ABSTRACT

Title of Thesis:

Experiments in Aerobic and Anaerobic Biodegradation of Methylene Chloride Using Activated Sludge

Biological removal of methylene chloride was studied under aerobic and anaerobic conditions using activated sludge cultures. Shaker flask experiments were conducted to test the ability of the microorganisms to degrade methylene chloride under aerobic conditions. Hydrogen peroxide was used a as source of dissolved oxygen to minimize physical removal of methylene chloride due to aeration. The effect of secondary substrates like glucose, cellulose acetate, ammonium acetate and nutrient broth on biodegradation of methylene chloride was studied. Biodegradation in the presence of surfactant and alkaline stress was also investigated.

No significant degradation was observed in all aerobic experiments.

Anaerobic sludge was obtained from a secondary wastewater treatment plant, and after digestion at 35 ^{o}C it was used for anaerobic experiments. Preliminary experiments were conducted in serum bottles to test the ability of the mixed cultures to biodegrade methylene chloride under anaerobic conditions. The effect on biodegradation due to the presence of glucose and sodium acetate was also studied. Very low methylene chloride removal rates were obtained in the serum bottles (0.0021 mg methylene chloride /day.mg biomass).

An effort was made to increase the degradation rates by immobilization. Two immobilized cell bioreactors namely, the Membrane Bioreactor and the Celite Carrier Packed Bed Reactor were developed and studied. Glucose was used to test the viability of the immobilized microorganisms in these reactors. The entrapped microorganisms

in the membrane reactor did not display activity; however, the attached microorganisms on the Celite carrier remained viable. Two hundred and fifty ppm methylene chloride was treated completely in the Packed Bed reactor in 8 days. A tenfold increase in the removal rate was observed (0.021 mg methylene chloride/day.mg biomass) in the Celite Carrier Packed Bed Reactor compared to that obtained in the serum bottles (0.0021 mg methylene chloride /day.mg biomass).

EXPERIMENTS IN AEROBIC AND ANAEROBIC BIODEGRADATION OF METHYLENE CHLORIDE USING ACTIVATED SLUDGE

by

Vivek Sinkar

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CHAPTER I

INTRODUCTION

Methylene chloride is designated as a priority pollutant by the U.S. Environmental Protection Agency (Silva, 1981). It is widely used as a solvent for cleaning and paint removal, for fumigation and refrigeration, in analytical applications, and as an aerosol propellant (Verscheren, 1977). Due to its versatility and its solubility in water (20 gm/l at 20°C), methylene chloride is found in many aqueous environments. Methylene chloride has been suspected of being carcinogenic because of its positive responses in the Ames test (Jongen et.al., 1978). It is a narcotic, which at high concentrations can damage the nervous system, and the respiratory organs (Galli, 1985). Consequently the entry of methylene chloride in the environment has to be prevented wherever possible.

Activated sludge has been recognized as one of the most versatile methods known for wastewater treatment. Sawyer (1965) has given an excellent account of the development in the activated sludge process in the fifty years of its existence. Both the activated sludge process and conventional physical/chemical processes have been used for methylene chloride removal and they have their advantages and disadvantages. The activated sludge process is not useful for high concentrations of methylene chloride, however at low concentrations it is economical and can completely mineralize methylene chloride. The conventional processes on the other hand cost more, they are not suitable for low concentrations of methylene chloride, and they (except ozonation) shift methylene chloride from one matrix to another without mineralizing it.

Rittmann et al. (1980) demonstrated for the first time that methylene chloride can be biodegraded and that it could support growth of bacteria enriched from sewage. Since then, efforts have been made to replace the conventional technologies, like steam

stripping, ozonation, adsorption on activated carbon by a biotechnological process to prevent the loss of methylene chloride in the atmosphere.

Both aerobic and anaerobic processes for degradation of methylene chloride have been studied. Both have their advantages and disadvantages. The aerobic process is relatively easy to control and is relatively fast. The anaerobic process requires a very close control of parameters like pH and temperature. However the removal of chloride is easier under reducing conditions compared to that under oxidizing conditions.

Literature on the anaerobic treatment of methylene chloride is scarce. Dienemann and coworkers, 1987 have reported sequential anaerobic/aerobic packed bioreactors for the treatment of a Superfund leachate containing methylene chloride. The authors were able to completely degrade 22 mg/lit methylene chloride by passing the leachate through the anaerobic bioreactor.

The biodegradation of methylene chloride using both pure and mixed cultures has been reported. Mixed cultures are easily available, they are economical, and due to their broad specificity are easily adaptive. Pure cultures on the other hand are expensive to grow and maintain.

In the present study, both aerobic and anaerobic experiments were carried out to determine the capability of activated sludge to degrade methylene chloride.

CHAPTER II

LITERATURE REVIEW

2.1 Aerobic Biodegradation

The possibility of mutagenicity associated with methylene chloride was investigated using the Ames mutation test by Jongen (1978). Mutation tests were carried out with TA 98 and TA 100 as tester strains. The investigators observed the mutation rate to increase at all the applied dose levels of methylene chloride. They concluded that methylene chloride was mutagenic for both tester strains and further indicated that methylene chloride induced frame shift mutations.

McCarty and coworkers (1980) showed that methylene chloride was biodegradable using mixed cultures enriched with methylene chloride for a period of over one year. Enrichment involved feeding the bottles at various concentrations of methylene chloride every two or three days. The investigators observed that methylene chloride utilization was significant only in the presence of sodium bicarbonate. The investigators were also able to immobilize the enriched bacteria on a glass column and grow the bacteria in presence of methylene chloride. They suggested the autotrophic pathway for biochemical breakdown of methylene chloride as they observed the growth to decrease in the absence of sodium bicarbonate from the mineral medium.

Brunner and coworkers (1980) isolated strain DM1 (genus Pseudomonas), a facultative methylotrophic bacterium utilizing methanol, formate as well as methylene chloride as a C1 substrate. The organism was strictly aerobic, gram negative, oxidase positive short rod, and motile by a single flagellum. The authors demonstrated that

methylene chloride was a growth inhibitory substrate and that a specific growth rate of 0.11 hr^{-1} could be reached between concentrations of 2 and 5 mM methylene chloride. The maximum dehalogenation activity of the cells was observed at pH 9. They suggested the halidohydrolase substitution reaction (substitution of a hydroxyl group for one chloride of methylene chloride) to be the most likely mechanism for dehalogenation of methylene chloride. The reaction would release two molecules of hydrochloric acid and one of formaldehyde.

In another study Stucki and coworkers (1981), isolated a facultatively methylotrophic bacterium from enrichment cultures containing methylene chloride as the sole carbon source and identified it as a hypomicrobium species. The investigators proved that in the presence of potassium sulfite as a trapping agent, the methylene chloride grown cells converted methylene chloride to formaldehyde, and that the conversion was strictly dependent on the presence of glutathione. They postulated the following stoichiometry for the dehalogenation of methylene chloride:

$$CH_2Cl_2 + H_2O \text{ -------} > 2Cl^- + 2H^+ + CH_2O$$

They further confirmed that the ability to use methylene chloride arose in natural microbial populations exposed to methylene chloride over extended periods and was not an inherent property of certain groups of methylotrophs.

In an interesting study Klecka (1982), described the fate and effect of methylene chloride in activated sludge that simulated a municipal wastewater plant. Activated sludge was acclimated to methylene chloride at concentrations between 1 and 100 mg/l by exposure for nine to eleven days. The acclimated cultures were shown to mineralize methylene chloride to carbon dioxide and chloride. He developed a hypothetical model to examine the significance of volatalization and biodegradation for the removal of methylene chloride from an activated sludge reactor. From the model he predicted that the biodegradation rate was approximately 12 times greater than the volatalization rate.

Galli and coworkers (1985) developed a flow through process for the mineralization of methylene chloride. They selected a bacterial strain with a high degradation capacity for methylene chloride by comparing the growth properties of ten isolates in batch and continous cultures. They found that several strains were unable to grow on media containing 120 to 170 mM methylene chloride due to the high concentration of the salt arising from the neutralization of the hydrochloric acid generated by the dehalogenation reaction. The organism with the maximum degradation capacity was characterized as Pseudomonas DM5, and the degradation rates were 0.62 g/l.h for 120 mM, and 0.86 g/l.h for 170 mM methylene chloride.

The authors could further increase the maximum degradation rate (0.86 g/l.h) of the strain DM5 by increasing the biomass concentration. To achieve this the authors developed a continous flow fluidized bed reactor as proposed by Shieh and coworkers (1981). They showed that the experiments with a fluidized bed reactor using immobilized cells provided encouraging results on the mineralization of methylene chloride. The investigators were able to develop a non-sterile system with a degradation capacity of 1.6 g/l.h that remained stable for extended periods of time.

In another study Kim and coworkers (1986) indicated that acclimated cultures capable of utilizing chlorinated organic substrates could be derived from activated sludge that were effective over a wide range of substrate concentrations. They conducted research to describe biodegradation kinetics of selected chlorinated organic chemicals and the resulting growth of microorganisms in presence of conventional metabolites. Two model compounds for the study were 2,4 dichlorophenoxyacetic acid and 3,5 dichlorobenzoate. The researchers used nutrient broth as an alternative multiple substrate mixture which contained common metabolites.

The investigators found that a lag phase occurred prior to the rapid utilization of the chlorinated substrate, and hence suggested an initial low level treatment concentration of the substrate. They further concluded that the addition of conventional meta-

bolites was not advantageous for acclimation, however an inoculum containing microorganisms with a diversity of metabolic capabilities was desirable to increase the probability of plasmid exchange.

In a recent study Fewson (1988) has described the biodegradation of xenobiotic and other persistent compounds, and the causes of their recalcitrance. He suggests that concentration and solubility of the substrate, the physical accessibility of the substrate, the chemical nature of the substrate, the availibility of oxygen, and the presence of other molecular species are the factors to be considered for the recalcitrance of a chemical substance. According to the author, polymerization and branching of the compound, formation of stable components linked by bonds which are not subject to facile hydrolysis, presence of chlorine, nitro and sulphonate groups are some of the molecular features that increased recalcitrance.

2.2 Anaerobic Biodegradation

Jeris and coworkers (1985) have described anaerobic digestion as a process employing facultative, and strictly anaerobic bacteria to decompose organic material in wastewater where the complex organic matter is degraded into two distinct stages. The first stage involves the hydrolysis, and fermentation of complex substances like fats and carbohydrates to simple compounds like volatile acids and ammonia. The second stage involves the fermentation of these simple compounds to produce methane and carbon dioxide.

According to the investigators, various upset signs like an increase in concentration of volatile acids, an increase in the amount of carbon dioxide, a decrease in the gas production, and a decrease in pH are seen due to overfeeding, changes in temperature, oxygen contamination and an increase in toxicity. Proper control can be achieved by

performing periodic tests of pH, alkalinity, volatile acids, gas production and consumption, temperature, liquid level, COD, grease, and organic and ammonia nitrogen.

Parkin and coworkers (1986) have discussed the fundamentals of anaerobic digestion of wastewater sludges. According to the authors the key factors to be considered for achieving optimum design and efficient operation of the anaerobic digester are optimum retention time, adequate mixing, proper pH, proper temperature control, adequate concentration of essential nutrients, absence of toxic materials and proper feed characteristics.

Bouwer and coworkers (1981) have studied the anaerobic biodegradation of halogenated 1 and 2 carbon organic compounds. They selected chloroform, trichloroethylene, tetrachloroethylene, dibromochloromethane and dibromodichloromethane as model organic compounds for their study. One milliliter of methanogenic mixed culture was grown in a laboratory digester. Along with a nutrient medium and the model organic compound, the culture was added to a series of serum bottles. The bottles were sealed and incubated in the dark at 20 ^{o}C. The authors concluded from their work that anaerobic biodegradation of chloroform was possible, whereas the removal of brominated trihalomethanes was due to chemical reaction, and that trichloroethylene and tetrachlororethylene could not be degraded under anaerobic conditions.

In an interesting study, Parkin and coworkers (1988) characterized the fate and effect of formaldehyde and methylene chloride on methane fermentation systems using propionate and acetate in the defined media. Formaldehyde and methylene chloride were added both as continuous and slug doses. Higher concentrations of formaldehyde could be tolerated when added continuously. The authors confirmed that 55-60 % of the total 80 % removal of formaldehyde was due to biodegradation. Methylene

chloride caused a greater effect on the acetate using methanogens than on the propionate using bacteria. Abiotic chemostats showed 20-25 % methylene chloride removal by volatilization. The investigators were able to degrade from 80 mg/l of methylene chloride to 1-2 mg/l in about 40 days.

2.3 Development of Bioreactors

Harper and coworkers (1987) compared several anaerobic treatment systems, each unique in the management of gaseous end products. Three anaerobic filter series were developed and studied. The first system consisted of a series of four anaerobic filters, stacked to form a single up flow filter. The second system consisted of four similar filters connected in series by liquid phase, the gas phase of the first three reactors was removed and passed through the last reactor in series. The third system was similar to the second one, except that the gases produced in each reactor were measured and released separately. The reactor systems were operated in continuous and pulsed loading mode.

In the pulsed loading mode, the third system exhibited the most stable pH and least volatile acid concentrations. The third system also exhibited best performance in the continuous loading mode by producing more gas.

In an interesting study, Kennedy and coworkers (1988) studied an Up Flow Blanket Filter Reactor (UBF) to treat land fill leachate and compared the results to those obtained from a well designed Down Flow Stationary Filter Reactor (DSF). Feed was continuously fed at the bottom of the UBF reactor, mixed with the recycle stream and the effluent was allowed to overflow. In the DSF reactor feed was continuously pumped in at the top of the reactor through a distribution manifold and the effluent was removed from the bottom for disposal and recirculation. The authors reported good treatment efficiencies for both the reactors; however, they indicated that the UBF reactor yielded a slightly greater overall performance.

Lin and coworkers (1986) have studied methanogenic digestion using various concentrations of mixed substrates, and various hydraulic retention times (HRT) in continuous-flow chemostat reactors maintained at 35 ^{o} C. The feed consisted of a mixture of acetic, propionic and butyric acids. The authors found that an increase in the feed substrate concentration adversely affected the propionate degradation, while a decrease in HRT adversely affected both the propionate and the acetate degradation . The authors further concluded that the methane production was independent of the feed concentration and HRT.

Lakhwala and coworkers (1990) of the NJIT Biotechnology Laboratory have reported a Polymeric Membrane Bioreactor for the biodegradation of phenol in wastewater. The authors indicate that the membrane reactor when used in a spiral configuration eliminates chanelling, and due to a high surface area and a high porosity it allows higher biomass loading, and consequently less reactor volumes.

The investigators immobilized microorganisms from activated sludge by attachment to the microporous sheets. Fifty ppm of phenol could be removed completely in 6 hours using the attached microorganisms.

CHAPTER III

OBJECTIVES

The main objectives of this research work are to:

1) Test the ability of the activated sludge cultures to degrade methylene chloride under aerobic and anaerobic conditions,

2) Study the effect of secondary substrates like, glucose, nutrient broth, ammonium acetate and cellulose acetate on the aerobic biodegradation of methylene chloride,

3) Study biodegradation of methylene chloride in the presence of glucose and sodium acetate under anaerobic conditions, and

4) Develop an immobilized cell bioreactor to enhance the removal rates of methylene chloride.

CHAPTER IV

MATERIALS AND METHODS

4.1 Aerobic Experiments

4.1.1) Microorganisms

Activated sludge was obtained from the Parsipanny wastewater treatment plant (NJ) for the present studies. The sludge was sieved through a 297 um opening screen and allowed to settle. The supernatant was removed and replaced by an equal volume of nutrient medium, the composition of which is described below. This sludge solution was acclimated to methylene chloride at room temperature. Usually, the activated sludge solution was fed with 100 ppm methylene chloride. Hundred milliliters of a 3% solution of hydrogen peroxide were added daily to maintain aerobic conditions through out the acclimation period. After acclimation, the culture was centrifuged at 3000 rpm and 5°C to obtain concentrated pellets which were stored in the refrigerator at 4 °C

4.1.2) Nutrient Medium

The nutrient medium used here was developed in an earlier study (Lakhwala, 1991) and consisted of the following chemicals:

Magnesium chloride	100.00 mg
Magnesium sulfate	10.00 mg
Ferric chloride	0.50 mg
Potassium phosphate	1.00 mg
Tap water	100.00 ml

The above solution was diluted to 1000 ml by adding distilled water, and then aerated for 15 minutes.

