., 1968).

In a well-operating digester partial pressures of hydrogen are usually very low (Smith, 1987). In this situation fermentation products of carbohydrates have been found to be principally acetic acid and hydrogen, with minor ammounts of other compounds as propionic acid, butyric acid, lactate, ethanol; the relative production of these last ones varying according to the situation (Gottschalk, 1986; Wolin, 1982a, 1982b; Mosey and Fernandes, 1988). Sykes (1970) found that during the anaerobic digestion of sewage sludge carbohydrates (cellulose) were the principal source of hydrogen in the system.

b. Protein and Amino Acid Fermentation.

Protein and peptides must be degraded to smaller molecules , amino acids, before they can be absorbed by microorganisms. Each amino acid has characteristic pathways by which it may be degraded. Amino acids may be utilized in different ways by different microorganisms (Kotze et al., 1968; Barker, 1961). The general processes of amino acid degradation are: transamination followed by deamination, oxidative deamination and

the Stickland reaction, the last reaction involves a pair of amino acids, with one being reduced and the other oxidized. (Kotze et al., 1968; Baker, 1961).

The end-products of amino acid fermentation are somewhat similar to carbohydrate fermentation, however some additional compounds are produced (e.g. valeric acid, caproic acid, ammonia and some branched fatty acids as isovaleric and isocaproic). Valeric and isovaleric acid have been found to play a significant role in overloaded anaerobic digesters treating secondary sludge from sewage treatment plants (Hickey, 1987). Ammonia plays a significant role in the pH regulation of a digester (Mc Carty, 1964).

Hydrogen can be produced and consumed in the amino acid fermentation (Kotze et al., 1968). Few studies exist that examine the effect of hydrogen in amino acid fermentation. Winter et al. (1987) found that in the presence of hydrogen-consuming microorganisms the preferred metabolic pathway of alanine-glycine mixtures switches from Stickland reaction to syntrophic utilization of alanine. Nagase and Matsuo (1982) found no significant effect in the degradation of several proteins during experiments that selectively inhibited the hydrogen consuming methanogens. Sykes (1970) found that proteinaceous material was not a significant source of hydrogen in sewage sludge digesters. According to these limited data it would seem that degradation of proteins is the least affected set of reactions by hydrogen concentrations in the environment, due primarily to the wide variety of metabolic pathways available.

c. Fats and Fatty Acid Degradation.

Fats are hydrolyzed in anaerobic digesters producing long chain fatty acids and glycerol. Short chain fatty acids are produced from the fermentation of carbohydrates and proteins. In the presence of oxygen, long chain fatty acids are completely degraded by beta-oxidation via acetyl-CoA units. Under anaerobic conditions, fatty acid oxidation probably occurs in a similar fashion (Stieb and Schink, 1985). The reducing equivalents obtained in the beta-oxidation are usually released as molecular hydrogen. Under standard conditions the Gibbs free energy of the reaction is unfavorable for the reaction to occur (Thauer et al., 1977). Molecular hydrogen is consumed by hydrogenotrophic methanogens or sulfate reducers, in some ecosystems (see Figure 1) maintaining the hydrogen partial pressure low and changing the free energy in such a way that the beta-oxidation reaction may occur (McInerney et al., 1979). This kind of association between hydrogen producers and consumers has been named by Bryant and his coworkers as syntrophic association (Bryant et al., 1967). Similar associations have been found for the degradation of isovalerate (Stieb and Schink, 1986), butyrate (McInerney et al., 1979; Stieb and Schink, 1985), and propionate (Boone and Bryant, 1980).

d. Hydrogen Production During Alcohol Degradation.

Alcohols are produced during the fermentation of carbohydrates, although in stable one-phase anaerobic digesters they are not considered an important intermediate (Gottschalk, 1986). Methanol is directly reduced with hydrogen to methane by methanogenic bacteria (Muller et

al., 1986; Miller and Wolin, 1985; Lovley and Klug, 1983; Smith and Mah, 1978). Other alcohols are found to be degraded by syntrophic associations involving interspecies hydrogen transfer (Bryant et al., 1967; Eichler and Schink, 1985).

e. Hydrogen Production During Acetoclastic Methanogenesis.

Methanogens are traditionally considered consumers rather than producers of hydrogen; nevertheless hydrogen is produced and consumed in trace amounts during methanogenesis from acetate and methanol by Methanosarcina species (Phelps et al., 1985; Lovley and Ferry, 1985; Boone et al., 1987; Bhatnagar et al., 1987; Krzycki et al., 1987). Hydrogen appears to reach an equilibrium level depending on the concentrations of products and reactants (acetate, methane), suggesting that molecular hydrogen is an intermediate in redox reactions during the metabolism of acetate by Methanosarcina (Krzycki et al., 1987). The effects of molecular hydrogen seem to vary from strain to strain of Methanosarcina and whether it is produced in catabolic or anabolic reactions is not clear (Boone et al., 1987; Krzycki et al., 1987; Bhatnagar et al., 1987).

Boone et al. (1987) proposed that although molecular hydrogen is a feasible substrate for Methanosarcina barkeri, growth occurs by catabolism of acetate when in mixed culture in a digester, with molecular hydrogen being used only when it is present at high concentrations. During growth on acetate M. barkeri may continually produce small amounts of molecular hydrogen. Some authors speculate that

the sarcina spatial arrangement of these bacteria helps them to recover the reducing equivalents in the hydrogen, by a kind of intraspecies hydrogen transfer (Krzycki et al., 1987).

f. Formate vs. Hydrogen Interspecies Transfer.

As has been mentioned low concentrations of hydrogen, or another electron carrier compound, are necessary in order to generate enough driving force to achieve a continuous evacuation of fatty acids and alcohols. This situation is usually referred to as syntrophism. Hydrogen has been traditionally considered the electron transporter in the syntrophic associations, although no conclusive evidence exists that other compounds (e.g. formate) could not be involved, (Thiele et al., 1988a, 1988b). Recently Thiele and Zeikus (1988b), have proposed that formate rather than hydrogen is the syntrophic electron carrier in the degradation of ethanol in an anaerobic digester treating whey. Formate apparently possesses a lower diffusion coefficient that permits it to be scavenged inside the biological floc before diffusion out can occur. According to their calculations, 10% of the actual formate production diffuses out of the floc where it is cleaved into hydrogen and carbon dioxide, with formate being the actual source of external hydrogen in the system. They also proposed that due to the involvement of bicarbonate in the reaction and the huge ratios of bicarbonate/formate (approx. 1000), extra energy can be drawn by the syntrophic acetogens. A coherent energy recovery mechanism was proposed for the syntrophic acetogens. It is not clear from the data presented by these researchers

whether the uncoupling of the external hydrogen pool and the actual kinetics of electron transfer inside the floc can be explained based on interspecies formate transfer. However, phenomena such as lack of inhibition of fatty acid degradation according to thermodynamic predictions based on hydrogen transfer (Denac et al., 1988), can certainly be explained by this mechanism.

The distinction of whether electrons are being transferred via formate or via hydrogen directly does not affect the utility of the gas phase hydrogen measurement as an indicator of anaerobic digestion performance, as hydrogen and formate reach equilibrium rapidly (Thiele and Zeikus, 1988).

2.2.2. Hydrogen as a Control Index in Anaerobic Digesters.

As can be seen from the above discussion, hydrogen is virtually present in all the main reactions taking place in an anaerobic reactor; this characteristic at the same time makes the interpretation of the hydrogen readings very difficult. In well-operating anaerobic digesters, hydrogen or formate, is produced and consumed at high rates with a concomitant low concentration in the digester environment. The purpose of good operation is to avoid the development of permanent imbalances between the different reactions taking place in the digester. There are several different causes of imbalances with the most common being toxic events and overloads of the system. The behavior of hydrogen when the cause of a potential imbalance in the process is present is not well

known; furthermore, the dependence of hydrogen on other environmental parameters such as pH, temperature, volatile fatty acids concentration it is also not known.

Sykes (1970) was the first to study the behavior of hydrogen in anaerobic digesters during overloading and inhibition scenarios. The sensitivity of hydrogen measurements was 1000 ppm, relatively high, limiting the utility of the results. Chynoweth and Mah (1971) measured the gas composition during the degradation of palmitic acid by an anaerobic sludge digester culture. No hydrogen accumulation was observed when the methanogens in the culture were selectively inhibited by chloroform or carbon tetrachloride.

Kaspar and Wuhrmann (1978) fully recognized the importance of hydrogen concentrations in the degradation of intermediate products and proposed hydrogen and alkalinity as key parameters for the monitoring of anaerobic digesters. Mosey (1983) developed mathematical expressions to describe the effects of hydrogen in a digester and proposed hydrogen as the "autopilot" of the process.

Scott et al. (1983) using membrane inlet mass spectrometry measured the behavior of hydrogen in samples from anaerobic digesters subjected to step inputs of glucose, acetate and propionate, and toxic levels of chloroform and carbon tetrachloride. In contradiction with theory, no significant increase in dissolved hydrogen was measured during the step inputs of the organic substrates although increased methanogenic activity was observed. Additions of chloroform inhibited methanogenesis and increased the concentration of hydrogen very rapidly,

15 minutes after the addition. Whitmore et al. (1986) using membrane inlet mass spectrometry compared the behavior of hydrogen in mesophilic and thermophilic anaerobic digesters. Step inputs of glucose, propionate, butyrate and isobutyrate were added to samples from the anaerobic digesters. Concentrations below 35 mM of glucose (aprox. 6,500 mg/l COD) did not produce a measurable response in the hydrogen concentration (detection limit 1 μ Mol or 1400 ppm); responses of hydrogen were observed in the first hour after the addition of higher concentrations. Whitmore and Lloyd (1986) tested control of a thermophilic anaerobic digester using a preset hydrogen concentration in order to regulate the supply of glucose to the digester. This strategy significantly delayed the onset of inhibition of the system; but was found to be dependent on the size of the input step.

Archer et al. (1986) monitored the concentration of hydrogen in the gas phase of a pilot scale digester treating brewery waste. The digester was subjected to volumetric overloads. Hydrogen response was quick, reaching maximum levels (2-5 times normal levels) between 3-6 hours and returning to normal concentrations at 24 hours. No significant acid accumulation was detected. Seng Chye Eng et al. (1986) administered shock-loads of sucrose to a laboratory scale upflow anaerobic sludge blanket (UASB) reactor and monitored hydrogen in the gas phase. An interesting pH effect was noticed; during similar overload shocks but different buffering capacity in the system the accumulation of products, including molecular hydrogen, were significantly different. Low pH in the media induced much higher hydrogen levels (5000 vs 300 ppm), higher

accumulation of intermediate acids and longer recovery time. McCarty and Smith (1986) and Smith (1987) studied the effects of shock-loads on the behavior of intermediate products during the anaerobic digestion of ethanol and propionate. Hydrogen showed again a rapid response to shock loads and had a pronounced effect on the kinetics of the metabolic products of ethanol and propionate degradation. Steady-state levels of hydrogen depended on the type of reactor and the energetic content of the waste. Collins and Paskins (1987) monitored the hydrogen partial pressure of 20 mesophilic sewage sludge digesters during normal operation and found it to vary between 15 and 199 ppm.

Hickey et al. (1987) and Hickey (1987) analyzed the effects of organic and inorganic toxicants, and organic overloads on the behavior of hydrogen in the gas phase of sewage sludge digesters. Hydrogen behavior showed different trends according to the nature of the inhibitor. However, a hydrogen dampening effect was detected. As hydrogen accumulates the reducing equivalents are deviated towards reduced organic compounds such as higher volatile fatty acids, limiting the sensitivity of hydrogen as an early warning indicator.

Mosey and Fernandes (1988) monitored on-line the hydrogen composition of the gas phase of laboratory scale anaerobic digesters with sucrose as a carbon and energy source. Hydrogen, concluded these researchers, could be used as loading indicator for feeding control, and as an alarm indicator for toxicity.

In summary, variations in hydrogen concentration in the gas phase rapidly indicate that a change in the reactor is occurring; however,

most important is to establish what is the cause of the change. It is of special importance to be able to distinguish the behavior in upset situations from natural variations of the indicator. Hydrogen should be used in concert with other indicators to define more precisely the different phenomena actually taking place in the system, and to decide upon the right corrective action.

2.3. CARBON MONOXIDE MONITORING IN ANAEROBIC DIGESTION.

The presence of carbon monoxide in the gas phase of anaerobic digesters is known. Stafford et al. (1978) reported concentration levels between 10 - 100 ppm. Nonetheless, its sources have only recently been suggested (Hickey, 1987), and most of the work on its metabolic role and interpretation of its variations as indicators of the general status of anaerobic digestion remain to be done. Carbon monoxide, like molecular hydrogen, is a poorly soluble gas a characteristic which makes gas phase monitoring favorable.

Hickey (1987) found a strong correlation between the CO concentration in the gas phase and the acetate concentration in the liquid phase. Based on this observation and a recently suggested biochemical pathway for acetate catabolism in methanogens (Krzycki and Zeikus, 1984), a mathematical expression that relates the concentration of acetate in the liquid phase with methane, hydrogen and carbon monoxide concentrations in the gas phase was proposed. This expression

is based on thermodynamic equilibrium calculations between the above mentioned compounds. The relationship is speculative because no direct evidence exists that the acetate degrading methanogens are the only source (or sink) of carbon monoxide, although strong circumstantial evidence exists. The expression however predicts with reasonable accuracy the acetate concentration; and most important, follows the accumulation trends of the acid.

The production and consumption of carbon monoxide by anaerobic bacteria is related to the recently proposed "Acetyl CoA pathway" for autotrophic growth in anaerobic microorganisms (Fuchs, 1986). The name of the pathway is a subject of discussion and some researchers have proposed "the Wood pathway of autotrophic fixation of CO_2 " (Ljungdahl, 1986).

Prior to the discovery of the acetyl CoA pathway, there were only two pathways known for autotrophic growth with CO_2 as the source of carbon; the reductive pentose cycle discovered by Calvin, and the reductive tricarboxylic cycle. Unlike these two pathways, the Acetyl CoA pathway is not a cycle; it occurs by the direct conversion of two molecules of CO_2 to Acetyl CoA, one of which is reduced to the methyl group.

The key enzyme of the pathway is the carbon monoxide dehydrogenase, which converts CO_2 to a CO group, accepts the methyl and CoA groups and condenses them to the acetyl CoA (Wood et al., 1986). Figure 4 illustrates proposed different uses of the Acetyl CoA pathway in anaerobic bacteria according to Fuchs (1986). It should be noted that

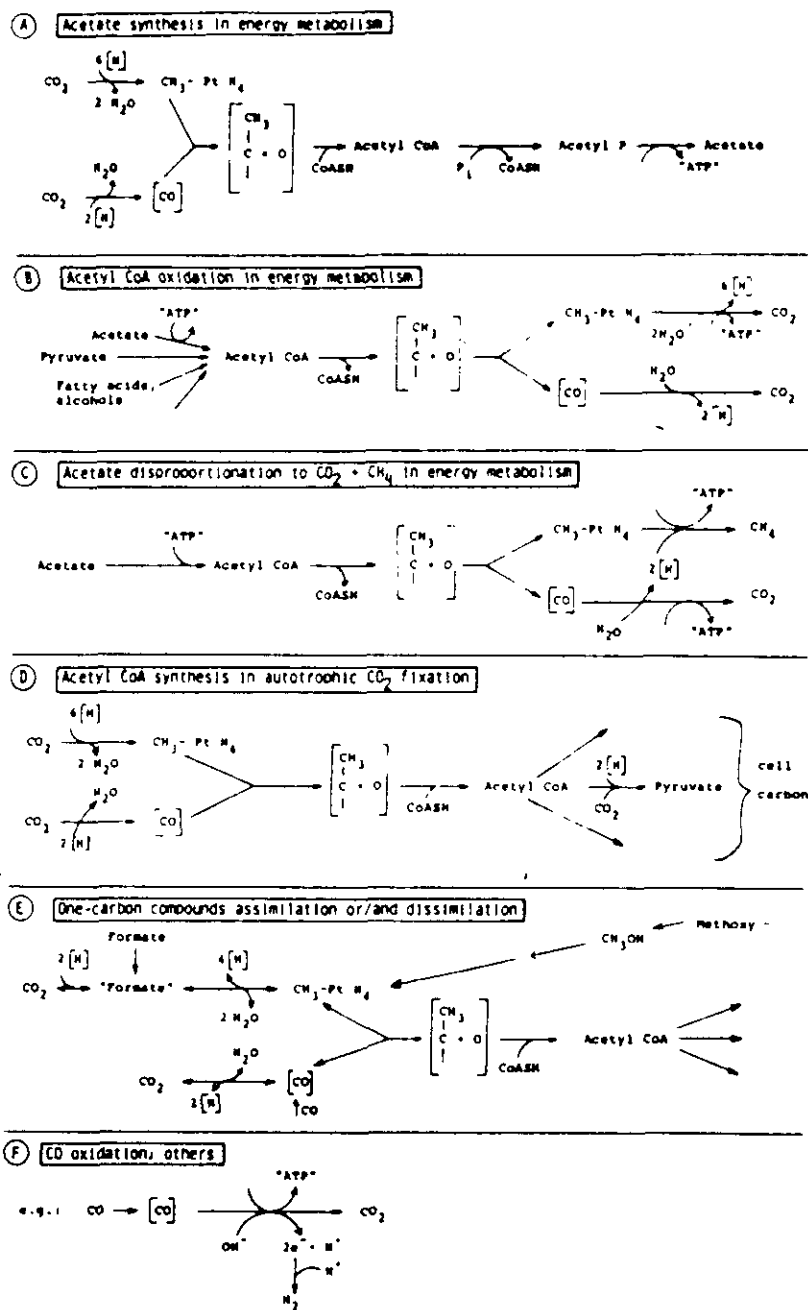


Figure 4. Uses of the Acetyl-CoA pathway by anaerobic microorganisms.

(After Fuchs, 1986)

all the different options presented in Figure 4 are potentially operative in anaerobic digesters.

Part A illustrates the use of the pathway in acetate synthesis during energy metabolism as it occurs in acetogenic bacteria (homoacetogenic bacteria) e.g. Acetobacterium Woodii, Clostridium thermoautotrophicum. (Diekert et al., 1984, 1986; Ragsdale and Wood, 1985; Fuchs, 1986).

Part B illustrates the use of the pathway in acetyl CoA oxidation during energy metabolism as it occurs in sulfate reducing bacteria e.g. Desulfovibrio vulgaris, Desulfococcus multivorans, Desulfobacterium autotrophicum and probably some syntrophic partners of methanogens oxidizing acetate, higher fatty acids and other compounds (Schauder et al., 1986; Lupton et al., 1984)

Part C illustrates the use of the pathway in acetate cleavage to CO₂ and CH₄ during energy metabolism as it occurs in methanogens growing on acetate e.g. Methanosarcina, Methanotrix (Kohler and Zehnder, 1984; Bott et al., 1986; Eikmanns and Thauer, 1984; Bhatnagar et al., 1987; Krzycki et al., 1985; Krzycki and Zeikus, 1984a, 1984b; Kenealy and Zeikus, 1982).

Part D illustrates the use of the pathway in acetyl CoA synthesis during autotrophic growth e.g. autotrophic acetogenic bacteria using the Acetyl CoA pathway, Autotrophic methanogenic bacteria (Methanosarcina when growing on H₂ and CO₂, Methanobacterium thermoautotrophicum), autotrophic sulfate reducing bacteria (Conrad and Thauer, 1983; Eikmanns

et al., 1985; Lange and Fuchs, 1987; Lupton et al., 1984; Bott et al., 1986).

Part E illustrates the use of the pathway in the assimilation into acetyl CoA and/or dissimilation to CO₂ of one-carbon compounds, such as CO, CH₃OH, formate, CH₃NH₂ e.g. the use of the pathway by organisms in parts A through D, which are able to grow on one-carbon compounds (Fuchs, 1986).

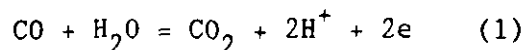
Part F illustrates the use of the pathway for purposes A-E or unknown functions in current research or which remain to be investigated (Fuchs, 1986).

One important characteristic of the different uses proposed for the Acetyl CoA pathway must be highlighted. In parts A,B and C (Figure 4) the pathway is used during catabolism of substrates, while in uses D,E it is used in anabolic reactions. This is an important observation in the postulation of the origin of CO in anaerobic digesters.

As has already been stated, the key enzyme of the acetyl CoA pathway is the carbon monoxide dehydrogenase, (CODH). Due to its protagonistic role in condensing the acetyl CoA molecule from its precursors a proposal to rename the enzyme to "Acetyl CoA Synthase" comes from Wood et al. (1986), in order to differentiate it from the carbon monoxide dehydrogenases of aerobic bacteria and photosynthetic bacteria. These bacteria use the Calvin cycle and the CODH enzyme play a different role.

CODH in anaerobic bacteria is a nickel-iron-sulfur protein rapidly

inactivated by O₂ and cyanide. The enzyme catalyzes the reaction:



with methylviologen as the electron acceptor (Diekert et al., 1985). The physiological electron acceptor is not definitively known; however, F-420 has been proposed for methanogens (Diekert et al., 1985). Electron carriers involved in the CODH reaction(s) are probably very different in different families of bacteria and may depend on whether the physiological role of CODH is to catalyze acetyl CoA synthesis or oxidation, and which electron donor/acceptor is available (Fuchs, 1986). Under physiological conditions the direction of the reaction seems to be to reduce carbon dioxide rather than to oxidize carbon monoxide. Nonetheless, the reversibility of the later reaction has been proven (Eikmanns and Thauer, 1984). The carbonyl group is metal-bound to the enzyme and equilibrates with the CO in the surroundings (Wood et al., 1986; Diekert et al., 1984). The specific activity of CODH in autotrophic methanogens is one order of magnitude lower than in other anaerobic bacteria, suggesting an anabolic rather than a catabolic function. This is also supported by the fact that methanogenesis from CO₂ were not affected by cyanide (Diekert et al., 1985; Stupperich and Fuchs, 1984). In Methanosarcina barkeri it has been proposed that in addition to the role of CODH in acetate synthesis and degradation, it produces hydrogen as a by-product of carbonyl group transformation (Bhatnagar et al., 1987).

Another interesting feature of the Acetyl CoA pathway is the conservation of energy through a chemiosmotic mechanism. Production and

consumption of CO by homoacetogens, autotrophic methanogens and Methanosarcina barkeri, has been shown to be coupled to energy conservation reactions (Diekert et al., 1986; Eikmanns et al., 1985; Bott et al., 1986; Krzycki and Zeikus, 1984). This is an important observation for CO monitoring, because it presents the possibility of accumulations of CO further than the equilibrium concentration in the reaction (1), where $\Delta G^{\circ} = -20.4 \text{ kJ/mol (30}^{\circ}\text{C)}$.

Populations of anaerobic bacteria in anaerobic digesters are highly specific for each particular reactor, and the present operational characteristics. As can be seen from the previous discussion carbon monoxide can potentially be produced by almost all the different trophic groups of microorganisms in anaerobic digesters, (see Figures 1 and 4) as almost all posses the Acetyl CoA pathway. Nevertheless, not all of them use the pathway in the same way, and the relative importance of each trophic group is different. It has been observed that CO is produced and consumed by microorganisms in pure culture using the Acetyl CoA pathway, apparently following a relationship with the concentration of products and reactants of the microorganism catalyzed reaction. This effect can be seen in the data of Lupton et al. (1984), Conrad and Thauer (1983), Eikmanns et al. (1985) and O'Brien et al. (1984). Therefore, the possibility exists that the measured CO in the gas phase is the result of a complex set of kinetic interactions between producers and consumers of CO.

In this case several factors would determine the equilibrium value of CO in the hypothetical production/consumption kinetics. These include:

- . Rates of CO consumption/production by each trophic group
- . Relative affinities for CO of each trophic group
- . Purpose of the Acetyl CoA pathway in each trophic group
- . Non-biological reactions of CO with the chemical matrix, and
- . Mass transfer retardation effects (Bacteria to liquid phase to gas phase or vice versa)

Several observations can help to simplify the picture. Apparently, microorganisms using the Acetyl CoA pathway in autotrophic carbon cell fixation, anabolism, have lower CODH activity and higher CO affinity constants e.g. Methanosarcina barkeri growing on H₂ and CO₂ vs acetate (Diekert et al., 1985; Zeikus et al., 1985). This implies that the fluxes of CO through the pathway are much lower than in the case of bacteria using the pathway in catabolism.

Not all the hydrogenotrophic methanogenic bacteria have carbon monoxide dehydrogenase activity e.g. M. ruminantium, M. smithii,

M. voltae or if they have it do not produce or consume CO during growth e.g. Methanospirillum hungatei (Bott et al., 1985). Cyanide, an specific inhibitor of CODH, inhibited Acetyl CoA synthesis in M. thermoautotrophicum but methane formation was unaffected (Stupperich and Fuchs, 1984). Based on this observations the possibility that hydrogenotrophic methanogens are a significant source or sink of carbon monoxide is excluded.

Additionally, considering the previous observations and the fact that homoacetogenic bacteria growing autotrophically on H₂ and CO₂ are believed to play a minor role in anaerobic digestion ecosystems (Zeikus, 198); the contribution of this group of bacteria can be neglected.

Organisms using the acetyl CoA pathway in catabolism and present in the microbial ecosystem of an anaerobic digesters are homoacetogenic bacteria growing on organic substrates, syntrophic bacteria oxidizing fatty acids and alcohols, sulfate reducing bacteria and acetoclastic methanogens,(see Figure 2). The relative importance of heterotrophic acetogens in anaerobic digestion is not known, although they can be significant in the degradation of certain compounds like phenols (Ljungdahl, 1986). CO production by syntrophic acetogens has not been reported ,to the author's knowledge, and this possibility is just a speculation (Fuchs, 1986).

Sulfate reducers are of varying importance in different anaerobic digestion ecosystems, and a significant ammount of the carbon and electron flow can be deviated through these versatile bacteria. Their importance in the anaerobic ecosystem reported by Hickey (1987) is

expected to be minor as the substrate was principally waste activated sludge from a municipal sewage treatment plant (relatively low in sulfate).

The last group of microorganisms are acetoclastic methanogens, which are responsible for 70% of the methane generated in anaerobic sludge digesters and consequently a major part of the carbon and electron flow through the acetyl CoA pathway.

In summary, the true origin of CO and interactions between CO producer/consumers in an anaerobic ecosystem are not precisely known. Here it is proposed that acetoclastic methanogens and sulfate reducers are the major organisms responsible for CO production and consumption. The role of the sulfate reducers in the dynamic of production and consumption of CO is highly unknown so far; therefore further research is needed.

The role of mass transfer retardation on the CO measurements in the gas phase is also unknown, but since CO is a highly insoluble gas it is expected that the mass transfer transport coefficient between the liquid and the gas phase of the reactor be controlled by the liquid phase resistance, according to two film transport model. Rates of consumption and production of CO at the low concentrations usually found in anaerobic digesters as well as diffusion coefficients for CO in biological flocs are not available, and a time relaxation study is not feasible at the moment in order to theoretically analyze the importance of the mass transfer effects. Further research is needed.

2.4. HEAVY METAL TOXICITY.

Heavy metals concentration in wastewaters, treated effluents, wastewater treatment plant sludges and composted sludges is a subject of intensive study due to toxicity of metals to all kinds of organisms. In anaerobic digesters, toxicity by heavy metals has been identified as a major cause of difficulty in the performance of the process (Swanwick et al., 1969). A recent review of the literature pertaining to occurrence of heavy metals in wastewater treatment plants has been done by Bacon (1988). Principal sources of metals in wastewater are residential, industrial and urban runoff. Table 2 summarizes typical information on the concentration of heavy metals in wastewater.

Heavy metals show strong affinity for ligands such as phosphates, cysteinyl and histidyl side chains of proteins, purines, pteridins and porphyrins (Vallee and Ulmer, 1972). Hence, heavy metals can potentially coordinate with a large number of biochemical sites. Some possible effects would be: inhibit enzymes having functional sulfhydryl groups, bind and affect the conformation of nucleic acids, and disrupt pathways of energy conservation. Heavy metals also cause substitution of prosthetic groups in metalloenzymes, and interact with phospholipids conforming extracellular and intracellular membranes. This last characteristic is of special importance, because it determines how much metal penetrates into the cytoplasm (Sterrit and Lester, 1980).

Several factors determine the toxicity of heavy metals. It is commonly accepted that the most toxic form of heavy metals is the free

TABLE 2. TYPICAL CONCENTRATIONS OF HEAVY METALS IN VARIOUS SOURCES.

METAL	RESIDENTIAL SOURCES (%)	URBAN RUNOFF (mg/L)	WWTP INFLUENT (μ g/L)	WWTP EFFLUENT (μ g/L)
Cd	40 - 70	0.025	0 - 40	0 - 16
Cr	20 - 30	0.016	30 - 800	10 - 20
Cu	40 - 100	0.46	150 - 400	30 - 70
Fe	--	--	1000 - 20000	200 - 400
Ni	20 - 70	0.15	20 - 1000	0 - 100
Pb	20	--	50 - 900	10 - 150
Zn	30 - 50	1.6	200 - 2000	100 - 300

[FROM BACON (1988)]

form, although this is not always true (Vallee and Ulmer, 1972). Hence, factors affecting the speciation of heavy metals affect the toxicity. Most commonly mentioned are pH, redox potential, temperature, concentration of chelating agents, concentration of inorganic anions, synergistic and antagonistic effects, and bacterial resistance. These factors are discussed below.

2.4.1. pH.

There are several effects of pH on the coordination of heavy metals under anaerobic conditions. Solubility of heavy metals is principally controlled by sulfide, carbonate and phosphate salts (Mosey, 1971). Sulfide, phosphate and carbonate activities are highly controlled by pH. Metals form hydrolysis species in aqueous solutions that are strongly dependent on pH (Stumm and Morgan, 1981). Gould and Genetelli (1978) report that the complexation capacity of anaerobic sludges is highly dependent on pH. They explain the behavior as a competition of sites between the hydrogen cation and the metal.

2.4.2. Redox Potential.

Redox potential affects the oxidation state of the metal. For example, Cu II is reduced to Cu I and Hg II is reduced to Hg⁰ under anaerobic conditions. Cu I is less soluble than Cu II (Mosey, 1976) and Hg⁰ can freely permeate the cell membrane (Vallee and Ulmer, 1972)

2.4.3. Temperature.

Gould and Genetelli (1982) studied the effects of temperature on heavy metal binding in anaerobically digested sludges. Zinc, nickel and cadmium were solubilized when the temperature was lowered; copper was not affected. The results were in accordance with the van't Hoff equation.

2.4.4. Concentration of Chelating Agents.

Soluble organic matter in anaerobic digesters significantly increased the solubility of heavy metals. A good correlation was found between solubility of zinc and soluble COD (Patterson and Hao, 1979). Toxicity of heavy metals has been shown to depend on the concentration of suspended organic material in anaerobic digesters (Mosey, 1976). Similar results were obtained by Jarrell and Saulnier (1987); these researchers found that 30 to 270 times higher concentration of zinc and copper were necessary to obtain similar inhibition of Msp. hungatei cultivated in sewage sludge compared with buffer solution. Patterson and Hao (1979) found that the affinity of heavy metals for the complexing material was variable, and the addition of one heavy metal to an sludge already exposed to other metals causes substitution and solubilization of the previously chelated metal.

2.4.5. Concentration of Inorganic Ions.

Ions of opposite charge form a soluble inorganic complex or ion pair and are no longer electrostatically effective. This phenomenon is known as "ion pair" (Stumm and Morgan, 1981). The denominated ion pair effect increases with increasing ionic strength and is responsible for increased solubility of metals (Callander and Barford, 1983).

Inorganic systems of singular importance in anaerobic digestion are the carbonate system, phosphate system, and sulfide system. Sulfide controls the solubility of iron, zinc, nickel, lead, cadmium and copper but not chromium (Lawrence and McCarty, 1965; Mosey, 1976). Sulfide is produced by the reduction of sulfates and degradation of amino acids such as cysteine and methionine. Addition of sulfide or sulfide precursors to anaerobic digesters has proven to be a successful strategy in the control of heavy metal toxicity (Lawrence and McCarty, 1965). The sulfide addition should be prior to the contact of the metals with the biological phase because reaction of metals with organic matter occurs more rapidly (Mosey et al., 1971). Phosphate can play a significant role in the solubility of lead (Mosey et al., 1971).

Sulfide in itself can be toxic and the toxicity appears to be more toxic in its free form (Koster et al., 1986; Karhadkar et al., 1987). Morper (1984) report successful operation of a UASB reactor treating Vinasses with a concentration of 40 mg/l of copper, 35,000 mg/l COD and sulfates as sulfide precursor. No inhibition of the process was observed and copper concentrations in the effluent were 0.8 mg/l (8 months

average). Concentrations of copper in the sludge were of 100.000 mg/l (> 10% of total solids).

Carbonate salts of iron, cadmium and zinc precipitate after sulfide, but present a higher dependence on pH. For example, for conditions prevailing in anaerobic digestion carbonate salts would precipitate if $\text{pH} > 7.2$ for cadmium; $\text{pH} > 7.7$ for zinc; $\text{pH} > 6.4$ for iron (Mosey, 1976).

2.4.6. Synergistic and antagonistic Effects.

Although antagonistic effects in the toxicity of heavy metals to bacteria (E. coli) exist, it is usually considered that the effects of heavy metal concentration are additive with no synergistic effects (Kugelman and Chin, 1970; Mosey, 1976). In order to evaluate the maximum level of metals allowable in the influent to an anaerobic sludge digester, Mosey (1976) proposed that the concentration of nickel, zinc cadmium, lead and copper should be expressed on an equivalent weight basis (i.e., as milliequivalents per liter) and these concentrations added. The sum should be divided by the solid concentration in the digesting sludge. If the resulting ratio is greater than 400, then digestion failure is considered as probable, and if greater than 800 as almost certain.

2.4.7. Bacterial Resistance.

Bacteria have two different types of resistance: non-specific resistance arising from the particular physiological state, and specific inheritable factors for particular heavy metals. Bacteria subject to permanent contact to heavy metals develop external capsules that complex the metals, or release chelating agents in the media that neutralize the metals (Sterritt and Lester, 1980). Methanogenic bacteria in sediments are able to methylate mercury, thereby solubilizing and returning it to the upper water layers (Vallee and Ulmer, 1972).

