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Complete Biological Dechlorination of Chlorinated Ethylenes to Non-toxic Ethylene under Methanogenic Conditions

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Civil Engineering

by

Chia-Ji Teng

1994

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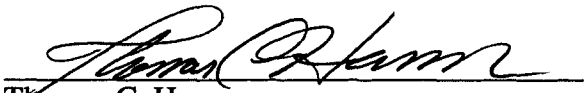

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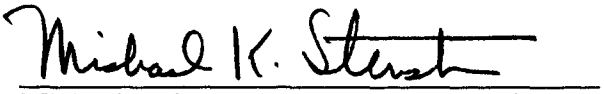

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ABSTRACT OF THE DISSERTATION

Complete Biological Dechlorination
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Professor Michael K. Stenstrom, Chair

Perchloroethylene-dechlorinating ability was examined first in seven different sludges, pond sediment, methanol-enrichment culture, and a mixture of Hyperion sludge and sediment. Hyperion sludge was then chosen to conduct the treatability, dechlorination progression, toxicological effect and semi-continuous operation tests for perchloroethylene (PCE), trichloroethylene (TCE), 3 isomers of dichloroethylene (DCE) and vinyl chloride (VC). The effect of methanol, mixing and activated carbon addition on PCE dechlorination was also evaluated with Hyperion sludge. Reductive dechlorination of PCE through TCE to cis-DCE was observed in all anaerobic test cultures. However, complete dechlorination of PCE to ethylene (ETH) was only observed in 6 sludges and the concentrated methanol-enrichment cultures. This indicates that PCE dechlorination may be a general characteristic of sludges and different sludges

may vary in their potential to completely dechlorinate PCE to ethylene. Complete dechlorination of all six chlorinated ethylenes to ethylene was also demonstrated in Hyperion sludge. Among them, PCE, TCE and cis-DCE were more likely to produce ethylene. From dechlorination progression test, it was further confirmed that all six compounds may be dechlorinated to ethylene through an identical co-metabolic route (i.e. PCE -> TCE -> DCE -> VC -> ETH). The less chlorinated ethylenes seemed only to utilize part of the route, while the fully chlorinated ethylene (PCE) utilized the whole route during the reductive dechlorination. For reductive dechlorination of each chlorinated compound, the total ethylene production increased with their initial concentration up to an upper limit. However, 100% complete dechlorination to ethylene was never observed for all test chlorinated compounds. On average, only about 30 to 40 percent of the cumulatively added chlorinated ethylenes were recovered as ethylene in long term semi-continuous operation. Excess added methanol or mixing conditions were further demonstrated to be more favorable for methanogenesis and thereby inversely affect the rate and extent of PCE dechlorination in acclimated sludge. Furthermore, complete dechlorination of PCE was also achieved in the biological activated carbon process (BAC) and the offline biological regeneration process (OBR).

1. INTRODUCTION

Perchloroethylene (PCE) and trichloroethylene (TCE) are two of the most frequently identified contaminants in groundwaters. Since both are suspected human carcinogens, thousands of small drinking water systems which rely on groundwater sources may be jeopardized. Consequently, a feasible means of controlling or treating chlorinated ethylenes in groundwaters is essential.

Perchloroethylene and TCE are halogenated aliphatic hydrocarbons, a particular class of chemicals representing one of the most important categories of industrial chemicals with respect to production volumes, dispersion in the environment, toxicological effects and population exposure. Both of them are widely used in industrial degreasing solvents, dry-cleaning fluids, and fumigants. The annual world production volumes of PCE and TCE (1.1 and 0.6 million tons, respectively) are among the 10 leading compounds of the halogenated aliphatic hydrocarbons (Leisinger, 1983). However, intensive worldwide application and spills from industry have resulted in extensive pollution of the environment. Contamination by PCE and/or TCE may occur in groundwater, surface water, soil, and air. The majority of such contamination is widespread in groundwaters because a significant fraction of these chemicals was discarded in waste dumps and then infiltrated into the groundwater (Westrick, Mello and Thomas, 1984; Travis and Doty, 1990). Concern about groundwater contamination with PCE and/or TCE is growing because it is potentially dangerous to human health even in very low concentration.

Groundwater represents more than 95 percent of all available freshwater in the United States. Approximately 80 percent of all public water suppliers in this country

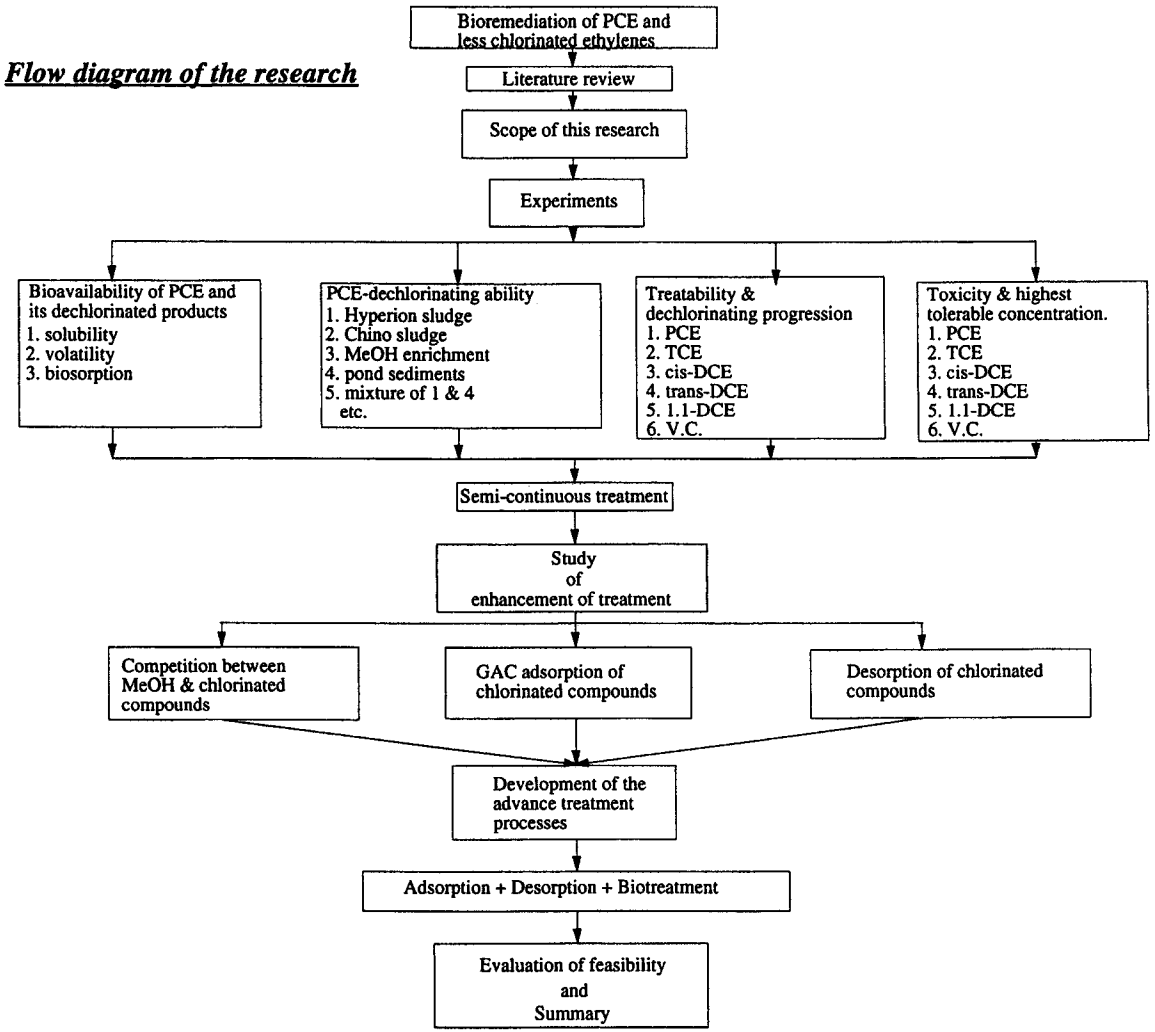
rely on groundwater for potable water sources, and about 96 percent of all water used for rural domestic purposes is obtained from groundwater. The continued value of groundwater as a future source of potable water will depend on controlling contaminants in groundwater, either through reduction of the source of the compounds or through the reclamation or treatment of groundwater supplies already affected. However, the effectiveness and widespread use of PCE and TCE in various applications makes a major reduction in their use unlikely in the near future. Techniques most frequently used for the treatment of contaminated groundwater include physical and chemical processes (e.g., air stripping and adsorption). However, there is considerable interest in biological remediation processes, especially in anaerobic environments, because they are capable of converting the heavily chlorinated compounds to harmless metabolites, rather than transferring them from one part of the environment to another.

Biological techniques that completely degrade contaminants without generating toxic end products may be best suited for treating large volumes of contaminated groundwaters and industrial wastewaters. For the halogenated aliphatic compounds, there are three general kinds of initial microbial transformation they may undergo: nucleophilic substitution, oxidation, and reductive dehalogenation (Leisinger, 1983). While the former two transformations have been demonstrated under aerobic conditions, reductive dehalogenation is probably responsible for the anaerobic transformation of halogenated aliphatic compounds. PCE and TCE, because of their volatility, are difficult to handle in aerobic systems. However, the anaerobic system is able to prevent volatilization of the added compounds. PCE dechlorination has been widely reported under anaerobic conditions, but it is converted only to tri- and dichlorinated compounds in most cases. These less chlorinated compounds tend to persist longer in anaerobic

environments than highly chlorinated compounds. However, if the environment becomes aerobic, the less chlorinated chemicals may be degraded by aerobic bacteria. Recently, a two-stage anaerobic-aerobic process was used to achieve complete destruction of chlorinated aliphatic hydrocarbons (Fathepure and Vogel, 1991); however this process suffered from the high volatilization of chemicals in the aerobic process. A lot of work must be done to prevent volatile loss of such chemicals from the treatment system. The present study concentrates on the feasibility of a completely anaerobic biotransformation of PCE and TCE to ethylene (ETH), a non-toxic and environmentally acceptable product, by a single-stage treatment process using anaerobic digested sludge. The conditions required for transformation by the digested sludge are also reported. The flow diagram for this research is depicted in Fig.1.

Because PCE and TCE are detected almost everywhere in the environment, it would be a promising strategy to use existing wastewater treatment plants for the reclamation or treatment of groundwater contaminated with PCE and TCE. This could be accomplished in anaerobic sludge digestors if degradability can be demonstrated.

Fig. 1 ***Flow diagram of the research***



2. LITERATURE REVIEW

Groundwater contamination with the organic solvents PCE and TCE is a serious problem, and concern about it is growing. Among various treatment technologies available to control groundwater contaminants, biological treatment is a promising method for completely degrading these recalcitrant compounds. Environmental contamination by PCE and TCE is of industrial origin and not due to natural occurrence of these compounds. These novel chemicals, to which microorganisms have not been exposed in evolutionary history, are not attacked by microbes and accumulate in the environment. Such compounds are called xenobiotics since they are foreign to the normal environment. To select bacteria capable of degrading previously nondegradable xenobiotics, successful adaptation of a mixed microbial population to metabolize the recalcitrant xenobiotics is a prerequisite. Furthermore, reductive dechlorination of chlorinated aliphatic compounds by microorganisms may not be sustainable unless an additional carbon substrate is provided because it is a co-metabolic process (Fathepure and Boyd, 1988a). Therefore, this review will cover definitions of " xenobiotics and recalcitrance ", " adaptation ", and " co-metabolism " first, then a summary of previous research on biotransformation of PCE and TCE under both anaerobic and aerobic conditions. Finally, the microbiological aspects of anaerobic digested sludge will be presented.

2.1. Xenobiotics and recalcitrance

A xenobiotic compound is a chemical to which microorganisms have not been exposed in evolutionary history (Leisinger, 1983). Hence, most of the anthropogenic

compounds (e.g. PCE, TCE, etc.) which do not occur naturally are categorized as xenobiotics. Most xenobiotics are not degraded under circumstances apparently adequate for microbial growth. This leads to their accumulation in the environment, i.e. to persistence or recalcitrance of the chemical. However, the recalcitrance of a given compound may also be due to insolubility, or limited adsorption onto biomass, or inhibition to microorganisms at the high concentration. At the enzymatic level, the compound may not be degraded because it is unable to enter the cell or the organism does not possess the appropriate enzyme (Painter and King, 1985). But it is believed that all organic compounds can be eventually biodegraded. A process, i.e. adaptation by which a mixed population of microorganisms develops the ability to degrade a substrate hitherto not biodegradable, must be performed.

2.2. Adaptation

Adaptation is a process to select bacteria capable of degrading previously nondegradable xenobiotics (Leisinger, 1983). It also covers the situation in which the populations develop tolerance to inhibitory substances (Painter and King, 1985). Therefore, determining if populations in anaerobic digested sludge can adapt to degrade PCE and TCE (novel compounds) is a prerequisite for this study. There are two general mechanisms by which acquisition of new degradative abilities can be achieved; first, the adaptation of existing catabolic enzymes and, second, the evolution of complete metabolic progressions (Painter and King, 1985). Usually, mixed microbial populations of many different species have a much greater probability of adapting to the degradation of new substrates than a single species. In most instances, the adaptation is the result of collective activity of a metabolically structured community. Except for this, nothing has

been reported as to why the various periods of adaptation were adopted and no agreed ways of adaptation exist. Little is known about the effects of factors such as time and pattern of exposure, concentration of the test substance, presence of other substrates, on the adaptive processes (Painter and King, 1985). After acclimation is achieved, the acclimated cultures can be further enriched to increase rates in degrading previously nondegradable xenobiotics.

2.3. Co-metabolism

Co-metabolism is the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound (Dalton and Stirling, 1982). Compared to the traditional biodegradation, it is a new phenomenon in which an organism grows on one substrate, but also has the ability to transform one or more other compounds, perhaps through only a few steps, without being able to derive energy or growth from the process. Although co-metabolizing organisms do not derive benefits from the metabolism of non-growth substrates, co-metabolism is thought to occur widely in nature and is probably more significant in the degradation of xenobiotics (Leisinger,1983). Co-metabolism can result from a simultaneous attack on the growth and non-growth substrates by the same enzyme or sequence of enzymes. It may also occur through the activity of enzymes not directly associated with the catabolism of the growth substrate (Painter and King, 1985). Generally speaking, co-metabolic transformation in the environment does not necessarily result in the complete oxidation of xenobiotics but may lead to the accumulation of transformation products with increased or decreased toxicity as compared to the original compound (Alexander, 1981). However, if the co-metabolic intermediate product from the xenobiotic by one

species can be completely oxidized for growth by another species, complete mineralization may be achieved.

For this reason, biodegradation of PCE and TCE can be considered complete if the carbon skeleton is converted to non-toxic metabolites and the chlorine is returned to the mineral state. Another issue related to co-metabolism is that co-metabolism of xenobiotics (e.g. PCE and TCE) usually occurs at a much slower rate than metabolism of growth substrates. When readily utilizable carbon sources are offered to these microorganisms together with xenobiotics, no selective pressure exists for growth on the xenobiotic compounds.

2.4. PCE-degradative ability

PCE is persistent in aerobic environments but degraded in anaerobic environments. Reductive dehalogenation is an important biodegradation mechanism for halogenated compounds under anaerobic conditions. Reductive dechlorination (i.e. the replacement of chlorine with hydrogen) of PCE is widely reported under a variety of anaerobic environments including methanogenic fixed-film reactors (Bouwer and McCarty, 1983; Vogel and McCarty, 1985; Fathepure and Vogel, 1991), anaerobic soils (Dooley-Dana, Fogel and Findlay, 1989), muck (Parsons et al., 1984), mixed methanogenic enrichments (Fathepure et al., 1987; Freedman and Gossett, 1989), and 10% anaerobic sewage sludge (Fathepure and Boyd, 1988b). In most cases, PCE was only partially dechlorinated to TCE or cis-DCE. The observed characteristics of reductive dechlorination of PCE are: (1) it typically occurs in a fashion of sequential removal of chlorine substituents from PCE; (2) the dechlorination rate decreases as the

number of chlorine atoms per molecule decreases, and (3) reductive dechlorination is not sustainable unless a carbon substrate is provided because PCE dechlorination by anaerobic bacteria is a co-metabolic process (Fathepure and Boyd, 1988a).

2.4.1. Carbon source

During methanogenic co-metabolism of PCE, electrons generated during the formation of methane may be diverted to PCE for reductive dechlorination. Hence, the primary carbon substrate is very likely to be the source of reducing equivalents for both methane formation and reductive dechlorination. Fathepure and Boyd (1988a) showed that the reductive dechlorination of PCE is directly proportional to the concentration of the primary carbon substrate and the number of methyl moieties associated with the primary substrate. This indicates dependence of PCE degradation on the methane-yielding capacity of a particular primary substrate. From a study on the dependence of PCE dechlorination on methanogenic substrate consumption, Fathepure and Boyd showed that adequate quantities of a carbon source and CH_4 biosynthesis were necessary for reductive dechlorination to occur. No additional dechlorination (TCE formation) was noted in experimental bottles after the substrate, methanol, was exhausted and CH_4 production ceased.

Fathepure and Boyd also found that different substrates (i.e., acetate, methanol, methylamine, and trimethylamine) affect the extent of PCE dechlorination due to different growth rates of methanogens. The appropriate substrate should provide conditions under which the most extensive reductive dechlorination can occur. Freedman and Gossett (1989) demonstrated that methanol provides the greatest ethylene

production in dechlorination of PCE. Acetate, formate, glucose, or hydrogen were not as effective. The higher dechlorination rate may be related to the greater reducing power of methanol metabolism (six reducing equivalents) than acetate metabolism (two reducing equivalents).

Furthermore, the extent of PCE dechlorination also depends on the concentration of cell mass (Fathepure and Boyd, 1988b). No change in the removal efficiencies of the halogenated hydrocarbons was observed while the feed substrate (acetate) was decreased (Bouwer and McCarty, 1983). They suggested that the removals were more a function of organism concentration than of the quantity of the primary substrate.

2.4.2. Sequential transformation

The principal intermediate in the anaerobic biotransformation of PCE is TCE. If continued, TCE can be further transformed to dichloroethylene (DCE) and then to vinyl chloride (VC) by sequential reductive dechlorination. Each chlorine is replaced by hydrogen. Fathepure and Vogel (1991) have shown that a significant amount of PCE underwent reductive dechlorination to less chlorinated products within a 37.5-h hydraulic residence time. Freedman and Gossett (1989) also indicated that it took only 2-3 days to convert PCE to VC, but, to further degrade VC to ethylene or CO_2 required a much longer retention time. The dechlorination of VC to ethylene was slow and only partially completed. In a follow-up study (DiStefano, Gossett, and Zinder, 1991), dechlorinating high concentrations (330 μM) of PCE to ethylene and small amounts of VC was achieved in a mixed anaerobic methanol-PCE enrichment culture. In general, the relative rate of dechlorination decreases as chlorine atoms are sequentially removed.

Thus, the transformation products of PCE, such as TCE, DCE, and VC with less chlorine atoms per molecule, tend to persist longer in anaerobic environments than PCE.

A biological process for remediation of groundwater contaminated with PCE and TCE can only be applied if the transformation products are environmentally acceptable (Bouwer and McCarty, 1983). Conversion of PCE to TCE or less chlorinated alkenes is of little or no benefit. Trichloroethylene, cis-1,2-DCE, trans-1,2-DCE and VC are also regulated under the 1989 Safe Drinking Water Act Amendments. In order to achieve the complete dechlorination of PCE, many researchers have attempted to couple anaerobic processes followed by aerobic processes, because the less chlorinated compounds are more likely to be degraded by aerobic bacteria. However, PCE and its biotransformation products are very volatile and hence would be difficult to handle in aerobic systems where aeration and stripping are possible. For anaerobic biological treatment to be useful and feasible, all of the possible intermediates from the reductive dechlorination of PCE have to be further degraded. Recently, DiStefano et al., (1991) showed that vinyl chloride, the most resistant intermediate of reductive dechlorination of PCE, can be further degraded to ethylene, a nonchlorinated and environmentally acceptable product under anaerobic conditions. This result suggests that reductive dechlorination under anaerobic conditions may be best suited for treating PCE and TCE contamination in groundwaters and industrial effluents.

2.4.3. Cultures

Methanogens / methanogenesis play an important role in the anaerobic dechlorination of PCE. Fathepure and coworkers (1987; 1988a,b) demonstrated that

pure cultures of two methanogenic bacteria (Methanosarcina strains) were able to convert PCE to TCE. Various other methanogenic environments were used to achieve the biotransformation of PCE. The reductive dechlorination of PCE may be caused by a member(s) of a methanogenic consortium, such as strain DCB-1, a chlorobenzoate-dechlorinating organism isolated from a methanogenic consortium that was able to degrade 3-chlorobenzoate (Fathepure and Boyd, 1987), and/or by methanogens themselves. PCE dechlorination occurred in both pure culture and in a methanogenic consortium. In pure culture, only a relatively small fraction of PCE underwent reductive dechlorination. This is consistent with the general observation that anaerobes are less active against xenobiotics in pure culture. In addition, the different strains of methanogens and cell mass could also significantly affect the extent of dechlorination. Generally, even though both the methanogens belong to the same genus, they differ in their ability to dechlorinate PCE. Several studies (Bouwer and McCarty, 1983a,b; Vogel and McCarty, 1985) also found more rapid dechlorination under methanogenic conditions than under sulfate-reducing environments and denitrifying conditions.

2.5. Degradability of TCE and less chlorinated compounds

As stated earlier, TCE and lower chlorinated compounds may still undergo reductive dechlorination but at much slower rates. Under aerobic conditions these compounds are also generally persistent in natural environments. However, certain aerobic microorganisms (e.g. methanotrophs) may degrade these less chlorinated compounds via oxidative mechanisms which are ineffective for heavily chlorinated compounds such as PCE. Aerobic environments have little chance to remediate waters contaminated with a mixture of chlorinated ethylenes.

Under aerobic conditions in the presence of methane, TCE was biologically oxdized to CO_2 (Wilson et al., 1985). The mechanism is TCE oxidation to trichloroethene epoxide by methane monooxygenase and then rapid hydrolysis (Fogel et al., 1986). This biodegradation process did not produce other volatile chlorinated compounds, and the degradation rates decreased for more-chlorinated compounds. Vinyl chloride was degraded more rapidly than TCE, and PCE was not degraded at all.

2.6. Microbiological aspects of anaerobic digested sludge

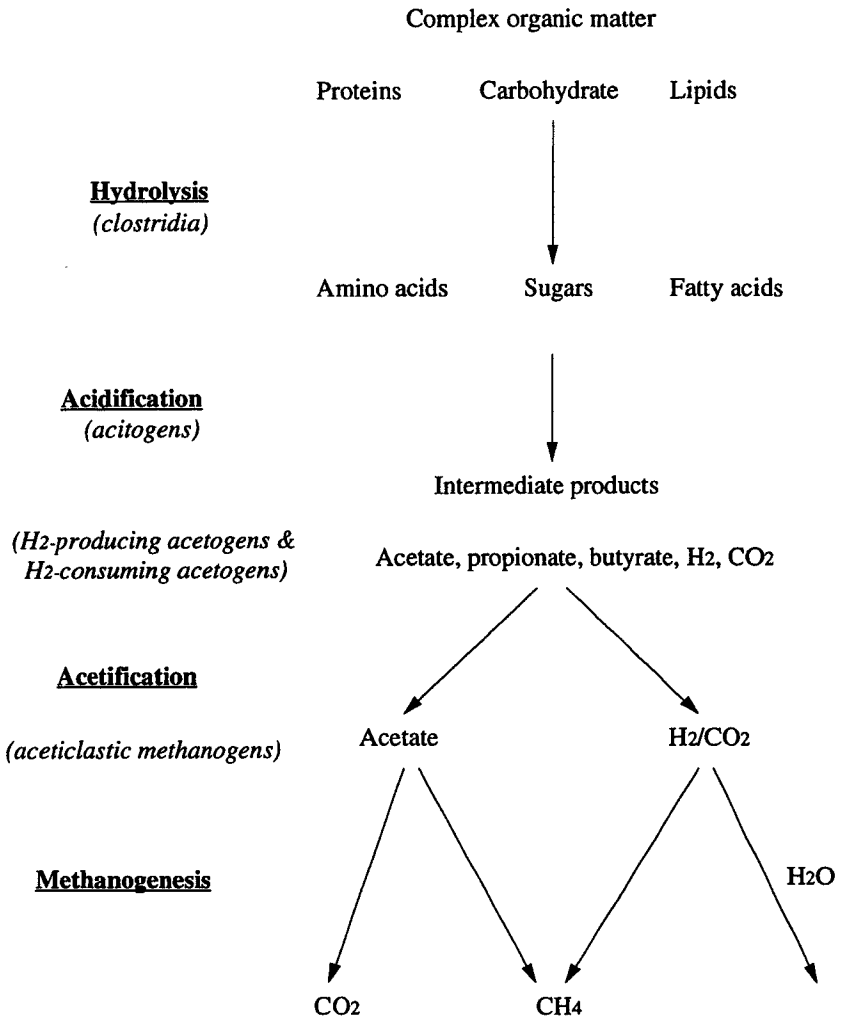
Methanogens / methanogenesis may be involved in reductive dechlorination in anaerobic communities. Anaerobic sludge digestors are important methanogenic sites where favorable growth conditions exist. Many anaerobic organisms are involved in anaerobic digestion. A common feature of anaerobic digestion is the formation of methane. This gas formation in anaerobic digestors is a syntrophic process depending upon the action of several types of anaerobic bacteria. The mechanism involved in this process includes four steps: (1) hydrolysis, (2) acidogenesis, (3) acetification, and (4) methanogenesis. Biodegradable organic compounds are first hydrolyzed and degraded to simpler compounds by a variety of facultative and anaerobic organisms. These simpler compounds are subsequently transformed to short-chain fatty acids, carbon dioxide and hydrogen gas by other non-methanogenic acidogens. Fatty acids are then metabolized by hydrogen-producing acetogenic bacteria to acetate and hydrogen. These organisms are unable to grow at partial pressures of hydrogen $>10^{-3}$ atm., therefore their maintenance within the methanogenic consortium depends on the continued removal of hydrogen by methanogens. Some hydrogen may be converted to acetate by hydrogen-consuming acetogens in this step. In the final step, methane is generated

mostly from acetate by the aceticlastic methanogens. Part of the methane production can be derived from hydrogen and carbon dioxide by hydrogenotrophic methanogens (Levett,1990).

Generally, the methanogenic bacteria are very substrate specific and are dependent on the non-methanogenic bacteria for their supply of substrate (Grady and Lim, 1980). Within digestors, methanogenesis occurs at an optimum rate in the range of pH 6-8. The optimum temperature for mesophilic digestion is 40°C. The multistep nature of anaerobic digestion is depicted in Fig. 2. In anaerobic sludge, many kinds of non-methanogenic bacteria may form hydrogen. Generally, this hydrogen serves as a source of the reductant required for reductive dechlorination of PCE and its partially dechlorinated intermediates if suitable enzyme(s) exist to divert hydrogen to dechlorinating processes.

Many dechlorinating bacteria potentially exist in anaerobic digestors. Methanogenic bacteria are an important group of anaerobic bacteria with dechlorinating capacity. Because of their extreme habitat diversity, these bacteria may be present in sludge digestors. However, sewage sludges from different sources vary in their potential to dechlorinate various substrates (Shelton and Tiedje, 1984)

Fig. 2 Multistep nature of anaerobic digestion of organic matter (Levett, 1990)



3. MATERIALS and METHODS

3.1. Chemicals

The halogenated organic compounds used were reagent-grade tetrachloroethylene (PCE), trichloroethylene (TCE), cis-dichloroethylene (cis-DCE), trans-dichloroethylene (trans-DCE), 1.1-dichloroethylene (1.1-DCE), and vinyl chloride (V.C.). All of them were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, at >99% purity except V.C., which was obtained from Fluka Company, Ronkon Koma, New York. The following non-chlorinated chemicals were used: methane (Aldrich Co.), ethane (Aldrich Co.), and ethylene (ETH; Aldrich Co.), and methanol (Fisher Scientific Co., Pittsburgh, Pa.).

3.2. Fresh sludge and pond sediment

Fresh anaerobic digested sludge for PCE-dechlorinating ability test at the outset was obtained from the anaerobic digestor at the Hyperion Wastewater Treatment Plant, LA., CA., and Chino Basin Wastewater Treatment Plant (RP2), Chino, CA. Both of the plants receive more than 70% of their wastewater from residential sources. The retention times of the anaerobic digestor are about 15 and 30 days for Hyperion and Chino Basin, respectively. The anaerobic sludge typically contains about 20,000 mg of solids per liter of mixed liquid. Unless otherwise stated, the sludge samples used in various tests throughout the course of this study were prepared as follows. After transport to the laboratory, fresh sludge (100 mL) was dispensed into 120 mL capacity serum bottles in an anaerobic hood. The serum bottle was then sealed with a Teflon-

lined rubber septum (Supelco, Inc., Belfonte, PA) and an aluminum crimp cap. These sludge samples contained no detectable levels of PCE. All experiments were conducted at 35°C under quiescent conditions. Fresh anaerobic pond sediment was obtained from the botanical garden at UCLA and transfered into serum bottles as described above.

After PCE-dechlorinating ability was demonstrated in the above cultures, five more sludges obtained from different wastewater treatment plants in California were examined to determine if PCE dechlorination was a general characteristic of anaerobic sludges. These five wastewater treatment plants are:

1. Terminal Island Wastewater Treatment Plant, San Pedro, CA.,
2. Chino Basin Wastewater Treatment Plant (RP1), Rancho Cucamonga, CA.,
3. Alvarado Wastewater Treatment Plant, Union, CA.,
4. Valencia Wastewater Treatment Plant, Valencia, CA., and
5. JWPCP Wastewater Treatment Plant, Carson, CA.

3.3. Methanol-enrichment culture

An anaerobic methaogenic methanol-enrichment culture was developed in two 2.1-liter Erlenmeyer flasks on stir plates. Initially, 630 ml of digested sludge obtained from an anaerobic digester (Hyperion Wastewater Treatment Plant, Los Angeles, CA) was used to seed each flask. Temperature was controlled at 35°C. Each flask was fed a non-sterile solution (Table 1) containing 100 mM methanol as the carbon source. Both flasks were completely mixed and operated semi-continuously (refed daily). The daily feed volume was increased gradually from 200 ml to 2000 ml. The mixing in both

flasks was discontinued for 3 hours prior to daily feeding to allow the biomass to settle and limit biomass wash-out.

After 6 months of operation, the MLSS in both flasks had increased to approximately 5000 mg/L and the MLVSS was 65-75 % of that. The methane production was about 65-70% of total gas production.

3.4. Anaerobic nutrient medium

To maintain the activity of the anaerobic sludge used in various dechlorination tests, an anaerobic mineral medium (modified from Freedman and Gossett, 1989) plus 50% of supernatant of fresh Hyperion anaerobic digestor sludge was prepared and used whenever the nutrients in the sludge treatment system needed replenishing. The anaerobic medium was made up of 50% deionized water and 50% fresh sludge supernatant. The supernatant was obtained by centrifuging the sludge liquid at 3500 rpm for 20 minutes. The mineral materials in the medium consisted of (per liter of liquid medium): NH_4Cl, 0.20 g; $K_2HPO_4 \cdot 3H_2O$, 0.01 g; KH_2PO_4, 0.055 g; $MgCl_2 \cdot 6H_2O$, 0.20 g; trace metal solution (per liter, 0.1 g of $MnCl_2 \cdot 4H_2O$; 0.17 g of $CoCl_2 \cdot 6H_2O$; 0.10 g of $ZnCl_2$; 0.20 g of $CaCl_2$; 0.019 g of H_3BO_4; 0.05 g of $NiCl_2 \cdot 6H_2O$; and 0.020 g of $Na_2MoO_4 \cdot 2H_2O$, adjusted to pH 7 with NaOH or HCl), 10 ml; resazurin, 0.001 g; $Na_2S \cdot 9H_2O$, 0.50 g; $FeCl_2 \cdot 4H_2O$, 0.10 g; $NaHCO_3$, 5.0 g; and yeast extract, 0.50 g. The first six components were boiled with deionized water and sludge supernatant to remove oxygen, and then cooled under an N_2 purge. After cooling, the remaining components except sodium sulfide were added and the purge gas was

switched to CO_2/N_2 (20%/80%). The medium was autoclaved and stored in 120 ml-serum bottles until they were needed. Sodium sulfide was then added before use.

Table 1: Composition of Feed Solution (Vogel and McCarty, 1985)

Compound	Concentration (mg/liter)
Inorganics	
$NaHCO_3$	3360
NH_4Cl	215
$MgSO_4.7H_2O$	150
K_2HPO_4	60
$CaCl_2$	25
KCl	25
$FeCl_2$	5.0
$CoCl_2$	0.5
$NiCl_2$	0.25
Organics	
Methanol	3200

Note: 6N HCl was used to adjust pH to 7.2 - 7.4

3.5. Analysis

3.5.1. Purgeable chlorinated compounds

Parent compounds (PCE/TCE) and dechlorinated products (TCE, DCE and VC) in the experimental serum bottles were identified and quantified using a 5890A Hewlett-Parkard gas chromatograph equipped with a flame ionization detector (FID) and a purge-and-trap device (Tekmar Model LSC-2, Cincinnati, Ohio). The bottles were vigorously shaken by hand and then centrifuged 5 min. at 3500rpm. before each time sampling. Water samples (5 mL) were prepared by adding 100 μL of supernatant to 4.9 mL of deionized water. 100 μL of supernatant was taken from each bottle with a 100-μL syringe and then transferred into a 5-mL gas-tight syringe in which 4.9 mL of deionized water has been filled. Samples (5 mL) were purged onto a Tenax TA absorbent trap (Supelco Co., Cat. No. 2-0294M) with helium (40 mL/min for 8 min) and desorbed (180°C for 4 min) onto a DB-624 capillary column (30 m by 0.53-mm inner diameter, J&W Scientific Co., Folsom, CA). The carrier gas was helium (8 to 10 ml/min). The temperature program was as follows: the initial temperature was 35°C with a 5 min hold, then increased to 70°C at 5°C/min, with a final hold of 1 min at 70°C. The detector temperature was 200°C. The air and hydrogen gas flow rates to the FID were 310 and 31 ml/min, respectively. Identification and quantification of chemicals was accomplished by comparison to carefully prepared external standards. The output signal was recorded using a Hewlett-Packard Model 3392 integrator. Retention times and detection limits for each compound of interest in this research under these GC conditions are listed in Table 2. Lower detection limits are possible with the

analytical system used, but the values given in Table 2 are adequate for the purposes of these experiments.

In order to evaluate the accuracy of the analytical method, control bottles were prepared with 50 μmol of each tested chemical per 100 mL and incubated for at least 12 hours. Both liquid and gas samples were analyzed as described above, and the total mass was compared to the added mass to calculate the percent recovery for each chemical. The analytical method yielded results that were within +/-5% of the correct amount of tested chemical added in each bottle.

Table 2 : Retention Times and Detection Limits

Parameter	Formula	Retention time(min)	Detection limit(μg/L)
Perchloroethylene	$Cl_2C=CCl_2$	9.99	0.7
Trichloroethylene	$Cl_2C=CHCl$	5.80	0.5
Cis-1,2-dichloroethylene	ClHC=CHCl	3.35	0.5
Trans-1,2-dichloroethylene	ClHC=CHCl	2.35	0.3
1.1-dichloroethylene	$Cl_2C=CH_2$	1.87	0.5
Vinyl chloride	$ClHC=CH_2$	1.56	0.3

3.5.2. Methane and ethylene

The methane and ethylene concentrations in the headspace of the serum bottles were measured by a Varian 3760 gas chromatograph equipped with a flame ionization detector and a glass column packed with 80/100 Porapak Q. The carrier gas was helium at a flow rate of 30 mL/min. A 100-μL gas-tight syringe was used to remove 100-μL gas sample for analysis.

3.6. Experiments

Unless otherwise indicated, biological experiments in this research were conducted with 120 mL serum bottles filled with 100 mL of fresh digested sludge that were sealed with Teflon-lined rubber septa and aluminum crimp caps.

3.6.1. PCE-dechlorinating ability test

At first, PCE-dechlorinating ability of several cultures obtained from different anaerobic habitats were tested. The tested cultures included:

1. Hyperion digested sludge.
2. Pond sediment.
3. Mixture (1:1) of Hyperion digested sludge and pond sediment.
4. Chino Basin (RP2) digested sludge.
5. Methanol-enrichment culture.

The experiments were carried out in 120 ml serum bottles containing 100 ml of tested culture liquid. Methanol was used as the carbon source and the electron donor. The tests of Hyperion sludge, pond sediment and their mixture were started with a low PCE concentration (2.5 μmol / L in liquid phase). The PCE concentration was achieved by adding 0.3 μmoles of PCE to each bottle. Once the added PCE was consumed, fresh PCE was added. After 28 days of operation, the amount of PCE added to each bottle was increased to test the dechlorinating ability under higher PCE concentrations. In testing of each of these three cultures, three different amounts (2.5, 25, and 123 μmol) of methanol were used in separate bottles to observe differences in PCE dechlorination.

For the Chino Basin sludge test, 369 and 4920 μmoles of methanol (in total) were applied to two separate bottles containing a high initial PCE concentration (49 μmoles per bottle). In the third bottle containing a low initial PCE concentration (0.6 μmoles per bottle), 369 μmoles of methanol (in total) was used. Two sludge samples obtained from two different digestors at the Chino Basin Treatment Plant (RP2) were tested. Identical experiments for each of these sludge samples were conducted.

The dechlorinating ability of methanol-enrichment cultures was investigated in the presence of methanol and hydrogen as the electron donor. Three bottles were prepared for the methanol-enrichment culture test. The first bottle contained 49 μmoles of PCE and 123 μmoles of methanol. The same amount of PCE and methanol was used in the second bottle but also under a H_2/CO_2 (80:20) atmosphere. In the third bottle, 49 μmoles of VC gas was incubated with 123 μmoles of methanol.

To evaluate if PCE dechlorination was a general characteristic of anaerobic sludges, five more fresh anaerobic digested sludges obtained from different wastewater treatment plants (Terminal Island, Chino Basin (RP1), Alvarado, Valencia and JWPCP Plant) were tested. Sludge samples used in this test were prepared as decribed before. For each sludge, the PCE dechlorination test was started with a high PCE concentration (about 67 mg/l in the liquid phase without accounting for biosorption) and performed in triplicate. Forty nine μmoles of PCE was added to each sample without adding any carbon source. For Valencia and JWPCP sludges, an additional bottle was prepared to test the dechlorinating ability under an even higher PCE concentration (about 134 mg/l). The PCE concentration was achieved by adding 98 μmoles of PCE to each bottle. All experiments were conducted at 35°C under quiescent conditions.

3.6.2. Treatability of chlorinated compounds

Hyperion digested sludge was chosen to conduct the treatability test for PCE, TCE, cis-DCE, trans-DCE, 1.1-DCE, and VC. The initial dose for all of the chlorinated compounds was 0.3 μmol per bottle. After the first chemical addition was consumed, the dose for each test chemical was doubled. Each test chemical was tested in separate bottles to avoid interference with each other. 5-10 μmoles of methanol was added to the bottles at the same time that PCE was added. For the PCE treatability test, two identical bottles, with and without methanol, were prepared. The purpose was to determine if the fresh digested sludge was capable of degrading PCE without adding methanol (electron donor). After 42 days of semi-continuous operation, the dose of each chemical was increased to 49 μmoles, and a higher amount of methanol was added (123 μmoles in

each bottle). This was done to determine if acclimation to a low concentration would facilitate the degradation under high concentrations.

The treatability of a high initial concentration of PCE and TCE was also evaluated. 49 μmoles of PCE and TCE were initially added into two separate bottles. Once the initial chemicals were consumed, the same amount of PCE or TCE was added again to observe the dechlorination in long-term operation. Each time, 246 μmoles of methanol was added with the PCE or TCE. An additional bottle was used to test treatability of high PCE concentration without methanol addition. These experiments were accompanied by a control sample of water and an autoclaved sludge sample with the same liquid phase volume (deionized water and sludge, respectively) and the addition of the chlorinated compounds.

3.6.3. Dechlorinated products and reductive dechlorination progression test

The sequential reductive dechlorination of chlorinated compounds (including PCE, TCE, 3 isomers of DCE, and V.C.) in fresh anaerobic Hyperion sludge was examined. Six identical fresh sludge samples were used to assess the dechlorination fate for each tested chemical under anaerobic conditions without any addition of methanol. For PCE dechlorination, one additional bottle (methanol bottle) received 5 μl of methanol (about 123 μmoles) as the added carbon source and electron donor. About 49 μmoles of each test chemical were added to separate bottles containing 100 ml of fresh sludge. These serum bottles were incubated in the dark without shaking at 35°C. Routine GC analysis of the supernatant was performed on all of the bottles to observe

the change in the concentration of tested compounds and formation of intermediates over time. A water control sample (100 ml of deionized water plus the same amount of each chlorinated compounds) was incubated under the same conditions in order to examine chemical losses from the serum-bottle system.

The effect of TCE and VC presence on PCE dechlorination was also studied at this stage. Dechlorination of a mixture of 49 μmoles of PCE and 49 μmoles of VC was conducted in a serum bottle with the same amount of fresh sludge, while the mixture containing 49 μmoles of each of PCE, TCE, and VC was incubated in an additional bottle. Both of them contained 246 μmoles of methanol as the electron donor.

3.6.4. Toxicity and highest tolerable concentration

All of the six chlorinated compounds were tested for their toxicity to methanogenesis in Hyperion sludge. For each test compound, 6 - 8 different dosages (approximately 0.55 to 250 μmoles per bottle) were injected to each of a series of bottles. After 3 weeks of incubation, 100 μl of headspace gas sample was removed from each bottle for methane production analysis. The methane production from each bottle was then compared with that of the blank control in each set of experiments (containing the same amount of identical sludge cultures without the addition of chlorinated compounds) to determine the relative inhibition of methane production at various chemical concentrations.