4.1.3) Measurement of Biomass Concentration

A known weight of pellets obtained after centrifugation was dried in an oven at 120 ^{o} C for 24 hours. The dry biomass so obtained was expressed as dry biomass per unit weight of pellets. The biomass concentration for the present set of experiments was around 52 mg dry biomass / g pellet.

4.1.4) Biodegradation studies

Shaker flask experiments were conducted to test the ability of the activated sludge cultures to degrade methylene chloride at low (50 ppm) and high (250 ppm) concentrations.

a) In absence of a secondary substrate

Two, 250 ml Erlenmeyer flasks containing 10 % (by weight) biomass in the nutrient medium were injected with methylene chloride so that the concentrations in the flasks was around 50 and 250 ppm methylene chloride. A 3 % hydrogen peroxide solution was added periodically (2 ml, twice in a day) to the flasks to maintain aerobic conditions through out the experiment. Control runs were conducted simultaneously to determine the losses, if any, of methylene chloride due to physical and chemical removal.

Flasks containing the samples were kept in an Orbit Environ Shaker at 27 ^{o} C and 160 rpm. Liquid samples were periodically taken and analyzed for methylene chloride on a gas chromatograph.

b) In presence of a secondary substrate

Kim and co-workers (1986) have postulated that biodegradation of chlorinated hydrocarbons is assisted by addition of a secondary substrate. Shaker flask experiments were conducted to test whether the addition of a secondary substrate enhanced the biodegradation of methylene chloride by the mixed cultures. Glucose, cellulose

acetate, ammonium acetate and nutrient broth were used as secondary substrates in this study.

Eight flasks containing 10 % biomass (by weight) in the nutrient medium were taken and labelled as L_1, L_2, L_3, L_4 and H_1, H_2, H_3, H_4. The starting concentration of methylene chloride in the flasks labelled "L" was around 50 ppm and in the flasks labelled "H" was around 250 ppm.

Glucose was added to the flasks subscripted 1, cellulose acetate was added to the flasks subscripted 2, ammonium acetate was added to the flasks subscripted 3, and nutrient broth was added to the flasks subscripted 4. The concentration of these secondary substrates was 50 ppm in all the eight flasks.

Similar flasks with control solutions, that is without biomass, were prepared to determine the losses of methylene chloride, if any, due to physical and chemical removal.

Hydrogen peroxide was added periodically as before to maintain aerobic conditions through out the experiment. The pH of the samples was adjusted around 7.0. All the 16 flasks were cork stopped and kept in an Orbit Environ Shaker at 27 ^{o} C. Liquid samples were periodically taken and analyzed for methylene chloride.

c) In presence of surfactant

Earlier studies have shown that use of surfactant enhances biodegradation of organic solvents, as the solubilty of the organic solvent of interest increases in aqueous phase, thereby, providing a better contact of the substrate with the microorganisms. Shaker flask experiments were carried out to check the effect of surfactant addition on biodegradation of methylene chloride. Triton X-405 was selected as a surfactant for this set of experiments.

Two flasks containing 10 % (by weight) biomass in the nutrient medium and, a starting concentration of 50 and 250 ppm methylene chloride were taken. Triton X-405 was added to each of these flasks to obtain 3 % (v/v) concentration of the surfactant in

the flasks. Control flasks (without biomass) were similarly prepared to determine the losses of methylene chloride due to physical and chemical removal. Hydrogen peroxide was added as before to maintain aerobic conditions through out the experiment. The pH of the samples was adjusted around 7.0.

The flasks were cork stopped and kept in an Orbit Environ Shaker at 27 ^{o} C and 160 rpm. Liquid samples were periodically taken and analyzed for methylene chloride.

d) Effect of alkaline stress

It was envisioned that the microorganisms when induced with an alkaline stress would degrade methylene chloride to yield chloride which would then neutralize the alkalinity. Shaker flask studies were carried out once again to determine weather the bacteria would degrade methylene chloride when under alkaline stress. The microrganisms may become inactive using a very high pH; so a moderate value of pH (9.0 to 9.5) was selected.

Twenty grams calcium alginate biobeads were added to the nutrient medium containing 100 ppm methylene chloride as starting concentration was taken. The method of preparation of calcium alginate has been described by Lakhwala (1991). A 1 N sodium hydroxide solution was added to make the solution alkaline (pH 9.4). Control flask contained control beads (beads without biomass) in the defined medium and 100 ppm methylene chloride, and the pH was adjusted to 9.4 using 1 N sodium hydroxide solution. Hydrogen peroxide was added as before to maintain aerobic conditions.

Both flasks were kept in the Orbit Environ Shaker at 27 ^{o} C and 160 rpm. Liquid samples were periodically taken and analyzed for methylene chloride.

e) Effect of Sodium Bicarbonate

Rittman and coworkers (1980) have observed, that the utilization of methylene chloride by suspended bacteria was significant only in the presence of sodium bicarbonate in the nutrient medium.

Experiments were conducted to test the effect of sodium bicarbonate on the biodegradation of methylene chloride by the waste cultures. The nutrient medium used is decribed by Rittmann and coworkers (1980) and is shown in **Table 4.1.** Methylene chloride was added to two shaker flasks containing the nutrient medium as described in **Table 4.1** to obtain a concentration of 100 ppm methylene chloride. Biomass was added to one of the flasks. Hydrogen peroxide was added periodically to maintain aerobic conditions. The bottles were sealed and kept in the shaker. Samples were periodically taken to determine methylene chloride concentration.

4.2 Anaerobic Experiments

4.2.1) Microorganisms

The anaerobic sludge used in this study was taken from the Secondary Treatment plant (STP, Livingston, NJ), and then digested in an anaerobic digester. The anaerobic digestion of sludge was done as described by Gourdon and coworkers (1989). The experimental set up of anaerobic digester is shown in **Figure. 4.1.** The digester was maintained at 35 ^{o} C using a water bath. One and a half kilograms of sludge were mixed with 2 litres of nutrient medium "A", and then digested in the digester. Nitrogen was bubbled through it for 20 minutes. The digester was sealed, and covered with aluminium foil to protect it from light. Periodically, the required amount of biomass was harvested for biodegradation studies, and then the same amount of nutrient medium "B" was added into the digester to keep the bacteria active. The nutrient

medium "B" contained higher concentrations of glucose and other compounds to provide enough nutrients to the microorganisms.

4.2.2)Nutrient Media

a) Nutrient medium "A" and "B"

The media A and B consisted of the same components but with different concentrations. The media were prepared using the solutions shown in **Table 4.2** and then diluted using tap water.

b) Nutrient medium "C" for anaerobic experiments

Nutrient medium C was used for anaerobic biodegradation experiments. The medium consisted of nutrients and vitamins for mixed anaerobic cultures and was modified from that used by Owen and McCarty (1979) in their studies.

The composition and the method of preparation of medium C is shown in **Table 4.3.**

4.2.3) **Anaerobic Experiments**

4.2.3.1) Biodegradation Experiments in Serum Bottles

The anaerobic sludge was digested over a period of two weeks after which 500 ml of biomass solution was removed. Thousand ml of nutrient medium C were added to this biomass solution, this was the working medium for anaerobic experiments. Forty milliliters of the working medium were added to a series of 70 ml serum bottles. Nitrogen gas was passed through these bottles. Various amounts of methylene chloride were added to these serum bottles to obtain the desired concentration of methylene chloride. Hundred ppm glucose and 100 ppm sodium acetate were added to four and three of the bottles respectively in the series to test the effect of addition of a secondary substrate on anaerobic biodegradation of methylene chloride. The bottles were sealed

and incubated in the dark at 35 ^{o} C. The bottles were shaken twice daily over the period of the experiment. The biogas generation was monitored every day, and liquid and gas samples were periodically taken to analyze for methylene chloride. Samples were analyzed for chloride ion at the end of the experiment.

Serum bottles with exactly similar conditions but without biomass solution, served as controls, and were stored simultaneously in the incubator to determine the losses of methylene chloride due to physical and chemical removal.

4.2.3.2.) Development of an immobilized anaerobic bioreactor.

Very low removal rates were observed in serum bottles. By immobilization the biodegradation rates can be increased because the biomass concentration increases. Glucose was used to test for the viability of immobilized microorganisms in the anaerobic bioreactor, and methylene chloride was used as a model compound. Two reactor configurations were studied which are described below.

a) Experiments in the Membrane Bioreactor

Lakhwala and coworkers (1990) have reported a polymeric membrane bioreactor for the biodegradation of phenol in wastewater. The spiral membrane configuration was thought to be suitable for entrapping microorganisms and providing well maintained anaerobic conditions.

Experimental Setup of the reactor.

The experimental setup of the membrane reactor is shown in **Figure. 4.2.** The polymeric membrane is a 1 inch wide and 36 feet long strip wound in spiral configuration with a spacing of 0.013 inches between adjacent layers and is fixed between two steel plates and a plastic housing which forms the complete reactor. The steel plates have openings for the inlet and outlet streams. A high pressure pump is used to pump the feed from the reservoir in inside-out direction. For batch recirculation experiments the working volume is typically 700 ml.

Inoculating the bioreactor

The reactor was initially flushed with sodium sulfide solution to create a reducing environment. The reservoir was filled with nutrient medium C. After adequate recycling to create anaerobic conditions, and to maintain a pH value of 7 in the reactor the feed pump was stopped. A mixture containing 300 ml biomass solution and 200 ml nutrient medium, was recycled through the reactor at a flow rate of 25 ml/min and a starting pressure of 1 psig. Inoculation was carried out by recycling a total volume of 900 ml biomass solution in 3 batches of 300 ml each. The attachment was evident from a gradual increase in pressure from 1 to 1.8 psig.

The reactor was then injected with 2 grams of glucose every 2 days, for 10 days, and the attached biomass was allowed to grow. Biogas generation was monitored, and a pH value of 7.0 was maintained through out the experiment.

b) Experiments in the Celite Carrier Packed Bed Reactor

The experimental setup of the Celite carrier packed bed reactor is shown in **Figure. 4.3**. Thirty grams of Celite beads were packed in a cylindrical reactor in two layers. Sodium sulfide solution was pumped through the reactor to create a reducing environment. The reservoir was filled with nutrient medium C and recycling was carried out to assure the prevalence of anaerobic conditions. In all, 900 ml of biomass solution was added to the reservoir in three batches of 300 ml each. Typically, 300 ml of biomass solution was added to 200 ml nutrient medium C, and was recyled through the reactor at 20 ml/min. A lower flow rate was selected to prevent the wash out of the attached biomass. Biofilm build up was clearly visible at the end of the third batch. The reactor was then injected with 2 grams of glucose every alternate day and the attached biomass was allowed to grow for one week. Biogas generation was monitored daily. At the end of the one- week period, the reactor was injected with around 250 ppm methylene chloride, and allowed to operate for a period of ten days. Liquid and gas samples were periodically taken to determine the methylene chloride concentration. The reactor was

then operated at a higher flow rate to ensure that all the attached biomass, was washed out. Methylene chloride was injected into the reactor, and the flow rate of the pump was maintained at 20 ml / min. Liquid samples were periodically taken and analyzed for methylene chloride. This experiment served as a control to determine the loss of methylene chloride due to physical and chemical removal.

4.3 ANALYTICAL METHODS

4.3.1) Methylene Chloride Analysis

Methylene chloride concentration was measured on a Perkin-Elmer 8500 gas chromatograph equipped with a FID. A stainless steel "Carbopak" column was used at oven temperature of 75 ^{o} C. Helium at 40 psig was used as a carrier gas. The injector was maintained at 200 ^{o} C and the FID was maintained at 250 ^{o} C.

4.3.2) Viability Check for Aerobic Microorganisms

The activity of the microorganisms was periodically checked in the microassay reactor, shown in **Figure 4.4**. The microassay reactor is small jacketed vessel of 1.8 ml capacity, with a provision for a Clark type dissolved oxygen probe. Water at the required temperature was circulated through the jacket and the reactor was mounted on a magnetic stirrer plate.

The reactor was initially washed with methanol and then rinsed with nutrient medium. Nutrient medium was added to the reactor, which was air sparged till it was saturated. Biomass whose viability was to be tested, was added to this saturated solution, and the reactor was capped and allowed to operate for a period of 20 minutes. The rate of oxygen consumption was evident from the slope recorded by the oxygen probe on the chart. This served as a viability check.

4.4.3) Chloride Analysis

Chloride analysis was conducted at the beginning and the end of anaerobic experiments. Silver nitrate was titrated aginst the sample, and the chloride ion concentration was determined using a Specfic Ion Electrode.

4.3.4) D.O Measurements

Dissolved oxygen was directly measured using an Orion (97-08 model) oxygen probe.

Table 4.1 Nutrient Medium containing Sodium bicarbonate

Compound	Concentration (mg/l)
KH_2PO_4	8.5
K_2HPO_4	28.5
Na_2HPO_4	33.4
$FeCl_3.6H_2O$	0.25
$NaHCO_3$	20.0
NH_4Cl	1.7
$MgSO_4$	22.5
$CaCl_2$	27.5
The pH of this solution was adjusted to 7.1	

Table 4. 2 Nutrient Medium for Anaerobic Digestion

Compound	Concentration (g / l)	
	A	B
Glucose	1.5	35
Yeast Extract	0.5	15
Peptone	0.25	None
NH_4Cl	0.15	3
$K_2HPO_4. 3H_2O$	0.05	1.5
$CaCl_2. 2H_2o$	0.025	0.50
$MnSO_4. H_2O$	0.005	0.1
$MgSO_4. 7H_2O$	0.01	0.2
$FeCl_3. 6H_2O$	0.005	0.1
Low viscosity sodium alginate	0.5	10
The pH of both solutions was adjusted to 7.0 by adding 0.1 N sodium hydroxide solution. Nitrogen was bubbled through the medium for 20 minutes to remove dissolved oxygen.		

Table 4. 3 Nutrient Medium for Anaerobic Experiments

Solution	Compound	Concentration (g / l)
S1	$(NH_4).2HPO_4$	26.7
S2	$CaCl_2.2H_20$	16.7
	NH_4Cl	26.6
	$MgCl_2.6H_2O$	120
	KCl	86.7
	$MnCl_2.4H_2O$	1.33
	$CoCl_2.6H_20$	2
	H_3BO_3	0.38
	$CuCl_2.6H_2O$	0.18
	$Na_2MoO_4.2H_20$	0.17
	$ZnCl_2$	0.14
S3	$FeCl_3.4H_2O$	370
S4	$Na_2S.9H_2O$	500

5.4 ml of S1 and 27 ml of S2 solutions were diluted to 1.8 litres and boiled for 15 minutes in a conical flask. This solution was cooled to room temperature and 1.8 ml of S3 and 1.8 ml of S4 solutions were added along with 1 gram of yeast extract and beef extract, and mixed well. Nitrogen gas was bubbled for 20 minutes. The flask was sealed to minimize the introduction of air. A pH value of 7 was adjusted.