The presence of excess metals in an anaerobic reactor causes selection for populations which are better adapted for resistance to metal toxicity. Ashley et al. (1982) report that continuous additions of nickel to an anaerobic digester selected for a population that degraded proteins over carbohydrates.

2.5. TOXICITY OF ORGANIC COMPOUNDS.

Organic compounds are usually specific inhibitors of microorganisms and are used in enzymatic and biochemical studies for that purpose. Organic toxic compounds also can generate bacterial resistance to their effect when the compound is permanently present in the system.

Swanwick et al. (1969) identified formaldehyde as one of the principal organic compounds responsible for toxic upsets in sludge

digesters. Several researchers have studied the toxicity of formaldehyde to anaerobic acetate enrichment cultures (Bhattacharya and Parkin, 1988; Parkin et al., 1983; Parkin and Miller, 1982; Chou et al., 1978). Free formaldehyde reacts spontaneously with the amino groups of proteins and nucleic acids (Zubay, 1988). Nevertheless, bound formaldehyde is an important intermediate in methanogenesis from hydrogen (Noll, Donnelly and Wolfe, 1987).

Bromoethanesulfonic acid (BES) is a structural analogous of coenzyme M (CoM), a C-1 carrier only found in methanogens (Gunsalus et al., 1978; McBride and Wolfe, 1971). CoM is involved in the last step of methanogenesis, the reduction of the methyl group to methane. BES reversibly inhibits the methyl reductase system of methanogens in pure and mixed culture (Gunsalus et al., 1978; Zehnder et al., 1980; Hickey, 1987; Zinder et al., 1984). Although BES is not a compound to be commonly found in wastewaters, it would serve as a benchmark with which to compare the effects of other toxicants.

CHAPTER III
METHODS AND MATERIALS

3.1. SCOPE OF THE WORK.

The purpose of this study was to examine the behavior of trace gas concentrations in the gaseous phase of sucrose-fed anaerobic digesters subjected to the presence of inhibitory concentrations of several organic and inorganic toxicants. The trace gases carbon monoxide and hydrogen were the principal focus of the study.

The heavy metals copper, zinc, nickel and cadmium and the organics formaldehyde and bromoethanesulfonic acid (BES) were tested. Metals and formaldehyde are found as common causes of operational difficulties in anaerobic digestors (Swanwick et al., 1969). BES is a specific inhibitor of methanogenesis (Zinder et al., 1984).

Behavior of trace gases was tested using serum bottle assays developed by Miller and Wolin (1974). The protocol for the assays is based on Hickey's modification of the anaerobic toxicity assay (ATA) (Hickey, 1987) developed by Owen et al. (1979).

Inoculum for these assays was provided by a sucrose-fed fill and draw reserve anaerobic digester.

3.2. RESERVE DIGESTER.

The digester used as inoculum source for the serum bottle assays was a 15-liter sucrose fed reactor, operated in a fill and draw mode with a solids residence time (SRT) of 20 days, and a temperature of $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$. This reactor was used as an inoculum source for a previous study, and was fed sucrose for more than one year (Robins, 1987). The reactor was stopped for a period of six months between both studies. It was fed again two months before the first serum bottle tests in this study (corresponding to three theoretical SRTs). The reactor started operation immediately, reaching steady state in less than one SRT.

The reactor was fed daily with 750 ml of synthetic media. Operational parameters such as gas production, gas composition, pH, volatile fatty acids and alkalinity were measured daily. COD removal efficiency and volatile suspended solids in the effluent were measured from time to time and before each serum bottle assay.

The composition of the synthetic feed was based on the feed used by Pause (1983). Calculation of the required amounts of nutrients were checked with theoretical microorganism composition, amounts of carbon source supplied and estimated yields for the culture. All nutrients were supplied in excess. The media was prepared daily according to the following composition:

- . 46 ml of Salt I.
- . 46 ml of Salt II.

- . 10 ml of Salt III.
- . 92 ml of Buffer solution.
- . 6 ml cysteine solution.
- . 1 ml of vitamins solution.
- . 4 gr of sugar.
- . Well water to complete 850 ml.

Composition of Salt I, Salt II, Salt III, vitamins solution, Buffer solution and cystein solution is presented in Tables 3 and 4. Vitamin solution was prepared according to Owen et al. (1979). The sucrose used was common table sugar.

3.3. SERUM BOTTLE ASSAYS.

The daily mixed-liquor waste from the reserve digester was anaerobically transferred into a sealed bottle with a 70% N₂, 30% CO₂ atmosphere and resarzurin indicator for oxygen presence. Synthetic media (2.5ml) was delivered into an empty 150-mL serum bottle along with a magnetic stir bar. With the help of a gas manifold, serum bottles were gased out with a mixture of 70% N₂, 30% CO₂ for several minutes. The sealed bottle with the inoculum was placed in a magnetic mixer to ensure that the content remained homogeneous. Inoculum from the reactor (47.5 mL) was transferred into the serum bottle using a repipeter. Headspace of the reserve bottle was connected to the gas mixture tank via the

TABLE 3. NUTRIENT SALTS.

<u>Salt I</u>	<u>Concentration (g/l)</u>
$(\text{NH}_4)_2\text{HPO}_4$	11.4
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	1.118
 <u>Salt II</u>	
NH_4Cl	14.0
KCl	2.0
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	5.0
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	5.0
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.3
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.61
 <u>Salt III</u>	
H_3BO_3	0.38
$\text{CuCl}_2 \cdot \text{H}_2\text{O}$	0.18
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.17
ZnCl_2	0.14

TABLE 4. VITAMINS AND ADDITIONAL NUTRIENTS.

<u>Buffer</u>	<u>Concentration (g/l)</u>
NaHCO ₃	50.0
<u>Vitamins solution.</u>	
Pyridoxine Hydrochloride	0.1
Biotin	0.02
Folic acid	0.02
Riboflavin	0.05
Thiamin	0.05
Nicotinic acid	0.05
Pantothenic acid	0.05
P-aminobenzoic acid	0.05
Thioctic acid	0.05
B ₁₂	0.001
<u>Cysteine solution.</u>	25.0

manifold to avoid air intake during the process. Serum bottles were continually gassed during the transfer, and after the transfer for the time necessary for the change in color of the resarzurin indicator from pink to colorless to occur (usually less than 1 minute).

Serum bottles were sealed with rubber septa and aluminum seals and transferred into a 35°C incubator for approximately one-half hour in order to reach a stable temperature of 35°C. Headspace pressure of the serum bottles was equilibrated to atmospheric with a lubricated glass syringe. Gas space was tested for content of CO₂, CH₄, CO and H₂. Immediately afterwards, the bottle was spiked with the desired amount of toxicant and incubated at 35°C with constant mixing in a pulley driven magnetic mixer. Concentration of the mentioned gases in the head space of the serum bottles was measured periodically, and the amount of gas produced was recorded. Gas production was determined with a lubricated glass syringe as suggested by Owen et al. (1979). Usually the test was run for 48 hours. At the end of the test pH was measured and samples were centrifugated and frozen for subsequent fatty acids analysis.

The amount of toxicant added to each bottle was initially estimated from literature values. Usually 2 or 3 runs were necessary to determine the toxicant concentrations necessary to achieve the desired levels of inhibition. All assays for a selected concentration were done in duplicate and the controls in triplicate. For a given toxicant four different concentrations were tested.

3.4. ANALYTICAL METHODS.

3.4.1. Gas Production Measurements.

Gas production in the reserve digester was measured with a wet gas meter with a precision of 1cl (10 ml). Gas production during the serum bottle assays was measured with a water lubricated glass syringe, according to Owen et al. (1979).

3.4.2. Gas Composition Measurements.

Methane and carbon dioxide were measured by gas chromatography in a GOW-MAC thermal conductivity gas chromatograph equipped with a Hewlett-Packard electronic integrator. Column characteristics and operational conditions of the instrument were the same as those reported by Pause and Switzenbaum (1983) and Hickey (1987). Relative standard deviations of the methane and carbon dioxide analysis were determined by Hickey (1987); the values obtained were 0.4% for methane and 1.28% for carbon dioxide. Gas samples from the reserve digester were collected with 1 mL disposable tuberculin syringes from the septum on the gas line. Gas samples from the serum bottles were collected in the same way, using a sample volume of 1 mL for methane and carbon dioxide content and 0.5 mL for hydrogen and carbon monoxide.

These last two gases were analyzed by gas chromatography in a TRACE ANALYTICAL RGA2 reduction gas analyzer with a sensitivity of parts per

million, ppm, for hydrogen and parts per billion, ppb, for carbon monoxide. Relative standard deviation for hydrogen measurements was 0.48%, and for carbon monoxide 0.38% according to Hickey (1987). Detection in this instrument is performed with a mercury oxide reducing bed and a mercury vapor UV photometer detector. Column characteristics and operational conditions of the instrument were the same as reported previously by Hickey (1987).

3.4.3. Volatile Fatty Acids (VFA).

Volatile fatty acids were determined following two different procedures depending on the purpose of the analysis. For daily monitoring of the reserve digester, acids were determined by titration following the procedure of O'Brien and Donland (1977). VFA determinations at the end of the serum bottle assays were determined by gas chromatography in a Varian 3700 gas chromatograph using a flame ionization detector. Column characteristics and operational conditions of the instrument were the same as reported previously by Hickey (1987).

3.4.4. Other Assays.

Volatile suspended solids, chemical oxygen demand, alkalinity, and pH were determined following the procedures outlined in Standard Methods (1985).

CHAPTER IV

EXPERIMENTAL RESULTS

The results of the serum bottle assays for the determination of the behavior of the trace gases hydrogen and carbon monoxide in an anaerobic sucrose fed digester under metabolic inhibitory concentrations of selected heavy metals and organic toxicants, are presented in this chapter. Pulse addition of the heavy metals cadmium, copper, nickel and zinc, and the organics formaldehyde and bromoethanesulfonic acid, BES, was investigated. These heavy metals are of common occurrence in domestic as well as industrial wastewaters (Bacon, 1988). Formaldehyde is the most toxic of the saturated aldehydes present in petrochemical wastewaters (Bhattacharya and Parkin, 1988), and of common use in industrial applications (Hickey, 1987). BES was selected because of its specific inhibitory effect on methanogens, and served as a reference point for interpretation of the results.

During the period of the experiment no attempt was made to quantify the chemical speciation, or partition of the toxicants in the different phases in the bottle. Hence, all the results will be presented and discussed in terms of applied dosage.

4.1. HEAVY METALS RESULTS.

4.1.1. Cadmium.

Concentrations of cadmium ranging from 4 to 40 mg/l were investigated. Cadmium was added as the chloride salt.

Increasing concentrations of cadmium progressively inhibited methane production (Figure 5). The dosage of 4 mg/l of cadmium shows a slight stimulation in the first 2 hours. For all the other concentrations the inhibition of methane production increases with time; no signal of recovery is detected in the period of the experiment.

Hydrogen evolution for the different concentrations of cadmium tested is presented in Figure 6. Two different patterns are suggested. For low concentrations of the metal, 4 and 8 mg/l of applied Cd, hydrogen behaves essentially as the controls. For higher concentrations, 20 to 40 mg/l of Cd, very high concentrations of hydrogen gas in the headspace of the serum bottles were recorded, with increasing concentrations with time.

The behavior of carbon monoxide is presented in Figure 7. Again, two different patterns seem to occur. At low concentrations of cadmium, 4 and 8 mg/l, carbon monoxide in the gas phase fluctuates around the control values. At high concentrations, 20 to 40 mg/l, carbon monoxide concentrations stay well below the control levels.

In an attempt to determine a typical pattern of response of carbon monoxide and hydrogen during cadmium inhibition of methane production, %

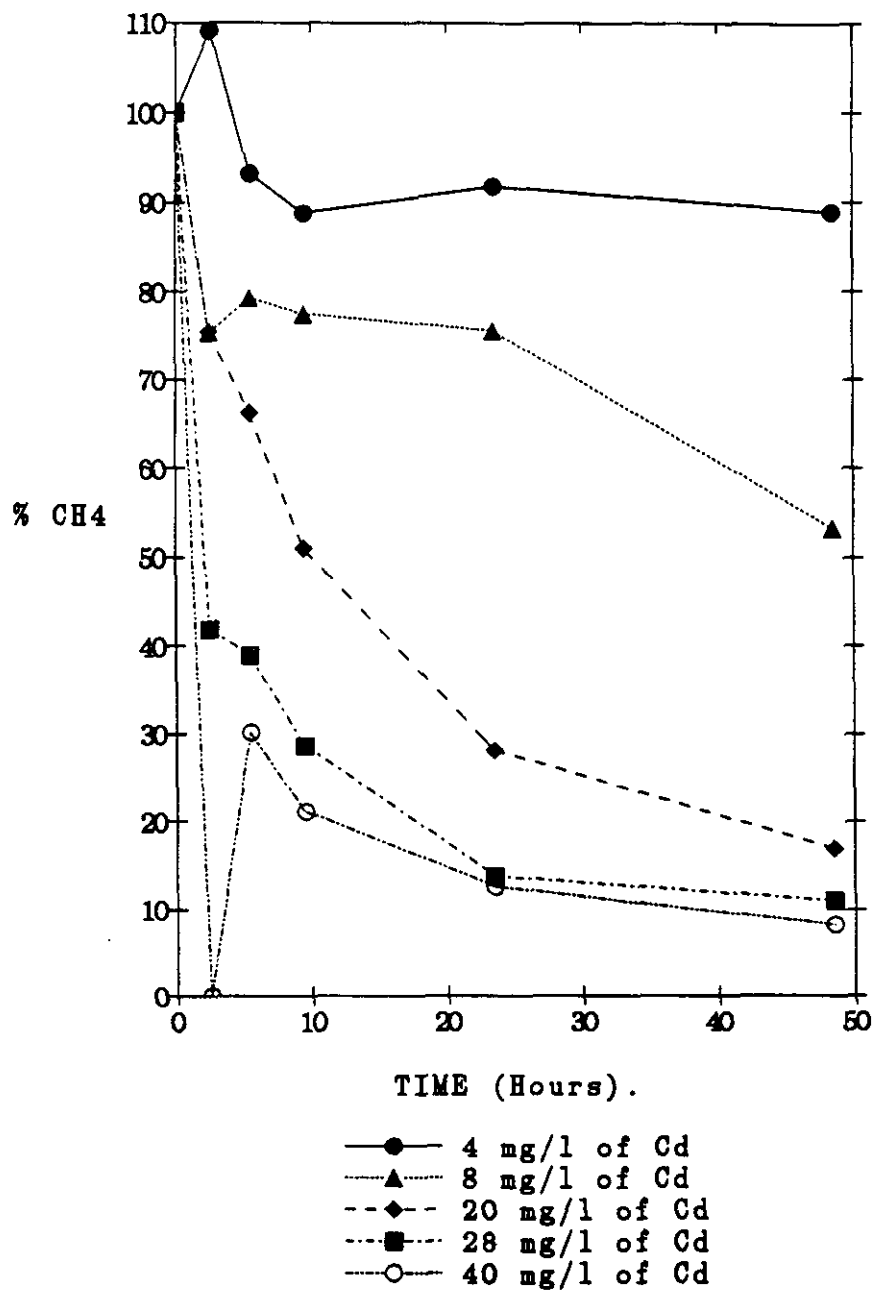


Figure 5. Effect of cadmium (Cd^{+2}) on the cumulative methane production as percent of control.

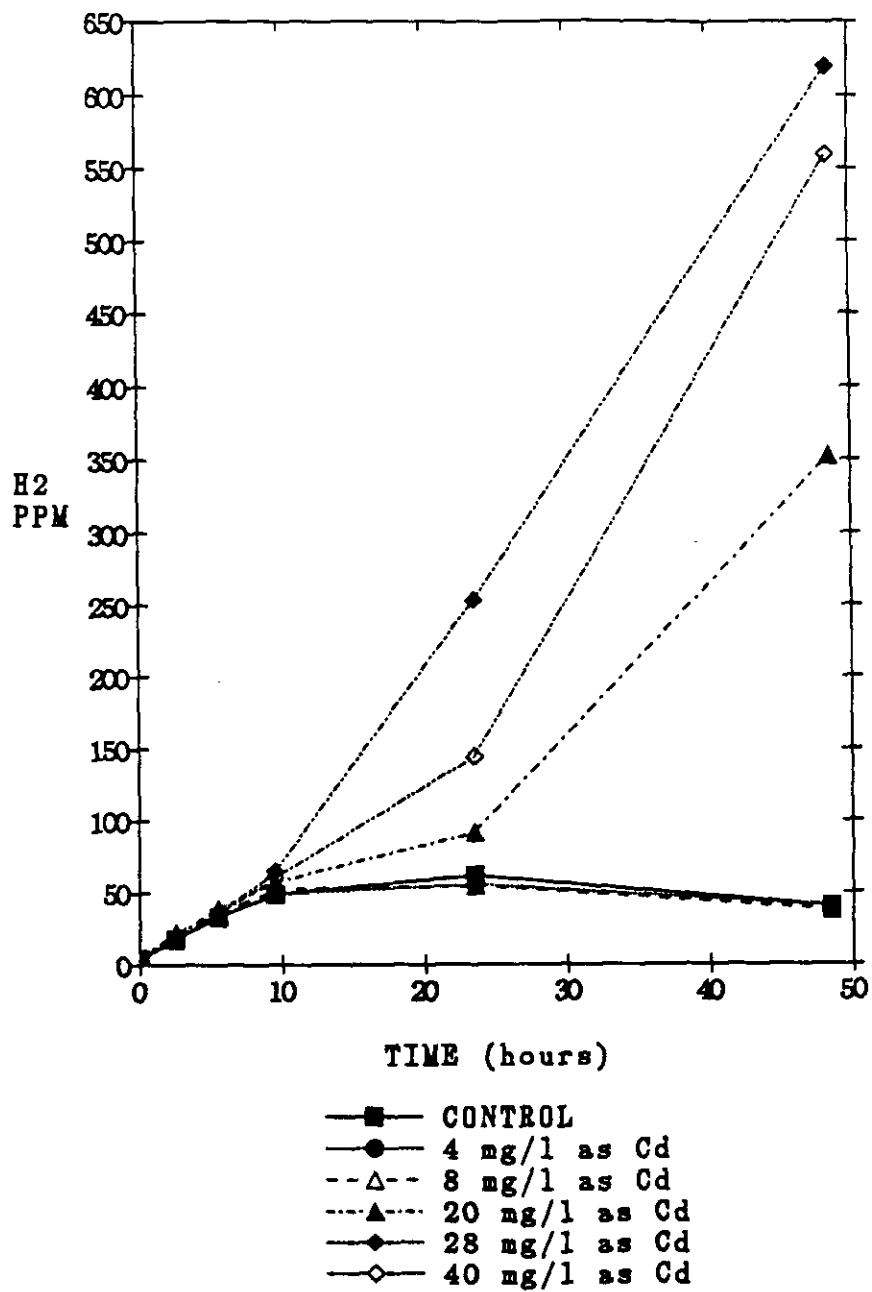


Figure 6. Effect of cadmium dosage (Cd^{+2}) on the headspace concentration of hydrogen over time.

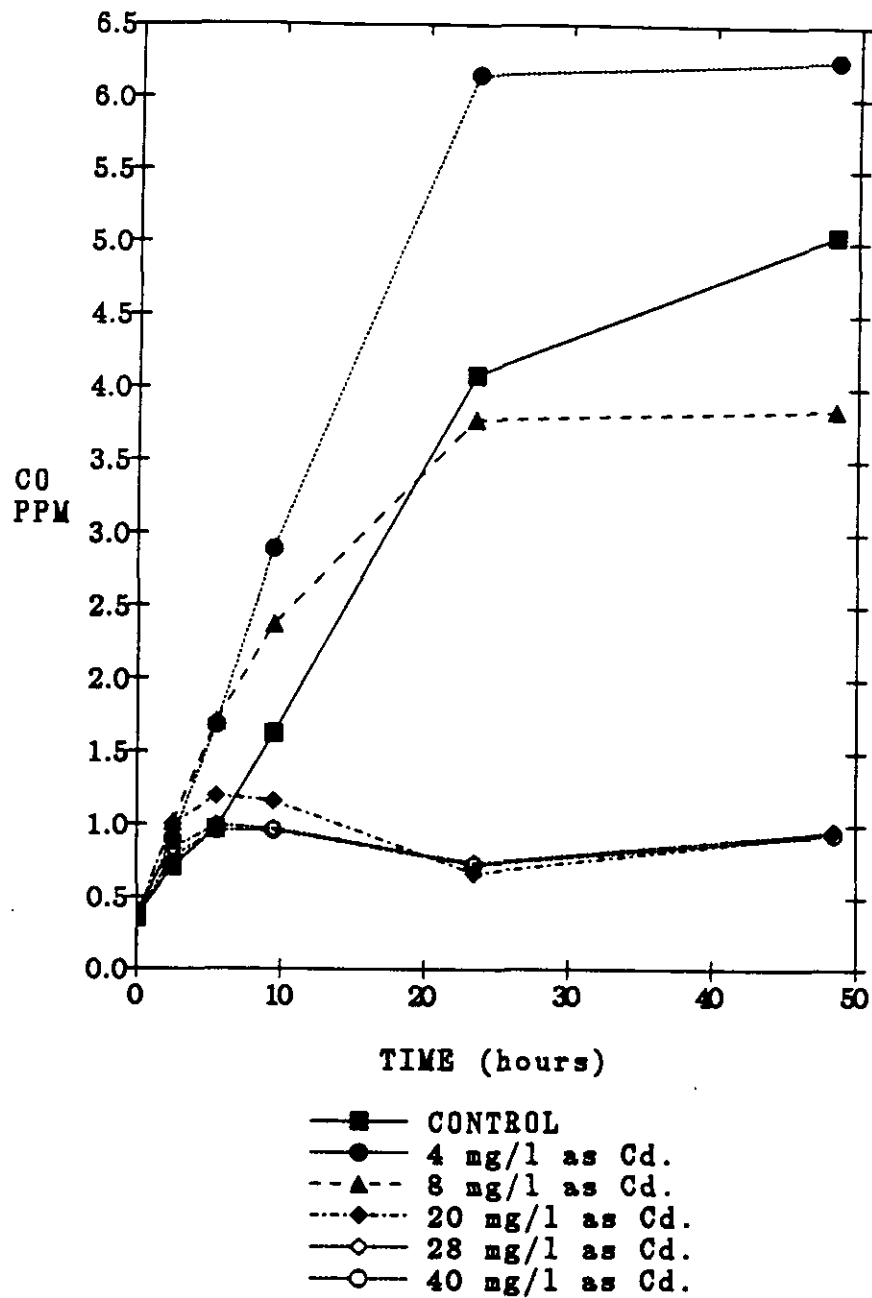


Figure 7. Effect of cadmium dosage (Cd^{+2}) on the headspace concentration of carbon monoxide over time.

of CO and % H₂ with respect to control values, vs, % of methane production to control, is presented in Figures 8 and 9. These two plots suggest that there is in fact a relationship between the level of inhibition by cadmium and the response of the trace gases; although the relation seems to be time dependent. Carbon monoxide might potentially be a better indicator of low level inhibition by cadmium than hydrogen.

Percentage of methane production at 48 hours, vs cadmium dosage in mg/l is presented in Figure 10.

4.1.2. Copper.

Concentrations between 2 and 10 mg/l of copper⁺² (as applied) were examined. Copper was applied as the chloride salt.

Increasing dosages of copper gave increasing levels of inhibition in general (Figure 11). Slight stimulation at the end of 48 hours is seen at an applied copper dose of 2 mg/l. A rapid and early stimulation in the first 2 hours was noticed in all the different concentrations tested. Recovery of inhibition was detected in all the different concent

rations as suggested by the positive slope of the curves.

The variation of hydrogen with time for the different dosages of copper tested is presented in Figure 12. Hydrogen concentrations are consistently lower than control levels for the dosages tested. Hydrogen follows the same pattern of control as carbon monoxide, but this time at a lower base level.

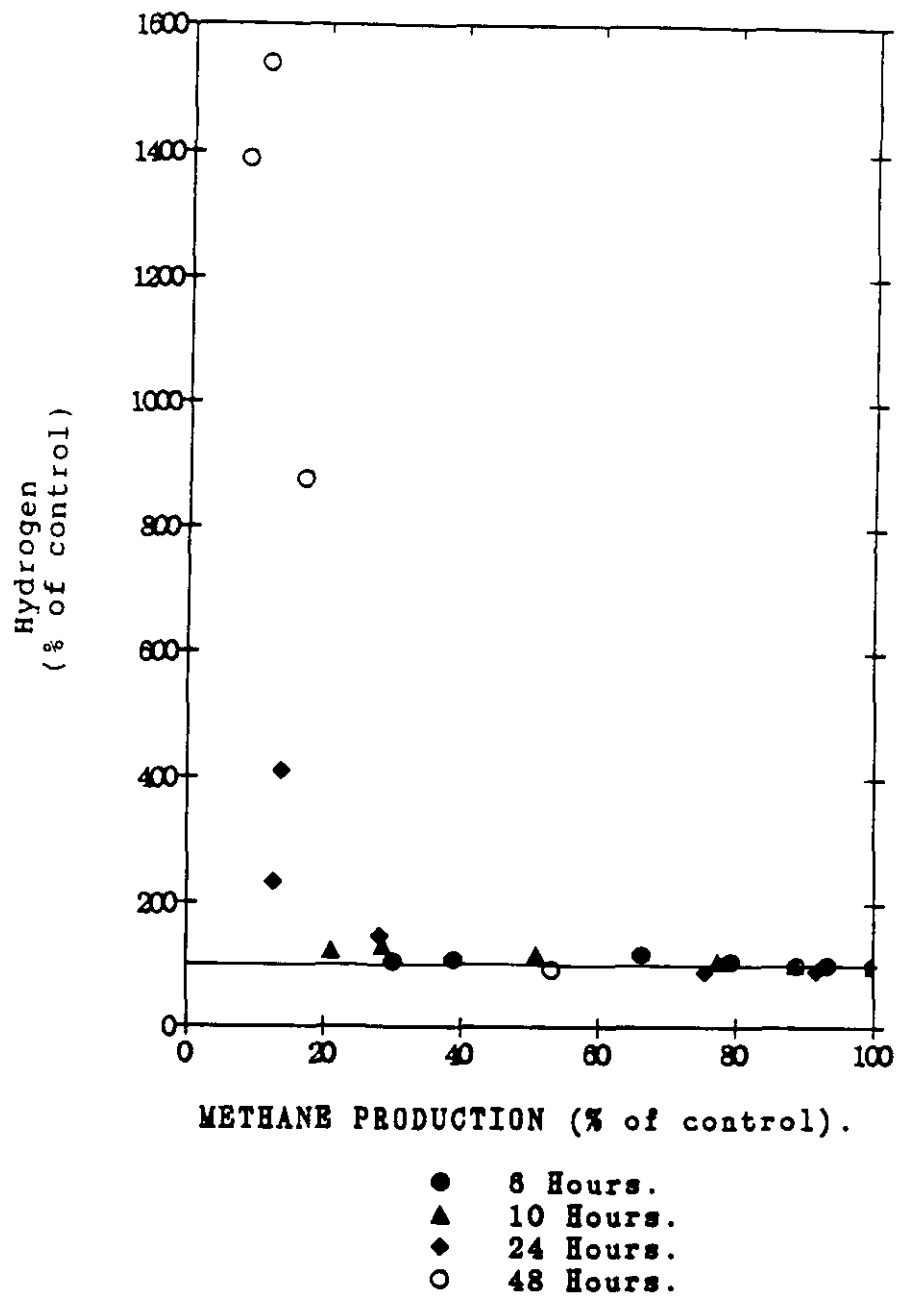


Figure 8. Comparison of hydrogen headspace concentration and cumulative methane production for cadmium inhibited samples. (% of control).

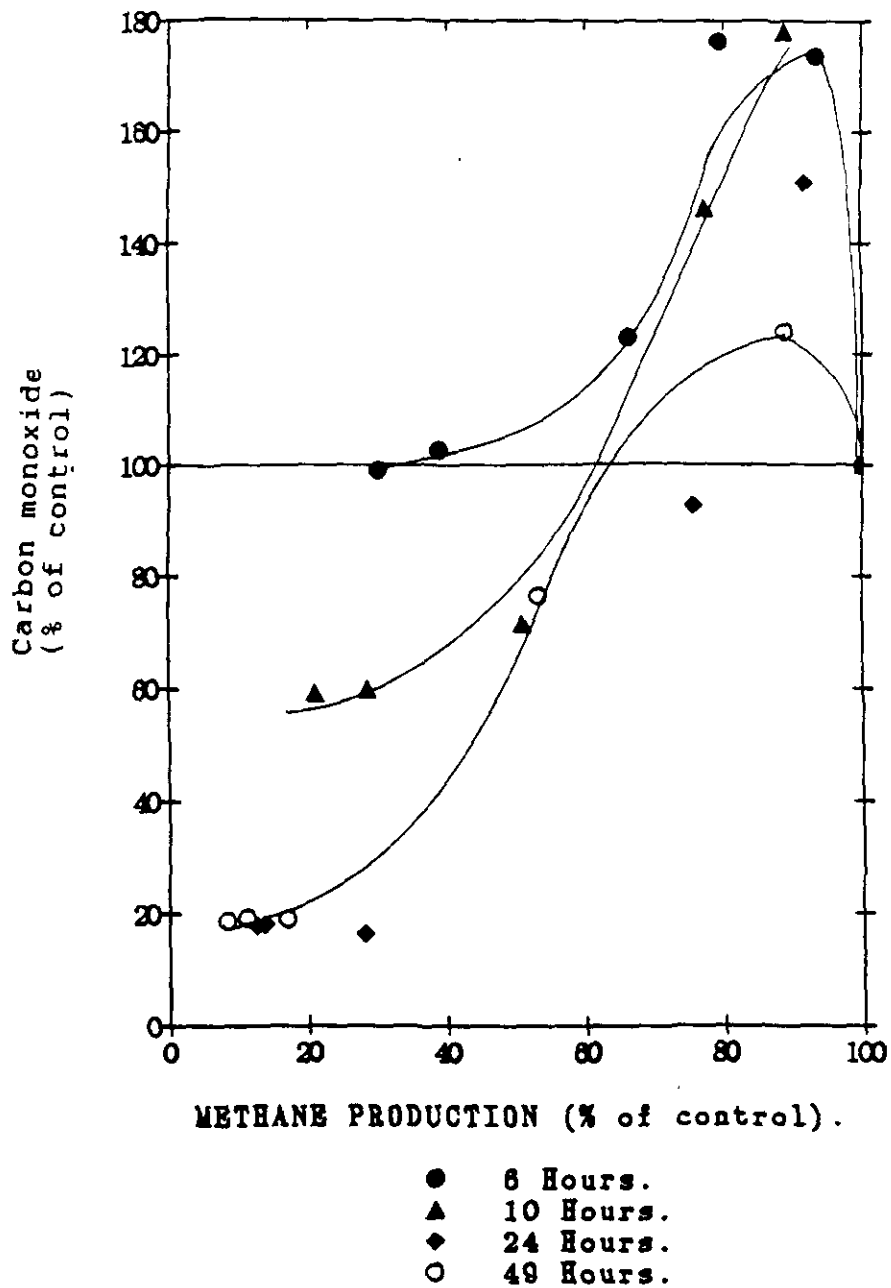


Figure 9. Comparison of the carbon monoxide headspace concentration and cumulative methane production for cadmium inhibited samples. (% of control).

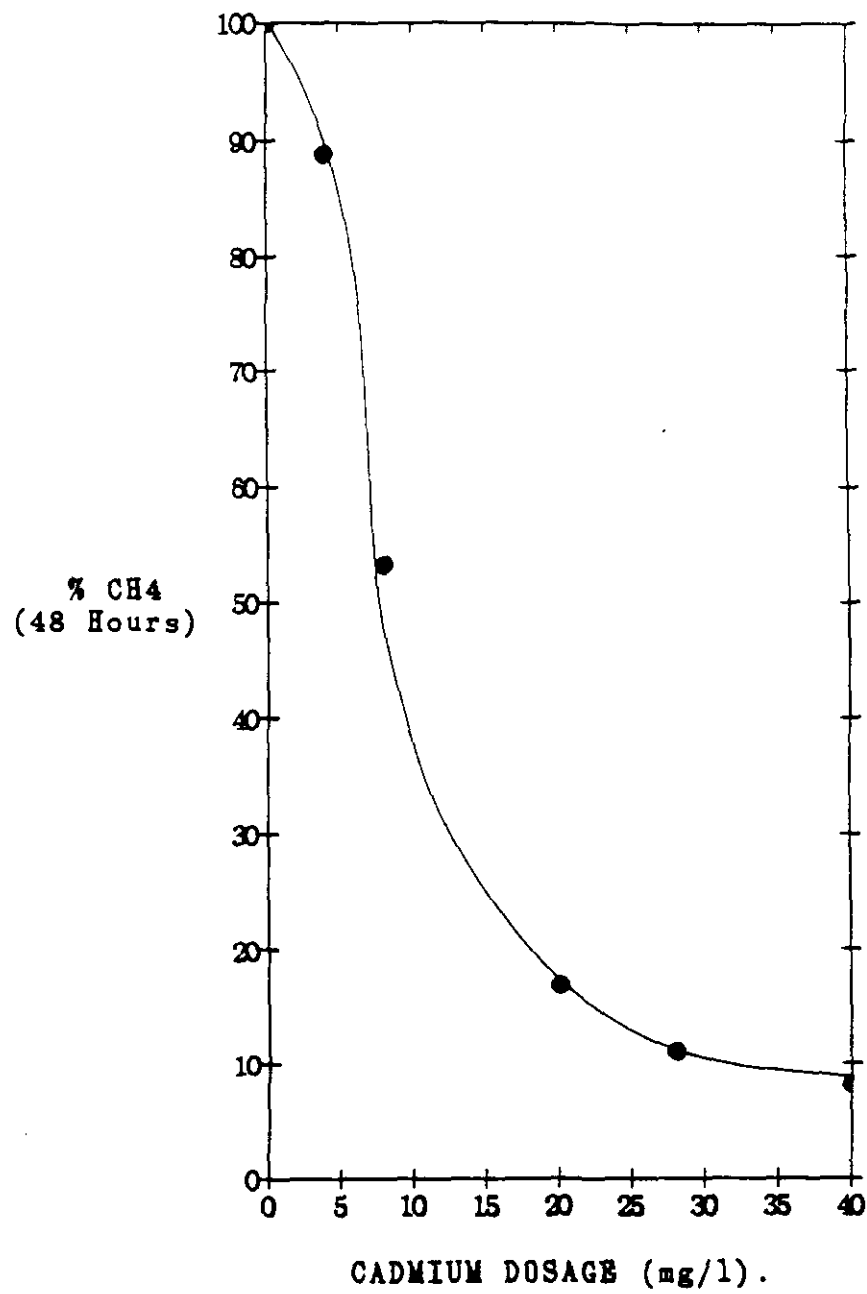


Figure 10. Effect of cadmium dosage on cumulative methane production as percent of control at t=48 hours.