In this experiment, supernatant liquid samples from all of the bottles were also periodically analyzed to observe the extent of dechlorination of each chlorinated

compound at various initial concentrations and the possible ethylene production from dechlorination of each chlorinated compound.

3.6.5. Semi-continuous operation

The six sludge bottles created in the dechlorination progression test for PCE, TCE, 3 isomers of DCE, and VC respectively, were periodically spiked with the same chemical to evaluate semi-continuous operation. When the added chlorinated compound in each bottle was degraded, the same amount of test chemical (49 μmoles) was repetitively added to the bottle accompanied with the addition of 123 μmoles of methanol. From the 59th day on, 5.0 ml of supernatant was also removed and replaced with fresh anaerobic nutrient medium (containing 50% of supernatant of fresh digested sludge and 500 mg of yeast extract per liter) in each addition of chlorinated compounds.

In addition to these cultures, two identical mixed-cultures were created. 10 mL samples from each of the six bottles (mentioned above) were used to create a mixture to determine if it could dechlorinate PCE better and faster than a single acclimated culture. The mixture bottles were operated semicontinuously. Whenever PCE was degraded to VC, 5 mL of mixed liquid was removed and replaced with fresh medium (containing 5 μL of MeOH) plus PCE. After 16 days, semi-continuous operation was followed by an incubation period (40 days) when PCE additions were stopped to see if all the VC can be further degraded to ethylene. During this period, replacement of mixed liquid with fresh medium and addition of methanol was continued. At the end of the incubation period, the pressure in the headspace of each bottle was measured and used to determine the total mass of ethylene and methane production.

3.6.6. Effect of Methanol (electron donor) on PCE dechlorination rate

The effect of methanol on PCE reductive dechlorination was examined by incubating the acclimated sludge with various amounts of methanol and PCE at 49 μmoles per bottle. The methanol concentrations applied to each bottle were 0, 123, 123, 1230, 2460, and 4920 μmoles per bottle (123 μmoles was chosen in two bottles). At the beginning, six bottles, each containing 100 ml of acclimated Hyperion sludge and 49 μmoles of PCE, were incubated with 123 μmoles of methanol. After repetitive PCE degradation in these six bottles was demonstrated, various amounts of methanol were added to each bottle with the same amount of PCE dose (49 μmoles). Whenever PCE was degraded, 5 ml of liquid was removed and replaced with the same amount of fresh medium plus PCE and methanol, except for one 123 μmoles-methanol bottle from which 20 ml of liquid was removed. The addition of PCE and methanol was repeated several times to allow cultures in each bottle reach stable condition. These results were used to evaluate the effect of methanol on the extent of PCE dechlorination and reaction rate.

3.6.7. Effect of mixing on PCE dechlorination test

Five identical bottles containing 100 ml of fresh Hyperion digested sludge were prepared. The fresh sludge in each bottle was incubated with 49 μmoles of PCE to acclimate to degrade PCE first. Once the added PCE was consumed, fresh PCE was added. At the same time, 5 ml of supernatant was also removed and replaced with fresh anaerobic nutrient medium plus 123 μmoles of methanol. After repetitive PCE

degradation in these bottles was demonstrated, all sludge liquid was mixed together with 25 ml of nutrient medium and then dispensed into 5 bottles again to create the homogeneity of mixed culture in each bottle. These five bottles designated as S1 - S5 were used for this series of experiments.

At first, while other samples were still incubated under quiescent conditions, S5 sample was placed in a 35°C shaker to observe differences in PCE dechlorination. After 10 days of incubation, the first run was ended. S1 - S4 samples and 10 ml of nutrient medium were mixed together and redispensed into original bottles. At the same time, 2.5 ml of sludge liquid was removed and replaced with nutrient medium for S5 sample. During the second run, S5 plus S4 were incubated in the 35°C shaker to test if the smaller amount of medium could improve PCE dechlorination because the partial dechlorination of PCE accompanying a much higher methane production occurred in S5 which was placed in the shaker during the last run.

After 6 days, S1 - S3 were mixed with 7.5 ml of medium as decribed above and 2.5 ml of sludge from S4 and S5 was replaced with medium. In addition, nitrogen was used to replace the gas in the headspace of S2 and S3 while other samples were still under a N2/CO2 (about 70:30) atmosphere. This was done for preventing the methane formation from carbon dioxide. The third run was conducted with 3 samples (S3 - S5) incubated in the shaker. After this run (6 days), 2.5 ml of sludge liquid was removed from each bottle and replaced with the same amount of medium. Nitrogen was still used for S2 and S3 samples. During the fourth run, S5 was removed from the shaker to test if the complete dechlorination ability could be recovered under quiescent conditions. The fourth run proceeded 6 days.

The fifth run was started with the same conditions as those done for the last run except no PCE being added to S5. In this experiment, 49 μmoles of PCE plus 123 μmoles of methanol were added to each bottle before each run was started.

3.6.8. Effect of temperature on PCE dechlorination test

Temperature is an important factor which can influence the microbial activity. The effect of temperature on the dechlorination process was tested by incubating the fresh digested sludge cultures with 49 μmoles of PCE under laboratory conditions without temperature regulation.

3.6.9. PCE-dechlorinating ability in old sludges test

To examine the reductive dechlorination ability in old sludge, eight fresh Hyperion sludge samples prepared as described previously were incubated for 1, 2, 3, 4, 12, 14, 20 and 21 days, respectively before PCE injection. For purposes of comparison, these experiments were accompanied by a control sample of fresh Hyperion sludge with the same liquid phase volume and the addition of PCE (49 μmoles).

3.6.10. Biological activated carbon process (BAC) test

Activated carbon is an amorphous form of carbon. It has been widely used for its excellent adsorptive capability for a variety of substances because of the characteristics of high porosity and high surface area. Nuchar granular activated carbon (grade WV-5, 10×25 mesh size), produced by Westvâco Corporation (Covington, VA) was used throughout this study.

Granular activated carbon (GAC) and/or powdered activated carbon (PAC) have been increasingly used for the treatment of water and wastewater through most of the twentieth century. At the beginning, influent water is applied to GAC column. In this case, biological growth always led to plugging problems. Nonetheless, these operation problems led to the idea of adding PAC to the aeration tank of an activated sludge plant. However, both of them were referred to as biological activated carbon. The biological activated carbon process (BAC) integrates biological treatment and adsorption into a single reactor. In complete mixing reactor, such system overcomes the plugging problems and lowers the adsorptive removals because the carbon tends toward equilibrium with the treated effluent (Benedek, 1980). The organic removals are also higher than those of separate biological and adsorption treatment .

This experiment concentrated on the effect of carbon addition on the PCE dechlorination in a biological treatment system. Six bottles designated as U1 -U6 were prepared for this series of experiments. Two grams of virgin activated carbon and 100 ml of anaerobic Hyperion digested sludge preacclimated with PCE degradation were used in each bottle. After bottles were sealed, 49, 98, 196, 294, 392, and 490 μmol of

pure PCE were injected into bottle U1 to U6, respectively. The above dosages resulted in PCE concentrations ranging from 490 to 4900 μmol/L (81.3 to 813 mg/L) in liquid phase without considering the headspace-liquid partitioning and adsorption on sludge and carbon. Both liquid and gas samples were analyzed over the length of the experiment.

3.6.11. Offline bioregeneration (OBR) test

The another way to combine biological treatment with carbon adsorption is offline biological regeneration (OBR). The process was proposed by Sigurdson and Robinson in 1978. In such a process, exhausted carbon is regenerated through biodegradation. Bacteria are supposed to degrade adsorbed organics. Biological regeneration of the spent carbon and subsequent recovery and recycle of the carbon to the adsorption process may be practiced because PCE has been demonstrated to be biodegradable in anaerobic digested sludge. Usually, regeneration of the spent carbon is more economical than replacement with virgin carbon. The conceptual difference between BAC and OBR is illustrated schematically in Figures 3 and 4.

To examine the feasibility of OBR for PCE laden carbon, activated carbon was initially saturated with PCE in adsorption bottles containing deionized water, then was regenerated in bioregeneration bottles by the acclimated anaerobic Hyperion digested sludge.

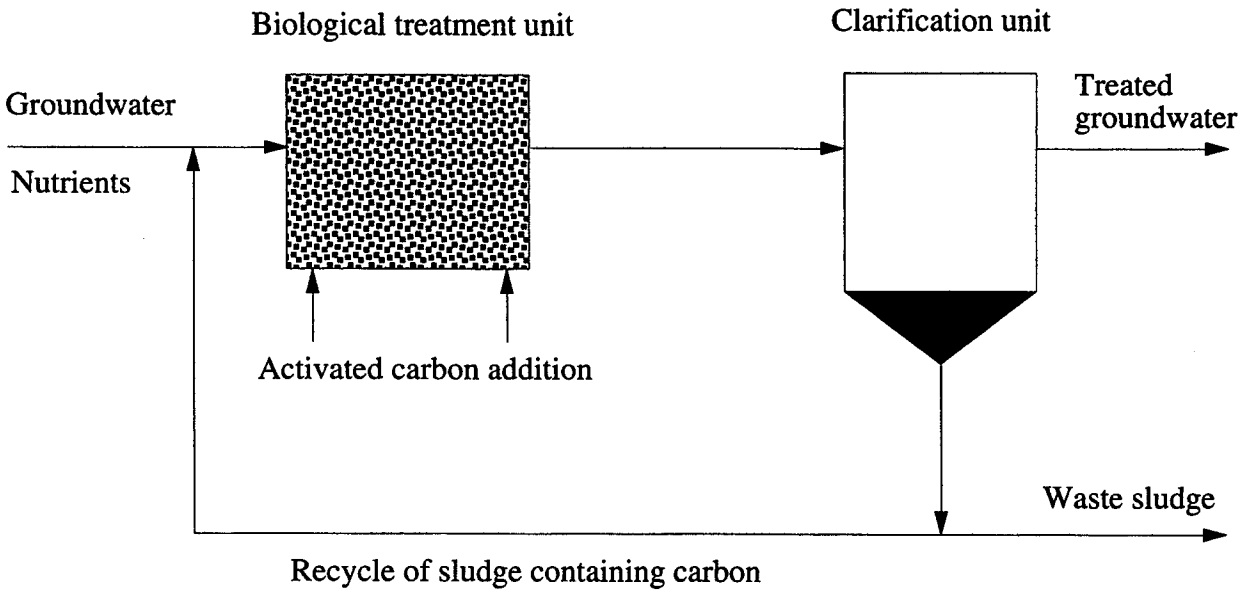


Figure 3. Schematic of Biological Activated Carbon Process (BAC)

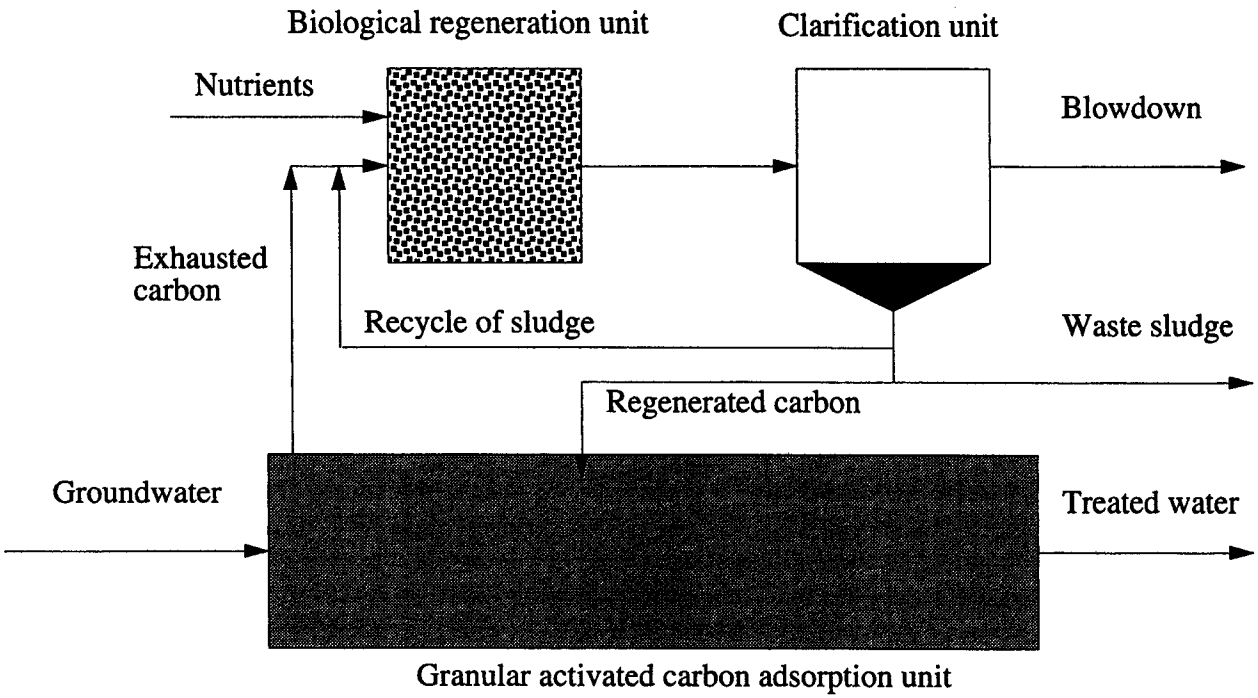


Figure 4. Schematic of offline biological regeneration process (OBR)

Six of two grams of exhausted granular activated carbon (preequilibrated separately with 49, 98, 196, 294, 392, and 490 μmol of PCE in 100 ml deionized water) were taken out of those PCE-preadsorption bottles and placed in six new bottles designated as S1 - S6. These amounts of PCE were chosen based on preliminary adsorption test results which provided an acceptable range of PCE liquid concentration for biodegradation. Each bottle contained 100 ml of acclimated sludge liquid and approximately 20 ml of headspace. At the beginning all PCE was adsorbed on the added carbon and no PCE was free in sludge liquid. Due to the non-equilibrium of PCE distribution between the liquid and carbon, desorption of previously adsorbed PCE from carbon to the liquid was supposed to occur to approach the equilibrium. This closed bottle system was designed to employ desorption and microbial activity for the regeneration of spent, PCE-bearing carbon. This was also used for the investigation of the concept of offline bioregeneration. Bottles were operated under batch condition and incubated at 35°C quiescently. Both liquid and gas samples (100μl) were withdrawn periodically to determine PCE and its dechlorinated product concentration in the bioregeneration bottle.

4. RESULTS

4.1. Chlorinated chemical loss control

First, the integrity of the serum bottle system sealed with Teflon-lined rubber septa and aluminum crimp caps to prevent sorption and volatile losses of chlorinated compounds was demonstrated. Over a period of 60 days, the decreases in total masses of PCE, TCE, cis-DCE, trans-DCE, 1.1-DCE, and VC in a water control (shown in Fig. 5) which contained 49 μmoles of each compound were 42, 37, 19, 36, 25, 25%, respectively. Over the same period, the losses were only 28, 26, 13, 28, 10, 15%, respectively, in the water control bottle wrapped with foil (shown in Fig. 6). The reduced loss of chlorinated compounds observed in the bottle wrapped with foil is consistent with the more stable chemical characteristics of chlorinated aliphatic compounds without exposure to light. By and large, chlorinated compounds are relative unstable under light.

PCE loss and abiological reductive dechlorination of PCE were also examined in an autoclaved-sludge control. Data are shown in Fig. 7. Over a period of 135 days, about 81% of 245 μmoles of cumulative PCE dose still remained. During the same period, 6.63 μmoles of TCE and trace amounts of cis-DCE (0.41 μmoles) were produced. This experiment also demonstrated that the integrity of the serum-bottle system is acceptable and the abiologically-mediated reductive dechlorination of PCE is insignificant in anaerobic digested sludge.

Fig. 5 The loss of volatile chlorinated chemicals in D.I.water control sample without aluminium foil.

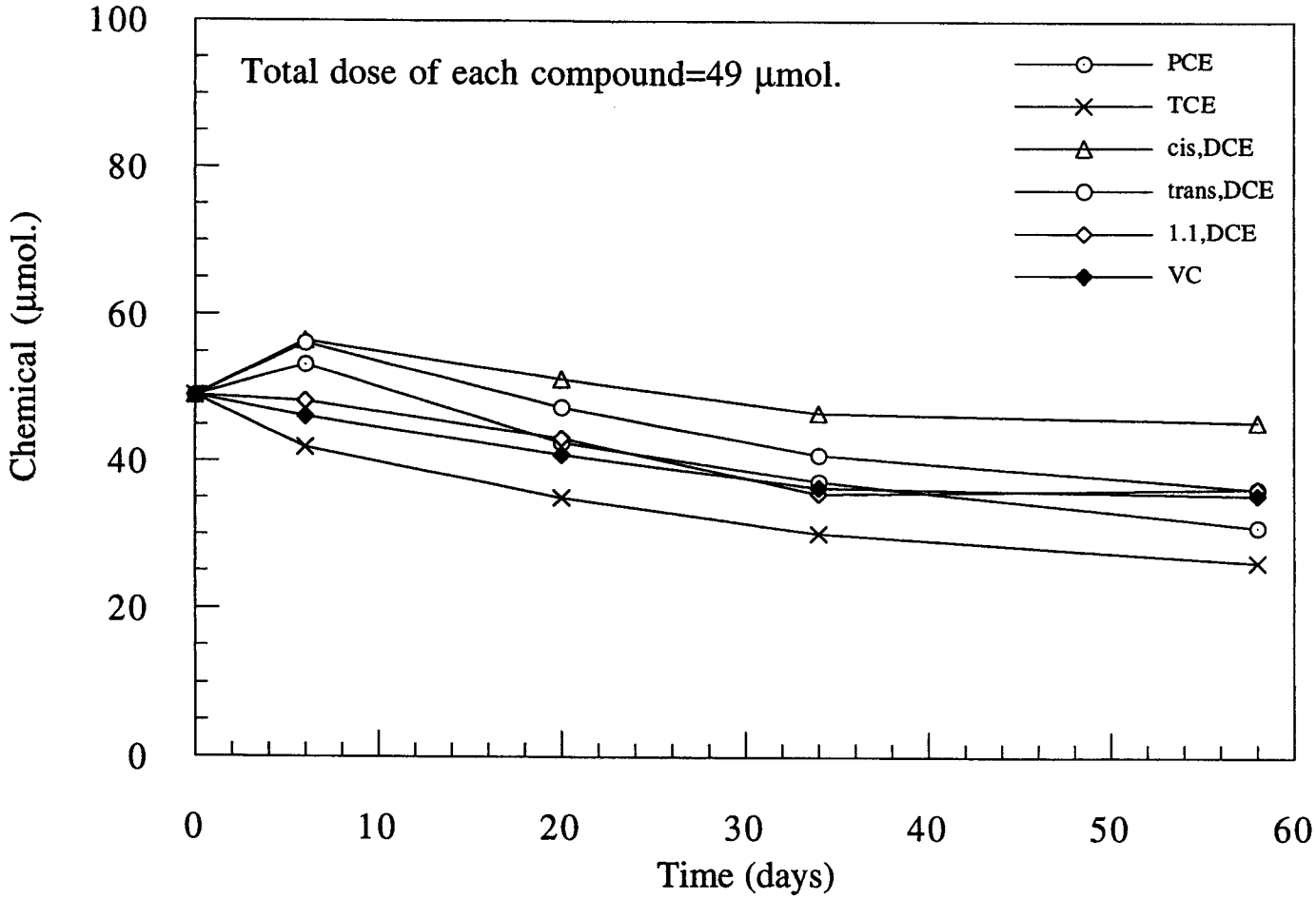


Fig. 6 The loss of volatile chlorinated chemicals in D.I.water control sample with aluminium foil cover to prevent degradation from light.

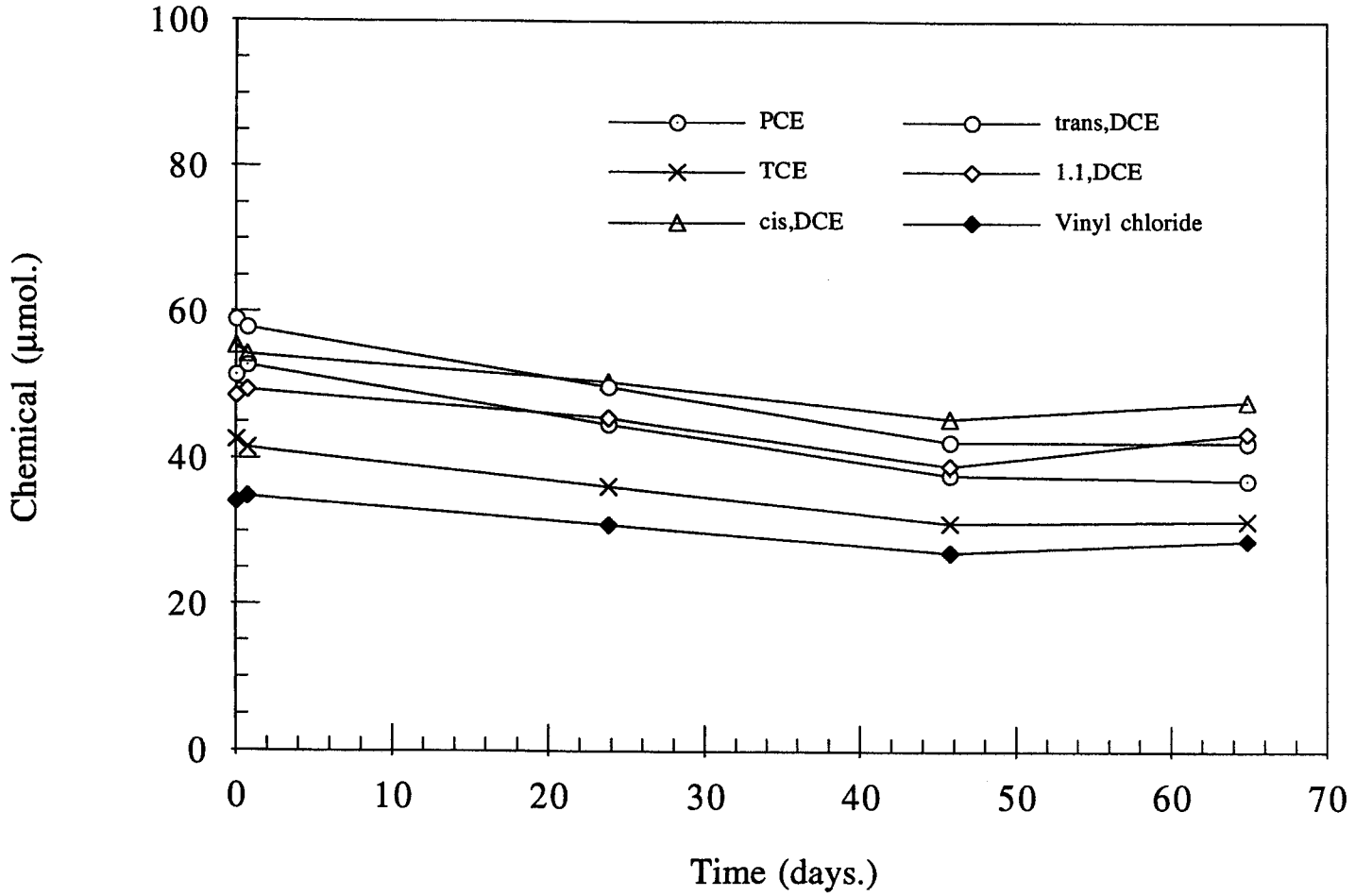
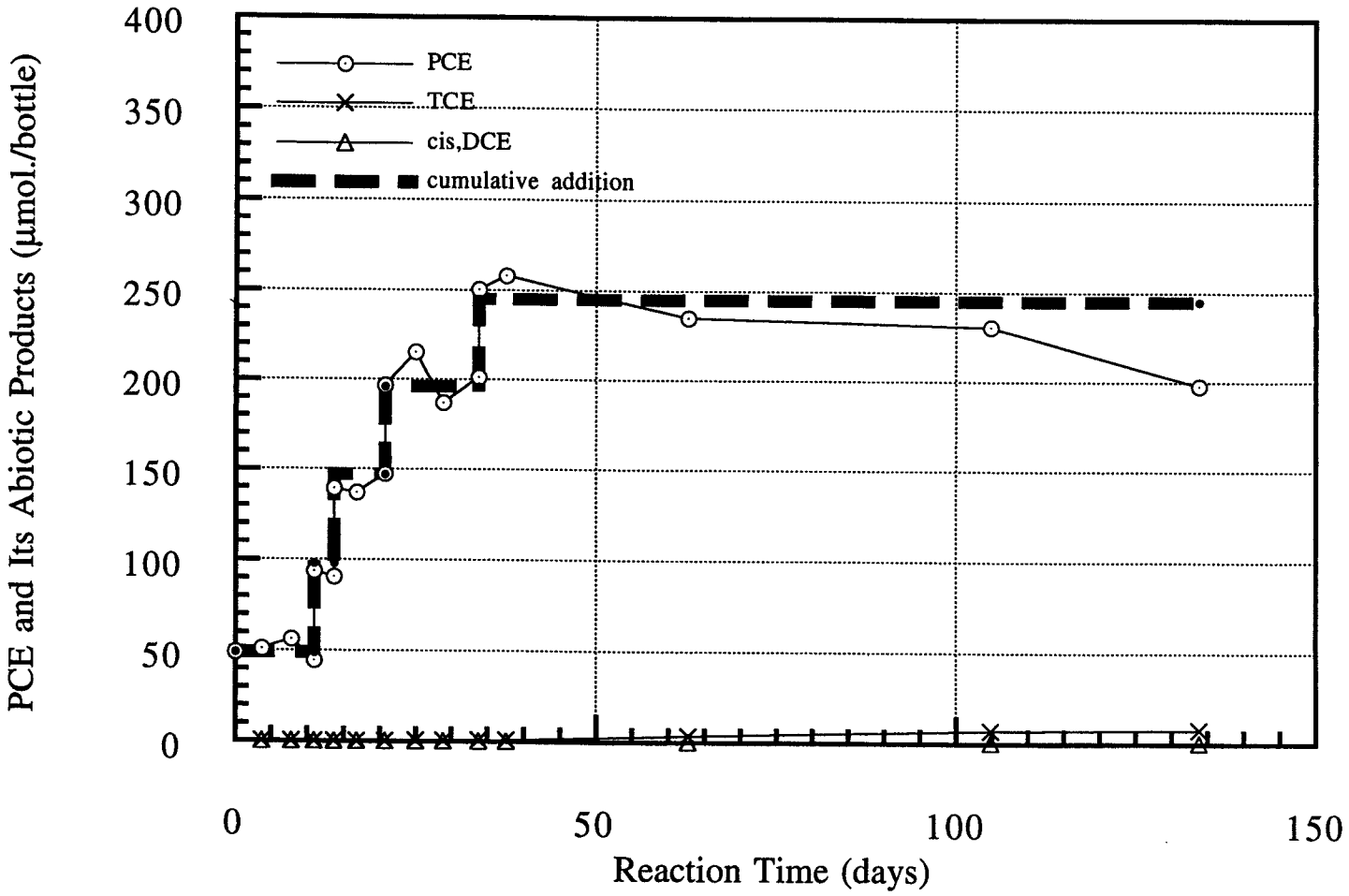


Fig. 7 PCE loss and its abiotic products in an autoclaved sludge control sample.



4.2. Mass balance

A methodology for performing mass balances of chlorinated compounds in the 120 ml-serum-bottle system containing 100 ml of digested sludge was developed by considering the headspace-liquid partitioning and biosorption within the bottle. Generally, the total mass of chlorinated compounds in an experimental bottle can be expressed as:

$$TM = \sum((C_{l,i}V_l + C_{g,i}V_g + K_i C_{l,i}^{(1/n_i)}M)/MW_i) \qquad (1)$$

where TM = total mass (μmole),
$C_{l,i}$ = concentration of chlorinated compound (i) in liquid phase (μg/l),
V_l = volume of liquid phase (liter),
$C_{g,i}$ = concentration of chlorinated compound (i) in gas phase (μg/l),
V_g = volume of gas phase (liter),
K_i = equilibrium constant indicative of adsorptive capacity,
n_i = constant indicative of adsorption intensity,
M = mass of bio-adsorbent (gram), and
MW_i = molecular weight of chlorinated compound (i) (gram/mole).

However, for dilute real solutions, a chemical concentration in gas phase is proportional to the concentration of that component in the liquid phase based on the Henry's law, expressed as:

$$C_{g,i} = H_{ci}C_{l,i} \qquad (2)$$

where H_{ci} = Henry's law coefficient of chlorinated compound (i)

By substituting equation (2) into equation (1), total mass can be expressed as:

$$TM = \sum((C_{l,i}V_l + H_{ci}C_{l,i}V_g + K_iC_{l,i}^{(1/n_i)}M)/MW_i) \quad (3)$$

To obtain the total mass of chlorinated compounds, volatility and biosorption of each chlorinated compound must be determined.

Henry's law coefficient

The modified EPICS (Equilibrium Partitioning in Closed Systems) procedure (Gossett, 1987) was applied to measure Henry's law coefficient for all chemicals at 35°C. For each chemical, Henry's coefficients were measured in six 60-ml serum bottles. Three contained 50-ml liquid (D.I.water) volumes; the other three, 5 ml. Bottles were prepared as follows: D.I. water (5 or 50 ml) was pipetted to each serum bottle, the bottles were sealed with teflon-lined rubber septa and aluminum crimp caps; the appropriate chemical was injected into each bottle with a gas-tight syringe. The liquid volume and the quantity of injected chemical were determined by gravimetric means. The six EPICS serum bottles were then incubated for 24 hours at 35°C. The liquid phase concentration in each bottle was analyzed for the determination of Henry's law coefficient. For each chemical, nine possible pairings of triplicate high and low liquid volume EPICS bottles provided nine possible estimates of Henry's law coefficient based on the following equation:

$$H_c = \frac{V_{l,2} - rV_{l,1}}{rV_{g,1} - V_{g,2}} \qquad (4)$$

where $$r = \frac{M_2 C_{l,1}}{M_1 C_{l,2}}$$

M = total mass of a volatile solute added to a serum bottle (μg),

V = volume (liter),

C = concentration (μg/l),

l, g = liquid and gas, respectively, and

1, 2 = bottle 1 and bottle 2, respectively.

Henry's law coefficients for all chlorinated ethylenes are listed in Table 3. Furthermore, Henry's law coefficients were also determined in the sludge system by direct measurement of gas and liquid equilibrium concentrations in a 120-ml serum bottle containing 100 ml anaerobic digested sludge.

Table 3. Summary of Henry's law coefficient (unitless)

Henry's coeff.	PCE	TCE	cis-DCE	trans-DCE	1.1-DCE	VC
in water system	1.06 (0.07)	0.61 (0.02)	0.26 (0.01)	0.65 (0.06)	1.40 (0.08)	1.45 (0.18)
in sludge system	0.81 (0.05)	0.52 (0.02)	0.22 (0.01)	0.53 (0.01)	1.63 (0.09)	1.39 (0.07)

( ) : standard deviation.

Biosorption

The characteristic constants of biosorption for each test chlorinated compound in the sludge system at 35°C were determined by using the Freundlich Isotherm. It has the following form

$$x/M = KC_e^{1/n} \tag{5}$$

where x = mass of adsorbate adsorbed on bio-adsorbent (μg),

M = mass of bio-adsorbent (gram),

K = equilibrium constant indicative of adsorptive capacity,

n = constant indicative of adsorption intensity, and

C_e = solution concentration at equilibrium after adsorption (μg/l).

The constants used in equation (5) were determined by plotting the mass adsorbed on per unit mass of bio-adsorbent versus the equilibrium concentration on log-log paper. The intercept is K, while 1/n is the slope. All of the six chlorinated compounds were tested for their biosorption in the 120 ml-serum-bottle system containing 100 ml of Hyperion digested sludge. For each test compound, 6 to 8 different masses (approximately 0.55 to 250 μmoles per bottle) were injected to each of a series of bottles. After 10 to 24 hours of incubation, both headspace gas and aqueous concentrations in each bottle were measured. The amount of chemical adsorbed on the sludge was obtained by subtracting the masses of the chemical in both gas and aqueous

phases from the total added chemical in each bottle. The experimental results are summarized in Table 4.

Table 4. Summary of Freundlich characteristic constants of biosorption

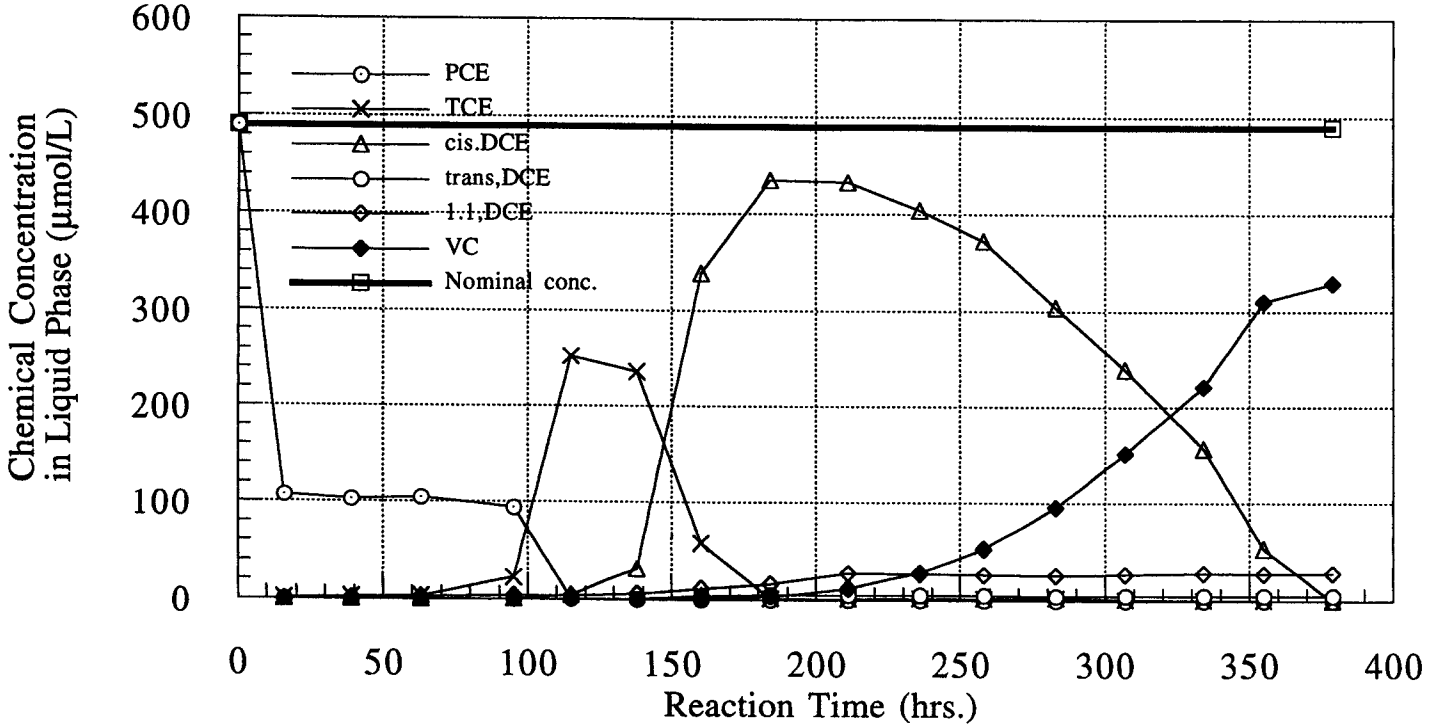
Constant	PCE	TCE	cis-DCE	trans-DCE	1.1-DCE	VC
K	0.2042	0.2907	0.1050	0.0185	0.0748	0.0910
n	1.0040	1.2309	1.3993	1.0821	1.1946	1.4186
Experimental range (Min.) (µg/l)	182.50	213.95	381.41	9993.42	273.83	2013.87
Experimental range (Max.) (µg/l)	60569	135161	180147	160443	113174	111741
Correlation coefficient (r)	0.9998	0.9928	0.9690	0.9874	0.9766	0.9234

Based on the Freundlich model, $x/M = K C_e \wedge (1/n)$, (Units: C_e = µg/l; x = µg; M = g).

Figure 8 shows typical results of the serum bottle experiment when sequential dechlorination of PCE to VC occurs. The measured concentration do not balance the added PCE concentration due to volatilization into the bottle's headspace and adsorption to the biosolids.

To account for volatilization and biosorption, the respective fractions were estimated using Henry's law and an adsorption isotherm. The amounts volatilized and adsorbed are significant, as the following example shows. If 49 µmoles PCE were added, it would correspond to 490 µmoles per liter (81.3 mg/l) in the liquid phase.

Fig. 8 The measured concentrations of chlorinated compounds in liquid phase without consideration of biosorption and volatility.



Note: When biosorption and volatility are not taken into consideration, the measured concentrations for each compound do not add up to the total nominal concentration.

Once these factors are taken into account, the sum of measured chemicals at each time point do add up to the cumulative additions into the system (compare Fig. 8 to Fig. 9).

Using Henry's law, this concentration would be reduced to only 421 μmoles/l (69.9 mg/l). The actual measured concentration was only 107 μmoles/l (17.8 mg/l). Using the isotherm shown in Table 4, to account for biosorption and volatilization, the calculated concentration in liquid phase is 104 μmoles/l, which is very close to the measured concentration of 107 μmoles/l. This indicates that the methodology is logically reasonable for performing mass balances of chlorinated compounds in the serum bottle system.

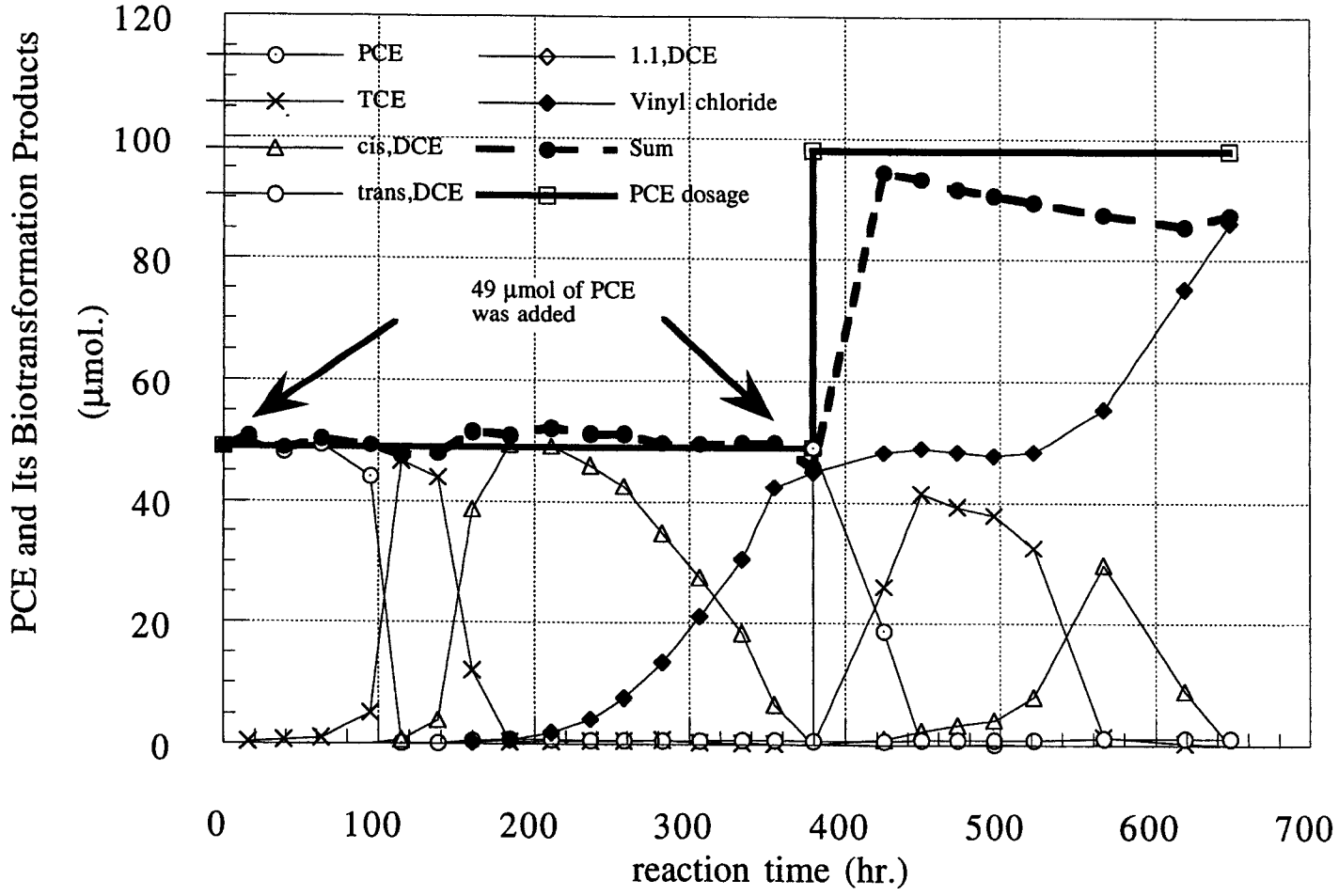
Figure 9 shows a calculated mass balance using the above procedures. It is observed that the total applied and measured plus calculated concentrations very closely balance. The small departure at the end of the test is probably due to ethylene production, which was not measured in this test.

4.3. PCE-dechlorinating ability and a general characteristic of sludges

PCE-dechlorinating ability:

PCE-dechlorinating ability was demonstrated in all of the first five anaerobic cultures tested. Vinyl chloride (VC) was the major accumulated dechlorination product in all of these cultures, excluding pond sediment (ethylene production was not measured at this stage because it was expected to be below the detection limit if there is any produced). PCE dechlorination only proceeded to cis-DCE in pond sediment culture; however, after mixing with Hyperion digested sludge, cis-DCE could be further dechlorinated to VC. This result suggests that the Hyperion sludge culture is mainly responsible for PCE dechlorination in the mixture.