FIG. NO 4.1 EXPERIMENTAL SETUP OF AN ANEROBIC DIGESTER

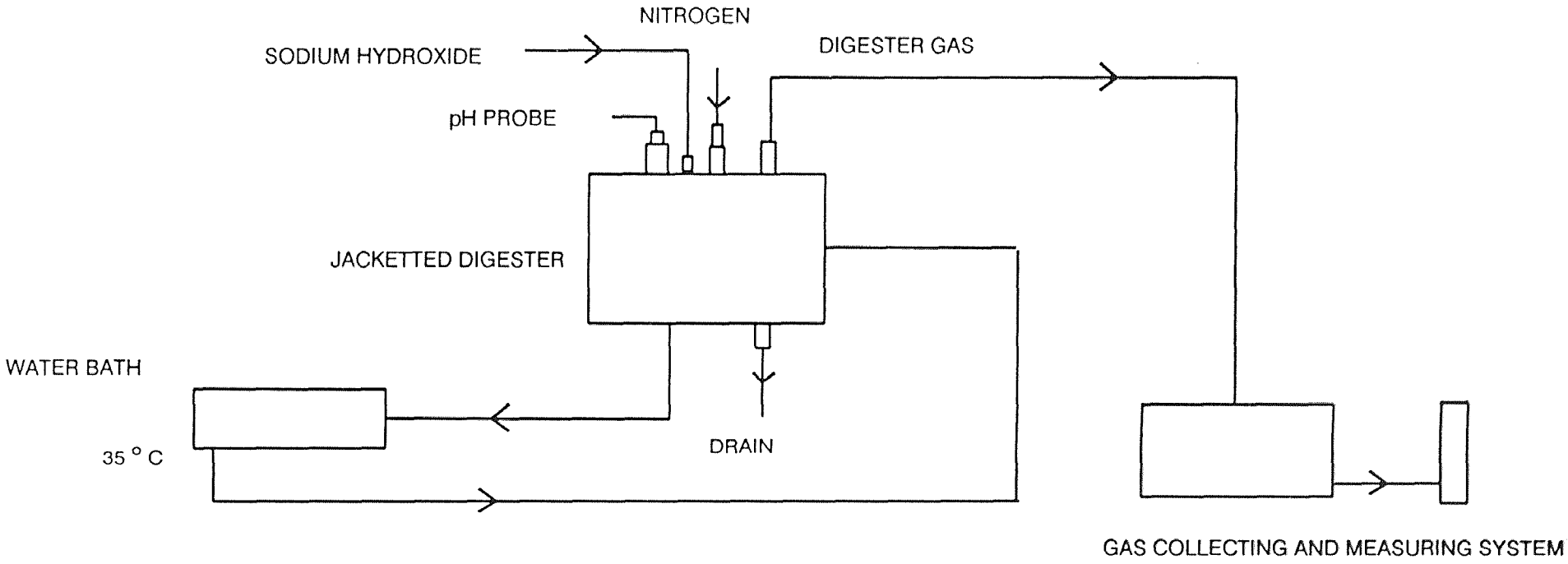
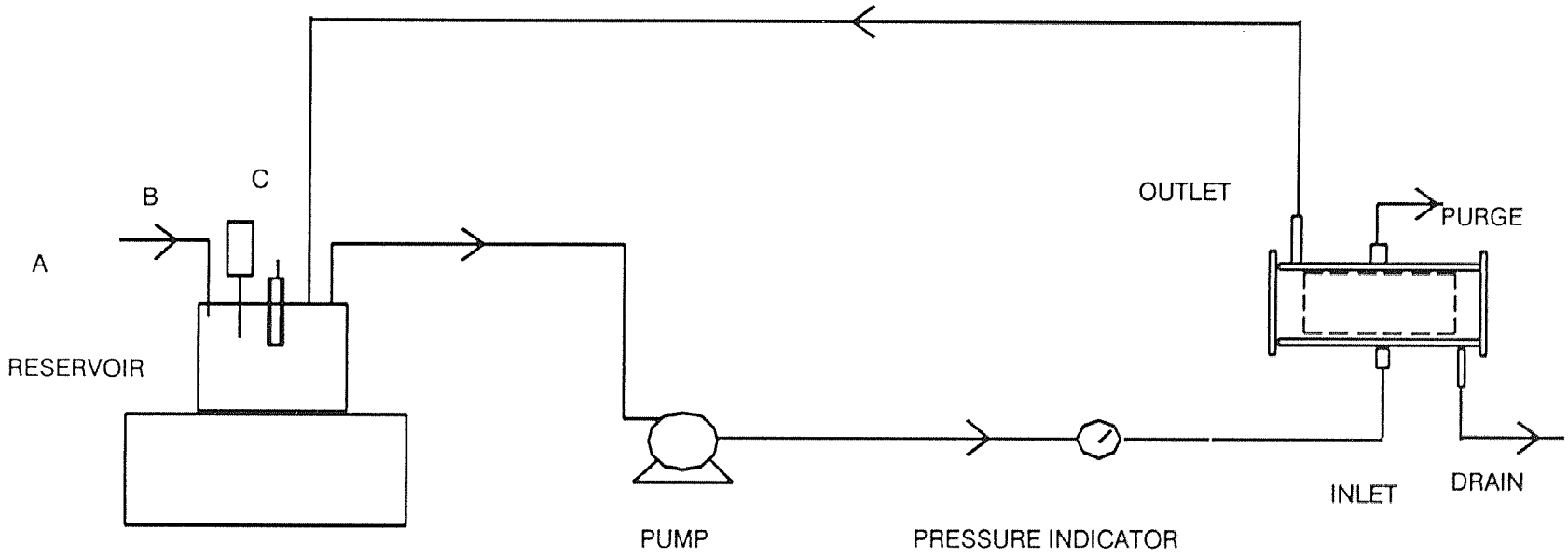


FIG. NO 4.2 EXPERIMENTAL SETUP OF THE MEMBRANE ANAEROBIC BIOREATOR



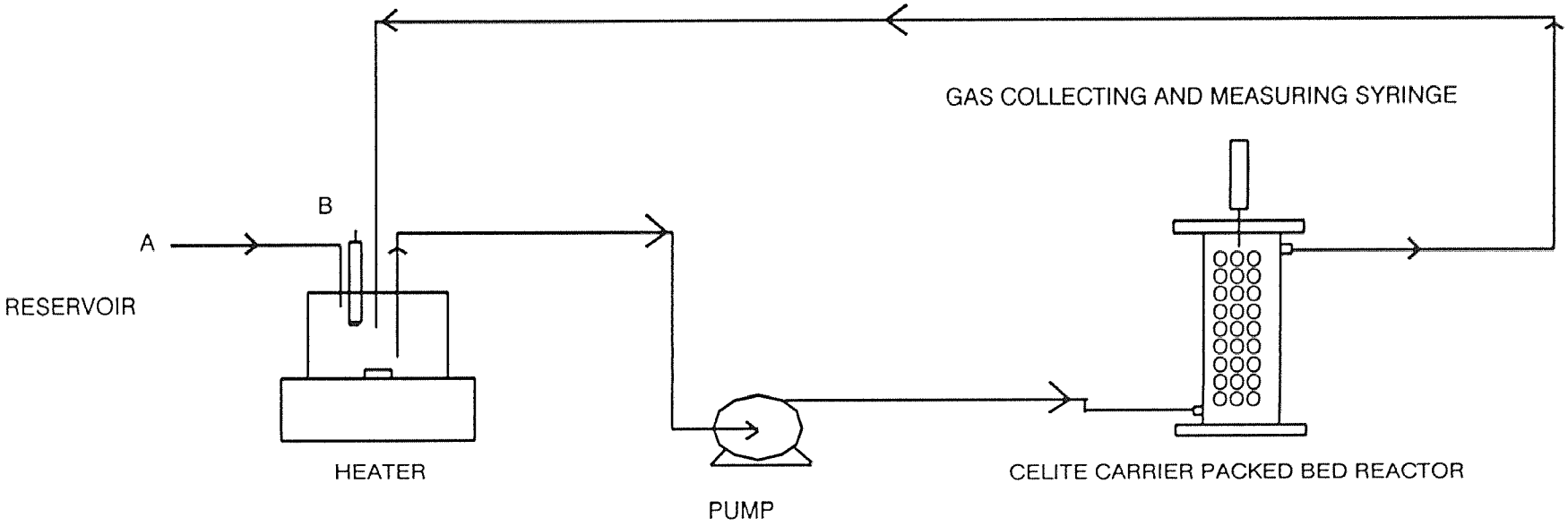
HEATER

A: SODIUM HYDROXIDE

B: GAS COLLECTING SYRINGE

C: pH PROBE

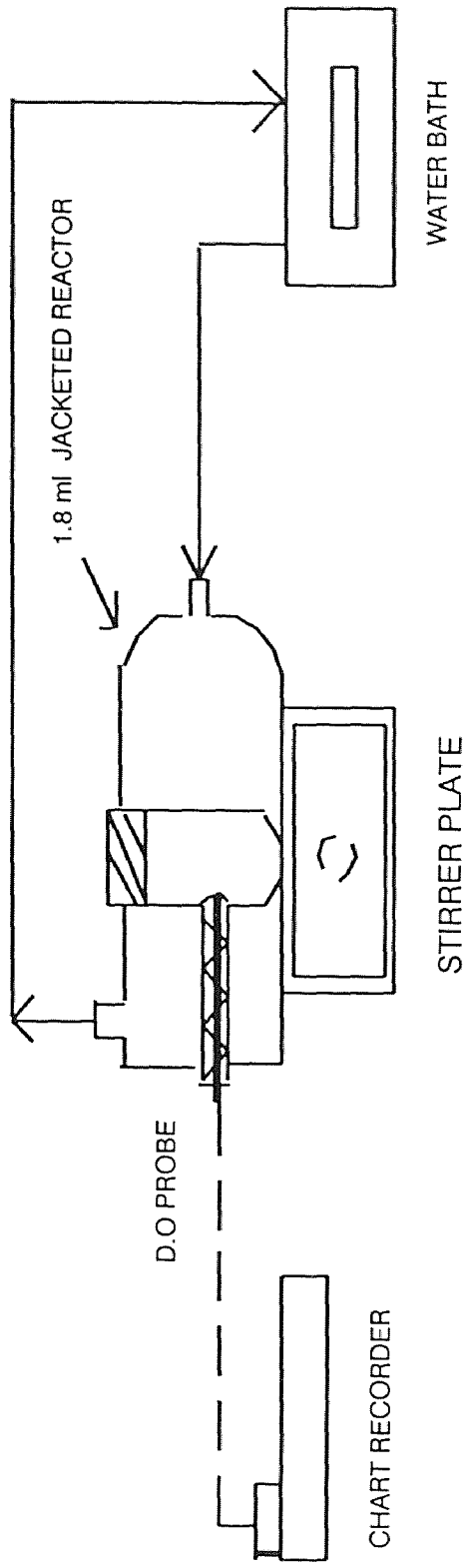
FIG. NO 4.3 EXPERIMENTAL SETUP OF THE CELITE PACKED BED REACTOR FOR ANAEROBIC BIODEGRADATION OF METHYLENE CHLORIDE



A: SODIUM HYDROXIDE

B: pH PROBE

FIG. NO 4.4 EXPERIMENTAL SETUP OF THE MICROASSAY REACTOR



CHAPTER V

RESULTS AND DISCUSSION

5.1 Aerobic Experiments

5.1.1) Shaker Flask Experiments

a) In absence of a secondary substrate

The results obtained for the biodegradation of methylene chloride in absence of a secondary substrate are presented in **Figure 5.1.** Starting with a methylene chloride concentration of around 50 ppm, the concentration decreased to 39 and 41 ppm in the control and bacterial flasks respectively in 40 hours. This clearly suggests that methylene chloride biodegradation was very insignificant in the absence of a secondary substrate. Similar results were obtained with a starting concentration of 250 ppm; the concentration of methylene chloride decreased to 184 and 175 ppm in the control and bacterial flasks respectively. The very small deviation from the concentrations in the control and bacterial flasks may be due to the adsorption of methylene chloride on activated sludge.

The inability of the microorganisms to degrade methylene chloride under the above conditions may be due to the lack of an appropriate environment to induce a metabolic pathway. Absence of a secondary substrate, was envisioned as one possible reason.

b) In presence of a secondary substrate

Kim and coworkers (1986) have reported the use of secondary substrates for biodegradation of chlorinated hydrocarbons. They report that an inoculum containing microorganisms with a diversity of metabolic capabilities was needed to increase the probability of plasmid exchange, and this diversity in metabolic capabilities could be

provided by adding a secondary substrate. Use of conventional metabolites like glucose, ammonium acetate and nutrient broth have been reported. The requirement of a co-substrate is also understandable from the fact that methylene chloride contains only 16.5 % by weight carbon which can be utilized by the microorganisms.

The results obtained using secondary substrates like glucose, ammonium acetate, cellulose acetate and nutrient broth for the biodegradation of methylene chloride using activated sludge microorganisms are shown in **Figures 5.2** through **5.5**. It is clearly seen from the figures that methylene chloride was not biodegraded. The decrease in the methylene chloride is due to physical removal only. It is also important to note that the secondary substrates were being consumed and the microbes were active.

c) In presence of Surfactant

Surfactants are known to increase the solubility of organics into the aqueous phase, thereby providing a better contact of the substrate with the microorganisms. The results obtained using a 3 % (v/v) solution of Triton X-405 in the working medium are shown in **Figure 5.6.**

It is clearly seen form the graphs that biodegradation of methylene chloride was not achieved. It shows that the proper environment was still not created for the microorganisms to degrade methylene chloride or the nutrient medium used did not provide the microorganisms with essential nutrients required for the biodegradation of methylene chloride.

d) Effect of alkaline stress

It was envisioned that the microorganisms when induced with an alkaline stress would degrade methylene chloride and release free chloride to overcome the alkalinity. Experiments were performed in shaker flasks maintaining a pH of 9.4. The results are presented in **Figure 5.7** which indicates that biodegradation of methylene chloride was

not possible under the alkaline stress. The pH dropped down to about 6.9 in both the control and the bacterial flasks. This drop in the pH may be due to the periodic addition of hydrogen peroxide to maintain aerobic conditions. Starting with a concentration of around 100 ppm of methylene chloride the concentration dropped down to 90 ppm in the control flasks and to 88 ppm in the bacterial flasks. The removal of methylene chloride was only due to abiotic losses.

e) In presence of Sodium Bicarbonate

Rittmann and coworkers (1980) have reported biodegradation of methylene chloride. The authors postulate that biodegradation of methylene chloride was significant only in the presence of sodium bicarbonate in the nutrient medium. They further reported that if sodium bicarbonate was omitted from the defined medium the culture turbidity changed, and the utilization of methylene chloride became insignificant. The results obtained using the defined medium as described by Rittmann and coworkers with our activated sludge microorganisms are shown in **Figure 5.8.** Starting with a concentration of around 100 ppm, the concentration of methylene chloride decreased to 84 ppm in the bacterial flasks, and to 87 ppm in the control flasks. Clearly, no biodegradation was achieved.

It seems that no proper environment was created for the microorganisms to biodegrade methylene chloride. It is also possible that the mixed culture does not contain the microorganisms which can induce a metabolic pathway for methylene chloride.

It is however interesting to note that the microorganisms remained very much active, throughout the above experiments. The decrease observed in the dissolved oxygen levels do indicate that oxygen was consumed by the microorganisms for respiration. This also indicates that methylene chloride is not a toxic substance to the activated sludge microorganisms.

5.2 Anaerobic Experiments

5.2.1) Biodegradation Studies

It is known that chlorinated hydrocarbons can be degraded under anaerobic conditions, because the removal of chloride ion is not very difficult under anaerobic conditions. The results obtained from the experiments carried in serum bottles to biodegrade methylene chloride under anaerobic conditions using the activated sludge microorganisms are presented in **Figures 5.9** through **5.17**. Starting concentrations of methylene chloride for the present study were 50, 100, 250, and 500 ppm. Glucose and sodium acetate provided a fermenter specific medium, and so the effect of these substances on the removal of methylene chloride was also studied.

Starting with 50 ppm of methylene chloride, more than 99 % removal was observed in the bacterial serum bottles compared to approximately 20 % removal in the control serum bottles in about 20 days, as seen in **Figure 5.9**. The effect of addition of glucose as an activator is shown in **Figure 5.10**. Glucose addition increased the amount of biogas production, but slowed down the methylene chloride biodegradation. This was obvious as glucose is much easier to degrade than methylene chloride. Seventy five percent of the initial amount of methylene chloride was removed in the bacterial serum bottles as compared to about 20 % removal in the control bottles in about 30 days. Chloride detected in the bottles without glucose was higher than that detected in the bottles with glucose. Chloride detection however confirms that mineralization of methylene chloride had actually taken place.

The results obtained using 100 ppm of methylene chloride as the starting concentration are shown in **Figure 5.11**. About 95 % removal of methylene chloride was observed in the bacterial serum bottles as compared to 25 % removal in the control serum bottles in about 35 days.