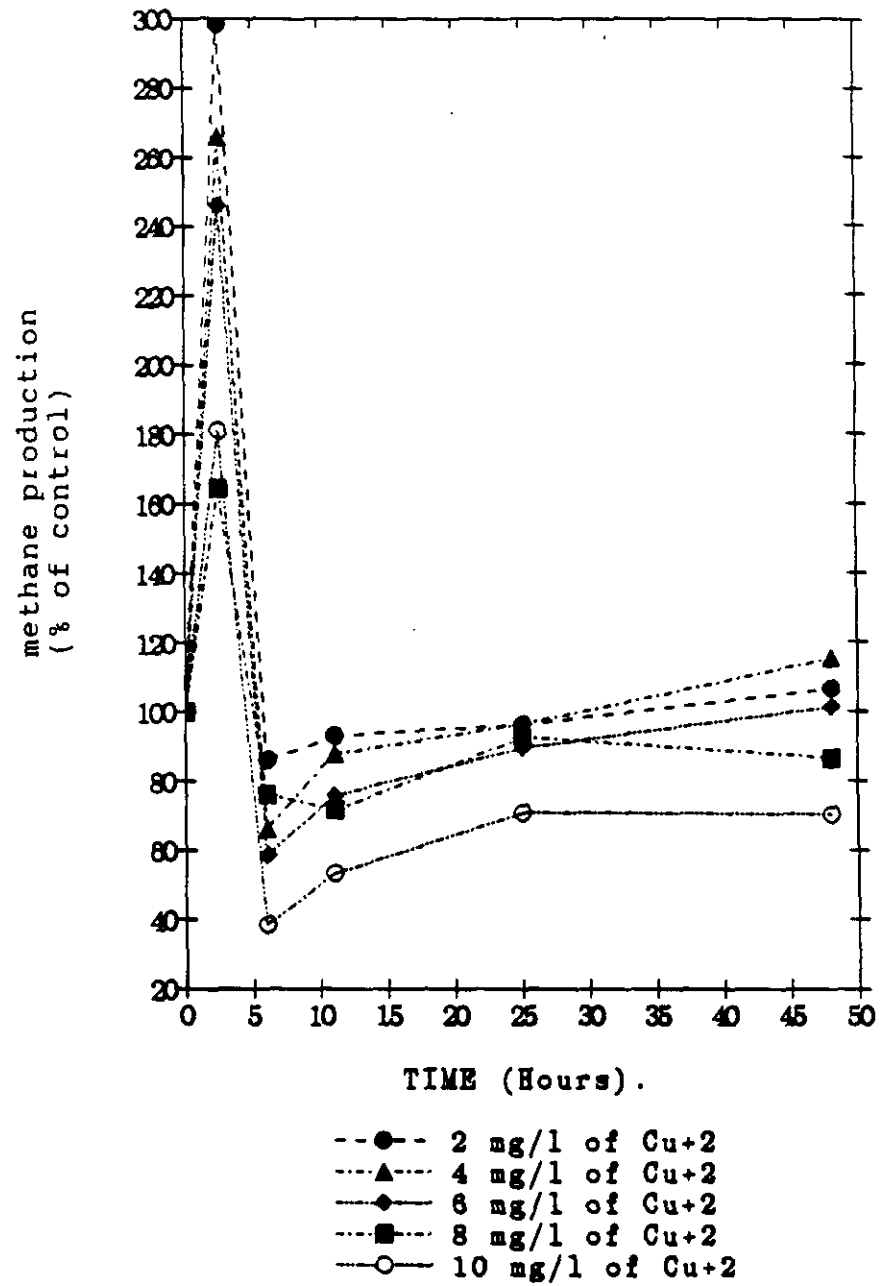


Figure 11. Effect of copper (Cu^{+2}) on the cumulative methane production as percent of control.

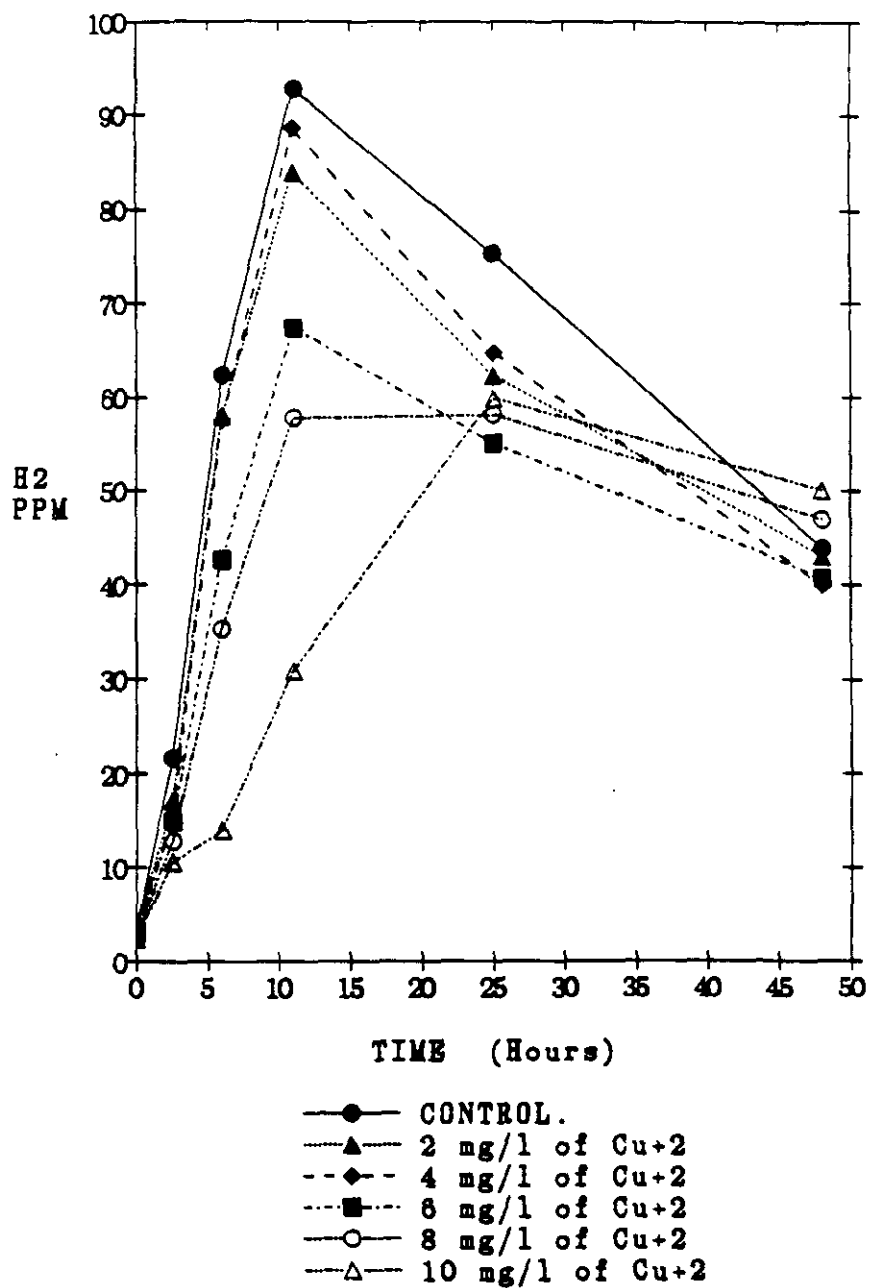


Figure 12. Effect of copper dosage (Cu^{+2}) on the headspace concentration of hydrogen over time.

Concentrations of carbon monoxide in the headspace were consistently higher than the controls (Figure 13). Carbon monoxide in all the different serum bottles seem to follow the same trend but at a different base level.

Trends of behavior for carbon monoxide and hydrogen with varying inhibition levels are presented in Figures 14 and 15. It can be seen the same pattern for carbon monoxide at the different times. For hydrogen the behavior seems to be more time independent, with consistently lower percent hydrogen variations at lower inhibitions. Again carbon monoxide might potentially be a better indicator than hydrogen for low inhibition levels caused by copper.

In Figure 16 the decrease in methane production activity at 12 hours, vs copper dosage is presented .

4.1.3. Nickel.

Concentration of nickel⁺² (as applied) between 150 and 1500 mg/l were studied. Nickel was added as the chloride salt.

Increased dosages of nickel progressively inhibited the methane production as illustrated in Figure 17. Slight recovery of the system was detected for the 300 mg/l dosage.

Hydrogen evolution in the head-space with time seems to follow a dual pattern (Figure 18). For dosages of 750 mg/l of Ni or higher, hydrogen partial pressure was even the same level or lower than the control. For dosages of nickel lower than 750 mg/l, hydrogen

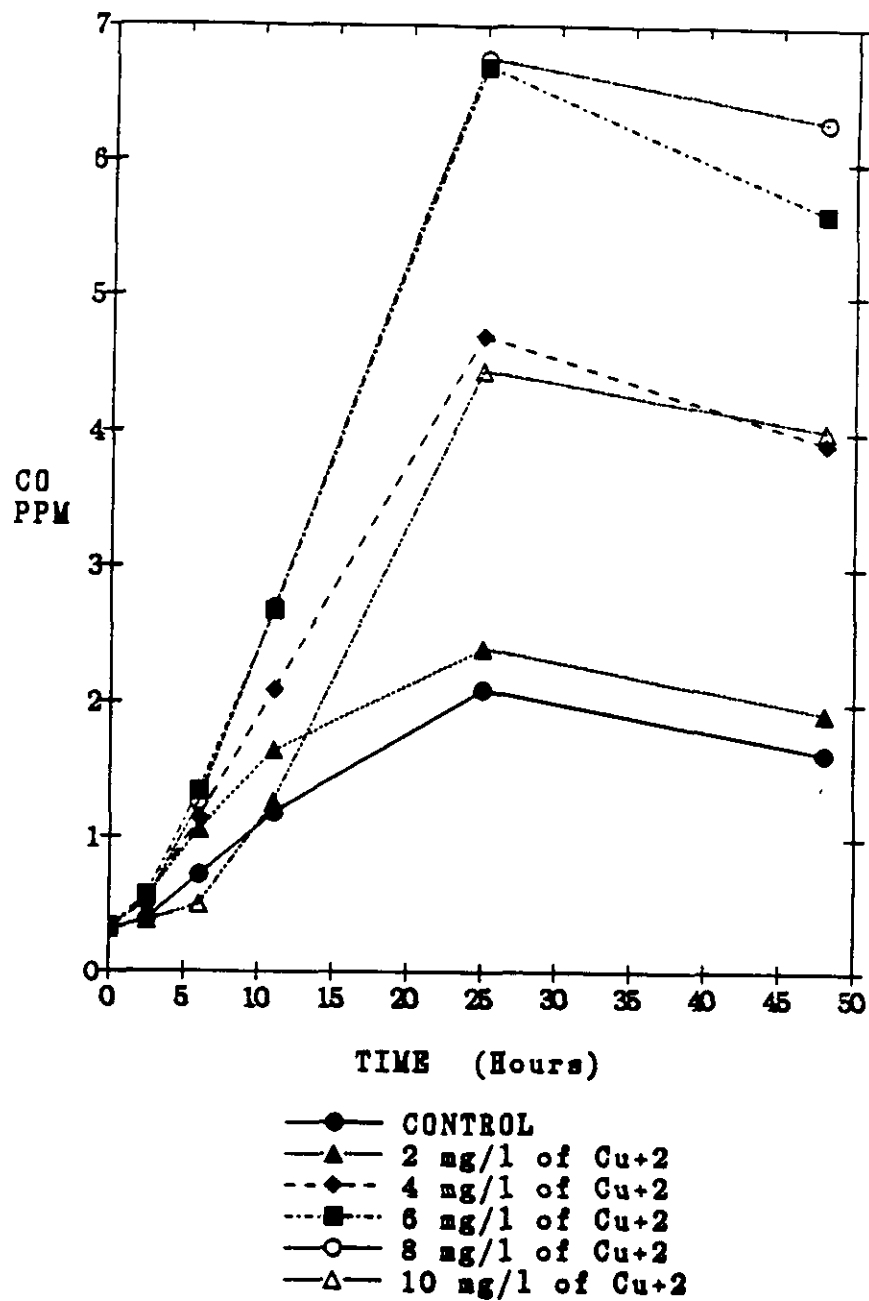


Figure 13. Effect of copper dosage (Cu^{+2}) on the headspace concentration of carbon monoxide over time.

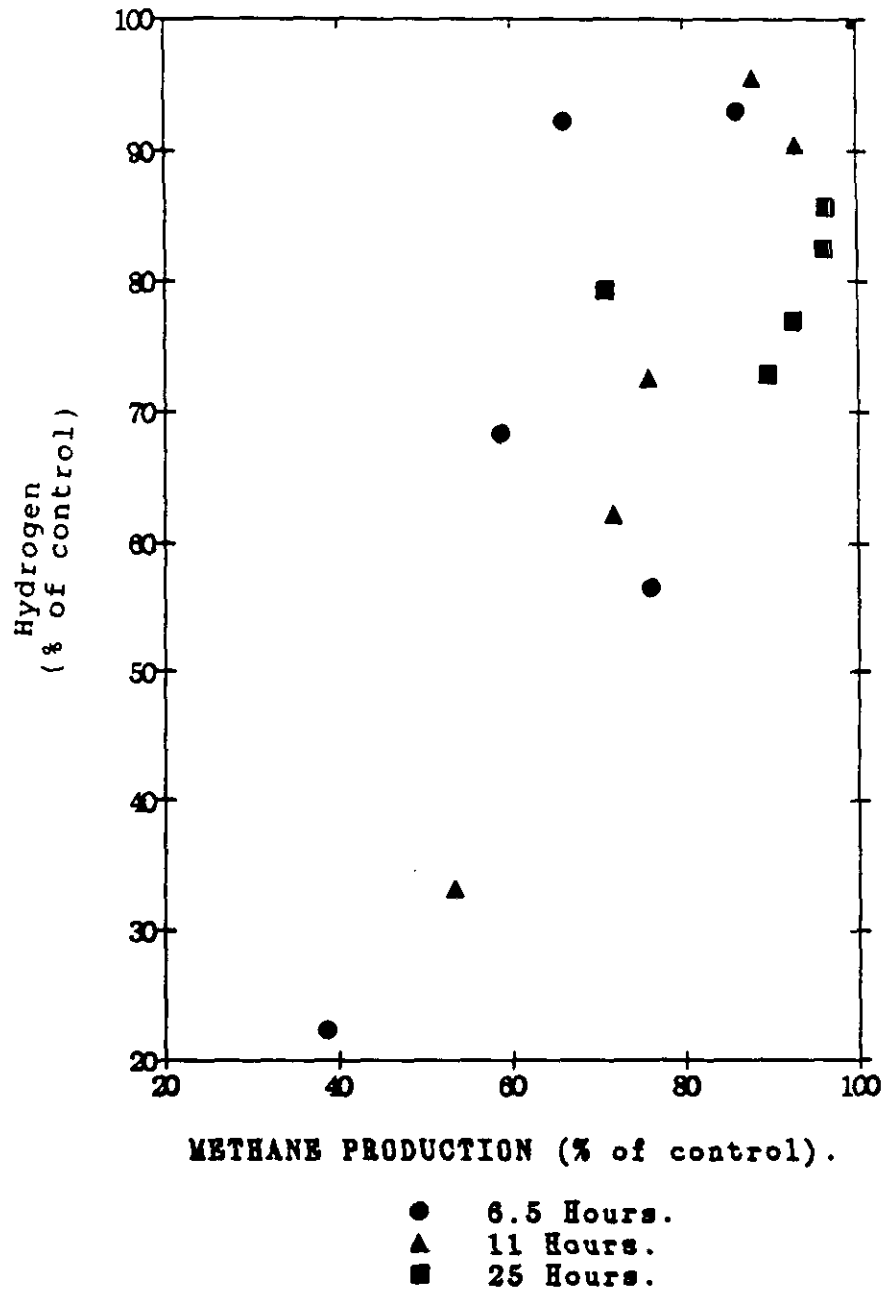


Figure 14. Comparison of hydrogen headspace concentration and cumulative methane production for copper inhibited samples. (% of control).

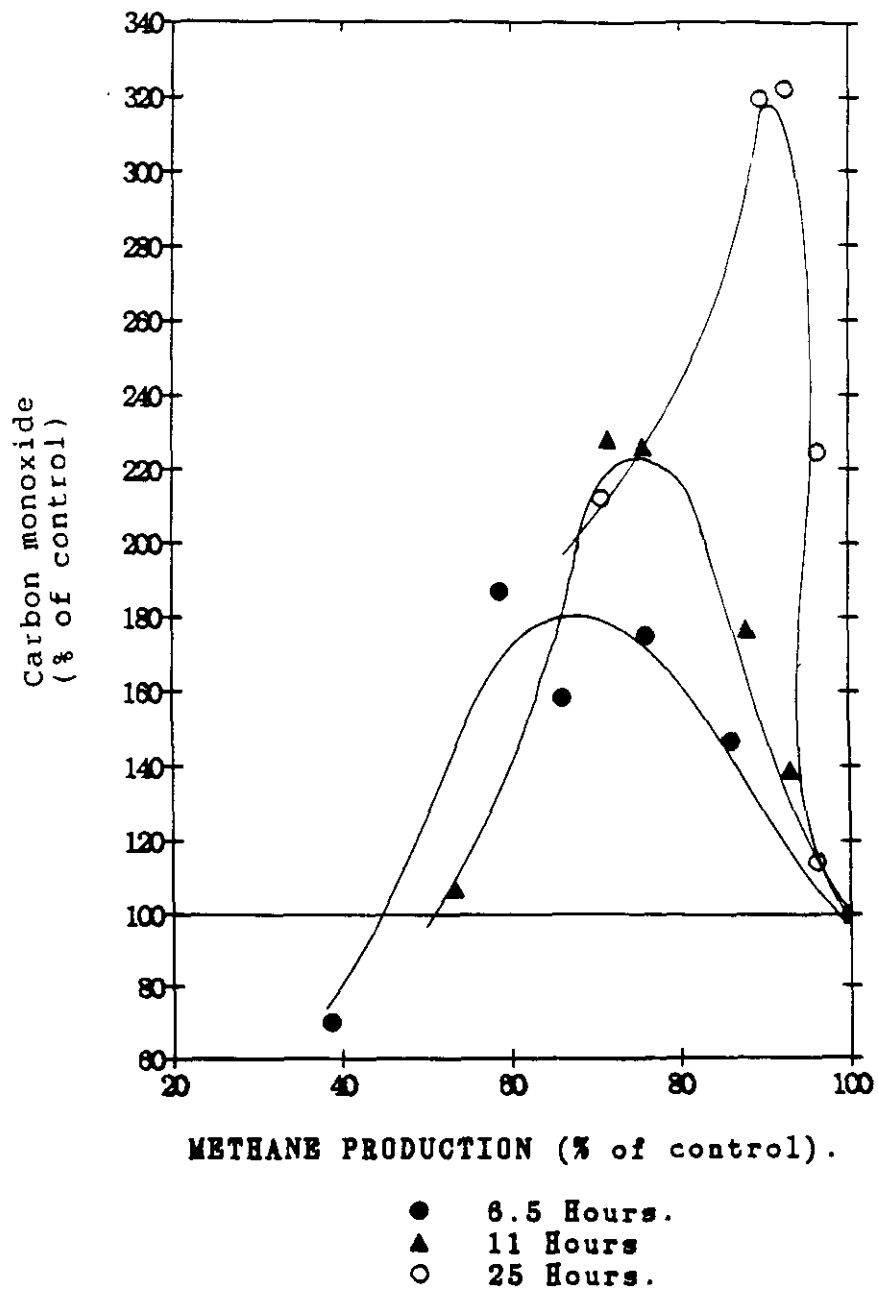


Figure 15. Comparison of the carbon monoxide headspace concentration and cumulative methane production for copper inhibited samples. (% of control).

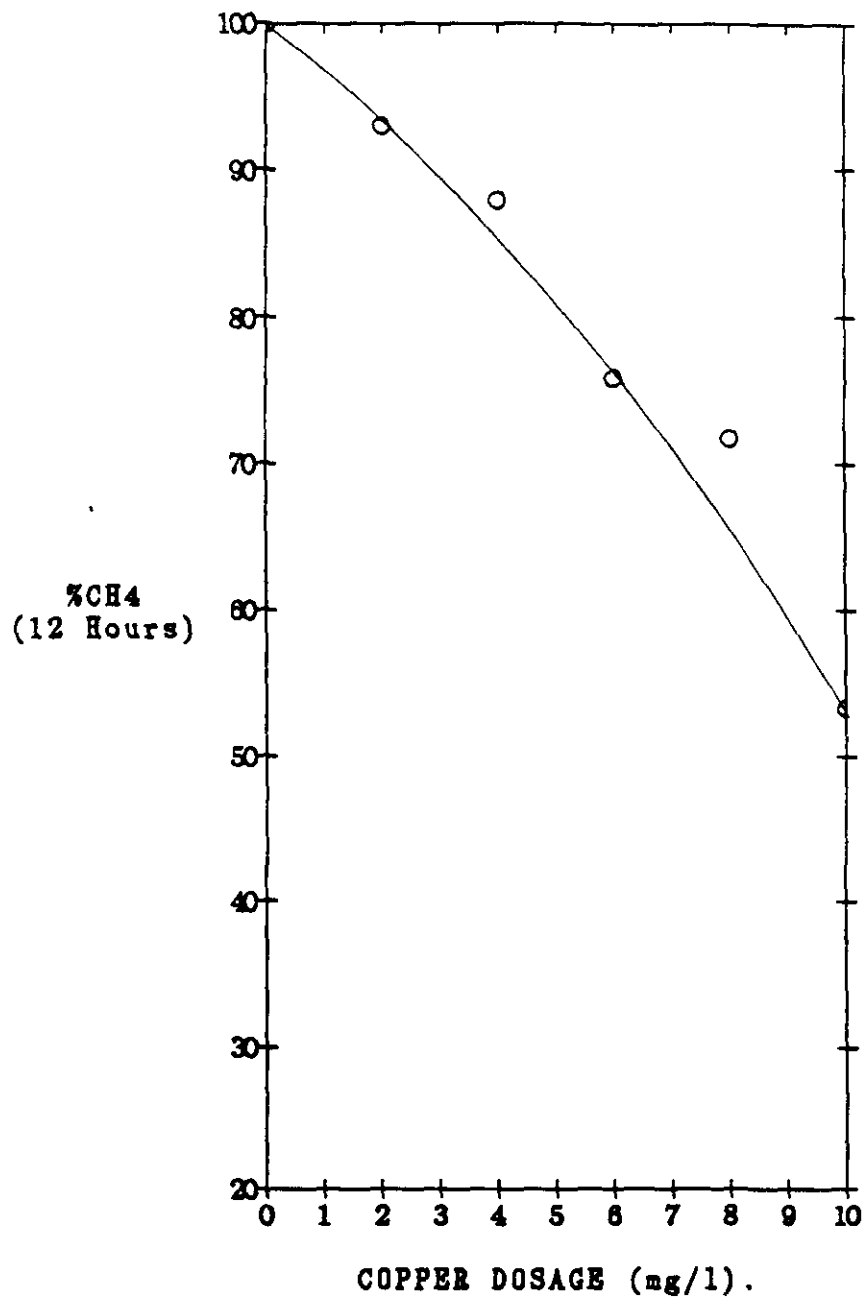


Figure 16. Effect of copper dosage on cumulative methane production as percent of control at t=12 hours.

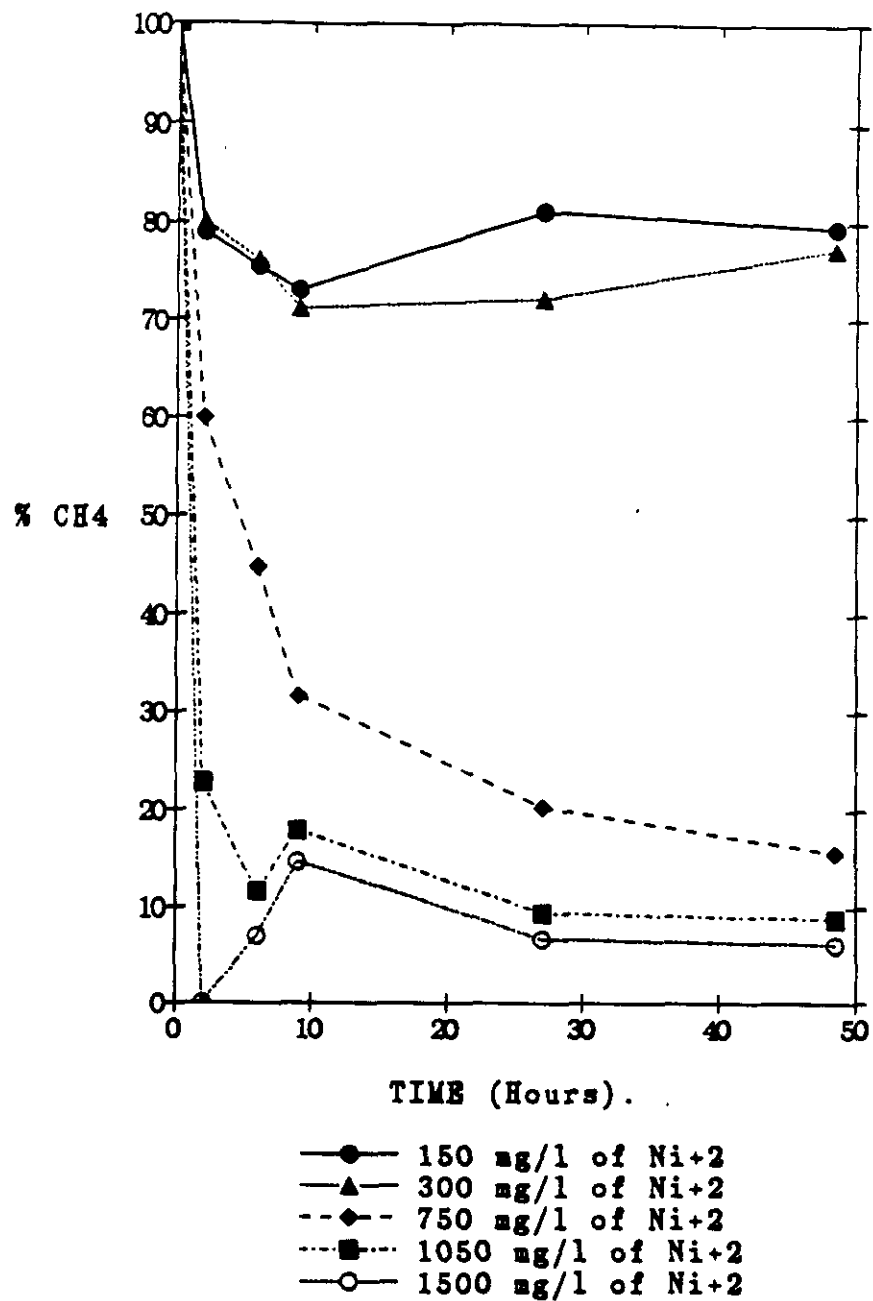


Figure 17. Effect of nickel (Ni^{+2}) on the cumulative methane production as percent of control.

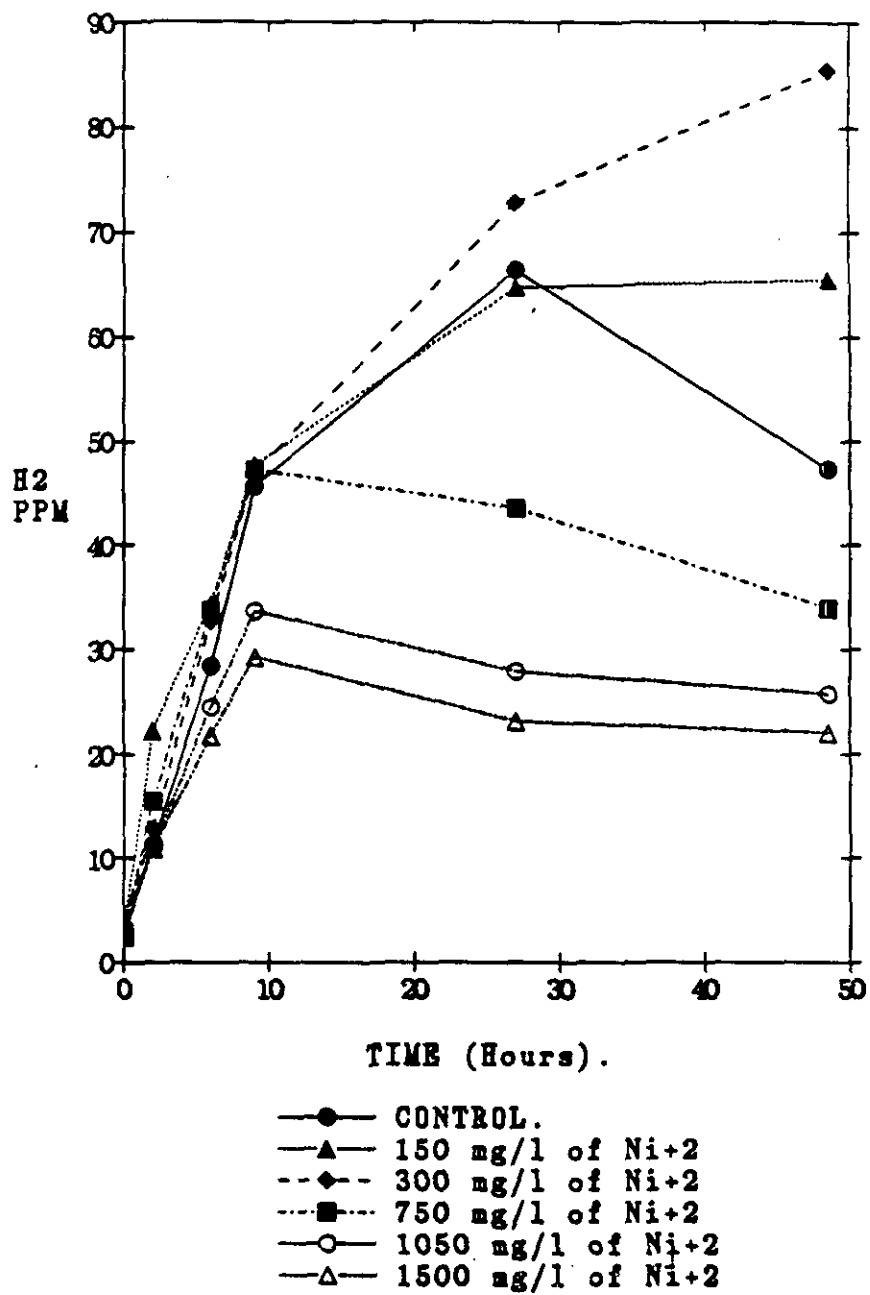


Figure 18. Effect of nickel dosage (Ni²⁺) on the headspace concentration of hydrogen over time.

partial pressure was in general higher than the control.

The behavior of carbon monoxide with time also shows a dual trend (Figure 19). For dosages of 750 mg/l or higher, carbon monoxide concentration always remains below the control level. Higher dosages of nickel produce a higher depression in the carbon monoxide levels. For concentrations of 150 and 300 mg/l, CO concentrations remain higher than the control during the first 24 hours.

Hydrogen concentrations in the head space as a function of methane production inhibition show a strong time dependent pattern (Figure 20). As the time from the initial pulse of toxicant passes, the hydrogen inhibition response pattern changes from higher than control values to lower than control values. The percent variation of the values relatively to the ones of the control is higher as the level of inhibition and time from the toxicant pulse become bigger.

Carbon monoxide concentration nickel-inhibition trends are also highly time dependent (Figure 21). In contrast with the hydrogen ones they present a peak curve for low inhibition levels with a higher percent variation than hydrogen. Hence, CO might potentially be, again, a better warning indicator than hydrogen for low level inhibitions by nickel.

Inhibition of methane production at 24 hours by applied nickel dosages is presented in Figure 22.