Fig. 9 Mass balance of chlorinated compounds in the Hyperion sludge system when biosorption and volatility are included.



Hyperion sludge culture was observed to have the greatest ability to dechlorinate PCE among all the tested cultures. The first addition of 0.3 μmoles of PCE was totally degraded to VC in 3 days without any significant lag period. Under the condition of low initial PCE concentration, a total (3.45 μmoles) of 8 successive PCE additions were degraded to VC in 29 days. After demonstrating PCE dechlorination at low concentrations, higher concentrations were tested. The sludge culture still transformed PCE to VC, but , it took longer. The results are shown in Fig. 10. Different amounts of methanol addition (123, 25, and 2.5 μmoles) in a series of bottles did not significantly change PCE dechlorination rates. The results for different amount of methanol addition are provided in Appendix A (Fig. A-1 and A-2).

Pond sediment (unlike Hyperion sludge) demonstrated only partial PCE-dechlorinating ability (see Fig. 11). In all three test bottles (with different methanol additions), cis-DCE was the major end product. A significant lag period (from 3 to 17 days) was observed before PCE transformation was observed. In the bottle with the least methanol added (2.5 μmoles, each time), the lag period is shortest but PCE-dechlorinating ability was decreased when PCE dose was increased, which was believed to be a result of TCE accumulation. The whole data are provided in Appendix A (Fig. A-3 and A-4).

Mixture (1:1) of fresh Hyperion sludge and pond sediment showed a similar ability of PCE dechlorination to that observed in 100% Hyperion sludge (see Fig. 12). PCE dechlorination resulted in VC formation and accumulation. However in the sample with 25 μmoles of methanol added each time, about 50% of PCE was degraded only to cis-DCE. After longer incubation, it was converted to VC. It appears that the Hyperion

Fig. 10 PCE-dechlorinating ability and formation of intermediates in a batch culture of fresh anaerobic Hyperion sludge after successive additions of PCE and 123 μmoles of methanol.

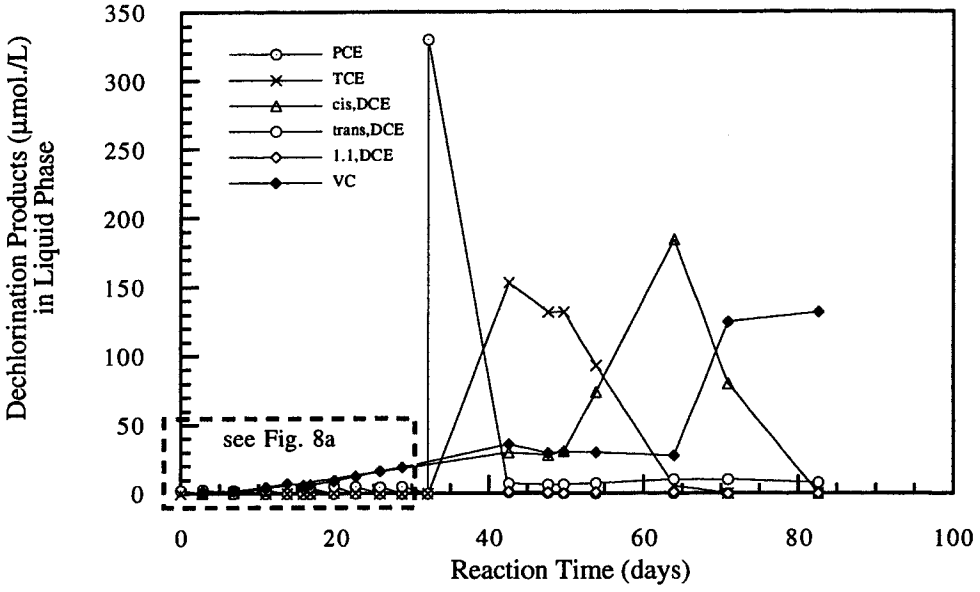
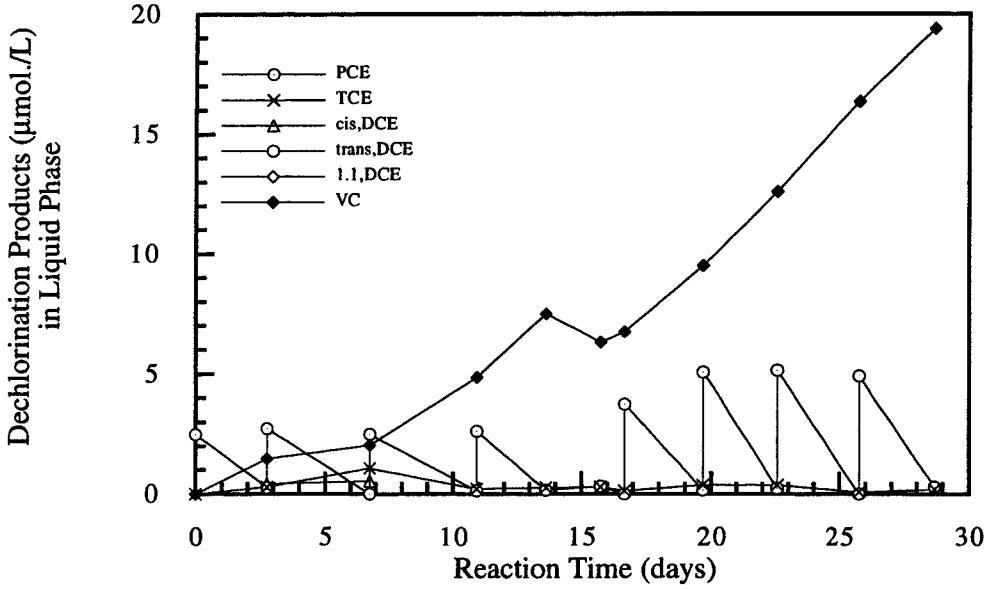


Fig. 10a PCE-dechlorinating ability and formation of intermediates in a batch culture of fresh anaerobic Hyperion sludge after successive additions of PCE and 123 μmoles of methanol.



Note: The major product from PCE dechlorination was VC.

Fig. 11 **PCE-dechlorinating ability and formation of intermediates in anaerobic pond sediment after successive additions of PCE and 123 μmoles of methanol.**

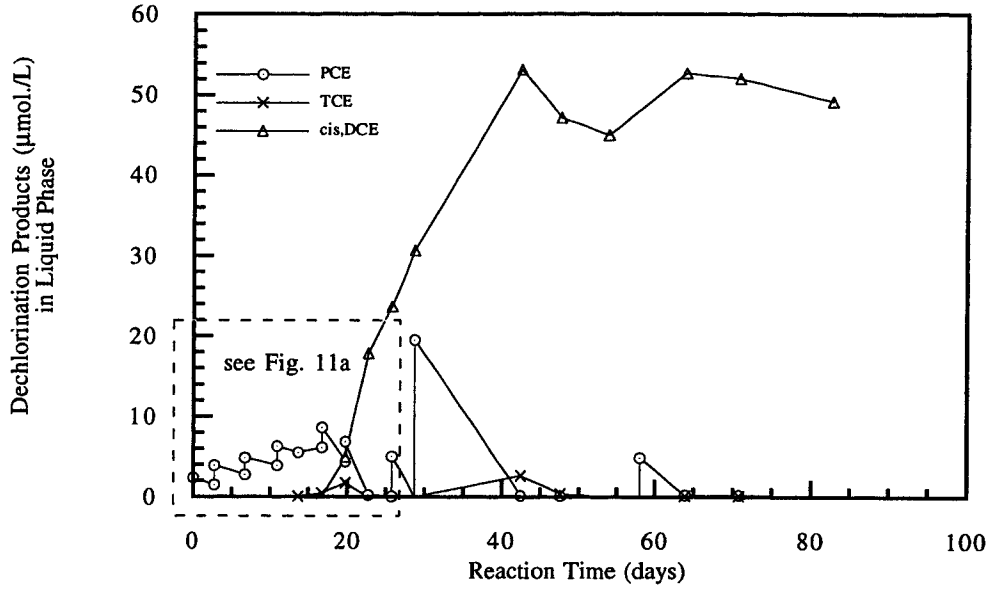


Fig. 11a **PCE-dechlorinating ability and formation of intermediates in anaerobic pond sediment after successive additions of PCE and 123 μmoles of methanol.**

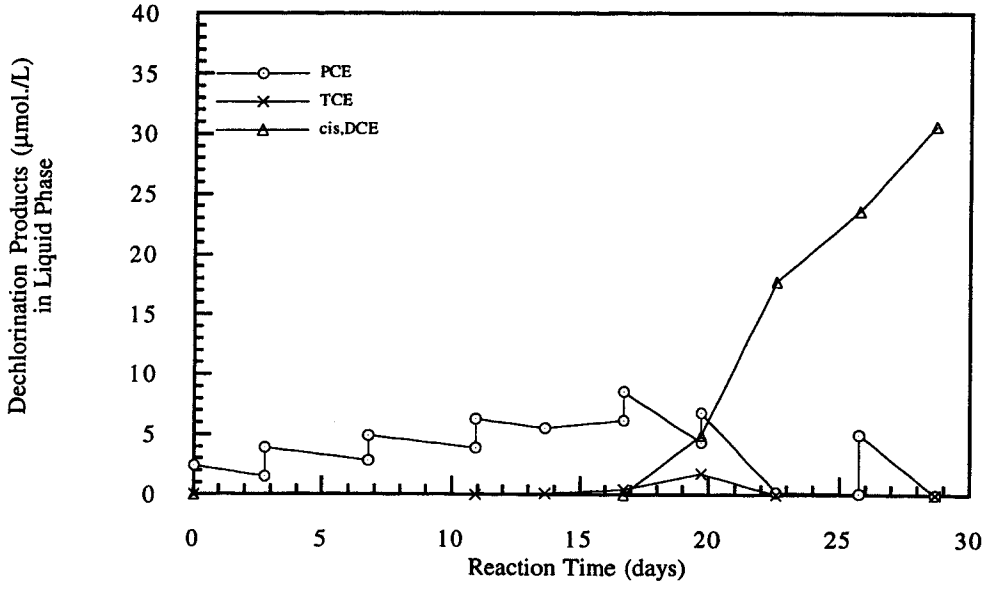


Fig. 12 PCE-dechlorinating ability and formation of intermediates in a mixture culture (1:1) of fresh anaerobic Hyperion sludge and anaerobic pond sediment after successive additions of PCE and 123 μmoles of methanol.

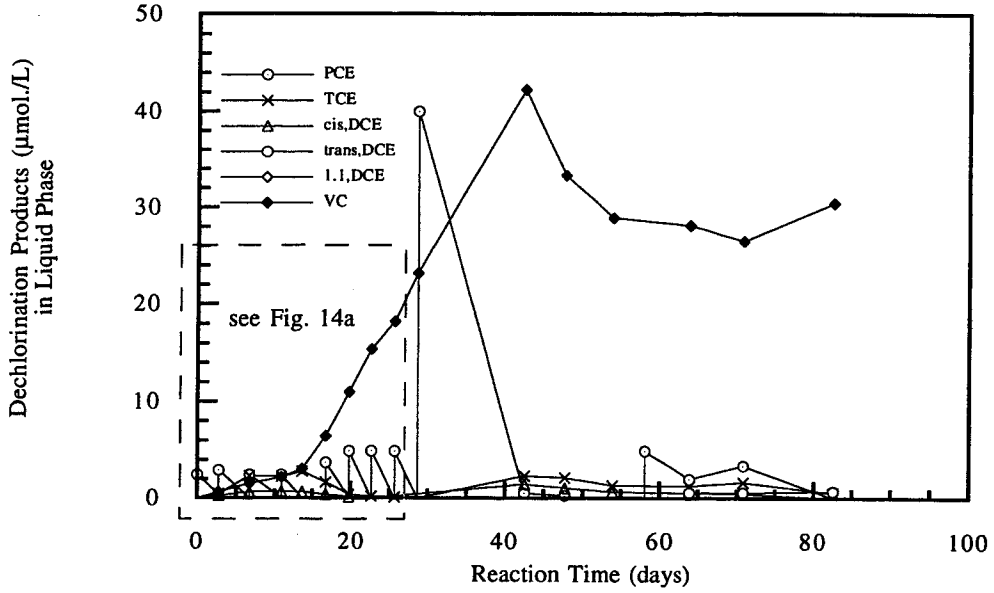
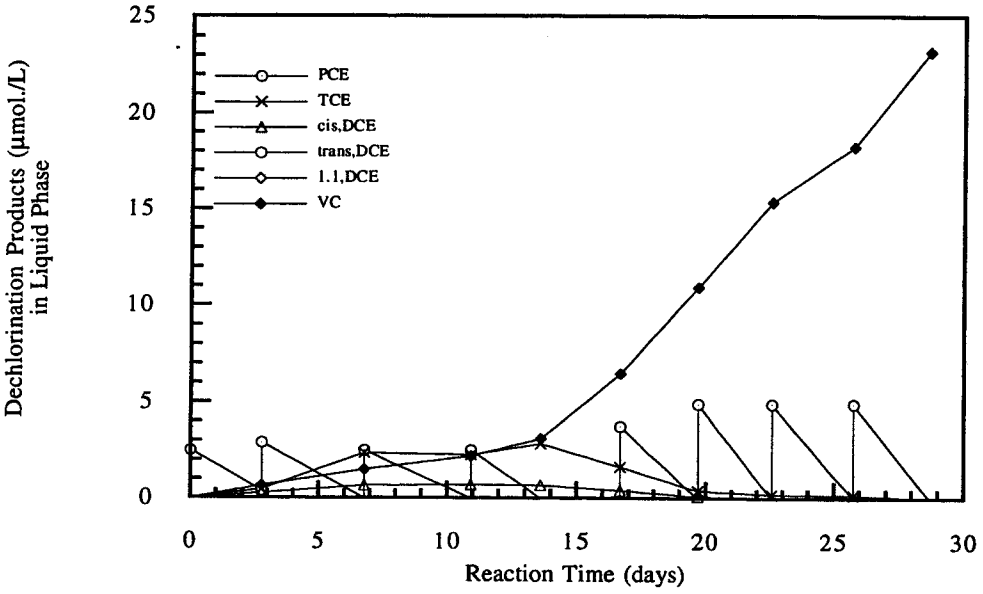


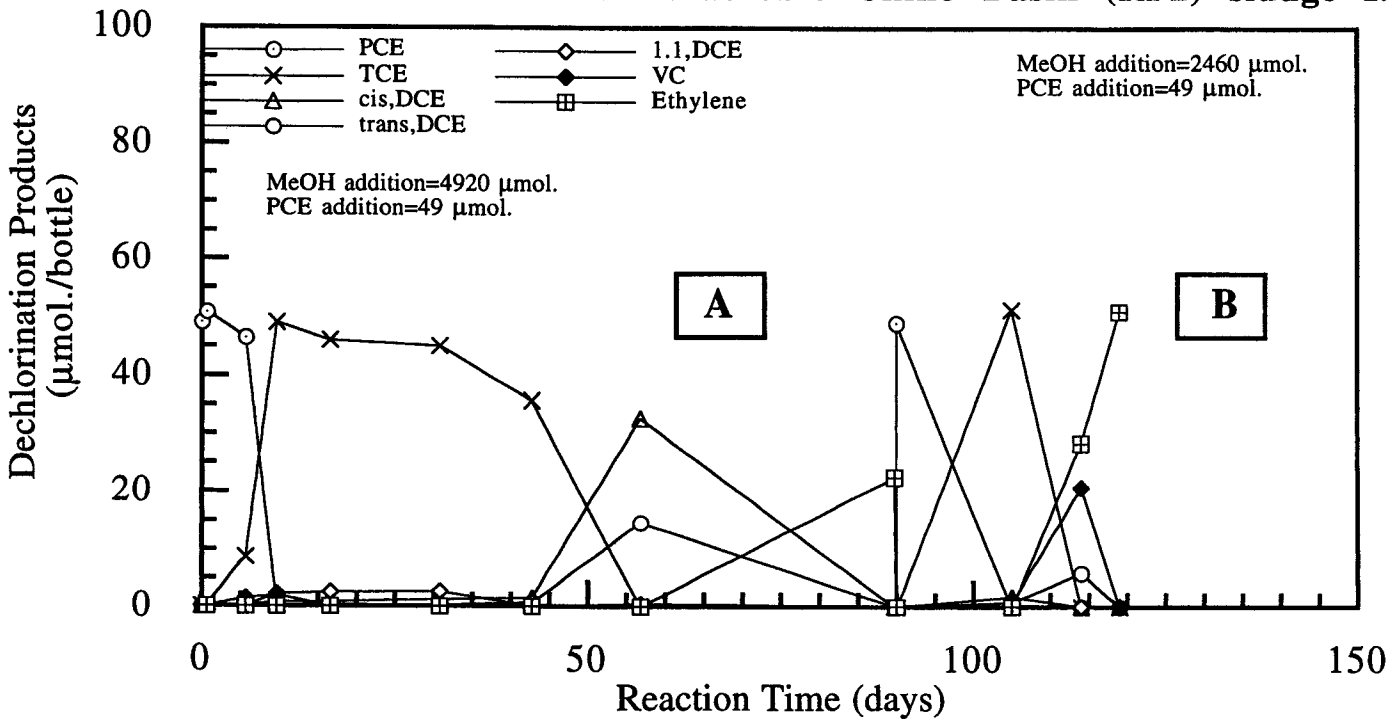
Fig. 12a PCE-dechlorinating ability and formation of intermediatess in a mixture culture (1:1) of fresh anaerobic Hyperion sludge and anaerobic pond sediment after successive additions of PCE and 123 μmoles of methanol.



sludge culture has contributed to the further dechlorination of cis-DCE. The dechlorination results for different amounts of methanol addition are provided in Appendix A (Fig. A-5 and A-6).

Chino Basin (RP2) sludge also showed partial PCE-dechlorinating ability in most cases. During the first 90 days, only one PCE addition was added to each bottle, and cis-DCE was found as the major dechlorination product in most of the bottles with various amounts of PCE and methanol added (Shown in Appendix A (Fig. A-7 through A-11)). However, from one of Chino Basin (RP2) sludge with 49 μmoles of initial PCE dose and a total methanol addition of 4920 μmoles, complete PCE dechlorination to non-toxic ethylene was observed at the end of the 90 day of incubation. About 22.6 μmoles of ethylene were produced from the dechlorination of 49 μmoles of PCE initially added. After that, PCE was completely dechlorinated to ethylene at even faster rates (51 μmoles of ethylene produced in 29 days). The results are shown in Fig. 13. To try to improve the PCE-dechlorinating ability after 90 days of operation, the supernatant in each bottle was replaced with the same volume of fresh anaerobic medium. The medium was prepared with 50% of fresh Hyperion sludge supernatant and 50% of D.I. water containing known concentrations of salts. Methanol was also used and the initial dose of PCE was 49 μmoles for each bottle. After two months of incubation, significant ethylene productions (50.9 and 44.3 μmoles, respectively) were observed from two more bottles, while cis-DCE was still the major end product of PCE dechlorination in three other bottles. The improvement of the dechlorinating-ability was achieved in part of these bottles. However, the factor(s) that has contributed to the improvement in complete PCE dechlorination ability is not clear.

Fig. 13 PCE-dechlorination ability and formation of intermediates in a batch culture of fresh anaerobic Chino Basin (RP2) sludge I.



Note: 1. 49 µmoles of PCE was added at the beginning of both stages (A & B).

2. Data obtained after 90 days indicates PCE dechlorination and formation of intermediates with initial addition of 49 µmoles of PCE, decreased concentration of methanol, and fresh medium replacement.

Methanol-enrichment culture was developed with the inoculum of Hyperion digested sludge. After one-year exposure of methanol (enriched by methanol (100 mM) as the only carbon and energy source), the enrichment culture was found not to degrade PCE any better than the original fresh Hyperion sludge; on the contrary, its ability to dechlorinate PCE is even worse. Over 79 days of incubation, PCE was mainly dechlorinated to TCE in methanol-enrichment culture with methanol (615 μmoles total) as the major carbon source (see Fig. 14). In the second bottle with the same amount of methanol under a H_2/CO_2 (80/20%) atmosphere (1.34 Kg/cm^2), a longer lag period (about 30 days) was observed before PCE transformation to TCE significantly occurred. TCE was the only observed end-product suggesting that the hydrogen can not induce later transformation steps.(see Fig. 15).

In the third methanol-enrichment culture bottle, VC was incubated with the same amount of methanol and hydrogen to test VC dechlorination. No detectable VC dechlorination was observed (Fig. 16).

Improved PCE-dechlorinating ability was found in concentrated mixture. After 80 days of incubation, the cell masses in the three methanol-enrichment culture bottles were centrifuged and transferred into one bottle, and then taken up to 100 ml by adding fresh anaerobic medium. 49 μmoles of PCE was completely dechlorinated to ethylene in 24 days. The same amount of PCE that was added thereafter was stoichiometrically transformed to ethylene at an even faster dechlorination rate within 9 days.(Fig. 17). The improvement of PCE-dechlorination ability was possibly due to the concentrated cell mass, replacement of nutrient medium, or induction.

Fig. 14 **PCE-dechlorination ability and formation of intermediates in a batch system of MeOH-enrichment cultures. (methanol was used as a sole carbon and energy source.)**

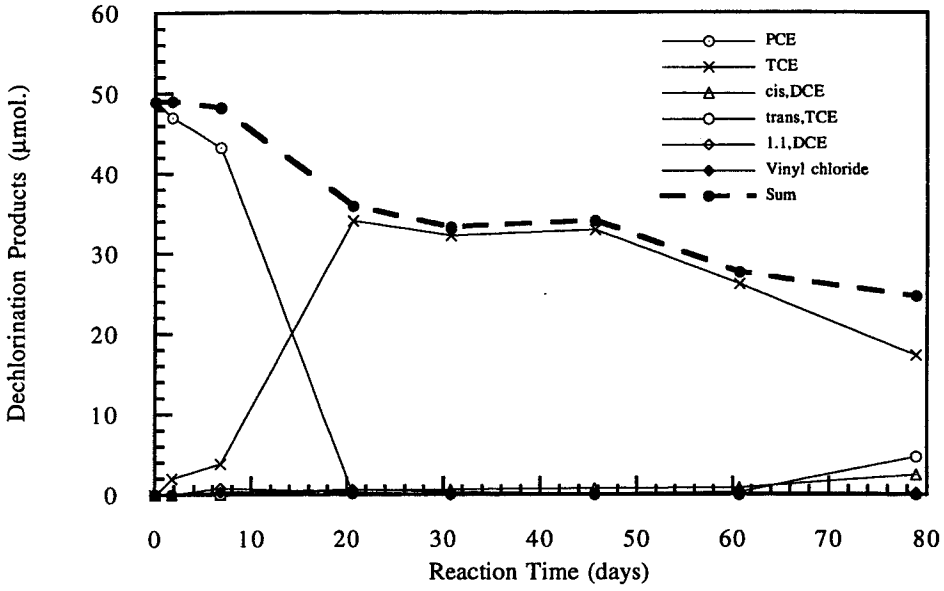
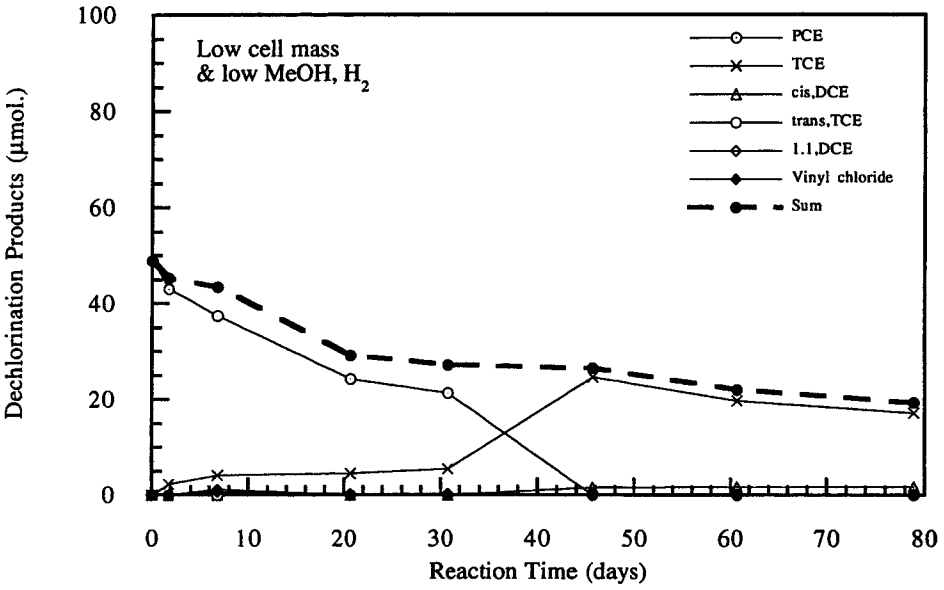


Fig. 15 **PCE-dechlorination ability and formation of intermediates in a batch system of MeOH-enrichment cultures. (methanol & H_2 were used as carbon and energy source.)**



Note: These results indicate that H_2 does not appear to have an effect on PCE dechlorination in the MeOH-enrich cultures.

Fig. 16 **Vinyl chloride-dechlorination ability and formation of intermediate in a batch system of MeOH-enrichment cultures.(methanol & H_2 were used as carbon and energy source.)**

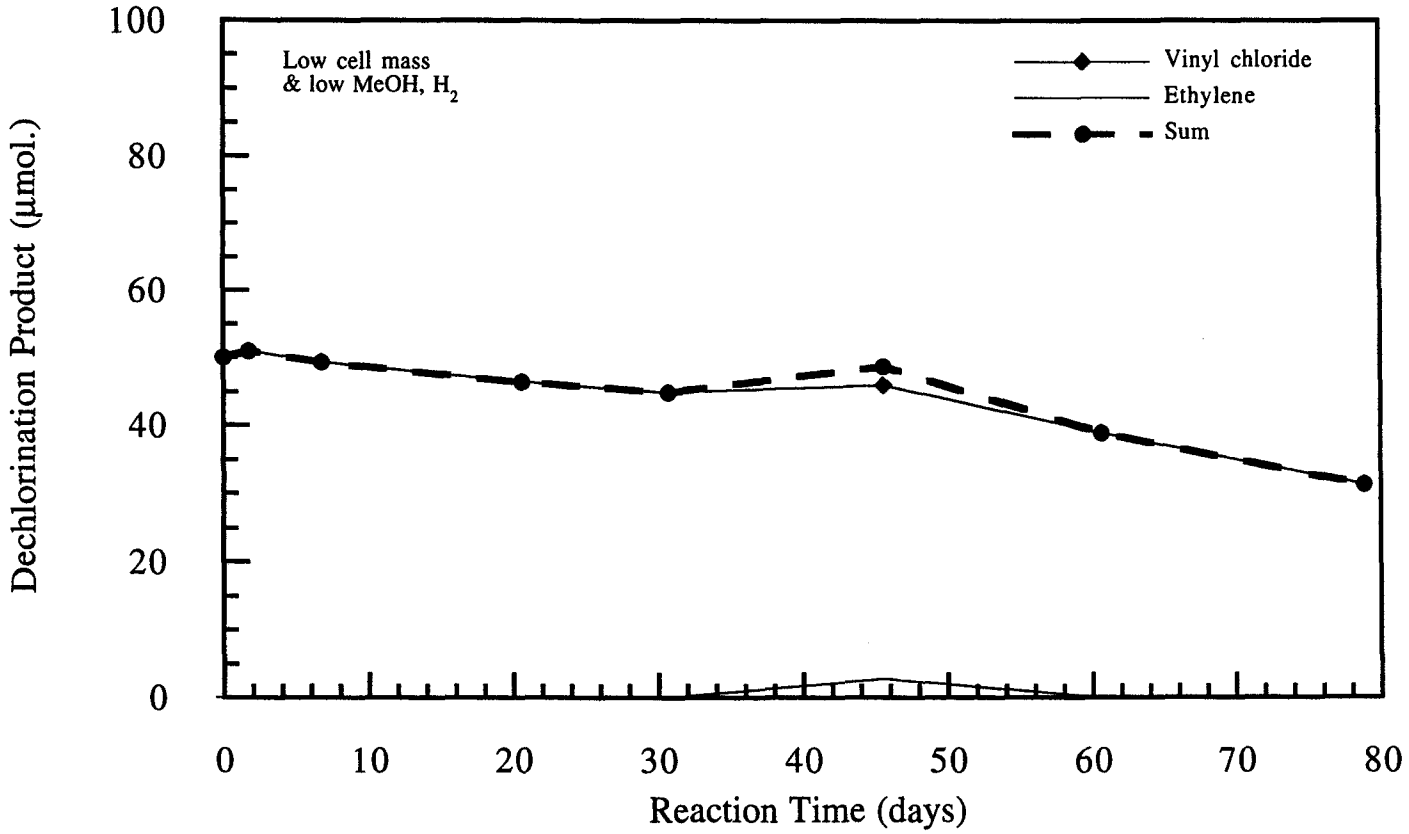
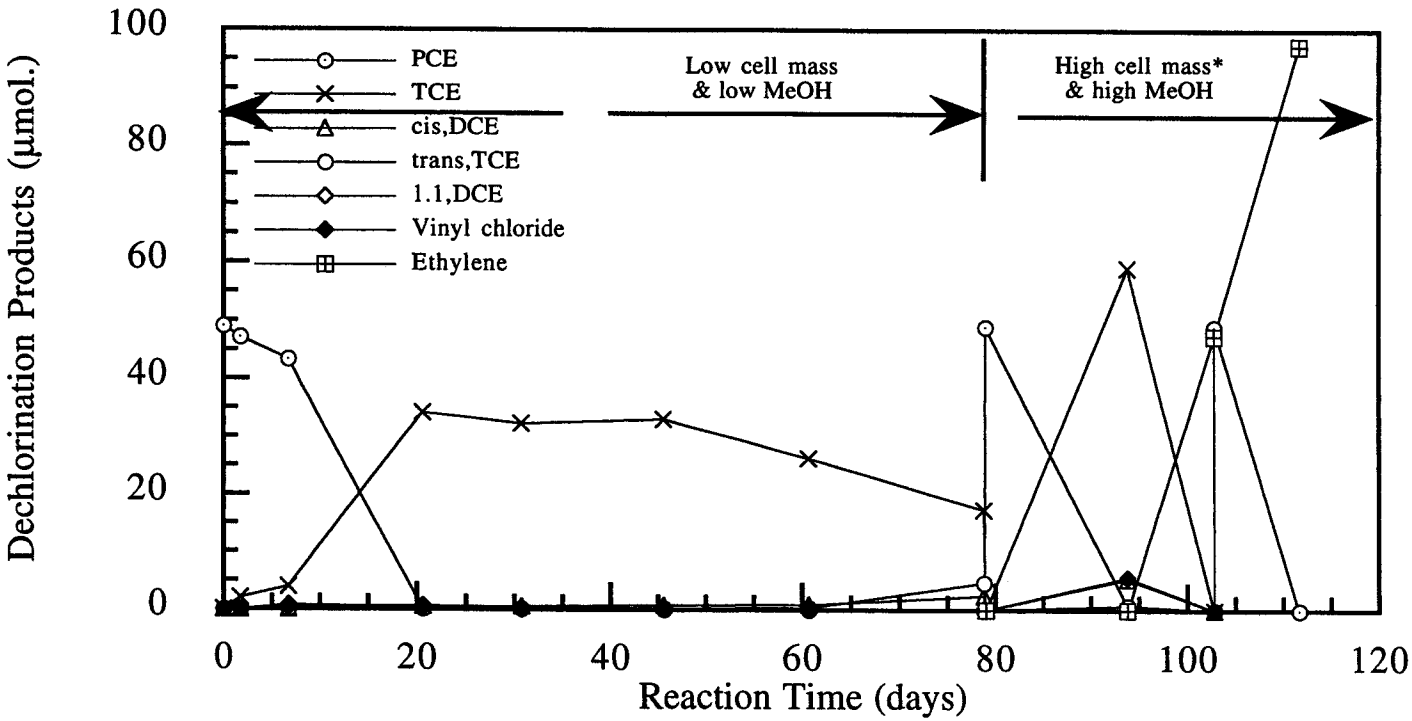


Fig. 17 **Improved PCE-dechlorination ability and formation of intermediates in a batch system of concentrated MeOH-enrichment cultures. (methanol was used as a sole carbon and energy source.)**



*: The high cell mass is the concentrated biomass of the 3 MeOH-enrichment cultures exposed to PCE+MeOH, PCE+MeOH+H_2, and VC+MeOH+H_2.

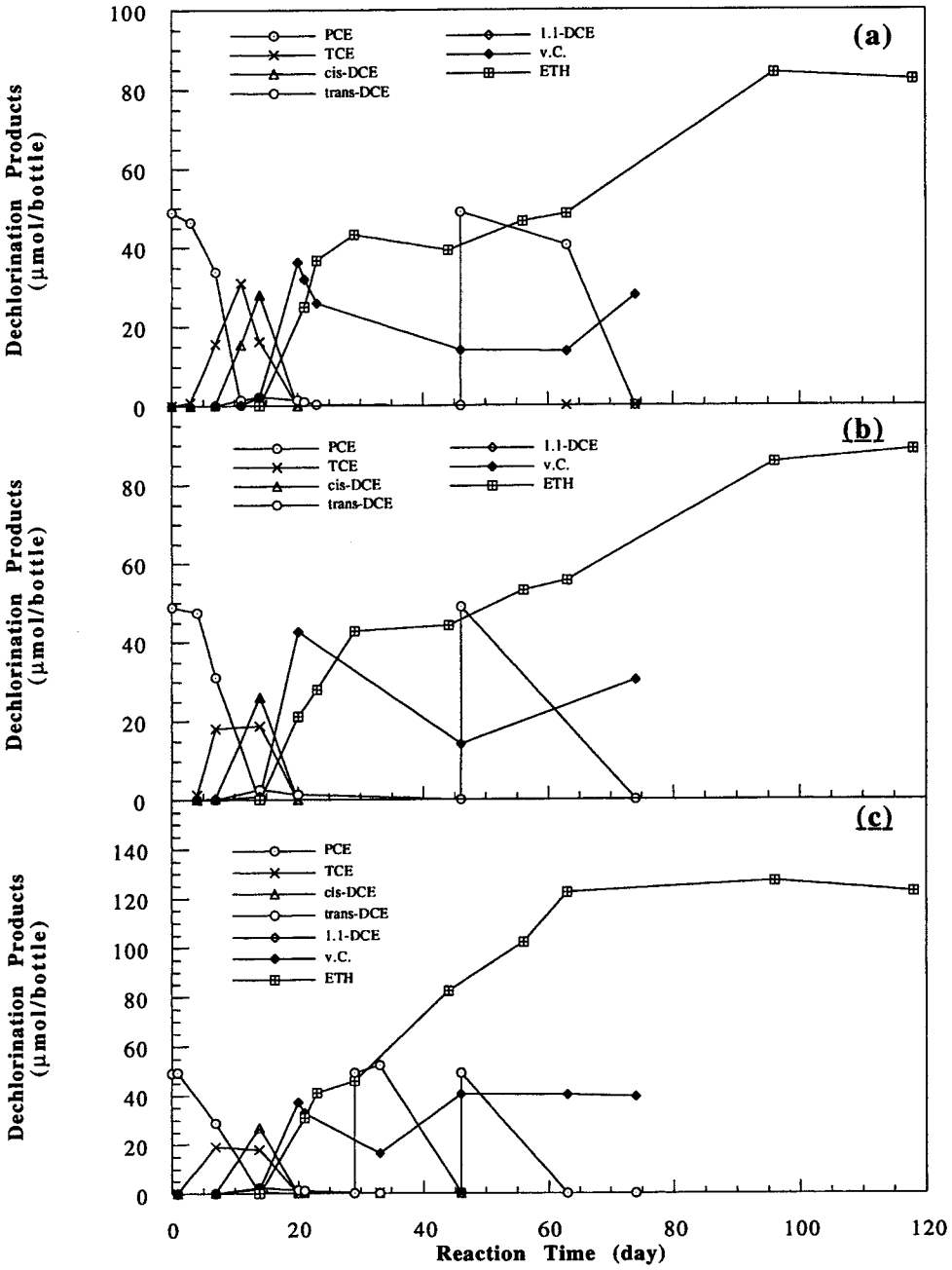
The improved PCE-dechlorination ability was possibly due to the concentrated cell mass, replacement of nutrient medium, or induction.

PCE dechlorination - A general characteristic of sludges:

The dechlorination of PCE in fresh anaerobic digested sludges obtained from five more different wastewater treatment plants (Terminal Island, Chino Basin (RP1), Alvarado, Valencia, and JWPCP Plant) was tested to evaluate if it is a general characteristic of sludges to metabolize PCE. Throughout the course of this study, the triplicate samples for each sludge performed similarly. The sequential reductive dechlorinations of PCE were observed in all sludges. Complete dechlorination of PCE to non-chlorinated ethylene was demonstrated in all test sludges, except for the Chino Basin (RP1) sludge culture in which PCE was only dechlorinated to cis-DCE after 4 months of incubation. The completely dechlorinating ability under a higher PCE concentration (162.6 mg/l) was also demonstrated in the cultures obtained from Valencia and JWPCP Treatment Plants. Without the addition of extra carbon source, PCE dechlorination was achieved in all sludge samples. This indicated that all fresh anaerobic digested sludges contain sufficient and adequate nutrients for PCE dechlorination.

Terminal Island sludge degraded PCE via sequential reductive dechlorinations. PCE was step-by-step dechlorinated to ethylene through TCE, cis-DCE and vinyl chloride. About 25 μmoles of ethylene was formed from the dechlorination of 49 μmoles of PCE after 20 days of incubation, while the rest of PCE was ending up as vinyl chloride. Over 30 days of incubation, at least 40 μmoles of ethylene was observed in all triplicate samples. More ethylene production was observed after repetitive PCE addition to these bottles. At the end of this experiment, 82, 88, and 142 μmoles of ethylene were accumulated in the bottles which received 98, 98, and 147 μmoles of PCE, respectively. The data are shown in Fig. 18.

Fig. 18 **The complete dechlorination of PCE in a batch culture of fresh anaerobic sludge obtained from Terminal Island Wastewater Treatment Plant.**

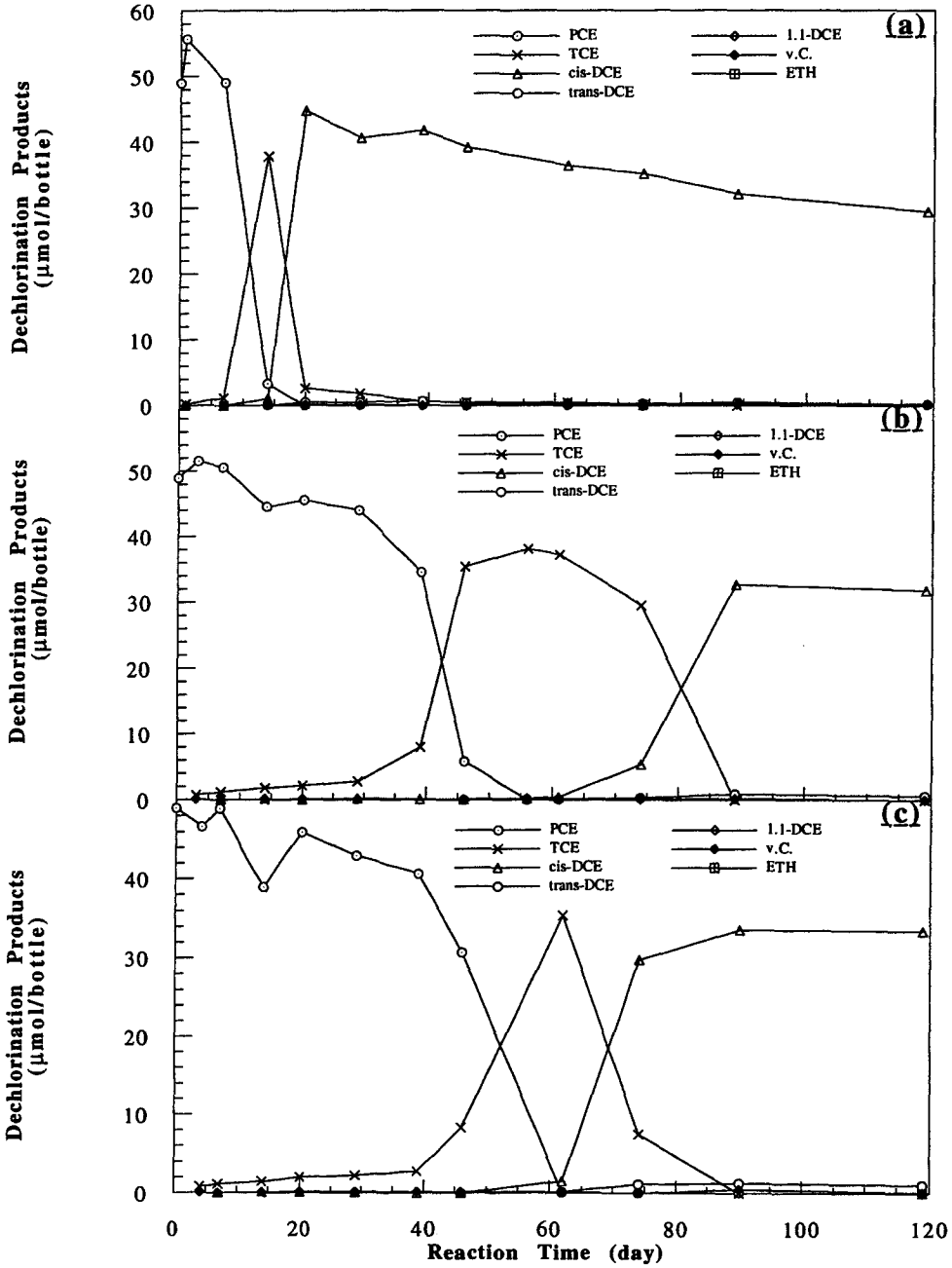


Note: figures a-c represent analyses of 3 Terminal Island wastewater sludge samples.