The anaerobic biodegradation of 250 ppm methylene chloride is shown in **Figure 5.12**. After a period of 43 days the control serum bottle had 210 ppm methylene

chloride, whereas the bacterial serum bottle had around 75 ppm methylene chloride. This indicates that 77 % removal was due to biodegradation. However, chloride analysis indicated the presence of only 36.5 ppm chloride in the medium which corresponds to 21 % of chloride balance. The degradation rate was **0.0024 mg methlene chloride / day mg biomass.**

The effect of addition of glucose on the biodegradation of 250 ppm methylene chloride is shown in **Figure 5.13.** After 48 days the bacterial serum bottle had about 35 ppm methylene chloride, and 107 ppm chloride was detected. Addition of glucose for the removal of 250 ppm methylene chloride however seems favorable. The degradation rate was **0.0026 mg methylene chloride / day mg biomass.**

On the other hand sodium acetate addition seems to inhibit the removal of methylene chloride at 250 ppm concentration as is seen from **Figure 5.14.**

The biodegradation of 500 ppm methylene chloride is shown in **Figure 5.15.** The effects of addition of glucose and sodium acetate are shown in **Figure 5.16** and **Figure 5.17** respectively. All the three graphs clearly indicate that methylene chloride biodegradation was insignificant at 500 ppm methylene chloride. Significant amounts of biogas were produced only in the bottle containing glucose. The amount of methylene chloride removed was almost equal in the bacterial and serum bottles for all the three cases. Also, significant quantities of methylene chloride were lost in the gas phase (detected while measuring biogas production) thereby preventing a proper contact with the microorganisms.

The results obtained for anaerobic degradation of methylene chloride in serum bottles are summarized on the next page.

With	50 ppm MCl	100 ppm MCl	250 ppm MCl	500 ppm MCl
	0.00147 78%	0.00173 75%	0.0024 77%	0.004 21%
+ Glucose	0.00173 75%	-	0.0026 81%	0.00475 7.35%
+Sod. Aceta.	-	-	0.00162 69%	0.0038 0%
The % value indicates percent biological removal Values in decimal indicate rate in mg methylene chloride/day mg biomass				

5.2.2)**Development of an immobilized cell anaerobic bioreactor.**

Very low removal rates were observed in the serum bottles. The biodegradation rates can be increased by immobilizing the microorganisms to increase the biomass concentration. Two anaerobic immobilized bioreactors namely, the Membrane Bioreactor and Celite Carrier Packed Bed Reactor, were developed and studied for this purpose.

a) Experiments in the Membrane Bioreactor

At the end of the innoculation period, the attachment was evident from the gradual increase in pressure from 1 to 1.8 psig. Glucose was fed periodically into this bioreactor. Glucose was added to test the viability of the attached microorganisms. The pH value decreased but no biogas was generated.

It was clear that the microporous membrane does help in the attachment of the anaerobic microorganisms. It is very much likely that, as the flow velocities in the reactor were very slow (82 cm/min), homogenous conditions (pH and concentration) were not maintained through out the reactor length. The methanogens are very much sensitive to pH. Because of the uneven pH conditions the methanaogens may have remained inactive. It is possible to maintain uniform conditions of pH within the reator

using higher flow rates, but then there is a risk of washout to occur. Optimum flow will have to be determined in the future.

b Experiments in the Celite Carrier Packed Bed Reactor

Biofilm build up was clearly visible at the end of the innoculation period, on the Celite Carriers. Glucose was added to test the viability of the attached microorganisms as discussed before. On an average, 15 cc of biogas was produced by adding 2 grams of glucose. The reactor was operated on glucose for a period over one week, at the end of which 250 ppm methylene chloride was injected into the reactor and the reactor was operated for a period of ten days. After ten days the biomass was washed out, and methylene chloride was again injected into the reactor, and operated to determine the losses of methylene chloride due to physical removal. The results obtained are shown in **Figure 5.18**. One hundred percent removal of methylene chloride was achieved in a period of 8 days in the reactor with the attached microorganisms. Ninety percent removal was observed in the reactor operated without the biomass over a period of 11 days.

Although abiotic losses were significant, due to a high biomass concentration the degradation rates were enhanced. The observed rate was **0.021 mg CH2Cl2 / day.mg dry biomass** which is almost 10 fold over that observed in serum bottles.

FIG. 5.1 BIODEGRADATION OF METHYLENE CHLORIDE USING H202

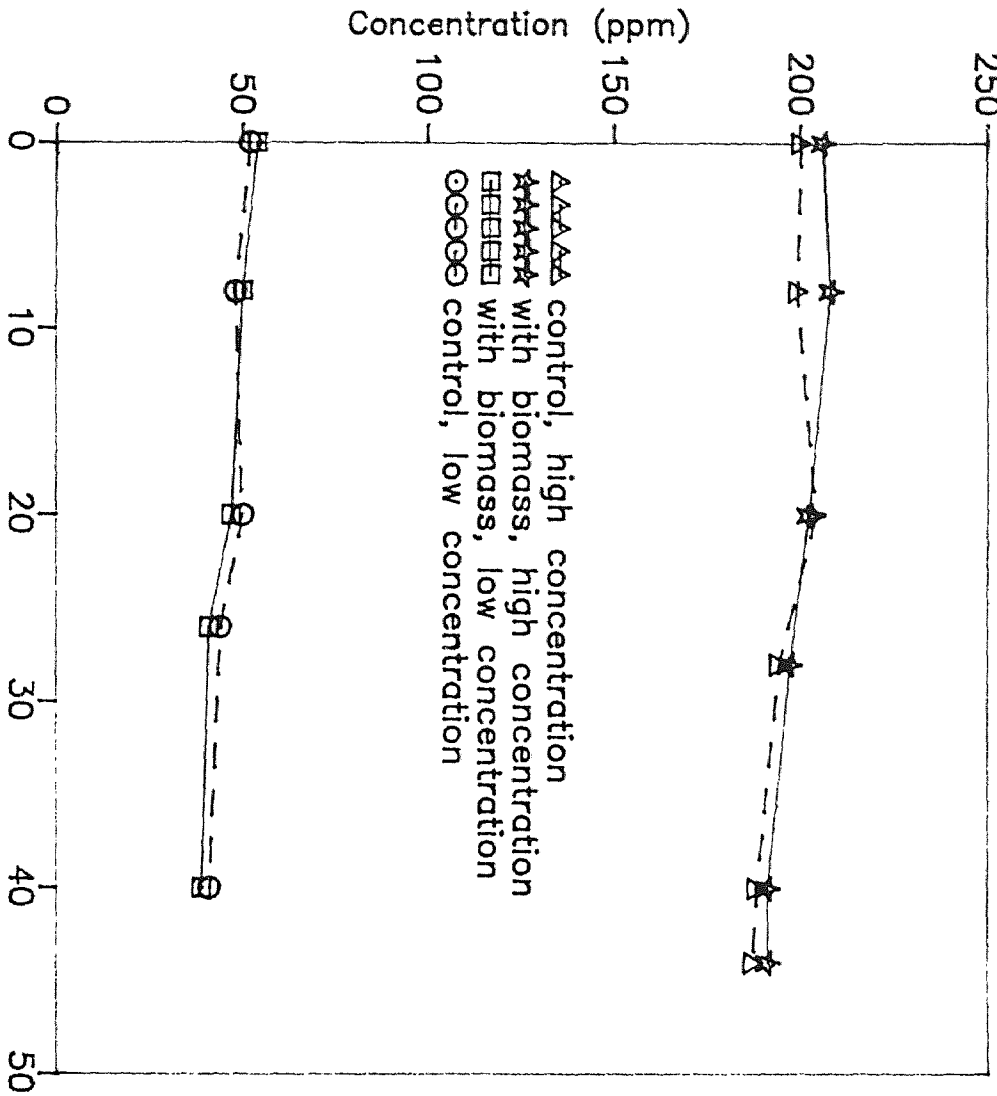
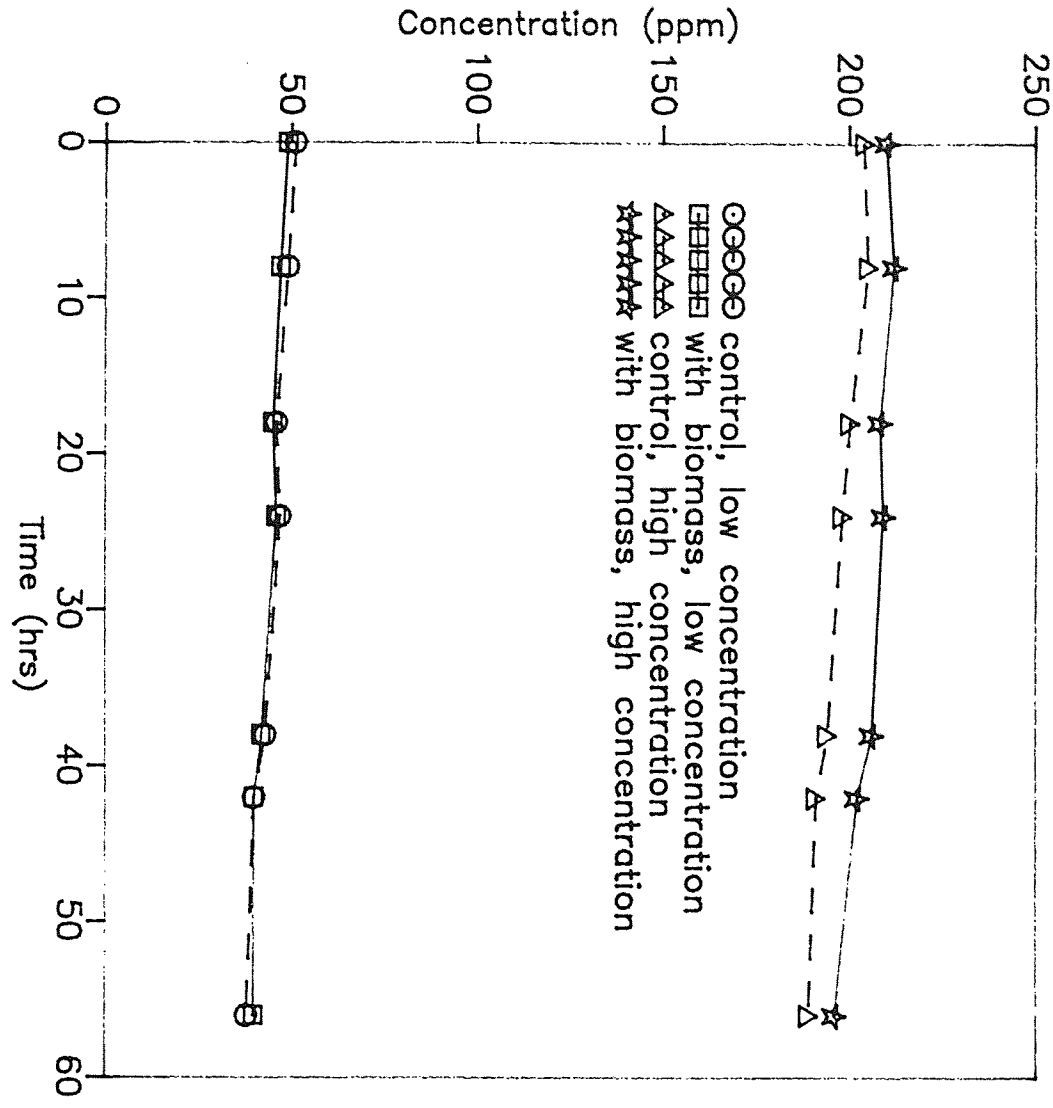