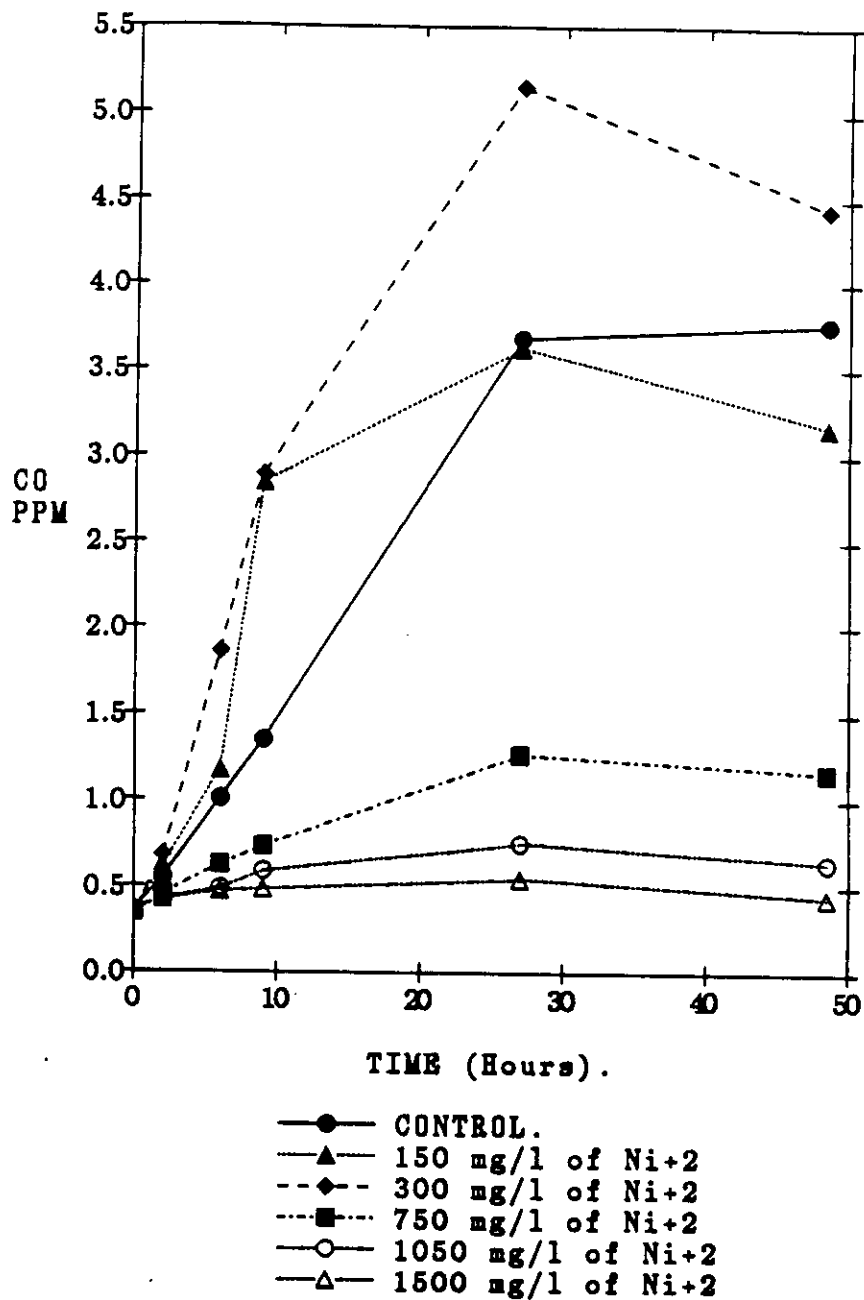


Figure 19. Effect of nickel dosage (Ni^{+2}) on the headspace concentration of carbon monoxide over time.

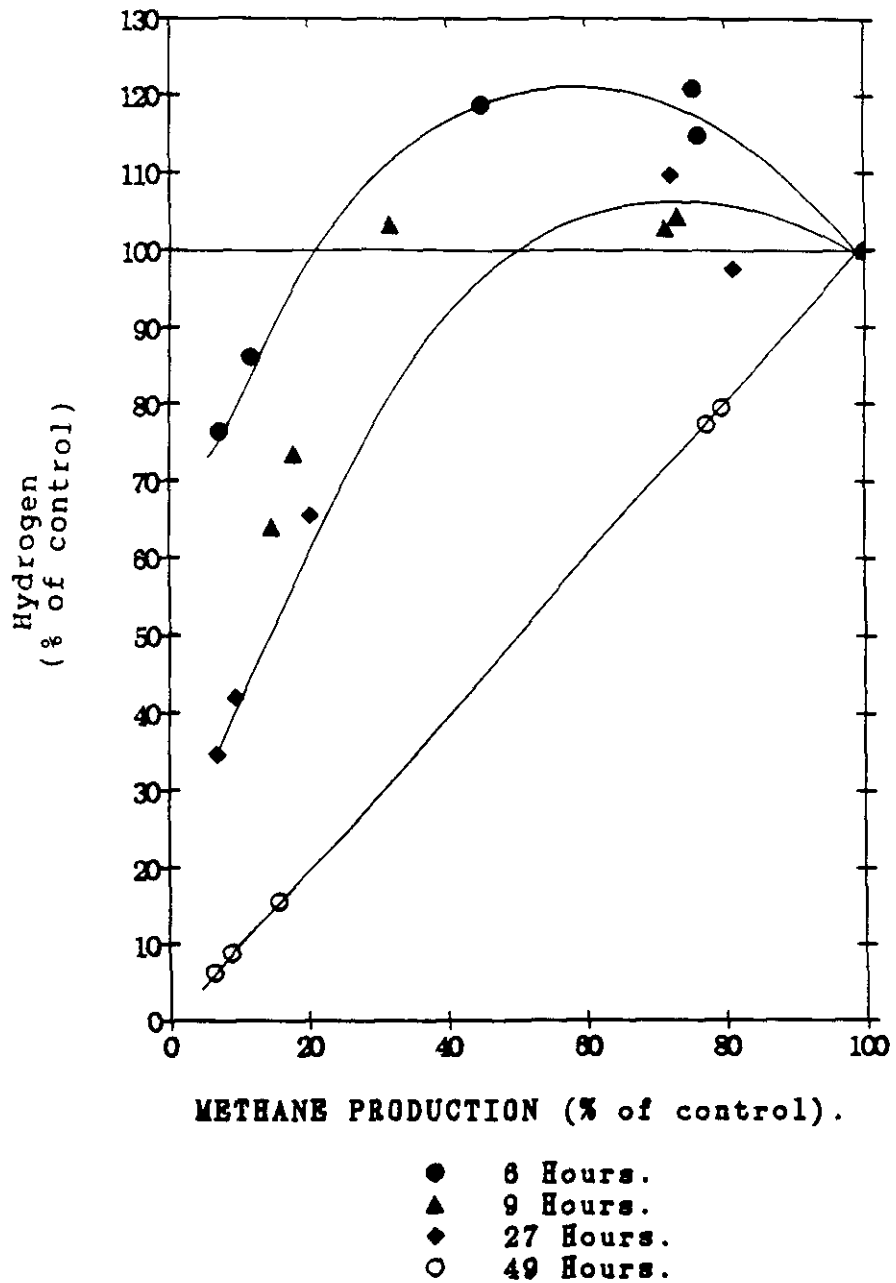


Figure 20. Comparison of hydrogen headspace concentration and cumulative methane production for nickel inhibited samples. (% of control).

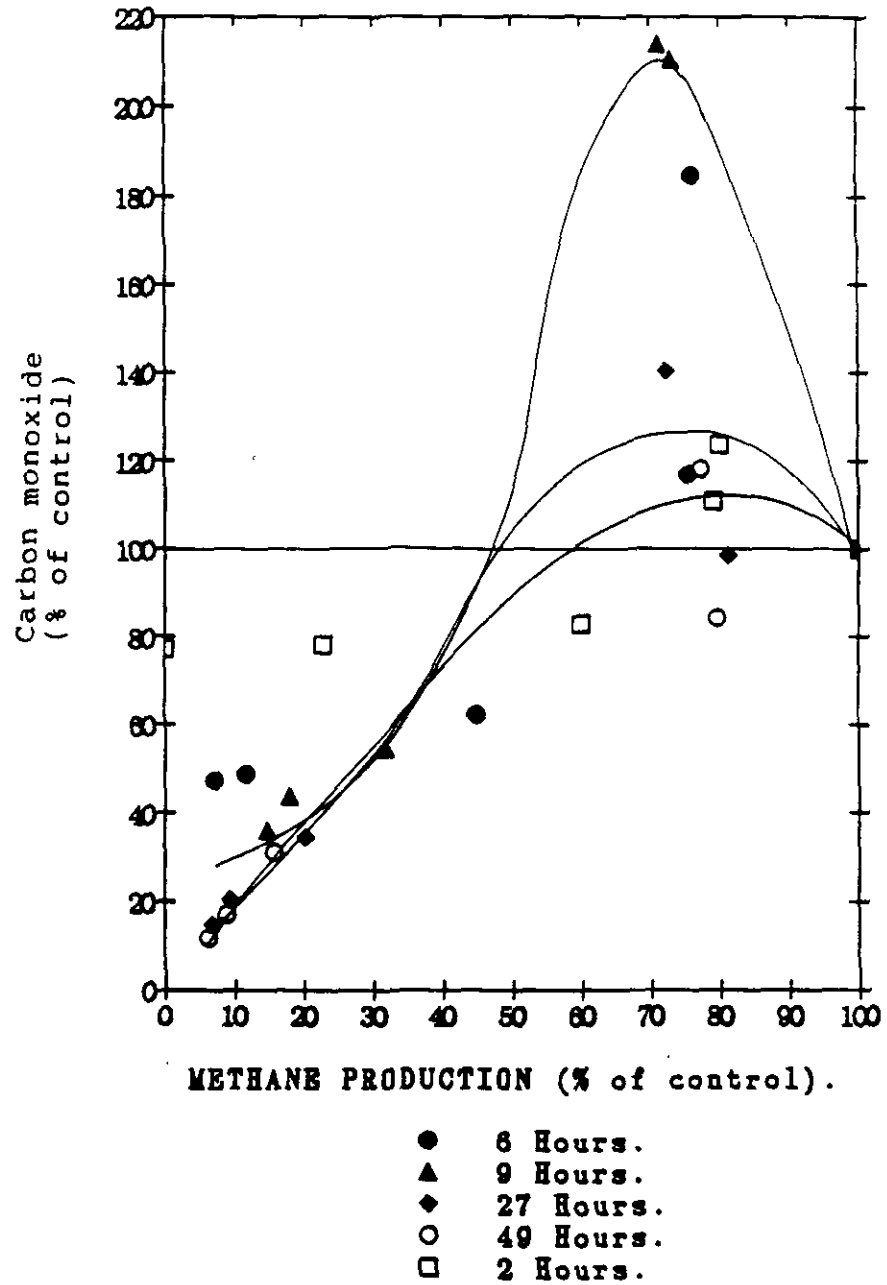


Figure 21. Comparison of the carbon monoxide headspace concentration and cumulative methane production for nickel inhibited samples. (% of control).

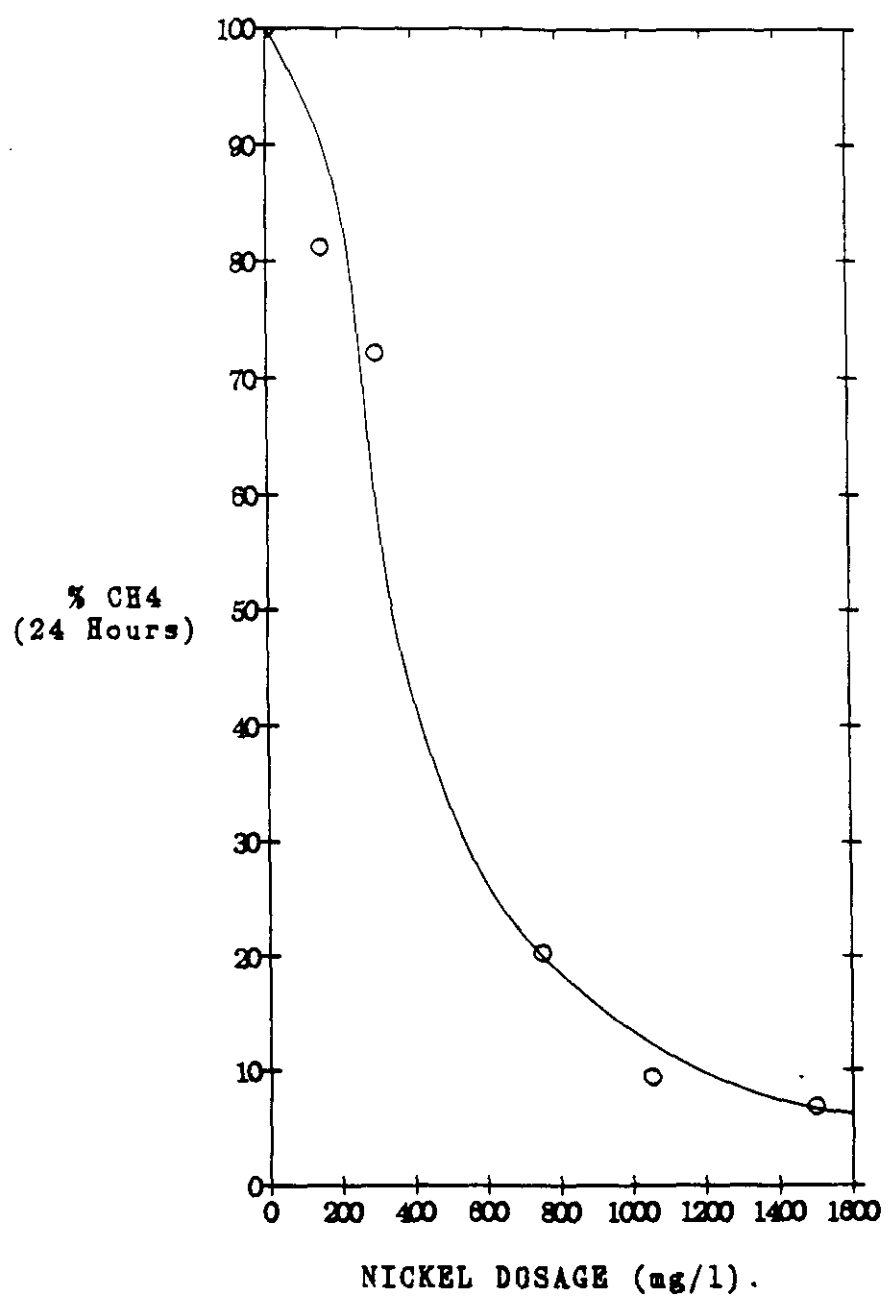


Figure 22. Effect of nickel dosage on cumulative methane production as percent of control at t=24 hours.

4.1.4. Zinc.

Concentration of Zn^{+2} (as applied) between 100 and 300 mg/l were examined. Zinc was added as the chloride salt.

In general higher dosages of zinc produced higher levels of inhibition, although 200 mg/l seems to produce less inhibition than 100 mg/l (Figure 23). Dosages of 100, 150 and 200 mg/l present some recovery with time.

Hydrogen concentrations in the headspace for the different doses of zinc investigated are presented in Figure 24. Hydrogen stays below control levels during the entire experiment, with the exception of one point, 48 hours and 300 mg/l. Hydrogen follows the same trend as the control for all the zinc dosages tested, but at a lower base level, and converging to a similar level at the end of the test.

CO concentrations are presented in Figure 25. CO seems to follow two different patterns. Until 10 hours CO is consistently at a higher level than the control, and after hour 10 at a consistently lower concentration than the control. As time passes, the difference between the control and the zinc-added systems becomes bigger, in contrast with the hydrogen pattern where all the values converge.

Variations of hydrogen with respect to control and inhibition levels are presented in Figure 26. Hydrogen trends are time dependent. Higher percent variations are noted at increasing times. Nevertheless, a trend of consistently lower levels of hydrogen than the control is suggested.

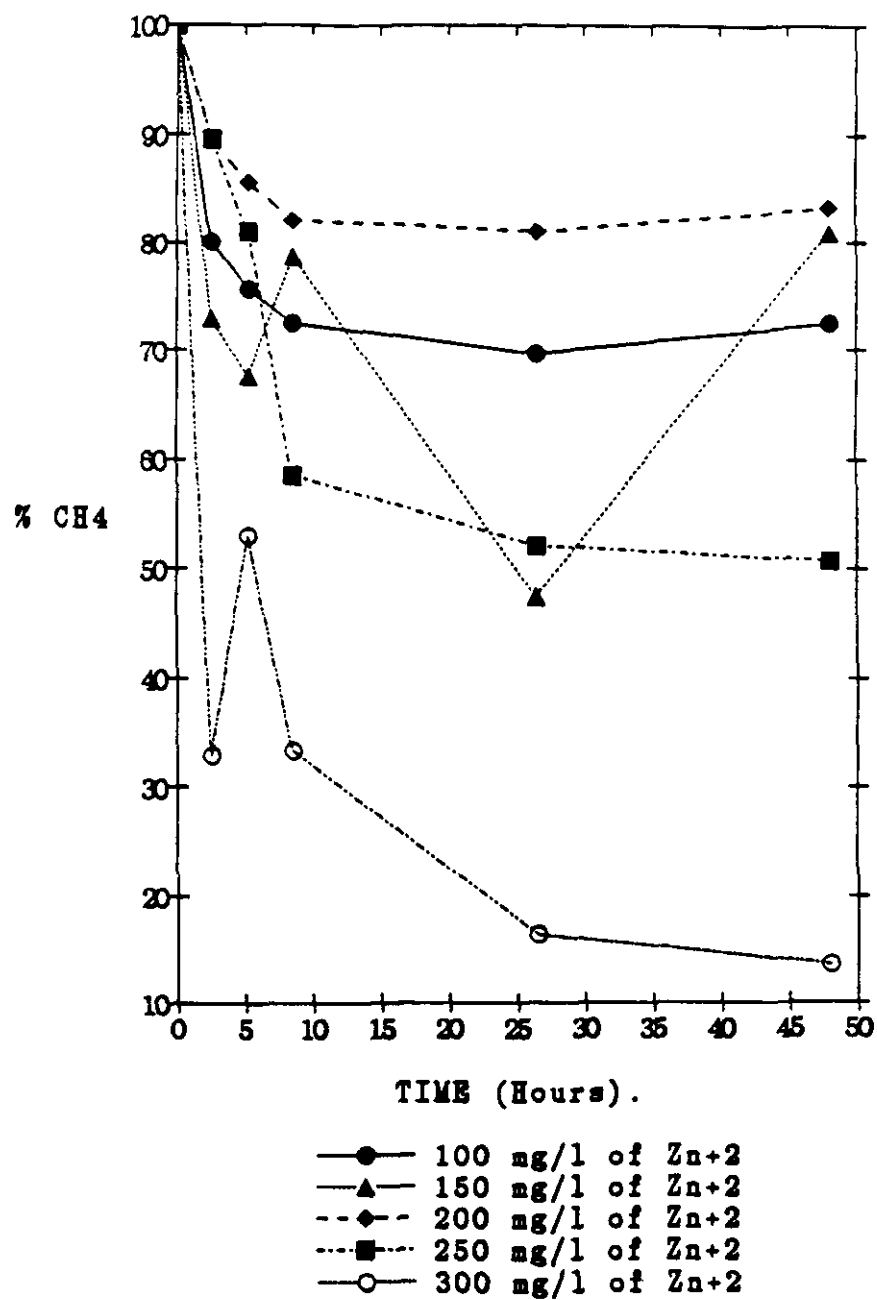


Figure 23. Effect of zinc (Zn^{+2}) on the cumulative methane production as percent of control.

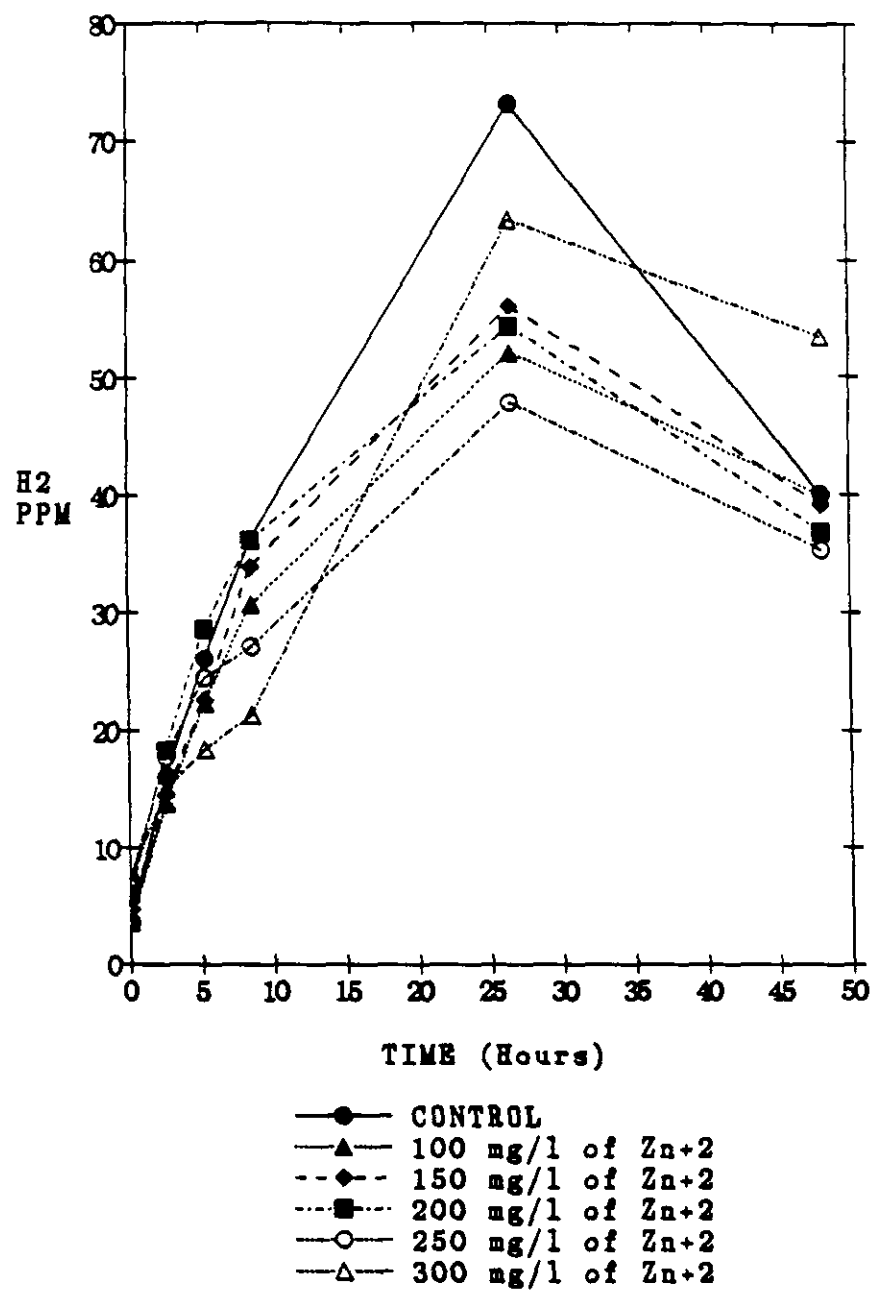


Figure 24. Effect of zinc dosage (Zn^{+2}) on the headspace concentration of hydrogen over time.

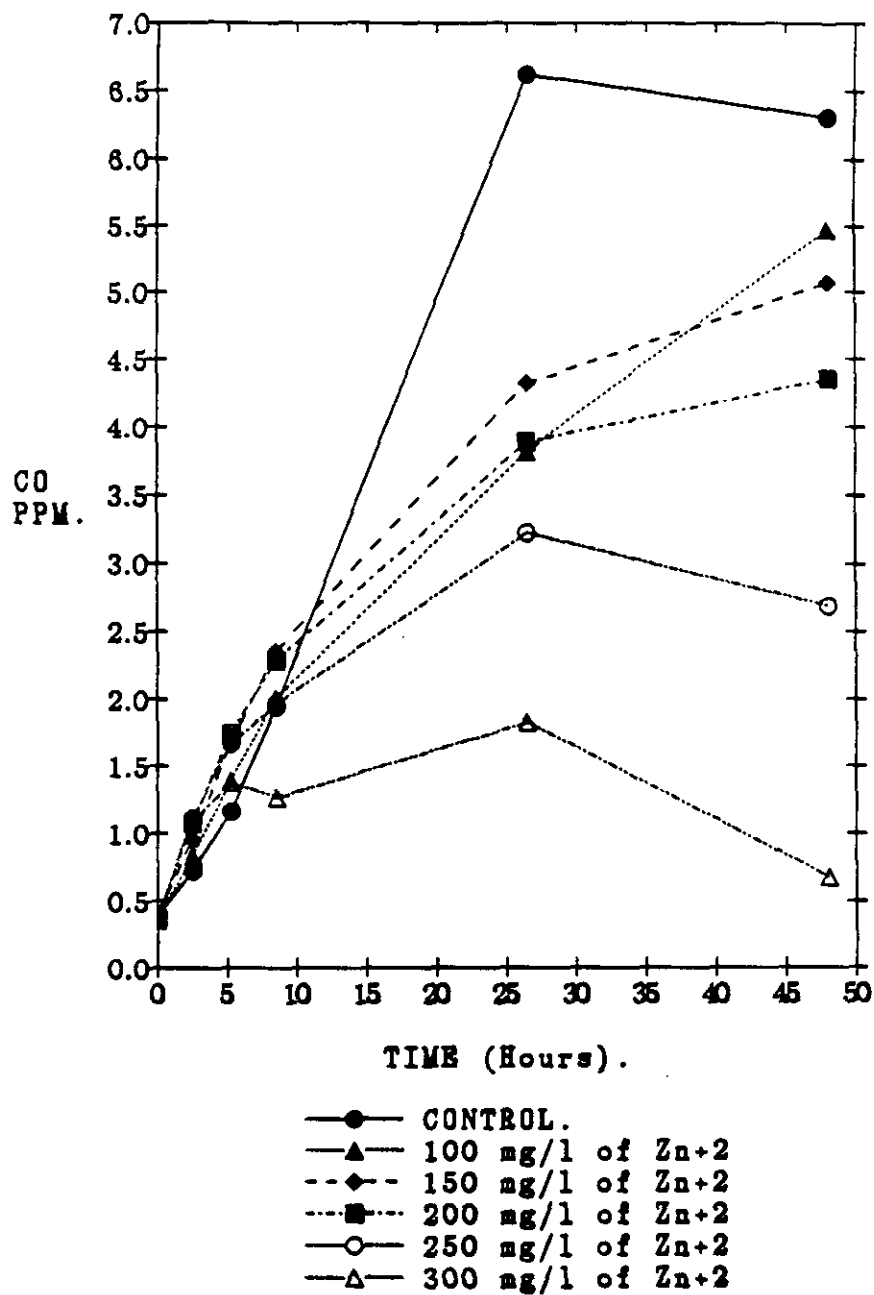


Figure 25. Effect of zinc dosage (Zn⁺²) on the headspace concentration of carbon monoxide over time.

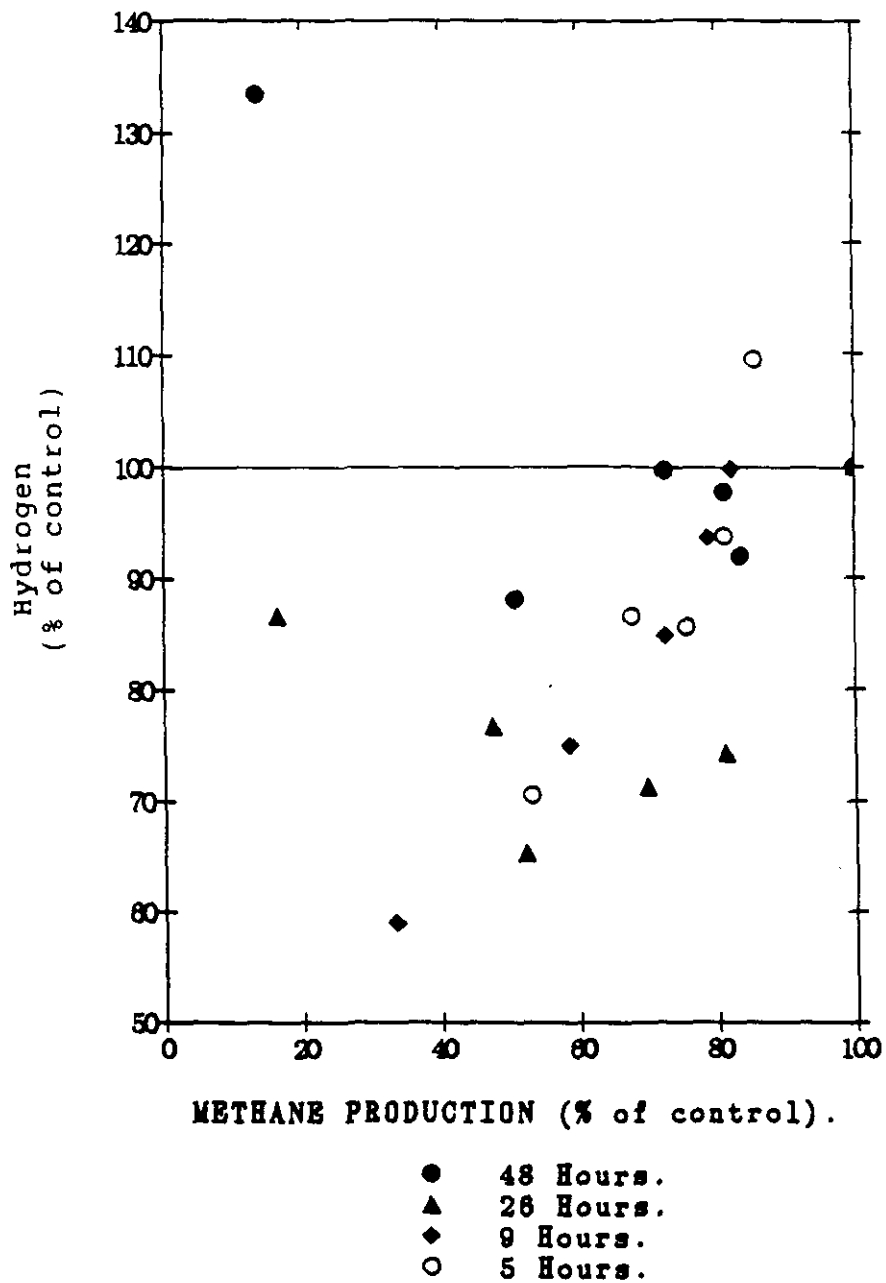


Figure 26. Comparison of hydrogen headspace concentration and cumulative methane production for zinc inhibited samples. (% of control).

Carbon monoxide behavior with respect to control and inhibition levels is presented in Figure 27. Trends are time dependent, and they show again the generation of a peak curve at early hours that flattens with time. Percentual variations with respect to control are higher than the ones of hydrogen.

Inhibition of methane production at 24 hours by different dosages of zinc used is presented in Figure 28.

4.2. ORGANIC TOXICANTS.

4.2.1. Bromoethanesulfonic Acid (BES).

BES concentrations between 2 and 20 mM (as applied) were examined. Inhibition of methane production as a function of time and the different dosages used is presented in Figure 29. BES increasingly inhibits the culture as the concentration added increases. No recovery was detected for any of the samples during the period of the experiment. The lowest dosage applied, 2 mM, presents a slight stimulation of the methane production during the first 2 hours of the experiment.

The hydrogen concentration in the headspace as a function of BES applied dosage is presented in Figure 30. Two different patterns are suggested. For low dosages of BES, 2 and 5 mM, the hydrogen concentration in the headspace is essentially the same as in the controls. Hydrogen increases very rapidly after the fifth hour for

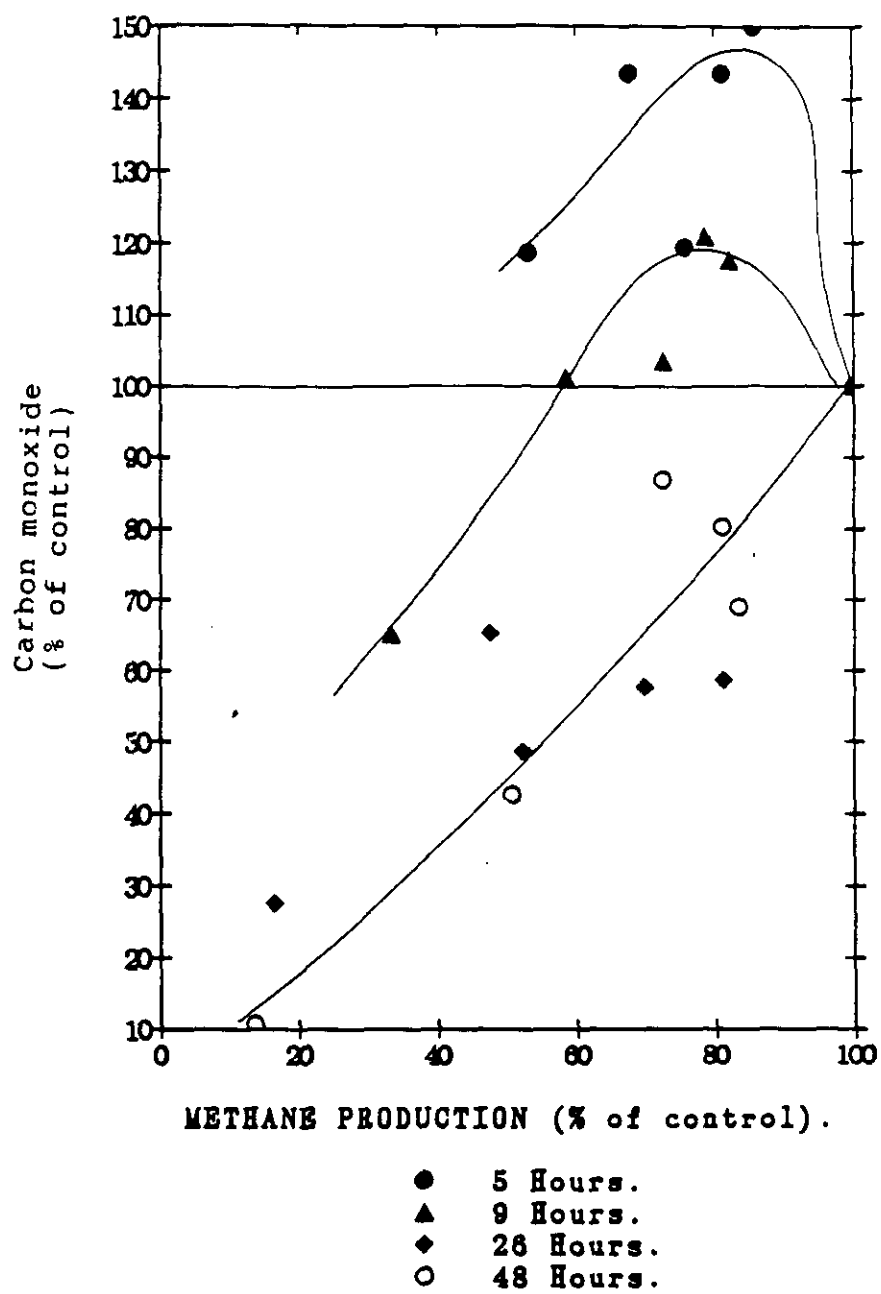


Figure 27. Comparison of the carbon monoxide headspace concentration and cumulative methane production for zinc inhibited samples. (% of control).