Chino Basin (RP1) sludge only dechlorinated PCE partially to cis-DCE. In triplicate samples, cis-DCE was the major end product. The complete dechlorination product, ethylene, was never detected. However, no any significant lag period was observed for PCE dechlorination even though it was dechlorinated to TCE very slowly during the first week (see Fig. 19). It took much longer (about 50 days observed from two of triplicate samples) to totally degrade PCE to TCE. The transformation of TCE to cis-DCE spent another 40 days. After that, no additional dechlorination (VC formation) was noted. Although cis-DCE was continuously declined from day 20 to 119 (see Fig. 19a), it was most likely due to leakage losses because no V.C. accumulation was observed. For Chino Basin (RP1) sludge, much more methane production was observed. After 20 days of incubation, a total of about 3000 μmoles of methane was yielded from Chino Basin sludge, while only 750 μmoles of methane production was observed from Terminal Island sludge samples. If it were the reason for the slow and partial dechlorination of PCE is not clear. The another factor which may cause the worse dechlorinating ability is that Chino Basin (RP1) Plant only receives less than 5% of their wastewater from industrial sources. The exposure to a low percentage of industrial wastewater may result in a dramatic reduction in the complexity of microorganisms involved in the sludge. Consequently, many dechlorinating bacteria may be excluded.

Alvarado sludge performed PCE dechlorination similarly to that in Terminal Island sludge. However, for an unknown reason, it also took longer (about 30 - 35 days) to completely dechlorinate 49 μmoles of PCE to VC. In addition, cis-DCE was no longer the only major product of TCE dechlorination. A significant amount of trans-DCE (15 μmoles; about 30% of TCE) was observed to accumulate in the sludge system during the transformation of TCE to DCE. Fortunately, it was not so persistent as

Fig. 19 **The complete dechlorination of PCE in a batch culture of fresh anaerobic sludge obtained from Chino Basin (RP1) Wastewater Treatment Plant.**

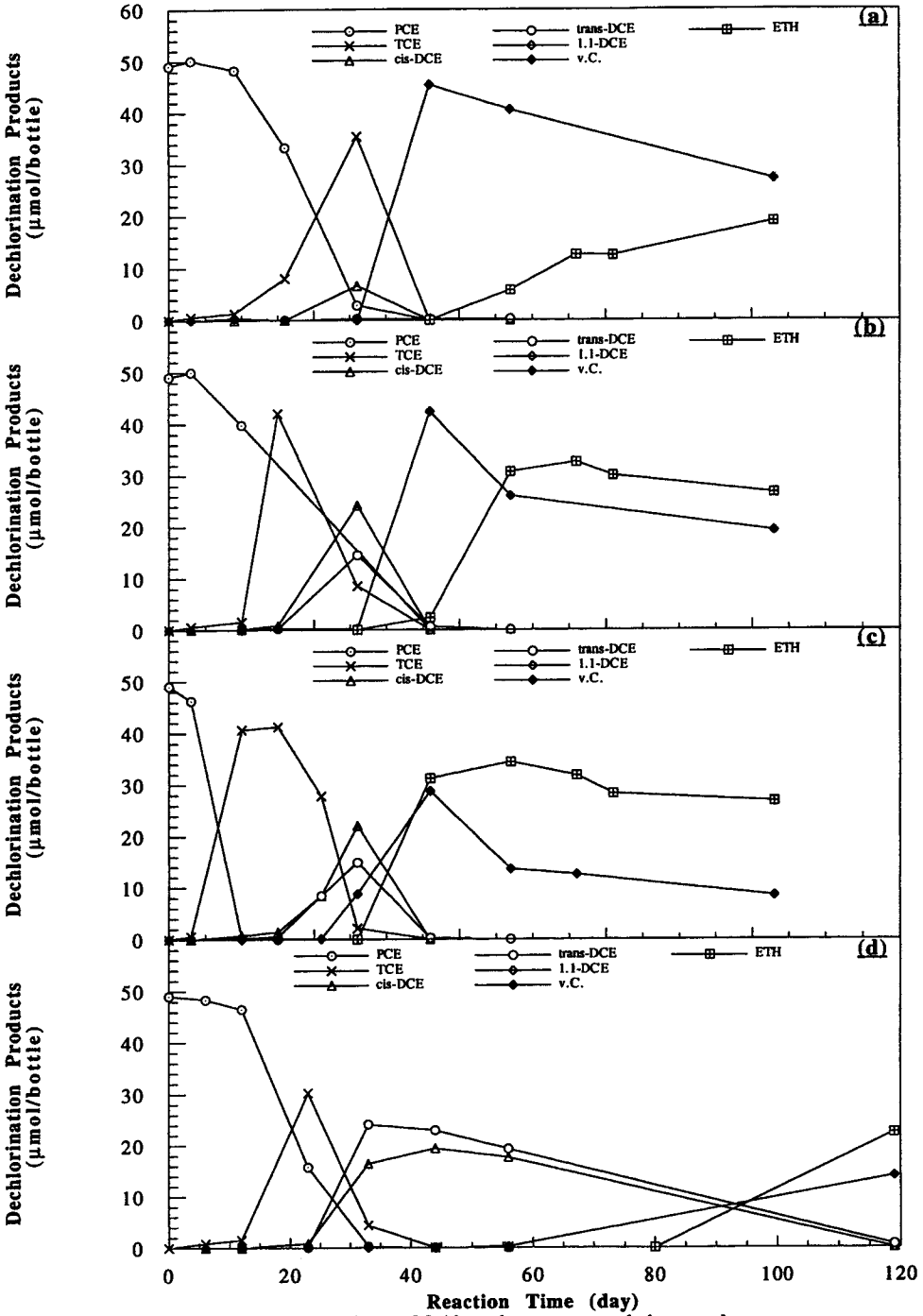


Note: The time needed for each sample to dechlorinate PCE and TCE is different. It may be due to the variation within the original sludge.

expected. Trans-DCE and cis-DCE were completely degraded to VC in 10 days. Over 83 days of incubation, 19 - 30 μmoles of ethylene were formed in the triplicate samples. To investigate the dechlorination ability in the old sludge, an additional sludge sample was incubated 3 days under the same conditions without PCE addition. After that, PCE was injected into the bottle. PCE still can be completely dechlorinated to ethylene but it took even longer in each step of PCE dechlorination. More trans-DCE (24 μmoles) accumulated during the transformation of TCE to DCE than that observed in fresh sludge samples. At the end of the experiment (119 days of incubation), a total of 22.5 μmoles of ethylene was measured. The data are shown in Fig. 20. During the period of experiment, an average of 2500 μmoles of methane production was observed with the fresh sludge samples, while the old sludge sample produced 3000 μmoles of methane in total.

Valencia sludge took only 7 days to dechlorinate 49 μmoles of PCE to cis-DCE, without any significant formation of the other two isomers (trans-DCE and 1.1-DCE). All of the cis-DCE was further dechlorinated to vinyl chloride in the next 14 days. About 4.5 to 6 μmoles of ethylene were produced from the triplicate samples on day 28. After that, ethylene was formed at an even slower rate. The average methane production from triplicate samples was around 2500 μmoles. The dechlorination ability under a higher initial PCE concentration (98 μmoles of PCE per bottle) was investigated in an additional sludge sample. Interestingly, the doubled amount of PCE did not take any longer for complete dechlorination. Within 7 days, PCE was totally degraded to cis-DCE. All of the cis-DCE was found to end up as vinyl chloride on day 21. However, much more ethylene (22.2 μmoles) was produced on day 28, compared to the ethylene production from the sample with a lower initial PCE concentration. This indicated that a

Fig. 20 **The complete dechlorination of PCE in a batch culture of fresh anaerobic sludge obtained from Alvarado Wastewater Treatment Plant.**



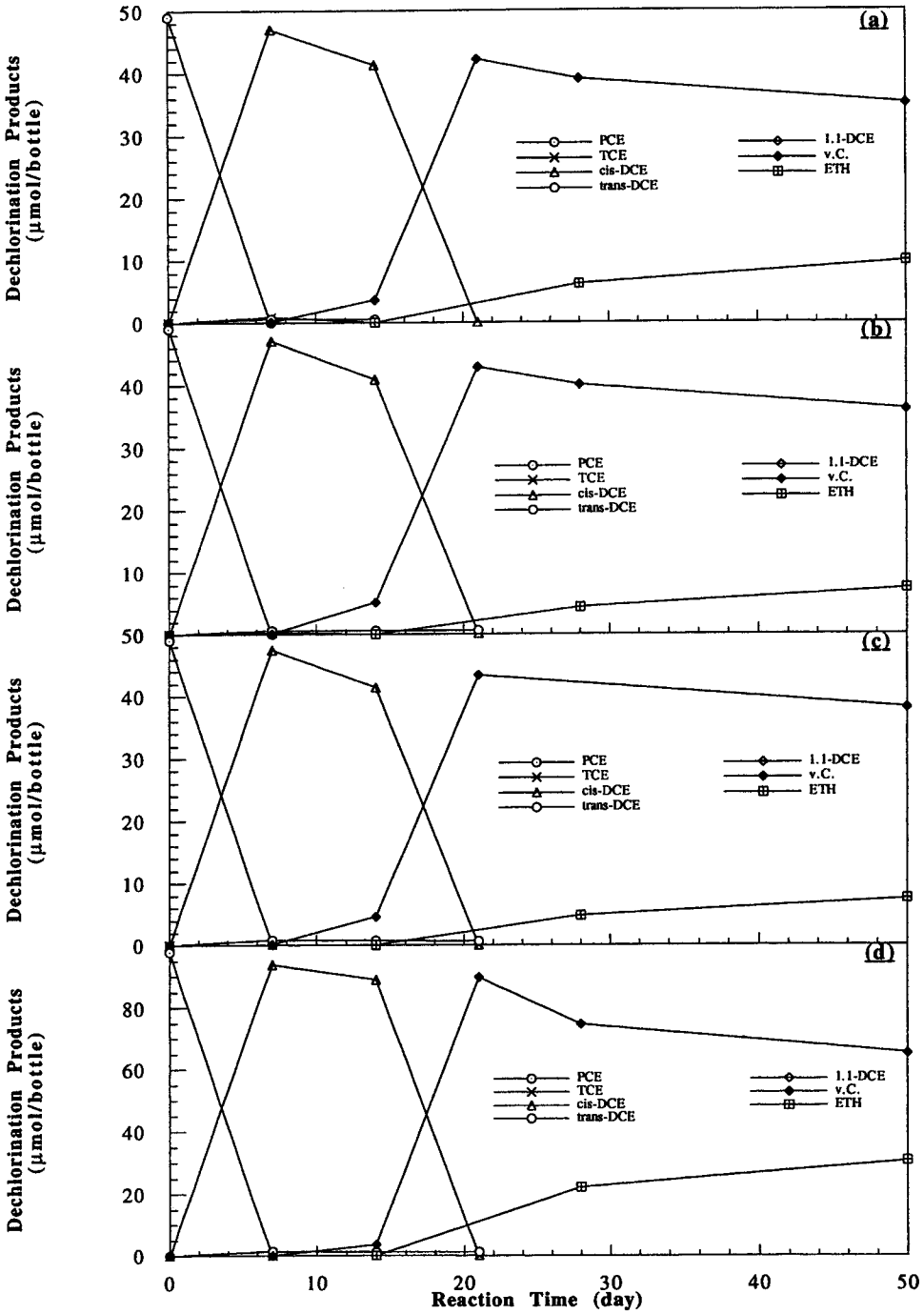
Note: 1. a-c represent analyses of 3 Alvarado wastewater sludge samples.
2. d represents the results from an older sludge sample (incubated 3 days before PCE inoculation).

higher concentration of vinyl chloride may not inhibit the formation of ethylene in the sludge system. However, during the same period, only a smaller amount of methane (about 1900 μmoles) was produced from the high PCE concentration sample. Whether it was due to the competition between methanogenesis and reductive dechlorination for electrons available in the system, or the toxicity of chlorinated compounds to methanogens is not known currently. The results are shown in Fig. 21.

JWPCP sludge took the shortest period of time (less than 13 days) to degrade 49 μmoles of PCE to VC and make ethylene (1.8 - 2.3 μmoles) emerge earliest from the complete dechlorination of PCE among all tested sludge. However, the ethylene production rates observed from JWPCP sludge were slower than that from Terminal Island sludge. Within 29 days, JWPCP sludge only yielded 18 - 19 μmoles of ethylene from the dechlorination of 49 μmoles of PCE, while about 43 - 45 μmoles of ethylene had been produced in Terminal Island sludge. At the end of the experiment (49 days), 34 - 40 μmoles of ethylene and an average of 2500 μmoles of methane were accumulated in triplicate samples. For the sample with a higher initial PCE concentration (98 μmoles per bottle), the complete PCE dechlorination proceeded similarly to that in Valencia sludge, but with a higher final ethylene production (83 μmoles). Again, the final methane production (1700 μmoles) was much lower than that produced from the samples with a lower initial PCE concentration. The data are shown in Fig. 22.

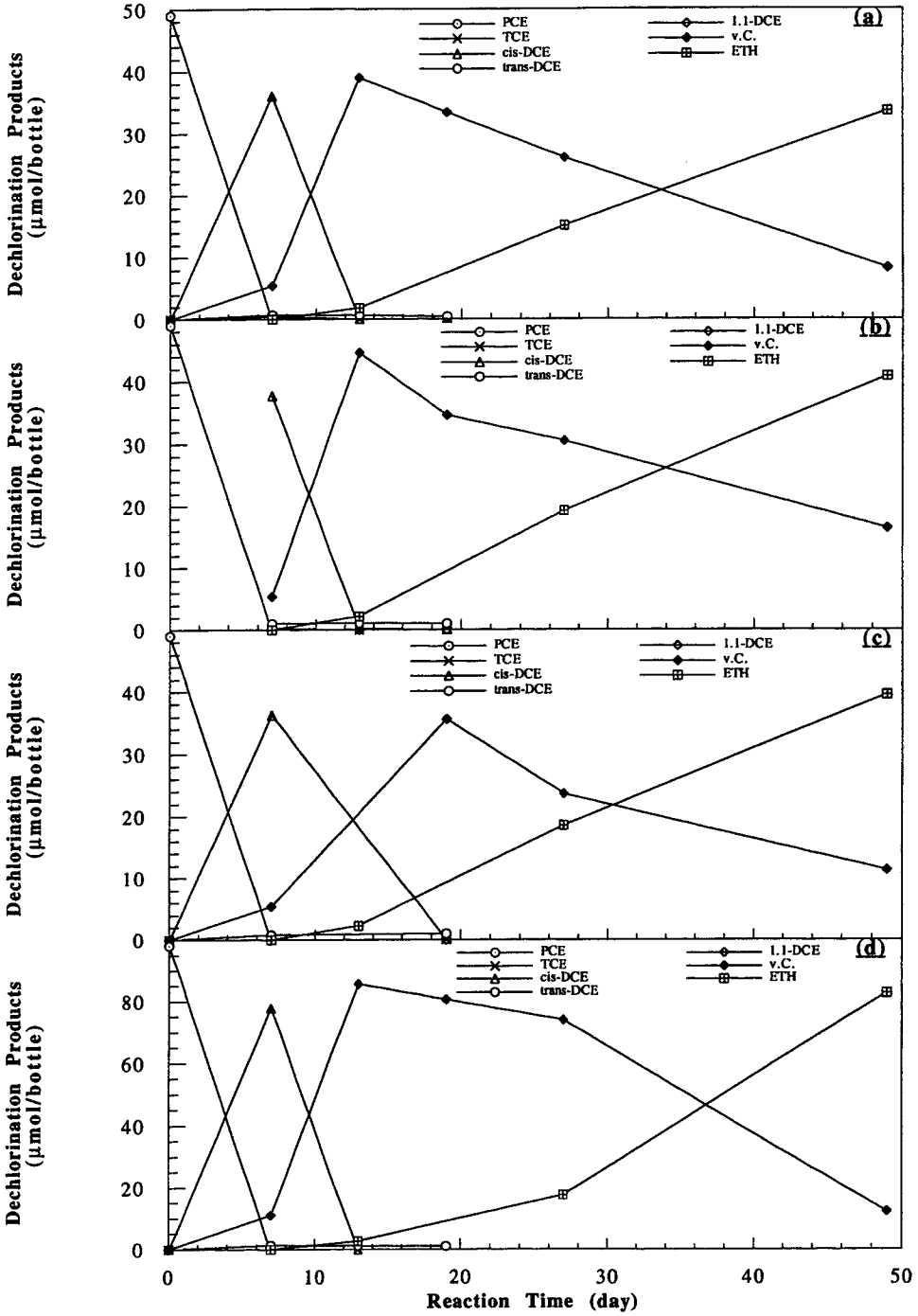
Based on the above results, reductive PCE dechlorination was observed with all tested anaerobic digested sludges. This indicates that the dechlorination of PCE may be a general characteristic of anaerobic digested sludges. However, due to an unknown

Fig. 21 **The complete dechlorination of PCE in a batch culture of fresh anaerobic sludge obtained from Valencia Wastewater Treatment Plant.**



Note: 1. a-c represent analyses of 3 Valencia wastewater sludge samples.
2. d represents the results of PCE dechlorination under a higher initial PCE concentration.

Fig. 22 **The complete dechlorination of PCE in a batch culture of fresh anaerobic sludge obtained from JWPCP Wastewater Treatment Plant.**



Note: 1. a-c represent analyses of 3 JWPCP wastewater sludge samples.
2. d represents the results of PCE dechlorination under a higher initial PCE concentration.

reason, digested sludges from different sources varied in their potential to completely dechlorinate PCE to ethylene.

4.4. Treatability of chlorinated compounds

The biodegradability (defined as the capacity of a substance to undergo microbial attack, Painter and King, 1985) of each chlorinated compound tested was significantly different from each other in fresh Hyperion digested sludge. However, when the period of incubation was long enough, all of the six tested chlorinated compounds were anaerobically biodegradable in Hyperion sludge. The observed lag periods for trans-DCE, 1.1-DCE, and VC were 12, 4, and 10 days, respectively under a low initial dose (0.3 μmoles of each tested compound plus 5 μmoles of methanol per bottle). No lag was observed in the degradation of PCE, TCE, and cis-DCE. After 6 additions into each bottle, PCE ,TCE, cis-DCE tended to accumulate. In order to determine if the dechlorination capacity in each bottle has been consumed, 49 μmoles (much higher than the dose (0.3-0.6 μmoles) used before) of each chlorinated compound plus 123 μmoles of methanol were added to each corresponding bottle and the remaining dechlorination capacity of the Hyperion sludge was tested in each bottle. After another 58 days of incubation, all of added compounds in each bottle were either partially or completely dechlorinated and ethylene was detected in all the test bottles. A summary of the results is given in Table 5. This suggested that all six chlorinated compounds can be degraded and the higher concentrations of each chlorinated compound may have contributed to the faster dechlorination rates observed in each bottle. Among all of the chlorinated compounds tested, dechlorination of PCE, TCE, and cis-DCE was more likely to result

Table 5. Ability of anaerobic Hyperion sludge to degrade various chlorinated ethylenes to ethylene.

Sample	Time (days)	Initial dose (µmol.)	Dechlorinated products PCE (µmol.)	TCE (µmol.)	cis-DCE (µmol.)	trans-DCE (µmol.)	1.1-DCE (µmol.)	V.C. (µmol.)	ETH (µmol.)
PCE	0	49.00	49.00						
	6		45.99	0.73	0.38	0.00	0.00	2.13	
	20		0.49	0.07	0.00	0.36	0.00	37.88	
	34		0.00	0.00	0.00	0.00	0.00	8.01	37.12
	58		0.00	0.00	0.00	0.00	0.00	3.05	41.48
PCE with	0	49.00	49.00						
higher conc.	6		48.80	0.37	0.04	0.00	0.00	3.61	
of MeOH	20		42.65	0.88	0.12	0.01	0.00	1.17	
(1230 µmoles)	34		15.44	1.01	0.11	0.00	0.00	18.95	0.30
	58		0.06	0.00	0.00	0.00	0.00	15.52	17.33
TCE	0	49.00		49.00					
	6			34.33	0.16	0.13	0.00	1.92	
	20			17.06	2.61	0.46	0.00	6.38	
	34			0.00	0.15	0.00	0.00	10.91	11.90
	58			0.00	0.00	0.00	0.00	2.94	22.66
cis-DCE	0	49.00			49.00				
	6				48.44	0.11	0.00	2.21	
	20				0.00	0.00	0.00	29.65	
	34				0.00	0.00	0.00	6.00	44.58
	58				0.00	0.00	0.00	4.71	53.13
trans-DCE	0	49.00				49.00			
	6					47.81	0.00	0.06	
	20					35.61	0.00	0.95	
	34					24.50	0.00	3.19	N.D.
	58					18.85	0.00	5.59	0.23
1.1-DCE	0	49.00					49.00		
	6						43.29	1.50	
	20						0.00	31.64	
	34						0.00	22.16	6.41
	58						0.00	19.01	9.02
V.C.	0	49.00						49.00	
	6							44.62	
	20							41.70	
	34							41.41	N.D.
	58							33.28	5.38

Note: the methanol addition in each sample was 123 µmoles.

in ethylene production. The detailed data for the treatability test of each compound are provided in Appendix A (Fig. A-12 through A-17).

Figure A-18 (in Appendix A) shows the result of reduced ethylene production from PCE (49 μmoles) dechlorination with higher methanol addition (1230 μmoles). With the higher added amount of methanol, PCE dechlorination was slower than that with lower methanol dosage (Fig. A-12 shown in Appendix A).

The PCE-dechlorinating capacity of the fresh Hyperion digested sludge was tested by successively adding PCE (49 μmoles each time) into each of two separate sludge bottles incubated with and without methanol addition, respectively. Similar results (as shown in Fig. A-19, Appendix A) were observed from both cases. After 245 μmoles of PCE were added, the sludge system appeared to lose the dechlorinating ability and the major end products accumulated were TCE and a lower than expected amount of VC. The accumulation of TCE may be due to nutrient deprivation even though 246 μmoles of methanol have been added accompanying with each PCE addition.

4.5. Reductive dechlorination progression

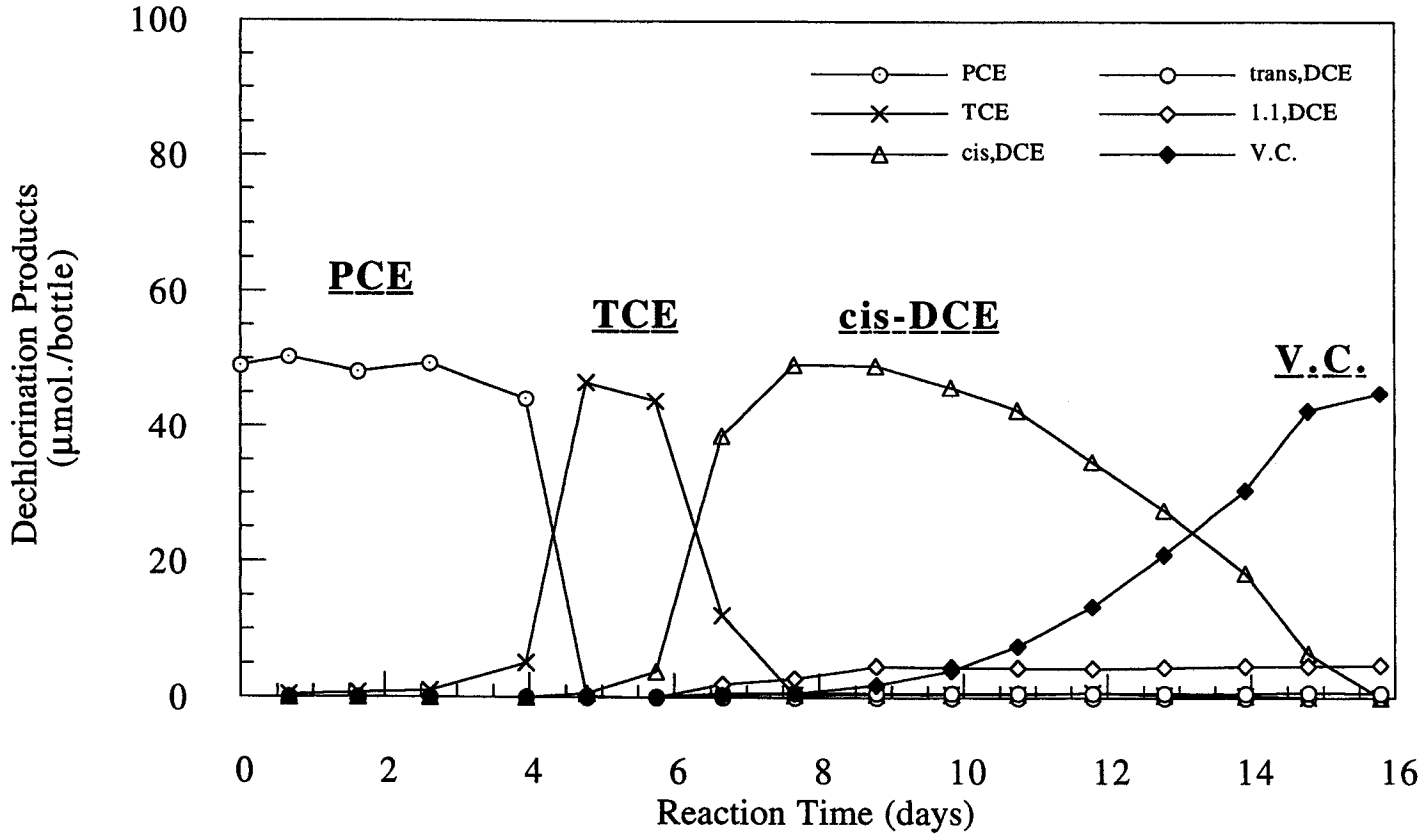
Vinyl chloride has been observed as a major intermediate in reductive dechlorination. The mechanism and path of PCE and other chlorinated compound dechlorination through VC to ethylene is still not clear. To further understand the mechanism of reductive dechlorination in the anaerobic digested sludge system, all of their possible dechlorinated products were investigated in fresh Hyperion sludge. The

results indicate that all of the tested chlorinated ethylenes may be dechlorinated to ethylene through an identical co-metabolic route (i.e. PCE -> TCE -> DCE -> VC -> ETH). The less chlorinated ethylenes seemed only to utilize part of the route, while the fully chlorinated ethylene (PCE) utilized the whole route during the reductive dechlorination.

Reductive dechlorination progression of PCE at a high PCE concentration in fresh Hyperion digested sludge is shown in Fig. 23. After a lag period of 4 days, PCE started to dechlorinate to TCE. After only one more day, 49 μmoles of PCE completely disappeared and 47 μmoles of TCE were created. TCE thereupon was transformed to cis-DCE (49 μmoles) in the following 3 days of incubation without any significant formation of the other two isomers (trans-DCE and 1.1-DCE). For an unknown reason, it took longer (8 days) to completely dechlorinate cis-DCE to VC. Only 45 μmoles of VC, less than expected, was measured. The difference between PCE consumption and VC production was most likely due to leakage losses and undetermined ethylene production. This high recovery (around 92%) suggests that PCE was stoichiometrically dechlorinated, through TCE and cis-DCE, to VC. It was sequentially reduced. In each step, one chlorine atom in the chlorinated compound was replaced with a hydrogen coming from an electron donor. A similar result (data not shown) was observed from the same experiment conducted under conditions without methanol. This suggested that sufficient nutrients contained in fresh digested sludge are adequate for PCE degradation.

The step-by-step dechlorination of PCE was also monitored from the sample with a low initial PCE concentration (see Fig. A-20 in Appendix A). There was no lag

Fig. 23 Reductive dechlorination progression of PCE at a high PCE concentration in a fresh anaerobic Hyperion sludge.



Note: The steps of PCE dechlorination appear to more defined at higher PCE concentration.

period observed and the characteristic step-by-step dechlorination was not as clear as in the high concentration PCE experiments. The steps of PCE dechlorination were more defined at a higher PCE concentration.

Reductive dechlorination progression of TCE also went through cis-DCE to VC. The result is shown in Fig. 24. Compared to PCE dechlorination, a longer lag period (about 12 days) was required. After the lag period, it only took 7 days to entirely dechlorinate TCE to VC. But, in PCE bottle, the same amount of TCE produced from the first step of PCE dechlorination took 11 days to terminate at VC.

Reductive dechlorination progression of three isomers of DCE is shown in Fig. 25 to 27. Interestingly, the transformations of all three isomers proceeded via the similar reductive dechlorination, all resulting in the formation of lower chlorinated ethylene, VC. Among them, trans-DCE was observed to be most resistant to reductive dechlorination. Trans-DCE took 17 days before its dechlorination was observed significantly, while cis-DCE and 1.1-DCE only took 6 and 5 days, respectively. After that, trans-DCE was dechlorinated at a much slower rate than that observed in cis-DCE and 1.1-DCE samples. The VC produced from dechlorination of trans-DCE was not accumulated as much as that from other two isomers. Most of it ended up as ethylene. This is possible due to the slower release of VC, a longer incubation period or different organisms and mechanisms involved in the formation of ethylene.

Reductive dechlorination progression of VC to ethylene was observed after 30 days of incubation. The data shown in Fig. 28 were taken after 50 days of

Fig. 24 **Reductive dechlorination progression of TCE at a high TCE concentration in a fresh anaerobic Hyperion sludge.**

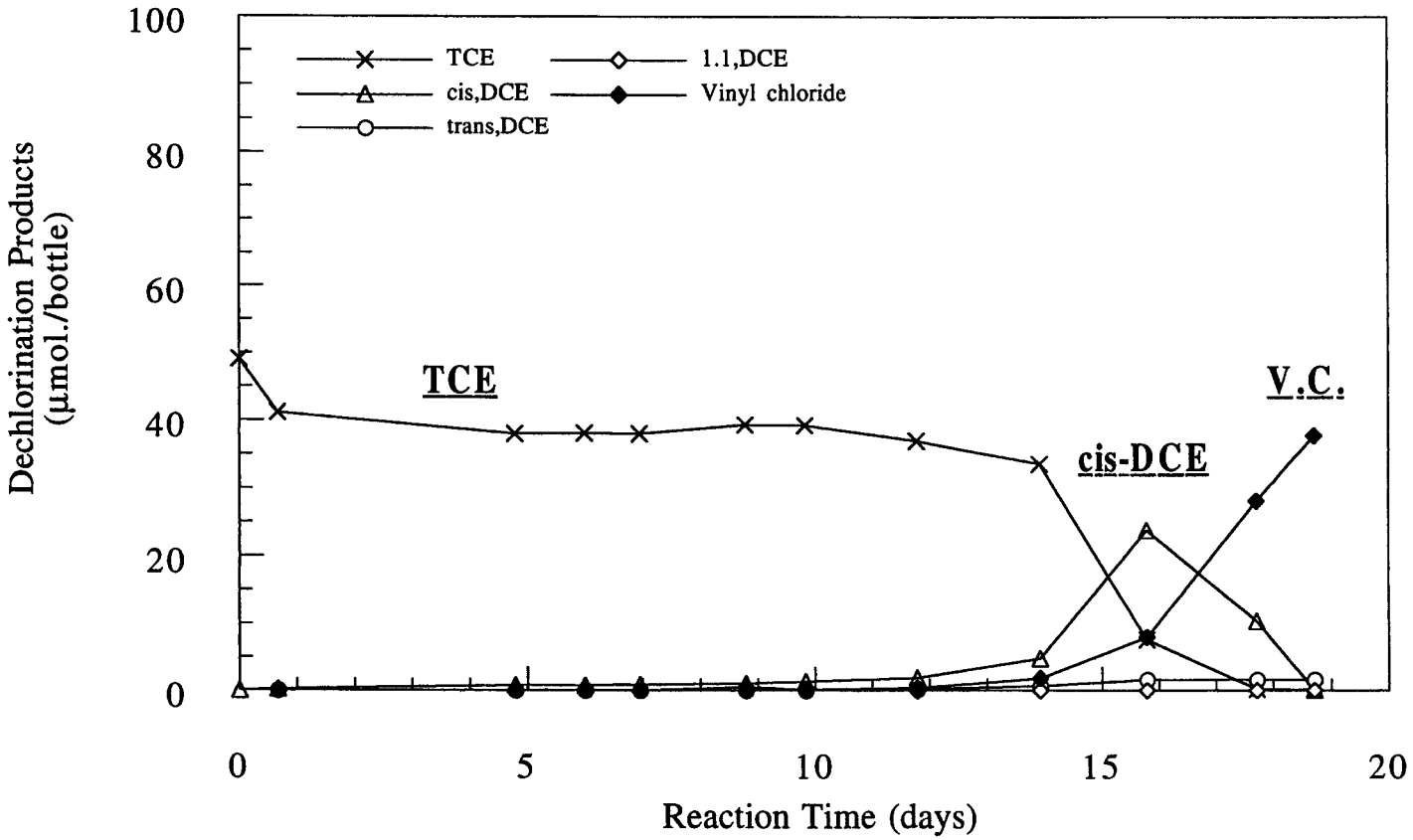


Fig. 25 **Reductive dechlorination progression of cis-DCE at a high cis-DCE concentration in a fresh anaerobic Hyperion sludge.**

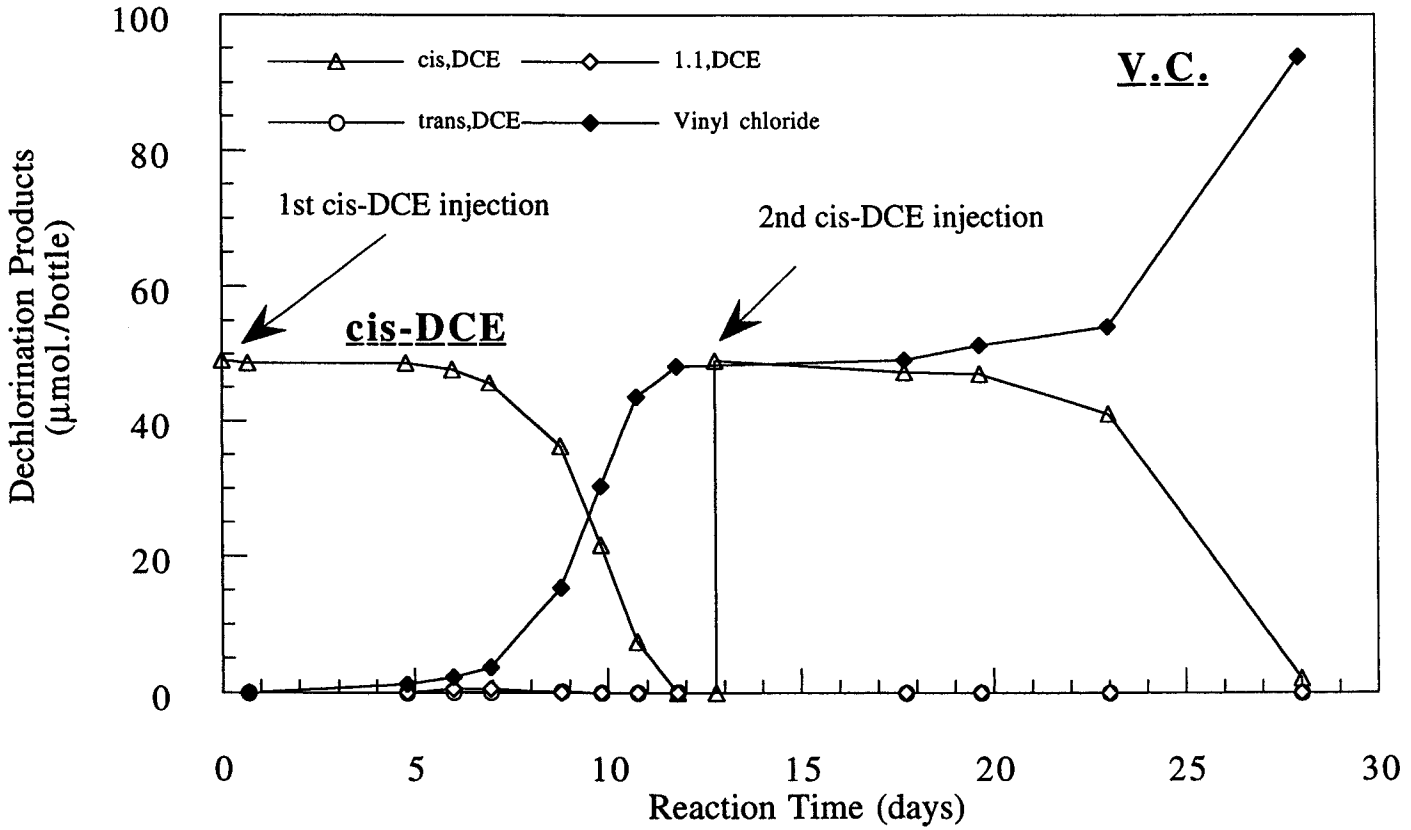
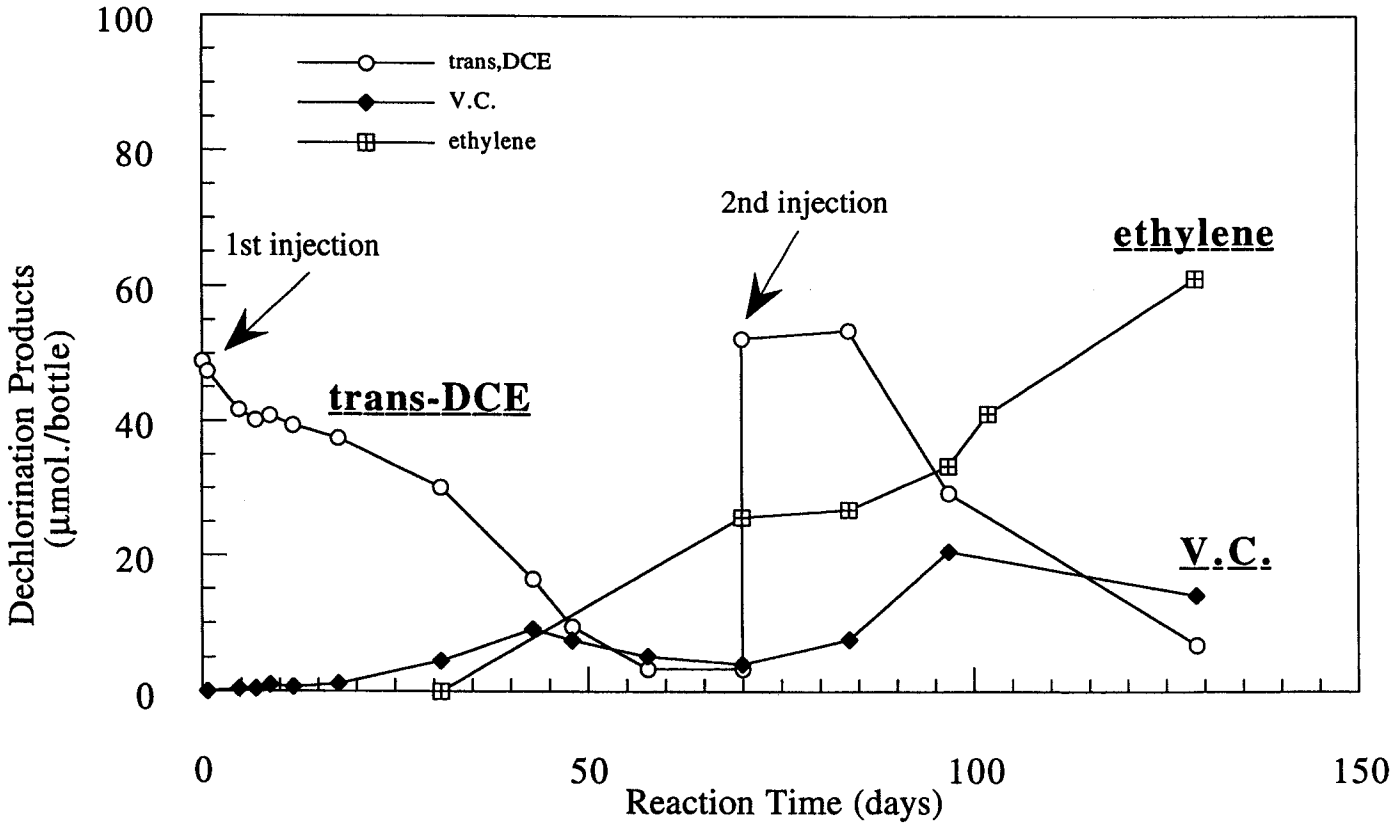


Fig. 26 **Reductive dechlorination progression of trans-DCE at a high trans-DCE concentration in a fresh anaerobic Hyperion sludge.**



Note: The major end product of trans-DCE dechlorination is ethylene, and not VC. This may be due to a longer incubation period that was observed with this culture.