FIG. 5.2 BIODEGRADATION OF METHYLENE CHLORIDE USING H2O2, IN PRESENCE OF GLUCOSE



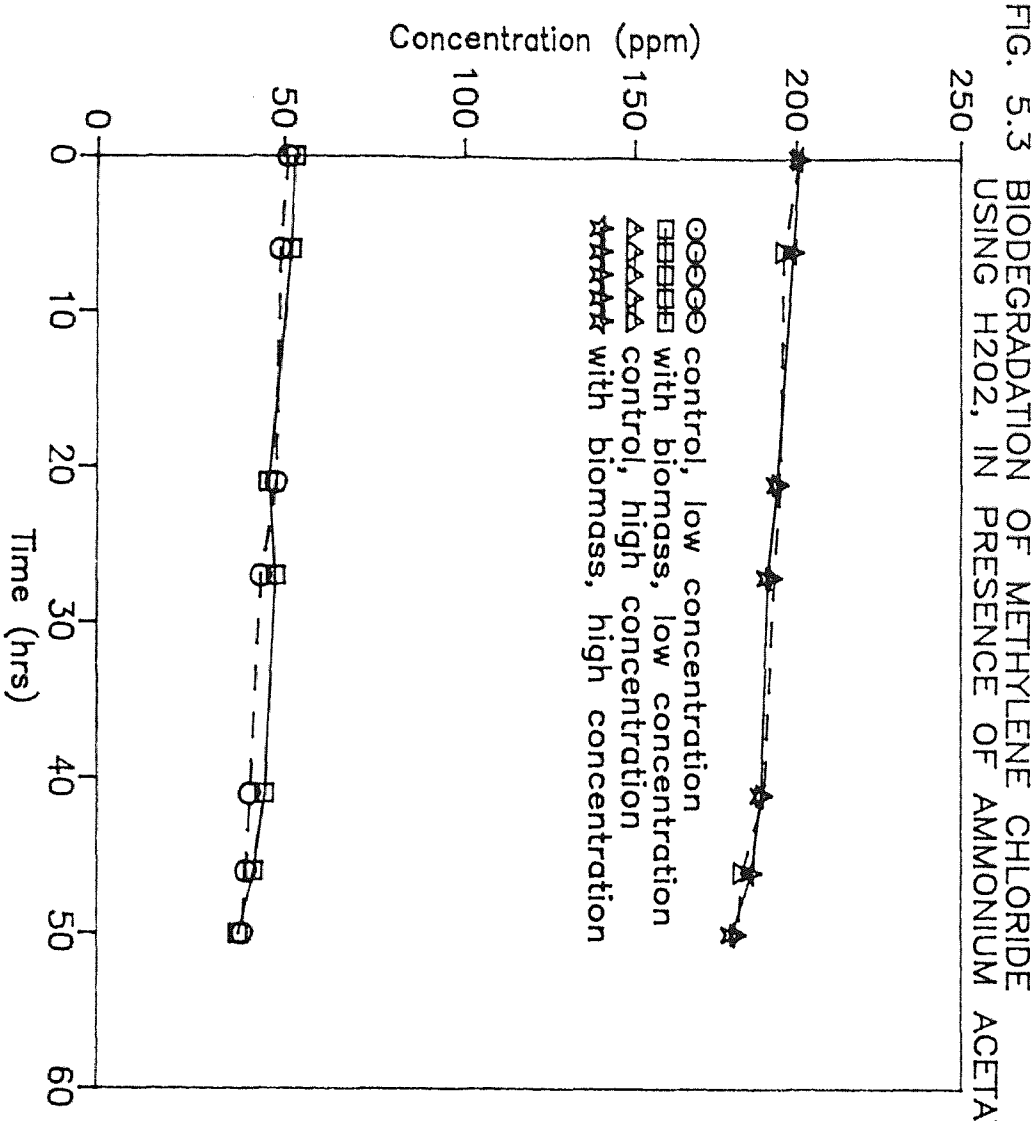


FIG. 5.3 BIODEGRADATION OF METHYLENE CHLORIDE USING H202, IN PRESENCE OF AMMONIUM ACETATE

FIG. 5.4 BIODEGRADATION OF METHYLENE CHLORIDE USING H202, IN PRESENCE OF CELLULOSE ACETATE

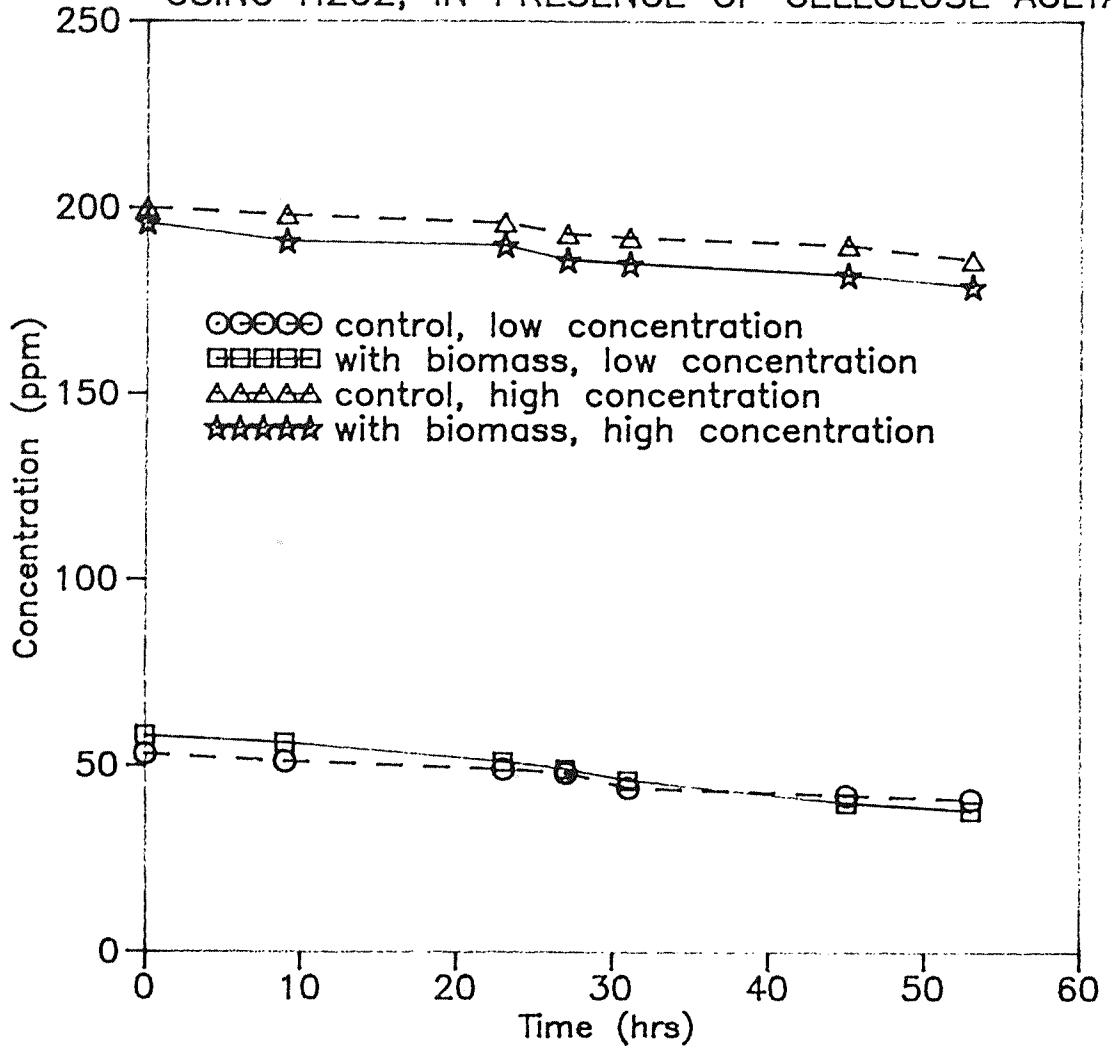
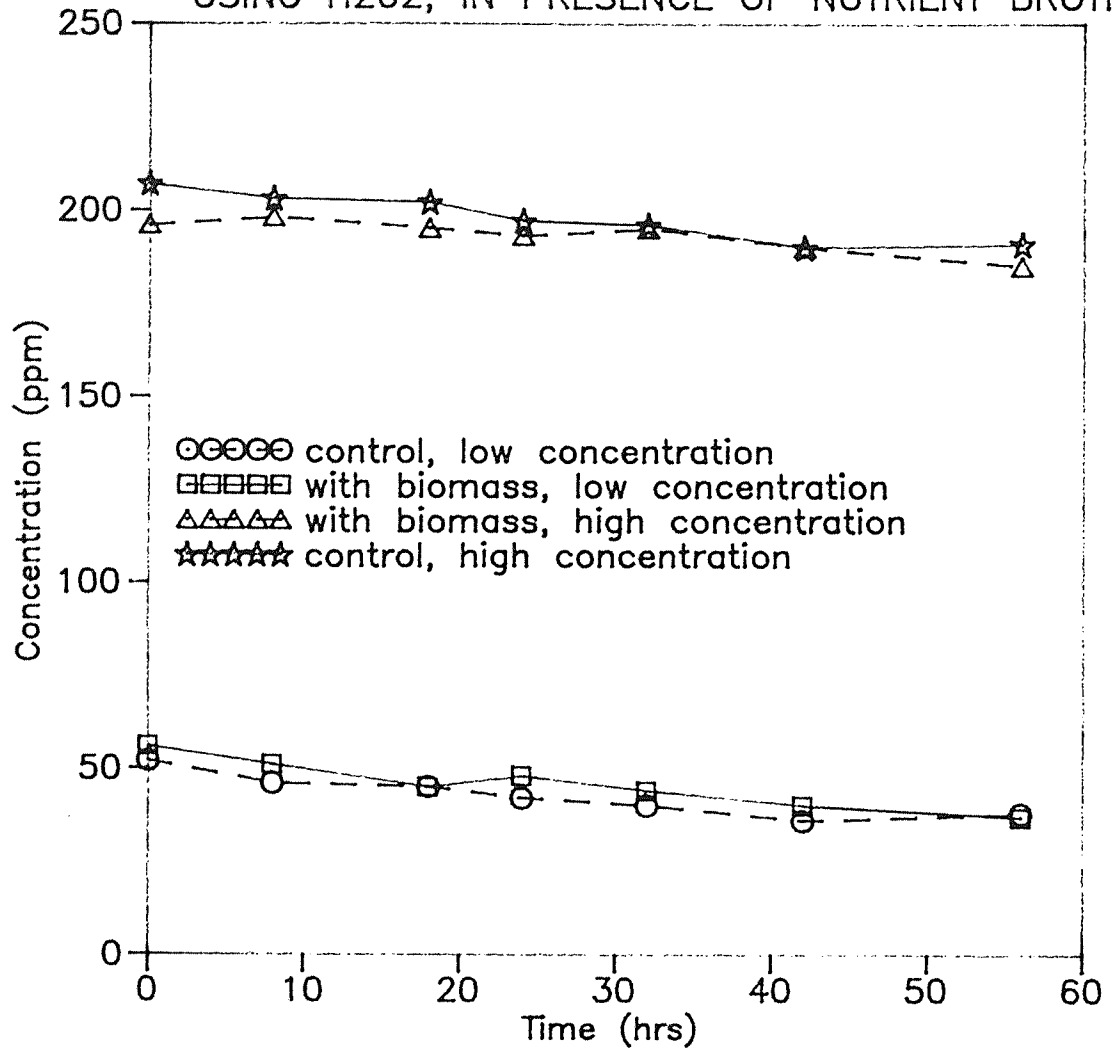