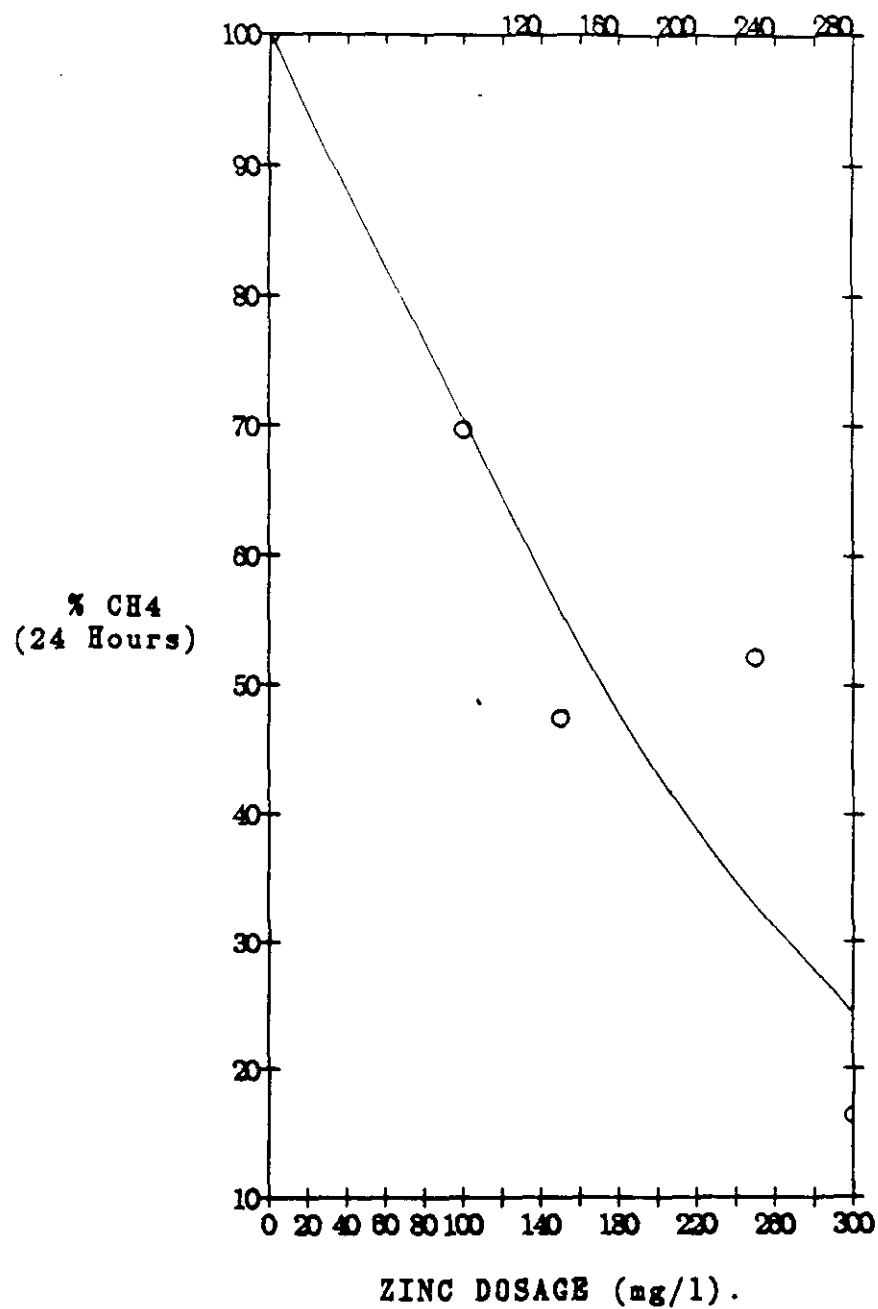


Figure 28. Effect of zinc dosage on cumulative methane production as percent of control at $t=24$ hours.

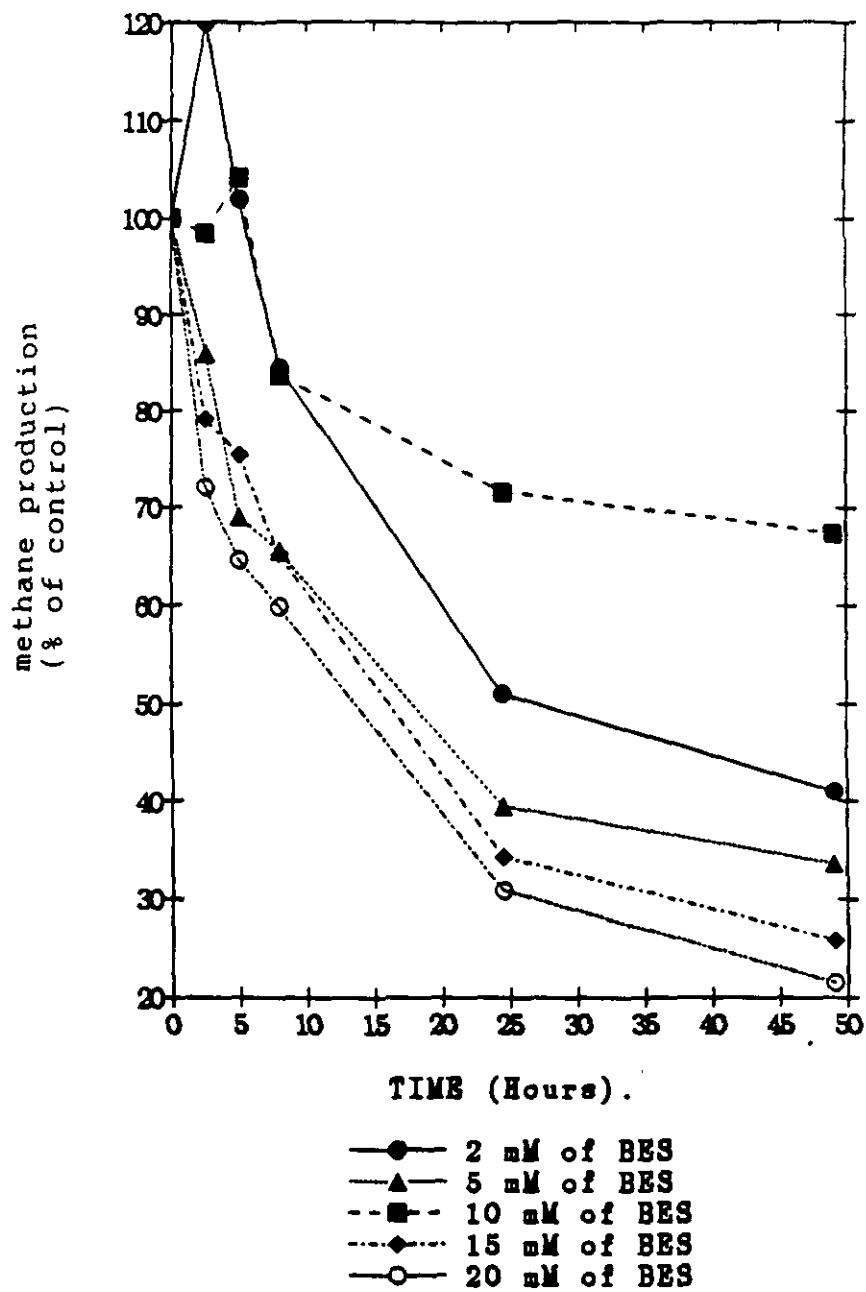


Figure 29. Effect of BES on the cumulative methane production as percent of control.

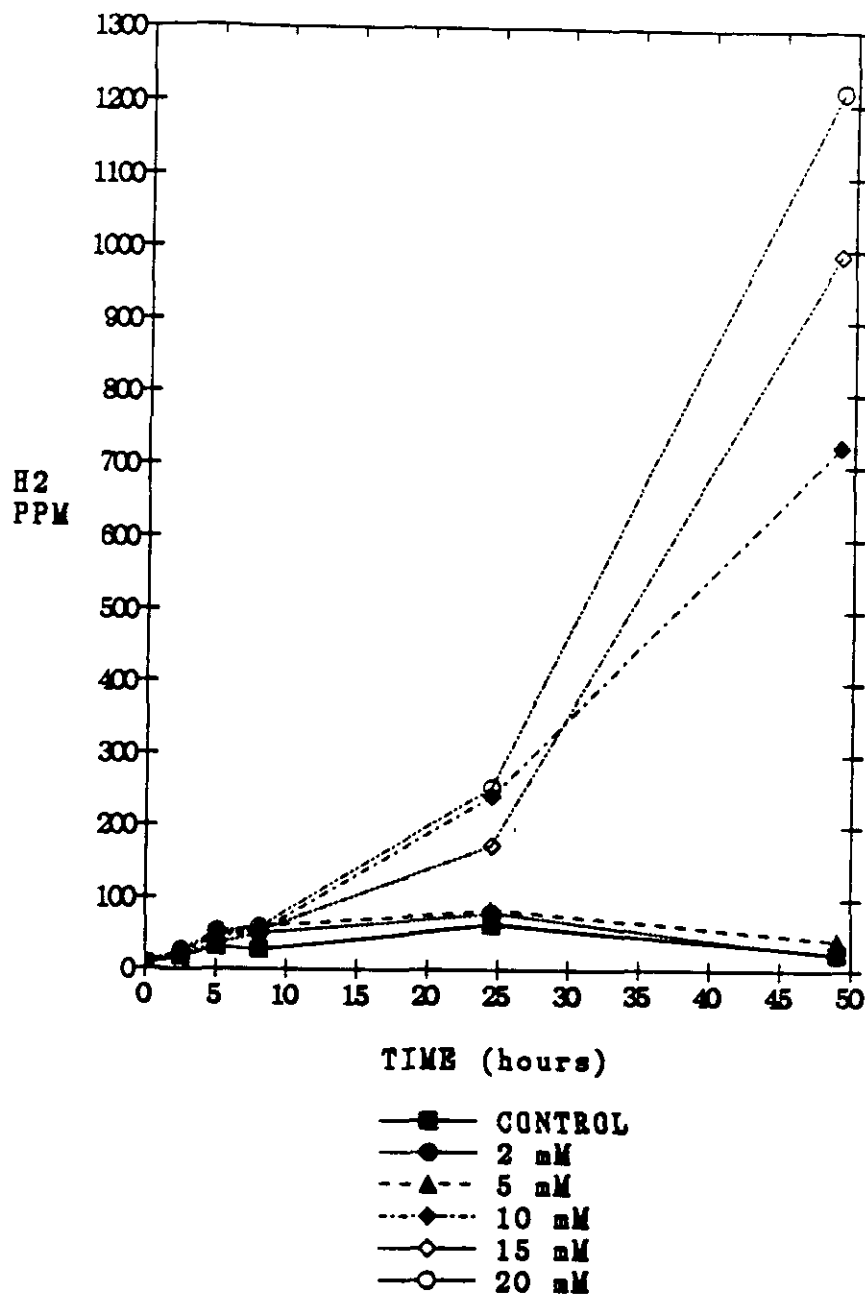


Figure 30. Effect of BES dosage on the headspace concentration of hydrogen over time.

higher dosages of BES, reaching levels on the order of 1000 ppm.

Variations of headspace concentration of carbon monoxide vs time are presented in Figure 31. Carbon monoxide levels remain lower than the controls during the entire experiment, for all the dosages of BES tested.

Figure 32 illustrates the relationships between level of inhibition and percent hydrogen relative to the control at different times. Although the pattern can be considered time dependent, it can be seen that hydrogen consistently increases with increasing inhibition levels and time.

Figure 33 illustrates the pattern for carbon monoxide. Again, trends are time dependent, although with a weaker dependence. Carbon monoxide consistently decrease in concentration as the levels of inhibition increase. Clear trends can be detected from the fifth hour.

The inhibition of methane production at 48 hours caused by the different levels of BES added is presented in Figure 34.

4.2.2. Formaldehyde.

Formaldehyde additions to achieve concentrations in the bottles between 16 and 64 mg/l were examined. Formaldehyde was applied in the same concentration as commercially supplied (37%).

Methane production inhibition is presented in Figure 35. All the different concentrations applied showed inhibition recovery during the time of the experiment. Inhibition by formaldehyde seems to act in a

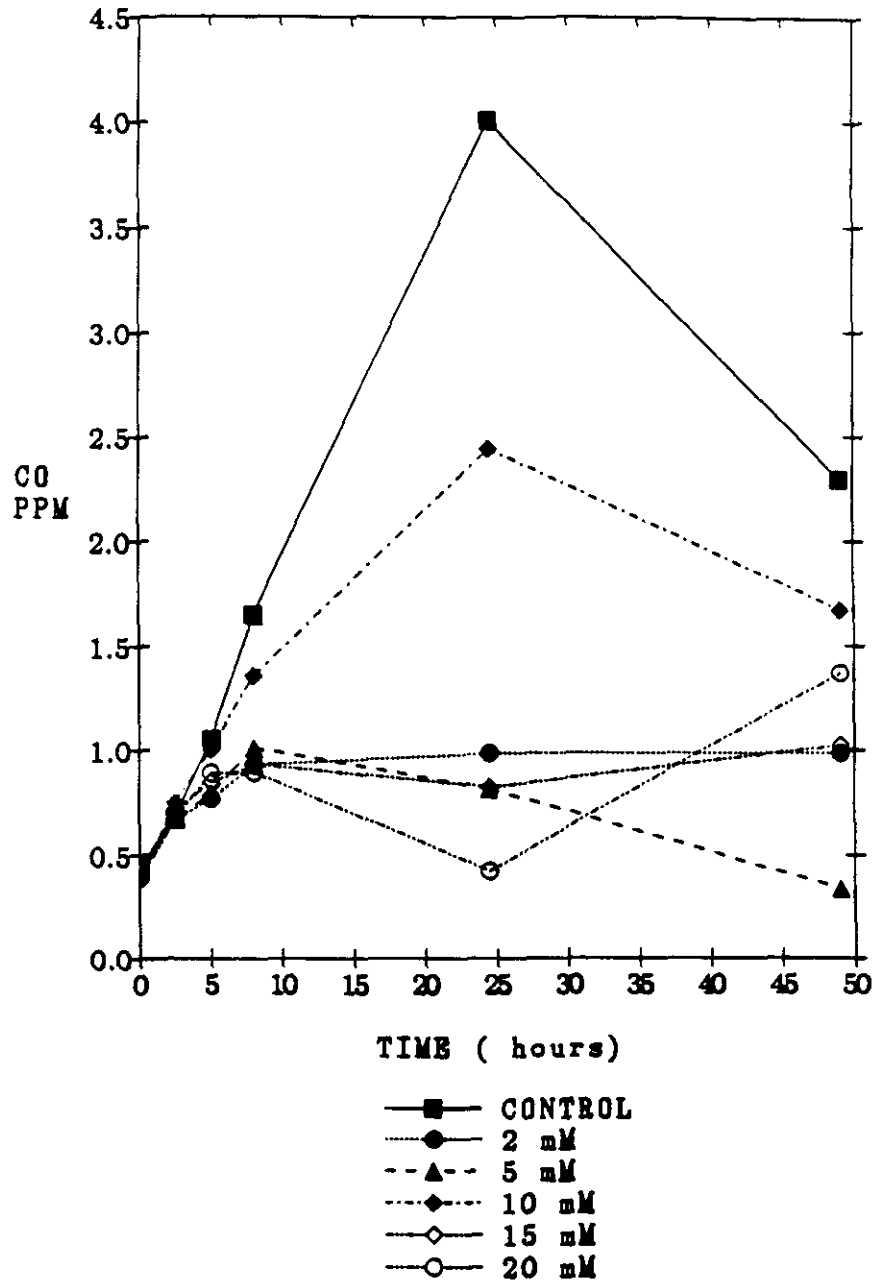


Figure 31. Effect of BES dosage on the headspace concentration of carbon monoxide over time.

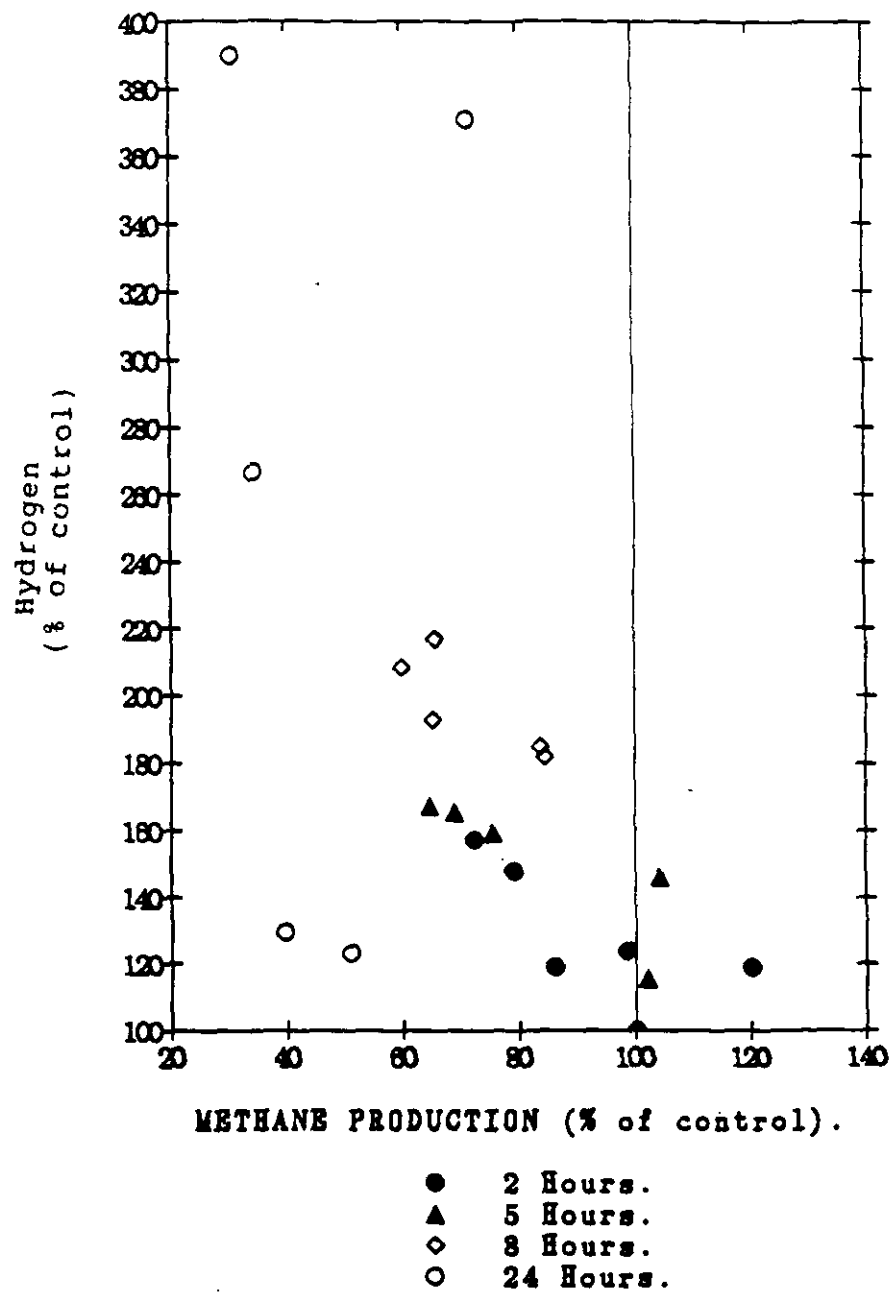


Figure 32. Comparison of hydrogen headspace concentration and cumulative methane production for BES inhibited samples. (% of control).

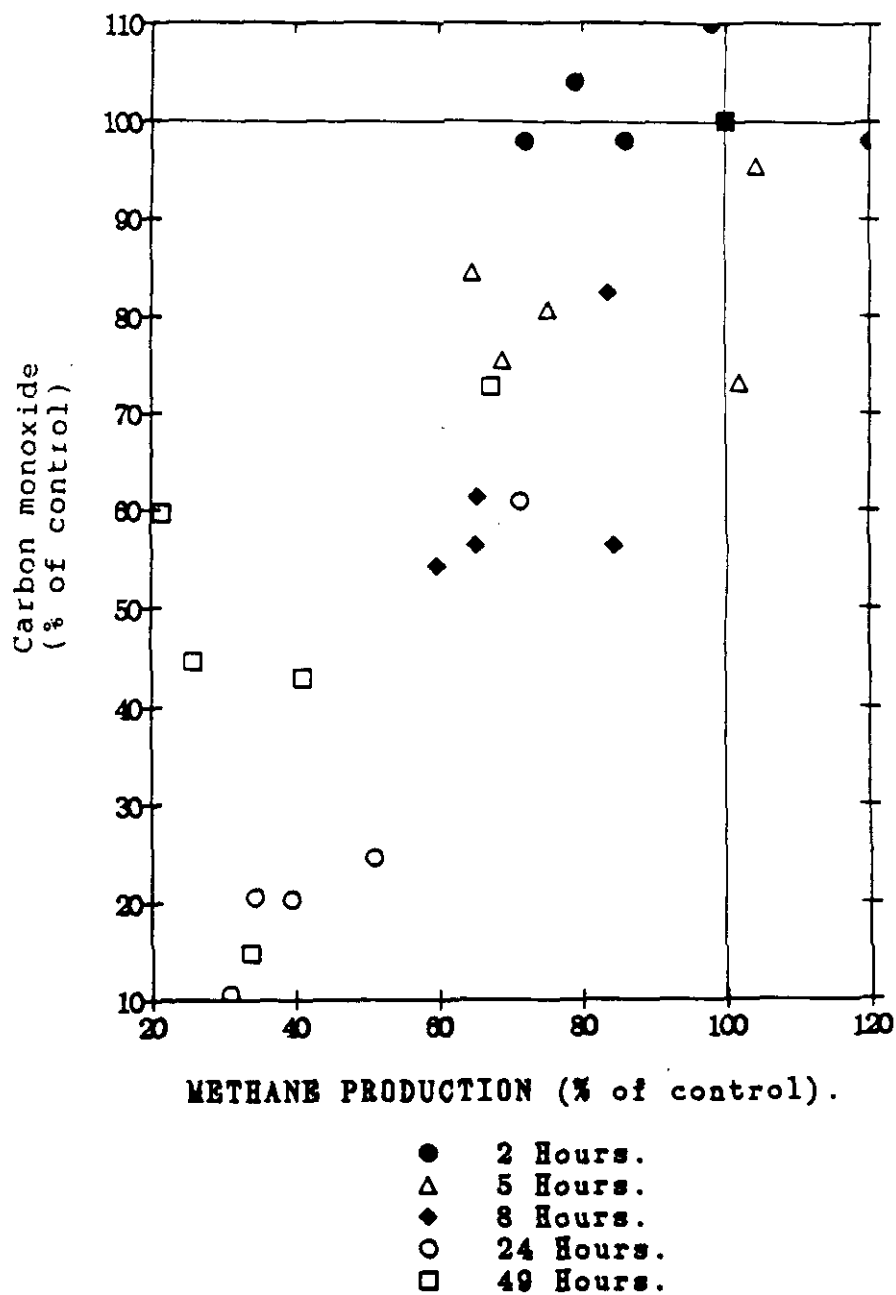


Figure 33. Comparison of the carbon monoxide headspace concentration and cumulative methane production for BES inhibited samples. (% of control).

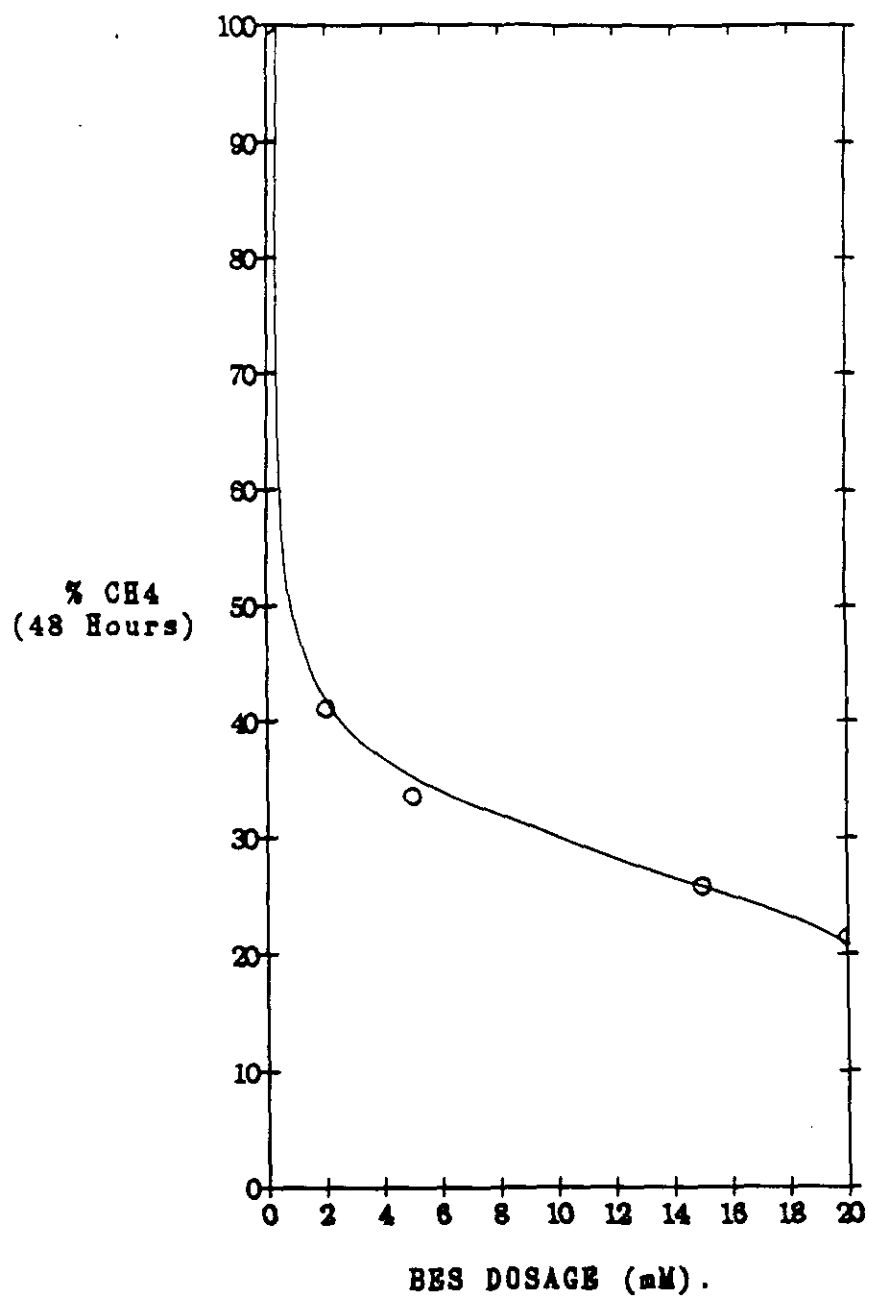


Figure 34. Effect of BES dosage on cumulative methane production as percent of control at t=48 hours.

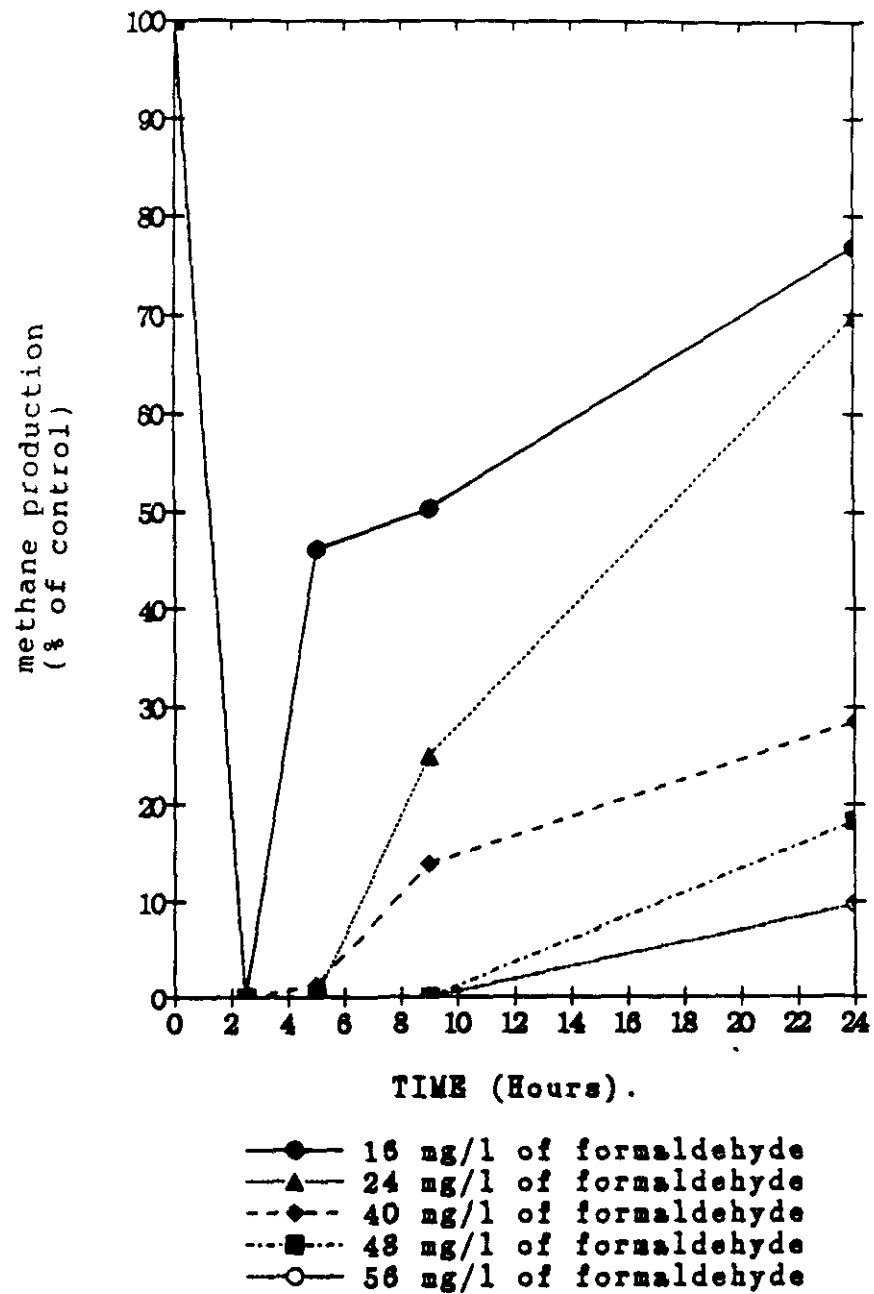


Figure 35. Effect of formaldehyde on the cumulative methane production as percent of control.

much quicker way than for the heavy metals. For these last ones, a couple of hours were usually necessary to fully see the inhibition potential of the applied dose. With formaldehyde, inhibition seems to occur instantaneously. Higher dosages of formaldehyde present longer times to start methane production and slower methane accumulation rates, that is, slower recovery.

Headspace hydrogen concentrations are presented in Figure 36. Two different trends are seen. For high dosages of formaldehyde, 48 to 64 mg/l, high hydrogen accumulation in the headspace occurs. For lower dosages accumulation occurs in a lower level, but still, in high percent levels compared to the controls. Comparison of hydrogen behavior with methane production inhibition presented in Figure 35, suggests that as the inhibition recovery is taking place, hydrogen levels approach control levels. Hence, production and consumption of headspace hydrogen occurs suggesting that headspace might be in equilibrium with the other phases.

Carbon monoxide patterns for different formaldehyde doses and times are presented in Figure 37. Carbon monoxide was consistently higher than control levels during the entire experiment. The low concentrations of formaldehyde tested during this experiments, 16 to 40 mg/l, stimulated carbon monoxide accumulation. Again as inhibition recovery is taking place carbon monoxide concentrations approach control levels. For higher formaldehyde dosages, 56 to 64 mg/l, carbon monoxide continuously accumulates during the entire experiment not showing any sign of recovery.

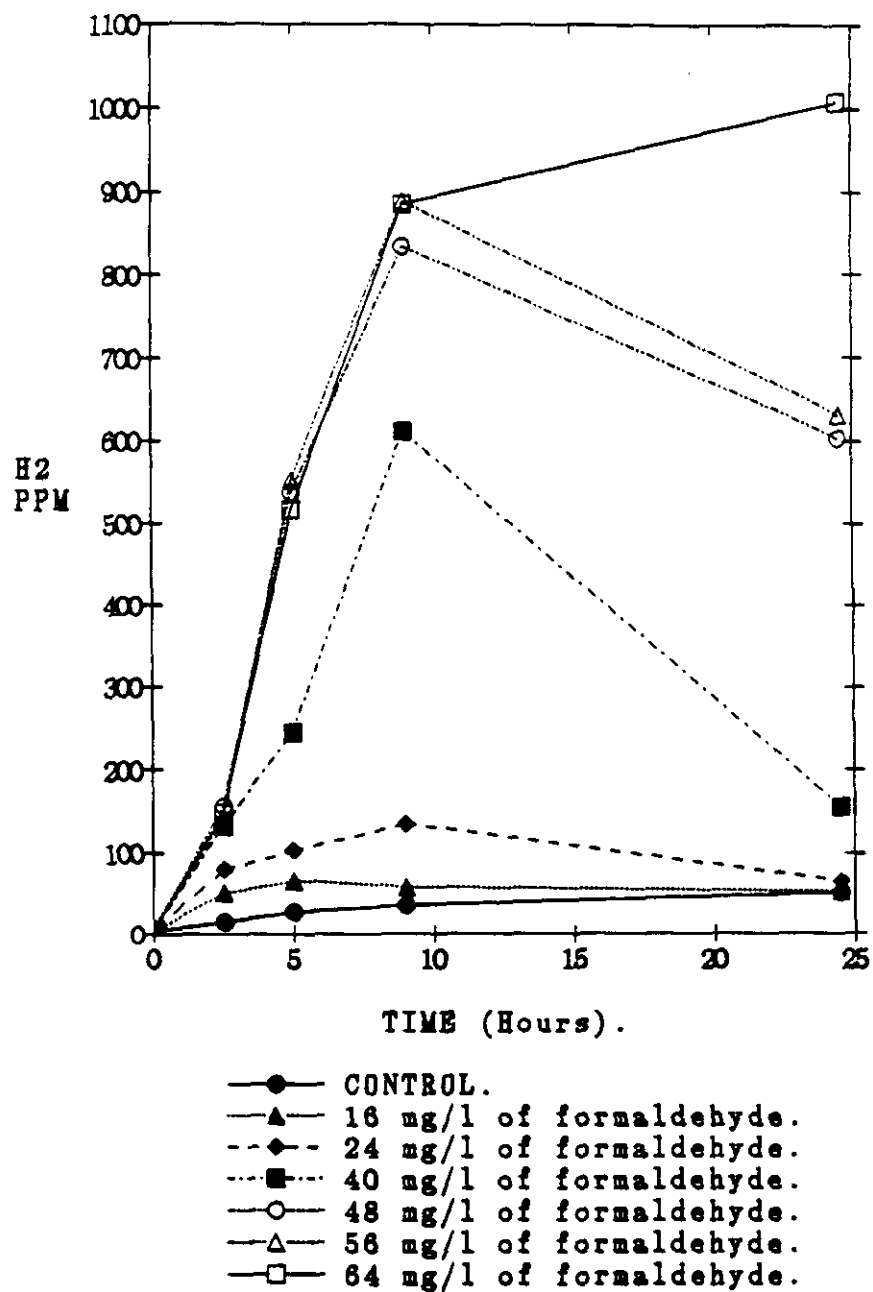


Figure 36. Effect of formaldehyde dosage on the headspace concentration of hydrogen over time.