Fig. 27 **Reductive dechlorination progression of 1.1-DCE at a high 1.1-DCE concentration in a fresh anaerobic Hyperion sludge.**

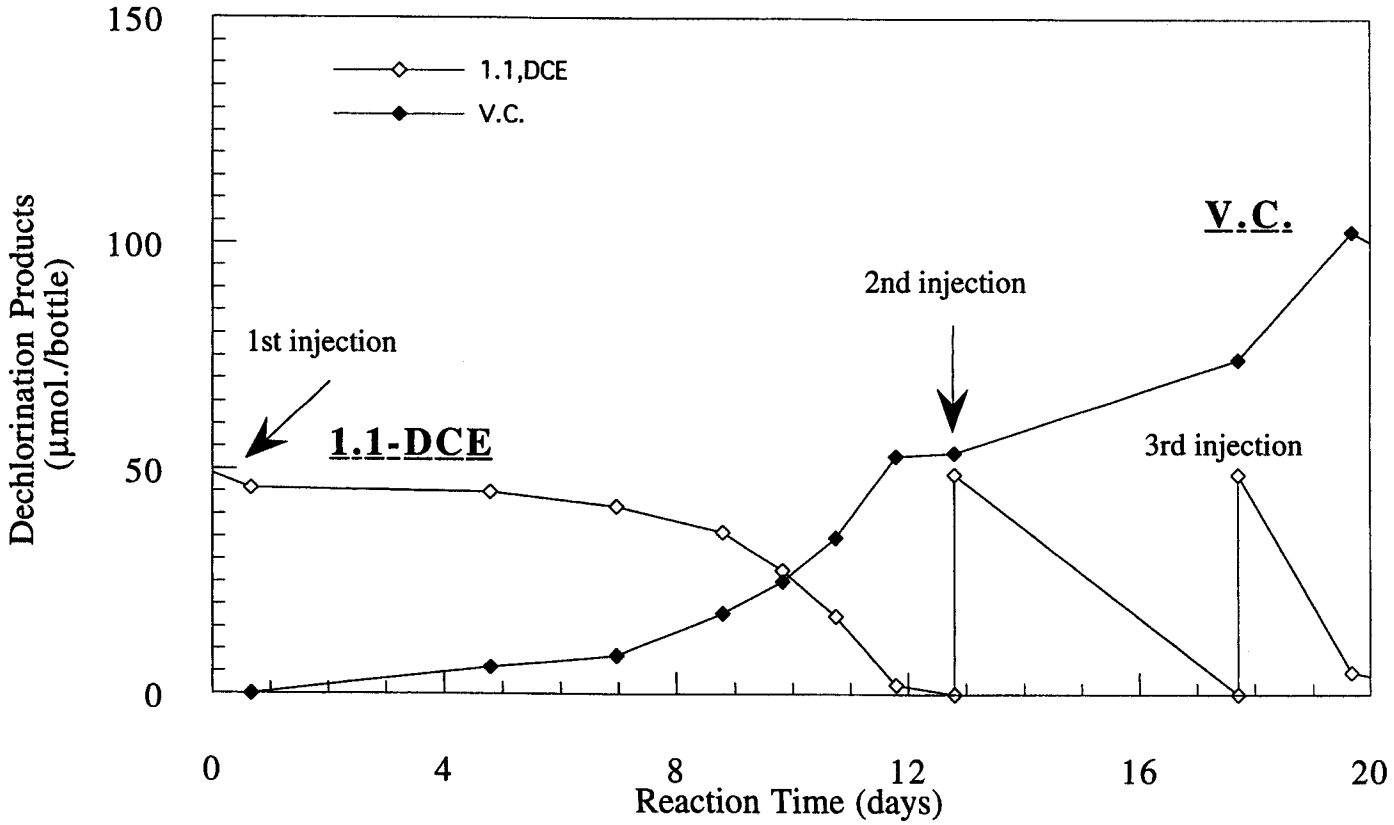
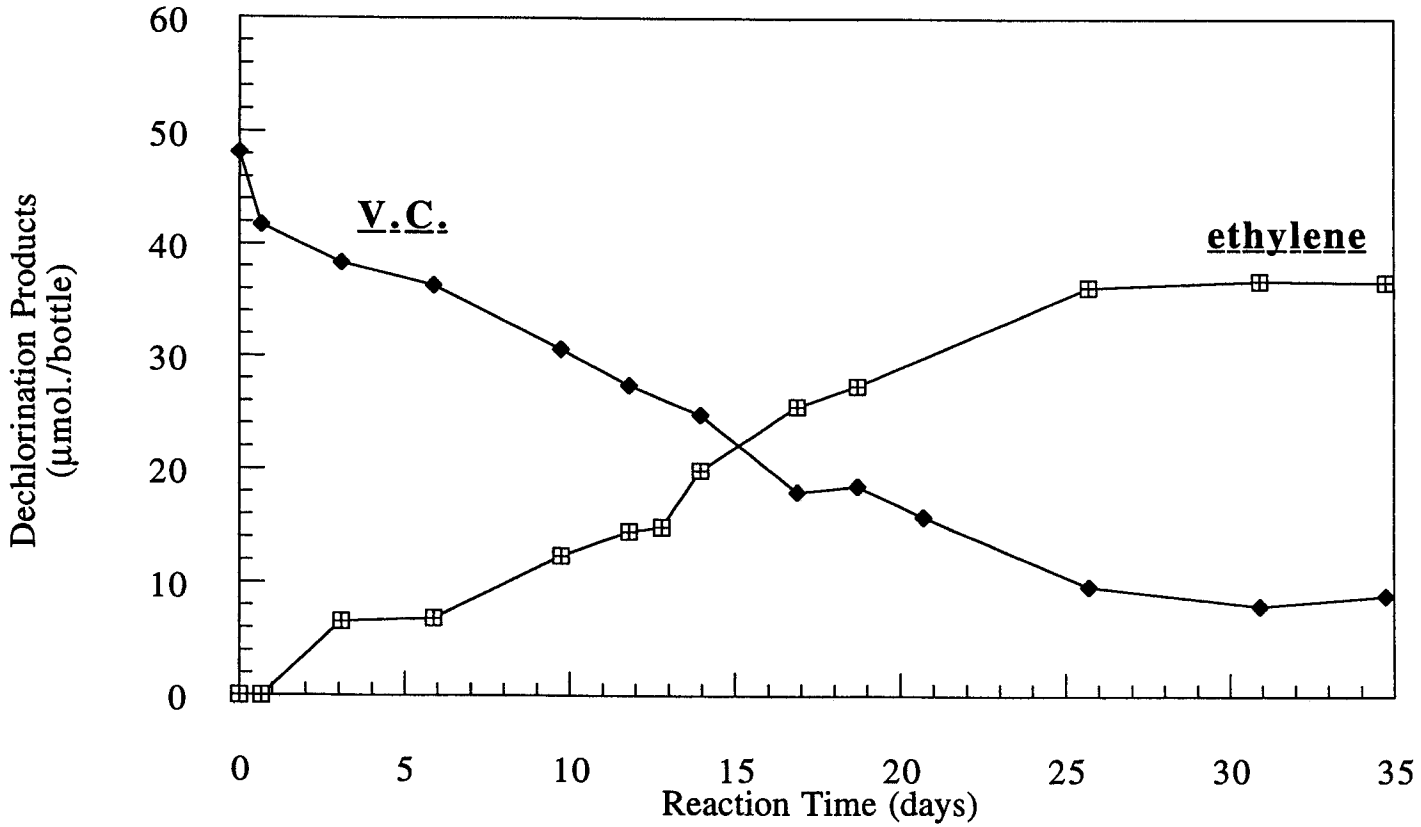


Fig. 28 Reductive dechlorination progression of VC at a high VC concentration in a fresh anaerobic Hyperion sludge.



Note: these data points were taken after 50 days initial incubation with 49 µmoles of VC.

semi-continuous operation fed with VC and methanol. With 49 μmoles of initial VC dose, 40 μmoles of VC was consumed and 36 μmoles of ethylene was produced in 35 days. This indicates that VC has undergone a reductive dechlorination ending up as ethylene.

Ethylene formation was detected from the complete dechlorination of all six chlorinated ethylenes in fresh anaerobic Hyperion sludge. To investigate the possible ethylene production from all six chlorinated ethylenes, a new series of six 120-ml serum bottles containing 100 ml of fresh Hyperion sludge were prepared again as decribed previously. Each bottle contained one kind of test compound and was incubated under the same conditions as described before. The initial dose was 49 μmoles for each chemical except VC. In order to enhance the ethylene production from VC dechlorination, a higher initial VC dose (122 μmoles) was applied to the last bottle. After 15 days, PCE, TCE, cis-DCE, and 1.1-DCE were observed to entirely end up as VC. Trans-DCE and VC showed a more persistent characteristic to reductive dechlorination as expected. Most of them continue to exist. From the 15th day on, ethylene analysis was conducted for each sample. Ethylene production (summarized in Table 6) was demonstrated from all test chlorinated compounds after 54 days of incubation. From Table 6, during the second run, much more ethylene production was observed from the dechlorination of trans-DCE and VC (the two most persistent compounds) after 12 days of incubation. This suggested that if the right environmental conditions were provided, even the most resistant compound can easily undergo complete dechlorination in the anaerobic sludge. The nutrient conditions (including the amount, type of electron donors and electron acceptors) and the concentration of target

Table 6.

Summary of ethylene formation from dechlorination of various chlorinated ethylenes

Time (day)	PCE	TCE	cis-DCE	trans-DCE	1.1-DCE	V.C.
	Accumulation of V.C. (μmol)					
1st Run						
15	43.3	30.78	46.47	1.28	44.7	104.02
				TD=43.35 μmol (residue)		
	Ethylene production (μmol)					
1st Run						
15	0.92	0.7	trace	N.D.	2.93	0.74
17	2.28	4.03	trace	trace	8.14	0.81
20	4.2	5.67	trace	trace	8.56	1.75
24	5.32	7.38	0.6	trace	9.06	2.13
46	9.39	10.52	0.6	1.13	8.42	14.85
54	12.46	11.36	2.4	5.55	8.21	20.62
2nd Run						
4			0.61	6.68	2.2	13.38
12			7.91	22.35	1.91	27.96

chlorinated compound may be two of the most important environmental factors for the reductive dechlorination in the anaerobic digested sludge.

The dechlorination patterns for each chlorinated compound tested in the fresh Hyperion sludge are summarized in Table 7.

Table 7. Summary of dechlorination pattern for each chlorinated compound in anaerobic digested sludge

Chlorinated compounds	Major products in sequential reductive dechlorination
PCE →	TCE→cis-DCE→V.C.→ETH
TCE →	cis-DCE→V.C.→ETH
cis-DCE →	V.C.→ETH
trans-DCE →	V.C.→ETH
1.1-DCE →	V.C.→ETH
V.C. →	ETH

The effect of the presence of TCE and VC on PCE dechlorination. When PCE was incubated with VC in the fresh Hyperion sludge, the dechlorination pattern for PCE was the same as observed with PCE alone. The dechlorination of PCE went through TCE, cis-DCE, VC and terminated at ethylene. Interestingly, the dechlorination of the initially added VC did not occur until all PCE was transformed to VC. In addition, a faster VC dechlorination rate was observed, compared to the result shown in Fig. 28 and Table 6. After the initial added PCE was entirely transformed to VC, 79 μmoles of VC, from a total of 99 μmoles of VC, were dechlorinated to 74

μmoles of ethylene in only 10 days. The result is shown in Fig. 29. A similar phenomenon also occurred in the sludge bottle which was incubated with PCE, TCE, and VC simultaneously. TCE dechlorination occurred after the completion of PCE dechlorination. When all of the TCE was transformed to VC, VC started to be degraded and ethylene emerged (shown in Fig. 30).

The results shown in Fig. 29 and 30 suggested that the dechlorination of less chlorinated ethylenes may be inhibited by the presence of higher chlorinated ethylenes. This was also commonly suggested in previous literature and lead people to believe that PCE is the most susceptible to the reductive dechlorination among six chlorinated ethylenes. However, if PCE can actually inhibit the dechlorination of less chlorinated ethylenes in the sludge ? We will discuss it later.

4.6. Toxicity effect

Methane production was used as an indicator to estimate the toxic effect of each chlorinated compound on the methanogenic activity of fresh Hyperion sludge. A variety of effects on methane production was observed with different chlorinated compounds. The term, LC_{50} was used to describe the concentration of a test chlorinated compound which reduced methanogenic activity by 50%. It is believed that the decrease in methane production may be partially or completely due to the competition for reduction between the electron acceptors used by methanogens in the sludge system and the test chlorinated compound. In some situations, it is possible that electrons are more easily diverted to chlorinated compounds than to the precursor materials of methane formation.

Fig. 29 The effect of vinyl chloride on PCE dechlorination in a fresh anaerobic Hyperion sludge cultures without methanol addition.

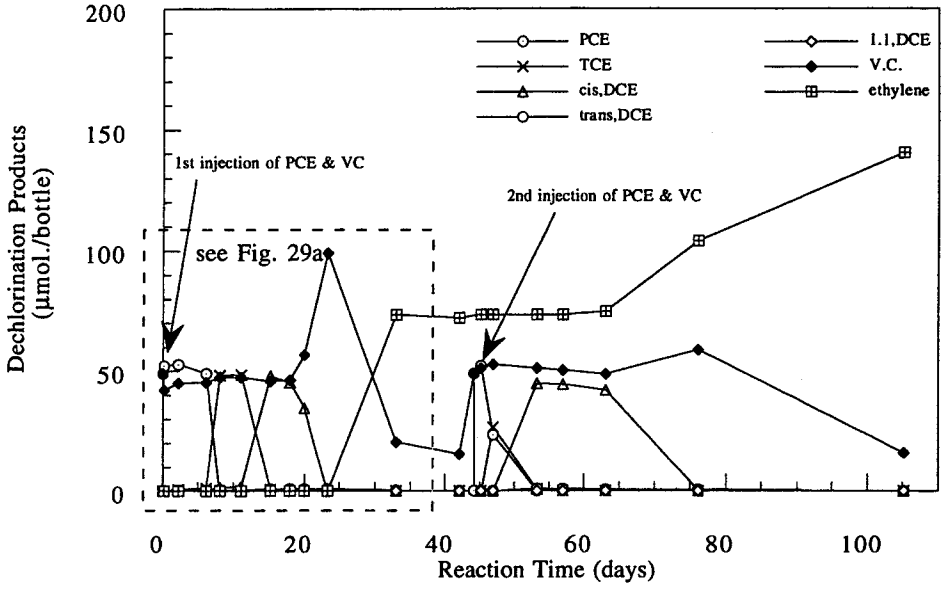


Fig. 29a The effect of vinyl chloride on PCE dechlorination in a fresh anaerobic Hyperion sludge cultures without methanol addition.

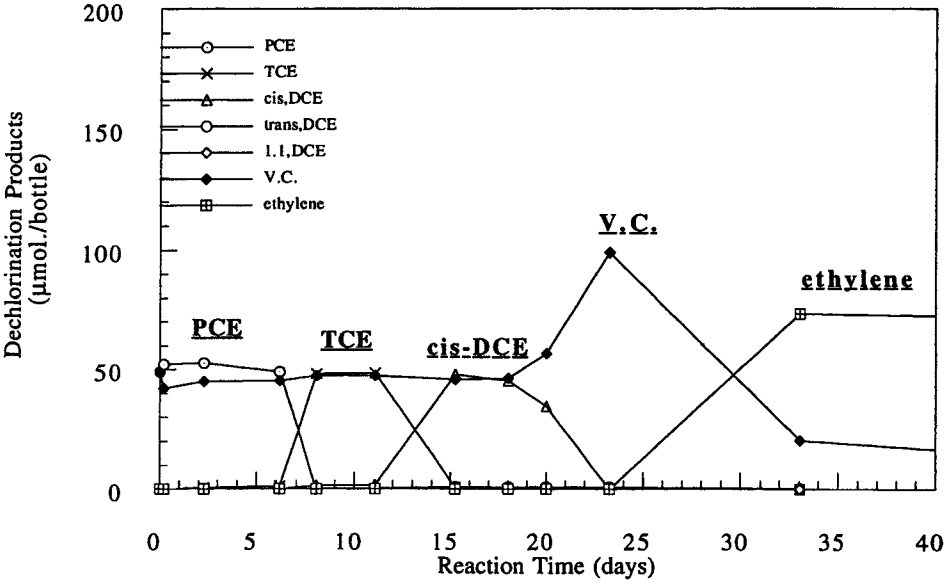


Fig. 30 The effect of the presence of TCE and VC on PCE dechlorination in a fresh anaerobic Hyperion sludge cultures without methanol addition.

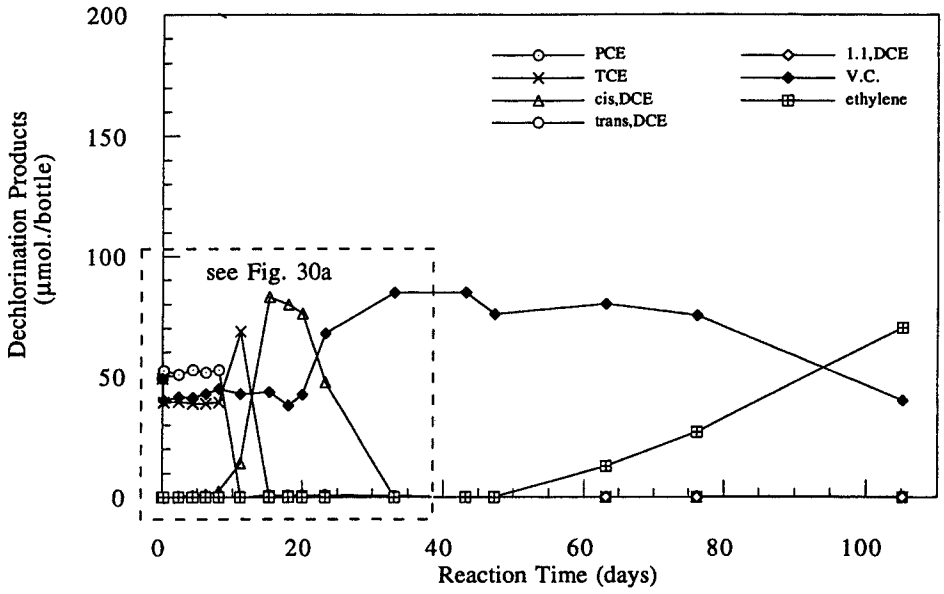
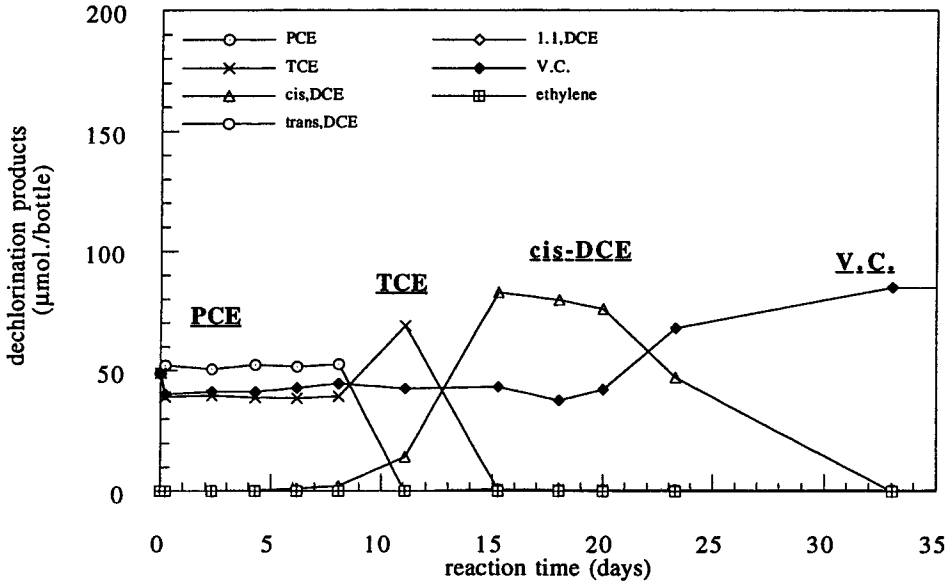


Fig. 30a The effect of the presence of TCE and V.C. on PCE dechlorination in a fresh anaerobic digested sludge cultures without methanol addition.



From Fig. 31 to 36, we found that the chlorinated compounds (including PCE, TCE, and cis-DCE) which were more rapidly dechlorinated, have lower LC_{50} values. Compounds with lower rates of dechlorination (such as trans-DCE, 1.1-DCE and VC) exhibited higher LC_{50} values. The LC_{50} values obtained from each chemical correlate with their treatability (the amenability of compounds to dechlorination during biological treatment).

4.7. Highest tolerable concentration and ethylene production

Table 8 to 13 show the change in total mass of each tested compound and their dechlorinated products over a 20-55 day period of incubation in the fresh Hyperion digested sludge. PCE showed the highest potential to produce ethylene, then TCE and cis-DCE. Trans-DCE, 1.1-DCE and VC were more resistant to degradation and ethylene productions. In these experiments, we did not observe 100% complete dechlorination at any initial dosage for each tested compound. The maximum level of ethylene production was observed from the sample with an initial dose of 97.86 μmoles of PCE. About 74% of the PCE was recovered as ethylene (72.4 μmol.) after 32 days. No instances of 100% transformation were observed during the incubation period, even for the sample with the lowest initial dosage of PCE. Vinyl chloride and trans-DCE were dechlorinated most slowly. The recovery for most of samples were less than 100%. This is due to the losses of chemicals from the released gas (for releasing pressure resulting from gas production in serum bottles containing fresh digested sludge).

PCE. After one month incubation, the highest dose of PCE that was most readily degraded to ethylene was 97.86 μmoles per bottle. Although degradation of PCE

Fig. 31 Toxicological Effect of PCE on Anaerobic Digested Sludge

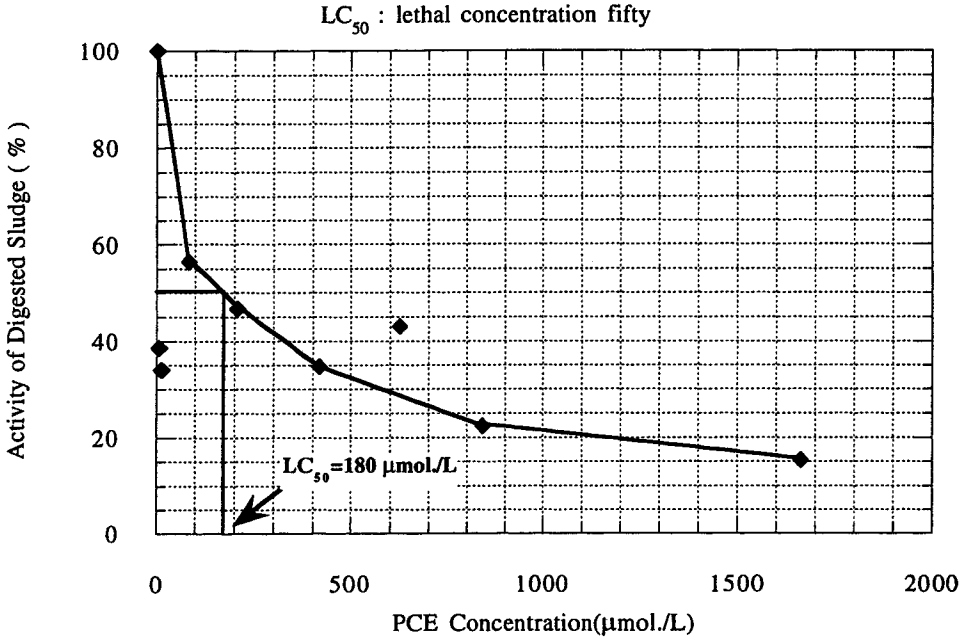


Fig. 32 Toxicological Effect of TCE on Anaerobic Digested Sludge

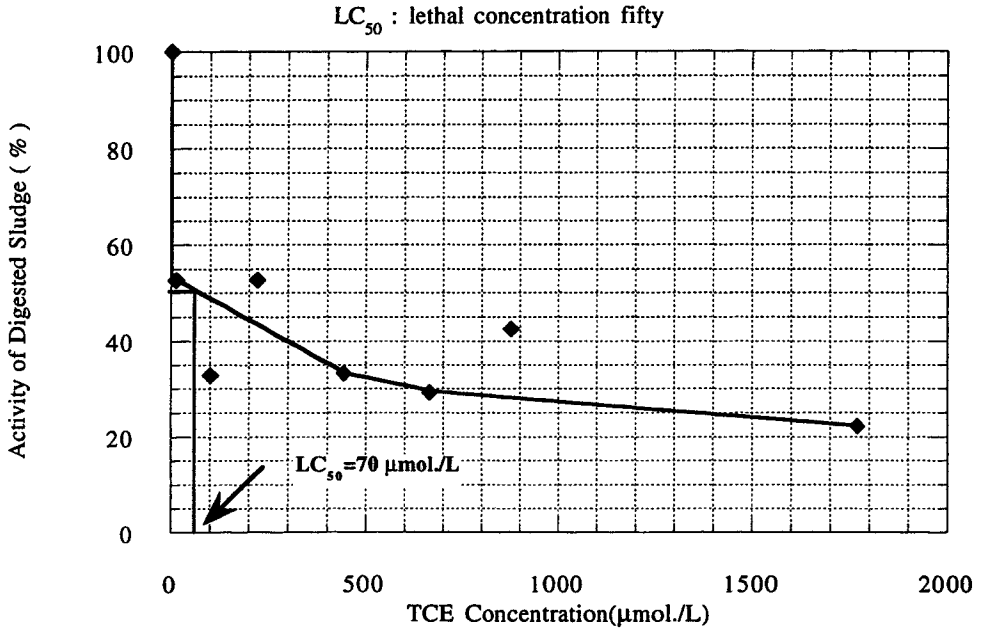


Fig. 33 Toxicological Effect of cis-DCE on Anaerobic Digested Sludge

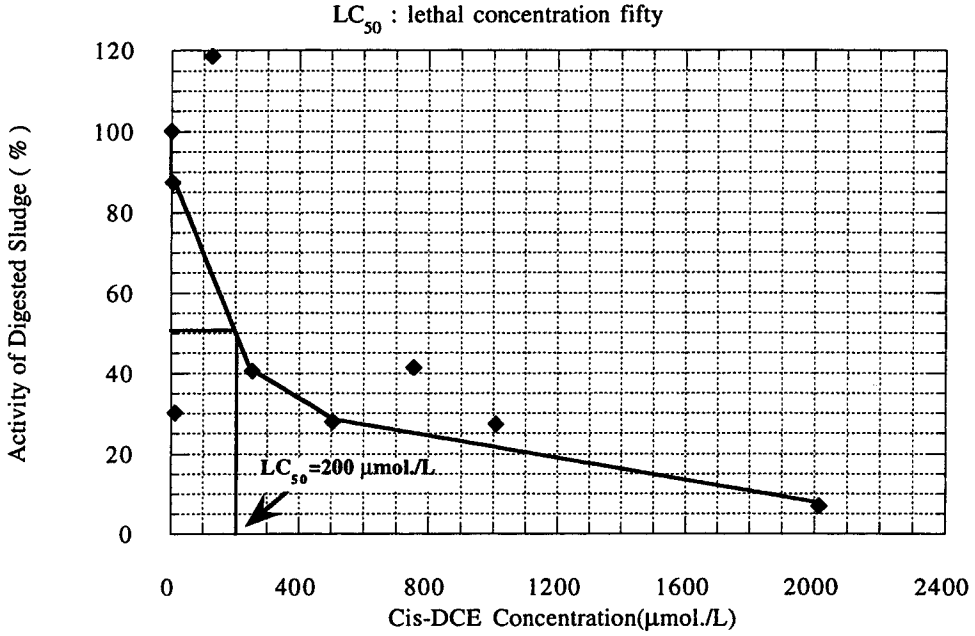


Fig. 34 Toxicological Effect of trans-DCE on Anaerobic Digested Sludge

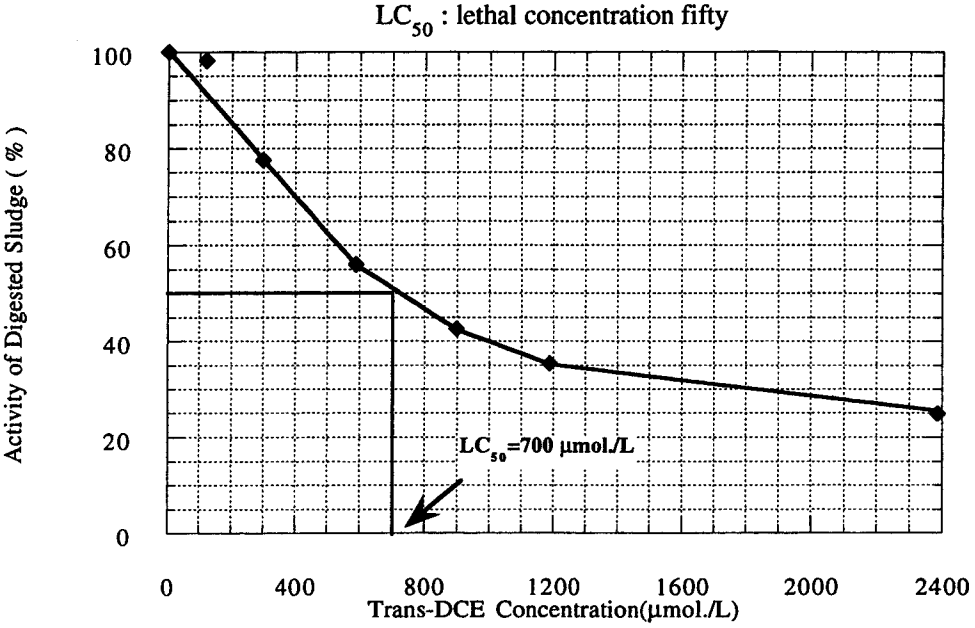


Fig. 35 Toxicological Effect of 1.1,DCE on Anaerobic Digested Sludge

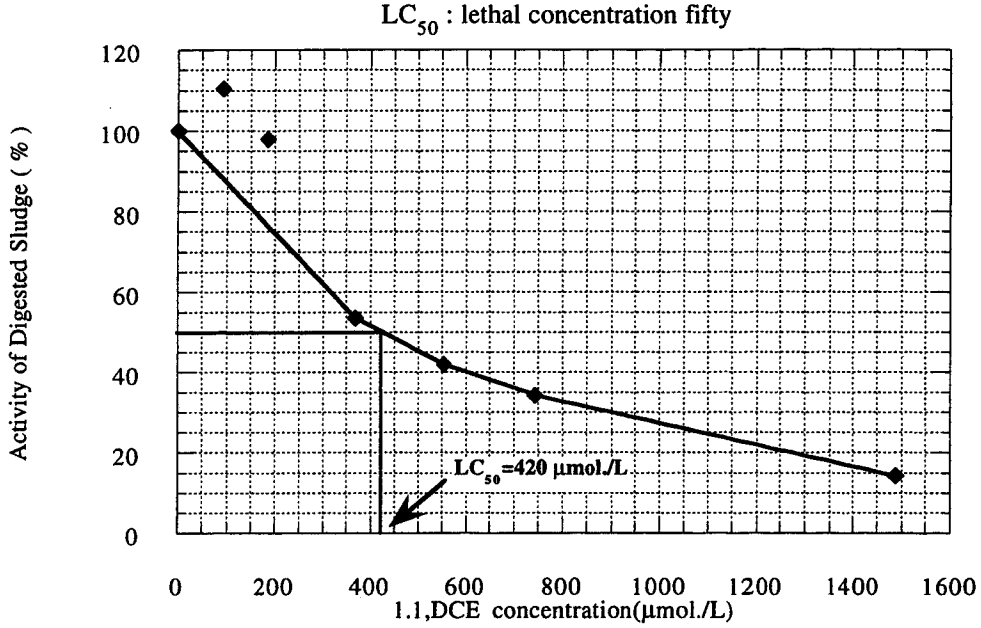
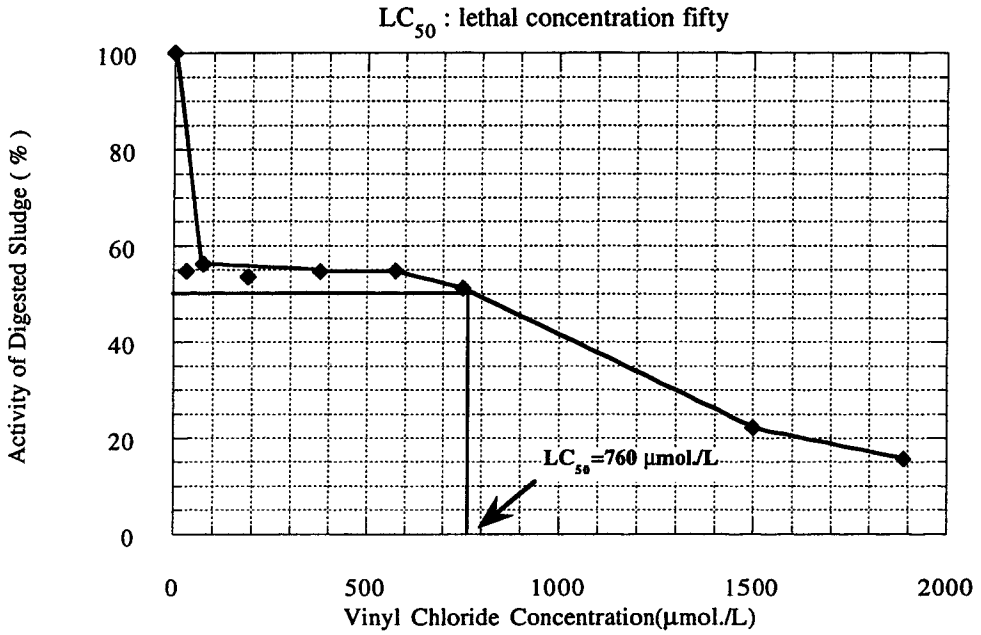


Fig. 36 Toxicological Effect of Vinyl Chloride on Anaerobic Digested Sludge



was still carried out at higher concentrations (up to 195.72 μmoles per bottle), the dechlorination that occurred within this period was not as complete. The major product of PCE dechlorination at 195 μmoles per bottle was vinyl chloride, instead of ethylene. The total ethylene production increased with the initial PCE concentration up to an upper limit. It seemed that the ethylene formation from the reductive dechlorination may be also influenced by the concentration of vinyl chloride. There was no any detectable ethylene produced from the samples with a low initial PCE concentration which resulted in a corresponding low VC concentration. Results of this experiment are shown in Table 8.

TCE. The results for TCE dechlorination were similar to those observed from PCE dechlorination. With up to 196.21 μmoles of TCE per bottle, dechlorination of TCE still occurred; however 91% of TCE was recovered as VC and only 9% of TCE was completely dechlorinated to ethylene. The maximum ethylene production (48.8 μmoles) was observed from the sample incubated with 98.11 μmoles of TCE, while the sample with 73.58 μmoles of TCE showed the highest percentage (about 56%) of transformation from TCE to ethylene (see Table. 9).

cis-DCE. The production of ethylene from cis-DCE was much more than those observed with the other two isomers. The highest tolerable cis-DCE concentration appeared to be 105.62 μmoles per bottle producing the maximum amount of ethylene (see Table 10). For the highest cis-DCE dosage (211.24 μmoles per bottle), only 112 μmoles of cis-DCE was degraded to VC after 39 days of incubation with no ethylene produced.

Table 8. Dechlorination of PCE and the formation of dechlorinated products at various initial dosages during anaerobic incubation in fresh Hyperion sludge.

Sample No.	Time (days)	Major dechlorinated products PCE (μmol.)	TCE (μmol.)	cis-DCE (μmol.)	trans-DCE (μmol.)	1.1-DCE (μmol.)	V.C. (μmol.)	Sub-total (μmol.)-(1)	PCE added (μmol.)-(2)	(1)/(2) (%)	ETH (μmol.)	Total-(3) (μmol.)	(3)/(2) (%)
1	12				0.03		0.68	0.72	0.56	128		0.72	128
	19						0.50	0.50	0.56	90	N.D.	0.50	90
	32						0.50	0.50	0.56	89	N.D.	0.50	89
2	12				0.03		1.02	1.05	1.40	75		1.05	75
	19				0.02		1.07	1.09	1.40	78	N.D.	1.09	78
	32						0.96	0.96	1.40	69	N.D.	0.96	69
3	12			1.35	0.09		6.86	8.29	9.79	85		8.29	85
	19		0.05		0.06		8.34	8.44	9.79	86	N.D.	8.44	86
	32						7.58	7.58	9.79	77	N.D.	7.58	77
4	12		0.20	10.65	0.66		11.15	22.66	24.46	93		22.66	93
	19				0.66		18.22	18.88	24.46	77	1.82	20.70	85
	32						15.50	15.50	24.46	63	4.20	19.70	81
5	12		3.36	22.13	2.03		10.91	38.43	48.93	79		38.43	79
	19						18.59	18.59	48.93	38	26.40	44.99	92
	32						12.44	12.44	48.93	25	35.63	48.07	98
6	12		31.41	20.11	3.37		7.38	62.28	73.39	85		62.28	85
	19						37.90	37.90	73.39	52	43.14	81.04	110
	32						23.47	23.47	73.39	32	59.12	82.59	113
7	12		61.32	10.15	4.57		7.61	83.65	97.86	85		83.65	85
	19	0.11			10.29		56.06	66.46	97.86	68	44.49	110.95	113
	32						26.78	26.78	**97.86**	27	**72.40**	99.18	101
8	12	0.77	154.82	3.41	1.09	1.01	0.66	161.76	195.72	83		161.76	83
	19	0.06	142.22	6.91	2.00	0.03	1.41	152.64	195.72	78	N.D.	152.64	78
	28		89.33	17.39	2.00	0.96	21.78	131.45	195.72	67		131.45	67
	32		49.76	15.13	2.29	1.18	65.80	134.16	195.72	69	N.D.	134.16	69
	35		11.57	2.06	2.54		126.95	143.13	195.72	73		143.13	73

N.D.: not detected; Sub-total=PCE+TCE+cis-DCE+trans-DCE+1.1-DCE+V.C.; Recovery= (3)/(2).

Table 9. Dechlorination of TCE and the formation of dechlorinated products at various initial dosages during anaerobic incubation in fresh Hyperion sludge.

Sample No.	Time (days)	Major dechlorinated products						Sub-total (μmol.)-(1)	TCE added (μmol.)-(2)	(1)/(2) (%)	ETH (μmol.)	Total-(3) (μmol.)	(3)/(2) (%)
		PCE (μmol.)	TCE (μmol.)	cis-DCE (μmol.)	trans-DCE (μmol.)	1.1-DCE (μmol.)	V.C. (μmol.)						
1	24						0.51	0.51	0.55	92	N.D.	0.51	92
	38						0.42	0.42	0.55	76	N.D.	0.42	76
2	24				0.01		0.76	0.77	1.40	55	N.D.	0.77	55
	38						0.57	0.57	1.40	41	N.D.	0.57	41
3	24				0.53		5.46	5.99	11.15	54	0.00	5.99	54
	38						4.13	4.13	11.15	37	N.D.	4.13	37
4	24				0.23		12.98	13.21	24.53	54	0.00	13.21	54
	38				0.50		10.85	11.35	24.53	46	N.D.	11.35	46
5	24				0.86		11.46	12.32	49.05	25	23.44	35.75	73
	38						5.85	5.85	49.05	12	18.35	24.20	49
6	24				1.72		26.02	27.74	73.58	38	41.22	68.96	94
	38						21.98	21.98	73.58	30	36.49	58.47	79
7	24				2.30		37.51	39.81	**98.11**	41	**48.82**	88.63	90
	38						32.55	32.55	98.11	33	47.93	80.48	82
8	24		1.22	47.79	2.00		143.89	194.91	196.21	99	N.D.	194.91	99
	38				2.23		176.81	179.04	196.21	91	18.80	197.84	101

N.D.: not detected; Sub-total=PCE+TCE+cis-DCE+trans-DCE+1.1-DCE+V.C.; Recovery= (3)/(2).

Table 10. Dechlorination of cis-DCE and the formation of dechlorinated products at various initial dosages during anaerobic incubation in fresh Hyperion sludge.

Sample No.	Time (days)	Major dechlorinated products PCE (µmol.)	TCE (µmol.)	cis-DCE (µmol.)	trans-DCE (µmol.)	1.1-DCE (µmol.)	V.C. (µmol.)	Sub-total (µmol.)-(1)	CD added (µmol.)-(2)	(1)/(2) (%)	ETH (µmol.)	Total-(3) (µmol.)	(3)/(2) (%)
1	25						0.45	0.45	0.56	80	N.D.	0.45	80
	39						0.30	0.30	0.56	53	N.D.	0.30	53
2	25						0.59	0.59	1.41	42	N.D.	0.59	42
	39						0.35	0.35	1.41	25	N.D.	0.35	25
3	25						12.61	12.61	13.20	96	N.D.	12.61	96
	39						11.60	11.60	13.20	88	N.D.	11.60	88
4	25						11.48	11.48	26.41	43	0.32	11.80	45
	39						8.00	8.00	26.41	30	0.59	8.59	33
5	25						13.37	13.37	52.81	25	31.23	44.60	84
	39						13.58	13.58	52.81	26	24.99	38.57	73
6	25						30.33	30.33	79.22	38	35.81	66.14	83
	39						27.25	27.25	79.22	34	29.80	57.05	72
7	25						60.13	60.13	105.62	57	31.72	91.85	87
	39						38.68	38.68	**105.62**	37	**49.69**	88.37	84
8	25			160.89	0.38		28.61	189.88	211.24	90	N.D.	189.88	90
	39			76.76	0.38		112.03	189.17	211.24	90	N.D.	189.17	90

N.D.: not detected; Sub-total=PCE+TCE+cis-DCE+trans-DCE+1.1-DCE+V.C.; Recovery= (3)/(2); CD=cis-DCE.

trans-DCE. Trans-DCE was highly resistant to dechlorination in the fresh digested sludge. After 54 days of incubation, no significant amount of trans-DCE was transformed in any sample tested (Table 11). This is consistent with earlier observations for the trans-DCE progression test. No ethylene production was observed even after 70 days of incubation (data not shown here).

1.1-DCE. At a dose of 50 μmoles of 1.1-DCE, the maximum amount of ethylene production was observed (16.7 μmoles). At higher concentrations of 1.1-DCE incomplete dechlorination was observed in all samples after 55 days of incubation period (see Table 12).

VC. Generally, VC is regarded as the most resistant compound to reductive dechlorination (Freedman and Gossett, 1989). In spite of this, we still found an optimum concentration at which the dechlorination of VC to ethylene was maximized, while VC was almost totally resistant at higher initial doses. VC was maximally dechlorinated at the initial dose of 73.57 μmoles per bottle. After 51 days, about 35 μmoles of ethylene was recovered from VC (refer to Table 13). This indicates that the concentration of VC influences its rate of dechlorination to ethylene.