FIG. 5.5 BIODEGRADATION OF METHYLENE CHLORIDE USING H202, IN PRESENCE OF NUTRIENT BROTH



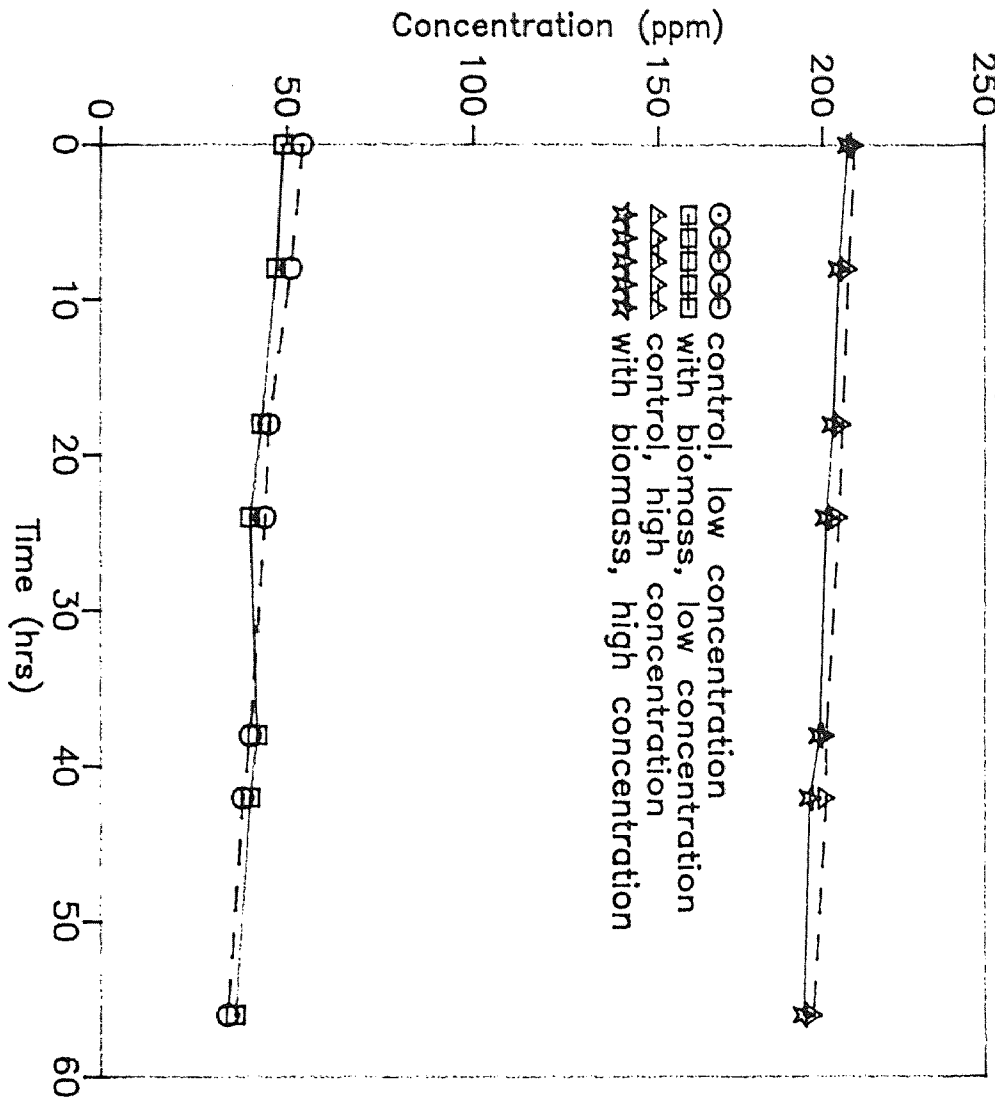


FIG. 5.6 BIODEGRADATION OF METHYLENE CHLORIDE USING H202, IN PRESENCE OF SURFACTANT

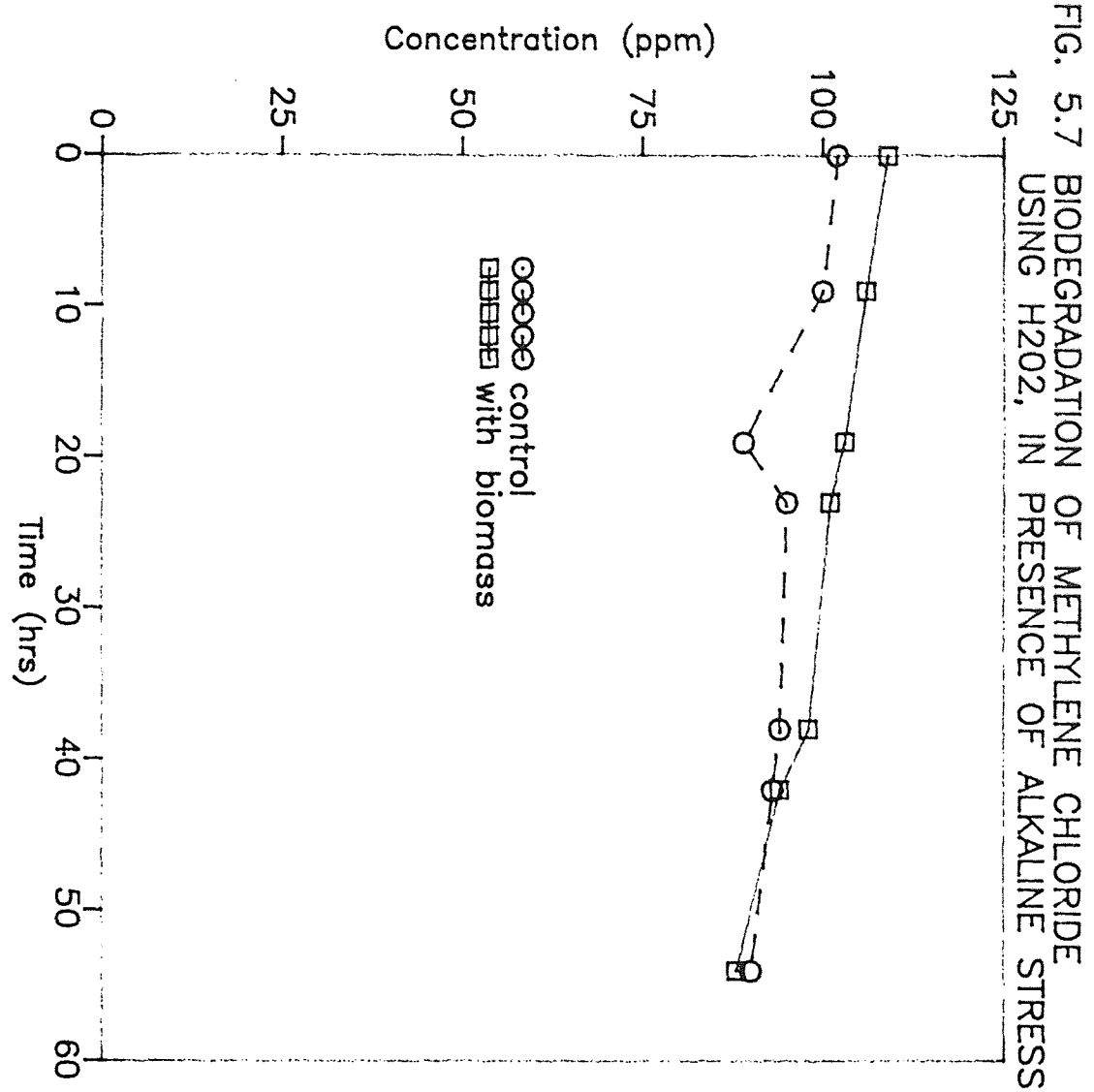


FIG. 5.7 BIODEGRADATION OF METHYLENE CHLORIDE USING H202, IN PRESENCE OF ALKALINE STRESS

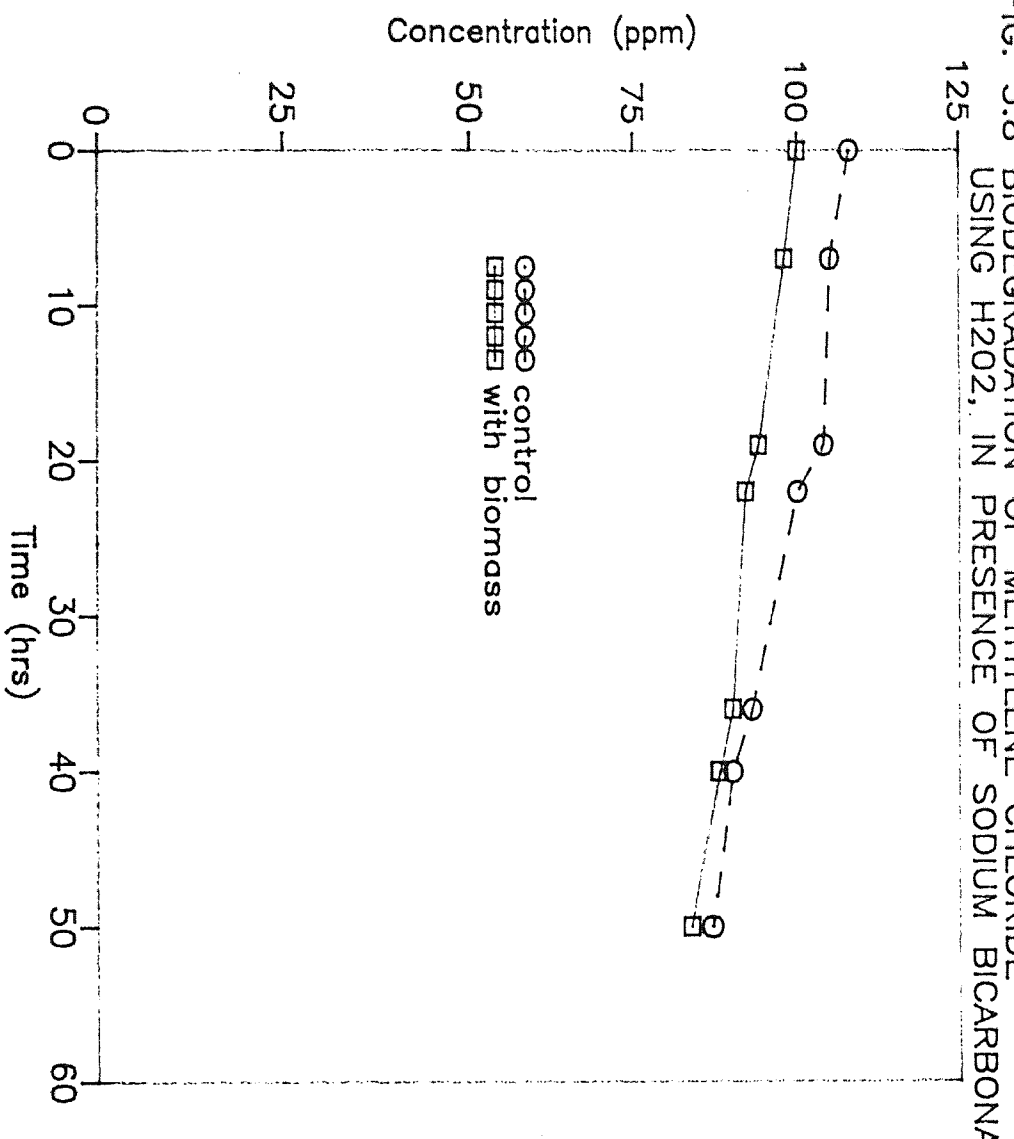


FIG. 5.8 BIODEGRADATION OF METHYLENE CHLORIDE USING H202, IN PRESENCE OF SODIUM BICARBONATE

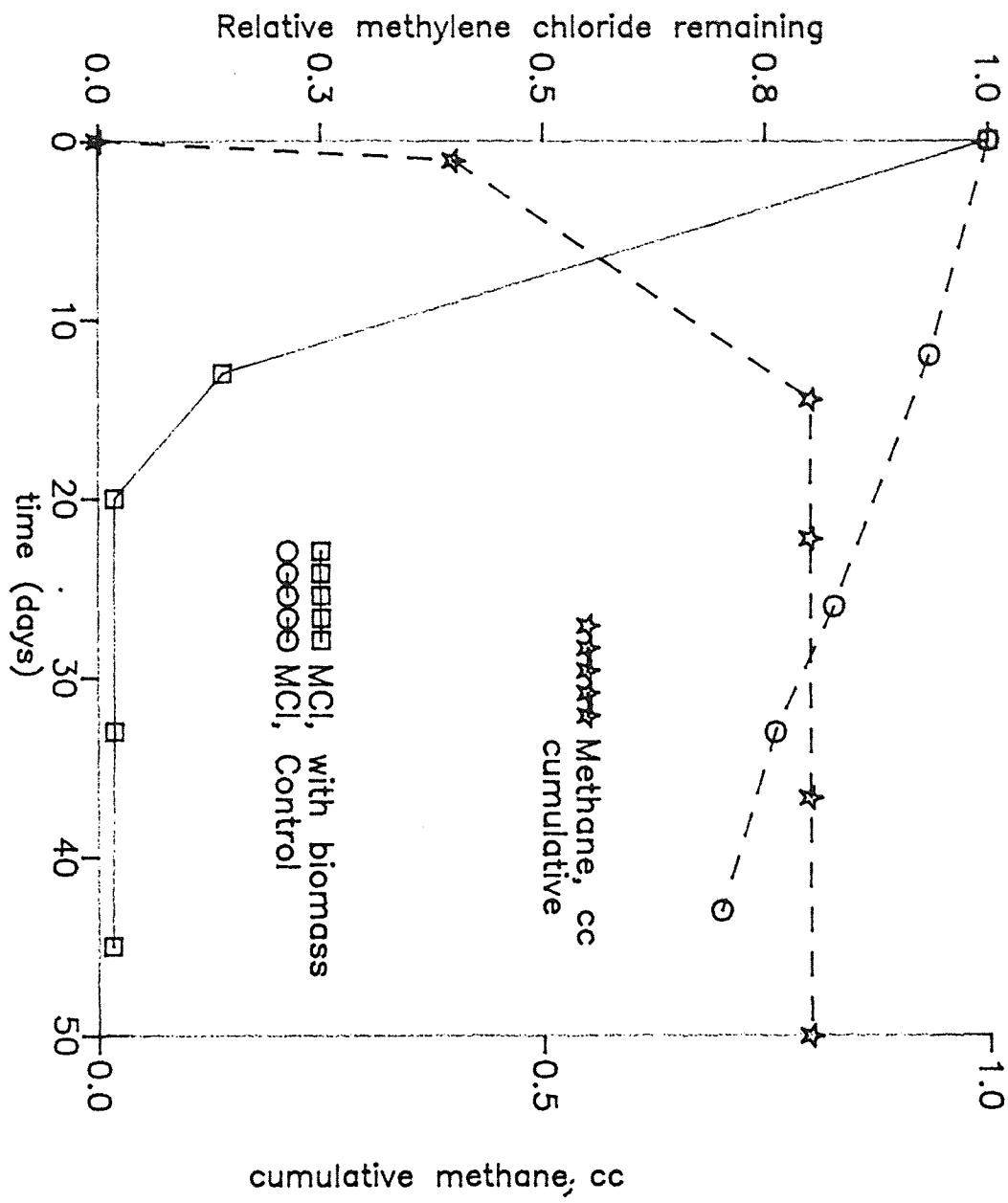


FIG. 5.9 ANAEROBIC BIODEGRADATION OF 50 PPM METHYLENE CHLORIDE

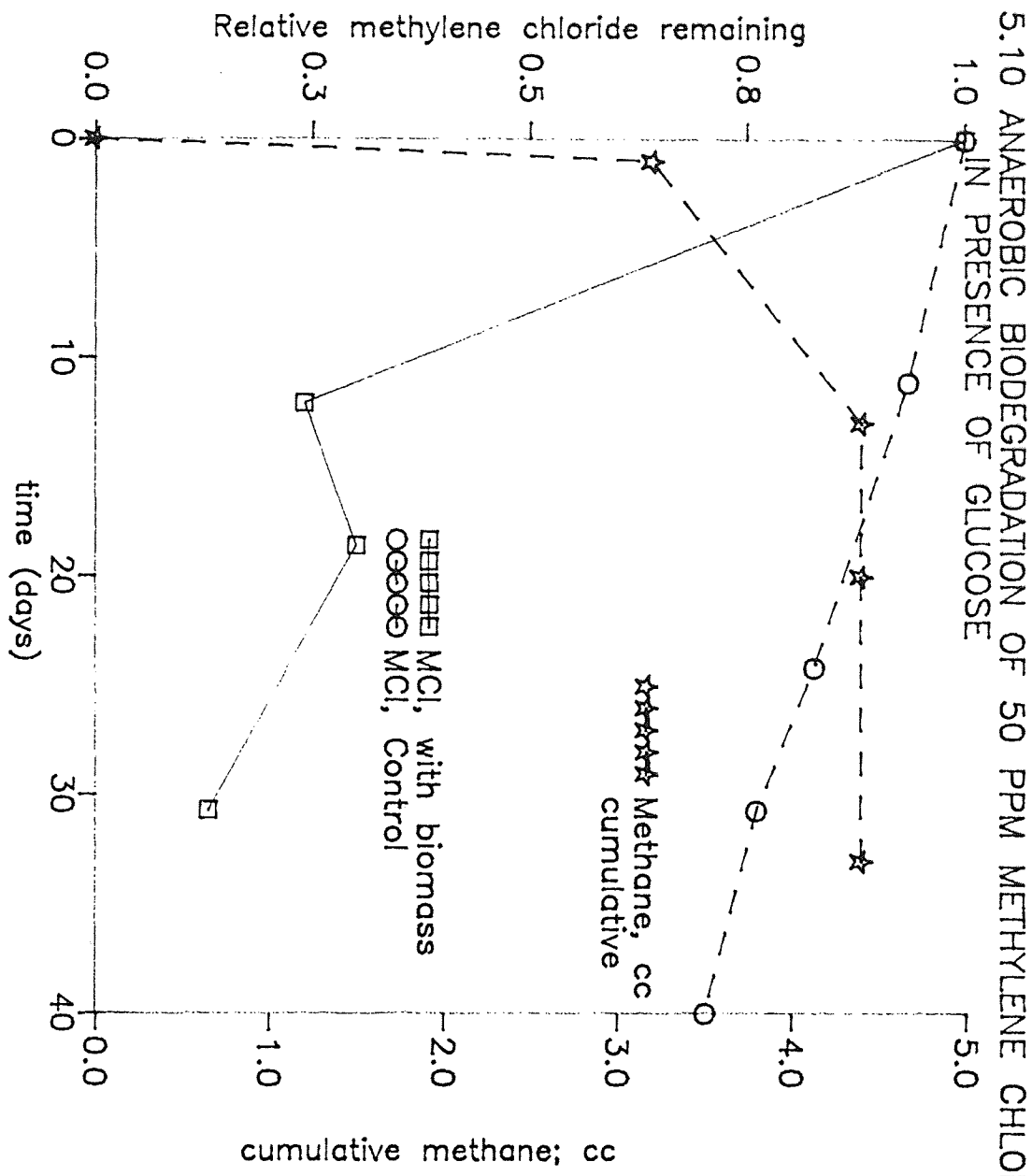