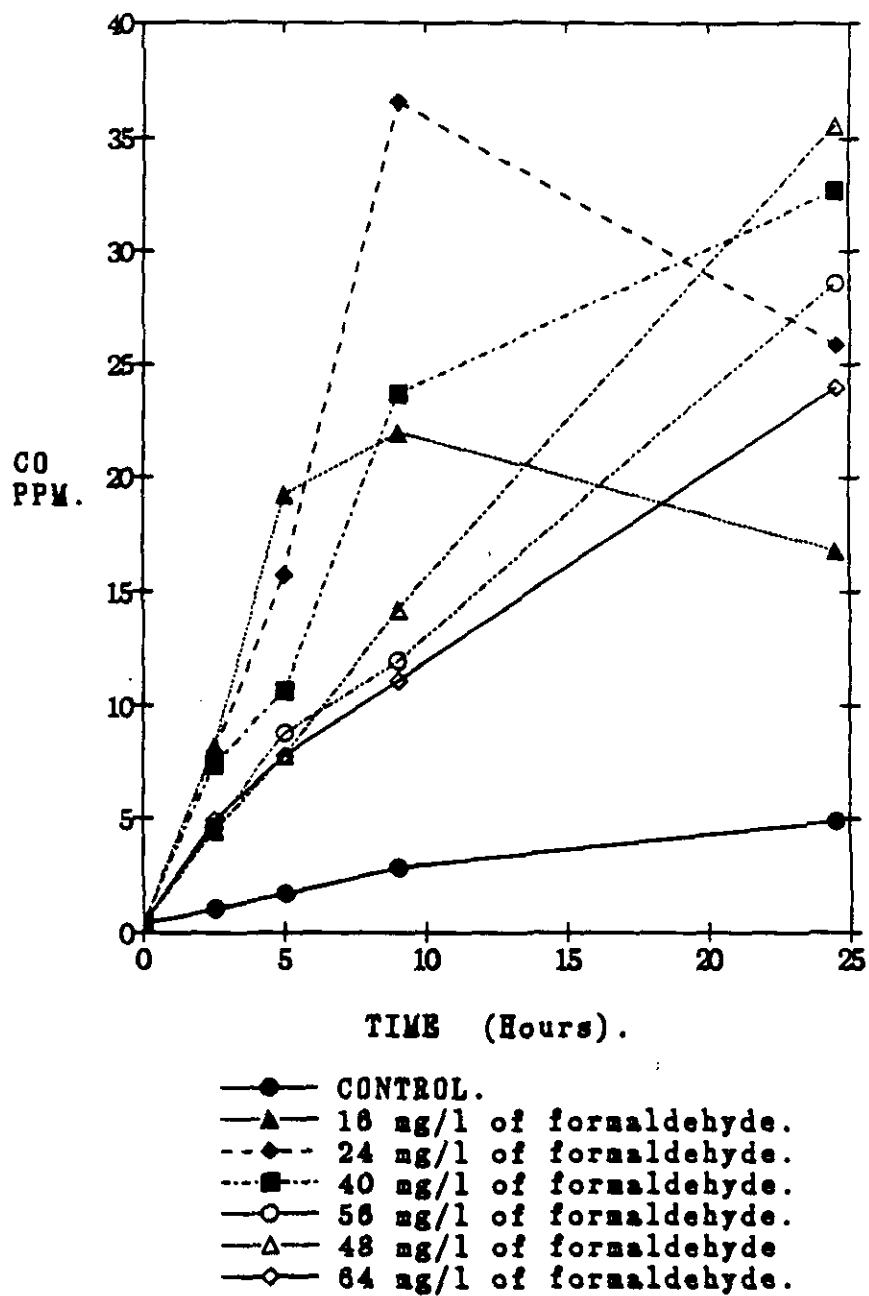


Figure 37. Effect of formaldehyde dosage on the headspace concentration of carbon monoxide over time.

Hydrogen patterns for the different inhibition levels and times examined during this experiment are presented in Figure 38. A clear pattern is shown, apparently time independent, suggesting again that hydrogen levels in the gas phase are in equilibrium with the other phases. As inhibition levels increase, percent hydrogen accumulation consistently increases.

Figure 39 illustrates CO trends during inhibition of methane production at different times. Patterns are not as well defined as hydrogen ones, nor are they time independent. Nonetheless a clear trend is seen, as inhibition of methane production increases, carbon monoxide percent accumulations rise and fall presenting a maximum around 40% activity (60% inhibition). The height of the peak is time dependent. Carbon monoxide is apparently a more sensitive indicator than hydrogen at low inhibition levels.

Inhibition of methane production at 24 hours by different dosages of formaldehyde is presented in Figure 40.

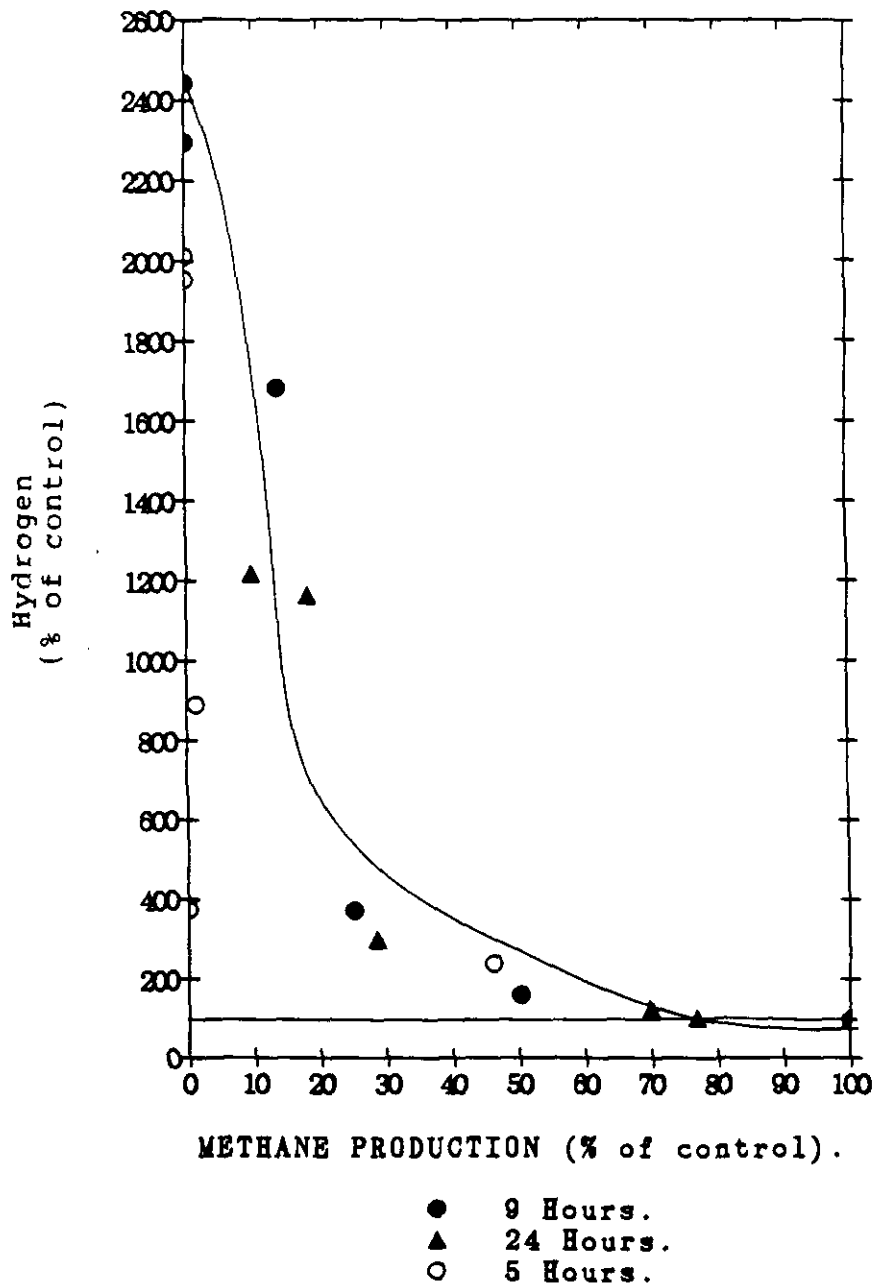


Figure 38. Comparison of hydrogen headspace concentration and cumulative methane production for formaldehyde inhibited samples. (% of control).

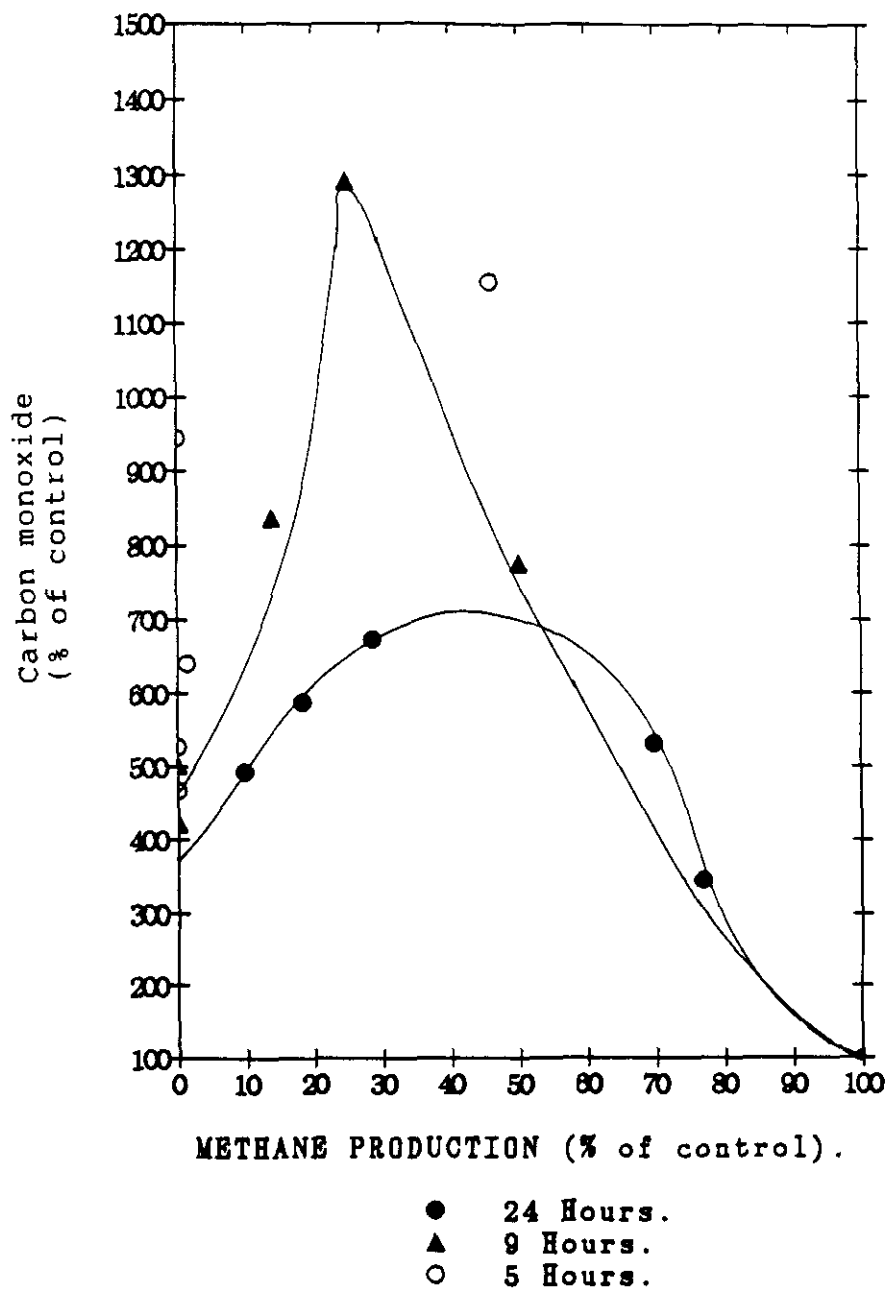


Figure 39. Comparison of the carbon monoxide headspace concentration and cumulative methane production for formaldehyde inhibited samples. (% of control).

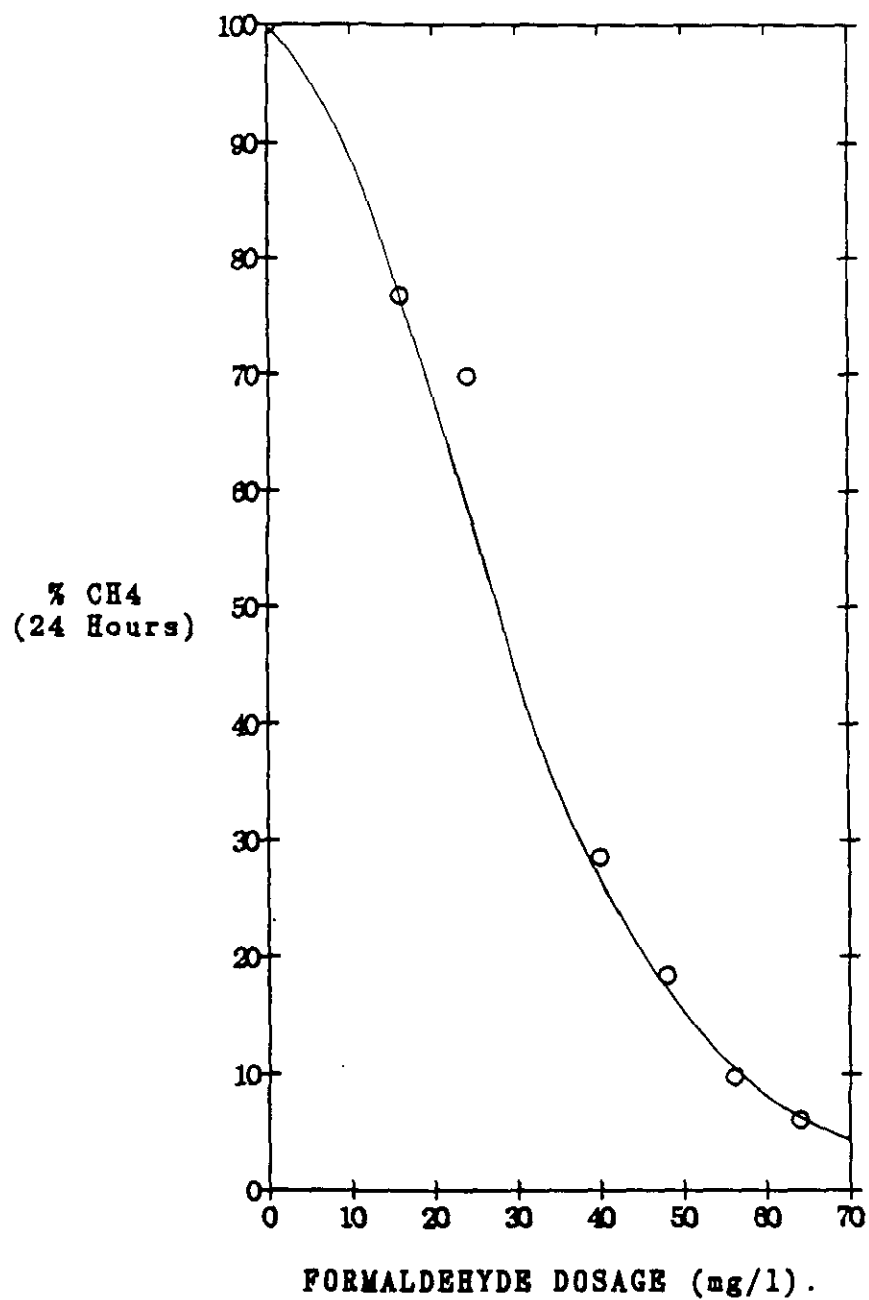


Figure 40. Effect of formaldehyde dosage on cumulative methane production as percent of control.

CHAPTER V
ANALYSIS AND DISCUSSION OF RESULTS

The reference frame for the present work is the achievement of stable operation of anaerobic digesters by improving the reliability of the process. The objective of anaerobic digestion applied to waste treatment is to consistently produce high levels of organic waste stabilization. In Chapter 2 of this work, the process control variables and the observable variables were discussed in some detail. It was proposed that in order to achieve the specific objective of organic matter stabilization and reliable operation, it was necessary to identify the occurrence of events that would produce strong imbalances in the digester dynamics, (e.g. toxic events). Additionally, it would be desirable to have a forecasting tool in order to compare the "normal" expected behavior of some selected indicator with the actual measured behavior of it, and from this comparison determine the occurrence of a toxic event.

Some variables of crucial importance in the definition of the actual state of an anaerobic digester (e.g., biomass activity) are poorly observed or evaluated with present technology. The feasibility of carbon monoxide and hydrogen concentration measurements in the gas phase of toxic inhibited anaerobic sucrose enrichment cultures as indicators of the event in the system is analyzed here. The analysis is based on the results presented in Chapter IV. In addition the theoretical

expectations from a mathematical model developed for this particular set of experiments are presented.

5.1. HYDROGEN AND CARBON MONOXIDE KINETIC RESPONSES.

In order to theoretically analyze the hydrogen and carbon monoxide evolution patterns in the gas phase of the serum bottles, a mathematical model was developed. The model is not an exact description of the intricate phenomena occurring in the bottles. Nevertheless, it is meant to show what the hydrogen and the carbon monoxide evolution patterns should look like, and the sensitivity of these responses to different parameters, according to reasonable assumptions and present knowledge of fermentation stoichiometries and kinetics.

5.1.1. Mathematical Model of Anaerobic Digestion.

The model was specifically developed for batch processes at 35° C. Mass balances for the different compounds of interest were done. Monod-type rate expressions for substrate consumption were used for all the mass balances. Rate expressions for propionic and butyric acid consumption were modified using the expression proposed by Mosey (1983) to take into account inhibition of the degradation of these compounds due to hydrogen accumulation. Glucose was considered as the substrate for acidogenic fermentative bacteria; sucrose was assumed to give two glucose equivalents. The model allowed the changing of stoichiometric

coefficients of the glucose fermentation products. Other stoichiometric coefficients used were according to Mosey (1983). Carbon dioxide production/consumption was assumed to be microbiological only; no attempt was made to model the carbonate system, with carbon dioxide production by acid titration. All the gases were assumed to transfer instantaneously to the gaseous phase, so the liquid and gas phase were always in equilibrium.

Five bacterial populations were considered: glucose fermenting, acetate consumers, propionic acid consumers, butyric acid consumers and hydrogenotrophic methanogens. Bacteria mass level was assumed to remain constant during the time frame of the assay (48 hours).

No attempt was made to consider internal and/or external mass transfer effects in the bacterial aggregates. According to values reported in the literature for affinity constants and maximum substrate utilization rates for hydrogen, this effect should not be important. However, as no measurement of these coefficients was done for this particular system, it is recommended to analyze this effect closer in future studies. The resultant model was a system of nine non-linear coupled differential equations corresponding to the variables: concentrations of glucose, acetate, propionate, butyrate, hydrogen, methane, carbon dioxide, and, cumulative methane production and cumulative biogas production. A summary of the equations used is presented in appendix B. Carbon monoxide concentrations were obtained from the relationship developed by Hickey (1987), and presented later in this work. The model was solved by linking the computer code with the

subroutine DGEAR of the IMSL library (IMSL, 1982) available in the VAX system of the College of Engineering of the University of Massachusetts. Elimination of the bacterial growth modelling considerably simplified the solution of the system. Inclusion of the bacterial growth may change the stiffness of the system, due to the big difference in time scales, making necessary adjustments in the way the subroutine DGEAR is used (IMSL, 1982). The computer code of the model is presented in the appendix B .

5.1.2. Model Predictions and Calibration.

In Figures 41 to 43 is presented methane production, hydrogen and carbon monoxide evolution patterns of the control bottles, (i.e. without any toxicant addition), of the several runs. The experimental data in these figures were collected over a six month period. These figures were used to callibrate the mathematical model. Parameters for the model were taken from the literature. The only parameter used to "adjust" the model to the real data (that is, methane and hydrogen evolution patterns) was the activity of the populations; all of the other parameters such as yield constants, maximum substrate utilization rates, and affinity constants were kept constant during the calibration. Laboratory evaluation of several parameters is recommended for future uses of this model.

Some interesting observation were noted during this curve fitting exercise. Foremost was, the strong dependence of the hydrogen response

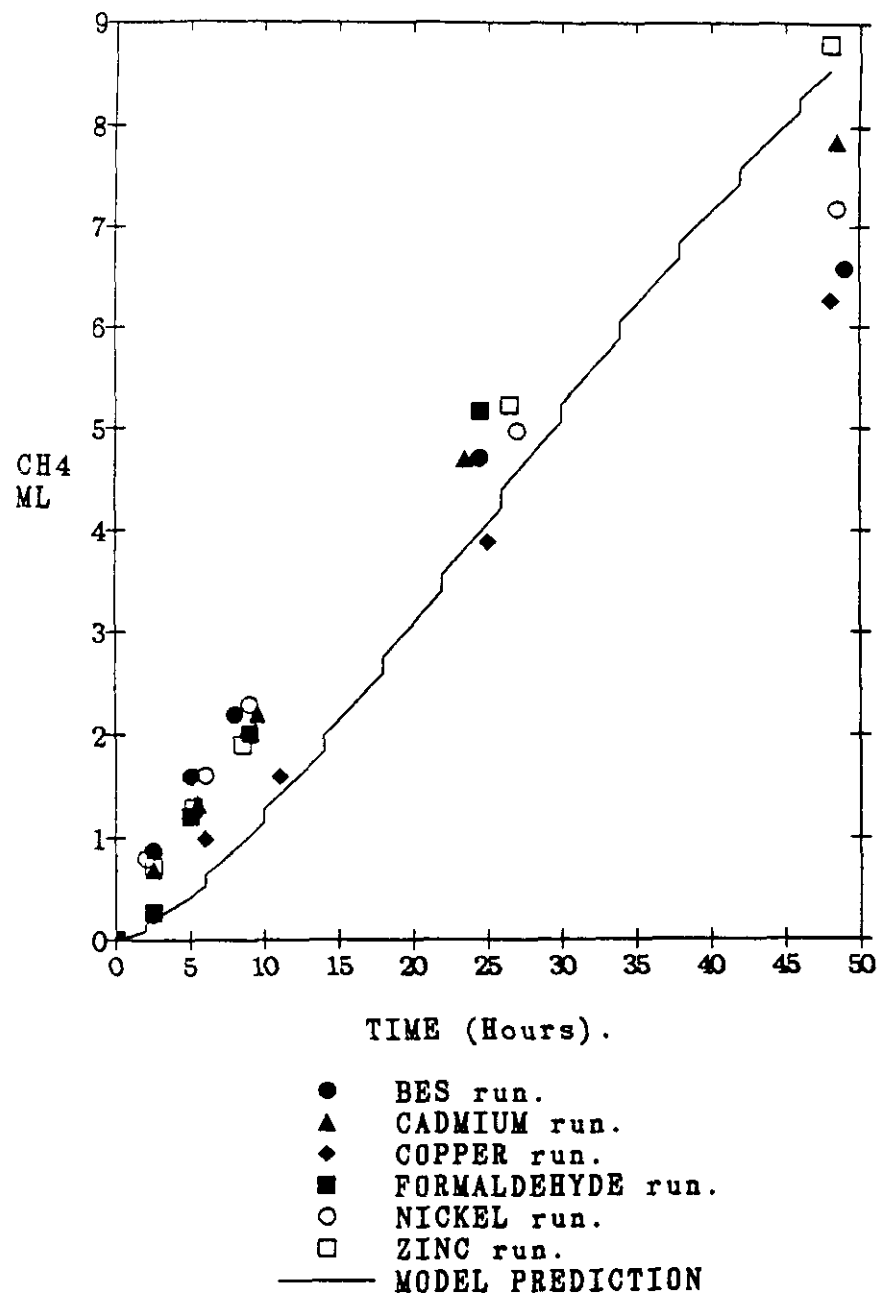


Figure 41 Variation in methane accumulation in the control bottles for the different runs.

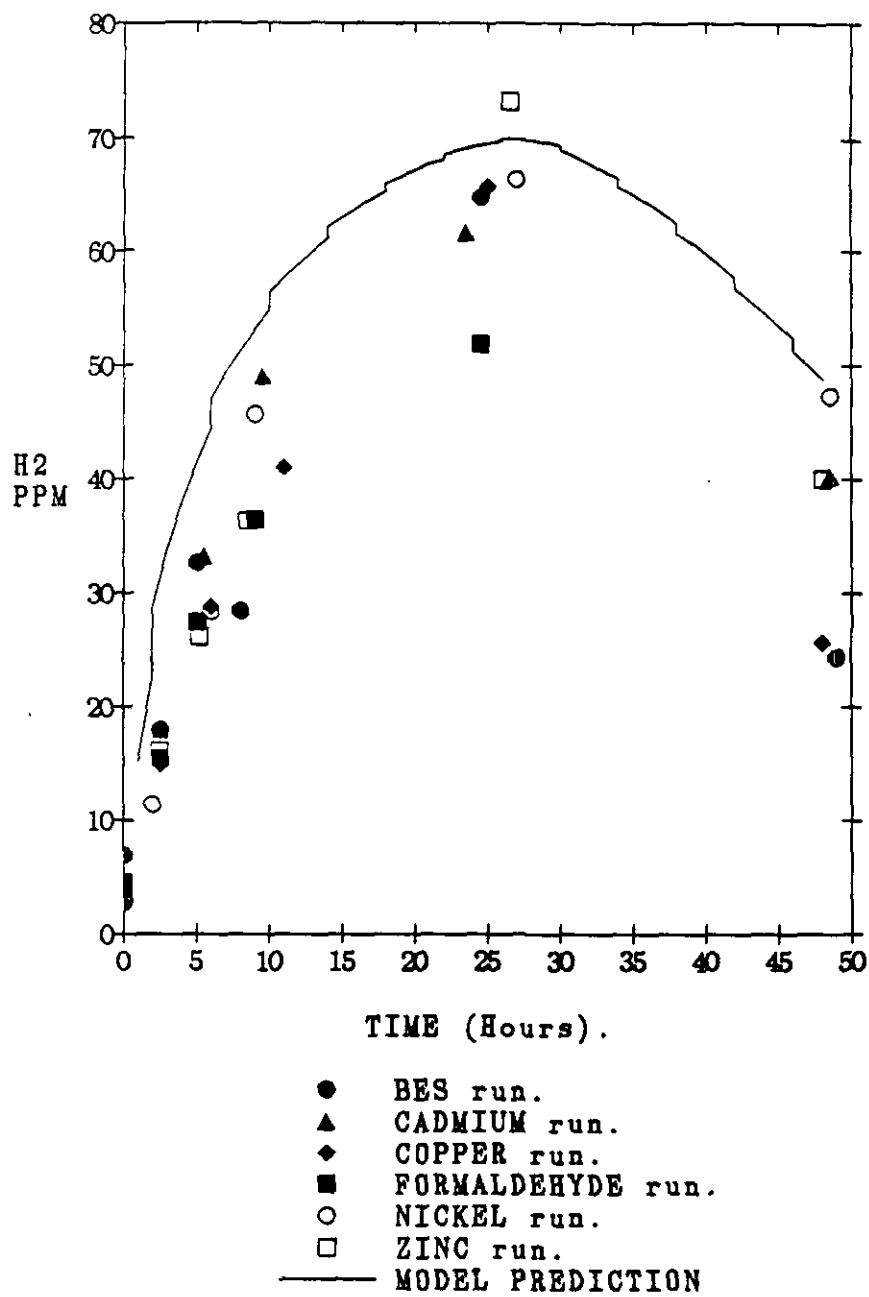


Figure 42 Variation in hydrogen partial pressure in the control bottles for the different runs.

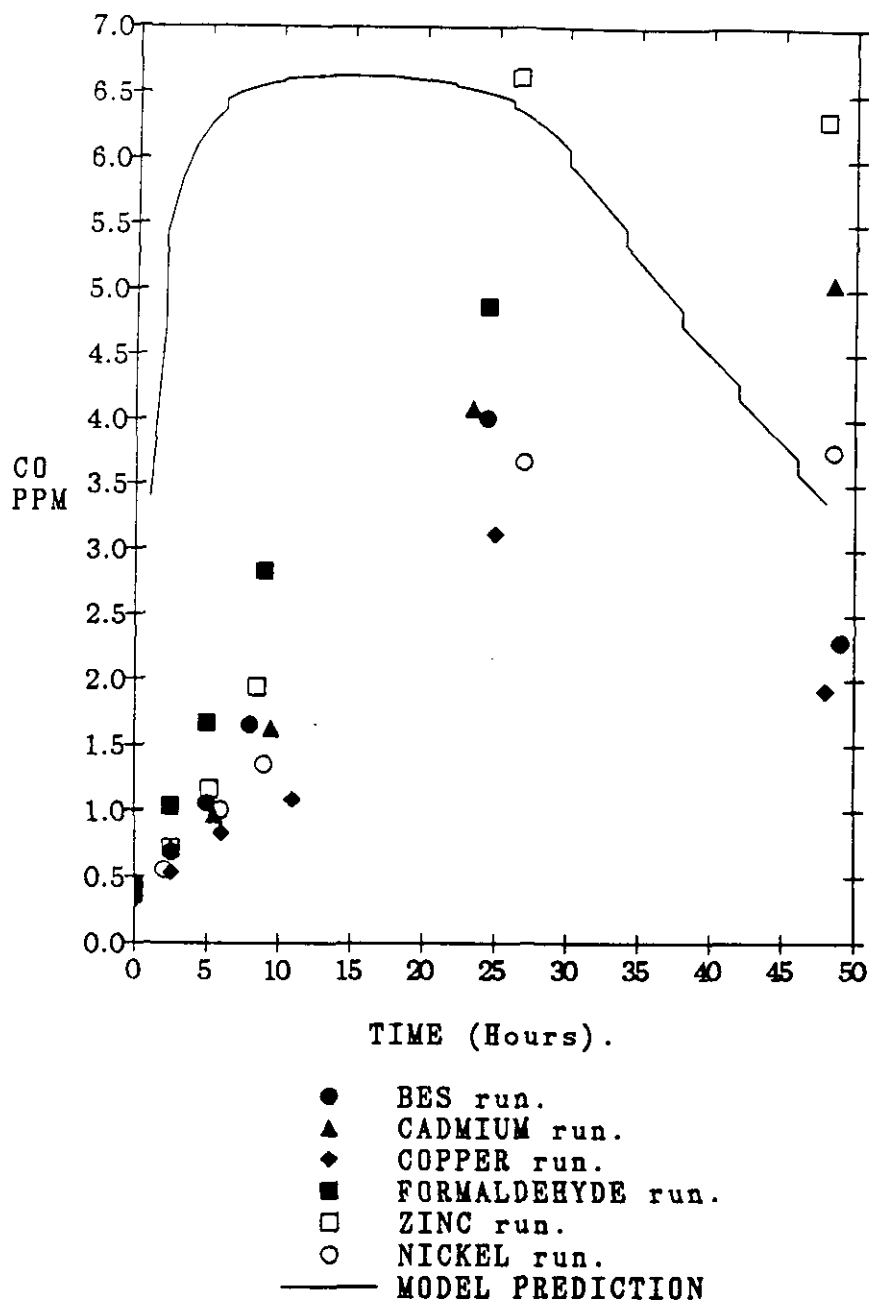


Figure 43 Variation in carbon monoxide partial pressure in the control bottles for the different runs.

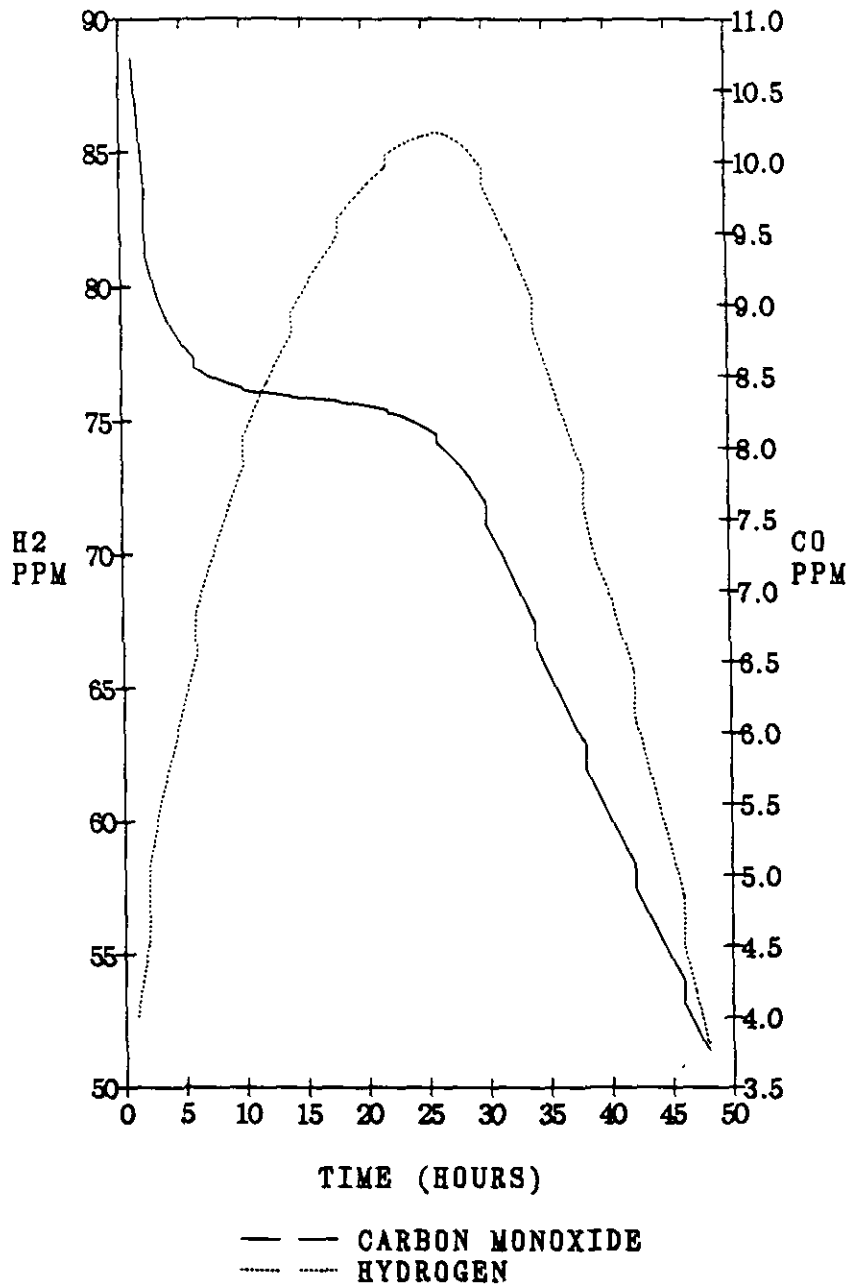
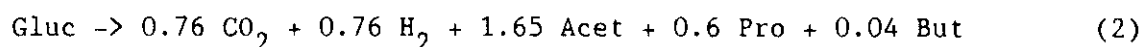


Figure 44 Hydrogen and Carbon monoxide model prediction behavior using the stoichiometry for glucose fermentation reported by Chynoweth and Mah (1971).