4.8. Semi-continuous operation

In order to evaluate long-term dechlorination efficiency for each chlorinated ethylene, the six sludge bottles created in the dechlorination progression test and two mixed-culture bottles were maintained under semi-continuous feeding. In PCE bottle, 49 μmoles of PCE was totally degraded to VC in 15 days at the beginning. After 5 runs,

Table 11. Dechlorination of trans-DCE and the formation of dechlorinated products at various initial dosages during anaerobic incubation in fresh Hyperion sludge.

Sample No.	Time (days)	Major dechlorinated products						Sub-total (μmol.)-(1)	TD added (μmol.)-(2)	(1)/(2) (%)	ETH (μmol.)	Total-(3) (μmol.)	(3)/(2) (%)
		PCE (μmol.)	TCE (μmol.)	cis-DCE (μmol.)	trans-DCE (μmol.)	1.1-DCE (μmol.)	V.C. (μmol.)						
1	30				10.30		0.05	10.35	13.20	78	N.D.	10.35	78
	54				7.16		0.19	7.35	13.20	56	N.D.	7.35	56
2	30				20.88		0.86	21.74	26.41	82	N.D.	21.74	82
	54				14.75		0.20	14.95	26.41	57	N.D.	14.95	57
3	30				45.05		0.73	45.78	52.81	87	N.D.	45.78	87
	54				38.13		0.11	38.24	52.81	72	N.D.	38.24	72
4	30				64.50		2.41	66.91	79.22	84	N.D.	66.91	84
	54				56.34		0.51	56.85	79.22	72	N.D.	56.85	72
5	30				87.54		2.60	90.14	105.62	85	N.D.	90.14	85
	54				72.61		2.86	75.47	105.62	71	N.D.	75.47	71
6	30				168.65		3.05	171.70	211.24	81	N.D.	171.70	81
	54				139.66		0.00	139.66	211.24	66	N.D.	139.66	66

N.D.: not detected; Sub-total=PCE+TCE+cis-DCE+trans-DCE+1.1-DCE+V.C.; Recovery= (3)/(2); TD=trans-DCE.

Table 12. Dechlorination of 1.1-DCE and the formation of dechlorinated products at various initial dosages during anaerobic incubation in fresh Hyperion sludge.

Sample No.	Time (days)	Major dechlorinated products						Sub-total (μmol.)-(1)	VD added (μmol.)-(2)	(1)/(2) (%)	ETH (μmol.)	Total-(3) (μmol.)	(3)/(2) (%)
		PCE (μmol.)	TCE (μmol.)	cis-DCE (μmol.)	trans-DCE (μmol.)	1.1-DCE (μmol.)	V.C. (μmol.)						
1	30						9.18	9.18	12.51	73	N.D.	9.18	73
	55						6.74	6.74	12.51	54	2.85	9.59	77
2	30						13.49	13.49	25.02	54	N.D.	13.49	54
	55						7.45	7.45	25.02	30	N.D.	7.45	30
3	30						39.45	39.45	50.04	79	6.46	45.91	92
	55						17.22	17.22	**50.04**	34	**16.67**	33.89	68
4	30					60.53	5.87	66.40	75.06	88	N.D.	66.40	88
	55					30.65	28.50	59.15	75.06	79	N.D.	59.15	79
5	30					85.87	7.56	93.43	100.08	93	N.D.	93.43	93
	55					46.40	40.97	87.37	100.08	87	4.77	92.14	92
6	30					158.33	4.31	162.64	200.17	81	N.D.	162.64	81
	55					134.02	5.92	139.94	200.17	70	N.D.	139.94	70

N.D.: not detected; Sub-total=PCE+TCE+cis-DCE+trans-DCE+1.1-DCE+V.C.; Recovery= (3)/(2); VD=1.1-DCE.

Table 13. Dechlorination of vinyl chloride and the formation of dechlorinated products at various initial dosages during anaerobic incubation in fresh Hyperion sludge.

Sample No.	Time (days)	Major dechlorinated products PCE (μmol.)	TCE (μmol.)	cis-DCE (μmol.)	trans-DCE (μmol.)	1.1-DCE (μmol.)	V.C. (μmol.)	Sub-total (μmol.)-(1)	V.C. added (μmol.)-(2)	(1)/(2) (%)	ETH (μmol.)	Total-(3) (μmol.)	(3)/(2) (%)
1	20						3.68	3.68	4.09	90	N.D.	3.68	90
	51						3.00	3.00	4.09	73	N.D.	3.00	73
2	20						8.71	8.71	9.81	89	N.D.	8.71	89
	51						6.93	6.93	9.81	71	N.D.	6.93	71
3	20						18.29	18.29	24.52	75	N.D.	18.29	75
	51						16.20	16.20	24.52	66	N.D.	16.20	66
4	20						40.29	40.29	49.05	82	0.50	40.79	83
	51						35.51	35.51	49.05	72	N.D.	35.51	72
5	20						63.97	63.97	73.57	87	8.79	72.76	99
	51						26.27	26.27	**73.57**	36	**34.87**	61.14	83
6	20						90.35	90.35	98.09	92	N.D.	90.35	92
	51						68.42	68.42	98.09	70	7.40	75.82	77
7	20						183.08	183.08	196.19	93	N.D.	183.08	93
	51						168.13	168.13	196.19	86	N.D.	168.13	86
8	20						221.09	221.09	245.24	90	N.D.	221.09	90
	51						210.06	210.06	245.24	86	N.D.	210.06	86

N.D.: not detected; Sub-total=PCE+TCE+cis-DCE+trans-DCE+1.1-DCE+V.C.; Recovery= (3)/(2).

only 3 days were required to degrade the same amount of PCE. A total of 686 μmoles of PCE was degraded over a period of 145 days. At the end of the experiment, a total of 33% of PCE was recovered as ethylene. VC was the major product accumulated in the serum bottle from PCE dechlorination. The results are shown in Fig. 37. Similar results (shown in Appendix, Fig. A-21) were obtained under conditions in which no methanol was added for the first 60 days. 275 μmoles of ethylene were produced from 735 μmoles of PCE cumulatively added in 145 days. Furthermore, long term semi-continuous tests carried out using TCE, cis-DCE, and 1.1-DCE showed similar results with large amounts of VC accumulation. The data are provided in Appendix A (Fig. A-22 to A-24).

In the trans-DCE bottle, 61 μmoles of ethylene were produced in 130 days from a total of 98 μmoles of trans-DCE cumulative addition. There were only 10 μmoles of VC accumulated in the bottle at the end of the experiment (see Fig. 26).

VC was also very slowly dechlorinated. During the period of 135 days, a total of 196 μmoles of VC was added and 141 μmoles of ethylene were recovered. At the end of the test, VC was still not completely dechlorinated. Although this result indicated that dechlorination of VC under anaerobic condition is possible, complete transformation of VC from the treatment system is more difficult and may take longer. The importance of methanol to VC dechlorination was also tested in this experiment by ending methanol addition on 85th day. Dechlorination of VC and production of ethylene were inhibited significantly until the 114th day, at which time methanol was added again (see Fig. 38). In this case methanol appears to be necessary for VC dechlorination to take place.

Fig. 37 Long term dechlorination of PCE and formation of intermediates in a semi-continuous operation serum bottle

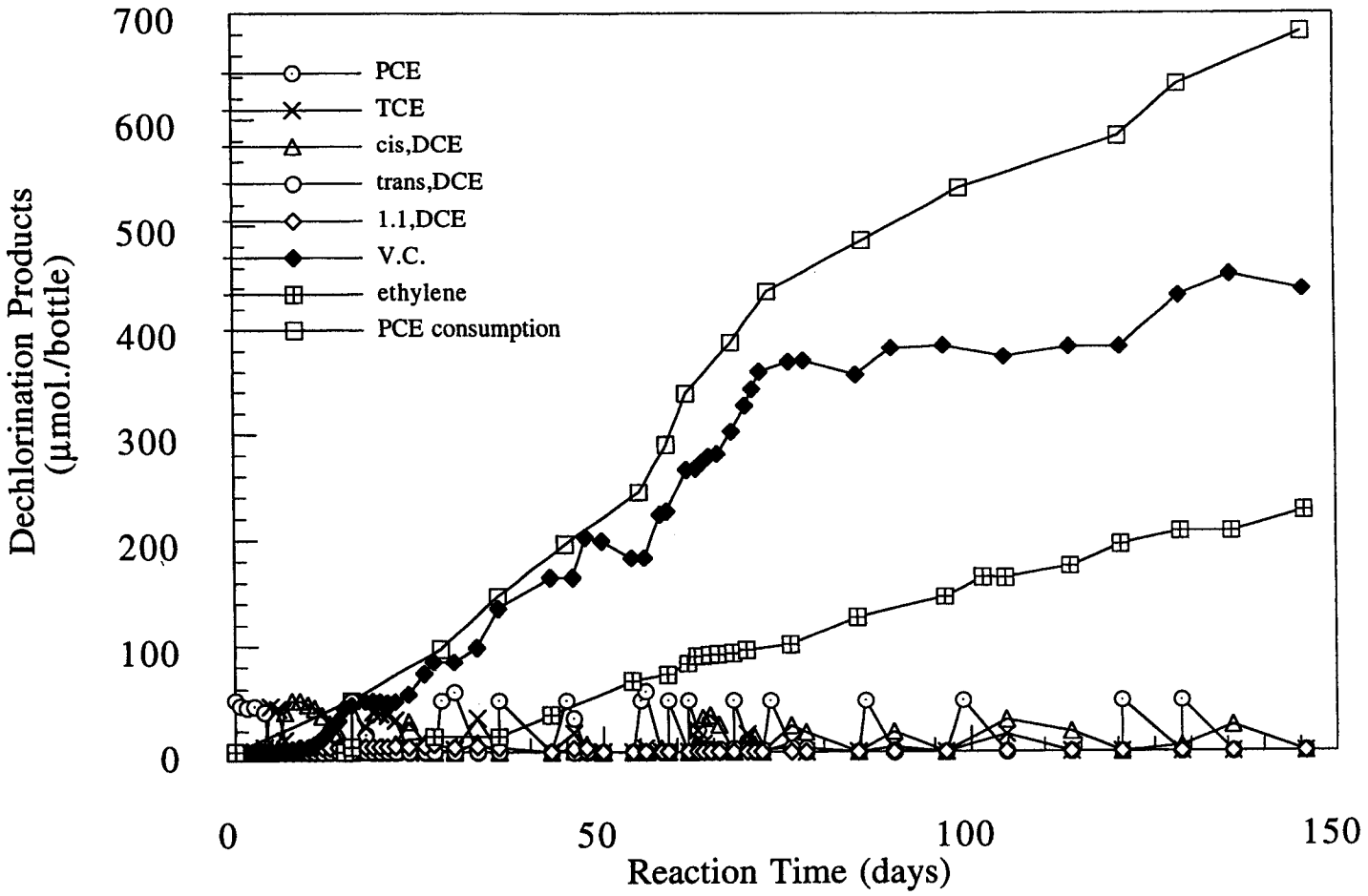
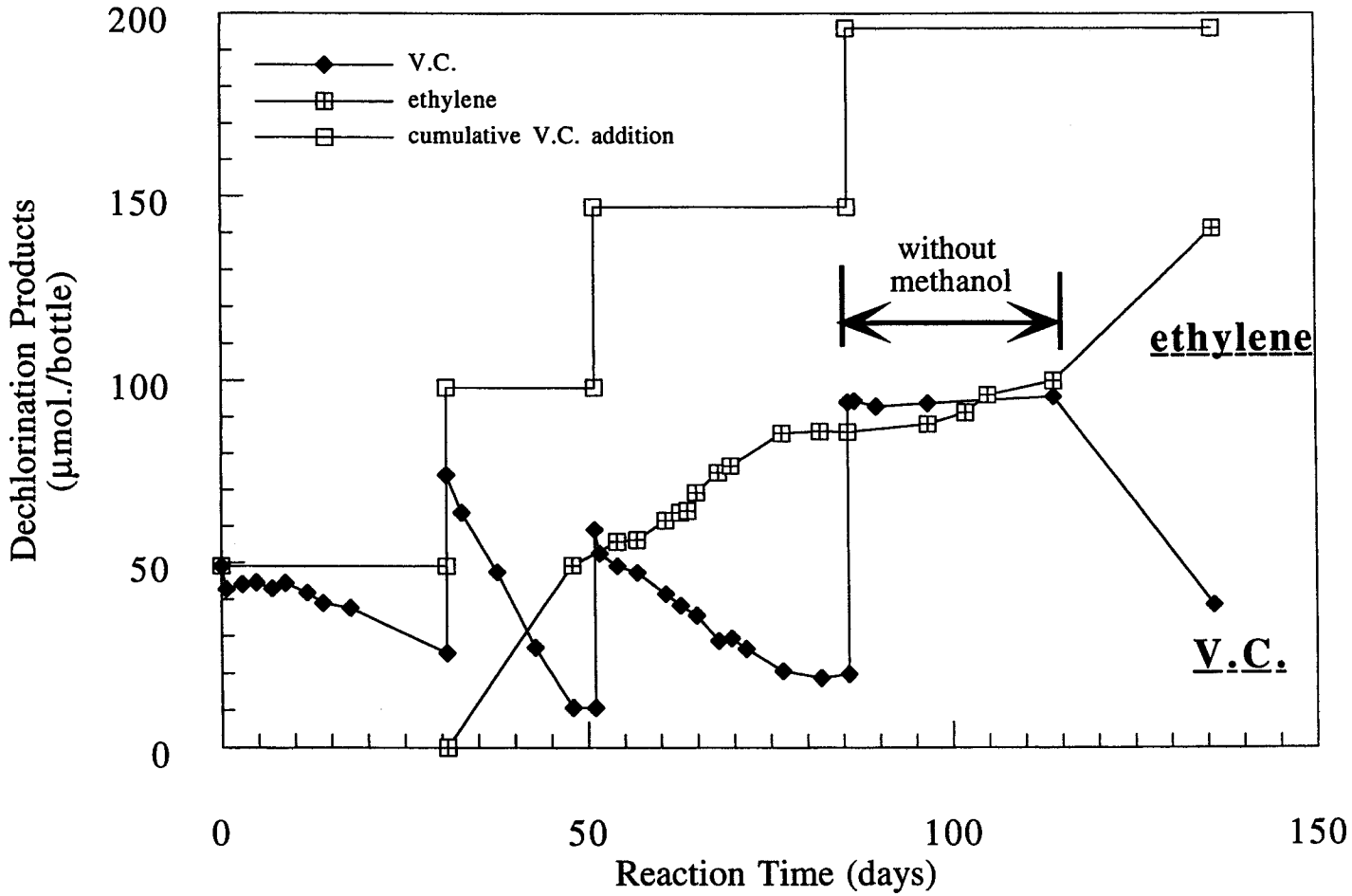
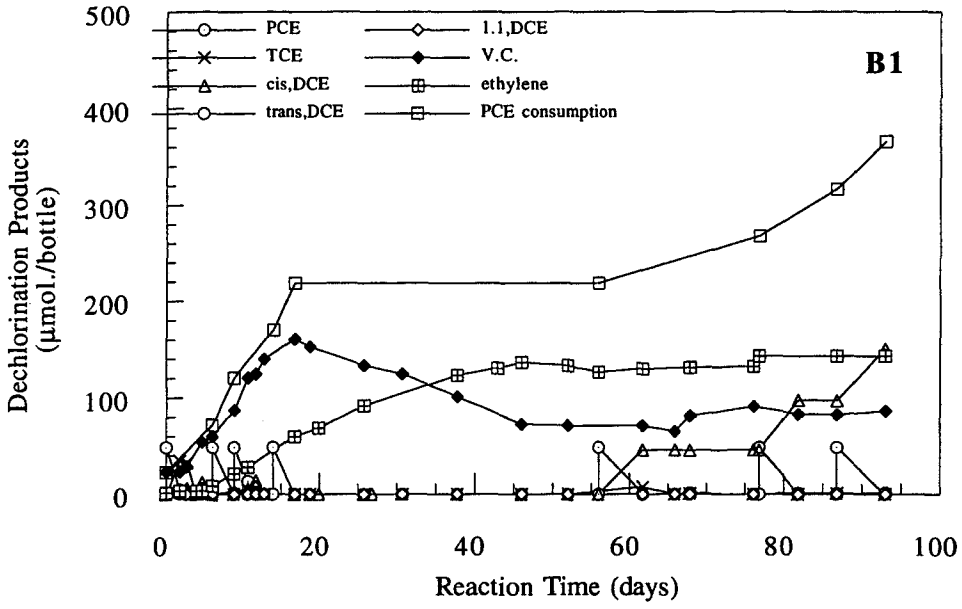


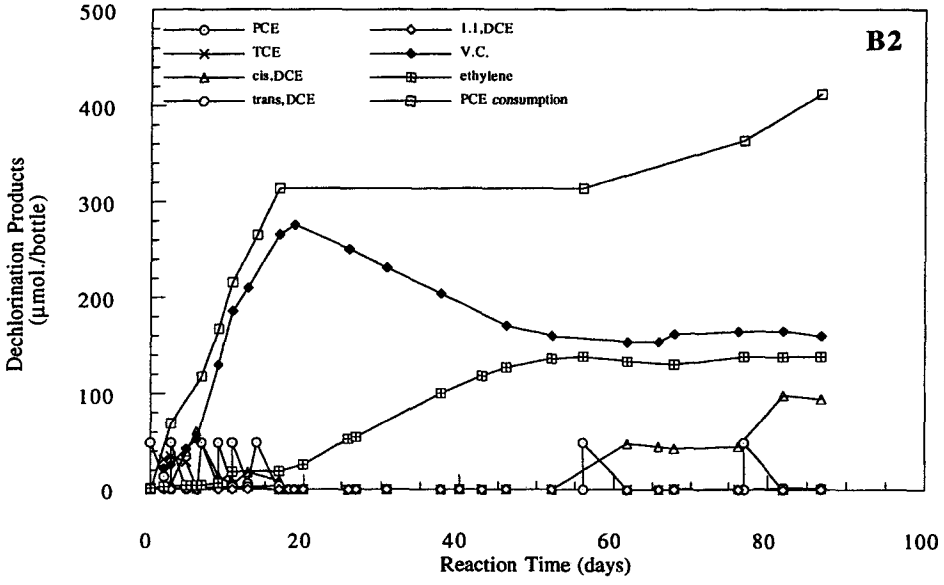
Fig. 38 Long term dechlorination of V.C. and formation of intermediate in a semi-continuous operation serum bottle.



For mixed-cultures, in bottle 1 (B1) the mixed-cultures degraded 219 μmoles of PCE during 16 days of semi-continuous operation. All of the PCE was converted to VC, which accumulated to a peak of 150 μmoles on day 16, at which time 60 μmoles of ethylene was produced. No further additions of PCE were made. Methanol (123 μmoles) was periodically added (every 10 days) to facilitate conversion of VC to ethylene. The mixed liquid in the bottle was also replaced with 5 mL of fresh medium at the same time. Routine analysis of liquid sample demonstrated that much more ethylene (133 μmoles) was formed in the mixed culture bottle during the incubation period (40 days) without further PCE addition, but it seemed to be very difficult to completely dechlorinate these remaining VC. At the end of this stage, 45 μmoles of VC remained. The difficulty in completely dechlorinating VC is similar to the observation from the VC bottle. PCE and methanol were added again on day 56. The added PCE was only dechlorinated to cis-DCE. No further dechlorination of cis-DCE was observed even with a longer incubation time. Ethylene levels in the bottle remained the same (see Fig. 39). This may have been due to either the long-term exposure of VC or methanol as only these two chemicals were present during that incubation period. Similar results were obtained from bottle 2 (B2) under conditions in which PCE was always respiked into the bottle whenever the previously added PCE was found to be degraded to TCE (also see Fig. 39), while the PCE was added to bottle 1 only after the previously added PCE was completely dechlorinated to VC.

Fig. 39 Long term dechlorination of PCE and formation of intermediates in a semi-continuous operation serum bottle in which 60 mL of mixture(*) of acclimated anaerobic digested sludge was used as the dechlorinating organisms.





(*): mixture is 10 ml from each long term experimental culture (60 ml total).

4.9. Effect of methanol.

In an attempt to establish a relationship between concentration of methanol (primary carbon source) and extent of reductive dechlorination of PCE, acclimated digested sludge was incubated with various amounts of methanol and PCE at 49 μmoles per 100 mL of sludge liquid. The results indicated the amount of added methanol will affect the rate and extent of dechlorination of PCE. As more methanol was added, the PCE dechlorination rate decreased. As a consequence, partial dechlorination and less ethylene production occurred. The additional methanol resulted in a proportional increase in methane production. This suggested that the excess methanol will compete with PCE for electrons derived from oxidation of methanol itself and methanogenesis had consumed most of electrons. Therefore, the increase in methanol had no benefit for the kinetics of dechlorination. But, the long term effect of methanol on PCE dechlorination is not clear yet.

The six acclimated sludge bottles were operated semicontinuously. The total operation time for each bottle was 230 days comprised of 23 successive runs. The shortest run took 4 days, while 30 days were expended in the operation of the longest run (the last run). Generally, 6 days were used for most of the runs because the complete dechlorination and stable results could be achieved within 6 days. At the beginning of each run, fresh medium, methanol and PCE (49 μmoles) are replenished. The detailed operation for each bottle is summarized in Table A-1 (Appendix A).

During the first two runs, there was no significant difference in PCE dechlorination and ethylene production between the bottles incubated with various

amounts of methanol. However, the effect of methanol on reductive dechlorination became more pronounced as time increased. From the 4th run on, a strong influence of methanol on ethylene production was observed with the samples receiving higher amount of methanol. During the 4th run, only about 8 - 19 μmoles of ethylene were formed from the samples with methanol addition more than 1230 μmoles, while 42.9 and 57.6 μmoles of ethylene were produced from the samples with 0 and 123 μmoles of methanol added, respectively. The data are shown in Table 14. The amount of added methanol was not only observed to affect ethylene production but also to influence the extent of PCE dechlorination. 20 μmoles of TCE and 3 μmoles of cis-DCE were still persistent in the sludge sample with the highest amount (4920 μmoles) of methanol after 4 days of incubation in the 3rd run, while PCE (49 μmoles)was entirely dechlorinated to either VC or ethylene in other samples. The deteriorated dechlorinating ability was also observed with the second highest methanol sample from the fifth run. After 6 days of incubation of that run, 49 μmoles of PCE were only partially dechlorinated to 32.7 μmoles of TCE and 15.4 μmoles of cis-DCE in that sample. After that, the added PCE was dechlorinated to cis-DCE only. The complete dechlorination never occurred again even though the frequency of PCE and methanol addition was decreased. The results of PCE dechlorination for each bottle are summarized as follows.

0 MeOH bottle. PCE dechlorination was tested under the conditions without methanol addition in this bottle. At the beginning of each run, 5 ml of supernatant was removed and replaced with the same amount of fresh medium plus 49 μmoles of PCE. After only one run's operation, a high ethylene producing ability was achieved in the acclimated sludge. 42.8 μmoles of ethylene were produced from the dechlorination of 49 μmoles of PCE and the residual VC (about 50 μmoles built up from previous

Table 14. Data showing the inhibitory effect of MeOH on ethylene production

Day	Run # (*)	Incubation time	0 MeOH	5 MeOH	5 MeOH + 20 Medium	50 MeOH	100 MeOH	200 MeOH
			Ethylene Production (µmol)					
22	2	4 days	42.76	45.26	34.17	48.96	43.66	47.86
32	4	6 days	42.87	57.62	26.69	7.91	18.94	0.91
49	8	0 day	0.00	0.00	0.00	0.00	0.00	0.00
52	8	3 days	14.40	22.26	3.79	17.31	0.00	0.00
53	8	4 days	23.21	33.17	9.79	21.86	0.00	0.00
54	8	5 days	31.04	41.50	14.89	27.29	0.00	0.00
55	**8**	**6 days**	**42.40**	**57.04**	**22.94**	**34.19**	**0.00**	**0.00**

(*) : At the beginning of each run, medium, PCE and MeOH are replenished.
The effect of MeOH becomes more prounced as time increases.

operation) in 4 days. During the same period, a total of 58 μmoles of methane were produced. After that, the average methane production was 83 μmoles for each run (1918 μmoles in total). Over 230 days of semi-continuous operation, a total of 1127 μmoles of PCE was consumed and 644 μmoles of ethylene were produced with an accumulation of 451 μmoles of VC. The ethylene yielding ratio is about 57%.

For all of the samples, the total ethylene production would have been higher and faster if the experiments had been conducted without any accident. The first accident that all samples were directly placed into the incubator without well shaking after samples were centrifuged for sampling during the 6th run (on day 41), resulted in zero ethylene production for that run. The second one was that all samples were incubated in a 35°C shaker for trying to speed up the PCE dechlorination and ethylene formation during the 11th and 12th runs. This (mixing) also resulted in no ethylene production and partial dechlorination for all samples. The last accident happened on day 104. The temperature of incubator was incidentally increased to 55°C for about 4 hours. After that, it took 10 days to dechlorinate 49 μmoles of PCE entirely to VC and obtain no any ethylene for all samples. The complete dechlorination ability of each sludge bottle was only partially recovered after 30 - 60 days.

5 MeOH bottle. 5 μl of methanol (123 μmoles) plus 5 ml of nutrient medium was replenished at each time of PCE addition. During the 2nd run, the complete dechlorination ability was demonstrated by the observation of 45 μmoles of ethylene production from the dechlorination of 49 μmoles of PCE in 4 days. The methane production was 65 μmoles. At the end of experiment, 775 μmoles of ethylene were yielded from 1127 μmoles of PCE, while the rest of PCE terminated at VC. The

ethylene yielding ratio is 68.8%. The average methane production for each run was 96 μmoles (2210 μmoles for the overall experiment).

5 MeOH+20 Medium bottle. 5 μl of methanol and 20 ml of nutrient medium were added at each time of PCE addition. The increase of the replenishing medium has caused the decrease in the ethylene production for this culture. At the beginning, a total of 85% of PCE was recovered as ethylene in 5 days. Then the total ethylene production in each run decreased gradually from 42 μmoles (at the beginning) to about 20 μmoles. However, a higher methane production was observed. On an average the methane production was 114 μmoles for each run. It is obvious that the more methane production is not beneficial to the complete dechlorination of PCE in the sludge. Over the same experimental period, a total of 1005 μmoles of PCE was consumed and resulted in the formation of 449 μmoles of ethylene. The ethylene yielding ratio is 44.7%.

50 MeOH bottle. A higher amount of methanol (50 μl, equivalent to 1230 μmoles) plus 5 ml of nutrient medium were replenished at each time of PCE addition. At the beginning, the complete dechlorination ability was not affected by the higher methanol dose. It was possibly due to the incomplete metabolism of methanol because a similar amount of methane production was observed during the first two runs. After that, the methane production increased to about 350 μmoles each run and ethylene formation became less (about 10 -20 μmoles each run). However, all of the added PCE still could be entirely dechlorinated to VC without any significant accumulation of other dechlorination intermediates in each run. In an attempt to improve the ethylene production, all bottles were placed in a shaker from day 67 to 80. Unfortunately, PCE

has been only dechlorinated to cis-DCE since. The complete dechlorination ability was never recovered and ethylene was not detected again from this bottle. During the whole period of experiment, methanol and PCE were added only 15 and 14 times, respectively. However, the decreased frequency of the addition of methanol and PCE which may result in a longer incubation time and a lower methanol loading failed to achieve further dechlorination of cis-DCE. A total of 686 μmoles of PCE was degraded and 229 μmoles of ethylene were formed over 230 days of period. At the end of the experiment, 5248 μmoles of methane were produced and the accumulation of cis-DCE and VC reached 156 and 308 μmoles, respectively. The ethylene yielding ratio is 33.4%.

100 MeOH bottle. 100 μl of methanol (2460 μmoles) plus 5 ml of nutrient medium were applied to the bottle each time when PCE was added. PCE dechlorination became more difficult in this bottle with the even higher methanol dose. At the beginning, PCE dechlorination ability and methane production were similar to those observed in other bottles. However, after the 4th run, TCE (32.7 μmoles) and cis-DCE (15.4 μmoles) started to accumulate. Even a longer period of incubation was applied, the further dechlorination of these compounds did not occur. After that, the added PCE tended to persist longer and was dechlorinated to cis-DCE only. Through the entire experiment, 392 μmoles of PCE and 19926 μmoles of methanol were applied. At the end of the test, 116 and 4436 μmoles of ethylene and methane, respectively were produced. The ethylene yielding ratio is 29.6%. However, if the ethylene produced during the period of the beginning had not been accounted for, the real yielding ratio would have approximated to zero.

200 MeOH bottle. 200 µl of methanol (4920 µmoles) plus 5 ml of nutrient medium were supplemented to the bottle at each time of PCE addition. A total of 39606 µmoles of methanol were added through the test. The highest amount of methanol resulted in the most methane production (7405 µmoles in total) among the test bottles. However, the worst progressive deterioration of PCE-dechlorinating ability was observed with this sludge. At the end of the 3rd run, part of added PCE (49 µmoles) terminated at TCE (20 µmoles) and cis-DCE (3.4 µmoles), while PCE was entirely ended up as either VC or ethylene in other bottles. At the same time, methane production in this bottle was observed to increase from 132 µmoles (last run) to 449 µmoles and ethylene production was decreased from 48 µmoles to 14 µmoles. Henceforth ethylene was never produced again and methane production was increased up to a maximum value of 2300 µmoles in a single run. Cis-DCE was the major end product of PCE dechlorination and PCE became highly resistant to reductive dechlorination. Usually, it took one month before PCE dechlorination started to occur in the sludge. Through the experiment, the dechlorination of 392 µmoles of PCE resulted in the production of 114 µmoles of ethylene and the accumulation of 142 and 96 µmoles of cis-DCE and VC, respectively. The ethylene yielding ratio is 29.1%. The detailed results are shown in Fig. 40a and b.

Generally speaking, a notable effect of methanol on PCE dechlorination was observed among the test samples after the methanogenesis had been increased with the increase of methanol addition. There seems to be an inverse relationship between the reductive dechlorination and the methanogenic reaction. The ethylene production and the extent of PCE dechlorination became smaller as the methanogenesis became larger. At the highest methanol dose, even PCE, the most readily degradable compound, was

Fig. 40(a)
The effect of methanol and fresh media on PCE dechlorination.

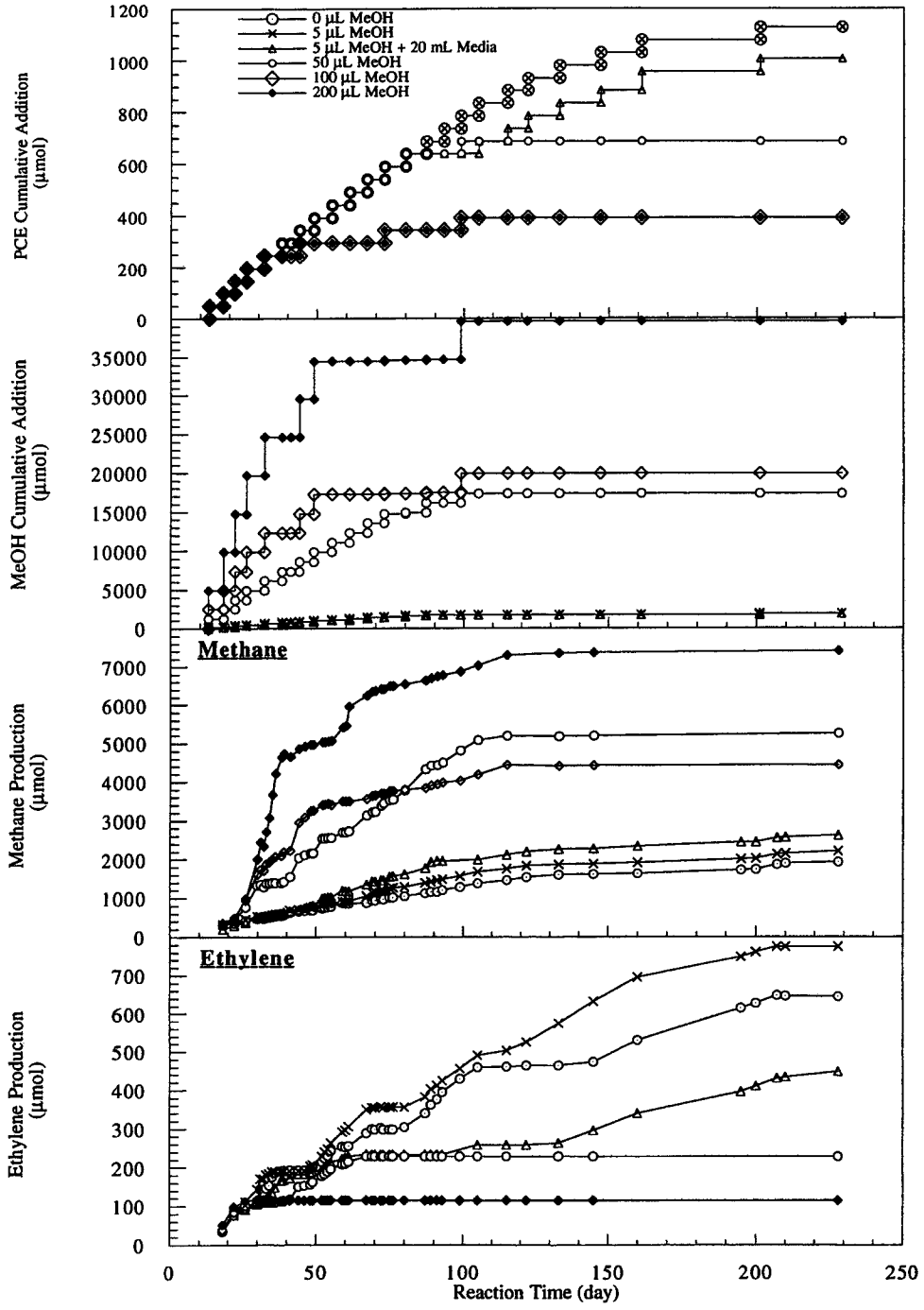
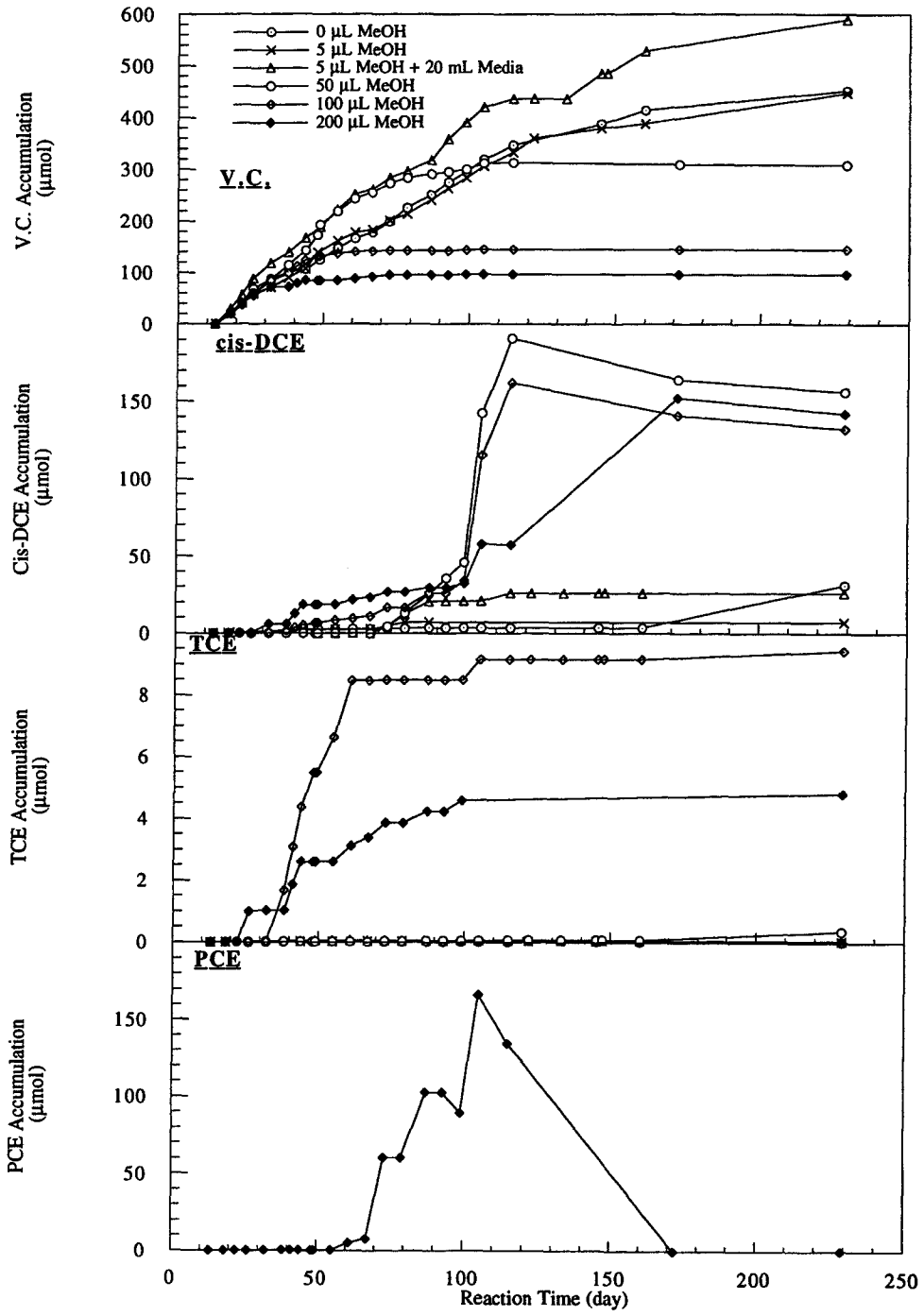


Fig. 40(b)

The effect of methanol and fresh media on PCE dechlorination.



inhibited from undergoing the reductive dechlorination and persisted longer. It is likely due to the existence of a competition for electrons between the dechlorination and methanogenesis because both are reductive reaction. In this test, not only methanol but also the added nutrient medium were observed to inhibit PCE dechlorination. Both of them have methane-yielding capability. However, the methane-yielding capacity of the fresh nutrient medium is not well known because it contains 50% of sludge supernatant and the composition of the supernatant can not be defined. This makes impossible to compare the two materials.

4.10. Effect of mixing on PCE dechlorination.

Proper mixing is one of the most important considerations in achieving optimum wastewater treatment efficiency. For biological treatment, mixing is used to accelerate the biological conversion process. However, biological dechlorination of PCE in anaerobic Hyperion digested sludge was observed to be retarded by mixing. Furthermore, partial dechlorination of PCE was noted under mixing conditions. Throughout the test, the samples incubated with mixing always produced more methane than those incubated under quiescent conditions. These indicated that mixing has indeed accelerated methanogenic activities. But it may be due to the same reason as observed in the methanol test to cause the retardation of PCE dechlorination.

Mixing effect on PCE dechlorination was clearly demonstrated during the 1st run. After 10 days of incubation, PCE terminated at cis-DCE in the sample (S5) with mixing, while the added PCE was entirely dechlorinated to VC in other samples without mixing. During the same period, a significant difference in methane production was also

observed. 302.2 μmoles of methane was produced from sample 5 (S5) and an average of 241 μmoles of methane was produced from other samples. This suggested that most electrons had been diverted to the electron acceptor for methanogenesis. The results are shown in Fig. 41 and Table 15. From Fig. 41, we can see that before PCE was dechlorinated to cis-DCE, there was no significant difference in the dechlorination between the samples with and without mixing. After that, cis-DCE was continuously dechlorinated in the sample without mixing but the dechlorination in the sample with mixing stopped. This suggested that mixing (or high methanogenesis) is relatively more tolerable to PCE and TCE dechlorination.

In order to reduce dechlorination inhibition due to methanogenesis, the volume of medium replaced was reduced from the 2nd run because it was believed that a larger medium replacement will favor methanogenesis. At the end of the 2nd run, PCE was entirely dechlorinated to VC (about 35 μmoles) and ethylene (14.6 μmoles) in the sample 4 (S4) which was moved into the shaker at the beginning of this run. It seemed that the smaller medium replacement, resulting less methanogenesis, has reduced dechlorination inhibition even under mixing conditions. Data are shown in Table 16. The better results of complete dechlorination were still observed in the samples without mixing. For sample 5 with mixing, PCE was still dechlorinated to cis-DCE only.

In order to further reduce methanogenesis, carbon dioxide was removed from the headspace of sample 2 and 3 at the beginning of 3rd run. After 6 days of incubation, the results of complete dechlorination observed from sample 3 (with mixing but without CO_2) were better than that observed from sample 4 (with mixing and with CO_2). This further indicated that methanogenesis may inhibit reductive dechlorination. The details

Fig. 41 The effect of mixing on PCE dechlorination and methane production. Data from 1st Run.