FIG. 5.10 ANAEROBIC BIODEGRADATION OF 50 PPM METHYLENE CHLORIDE IN PRESENCE OF GLUCOSE

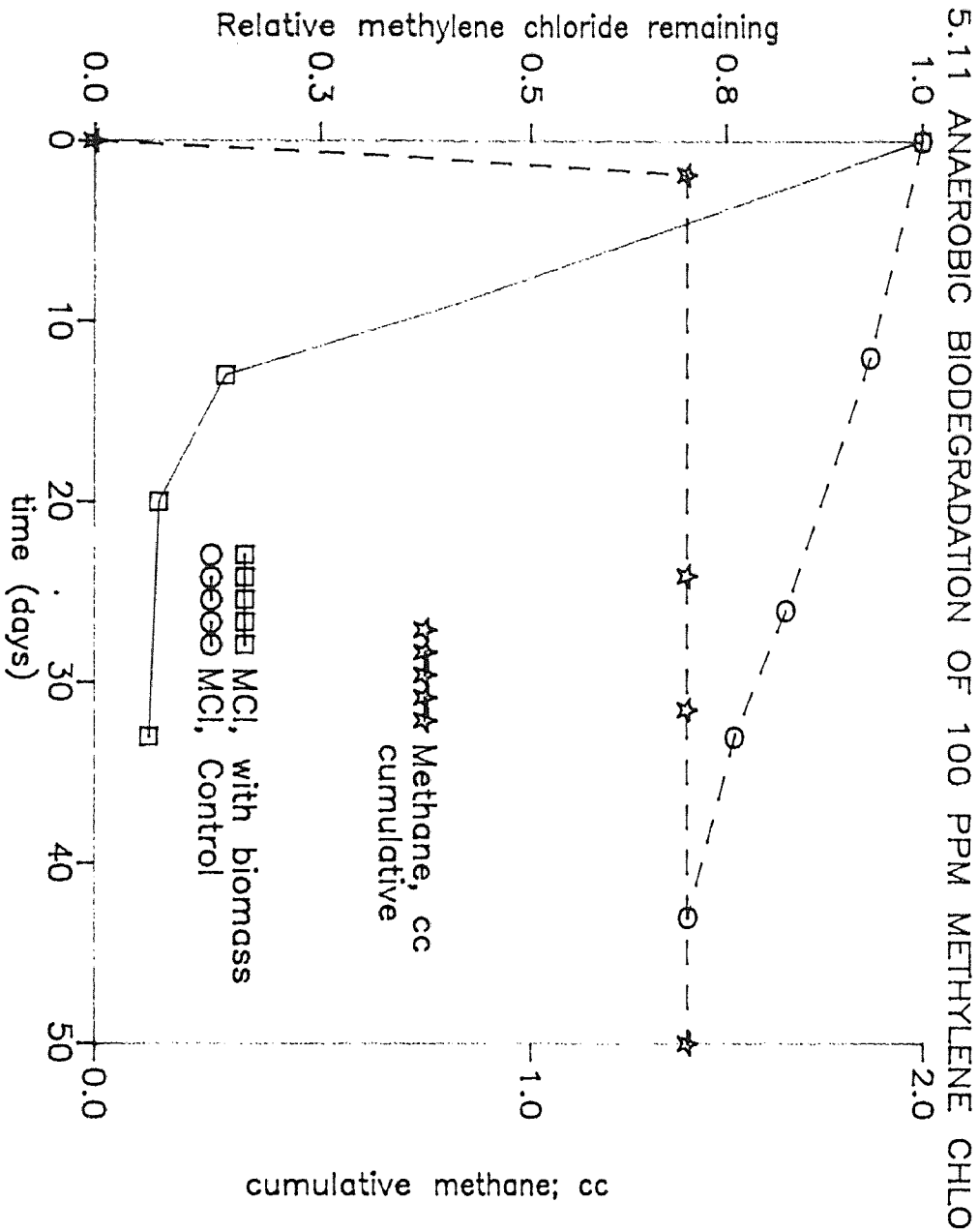


FIG. 5.11 ANAEROBIC BIODEGRADATION OF 100 PPM METHYLENE CHLORIDE

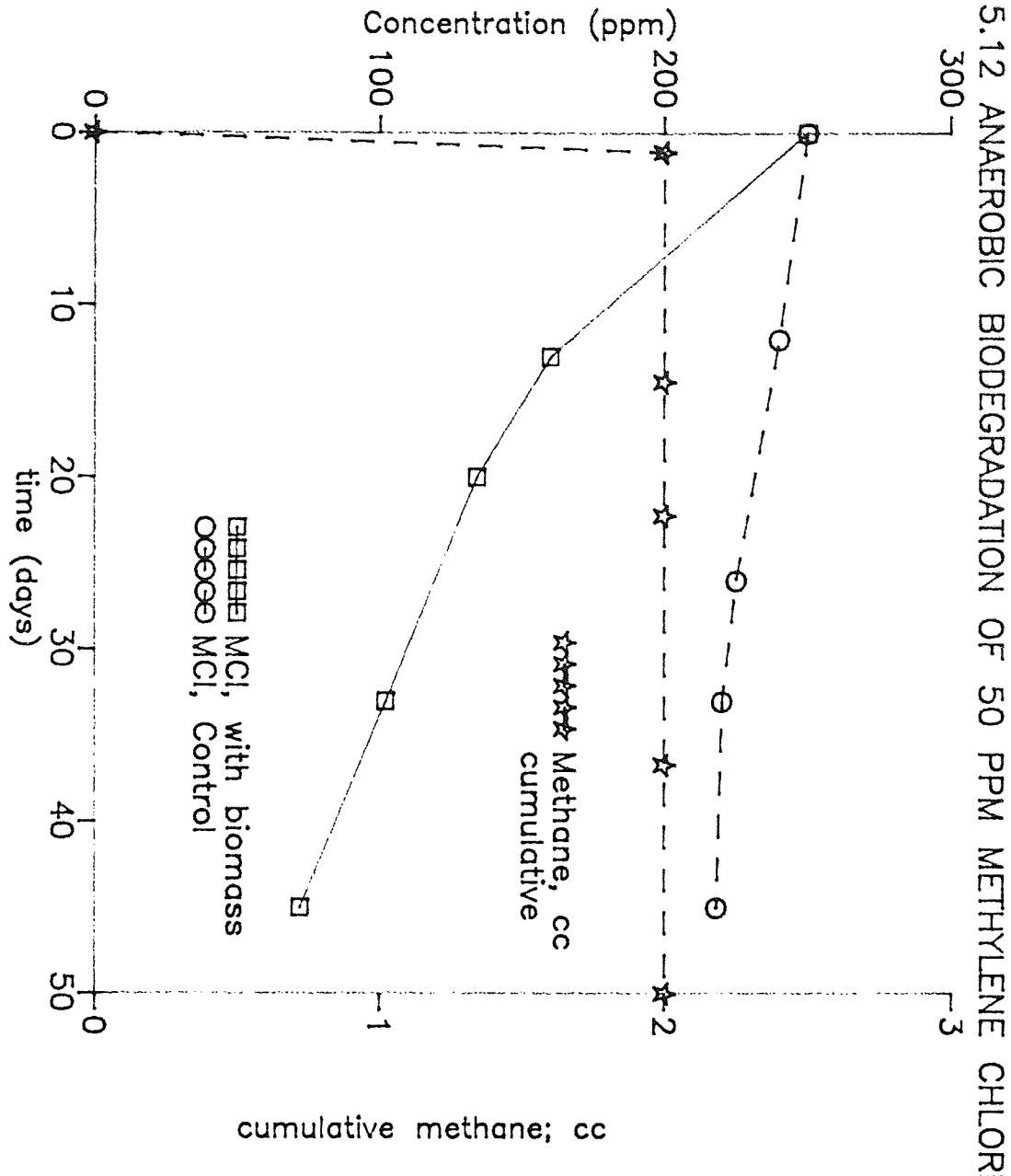


FIG. 5.12 ANAEROBIC BIODEGRADATION OF 50 PPM METHYLENE CHLORIDE

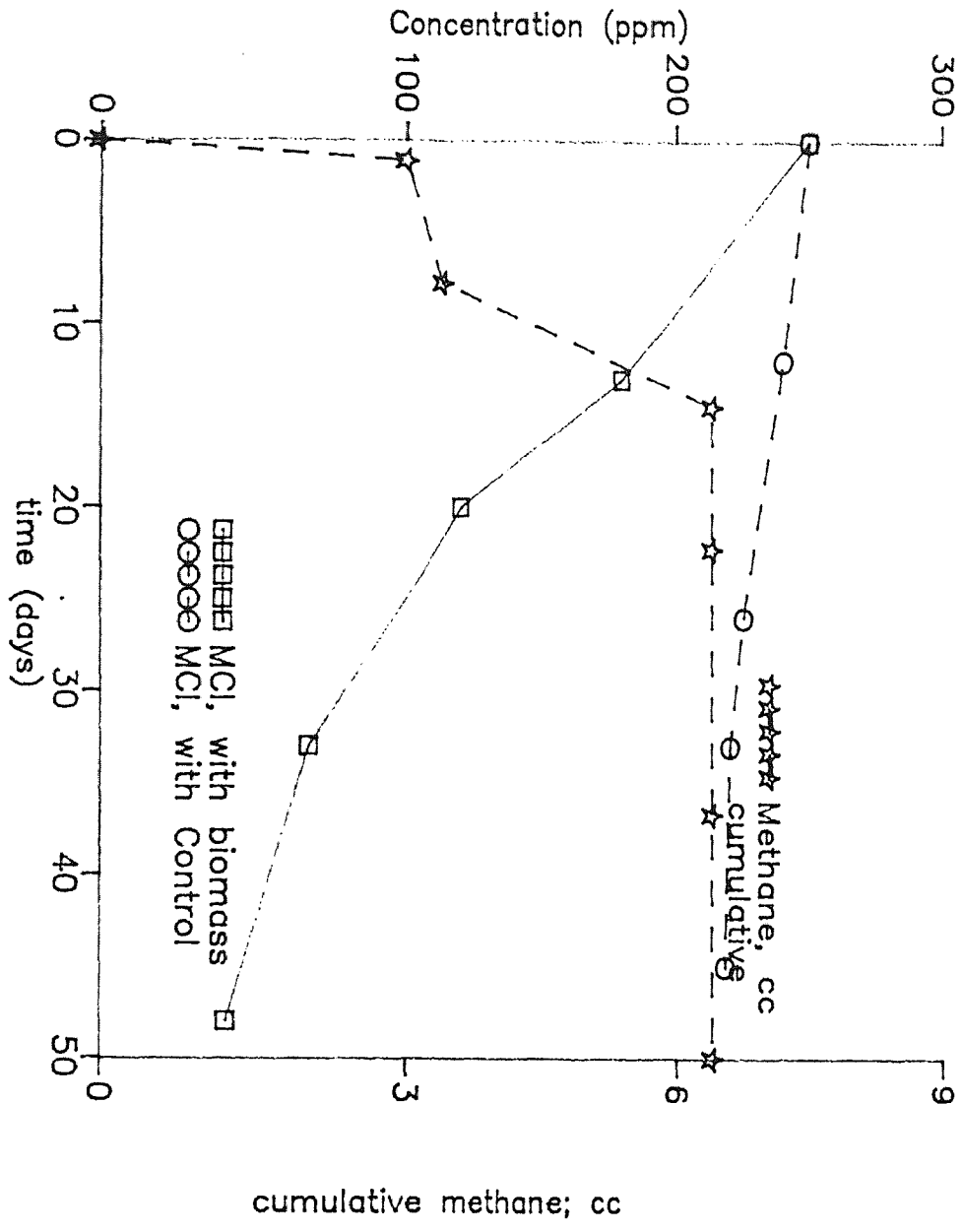


FIG. 5.13 ANAEROBIC BIODEGRADATION OF 250 PPM METHYLENE CHLORIDE IN PRESENCE OF GLUCOSE

FIG. 5.14 ANAEROBIC BIODEGRADATION OF 250 PPM METHYLENE CHLORIDE IN PRESENCE OF SODIUM ACETATE

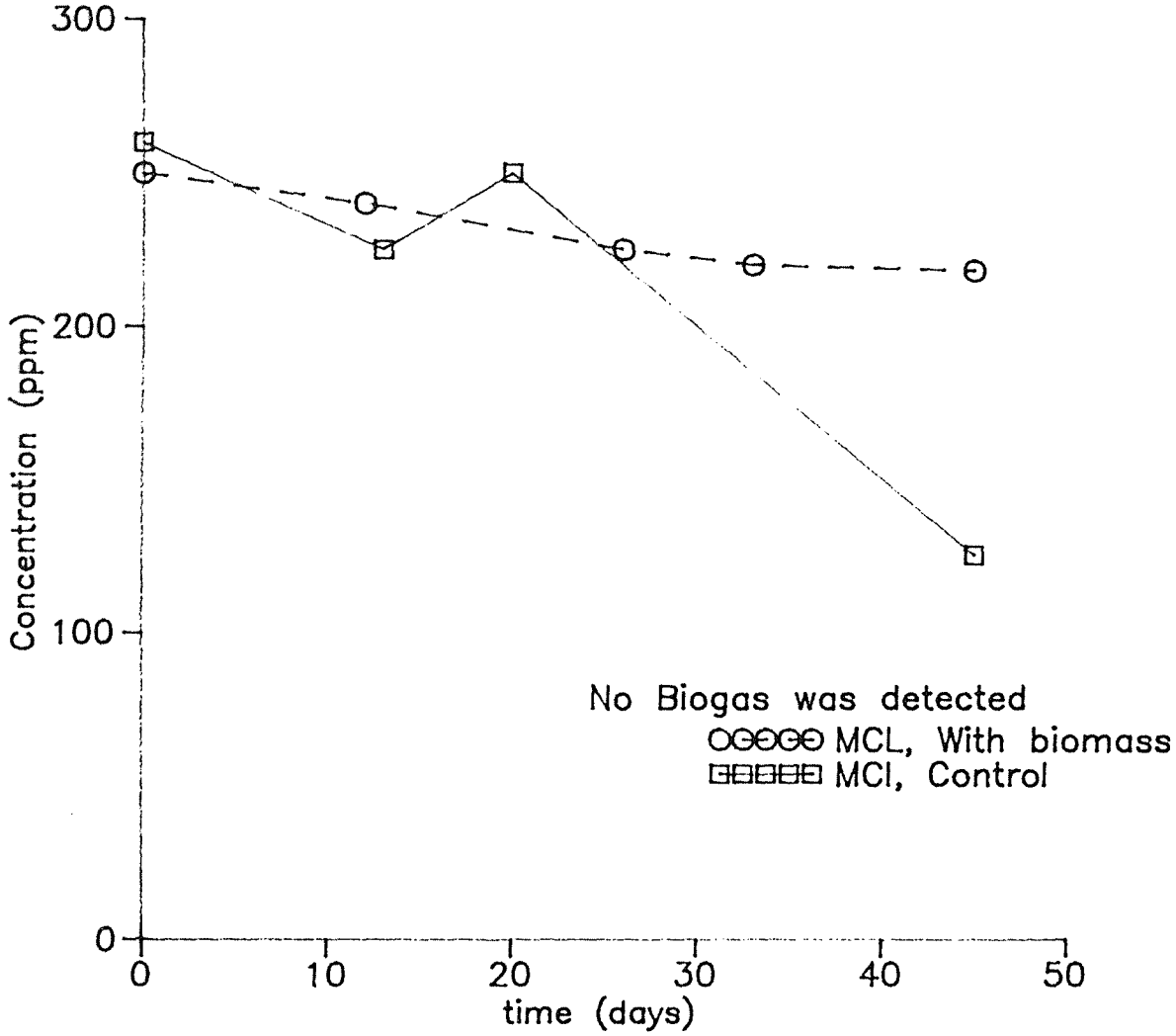
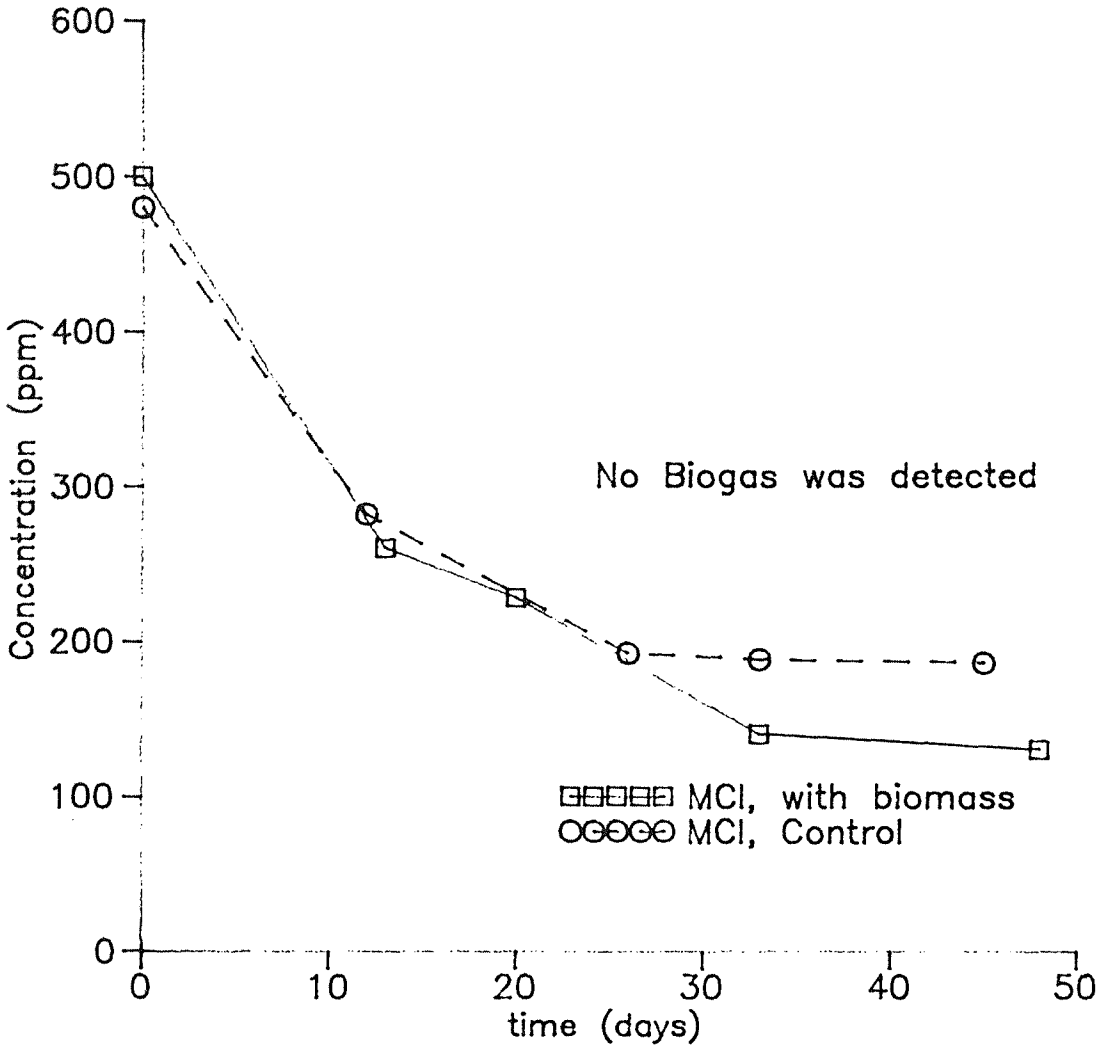