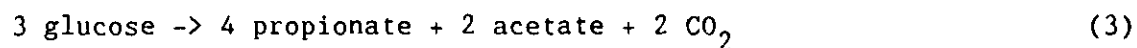
on the prevailing glucose fermentation type. Figure 44 illustrates this point. This simulation was generated using the glucose fermentation stoichiometry presented by Chynoweth and Mah (1971). This glucose fermentation stoichiometry is:



As can be seen, hydrogen is produced in considerable stoichiometric amounts from glucose. The higher the rate of glucose degradation, the higher the rate of hydrogen production. As a result of the relative rates of hydrogen production and consumption, hydrogen is rapidly accumulated in the headspace of the bottle during the degradation of the glucose. Once the glucose is depleted, hydrogen concentrations drop very quickly, and appear to reach equilibrium with the propionate degradation as hydrogen source. Most interesting is the observation that the hydrogen concentration does not increase again.

As the relative kinetics and population of fermentative bacteria are higher in an anaerobic digester ecosystem, the hydrogen accumulation rates when hydrogen is produced during the fermentation of carbohydrates, is expected to be much higher than the accumulation when hydrogen is produced from intermediate products such as propionate, butyrate, lactate, ethanol etc.. Mosey and Fernandes (1988) report very short times, on the order of 10 to 30 minutes, for hydrogen accumulation/consumption transients during the anaerobic digestion of milk-sugars. As it is shown in Figure 42, hydrogen accumulation patterns

for the experiments we conducted present a different time scale; hydrogen peaks occur around 24 hours in a mild transient. This behavior suggests that hydrogen accumulation is the result of intermediate products degradation (i.e. propionate, butyrate, lactate etc.) rather than hydrogen being produced in the initial glucose fermentation step. In fact, when the stoichiometric coefficients were changed to a fermentation of the kind:



the model predictions better followed the observed laboratory results as shown in Figures 41 and 42. This analysis suggests that with the microbial population established in our laboratory reactor a different type of fermentation, and consequently, different intermediate products flow to methane and carbon dioxide might be taking place. Unfortunately no experimental data exist to support this hypothesis; nevertheless, the subsequent analysis of results will be done based on it.

In Figure 43 is presented the data of the carbon monoxide evolution pattern for the controls of the different runs. The connected line shows the prediction of the mathematical model. Carbon monoxide calculations in the model were done using the approach for acetate concentration calculations developed by Hickey (1987). The following equations were developed by Hickey:

$$\Delta G'_9 = -15.8 + 5.9 \log \frac{[\text{CH}_4][\text{CO}]}{[\text{CH}_3\text{COO}^-][\text{H}_2]} \quad (4)$$

and,

$$\Delta G'_{10} = -20 + 5.9 \log \frac{[\text{CO}_2][\text{H}_2]}{[\text{CO}]} \quad (5)$$

Where $\Delta G'$ symbolizes the physiological free energy of reaction. The subscripts 9 and 10 in the equations refers to equations (9) and (10) on Hickey (1987). If it is assumed that the acetogenic bacteria Acetyl CoA pathway (see chapter 2) operates at a point in which these two free energy values are equal, the concentration of carbon monoxide, CO, as a function of methane fugacity, hydrogen fugacity, acetate activity and carbon dioxide fugacity can be obtained from the resulting equation:

$$[\text{CO}] = [\text{H}_2] * \left(\frac{[\text{CO}_2][\text{CH}_3\text{COO}^-]}{5.15 * [\text{CH}_4]} \right)^{0.5} \quad (6)$$

During the "curve fitting" exercise no attempt was made to reproduce carbon monoxide patterns. The fitting was done using methane production and hydrogen evolution data as a criteria. Considering these factors, it is encouraging that the relationship gives results on the same order of magnitude as the ones measured, and qualitatively describes the pattern of carbon monoxide. This observation suggests that the CO is in fact in equilibrium with hydrogen, methane, carbon dioxide and acetate.

5.1.3. Sensitivity Analysis of H₂ and CO to the Activity of Different Populations of Microorganisms.

In order to examine the influence of the activity of the different populations of microorganisms have on the hydrogen and carbon monoxide responses, a sensitivity analysis was performed.

After the model was "adjusted" to the data from the laboratory experiments, as explained in the previous section, the activity of the different populations was varied independently. An inhibition coefficient that affected V_{max} (maximum specific rate of substrate utilization) was used. This is also equivalent to "inactivating" part of the population of microorganisms. Theoretical models for the action of toxic substances on microorganisms are usually an extrapolation of models for enzyme kinetics to microbial kinetics (Grady and Lim, 1980). Three different cases of inhibition are distinguished: competitive, uncompetitive and noncompetitive. In the first two classes, the inhibitor reversibly binds to the enzyme or to the enzyme-substrate complex. In noncompetitive, it binds to both of them. In this last case, the effect of the toxicant on the kinetic expression is a decrease in the value of the V_{max} coefficient. When dealing with microorganisms and metals, it seems more realistic to select this kind of inhibition mechanism.

It is important to remark that all the calculations for carbon monoxide levels are done under the assumption of thermodynamic equilibrium. In some circumstances, and depending on the specific

toxicant, this equilibrium may not hold, and the actual behaviors of hydrogen and carbon monoxide are uncoupled. Also, a main hypothesis for the behavior of hydrogen in this model is that hydrogen is an end product of the metabolism of the microorganisms involved. In some instances the effect of one toxicant is in an internal metabolic pathway, and hydrogen that under normal circumstances would be internally produced and consumed is liberated by the bacteria (e.g. heavy metal inactivation of an specific hydrogen carrier in dehydrogenase action). In these cases the model in its present form would be useless.

In Figure 45 is presented the effect of the activity of sucrose fermenting microorganisms on methane accumulation, hydrogen partial pressure, and carbon monoxide partial pressure in the gas phase. It is important to notice the effect on the hydrogen pattern. As inhibition increases (activity decreases), the hydrogen profile is flattened and the peak-time is delayed. Eventually the rate of production of hydrogen is smaller than the rate of consumption and the peak never occurs. Carbon monoxide follows the hydrogen trends. Another important aspect to notice is the non-linearity of the methane accumulation rate and the activity (or inhibition). It is usually tacitly implied in the literature that an increase in inhibition, measured as a decrease in methane accumulation rate, corresponds to an equivalent decrease in microbial activity. This is only true if all the microbial populations were equally inhibited, and would not be true if there is a selective inhibition action by the toxicant. This nonlinearity aspect observed

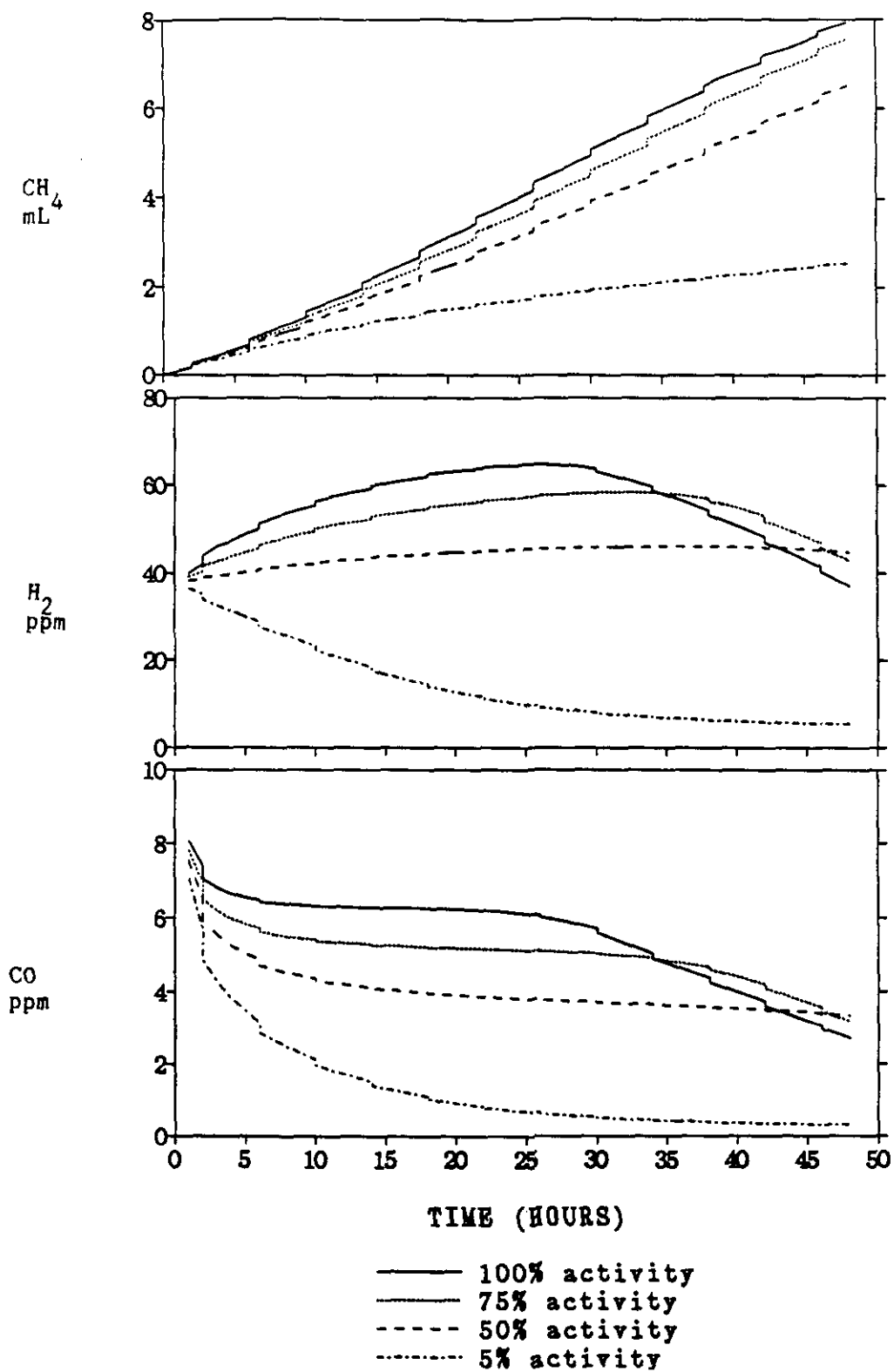


Figure 45

Effect of variation in activity of fermentative population on (a) methane (b) hydrogen (c) carbon monoxide during a batch run.

here will be a common factor in all the following analysis and will be treated in more detail later.

The effect on the propionate consumers activity (obligate proton reducers) is presented in Figure 46. This time the effect on the hydrogen profile is merely an intensity effect (that is, lowering the height of the peak without displacing it). The carbon monoxide response does not present as wide variations as hydrogen. Methane accumulation is more affected than the latter case, but nevertheless, shows the non-linear behavior with increasing inhibition.

Figure 47 illustrates the effect of inhibition of the activity of acetate consuming methanogens (acetoclastic methanogens) on hydrogen, carbon monoxide and methane concentrations. Methane accumulation rate behavior is slightly sensitive to variations in acetoclastic methanogens activity. The hydrogen profile is not affected at all. This last point deserves an explanation. It is usually considered that the acetoclastic methanogens are neither a source nor a sink of hydrogen, in fact most of the engineering literature in anaerobic digestion agrees with this statement. The actual behavior of acetoclastic methanogens in a mixed population, such as in an anaerobic digester, as hydrogen producers/consumers is not yet clear. In section 2.3.1.5 of this report this aspect was discussed. In the modeling attempt used here, it was assumed that the main and determining sources/sinks of the actual level of hydrogen in the digester were the propionate consumers and the hydrogenotrophic methanogens. This explains why there is no variation in hydrogen with acetate consuming activity. In some instances this

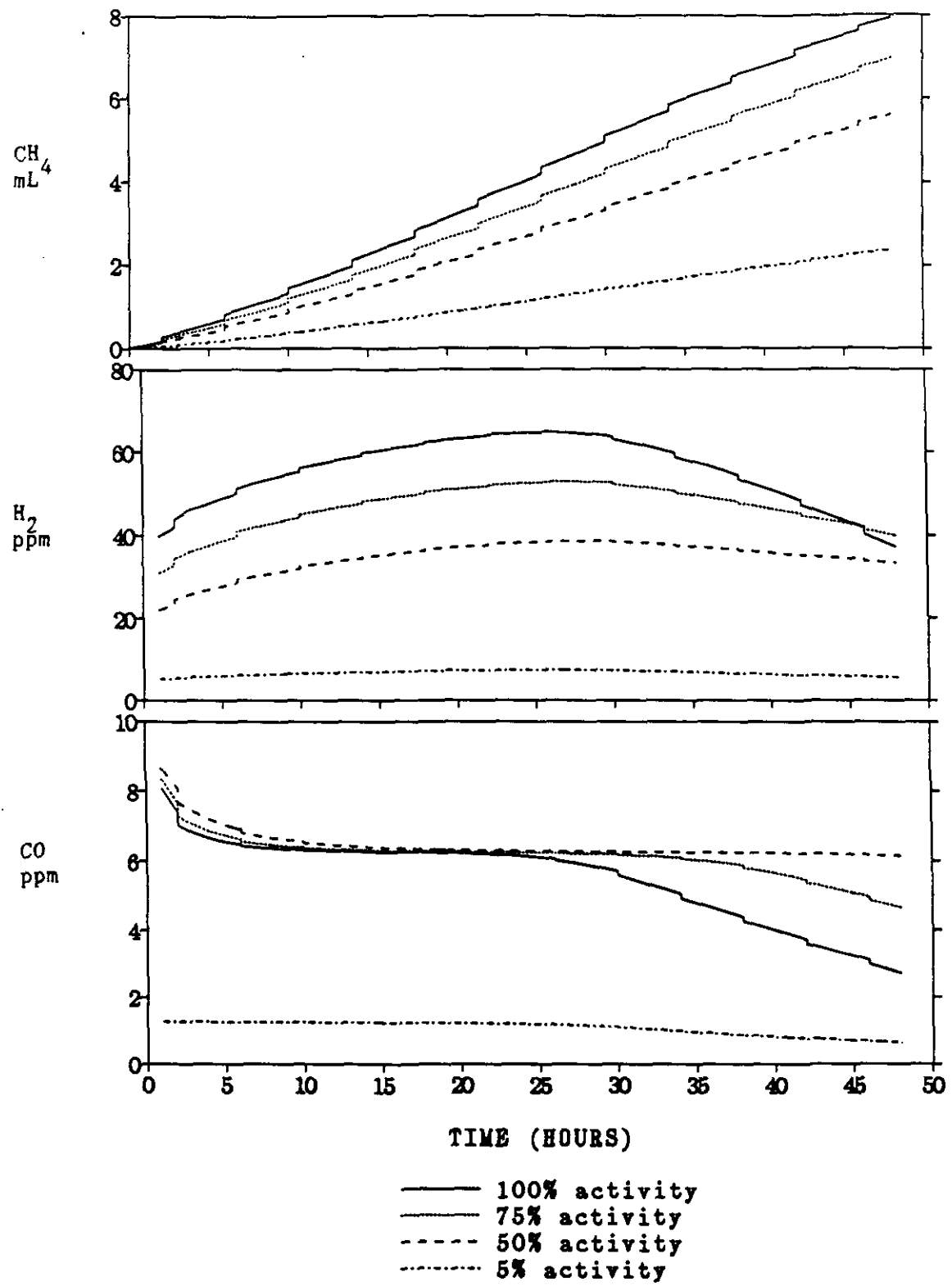


Figure 46 Effect of variation in activity of propionate degrading population on (a) methane (b) hydrogen (c) carbon monoxide during a batch run

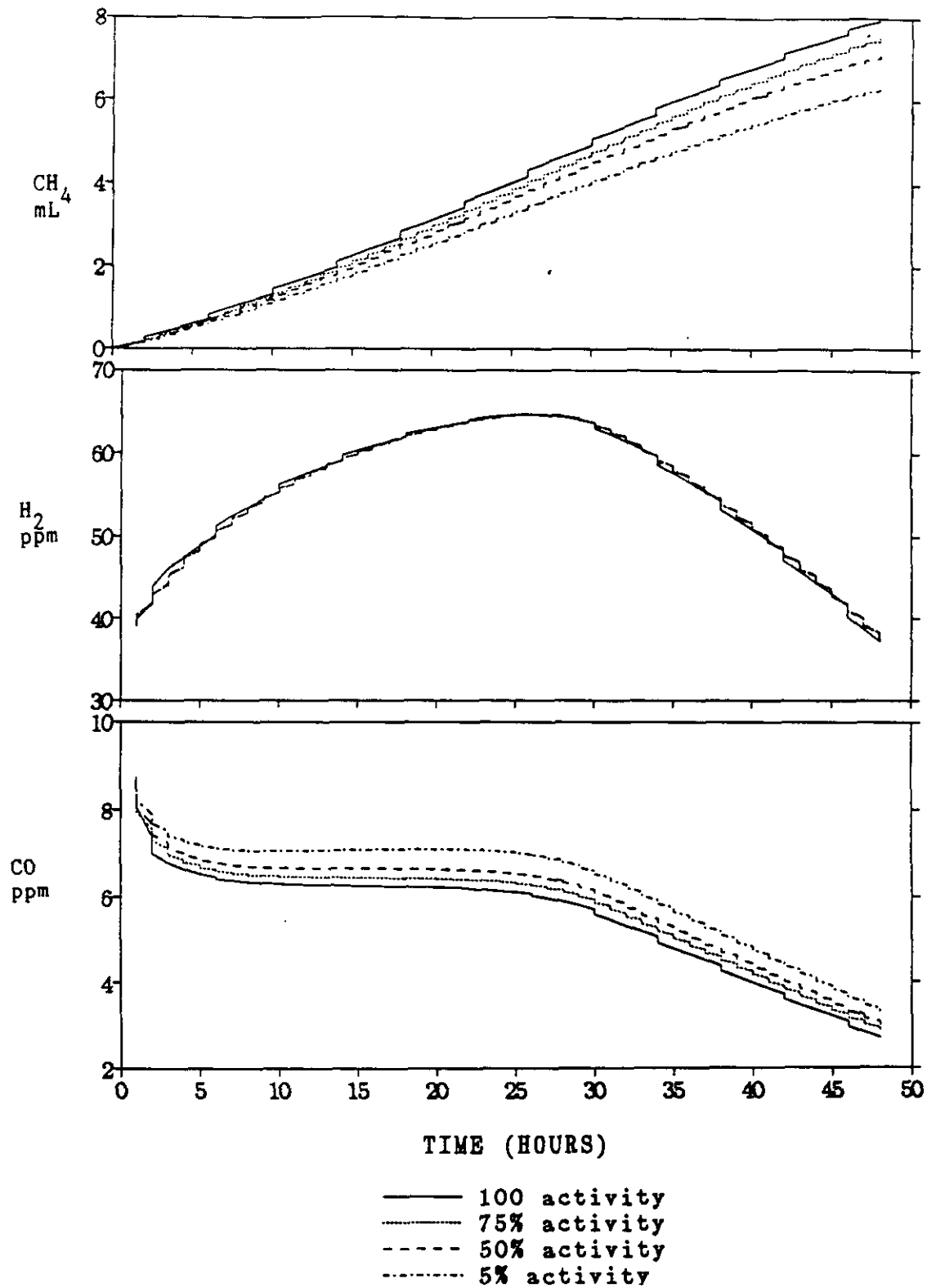


Figure 47 Effect of variation in activity of acetoclastic methanogens population on (a) methane (b) hydrogen (c) carbon monoxide during a batch run

assumption may not be a good one, as suggested by the results of Norgren (1988). Carbon monoxide in this case, Figure 47, presented a higher variation than hydrogen, basically as a result of the variation in the methane/acetate ratios.

The effect of hydrogen consuming methanogens (hydrogenotrophic methanogens), is presented in Figure 48. The methane accumulation rate again appears to be nonsensitive to inhibition levels. Hydrogen, as was expected, shows a high dependence on hydrogenotrophic methanogenic activity. The height of the peak increases as activity decreases, and for the highest inhibition, 5% activity, hydrogen reaches levels of 1200 ppm. Carbon monoxide, consequently, increases with inhibition. It is important to emphasize that all the calculation of carbon monoxide concentrations were done under the assumption of thermodynamic equilibrium.

In Figure 49 is presented the effect of uniform inhibition in all of the different populations of microorganisms in the consortium. In this situation a linear relationship occurs between the methane accumulation rate and population activity. This Figure is useful to illustrate another point, namely the dependence of the hydrogen peak-time on the methane production rate of an ecosystem (e.g., higher carbon and electron flow through the ecosystem by shorter solids retention time, or higher influent substrate concentration). As the activity of the consortium increases, the hydrogen profile sharpens and the time where the peak value occurs shortens. As a consequence, anaerobic digesters fed with particulate material such as waste activated sludge (Hickey,

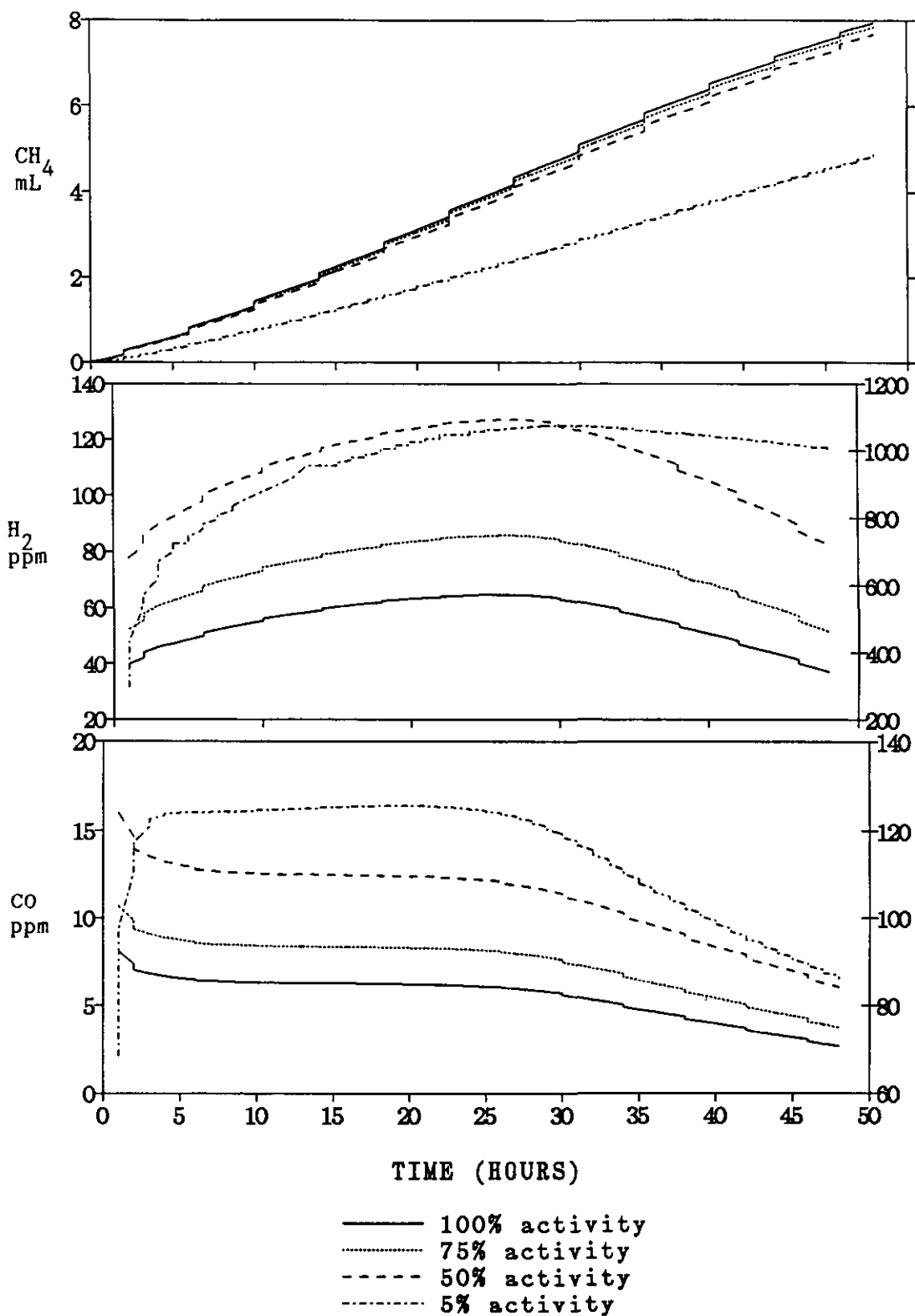


Figure 48 Effect of variation in activity of hydrogenotrophic methanogens population on (a) methane (b) hydrogen (c) carbon monoxide during a batch run

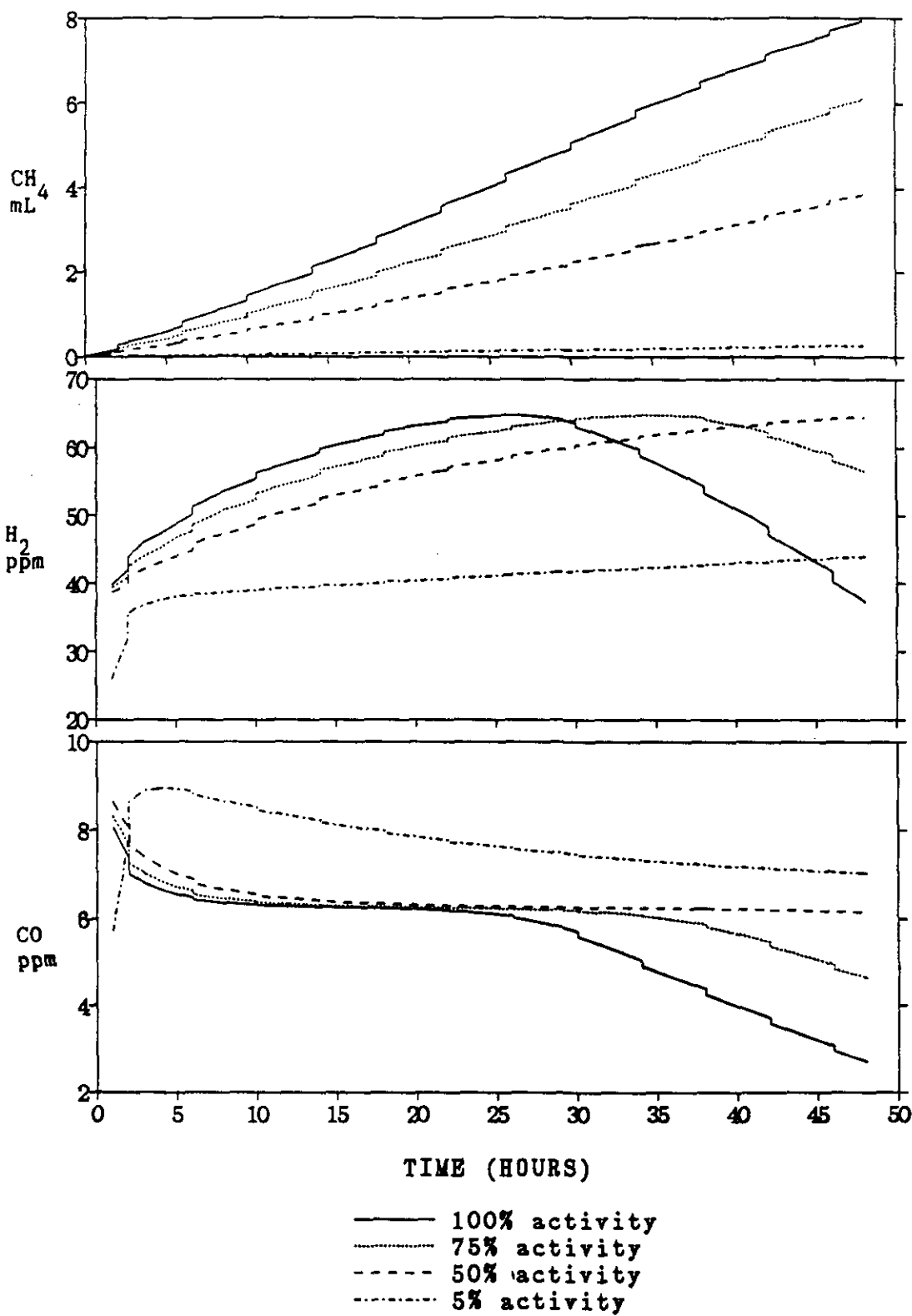


Figure 49 Effect of variation in activity of all populations (a) methane (b) hydrogen (c) carbon monoxide during a batch run

1987) and digesters fed soluble substrate as in this study may present similar peak times depending on the relative carbon and electron flux through the ecosystem. It would be interesting to analyze the ratio of hydrogen accumulation rate to the methane accumulation rate for different systems.

To illustrate more explicitly the behavior of methane accumulation rate as a function of the activity of the different populations, Figure 50 was prepared. It can be seen how a decrement in activity of the population corresponds to a similar decrement of methane accumulation rate only when all the members of the consortium are equally affected. In the case of selective inhibition of one population the responses are different. In these cases a kind of "buffering" effect is observed. Even if the activity of the population were severely inhibited, the methane accumulation rate would not have varied significantly. For instance, in the case of acetoclastic methanogens a 95% decrease in the activity generated just a 20% decrease in the methane accumulation rate (relative standard deviations for methane accumulation rate in this study fluctuated between 10 and 22%, see Table 5).

This kind of behavior puts a severe limitation for the use of methane production rate and volatile fatty acids accumulation as monitoring parameters in a scenario of potential toxic events; they would only reflect a problem when there were an expiring population of microorganisms and by then, few remedial measures can be taken. It is important to remark here that Figure 50 was developed for the type of carbon and electron flow from glucose to methane and carbon dioxide,

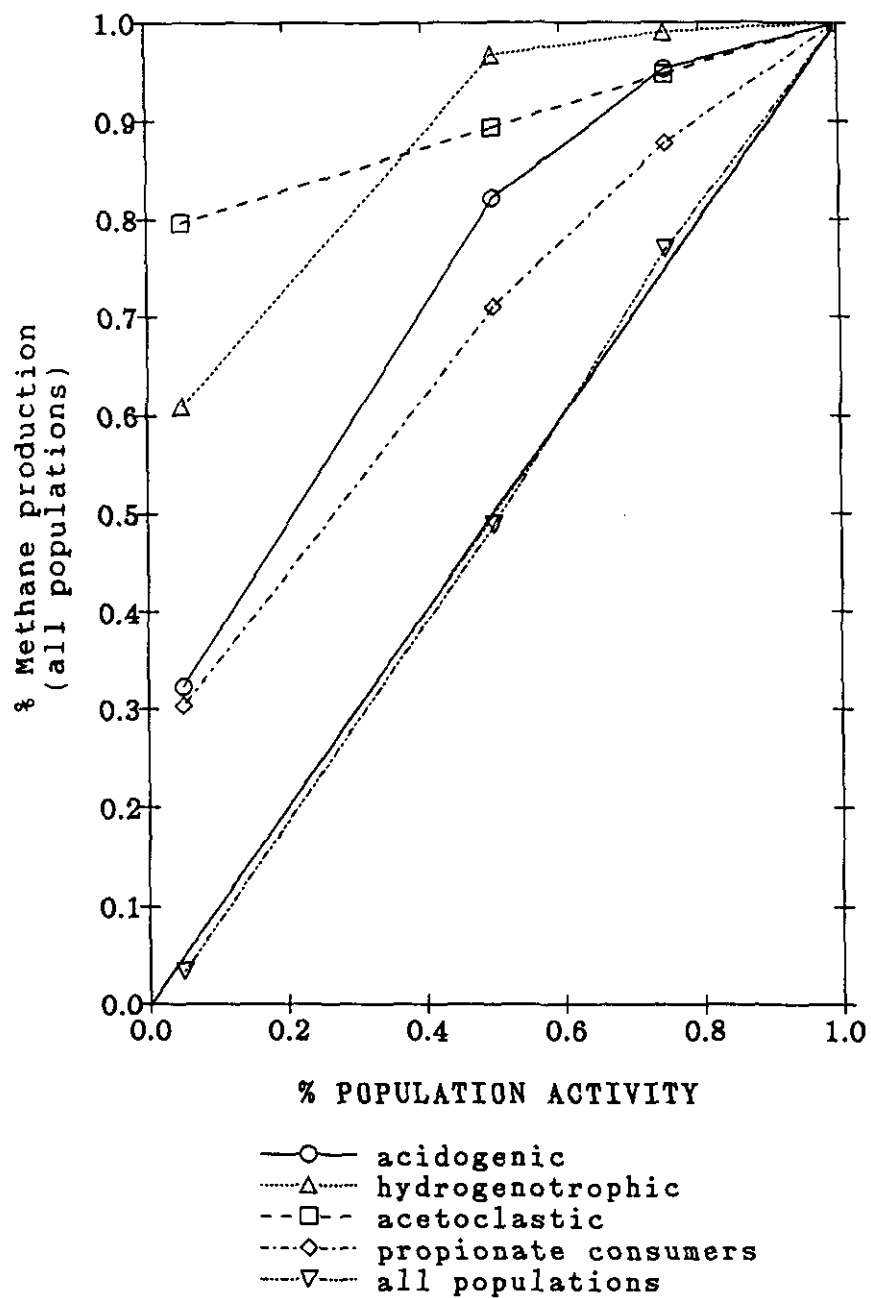


Figure 50 Effect of variation in activity of the different individual populations on methane accumulation (percent of total activity)

TABLE 5. STATISTICAL PARAMETERS OF CO, H₂ AND CH₄ PRODUCTION

TIME (hours)	μ ⁽¹⁾ CH ₄ ml	Cv ⁽²⁾ CH ₄	μ H ₂ ppm	Cv H ₂	μ CO ppm	Cv CO
0	0	0	5	0.44	0.379	0.12
2.5	0.6	0.46	16	0.19	0.705	0.26
5	1.2	0.28	28	0.17	1.06	0.32
9	2	0.21	43	0.21	1.76	0.38
24	4.6	0.14	63	0.12	4.19	0.28
48	7.3	0.14	35	0.29	3.96	0.44
Cv MEAN		0.24		0.24		0.30
1.5 Cv MEAN		0.37		0.35		0.45

(1) μ = mean value of the observations.