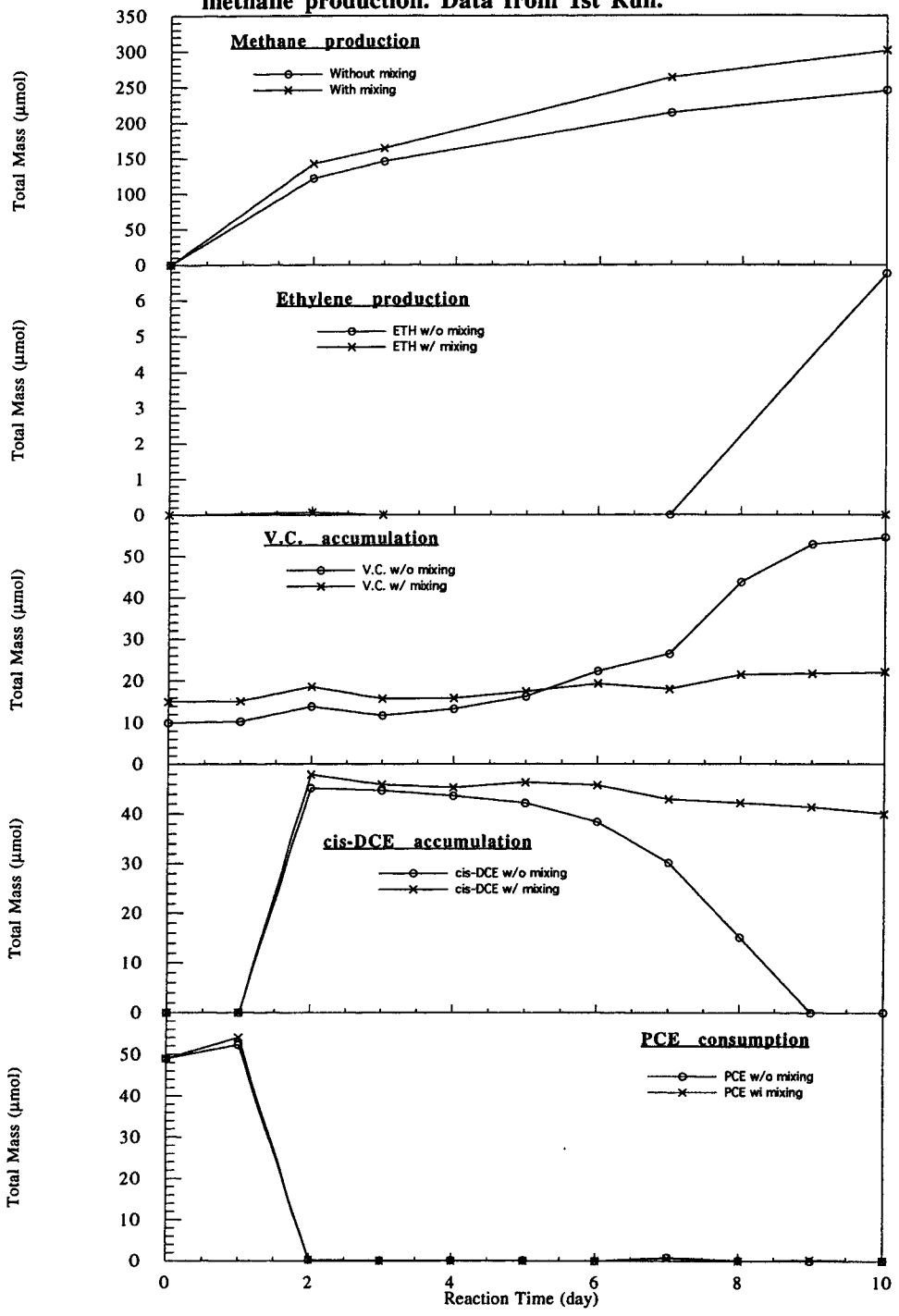


Table 15.
The effect of mixing on PCE dechlorination - 1st Run

Time (day)	PCE (μmol)	TCE (μmol)	CD (μmol)	VC (μmol)	ETH (μmol)	CH4 (μmol)
Sample 1 (without mixing)						
2					0.00	127.34
3					0.00	140.62
7					0.00	199.62
10	0.00	0.00	0.00	56.79	4.61	249.63
Sample 2 (without mixing)						
2						108.99
3						130.16
7					0.00	204.18
10	0.00	0.00	0.00	54.47	3.71	241.36
Sample 3 (without mixing)						
1	52.30	0.65	0.08	10.31		
2	0.36	0.15	45.11	13.94		121.58
3	0.23	0.00	44.73	11.78		146.28
4	0.16	0.00	43.56	13.26		
5	0.23	0.00	42.21	16.36		
6	0.00	0.00	38.42	22.27		
7	0.92	0.00	30.14	26.49	0.00	214.07
8	0.18	0.00	15.14	43.82		
9	0.11	0.00	0.00	52.87		
10	**0.00**	**0.00**	**0.00**	**54.57**	**6.76**	**246.08**
Sample 4 (without mixing)						
2						106.47
3						126.80
7					1.18	187.93
10	0.00	0.00	0.00	57.04	5.56	225.78
Sample 5 (with mixing)						
1	54.06	0.69	0.09	15.18	0.00	
2	0.29	0.00	47.93	18.61	0.07	143.19
3	0.09	0.00	45.91	15.71	0.01	164.18
4	0.09	0.00	45.28	15.91		
5	0.12	0.00	46.31	17.52		
6	0.00	0.00	45.72	19.30		
7	0.74	0.00	42.90	18.06		263.27
8	0.00	0.18	42.19	21.51		
9	0.30	0.00	41.32	21.77		
10	**0.00**	**0.00**	**39.93**	**22.15**	**0.00**	**302.20**

Note: 1. The initial dosage of PCE for each sample is 49 μmol.
2. The residue of V.C. in each sample at the beginning is about 10 - 15 μmol.

Table 16. The effect of mixing and reduced medium replacement on PCE dechlorination - 2nd Run

Time (day)	PCE (μmol)	TCE (μmol)	CD (μmol)	VC (μmol)	ETH (μmol)	CH4 (μmol)
Sample 1 (without mixing)						
0	*49.00*			*21.00*	*0.00*	*0.00*
4					7.77	136.11
6	0.00	0.00	0.00	54.44	16.11	177.82
Sample 2 (without mixing)						
0	*49.00*			*25.00*	*0.00*	*0.00*
4					8.71	126.65
6	0.00	0.00	0.00	51.99	22.34	183.61
Sample 3 (without mixing)						
0	*49.00*			*30.00*	*0.00*	*0.00*
4	0.00	0.00	0.61	64.09	12.78	136.19
6	**0.00**	**0.00**	**0.00**	**48.65**	**31.25**	**168.40**
Sample 4 (with mixing)						
0	*49.00*			*20.00*	*0.00*	*0.00*
4	0.00	0.00	0.42	65.06	5.22	143.82
6	0.00	0.00	0.00	55.38	14.58	212.46
Sample 5 (with mixing)						
0	*49.00*		*32.00*	*18.00*	*0.00*	*0.00*
4	0.16	0.00	81.14	17.85	0.00	163.95
6	**0.00**	**0.00**	**77.33**	**18.02**	**0.00**	**207.67**

Note: 1. The volume of fresh medium replacement for each bottle was reduced from 5 mL to 2.5 mL.

2. The concentration of PCE and V.C. of each sample at the beginning were estimated based on the initial PCE dose and V.C. residue.

are shown in Table 17. At this time, PCE was still dechlorinated to cis-DCE only from sample 5 and an even better dechlorination result was obtained from samples without mixing.

To test if the deteriorated dechlorination ability under mixing was a reversible effect, sample 5 was removed from the shaker to the quiescent incubator at the beginning of 4th run. After 6 days of incubation, about 21 μmoles of cis-DCE were found to be further dechlorinated to VC. During this run, more cis-DCE was observed to accumulate in sample 3 and 4 (under mixing). However, a relatively better result was still obtained from sample 3 in which CO_2 was not present. The results are summarized in Table 18.

Finally, to achieve a better dechlorination, extended incubation was applied to each sample and PCE addition was stopped for sample 5 in 5th run. After one month of incubation, the deteriorated dechlorination ability was demonstrated as a reversible effect because a lot of ethylene production (64 μmoles) was measured from sample 5. The dechlorination in all other bottles was also improved after the extended incubation period. Much more ethylene production was observed from sample 1 to 3. At the end of the experiment, PCE was dechlorinated to cis-DCE and VC only without any ethylene production in sample 4 which contained CO_2. Compared to the results of sample 3, it seemed that the presence of carbon dioxide is also inhibitory to the reductive dechlorination. Data are shown in Table 19.

Table 17. The effect of mixing and carbon dioxide on PCE dechlorination - 3rd Run

Time (day)	PCE (μmol)	TCE (μmol)	CD (μmol)	VC (μmol)	ETH (μmol)	CH4 (μmol)
Sample 1 (without mixing; with carbon dioxide)						
0	*49.00*			*34.00*	*0.00*	*0.00*
2					5.82	80.83
6	0.00	0.00	0.00	57.73	25.72	129.49
Sample 2 (without mixing; without carbon dioxide)						
0	*49.00*			*23.00*	*0.00*	*0.00*
2					2.25	24.62
6	0.00	0.00	0.00	56.02	16.07	61.76
Sample 3 (with mixing; without carbon dioxide)						
0	*49.00*			*21.00*	*0.00*	*0.00*
2					3.53	48.12
6	**0.11**	**0.00**	**0.00**	**60.65**	**10.06**	**102.75**
Sample 4 (with mixing; with carbon dioxide)						
0	*49.00*			*31.00*	*0.00*	*0.00*
2					7.15	96.27
6	**0.08**	**0.00**	**0.00**	**75.95**	**4.92**	**143.62**
Sample 5 (with mixing; with carbon dioxide)						
0	*49.00*		*65.00*	*16.00*	*0.00*	*0.00*
2					0.00	99.35
6	**0.08**	**0.00**	**114.27**	**16.61**	**0.00**	**173.72**

Note:
1. The volume of fresh medium replacement for each bottle was 2.5 mL.
2. The headspace of Sample 2 and 3 was filled with nitrogen, while other samples were filled with 30% carbon dioxide and 70% nitrogen.
3. The concentration of PCE and V.C. of each sample at the beginning were estimated based on the initial PCE dose and V.C. residue.

Table 18. The effect of mixing and carbon dioxide on PCE dechlorination - 4th Run

Time (day)	PCE (μmol)	TCE (μmol)	CD (μmol)	VC (μmol)	ETH (μmol)	CH4 (μmol)
Sample 1 (without mixing; with carbon dioxide)						
0	*49.00*			*28.00*	*0.00*	*0.00*
6	0.00	0.00	0.02	68.76	8.92	39.15
Sample 2 (without mixing; without carbon dioxide)						
0	*49.00*			*32.00*	*0.00*	*0.00*
6	0.00	0.00	0.00	64.48	17.08	37.47
Sample 3 (with mixing; without carbon dioxide)						
0	*49.00*			*30.00*	*0.00*	*0.00*
6	**0.00**	**0.00**	**19.75**	**59.28**	**0.14**	**94.39**
Sample 4 (with mixing; with carbon dioxide)						
0	*49.00*			*45.00*	*0.00*	*0.00*
6	**0.00**	**0.00**	**40.44**	**54.24**	**0.00**	**77.47**
Sample 5 (without mixing; with carbon dioxide)						
0	*49.00*		*103.00*	*16.00*	*0.00*	*0.00*
6	0.00	0.00	131.60	37.24	0.00	144.27

Note:
1. The volume of fresh medium replacement for each bottle was 2.5 mL.
2. The headspace of Sample 2 and 3 was filled with nitrogen, while other samples were filled with 30% carbon dioxide and 70% nitrogen.
3. Sample #5 was moved back to the incubator without mixing to test if cis-DCE can be further dechlorinated.
4. The concentration of PCE and V.C. of each sample at the beginning were estimated based on the initial PCE dose and V.C. residue.

Table 19. The effect of mixing carbon dioxide on PCE dechlorination - 5th Run

Time (day)	PCE (μmol)	TCE (μmol)	CD (μmol)	VC (μmol)	ETH (μmol)	CH4 (μmol)
Sample 1 (without mixing; with carbon dioxide)						
0	*49.00*			*44.00*	*0.00*	*0.00*
6	0.00	0.00	9.36	84.62		
9	0.00	0.00	0.00	87.39		
11	0.00	0.00	0.00	84.38	8.17	103.53
21	0.00	0.00	0.00	77.36	13.11	126.80
32					47.96	68.24
Sample 2 (without mixing; without carbon dioxide)						
0	*49.00*			*42.00*	*0.00*	*0.00*
6	0.00	0.06	15.02	69.05		
11	0.00	0.00	0.83	83.43	8.81	113.85
21	0.00	0.00	0.83	83.43	17.18	140.90
32					49.65	85.05
Sample 3 (with mixing; without carbon dioxide)						
0	*49.00*		*15.00*	*38.00*	*0.00*	*0.00*
6	0.07	0.00	53.94	48.61		
11	0.00	0.00	40.35	63.65		
16	0.00	0.00	19.71	81.59		
21	0.00	0.00	1.81	98.15	0.00	190.54
32					**36.00**	**100.32**
Sample 4 (with mixing; with carbon dioxide)						
0	*49.00*		*35.00*	*34.00*	*0.00*	*0.00*
6	0.00	0.00	70.41	47.98		
11	0.00	0.00	57.13	59.99		
16	0.00	0.00	41.07	71.17		
21	0.00	0.00	28.18	83.19	0.00	196.66
32					**0.00**	**103.96**
Sample 5 (without mixing; with carbon dioxide)						
0	*0.00*		*124.00*	*23.00*	*0.00*	*0.00*
6	0.10	0.05	53.50	94.42		
11	0.00	0.00	41.69	105.98	0.00	204.21
14	0.00	0.00	6.37	140.06		
21	0.00	0.00	0.00	99.38	44.55	209.97
32					64.33	84.18

Note:
1. The volume of fresh medium replacement for each bottle was 2.5 mL.
2. The headspace of Sample 2 and 3 was filled with nitrogen, while other samples were filled with 30% carbon dioxide and 70% nitrogen.
3. PCE was not added to sample #5 to test if the accumulated cis-DCE can be completely dechlorinated to ethylene.
4. The concentration of PCE and V.C. of each sample at the beginning were estimated based on the initial PCE dose and V.C. residue.

4.11. Effect of temperature on PCE dechlorination.

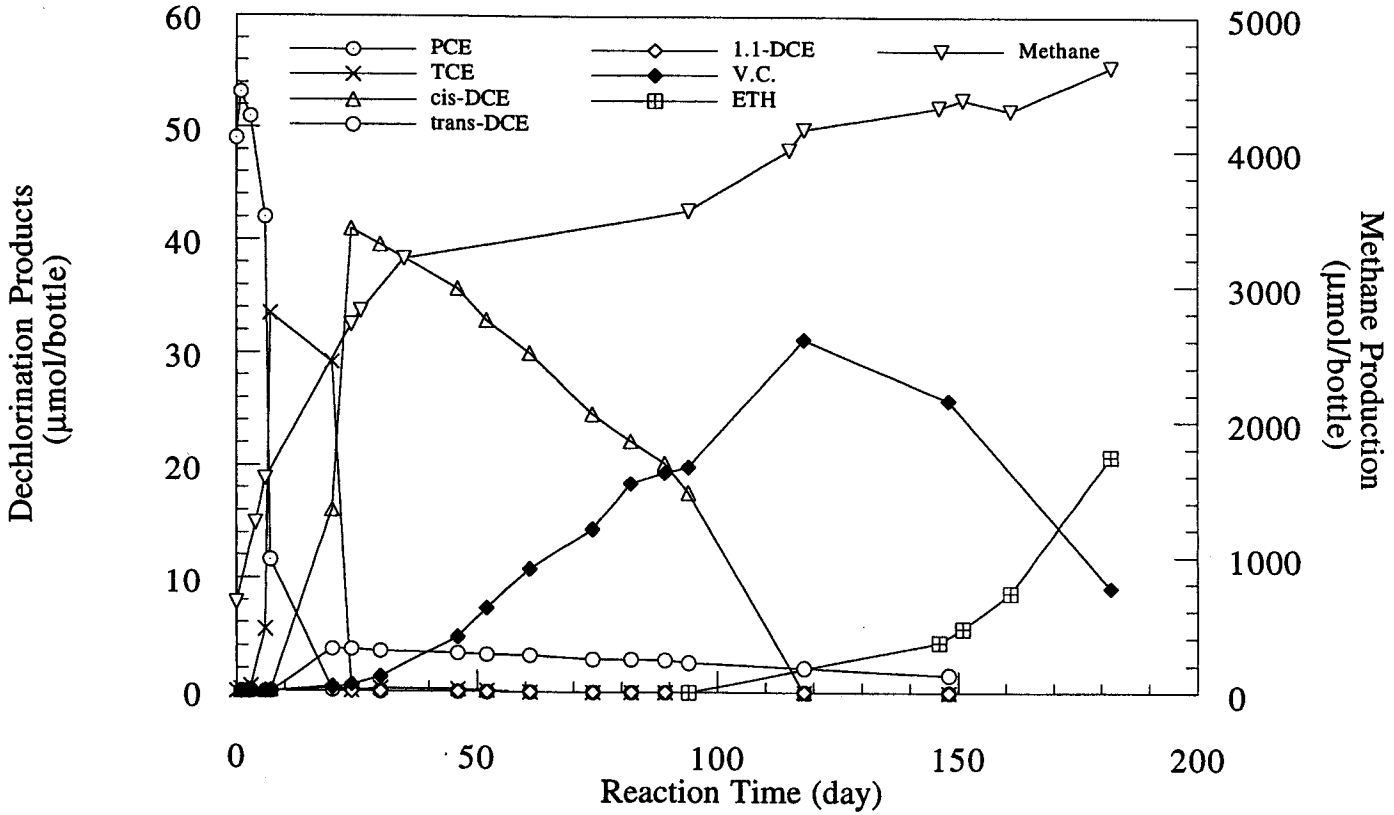
Temperature is an important environmental parameter. To optimize the efficiency of the mesophilic anaerobic digestion, operation temperature is usually controlled in the ranges of 30 to 38°C. For unheated digestors, bacteria may function worse. In this experiment, we test PCE dechlorination under the room temperature which fluctuated with local climatic conditions. As with all biological systems, lower temperatures retarded the dechlorination process.

PCE dechlorination proceeded more slowly in the fresh Hyperion sludge under laboratory conditions without temperature regulation. It took 6 days to start to significantly degrade PCE. In the following 18 days, PCE was entirely dechlorinated to cis-DCE. After that, cis-DCE was dechlorinated even more slowly. It took 3 months to dechlorinate cis-DCE to VC. Ethylene was not detected until day 146. At the end of the test (182 days), a total of 21 μmoles of ethylene was recovered from the dechlorination of 49 μmoles of PCE (see Fig. 42). However, for the same sludge incubated in a 35°C incubator, it only took 23 days to produce this amount of ethylene (see Fig. A-25, in Appendix A).

4.12. Retardation of PCE dechlorination in old digested sludges.

It was very often for the previous researchers to use refrigerated sludge sample or old sludge as an inoculum in their dechlorination system. In most cases, PCE and TCE were dechlorinated very slowly. It is the inspiration for the old sludge test. Eight sludge samples (1, 2, 3, 4, 12, 14, 20, and 21-day old, respectively) were tested. An

Fig. 42 PCE dechlorination in a batch culture of fresh anaerobic Hyperion digested sludge under laboratory conditions without temperature regulation.



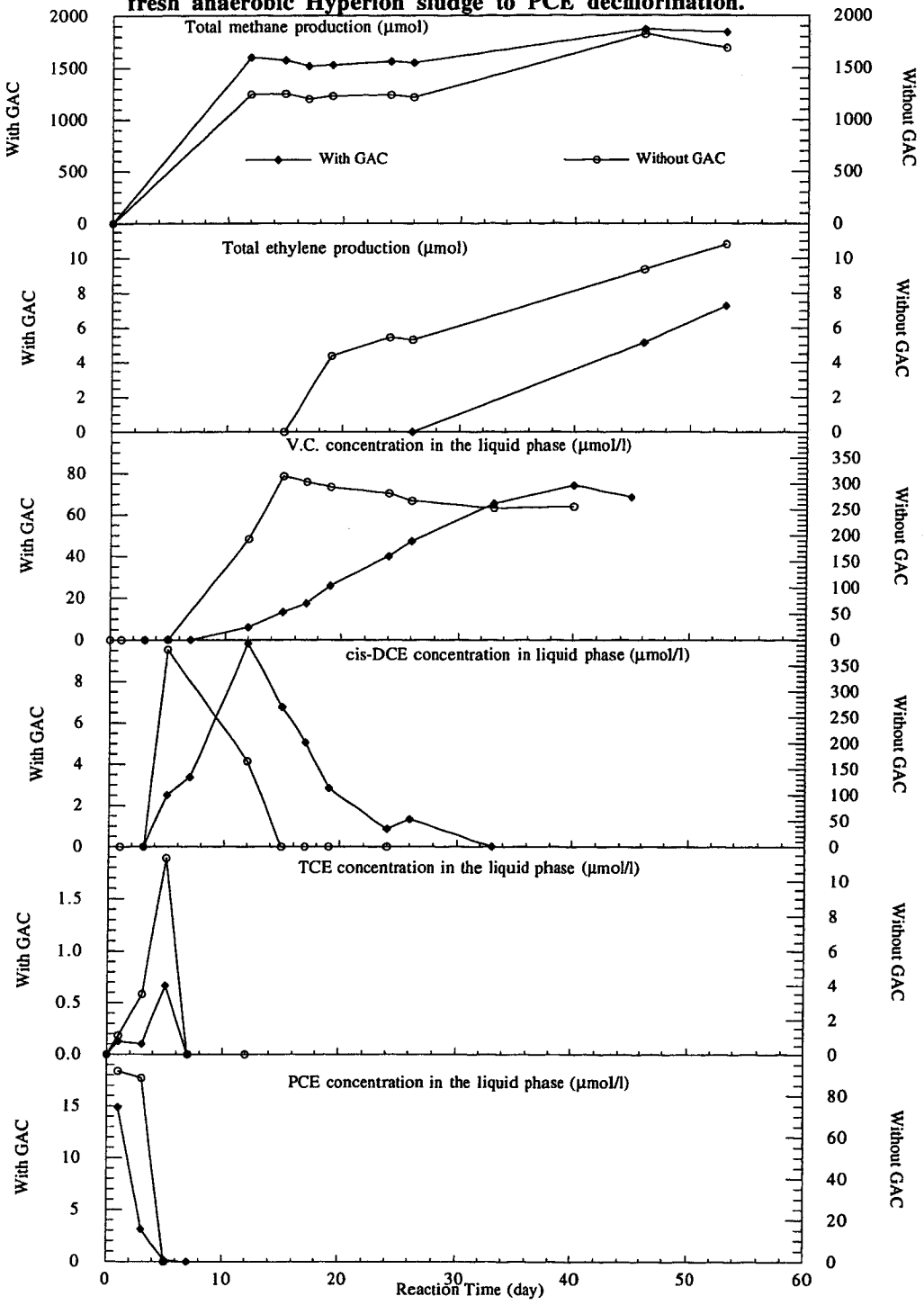
additional fresh sludge sample served as the control. Collectively, slower dechlorination was observed in old sludges and the older the sludge was, the slower the dechlorination was. The detailed results are provided in Appendix A (Fig. A-25 to A-33).

In addition, the transitional accumulation of TCE and cis-DCE was not detected from most of the old samples. Referring to Fig. A-28, 18.4 μmoles of PCE was coexisting with 12.0 μmoles of VC and 8.6 μmoles of ethylene on day 38. This has obviously deviated from what people used to think. It is not necessarily the presence of higher chlorinated compounds that inhibits the dechlorination of lower chlorinated compounds. Another possible explanation for this phenomenon may be due to decreasing methanogenic activity allowing the lower chlorinated compounds to successfully compete for electrons in the system.

4.13. Effect of activated carbon on PCE dechlorination.

At the beginning of activated carbon tests, a series of preliminary experiments was conducted to investigate the possible effects of carbon addition on PCE dechlorination in the anaerobic sludge system. At first, the difference in the acclimation of fresh sludge to PCE dechlorination between the samples with and without carbon addition was tested. As expected, the chemical concentration in the liquid phase was dramatically decreased in the sample with carbon addition due to carbon adsorption. For the same sample, a slower dechlorination but higher methane production were observed (see Fig. 43). The higher methanogenic activity may have resulted from the reduced concentration of chlorinated compounds in the liquid phase. However, after successive additions of PCE, a higher ethylene production was observed from the sample with

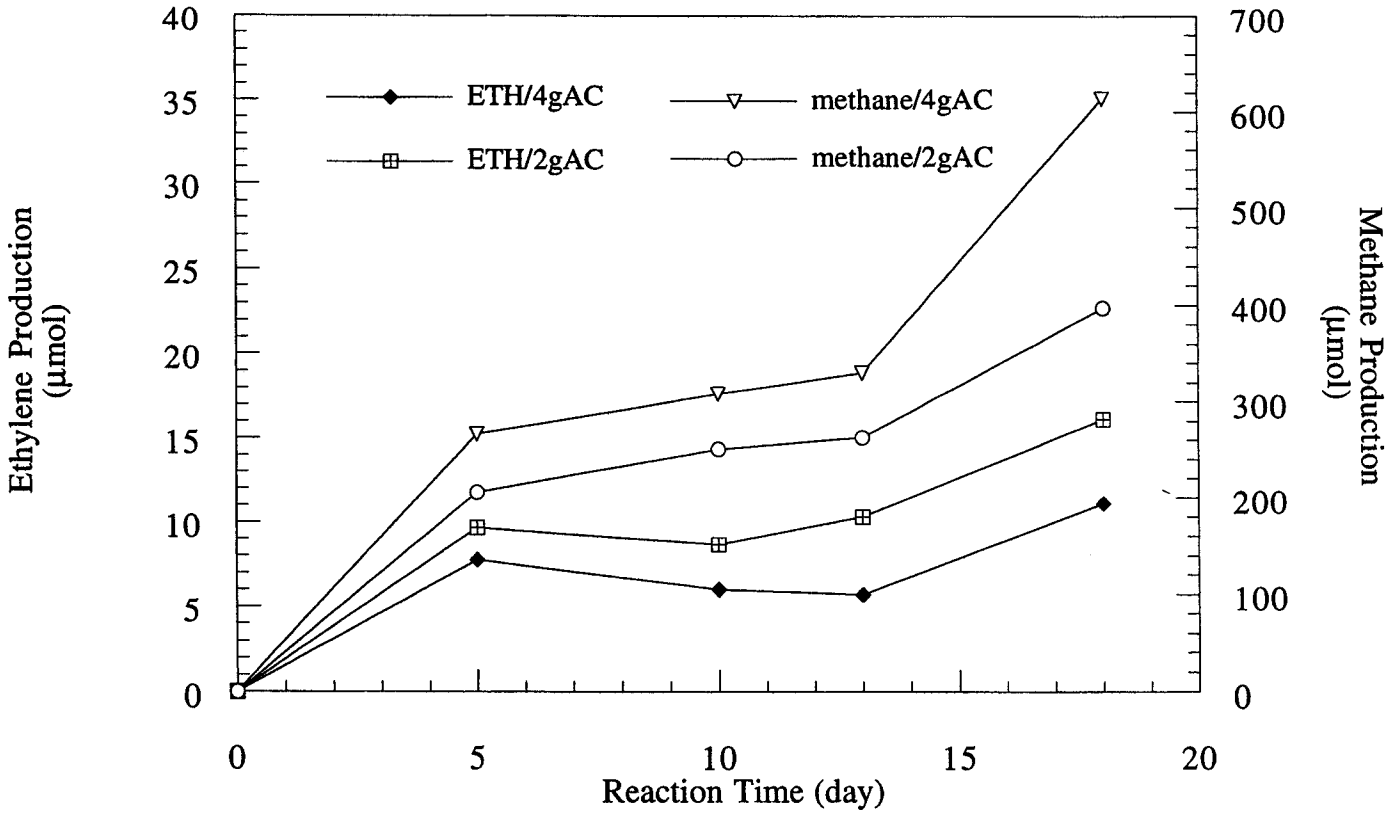
Fig. 43 **The effect of activated carbon on the acclimation of fresh anaerobic Hyperion sludge to PCE dechlorination.**



carbon added. After that, PCE dechlorination was stuck between cis-DCE and VC in the sample without carbon addition due to an unknown reason when complete PCE dechlorination still occurred as usual in the sample with carbon added (see Fig. A-34, in Appendix A). It would not be a surprise because as always for the combined biodegradation and carbon adsorption system, activated carbon can protect bacteria from toxic or inhibitory substances.

In a follow-up test, too much carbon was demonstrated to be an additional retardation factor for complete dechlorination. When 2 more grams of carbon was added to a culture with 2 grams of carbon addition initially, the culture (with more carbon) produced more methane but less ethylene than those produced from the culture with 2 grams of carbon throughout the experiment. The results are shown in Fig. 44 and Fig. A-35 (in Appendix A). The retardation may due to that compounds bind more tightly to the activated carbon when more carbon is introduced. This will result in a slower desorption of chemicals. Therefore, the bioavailability of chemicals decreases. The similar results were observed with an another acclimated sludge sample in which 8 grams of carbon and 196 μmoles of PCE were added. After 85 days of incubation, there was no ethylene produced from that sample. However, during the same period 75 μmoles of ethylene was produced from the sample with less amount (4 grams) of carbon and the same amount of PCE addition (see Fig. A-36 to A-37, in Appendix A). These results indicated that the amount of added carbon will influence the effectiveness of carbon addition on PCE dechlorination in the carbon-sludge system.

Fig. 44 Comparison of methane production and ethylene formation during PCE dechlorination when two different amounts of GAC are introduced to acclimated sludge.



4.14. Complete PCE dechlorination in biological activated carbon system.

The complete dechlorination proceeding via sequential removal of chlorine substituents from PCE was also observed in BAC process. After 66 days of incubation, the total ethylene productions from the six tested bottles with 49, 98, 196, 294, 392, and 490 μmoles of initially added PCE, were 34.5, 44.2, 85.2, 108.2, 142.4, and 188.9 μmoles, respectively. At the same time, all remaining PCE in each bottle had terminated at VC. The amount of PCE degraded increased with the added amount of PCE. Most of PCE was degraded at a concentration much higher than the highest tolerable PCE concentration observed in the toxicity test. This suggested that the adsorptive capacity of activated carbon has effectively dampened concentration fluctuations of PCE in each bottle and protected microorganisms from poisonous PCE. In this test, an inverse relationship between the total methane production and the total added PCE was observed. The more PCE was added, the more electrons were diverted to PCE for reductive dechlorination.

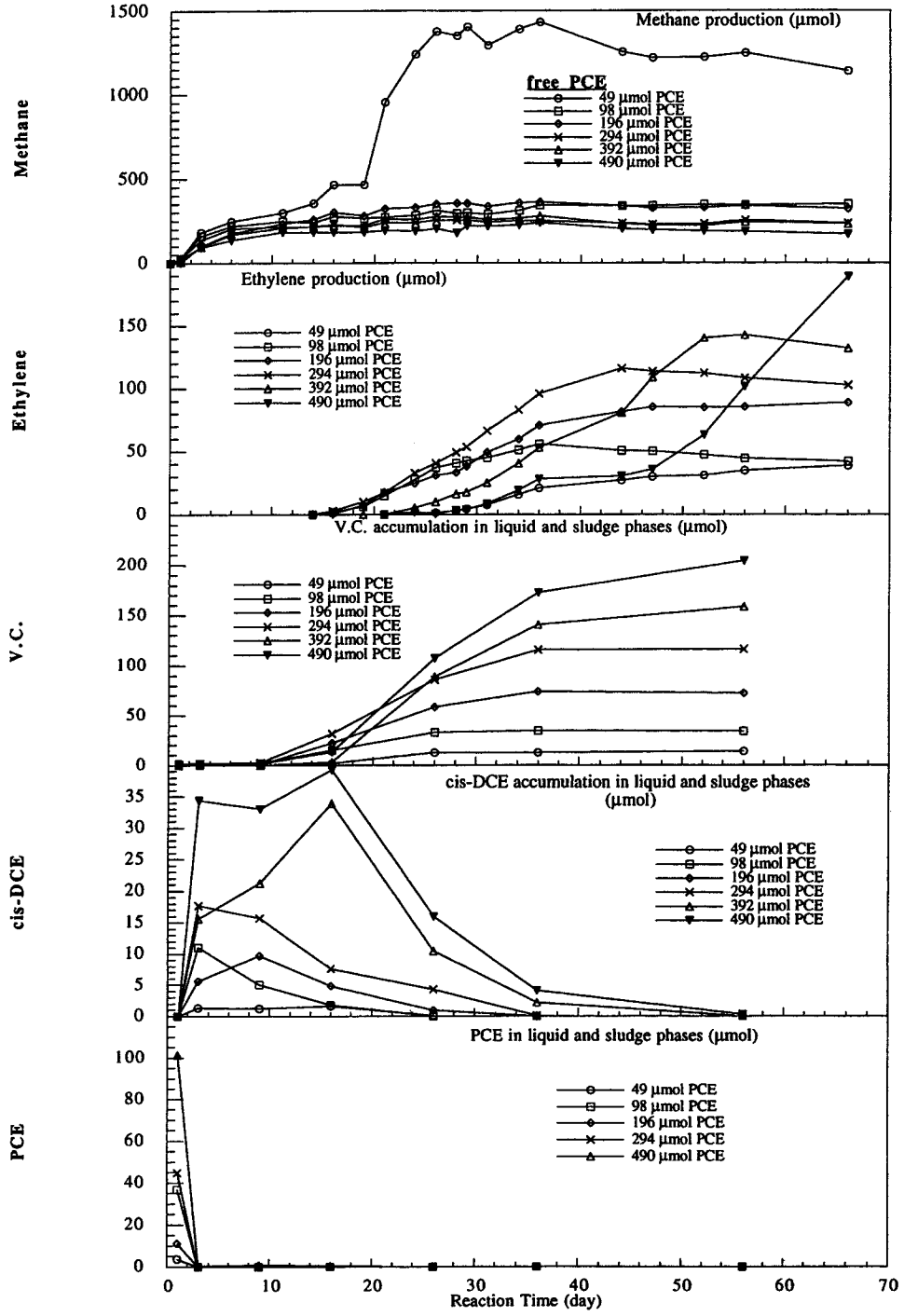
From all test bottles, a significant formation of cis-DCE was observed in only three days of incubation. On day 3, the amount of cis-DCE produced in each bottle increased with the amount of PCE added. This indicated that the concentrations of PCE resulting from the initial doses applied to each bottle were all below the inhibitory concentration of PCE. However, the time needed for entire transformation of PCE to VC was different from each bottle because the total amount of PCE added to each bottle was different. The times taken for entire transformation of PCE to VC were: 26 days for U1 bottle with 49 μmoles of PCE, 36 days for U2 to U4 bottles with 98 to 294 μmoles

of PCE, and 56 days for U5 and U6 bottles with 392 and 490 μmoles of PCE. It increased with the initial PCE dose.

An earlier ethylene formation was observed from the higher PCE dose bottles (U2 to U6). Ethylene was formed before chlorinated compounds entirely terminated at VC in these bottles. However, for the bottle (U1) with the least amount of PCE added, ethylene was not detected until all PCE has ended up as VC. The formation of ethylene may be related to the concentration of VC in the sludge system. For all bottles, bacteria started to produce ethylene when the concentration of VC was increased up to about 90 μmoles/l (5.6 mg/l) in the liquid phase. In addition, the presence of too much other chlorinated compounds, e.g. PCE and cis-DCE, may be an another factor to retard ethylene formation because only trace amount of ethylene was produced in U6 bottle when VC concentration had reached 50 mg/l in the liquid phase. At that time (on day 26), the concentrations for PCE, TCE, and cis-DCE in the liquid phase of U6 bottle were 41, 106, and 1550 μg/l, respectively. However, ethylene production from that bottle was increased dramatically after PCE and TCE disappeared. The detailed results of PCE dechlorination for each bottle are provided in Appendix A (Fig. A-38 through A-43). The effect of varying PCE concentrations on dechlorinating efficiency in BAC system is shown in Fig. 45.

Generally speaking, the total ethylene production increased with the amount of PCE added if the incubation time was long enough. In addition, a significant amount of PCE in each bottle was ended up as VC at the end of the experiment. The accumulation of VC in each bottle was also increased with the amount of PCE added. However, the

Fig. 45 **Effect of varying PCE concentrations on dechlorinating efficiency. (Biological activated carbon system)**



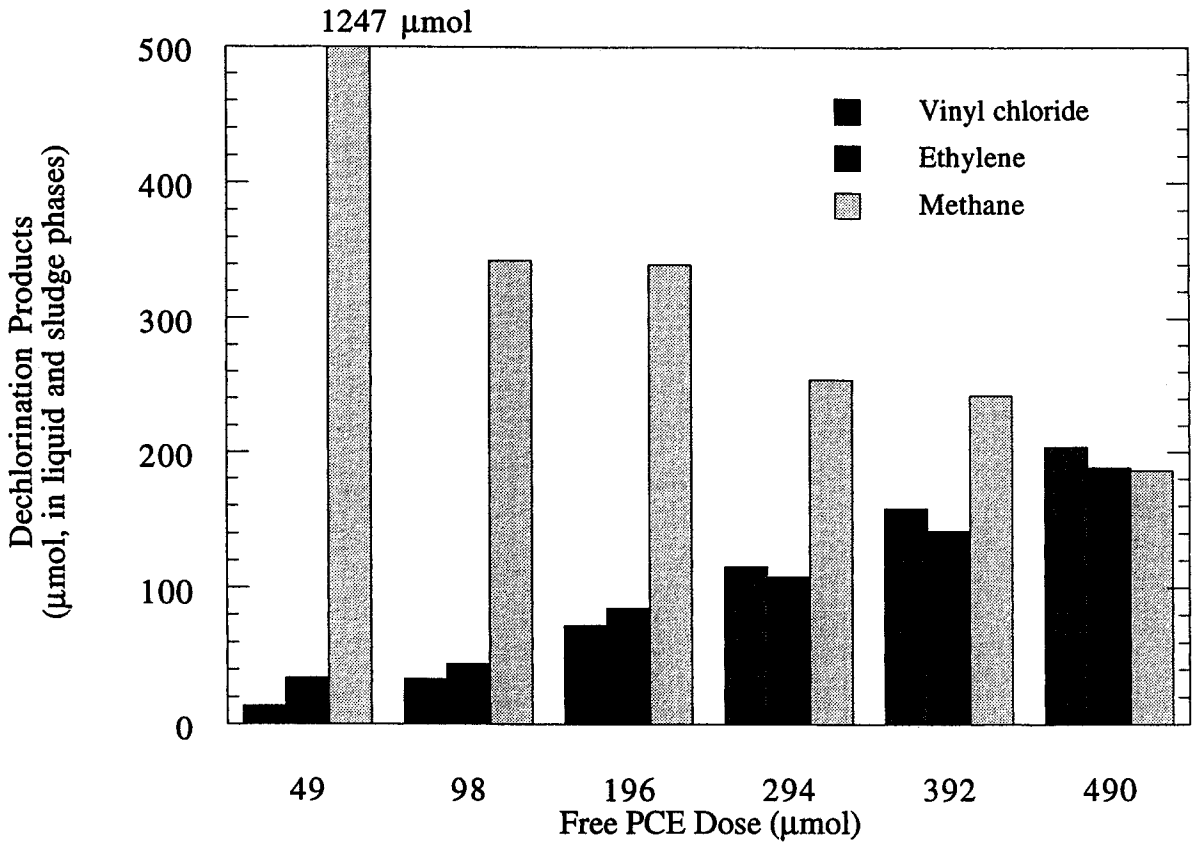
opposite trend was observed for methane production. The final data are shown in Fig. 46.

4.15. Biological regeneration of PCE laden carbon.

The complete dechlorination of PCE to ethylene has been demonstrated in anaerobic digested sludge. This makes bioremediation an attractive method to remediate groundwater contaminated with PCE. To sustain an adequate amount of groundwater, treated groundwater usually has to be conserved and recycled for groundwater recharge. It is difficult for the conventional biological treatment processes to make treated groundwater meet the criteria of groundwater recharge. However, activated carbon is an excellent adsorbent for the removal of a wide range of substances. If a sequential preadsorption, bioregeneration and postadsorption is applied to the treatment of contaminated groundwater, the potential capabilities of the adsorption and biodegradation in the first two steps may make treated groundwater free of secondary pollution, and totally remove PCE from groundwater. Postadsorption is designed as a polishing step.

For the dechlorination of PCE adsorbed on carbon, anything, except methane production, was similar to that observed in BAC system. The methane production from OBR system was higher than that from BAC system and it was observed consistently for each test bottle. The comparisons of bioregeneration of PCE laden carbon with the dechlorination of PCE in BAC dechlorinating system for each initial PCE dose are shown in Fig. A-50 to A-55 (in Appendix A).

Fig. 46 Ethylene production and accumulation of PCE dechlorination products in the BAC system with various amounts of PCE added. (after 60 day incubation)



The complete dechlorination of PCE adsorbed on carbon by anaerobic digested sludge cultures was demonstrated in all test samples. The dechlorination results for each initial dose are provided in Appendix A (Fig. A-44 through A-49). At the end of the experiment, the total production of ethylene in each bottle was increased with the amount of added PCE. However, the higher PCE dose also retarded the production of ethylene and resulted in a higher accumulation of VC . The effect of varying PCE doses on dechlorinating efficiency is shown in Fig. 47. For an unknown reason, PCE dechlorination was proceeding very slowly in the sample with 392 μmoles of PCE added during the first 30 days. After that, the dechlorinating ability was recovered. The final results for each PCE dose at the end of the experiment are summarized in Fig. 48.