FIG. 5.15 ANAEROBIC BIODEGRADATION OF 500 PPM METHYLENE CHLORIDE



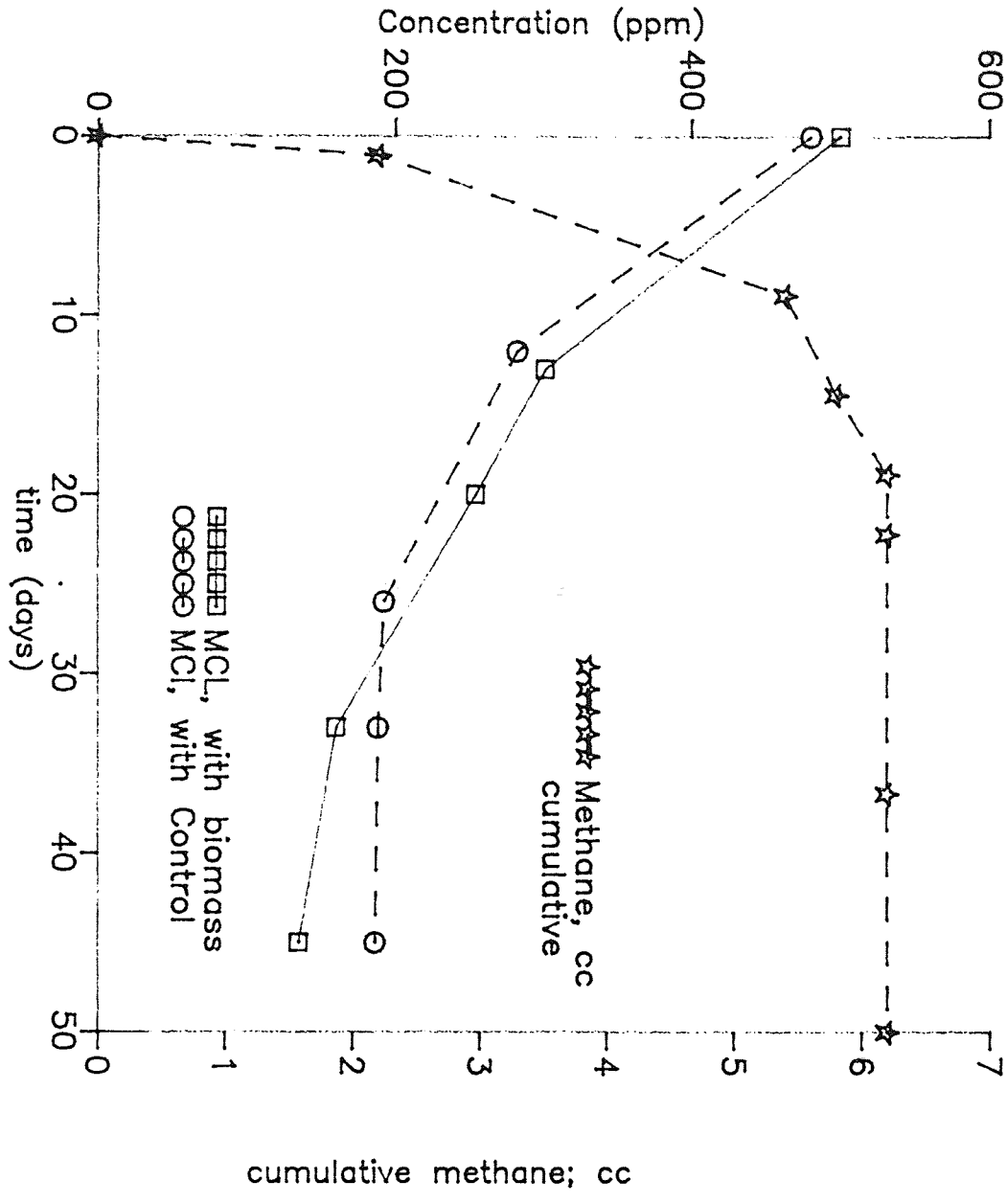


FIG. 5.16 ANAEROBIC BIODEGRADATION OF 500 PPM METHYLENE CHLORIDE IN PRESENCE OF GLUCOSE

FIG. 5.17 ANAEROBIC BIODEGRADATION OF 500 PPM METHYLENE CHLORIDE IN PRESENCE OF SODIUM ACETATE

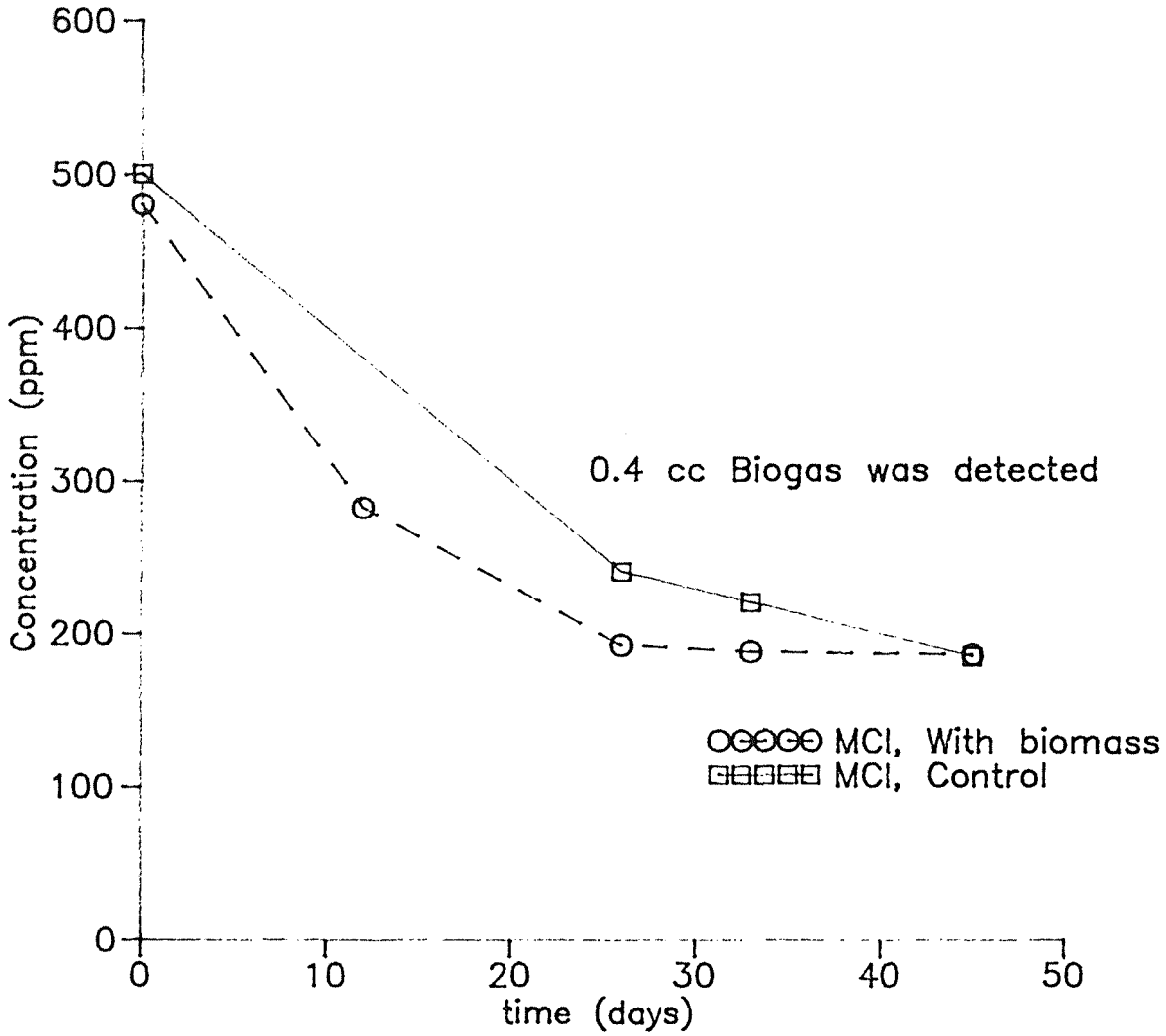
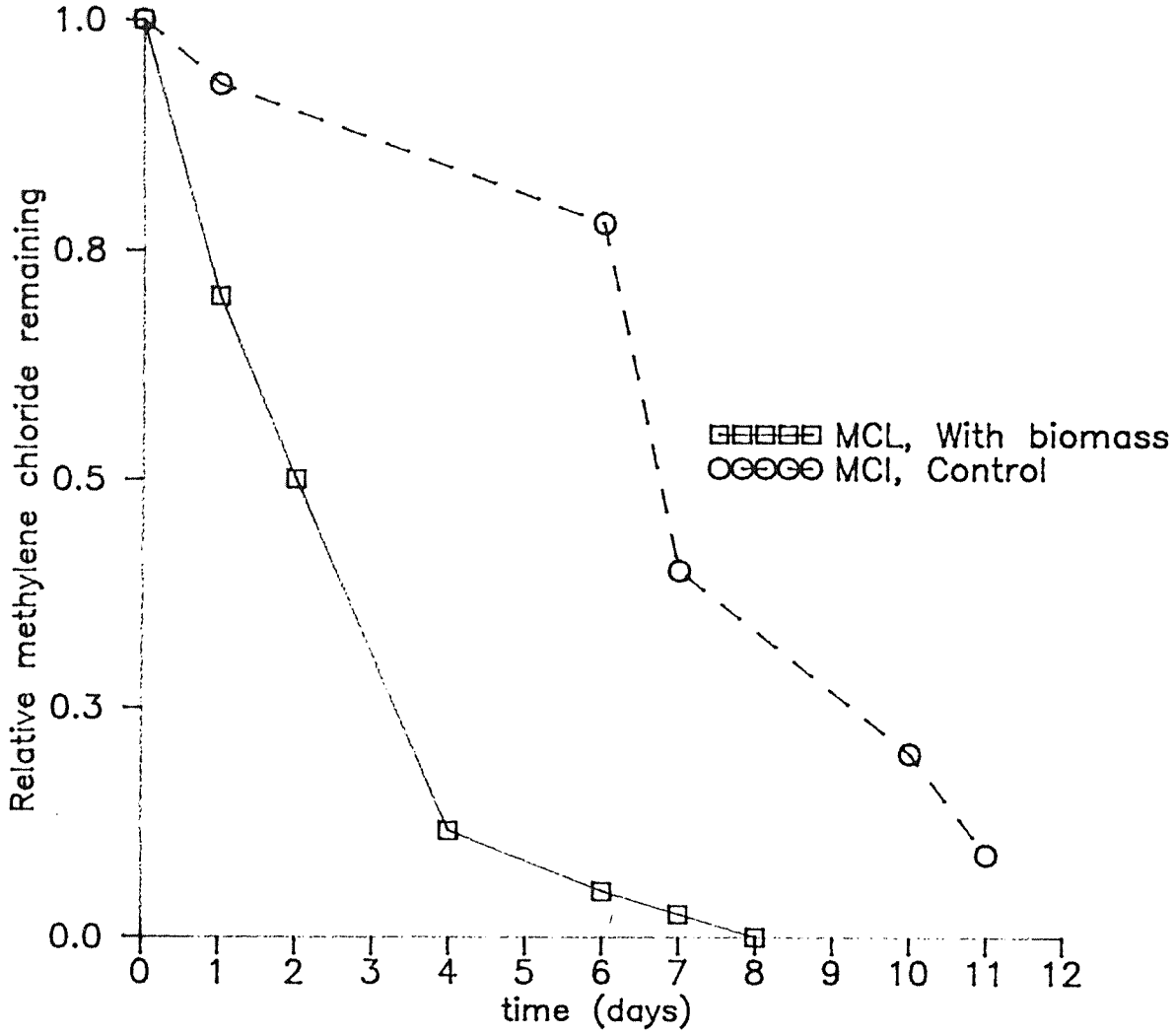


FIG. 5.18 BIODEGRADATION OF 250 PPM METHYLENE CHLORIDE USING ATTACHED MICROORGANISMS IN CELITE PACKED BED REACTOR



CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Conclusions derived from the experiments conducted with the activated sludge microorganisms to degrade methylene chloride under aerobic and anaerobic conditions are listed below:

1) The aerobic biodegradation of methylene chloride using activated sludge microorganisms in absence of a secondary substrate is insignificant.

2) Addition of secondary substrates like glucose, nutrient broth, ammonium acetate and cellulose acetate did not help in the aerobic biodegradation of methylene chloride.

3) Methylene chloride can be removed to a greater extent under anaerobic conditions. The biological removal of methylene chloride, is evident from the enhanced removal in the bacterial serum bottles compared to the control bottles and is supported by chloride formation.

4) Addition of glucose enhances the methylene chloride removal, whereas sodium acetate is found to inhibit methylene chloride degradation under anaerobic conditions.

5) The Membrane Bioreactor is not suitable for the anaerobic microorganisms. The methanogens require a very close control of pH, and the improper pH conditions through out the length of the reactor may have inhibited their growth and performance.

6) The attached microorganisms on the Celite Carrier in the Packed Bed Reactor remained viable. A ten fold increase in the removal rate of methylene chloride was observed in the Celite Carrier Packed Bed Reactor when compared to the rates obtained in the serum bottles.

6.2 Recommendations

The following recommendations are made for the aerobic biodegradation of methylene chloride.

1) The composition of the nutrient medium should be altered: The nutrient medium used by Klecka (1982) containing glucose and nutrient broth should be attempted. Stucki and coworkers (1981) have used a specific nutrient medium containing potassium sulphite as a trapping agent, to biodegrade methylene chloride. The medium used by Stucki and coworkers may also be attempted.

2) Biodegradation in presence of a secondary substrate and surfactant together, should be attempted.

3) Acclimation over a longer period of time may be necessary.

Suggestions made for the anaerobic biodegradation of methylene chloride include,

1) Surfactant should be added to the serum bottles. Presence of a surfactant may decrease the abiotic losses of methylene chloride as the surfactant has the capability to retain the organic compound of interest, in the liquid phase,

2) Anaerobic experiments should be carried out at a lower temperature to minimize the abiotic losses. This will require anaerobic digestion to be conducted at a lower temperature, so that the bacteria get acclimated to that low temperature,

3) For proper maintenance of pH in the membrane reactor, a higher flow rate should be tried. However, an optimum flow rate will have to be determined to prevent the washout of microorganisms. Alternatively, a higher spacing microporous module with a higher cross sectional area may also be used, and

4) The calcium alginate Biobead Reactor has been shown to be very efficient for aerobic biodegradation of organic compounds. A similar kind of a Biobead reactor

should be tried for the anaerobic microorganisms to degrade methylene chloride. This reactor will provide an higher biomass concentration, than that obtained in the celite reactor.

REFERENCES

Bouwer E. J., Rittmann B. E, McCarty P. L. "Anaerobic Degradation of Halogenated 1- and 2-Carbon Organic Compounds." Environmental Science & Technology. **15**: 596-599, 1981.

Brunner W., Staub D., and Leisinger T. "Bacterial Degradation of Dichloromethane." Applied and Environmental Microbiology. **40** : 950-958, 1980.

Fewson C. A."Biodegradation of Xenobiotic and Other Persistent Compounds: The Causes of Recalcitrance." TIBTECH. **6** : 148-153 1988.

Galli R., and Leisinger Th."Specialized Bacterial Strains for the Removal of Dichloromethane from Industrial Waste." Conservation & Recycling. **8** : 91-100, 1985

Gourdon R., Mahamat A.Y., Leger K., and Vermande P. " Methane Recovery by Anaerobic Digestion of Cellulosic Materials Available in Sahel." Biological Wastes. **30** : 181-197, 1989.

Harper S. R., and Pohland F. G."Enhancement of Anaerobic Treatment Efficiency Through Process Modification." Journal WPCF. **59** : 152-161, 1987.

Jeris J. S., and Kugelman I. J."Secrets to the Success of Anaerobic Digestion." WATER/Engineering & Management. 32-35, 1985.

Jongen W. M. F., Alink G. M., and Koeman J. H."Mutagenic Effect of Dichloromethane on Salmonella Typhimurium."Mutation Research. **56** : 245-248, 1978.

Kennedy K. J., Hamoda M. F., and Guiot S. G.

"Anaerobic Treatment of Leachate Using Fixed Film and Sludge Bed Systems." Journal WPCF. **60** : 1675-1683, 1988.

Kim C. J., and Maier W. J."Acclimation and Biodegradation of Chlorinated Organic Compounds in the Presence of Alternate Substrates." Journal WPCF. **58** : 157-164, 1986.

Klecka G. M."Fate and Effects of Methylene Chloride in Activated Sludge."Applied and Environmental Microbiology. **44** : 701-707 1982, 1982.

Lakhwala F."A Combined Gel Entrapped and Membrane Attached Microbial Process for Biodegradation of Organic Compounds". Doctoral Dissertation, New Jersey Institute of Technology Newark NJ, 1991.

Lakhwala F., Sinkar V., Sofer S., and Goldberg B."A Polymeric Membrane Reactor for Biodegradation of Phenol in Wastewater." Journal of Bioactive and Compatible Polymers. **5** : 439-452, 1990.

Lin C. Y., Sato K., Noike T., and Matsumoto J."Methanogenic Digestion Using Mixed Substrate of Acetic, Propionic and Butyric Acids." Wat. Res. **3** : 385-394, 1986.

Liao Qiong." Experiments in Aerobic and Anaerobic Biodegradation of Trichloroethylene". Masters Thesis, New Jersey Institute of Technology Newark NJ, 1991.

Parkin G. F., and Bhattacharaya S. K."Fate and Effect of Methylene Chloride and Formaldehyde in Methane Fermentation Systems." Journal WPCF. **60** : 531-538, 1988.

Parkin G. F., and Owen W. F."Fundamentals of Anaerobic Digestion of Wastewater Sludge."Journal of Environmental Engineering. **112** (1986).

Polasko L. T. L., McCarty P. L., and Zehnder A. J. B."Secondary Substrate Utilization of Methylene Chloride by an Isolated Strain of Pseudomonas sp." Applied and Environment Microbiology. **47** : 825-832, 1984.

Rittmann B. E., and McCarty P. L."Utilization of Dichloromethane by Suspended and Fixed-Film Bacteria." Applied and Environment Microbiology. **39** : 1225-1226, 1980.

Shieh, W.K., Sutton P.M., and Kos P. " Predicting Reactor Biomass Concentration in a Fluidized Bed System ". J. WPCF., **53**, 1573- 1584, 1981.

Silva, S. J. "EPA Moving to Control Industrial Toxic Pollutants with New NPDES Permits". Civil Engg. **51** : 76-78, 1981.

Stucki G., Galli R., Ebersold H. R., and Leisinger T ."Dehalogenation of Dichlotomethane by Cell Extracts of Hyphomicrobium DM2." Archives of Microbiology. **130** : 366-371, 1981.

Verschueren, K. Handbook of Environmental Data of Organic Chemicals. Van Nostrand Reinhold Co., New York, 1977.