(2) Cv = coefficient of variation (standard deviation/mean).

according to the curve fitting procedure previously explained. In fact, the small effect seen for the acetogenic population can be explained if it one assumes that the propionate degradation is the limiting step, with the stoichiometry used. Because of this fact, the relative importance of each population, and as a consequence the effect on the parameters investigated, is particular to this system and extrapolations to other systems can not be made directly. Nonetheless, similar behavior patterns can be expected for other ecosystems. In order to explore this possibility in more detail it is necessary to measure in the laboratory the actual kinetic constants of the mixed population of microorganisms, and to conduct experiments of selective inhibition behavior.

5.2. VARIABILITY OF PARAMETERS IN THIS STUDY.

An important fact to determine in evaluating the usefulness of an early warning indicator of bacterial activity in an anaerobic digester is the intrinsic variability of this parameter under natural circumstances. Table 5 was developed from the data collected over seven months for the control serum bottles during the experimentation stage of this study, in order to explore this aspect. In this table are presented the mean value and the relative standard variation for different times of the three process indicators- methane accumulation rate, hydrogen partial pressure and carbon monoxide partial pressure. It can be seen that methane and hydrogen show a smaller coefficient of variation

(relative standard deviation) than carbon monoxide, but nonetheless not much difference was observed. If a normal probability distribution is assumed, 1.0 standard deviation from the mean value covers 68% of the possible events, 1.5 standard deviations covers 86% of the possible events and 2.0 standard deviations covers 95% . It seems reasonable to select 1.5 standard deviation from the mean value as a criterion to establish the potential of a toxic event during the analysis of toxic indicators. With this selection the following threshold values for toxicity evaluation criteria for carbon monoxide and hydrogen result: 31% deviation from control values for hydrogen, and 42% variation for carbon monoxide.

In an actual situation in the field, analogous statistical analysis for natural variability based on historical records should be developed in order to establish a criterion. The measurement obtained from the anaerobic digester sampled should be compared with some predicted value considered as normal. The prediction can be based on historical records of successful operations or by using a model that relates the behavior of the indicators to the actual waste input to the process such as the one developed here.

5.3. ORGANIC TOXICANTS.

Organic compounds are usually specific inhibitors of microorganisms, and are used in enzymatic and biochemical studies for that purpose.

Another important feature of toxicity by organic compounds is the adaptation potential of the microorganisms to the compound when the compound is endemic in the system. In this section the results of toxicity by Bromoethanesulfonic acid and formaldehyde are analyzed.

5.3.1. Bromoethanesulfonic acid (BES).

BES is a structural analogous of coenzyme M (CoM), a C-1 carrier only found in methanogens (Gunsalus et al., 1978; McBride and Wolfe, 1971). CoM is involved in the last steps of methanogenesis, the reduction of the methyl group to methane. BES reversibly inhibits the methyl reductase system of methanogens (Gunsalus et al., 1978). BES has been found to inhibit methanogens in pure and mixed cultures (Zehnder et al., 1980; Hickey, 1987; Zinder et al., 1984; Healy et al., 1980; Wildenauer et al., 1984). Acetoclastic methanogens are considered more sensitive to BES action than hydrogenotrophic methanogens as the results of Zinder et al. (1984) and Hickey (1987) suggest. Eikemanns et al. (1985) comparing the effects of BES on cell extracts and whole cells of Methanobacterium thermoautotrophicum proposed that the cytoplasmic membrane of this organisms is impermeable to BES.

In Figure 29 is presented the effects of BES on hydrogen partial pressures on the sucrose enrichment culture. At low BES dosages the results are equivalent to the results obtained by Norgren (1988) using acetate as electron donor; slight increase in the hydrogen levels but not in the range of statistical significance. When higher dosages of BES

were applied, the partial pressure of hydrogen rose to very high levels. Comparison of these results with those obtained by Norgren (1988) and with the theoretical prediction for hydrogenotrophic methanogens inhibition (see Figure 48), suggests that a situation of selective inhibition of different microorganism populations is taking place; acetoclastic methanogens being more sensitive to BES concentrations than hydrogenotrophic methanogens. This is in agreement with the results of Hickey (1987).

Carbon monoxide data is presented in Figures 30 and 32. Carbon monoxide concentrations were consistently lower than the controls during most of the assay. It can be seen that after 8 hours carbon monoxide would deviate more than 40% from the expected value, signaling the toxic event with 85% likelihood. Comparison of these results with those obtained by Norgren (1988) does not reflect the selective inhibition mechanism of BES like hydrogen does; however, they do add additional evidence to the hypothesis that the source of carbon monoxide in methanogenic ecosystems is principally the acetoclastic methanogens (Hickey, 1987). Additionally, the comparison suggests that the carbon monoxide pool equilibrates with the hydrogen pool (higher hydrogen partial pressures produce higher carbon monoxide partial pressures). In the sucrose mixed culture hydrogen partial pressure is the result of the interaction of several microorganisms, in contrast to the results of Norgren (1988) where hydrogen pressure was determined by the acetoclastic methanogens.

5.3.2. Formaldehyde.

Formaldehyde reacts spontaneously with the amino groups of proteins and nucleic acids, hydroxymethylating them and forming methylene-bridged cross-links between them (Zubay, 1988). Hence, free formaldehyde is a very toxic compound for microorganisms. Nevertheless, bound formaldehyde is an important intermediate in methanogenesis from hydrogen. Formaldehyde is bound to the carrier tetrahydromethanopterin in the form of methylene radical (Noll, Donnelly and Wolfe, 1987). Several researchers have studied the formation of methane from formaldehyde and molecular hydrogen in acetoclastic and hydrogenotrophic methanogens (Blaut and Gottschalk, 1984; Poirot et al., 1987).

Recently, Bhattacharya and Parkin, 1988 reported that formaldehyde was a stronger inhibitor of acetoclastic methanogenesis than acetogenesis from propionate, when it was continuously added allowing acclimatization, or in slug dosages. They also reported significant acclimatization of the populations to the toxicant. Several reports exist on the effect of formaldehyde in the methane accumulation rate of acetate enrichment cultures (Parkin et al., 1983; Parkin and Miller, 1982; Chou et al., 1978).

Figures 34 to 39 illustrate the response of hydrogen and carbon monoxide to different dosages of formaldehyde. The data presented in Figure 34 show a well defined pattern for the action of formaldehyde. It acts instantaneously in comparison to metals which usually take several hours to progressively inhibit the consortium. This observation could

be a result of the inhibition of the acidogenic bacteria that never get to degrade the sucrose. All the batches exhibit recovery after that instantaneous inhibition, the time for recovery being dose dependent. It is interesting to notice that, again, two different response patterns are observed for hydrogen (see Figure 35), suggesting selective inhibition behavior. Comparison with the results of Norgren (1988), however, shows that this could be an inhibition of just acetoclastic bacteria.

Comparison of the results of hydrogen evolution with the results of Hickey (1987), for particulate substrate, shows that the same dual response pattern is exhibited for sucrose and waste activated sludge systems; however, there is a difference in the peak-time. The methane accumulation rate for the waste activated sludge system is roughly twice that of the sucrose system; the peak-time for the sucrose system is twice the time for the activated sludge system. This result illustrates the prediction of the mathematical model that peak-times are dependent on the flux of carbon and electrons through the microbial ecosystem. Another interesting aspect from the comparison with the results of Hickey (1987), is the evident recovery of the systems. As the system recovers, the hydrogen is utilized by the microorganisms in both experiments.

Figure 37 illustrates the relationship between the methane accumulation rate and hydrogen. Hydrogen shows a very clear relationship; as the inhibition increases, hydrogen accumulates to

values of statistical significance in the first 5 hours of the experiment.

Results for carbon monoxide are presented in Figures 36 and 38. An opposite trend when compared with results for BES is observed, suggesting that mechanisms of action of both toxicants are different. Carbon monoxide shows greater sensitivity than hydrogen for low inhibition levels measured as methane accumulation rate. It reaches statistical significant levels in the first 2.5 hours even for the less inhibited samples.

Comparison with the data for acetoclastic methanogens obtained by Norgren (1988), shows that carbon monoxide behavior in both systems is totally alike, suggesting one more time that the principal source for carbon monoxide in the sucrose enrichment culture are the acetoclastic methanogenic population. It seems, though, that the effect of other populations in terms of the behavior of carbon monoxide when the acetoclastic methanogens are in mixed culture is only quantitative rather than qualitative.

Comparison of carbon monoxide results of the sucrose enrichment with the results from Hickey (1987) shows the same peak-time effect discussed for hydrogen, and the same pattern behavior with inhibition.

5.4. INORGANIC TOXICANTS - METALS.

As was discussed in Chapter 2 of this report, heavy metals can coordinate with a large number of important biochemical sites changing the structural conformation of enzymes, nucleic acids, and membranes of the cell. Additionally, it is believed that the chemical speciation of the metals strongly influences their toxicity. Factors that influence the speciation of metals in an anaerobic digester are pH, redox potential, temperature, concentration of chelating agents and concentration of inorganic anions. These factors were discussed in Chapter 2. As a result of these two characteristics of metals, their toxicity can vary notably from system to system, and the complete characterization of the status of the system is a difficult task.

Inhibition of heavy metals seems to act at a slower pace than inhibition by organic compounds. Inhibition seems to increase in a gradual progressive way, with hours as the time scale, and eventually some recovery is usually seen.

Analysis of the results of inhibition by heavy metals presented in Chapter 4 allows some generalizations on the behavior of carbon monoxide to be made. Comparison of Figures 8,14,20, and 26 shows that carbon monoxide presents a fairly constant response pattern as a result of the inhibition of methane accumulation rate, for all of the heavy metals tested in this study (Cu, Cd, Zn, Ni). This result is consistent with the previous study of Hickey (1987) where Cd, Cu and Zn were tested for an anaerobic digester fed with waste activated sludge.

In general, carbon monoxide reaches statistical significant values for low inhibition levels of the methane accumulation rate (Inhibition < 70%) during the first six hours of the assay.

Carbon monoxide exhibits a s-shaped curve that evolves with the time. At low inhibition levels of the methane accumulation rate the carbon monoxide takes values higher than the control ones. At high inhibition levels the carbon monoxide remains consistently under the control values. At intermediate inhibition levels (30% < inhibition < 60%) carbon monoxide is not statistically significant.

By contrast, hydrogen partial pressure results are not homogeneous. Comparison of Figures 7, 13, 19 and 25 shows different patterns, suggesting different mechanisms of action for the different metals. In general hydrogen is not a good indicator of low toxicity levels of the metals tested given the statistically significant level determined for this study. Hydrogen is a good indicator only at high inhibition levels of the methane accumulation rate, inhibition > 60%, and in cases like zinc, hydrogen does not show significant variation in any range.

Comparison of the hydrogen and carbon monoxide results with the results obtained by Norgren (1988) for acetoclastic methanogens and the predictions of the theoretical model for the inhibition of the different trophic groups of the ecosystem, suggest that inhibition by heavy metals affects several populations in the consortium. The groups affected and the mechanism of action of the different metals varies from one to the other.

This last result suggests that a simple mechanism, such as noncompetitive inhibition, is not enough to model the complex responses of hydrogen and carbon monoxide in a system exposed to toxic concentrations of heavy metals. The high potential range of action of the heavy metals in the biochemical machinery of the cell, and the ubiquitous presence of hydrogen in the internal metabolic pathways places any explanation in the realm of speculation.

5.5. ANALYSIS OF APPLIED DOSAGES.

It is usual in the reports of toxicity studies to present the toxic dosage of the compound tested that produced a determined level of toxic effect (such as LC_{50} , EC_{50} etc.). The definition of the toxic effect can be done in several ways, and in anaerobic digestion toxicity studies the methane production rate is commonly used. From the previous discussion on the selective effect of the different chemicals in the different trophic groups of the consortium, it is seen that this definition of the toxic effect may not be the most appropriate in certain circumstances. On the other hand, the results presented in this report suggest that the action of the different compounds is highly time dependent (e.g., some compounds act instantaneously, some present a gradual progression in the inhibition, other show evidence of recovery of the population with time etc.). Additionally, in the case of the metals, the chemical speciation is expected to have a definite influence in the toxic effect. It is

discussed in chapter 2 the physical-chemical-biological parameters that affect the speciation of metals in an anaerobic reactor. All these factors combined make it difficult to do comparisons of the type applied dosage vs inhibition of the methane accumulation rate, among different systems. Aware of the complications, one of these comparisons is presented in Table 6, taken from Norgren (1988).

It is important to note one point. Inhibition in the methane accumulation rate of the acetoclastic methanogens corresponds to a lower inhibition of the methane accumulation rate of a complete ecosystem where other populations are contributing to the overall methane production. Hence, same dosages would present lower inhibition in the methane accumulation rate, or in other words, higher dosages are necessary to reach the same inhibition. Additionally, if a trophic group earlier in the degradation chain of the initial substrate is more susceptible to the action of the toxicant than the methanogens, lower amounts of methane accumulation would be reported for the same applied dosages of the toxicant (if all the other variables are held constant). Thus, comparing the results presented in Table 6 for acetate fed microorganisms and sucrose fed it can be seen that acetoclastic methanogens are as resistant or more to heavy metals as the other trophic groups in the system. This statement agrees with the results of the analysis of carbon monoxide and hydrogen data. Comparison of results with the waste activated sludge is more difficult given the different rate limiting steps in the overall degradation and the different chemical matrixes of the assay e.g. high ammonia concentration in waste

TABLE 6. COMPARISON OF TOXICANT DOSAGES CAUSING
50% INHIBITION OF METHANE PRODUCTION
AFTER 24 HOURS.

DIGESTER SYSTEM	Cu mg/gVSS	Cd mg/gVSS	Ni mg/gVSS	Zn mg/gVSS	BES mM	HCHO mg/l
ACETATE	10 ¹	20	100	350 ²	2.0 ³	35
SUCROSE	10	9	380	150	1.0 ²	30
WASTE ACT. SLUDGE	15	28	-	70	1.8	40

(1) Time= 23 hours.

(2) Time= 48 hours.

(3) Time= 53 hours.

activated sludge. Nonetheless, the relative toxicity of the different metals seems to agree.

CHAPTER VI
CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

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

. The mathematical model developed described hydrogen and carbon monoxide trends satisfactorily, under the limitations discussed. The model, when calibrated, could serve as a comparison landmark between predicted and measured hydrogen and carbon monoxide partial pressures.

. A simple mechanism for inhibition modelling, such as noncompetitive inhibition employed here, is not enough to model the complex responses of hydrogen and carbon monoxide in this system.

. Conventional modelling of does not consider acetoclastic methanogens as a source or sink of hydrogen. The results of this study suggest that in some circumstances it should be considered.

. Hydrogen peak-time theoretically shows a strong dependence on the electron and carbon flux through the microbial ecosystem (volumetric load). Thus, systems treating particulate and soluble wastes may potentially show similar time-scale responses. This is important in determining hydrogen monitoring time frequency.

. Comparison of carbon monoxide evolution behavior with thermodynamic equilibrium calculations, suggests that CO is in fact equilibrated with acetate, methane and hydrogen concentrations. This can also be concluded from a comparison with the results of Norgren (1988).

. Comparison of the results obtained for the hydrogen evolution with theoretical predictions, suggests that the main source of hydrogen in the ecosystem is not the fermentation of sucrose (glucose equivalents) by the acidogenic population, but the degradation of intermediate compounds (e.g. propionate).

. The results show clearly that different population groups are affected differently by the different toxicants. This selective inhibition behavior raises a serious objection to the use of conventional indicators such as methane yield, volatile fatty acids or alkalinity in toxic scenarios. This is due to the small sensitivity of these parameters to the activity of one individual population in the microbial ecosystem.

. Comparison of the results of carbon monoxide evolution with the ones obtained by Norgren (1988), suggests that the source of carbon monoxide in the ecosystem was in fact the acetoclastic methanogenic population.

. The action of organic toxicants was quicker than the action of heavy metals. The former act almost instantaneously, while the latter take some time (hours) to exert their full action. This fact was reflected in the time responses of the gases, especially carbon monoxide.

. Carbon monoxide is a better indicator of toxicity at low methane accumulation inhibition (< 30%) than hydrogen. Hydrogen only reaches statistically significant variations at methane accumulation inhibitions higher than 60%.

. In general, carbon monoxide partial pressures reach statistically significant variation during the first 2.5 hours of a toxic event from organics and 6 hours of a toxic event from heavy metals. This result is specific for this system; other systems may behave according to the carbon and electron flux through the bacterial consortium.

. The relative toxicity of the heavy metals agrees qualitatively with previous results of Hickey (1987) and Norgren (1988) (i.e., Cu > Cd > Zn > Ni).

6.2. RECOMMENDATIONS.

Based on the results of this study the following are suggested as topics for further examination:

. Study the influence of speciation of metals on the toxic effect to anaerobic digestion.

. Determine the kinetic coefficients for the different populations of microorganisms in the consortium. Calibrate and validate the mathematical model.

. Develop a historical data base on natural variations of carbon monoxide and hydrogen, in order to obtain a probability distribution that permits the establishment of better criteria for upset situations based on these two indicators.

. Investigate the effect of volumetric load on the time-scale of hydrogen monitoring (e.g., high volumetric load anaerobic biofilm reactors).

. Investigate the effects of selective inhibition on the behavior of conventional indicators like methane yield, alkalinity and volatile fatty acids.

APPENDIX A

DATA FOR SERUM BOTTLE RUNS

DATA FOR CADMIUM RUN.

(*)	CH4 mL 0.0	H2 ppm 0.0	CO ppm 0.0	CH4 mL 2.0	H2 ppm 2.0	CO ppm 2.0	CH4 mL 6.0	H2 ppm 6.0	CO ppm 6.0
Control	0.0	4	0.4	0.7	18	0.7	1.3	33	1.0
4 mg/l	0.0	4	0.4	0.8	19	0.9	1.2	34	1.7
8 mg/l	0.0	5	0.4	0.5	23	1.0	1.0	36	1.7
20 mg/l	0.0	6	0.4	0.5	21	1.0	0.9	39	1.2
30 mg/l	0.0	3	0.4	0.3	17	0.8	0.5	37	1.0
40/mg/l	0.0	3	0.4	0.0	18	0.8	0.4	35	1.0

(*)	CH4 mL 10.0	H2 ppm 10	CO ppm 10	CH4 mL 24	H2 ppm 24	CO ppm 24	CH4 mL 48	H2 ppm 48	CO ppm 48
Control	2.2	49	1.6	4.7	62	4.1	7.9	40	5.1
4 mg/l	2.0	50	2.9	4.3	56	6.2	7.0	40	6.3
8 mg/l	1.7	53	2.4	3.5	55	3.8	4.2	38	3.9
20 mg/l	1.1	58	1.2	1.3	92	0.7	1.3	353	1.0
30 mg/l	0.1	65	1.0	0.7	253	0.7	0.9	620	1.0
40/mg/l	0.5	61	1.0	0.6	144	0.7	0.6	560	0.9

(*) numbers represents times in hours after toxicant a

DATA FOR COPPER RUN

(*)	CH4 mL 0.0	H2 ppm 0.0	CO ppm 0.0	CH4 mL 2.5	H2 ppm 2.5	CO ppm 2.5	CH4 mL 6.5	H2 ppm 6.5	CO ppm 6.5	CH4 mL 11
Control	0.0	3	0.3	0.3	22	0.4	2.5	62	0.7	3.5
2 mg/l	0.0	3	0.3	1.0	17	0.6	2.2	58	1.1	3.3
4 mg/l	0.0	3	0.3	0.9	16	0.5	1.7	58	1.1	3.1
6 mg/l	0.0	3	0.3	0.9	15	0.6	1.5	43	1.3	2.7
8 mg/l	0.0	3	0.3	0.6	13	0.5	1.9	35	1.3	2.5
10 mg/l	0.0	2	0.3	0.6	11	0.4	1.0	14	0.5	1.9

(*)	H2 ppm 11	CO ppm 11	CH4 mL 25	H2 ppm 25	CO ppm 25	CH4 mL 48	H2 ppm 48	CO ppm 48
Control	93	1.2	5.7	75	2.1	8.3	44	1.6
2 mg/l	84	1.6	5.5	62	2.4	8.9	43	1.9
4 mg/l	89	2.1	5.5	65	4.7	9.6	40	3.9
6 mg/l	67	2.7	5.1	55	6.7	8.4	41	5.6
8 mg/l	58	2.7	5.3	58	6.8	7.2	47	6.3
10 mg/l	31	1.3	4.1	60	4.5	5.9	50	4.0

(*) refers to time in hours after toxicant addition.

DATA FOR THE NICKEL RUN.

(*)	CH4	H2	CO	CH4	H2	CO	CH4	H2	CO
	mL	ppm	ppm	mL	ppm	ppm	mL	ppm	ppm
	0.0	0.0	0.0	2.0	2.0	2.0	6.0	6.0	6.0
Control	0.0	3	0.4	0.8	11	0.6	1.6	28	1.0
150 mg/l	0.0	3	0.4	0.6	22	0.6	1.2	34	1.2
300 mg/l	0.0	4	0.3	0.6	13	0.7	1.2	33	1.9
750 mg/l	0.0	2	0.4	0.5	15	0.5	0.7	34	0.6
1050 mg/l	0.0	3	0.4	0.2	11	0.4	0.2	24	0.5
1500 mg/l	0.0	3	0.4	0.0	11	0.4	0.1	22	0.5

(*)	CH4	H2	CO	CH4	H2	CO	CH4	H2	Co
	mL	ppm	ppm	mL	ppm	ppm	mL	ppm	ppm
	9.0	9.0	9.0	27	27	27	49	49	49
Control	2.3	46	1.4	5.0	66	3.7	7.2	47	3.8
150 mg/l	1.7	48	2.8	4.0	65	3.6	5.7	66	3.2
300 mg/l	1.6	47	2.9	3.6	73	5.2	5.6	85	4.4
750 mg/l	0.7	47	0.7	1.0	43	1.3	1.1	34	1.2
1050 mg/l	0.4	34	0.6	0.5	28	0.8	0.6	26	0.6
1500 mg/l	0.3	29	0.5	0.3	23	0.5	0.5	22	0.4

(*) Numbers refer to time in hours after toxicant addition.

DATA FOR THE ZINC RUN.

(*)	CH4	H2	CO	CH4	H2	CO	CH4	H2	CO
	mL	ppm	ppm	mL	ppm	ppm	mL	ppm	ppm
	0.0	0.0	0.0	2.0	2.0	2.0	5.0	5.0	5.0
Control	0.0	4	0.4	0.7	16	0.7	1.3	26	1.2
100 mg/l	0.0	4	0.4	0.6	14	0.8	1.0	22	1.4
150 mg/l	0.0	5	0.4	0.5	15	1.0	0.9	23	1.7
200 mg/l	0.0	6	0.4	0.6	18	1.1	1.1	29	1.7
250 mg/l	0.0	7	0.4	0.6	18	1.1	1.0	25	1.7
300 mg/l	0.0	7	0.4	0.2	15	1.1	0.7	18	1.4

(*)	CH4	H2	CO	CH4	H2	CO	CH4	H2	CO
	mL	ppm	ppm	mL	ppm	ppm	mL	ppm	ppm
	9.0	9.0	9.0	26	26	27	48	48	48
Control	1.9	36	1.9	5.2	73	6.6	8.8	40	6.3
100 mg/l	1.4	31	2.0	3.6	52	3.8	6.4	40	5.5
150 mg/l	1.5	34	2.4	2.5	56	4.3	7.1	39	5.1
200 mg/l	1.6	36	2.3	4.2	54	3.9	7.3	37	4.4
250 mg/l	1.1	27	2.0	2.7	48	3.2	4.5	35	2.7
300 mg/l	0.6	21	1.3	0.9	63	1.8	1.2	54	0.7

(*) Numbers refer to time in hours after toxicant addition.

DATA FOR THE BES RUN.

(*)	CH4 mL 0.0	H2 ppm 0.0	CO ppm 0.0	CH4 mL 2.5	H2 ppm 2.5	CO ppm 2.5	CH4 mL 5.5	H2 ppm 5.5	CO ppm 5.5
Control	0.0	7	0.4	0.9	18	0.7	1.6	33	1.1
2 mM	0.0	6	0.4	1.0	21	0.7	1.6	38	0.8
5 mM	0.0	5	0.4	0.8	21	0.7	1.1	54	0.8
10 mM	0.0	6	0.4	0.9	22	0.8	1.7	48	1.0
15 mM	0.0	7	0.4	0.7	27	0.7	1.2	52	0.9
20 mM	0.0	9	0.4	0.6	28	0.7	1.0	54	0.9

(*)	CH4 mL 9.5	H2 ppm 9.5	CO ppm 9.5	CH4 mL 23	H2 ppm 23	CO ppm 23	CH4 mL 48	H2 ppm 48	CO ppm 48
Control	2.2	28	1.7	4.7	65	4.0	6.6	24	2.3
2 mM	1.9	52	0.9	2.4	80	1.0	2.7	21	1.0
5 mM	1.4	62	1.0	1.9	84	0.8	2.2	44	0.3
10 mM	1.8	52	1.4	3.4	240	2.4	4.4	728	1.7
15 mM	1.4	55	0.9	1.6	173	0.8	1.7	994	1.0
20 mM	1.3	59	0.9	1.4	253	0.4	1.4	1217	1.4

(*) Numbers refer to time in hours after the toxicant

DATA FOR THE FORMALDEHYDE RUN.

(*)	CH4	H2	CO	CH4	H2	CO	CH4	H2	CO
	mL	ppm	ppm	mL	ppm	ppm	mL	ppm	ppm
	0.0	0.0	0.0	2.5	2.5	2.5	5.0	5.0	5.0
Control	0.0	4	0.4	0.3	15	1.0	1.2	27	1.7
16 mg/l	0.0	5	0.4	0.0	52	8.2	0.6	66	19.2
24 mg/l	0.0	6	0.4	0.0	80	7.9	0.0	103	15.7
40 mg/l	0.0	5	0.4	0.0	133	7.4	0.0	244	10.7
48 mg/l	0.0	7	0.4	0.0	156	4.5	0.0	537	7.8
56 mg/l	0.0	6	0.4	0.0	142	4.6	0.0	553	8.8
64 mg/l	0.0	5	0.4	0.0	148	4.9	0.0	517	7.8

(*)	CH4	H2	CO	CH4	H2	CO
	mL	ppm	ppm	mL	ppm	ppm
	9.0	9.0	9.0	24	24	24
Control	2.0	36	2.8	5.2	52	4.9
16 mg/l	1.0	58	22.0	4.0	54	16.8
24 mg/l	0.5	135	36.6	3.6	66	25.9
40 mg/l	0.3	611	23.7	1.5	156	32.8
48 mg/l	0.0	834	14.2	4.4	603	35.6
56 mg/l	0.0	888	12.0	5.6	632	28.6
64 mg/l	0.0	886	11.1	3.1	1008	24.0

(*) Numbers refer to the time in hours after toxicant addition.

A chemical reaction such as:

1 glucose = 1.65 acetat + 0.6 prop + 0.04 but + 0.76 hydr + 0.76 CO₂ (1)
can be written as:

-1 glucose = 1.65 acet + 0.6 prop + 0.04 but + 0.76 hydr + 0.76 CO₂ (1')
where each of the stoichiometric coefficients of the substrates change sign indicating that each time the reaction occurs substrate is consumed. It should be noticed also, that the rate limiting substrate in the reaction is normalized to one.

When there are n different reactions and m different compounds of interest, the system of equations can be written in matrix notation:

$$Y^T * S \quad (2)$$

Where Y^T ($m \times n$) is the transpose matrix of stoichiometric coefficients of each of the reactions, and S ($n \times 1$) is the vector of the n rate limiting compounds present in the system. For example:

$$Y^T = \begin{matrix} & -1 & 0 & 0 & 0 & 0 \\ & 1.65 & -1 & +1 & +2 & 0 \\ & 0.6 & 0 & -1 & 0 & 0 \\ & 0.04 & 0 & 0 & -1 & 0 \\ & 0.76 & 0 & 3 & 2 & -1 \\ & 0 & 1 & 0 & 0 & 1 \\ & 0.76 & 1 & 3 & 4 & -1 \end{matrix} \quad (3)$$

In the model of anaerobic digestion developed in this research project, 8 different compounds were modeled. These were: glucose, acetate, propionate, butyrate, hydrogen, methane, carbon dioxide and carbon monoxide. Five different reactions were considered. These were: glucose fermentation, propionate degradation, butyrate degradation, methanogenesis from acetate and methanogenesis from hydrogen and carbon dioxide. The stoichiometric coefficients used are contained in matrix Y^T . For example, column 1 contains the stoichiometric coefficients of reaction one (glucose limiting substrate), column 2 the stoichiometric coefficients of reaction 2 (acetate limiting substrate), column 3 of reaction 3 (propionate limiting substrate), column 4 of reaction 4 (butyrate limiting substrate) and column 5 of reaction 5 (hydrogen limiting substrate).

When a mass balance on each of the compounds considered is done, the following equation results:

$$\frac{dS}{dt} = Q + Y^T * r \quad (4)$$

Where Q represents the vector of net fluxes, of each of the compounds considered, into the system (in the case of a batch system is zero, except for methane and carbon dioxide that are released out as gases). The vector r represents the rates of each of the reactions considered (in our case 5).

Equation 4 is the one solved numerically in the computer code that follows.

This is the computer code of the anaerobic digestion model
for sucrose degradation under batch conditions. Will provide 157
Hydrogen and Carbon Monoxide predictions as principal features.
The integration is done numerically using the IMSL subroutine
DGEAR linked to the program. No attempt is done for modelling
growth neither the inhibitory effects of hydrogen on glucose
fermentation. Hydrogen effects on propionate and butyrate degradation
kinetics are modeled according to Mosey (1983) War.Sci.Tech.
It is assumed also that mass transfer kinetics between biomass and
liquid and liquid/gas are not limitant in any aspect. Sucrose is
assumed to hydrolyse in two glucose (equivalent) molecules, and this
hydrolysis is not kinetically limiting either.

```
-----  
CHARACTER FILENAME*20,RESULTS*20  
REAL KS,K,VGAS,VLIQ,X,NUMMIN,SS,S,RES,T,H,TOL,WK(243),TEND,ACH4,STEMP  
REAL INH(5),PHI(7)  
INTEGER N,METH,MITER,INDEX,IWK(9),IER,L  
DIMENSION KS(5),K(5),X(5),S(9),SS(2),RES(11,600)  
COMMON/VAR/KS,K,VGAS,VLIQ,X,SS,INH,PHI  
EXTERNAL DIG,FCNJ  
N=9  
T=0  
METH=1  
MITER=0  
INDEX=1  
TOL=.0001  
H=.0001  
S(8)=0  
S(9)=0  
PRINT*,'ENTER DATA FILE NAME IN QUOTATIONS'  
READ*,FILENAME  
OPEN(UNIT=10,FILE=FILENAME,STATUS='OLD')  
READ(10,*,END=15)(KS(I),I=1,5)  
READ(10,*,END=15)(K(I),I=1,5)  
READ(10,*,END=15)((S(I),I=1,7),(SS(I),I=1,2))  
READ(10,*,END=15)(X(I),I=1,5)  
READ(10,*,END=15)VLIQ,VGAS  
READ(10,*,END=15)(INH(I),I=1,5)  
READ(10,*,END=15)(PHI(I),I=1,7)  
GO TO 16  
PRINT*,'ERROR IN DATA LECTURE'  
PRINT*,'ENTER TIME PERIOD TO BE SIMULATED IN HOURS'  
READ*,NUMMIN  
NUMMIN=NUMMIN*60  
PRINT*,'ENTER STEP INCREASE IN MINUTES'  
READ*,STEP  
PRINT*,'ENTER OUTPUT FILE NAME SURROUNDED BY QUOTES'  
READ*,RESULTS  
RESULTS='DIG.OUT'  
OPEN(UNIT=11,FILE=RESULTS,STATUS='NEW',RECL=85)  
OPEN(UNIT=12,FILE='CO_H2',STATUS='NEW',RECL=35)  
TEND=TEND/60  
WRITE(11,25)(TEND,(S(J),J=1,8),H,ACH4)  
L=0  
DO 30 I=STEP,NUMMIN,STEP  
TEND=I  
CALL DGEAR(N,DIG,FCNJ,T,H,S,TEND,TOL,METH,MITER,INDEX,IWK,WK,IER)  
  
LIMITING CONDITION FOR GLUCOSE REMOVAL  
IF (S(1).LE..001)S(1)=0  
  
CALCULATION OF CARBON MONOXIDE CONCENTRATION (PPM)  
SS(1)=352.258*S(5)*(S(7)*S(2)/S(6))**0.5  
  
CALCULATION OF CUMULATIVE METHANE PRODUCTION  
ACH4=ACH4+VGAS*(S(6)-STEM)+S(8)*S(6)
```


RETURN
END

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DUMMY SUBROUTINE FOR FCNJ
SUBROUTINE FCNJ(N,X,Y,PD)
INTEGER N
REAL Y(N),PD(N,N),X
RETURN
END

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