The major purpose of this study is to examine the feasibility of the offline biological regeneration process (OBR) for regenerating granular PCE laden activated carbon. The regeneration of spent, PCE-bearing granular activated carbon by anaerobic digested sludge cultures was achieved in all test samples. Theoretically speaking, the PCE adsorbed on carbon was completely removed because PCE has totally vanished in the liquid samples. However, all partial dechlorination products of PCE are still adsorbable species. These products can not be used as an indicator to evaluate bioregeneration efficiency. To examine bioregeneration efficiency correctly, the total renewal of the adsorptive capacity of activated carbon through the removal of adsorbable chlorinated species has to be evaluated. The production of ethylene, adsorbable neither on carbon nor on sludge, plus the total mass of chlorinated compounds present in the liquid and sludge phases would be an effective indicator for this purpose. At the end of

Fig. 47 Effect of varying PCE concentrations on dechlorinating efficiency. (Bioregeneration of PCE laden carbon)

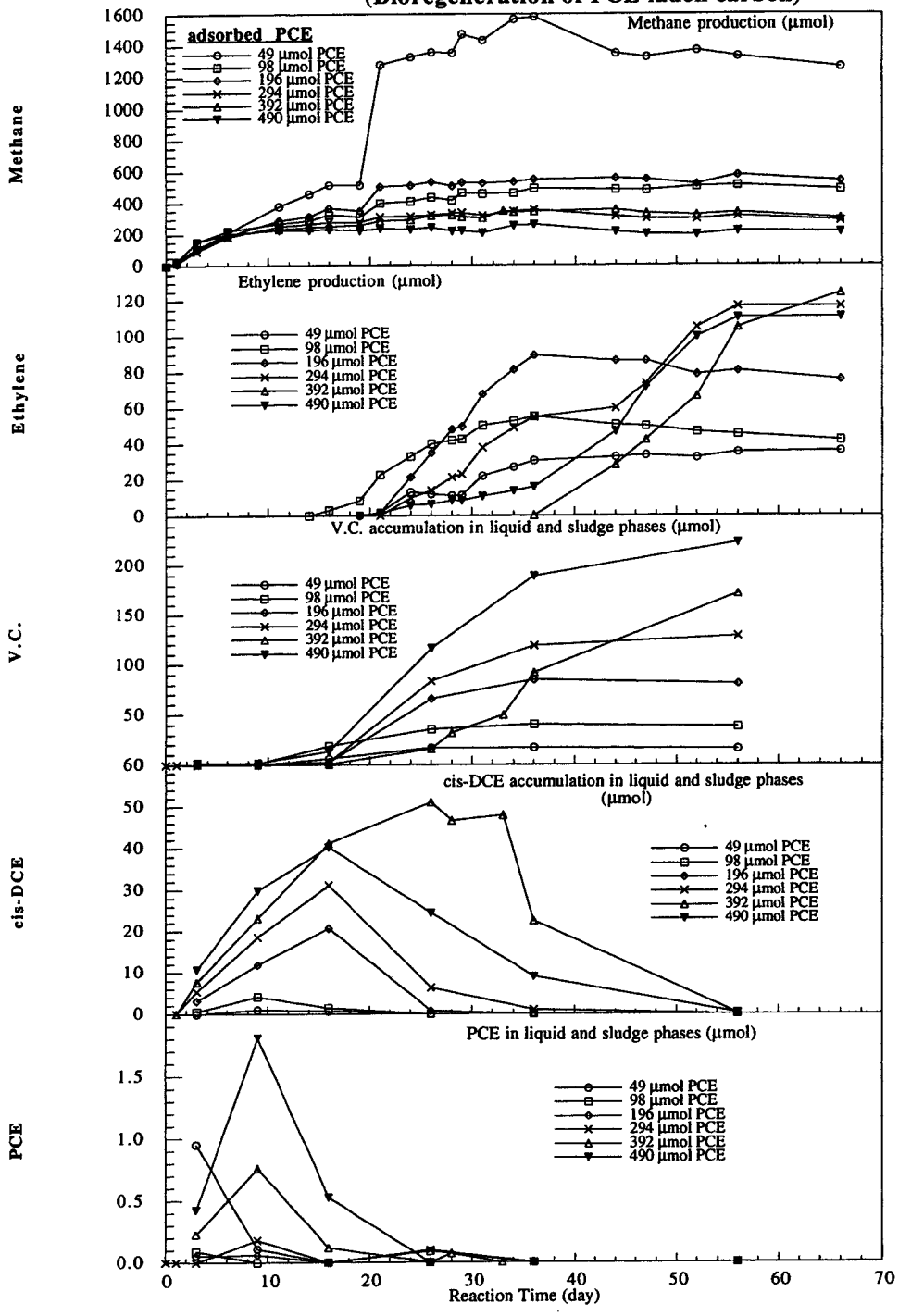
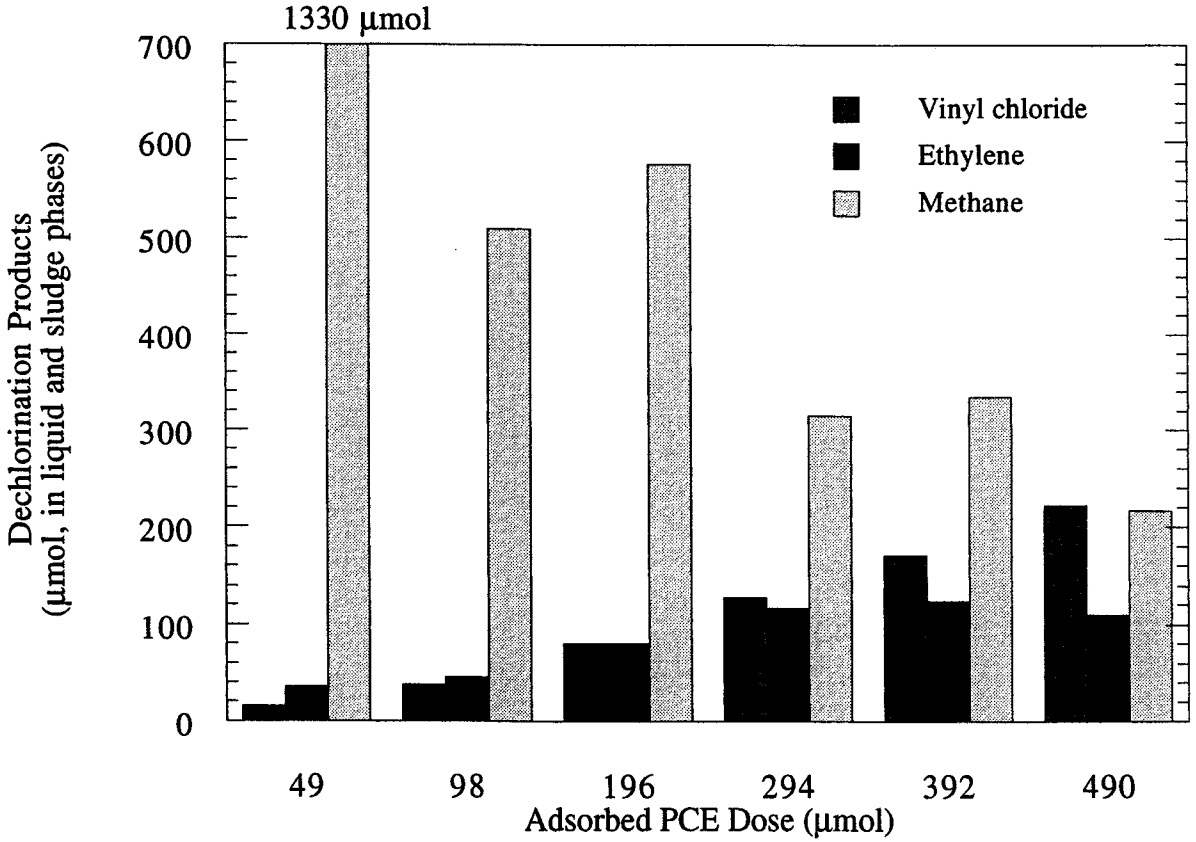


Fig. 48 Ethylene production and accumulation of PCE dechlorination products in OBR system with various amounts of PCE adsorbed on carbon. (after 60 day incubation)



this study, PCE in each bottle was either dechlorinated to ethylene or VC. The data for each bottle are summarized in Table 20.

Table 20. Bioregeneration efficiency of PCE laden carbon.

Adsorbed PCE (μmole) - 1	V.C.* (μmole) - 2	ETH (μmole) - 3	ETH / PCE (%)	Total efficiency (%)
49	15.6	35.3	72.0	103.9
98	37.3	45.2	46.1	84.2
196	80.3	80.5	41.1	82.0
294	127.7	116.8	39.7	83.2
392	170.6	123.8	31.6	75.1
490	222.3	110.4	22.5	67.9

* :Total amount of VC calculated in the liquid phase and adsorbed on sludge materials.

Total efficiency = (2 + 3) / 1 %.

From Table 20, a high efficiency of bioregeneration of PCE laden carbon was achieved in all test samples. Based on the results, regeneration efficiency depends on the initial amount of adsorbed PCE. Of course, the percentage of bioregeneration should be also dependent on regeneration time. The above results suggested that the concept of the offline biological regeneration is feasible for the practical bioremediation of groundwater contaminated with PCE and TCE.

5. DISCUSSION

5.1. PCE dechlorination - A common characteristic of sludges.

PCE-dechlorinating ability has been demonstrated in all of the tested cultures. Furthermore, the complete dechlorination of PCE to ethylene, a non-toxic and environmentally acceptable product, was also significantly achieved in all of the test sludges, except for the sludge obtained from Chino Basin (RP1) Wastewater Treatment Plant (see Table 21). That the dechlorination of PCE occurred in different sludges and MeOH enrichment cultures is an ecologically important characteristic. This suggests that PCE dechlorination is a general characteristic of anaerobic habitats, especially for anaerobic digested sludges. The reductive dechlorination activity of PCE may be general and widespread in anaerobic habitats. This would account for the observations that PCE was degraded in all tested anaerobic cultures and that acclimation periods were not typically required for PCE reductive dechlorination activity. However, it is not known which organisms are responsible for the complete dechlorination of PCE. We also do not know whether the culture(s) dechlorinating PCE in all of the tests is the same culture or different cultures. The another reason for the observations of readily dechlorination of PCE is that all the tested cultures were obtained from complex and mutualistic communities. Generally speaking, reductive dechlorination of PCE is more likely to occur in complex and mutualistic communities. Such complex cultures are more likely than pure cultures to behave like populations in natural habitats or habitats engineered for bioremediation. Therefore, more investigations with complex communities are necessary in understanding ecological factors that affect reductive dechlorination and in using reductive dechlorination in engineered treatment processes. Besides, the

ubiquitous nature of PCE dechlorination may also be due to a lack of substrate specificity for reductive dechlorination under anaerobic habitats. If it is true, it also can be useful in explaining why the sludge cultures can degrade all of the chlorinated ethylenes tested.

Table 21. Summary of PCE dechlorination and formation of intermediates in all test anaerobic cultures.

	Major dechlorinated intermediates					
Anaerobic sample	**TCE**	**cis-DCE**	**trans-DCE**	**1.1-DCE**	**VC**	**Final end product**
Hyperion	√	√	trace		√	ETH
Chino-RP1	√	√	trace			cis-DCE
Chino-RP2	√	√	minor		√*	ETH*
Terminal Isl.	√	√			√	ETH
Alvarado	√	√	minor$^{\Delta}$		√	ETH
Valencia	***	√			√	ETH
JWPCP	***	√			√	ETH
Pond	√	√				cis-DCE
Mixture	√	√			√	VC/ETH (?)
MeOH	√	√	trace		√**	ETH**

Note:
1. * detected only in 3 of the six test bottles.
2. ** detected only in concentrated mixture of three original test cultures.
3. $^{\Delta}$ trance-DCE became the more important dechlorinated intermediate in an older sludge sample.
4. *** not detected because the interval of sampling is too long to catch the right point for TCE production.
5. (?) it is not sure if ETH has been produced because it was not measured in this test.

There is no attempt to identify which organism(s) is responsible for the reductive dechlorination in this study. In the literature, the occurrence of reductive dechlorination has been demonstrated in both pure culture and mixed microbial population. However, only a relatively slow rate and partial dechlorination was achieved in most cases studying with pure cultures (e.g. 0.25 - 0.45 μmoles of PCE per liter per day observed in Fathepure and Boyd's work, 1988b). In the present study, we observed that PCE dechlorination proceeded at a much faster rate in sludges. On average, 49 μmoles of PCE can be entirely dechlorinated to VC with a significant amount of ethylene accumulated in 15 days for most test sludges. The dechlorination rate corresponding to these reactions is about 32.7 μmoles per liter per day. Furthermore, PCE was dechlorinated not only to TCE but also further to VC and ethylene in this study. Therefore, both the rate and the extent of PCE dechlorination have been improved. Usually, pure cultures are more unlikely to degrade a recalcitrant compound so completely. The complete dechlorination of PCE in digested sludges is more likely to be the co-metabolic result of a methanogenic consortium.

In anaerobic digested sludge, many kinds of non-methanogenic bacteria may form hydrogen. Generally, this hydrogen serves as a source of the reductant required for reductive dechlorination of PCE and its partially dechlorinated intermediates. The biological reductive dechlorination of PCE may be a nonspecific reaction and mediated nonspecifically by coenzymes involved in methanogenesis. In addition, because PCE is dechlorinated sequentially, the conditions (nutritional and process requirements) for each step of the complete dechlorination of PCE may be different. To achieve optimum dechlorinating efficiency, the environmental conditions for each step should be optimized separately. However, it would be complex and difficult to elucidate.

5.2. Dechlorination progression and complete dechlorination.

The fresh anaerobic Hyperion sludge incubated with PCE exhibited a typical result for the reductive dechlorination pattern (Fig.23). The complete reduction of 49 μmoles of PCE per 100 mL occurred within 5 days. The transient accumulation of TCE reached its maximum level on day 5. After this time, the total mass of TCE started to decrease and cis-DCE started to increase. Then cis-DCE decreased and V.C. began to accumulate in the system. The digested sludge incubated with PCE contained TCE, cis-DCE, and V.C. after 4, 6, and 9 days, respectively, suggesting that TCE, cis-DCE, and V.C. were sequential intermediates of PCE dechlorination (PCE was dechlorinated stepwise via TCE, cis-DCE, and VC to ethylene).

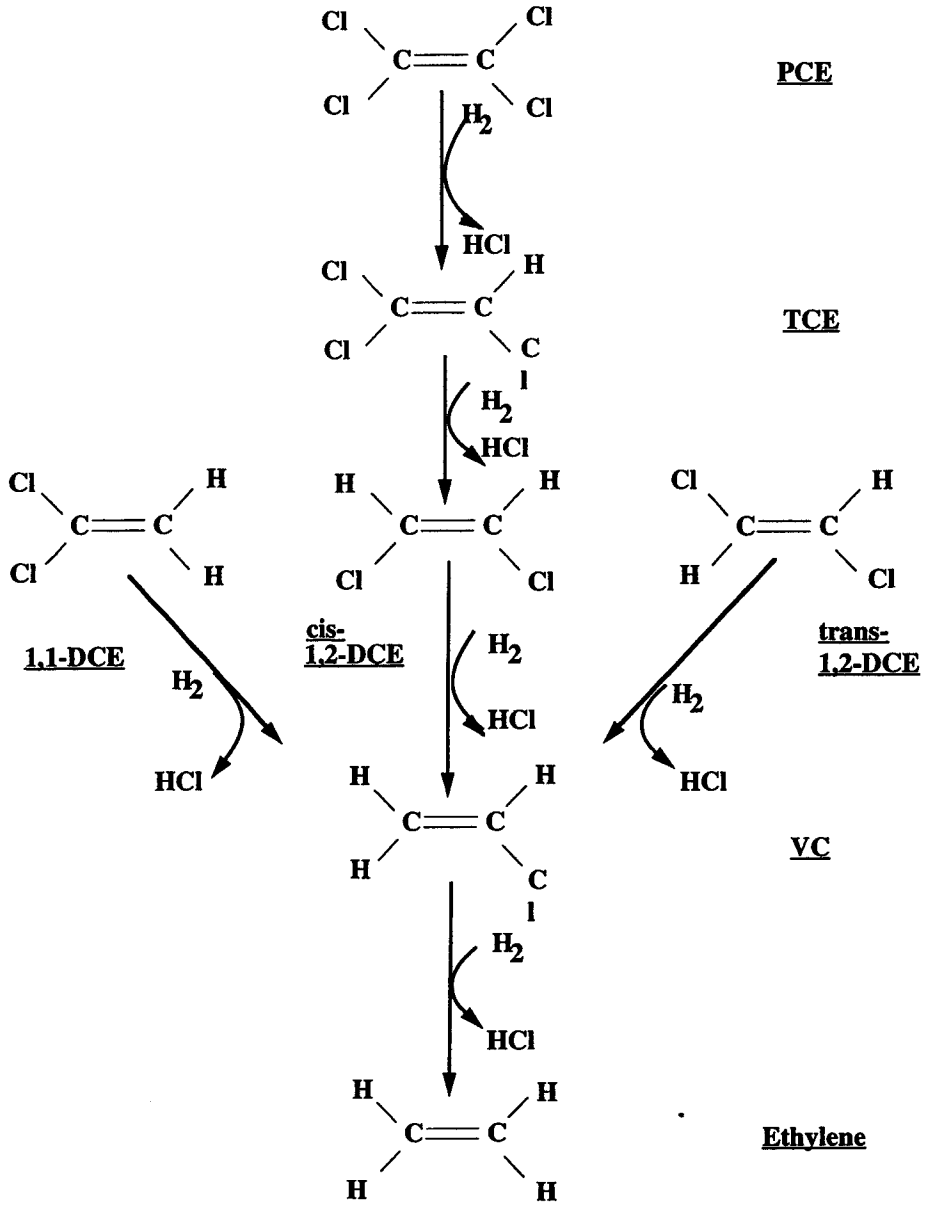
Ethane (a possible reduced product of ethylene) has never been detected in any significant amount throughout the course of this study. Apparently, the methanogenic consortium in the anaerobic digested sludge is not only capable of degrading PCE but also capable of degrading all of its possible biotransformation products (i.e. TCE, 3 isomers of DCE, and V.C.). However, it is still not known whether the same microorganisms are involved in the dechlorination of different compounds or different species are needed for each step of dechlorination. With the lag periods observed in each step of PCE dechlorination, it was very possible that the different dechlorination steps might be effected by different microorganisms. Several different microorganisms are needed to achieve complete dechlorination of PCE. To identify the species that posses dechlorinating activity is most important. The dechlorinated intermediates for each parent target compound listed in Table 7 and their sequence of appearance and

disappearance of the dechlorinated products shown in Fig. 24 to 28 appeared to indicate that all the tested chlorinated compounds have undergone a dechlorination progression that is consistent with a similar mechanism which leads to the same intermediates and end products. The common observed dechlorination pattern is summarized in Fig. 49. The dechlorination pattern is similar to that observed previously in other methanogenic environment (Vogel and McCarty, 1985; Barrlo-Lage et al., 1986).

The findings of complete reductive dechlorination of PCE to ethylene in anaerobic digested sludges obtained from six different wastewater treatment plants and in methanol-enrichment cultures enriched from Hyperion sludge are of great importance for bioremediation applications. This is the first report of such a complete dechlorination of PCE occurring in fresh anaerobic digested sludge at such a high rate. These findings make reductive dechlorination in anaerobic digested sludge an attractive method for removal of PCE in bioremediation processes.

In this study, not only PCE but also TCE, 3 isomers of DCE, and VC were demonstrated to readily undergo the complete reductive dechlorination in anaerobic digested sludge. It is apparent from these studies that reductive dechlorination is the primary mechanism in the degradative sequence of chlorinated compounds in anaerobic digested sludge. All of tested chlorinated compounds were dechlorinated in a stepwise fashion and all of them can be dechlorinated to ethylene. These results are consistent with recently observed dechlorinations under anaerobic conditions (Freedman and Gossett, 1989, 1991; Bruin and Zehnder, 1992). The evidence of these dechlorination are based on the disappearance of parent compounds (i.e. PCE, TCE, DCE and V.C.) and the formation of dechlorinated products under strict anaerobic conditions. Whether

Fig. 49 **Schematic for the common observed dechlorination pattern for conversion of chlorinated compounds to ethylene.**



biooxidation mechanisms were also in part involved in the transformations of chlorinated compounds was not determined because CO_2 was not measured in this study.

In this study, we did not conduct radioisotope studies to determine whether the ethylene formed was a consequence of PCE degradation. Indirect evidences suggest that the formation of ethylene is directly related to the dechlorination of PCE. These evidences are: (1) ethylene was detected only from the time when VC started to disappear, (2) in most of cases, the amount of ethylene formed was nearly stoichiometrically equal to the responding decrease in VC, and (3) there was no any detectable ethylene observed in the sludge sample which was incubated without PCE under the same conditions. Although the transformation of PCE to ethylene, and eventually to ethane was monitored in Bruin et al.'s report (1992), there was no significant amount of ethane being found throughout this study. Furthermore, we have not tried to determine whether products not detectable by the purge-and-trap method were also being formed.

PCE-dechlorinating ability may vary with the microorganisms present and physicochemical environment. Accordingly, reductive dechlorination may degrade PCE to various extents under different conditions. In most previous studies of biological dechlorination of PCE, only partial dechlorination was reported. The partial PCE-dechlorinating ability was also found in the pond sediment obtained from UCLA's botanical garden. In that case, PCE was initially dechlorinated to TCE and cis-DCE. After one month of semi-continuous operation cis-DCE was the only end product. The cis-DCE did not further dechlorinated even after 6 months of extended incubation. The

less chlorinated products may not be further degraded under the same conditions. Not surprisingly, in contaminated anaerobic environments, such as waste dumping sites, subsoil, and groundwater, partial dechlorinated products of PCE are often found. For example, cis-DCE is frequently the major environmental contaminant when PCE or TCE has been released in a dump. Therefore, in assessing the biodegradation of PCE, one must also assess the partial dechlorination products formed and their possibility to further biodegradation, not simply the disappearance of PCE.

5.3. Dechlorination rate

In this study, PCE dechlorination commenced mostly after a short initial lag. The insignificant lag time might be due to the sufficient, appropriate primary carbon source, electron acceptor, or larger cell population in the anaerobic digested sludge. The initial lag time will also depend upon the chlorinated compound. For example, V.C. and trans-DCE needed much longer period of time to start being degraded. If PCE dechlorination were biologically catalyzed, the relatively short acclimation time is also probably due to the continual presence of dechlorination catalyst, that may be a non-specific catalyst, in anaerobic digested sludge. The possible explanations for the periods of acclimation are: (i) genetic change, (ii) induction, (iii) exhaustion of preferred substrates, or (iv) growth of the active population from a very low initial density (Mohn and Tiedje, 1992). Exhaustion of preferred substrates may be responsible for the lag period observed in the reductive dechlorination because PCE dechlorination occurred at the initial period, but only at a very slow rate (a typical data from PCE dechlorination was shown in Table 22). This appeared to indicate that microorganisms prefer to degrade non-PCE materials other than PCE at initial stage. For both cases of trans-DCE

and VC with longer acclimation periods, their methane productions observed at initial stage were relative higher than those observed in the sludge acclimated to other compounds causing shorter acclimation periods.

Induction may not be used to explain the reductive dechlorination observed in the digested sludge because there was a detectable dechlorination occurred at the initial period and hence the PCE-dechlorination activity in the sludge cultures should be expected initially. Genetic change, which might involve mutation or genetic exchange, was also unlikely because of the reproducibility of the acclimation periods. The last explanation may also be applied to explain the case of dechlorination because the dechlorination was initially present at a low rate. If it is true, the lag period in PCE dechlorination is not true acclimation. The short acclimation period required for the reductive dechlorination of PCE also suggests that populations capable of dechlorinating PCE are popular in anaerobic digested sludge.

In this study, PCE was very quickly dechlorinated to V.C., which was dechlorinated to ethylene at a much slower rate than previous steps. On average, after the lag time 49 μmoles of added PCE could be degraded to VC in 4 days in the 100 ml-sludge system (that is, the calculated maximum dechlorination rate was about 122.5 μmoles of PCE per liter per day), while only about 30% of PCE was recovered as ethylene. It takes much longer to produce more ethylene. Over the 40 days of extended incubation observed from the mixed-culture bottle (B2), 119 more μmoles of ethylene was produced from dechlorinating VC. The VC dechlorination rate corresponding to this reaction is only about 3.0 μmoles per liter per day. Therefore, the dechlorination of VC to ethylene would be the rate-limiting step in complete dechlorination of PCE.

Optimizing the dechlorination conditions to enhance the dechlorination in this step is important in using reductive dechlorination in bioremediation processes.

Table 22 The increasing rate of PCE dechlorination by fresh Hyperion sludge at initial lag period.

reaction time (days)	PCE* (μmoles)	TCE (μmoles)	cis-DCE (μmoles)	VC (μmoles)	CH_4 (μmoles)
0	49.0**	0.00	0.00	0.00	0.00
0.67	48.66	0.34	0.00	0.00	1046
1.63	48.30	0.68	0.02	0.00	1438
2.63	47.03	0.94	0.03	0.00	1621
3.96	43.82	5.10	0.08	0.00	1776
4.79	1.78	46.6	0.62	0.00	1831

* PCE = 49 - (TCE + cis-DCE + VC); ** Initial PCE dosage.

When PCE was dechlorinated in digested sludge, a series of less chlorinated intermediates have been observed to accumulate transiently. Such sequential dechlorination not only revealed the anaerobic biodegradation progression of PCE, but also provided the information that the more chlorinated congeners (such as PCE and TCE) are thermodynamically more favorable to be dechlorinated. After a short lag time, PCE was totally degraded to TCE in 0.8 days in the Hyperion sludge, then two more days were required to convert to cis-DCE, and then cis-DCE took 7 days to become VC. An even longer period is generally expected to carry out the dechlorination of VC to ethylene. The dechlorination of pure VC to ETH was observed to be much more

resistant than that for any other tested compounds. During the process of sequential reductive dechlorination of all tested compounds, the conversion of VC to ETH was also slow, but it was notably faster than the pure VC conversion to ETH. The possible reason has been suggested in DiStefano's dissertation(1992) that the presence of PCE and/or its lesser chlorinated products (TCE, DCEs) may facilitate transformation of VC to ETH.

However, as suggested previously in this study, the required environmental conditions may be different for each compound's dechlorination. Therefore, perhaps, the resistance of VC to reductive dechlorination has resulted from the presence of inappropriate environmental conditions. After the dechlorination of more chlorinated compounds the environment may undergo changes to fit the required conditions for dechlorinating less chlorinated compounds. If so, it should not be the presence of higher chlorinated compounds to inhibit the dechlorination of less chlorinated compounds. Although this inhibition was indeed observed in the fresh Hyperion sludge cultures to which PCE, TCE and VC were simultaneously added at the beginning (see Fig. 29 and 30), the concurrent dechlorination of PCE, TCE, cis-DCE and VC was also observed in many old sludge samples (see Fig. A-27, 28 and 29 in Appendix A). It indicated that before PCE is completely dechlorinated to TCE, the dechlorination of TCE is possible to occur. Dechlorination of VC is also achievable with the presence of TCE and cis-DCE. It is not necessary to always expect the slowest rate for VC dechlorination if the right environmental conditions are provided. Based on the observations of this study, the factor most possibly responsible for the tardy VC dechlorination is methane-yielding capacity of the sludge used. For the old sludge samples, after most methane-yielding capacity has been consumed (i.e. there is no excess electron acceptor existing for

methanogenesis), VC would become more competitive for reductive reaction and thereby result in a faster dechlorination.

5.4. Nutrient requirements

The presence of a suitable electron donor is necessary to prevent the accumulation of dechlorinated intermediates. The dechlorination of PCE has to be stimulated by electron donors like lactate, formate, glucose and methanol. But in most previous studies, a complete dechlorination was not achieved. This is possibly because reducing equivalents were not available for reductive dechlorination resulted from competitions for electrons by other species in the same system. For example, PCE dechlorination was partially inhibited in Hyperion sludge cultures incubated with methanol more than 1230 μmoles (comparing Fig. A-12 and Fig. A-18). Experiments conducted with sludge obtained from Chino Basin Wastewater Treatment Plant showed slower dechlorination. This was probably due to carbon source deficiency in this sludge because much less methane production was observed in these samples than that in the bottles containing the fresh sludge obtained from Hyperion Wastewater Treatment Plant. The fresh Hyperion sludge was also found to gradually lose their dechlorinating ability from the bottle without replenishing nutrients. The apparent inability of digested sludge to dechlorinate PCE in the absence of carbon substrate suggests that PCE dechlorination by digested sludge is a co-metabolic process.

However, autoclaved Hyperion sludge (possessing plenty of nutrients) amended with PCE, TCE, DCE, V.C. showed no significant transformation, suggesting that dechlorination is biologically dependent and no significantly abiotic dechlorination have

occurred. In digested sludge cultures reductive dechlorination of PCE is biologically dependent, but it is not clear that the activity is biologically catalyzed because we found 6.5 μmoles of TCE and 0.4 μmoles of cis-DCE productions in the autoclaved digested sludge incubated with PCE after 4 months. Mohn and Tiedje (1992) have pointed out that the inhibition of reductive dechlorination by sterilization only implies either biological or biologically dependent catalysis. Sterilization may abolish the catalyst of reductive dechlorination or the source of reductants, which are products of biological activity and needed for the reductive dechlorination. If PCE dechlorination were not biologically catalyzed, it is possible to occur in autoclaved sludge containing the reductant(s).

In dechlorination of PCE, only the chlorine substituents were removed and degradation of the remaining hydrocarbon compound (i.e. ETH) did not occur. Therefore, we can conclude that such substrate did not support growth. In order to maintain the activity of microorganisms, nutrients also have to be replenished. In the study of dechlorination of chlorinated ethylenes by fresh digested sludge, major required nutrients were presumably supplied initially by the source sludges. When PCE and other tested compounds were slowly degraded, these existing nutrient materials were also metabolized to provide electrons and carbon. Later on, an organic substrate (methanol) plus autoclaved anaerobic medium solution (containing 50% of supernatant of fresh digested sludge and 500 mg per liter of yeast extract) was provided as the potential electron donor and carbon source. The purposes of the addition of substrate are to serve as an electron donor for reductive dechlorination and to support the growth of the dechlorinating organisms. Throughout this study, yeast extract was used to provide essential amino acids, vitamins, and trace elements. But the nutritional requirements of

reductively dechlorinating communities are still not understood yet. For their use in bioremediation, the knowledge of nutritional requirements is essential.

5.5. Electron donor

As mentioned above, the presence of the suitable electron donor is necessary to reductive dechlorination. The availability of reducing equivalents will possibly affect the extent of reductive dechlorination. H_2 is the most possible source of reducing equivalents for the reductive dechlorination of chlorinated compounds by an anaerobic consortium (Dolfing, J., and Tiedje, J.M., 1986). H_2 can be obtained from the oxidation of organic substrates, e.g. from the acetogenic oxidation . But very little information about the origin of the reducing equivalents needed for the dechlorination in monoculture has been deduced.

Differences in the nature of reducing equivalents used for reductive dechlorination may account for the enhanced rates of PCE dechlorination in the consortium (Fathepure and Boyd, 1987). Under methanogenesis, it is proposed that the reducing equivalents for PCE dechlorination are derived from methane biosynthesis and the electrons can be diverted to PCE by a reduced electron carrier involved in methane formation (Fathepure and Boyd, 1988a). If electrons from the primary carbon source are being shared between methanogenesis and reductive dechlorination processes, a competition for grabbing the electrons between these two reductive reaction processes is expected. According to our results, that too much methanol added to the sludge did not result in more or faster dechlorination reaction in the sludge treatment system, may be due to the predomination of methanogenesis. Most of electrons generated from methanol

have flowed to the methane formation. This is confirmed by the observation (from the sludge bottle containing 49 μmoles of PCE and 4920 μmoles of methanol in MeOH effect test) that the calculated rate of methane formation was 73 times higher (600 μmoles per day per 100 mL) than the rate of PCE dechlorination (8.2 μmoles per day per 100 mL). Furthermore, in this bottle, reductive dechlorination was obviously inhibited and PCE was partially dechlorinated to cis-DCE only.

However, based on the similar results, Fathepure and Boyd (1988a) deduced that the reductive dechlorination of PCE is directly proportional to the concentration of the primary carbon substrate because it is the source of reducing equivalents for both methane biosynthesis and reductive dechlorination. However, when the amount of TCE formed, shown in the Table 1 of Fathepure and Boyd's report (1988a), is normalized on the basis of methanol consumed, we will get TCE production rate per millimole of methanol consumed from 52 to 31 nmol per millimole of methanol, for the total added mass of methanol from 0.25 to 5.0 mmoles, respectively. It is apparent that the dechlorination of PCE per unit of methanol is inversely proportional to the concentration of methanol. Besides, the incubation period (2 weeks) is too long to observe the inverse effect of high methanol concentration on the PCE dechlorination.

In fact PCE dechlorination is indeed linked to the methanol consumption during the methanogenesis. But too much methanol added will inhibit the chlorinated compounds to undergo reductive dechlorination. In reductive dechlorination, all chlorinated compounds are electron acceptors. The presence of electron acceptors will affect the composition of species present in the digested sludge. Therefore, the presence of reductively dechlorinating organisms in the digested sludge may be affected by

electron acceptors. Electron acceptors do affect the dechlorination activity in anaerobic digested sludge. Also it is possible to have a competition for electron donors between different electron acceptors. So, the flow of electrons may be affected by the availability of electron acceptors. This effect might occur via intracellular channeling of electrons or via interspecific competition for electron donors (Mohn and Tiedje, 1992). Methanol-utilizing methanogens might compete with PCE-dechlorinating populations for available electron donors. This may be a direct inhibition of PCE dechlorination by the electron acceptor because methanol might have higher potential being reduced to produce methane gas.

5.6. Competition and inhibition

Competition between dechlorination and methanogenesis.

Reductive dechlorination of PCE is mainly known under anaerobic conditions. Reductive dechlorination involves the replacement of chlorine substituent with hydrogen. This process requires a reductant. The reductant may be derived from numerous microbiological activities in anaerobic sludge systems. However, in such systems the reductant is also strongly required by methanogenic reactions and it may be ecologically or thermodynamically more favorable for methanogenesis. Therefore, a competition for reductants between dechlorination and methanogenesis would be expected to occur in the systems. Furthermore, it is possible that the availability of various electron acceptors will determine the flow of electrons. Many evidences

observed in this study indeed showed that electron acceptors used by methanogens do affect dechlorination activity in digested sludge.

Reductive dechlorination rates were found to be inversely correlated to methane productions in the test of methanol effect on PCE dechlorination. The higher amount of added methanol resulting a more methane production did not stimulate a faster dechlorination. Instead, PCE dechlorination was only slower and partial under higher initial methanol concentrations while it was still complete for other samples with lower methanol concentrations. The high methane production was the evidence of electrons having mostly flowed to methanogenic process, rather than reductive dechlorination. Although it has been widely suggested in the literature that methanogens/methanogenesis are required for dechlorination of PCE under methanogenic conditions, a high methanogenic activity still need to be avoided.

In order to enhance the dechlorination of PCE, mixing was applied to some test sludge samples to provide sufficient interaction between the treated compound and microorganisms. However, it seemed that mixing was more favorable for methanogenesis. As similarly observed in methanol test, reductive dechlorination was significantly inhibited by the higher methane production under mixing conditions. In addition, the presence of CO_2, a methanogenic electron acceptor (used by methanogens), also showed a significant influence on PCE dechlorination under mixing conditions. The presence of CO_2 resulted in a higher methane production but a smaller ethylene formation during PCE dechlorination under mixing conditions. However, there

was no significant difference observed between the samples with and without CO_2 incubated under quiescent conditions. This may be due to the much slower gas transfer efficiency for CO_2 under quiescent conditions.

In the test of old sludges, a favorable environmental conditions may have been created through exhaustion of substrates and reduction of redox potential because all test sludges have been stored anaerobically in a 35°C incubator for a few days to allow methane evolution before their uses. However, an apparent longer lag time was found in old sludges. This is likely due to the decrease in viability during storage. After the lag time, dechlorination of PCE, TCE, cis-DCE and even VC (the most resistant one) was observed to occur concurrently under the less actively methanogenic conditions. That the higher chlorinated ethylenes inhibit dechlorination of less chlorinated ones was no longer observed in old sludges. The similar results were also commonly observed in acclimated Hyperion sludge. The step-by-step dechlorination in acclimated sludge was not as clear as in the fresh anaerobic digested sludge samples. This phenomenon might be brought about by various possible reasons; e.g., (i) different chlorinated substrates might have different ability to compete with methanogenic electron acceptors for electrons and (ii) the same organism(s) catalyze the different dechlorination steps but different environmental conditions are required for each step. Based on these results, reductive dechlorination of each chlorinated ethylene appeared to have variable responses to the concentration of electron acceptors used by methanogens. For fresh digested sludges, a plenty of electron acceptors is available for methanogenesis. Methanogenic reactions might outcompete reductive dechlorinations of less chlorinated ethylenes by virtue of a higher potential for electron consumption. However, at this

time, PCE and/or TCE might have a higher competing ability for electrons and still can undergo reductive dechlorination. For old or acclimated sludges, in contrast, only a few methanogenic electron acceptors are available. The lower concentration of methanogenic electron acceptors might reduce the redox potential of the system and make less chlorinated ethylenes (e.g. cis-DCE and VC with a lower competing ability for electrons) more readily to undergo dechlorination. Therefore, the tolerable concentration of methanogenic substrate may be different for dechlorination of each compound. To achieve complete dechlorination of PCE, the required environmental conditions for each step may have to be created separately.

The different ability of competition for electrons with methanogenesis for each chlorinated ethylene was demonstrated more obviously in the toxicity test. The ability of competition for electrons for different chlorinated ethylenes had the following order: TCE > PCE > cis-DCE > 1.1-DCE > trans-DCE > VC. The sludge sample incubated with more readily degradable chlorinated compounds produced less methane; on the contrary, more methane production was observed from the samples with more persistent chlorinated compounds.

However, for the same test compound, the competing ability and dechlorination rate were observed to be proportional to the concentration of chlorinated compound present, up to some maximum value (see Table 8 to 13). This suggested that the concentration of each chlorinated compound is also an important factor which may affect the competition between dechlorination and methanogenesis.

Methanogenesis has been widely implied to play an important role in reductive dechlorination of PCE under methanogenic conditions. However, methanogens may not necessarily be involved directly in the transformation of chlorinated ethylenes if they can support conditions where the available electrons may be diverted to PCE dechlorination process (Baker and Herson, 1994). In the sludge system, redox conditions may determine the type of biological community that develops. However, the concentrations of biologically produced reductants and methanogenic electron acceptors would affect the redox potential of the system. Therefore, the concentrations of reductants and methanogenic electron acceptors are two of the most important factors to be controlled. For complete dechlorination of PCE, the concentration of methanogenic electron acceptors must be decreased below a certain level before the use of PCE or other less chlorinated compounds as an electron acceptor starts.

Toxicity and inhibition.

PCE and other less chlorinated ethylenes are usually mentioned in regard to their environmental persistence and toxicity. However, it is a difficult question to answer properly if PCE or other chlorinated ethylenes are toxic materials. Many factors may influence their toxicity to sludge cultures; e.g., characteristics of these compounds, concentration, ability of microorganisms to adapt to their presence, and time of exposure (Baker and Herson, 1994). Usually, for a non-inhibitory substrate, the biodegradation rate increases with the substrate concentration, up to an upper limit. Beyond this limit, the rate remains constant. However, for an inhibitory substrate, the degradation rate will

decline when the substrate concentration is beyond the maximum value. Based on this criterion, we may categorize all these compounds as toxic materials. In the highest tolerable concentration test, dechlorination rates for each test compound were observed to increase with their concentrations, up to an upper value. Beyond that limit, dechlorination rate was decreased significantly for all test compounds. In addition, the methane productions for each compound were also decreased. It suggested that the high concentrations of chlorinated compounds have also inhibited methanogenic activity in the sludges.

The toxicity of PCE was also demonstrated in the sludge sample with activated carbon added. After the system has reached a stable condition, a faster dechlorination accompanying a higher methane production was observed. This may be due to the lower toxicity because most PCE was adsorbed on carbon and PCE concentration in liquid phase has been dramatically decreased. Due to the toxicity, the concentration of each compound applied to the biological treatment process must be largely restricted. However, the highest tolerable concentration for all chlorinated ethylenes can be further enhanced in the sludge system with activated carbon addition because all of these compounds are adsorbable on activated carbon.

5.7. Carbon addition and dechlorination substrate availability

The availability of dechlorination substrates to microorganisms has been demonstrated to affect their dechlorination rates in the highest tolerable concentration test (see Table 8 through 13). The dechlorination rate for each test compound was observed to be proportional to their initial concentration, up to an upper limit. To increase substrate availability, activated carbon is often added to liquid treatment systems because it can concentrate substrates on the carbon surface and thereby enhance the contact of microorganisms and substrates. The concentration enhancement may occur when a significant fraction of the bacteria can live in the pores and these bacteria can directly metabolize adsorbed chemicals on the carbon. However, most bacteria are several orders of magnitude larger than the average pore size and can not enter the pores. Therefore, it is still questionable if bacteria can directly attack the chemical adsorbed on carbon. In this experiment, no direct evidence showed that the addition of activated carbon has improved the dechlorination rate in the acclimated sludge system. Nevertheless, the carbon addition indeed offered significant protection against inhibitory factors to complete dechlorination.

For an unknown reason, partial dechlorination of PCE to cis-DCE followed a retardation of further dechlorination was observed in several parallel test sludge bottles without any carbon addition. However, in the bottles with carbon added, PCE was dechlorinated to ethylene with no exception. The introduction of activated carbon into

the acclimated sludge system may have adsorbed inhibitory or toxic substances, thereby prevent bacteria from toxicity.

In addition, the overall dechlorinating ability observed in both BAC and OBR systems exceeded the ability of the pure sludge system alone. With only one time of nutrient replacement, the sludge culture with carbon added degraded PCE as much as 490 μmoles. There are two things here which are not achievable in the pure sludge system. Firstly, the reductive dechlorination would have been totally inhibited if 490 μmoles of PCE had been added to the pure sludge system. However, it is not so surprising because the adsorptive capacity of activated carbon is expected to reduce the concentration of adsorbable species in the liquid phase. Secondly, it is impossible for the sludge to entirely dechlorinate 490 μmoles of PCE with such a small amount of supplementary nutrient (electron donors). Compared to the methane production (about 1300 μmoles) from the samples (U1 and S1) with 49 μmoles of PCE addition, a much smaller amount (about 200 μmoles) of methane was produced from the highest initial PCE dosage samples (U6 and S6). This indicated that most electrons available in the system have been diverted to the reductive dechlorination. The synergism of the presence of activated carbon and biodegradation may have created an environmental condition which is more favorable for reductive dechlorination. The toxicity of PCE was reduced through carbon adsorption. Consequently, biological activity was enhanced. In addition, the biodegradation of PCE in liquid phase disturbed the equilibrium of PCE between carbon and aqueous phases and resulted in the desorption of PCE from carbon. This continuous supplement of PCE may also result in dechlorination occurred more competitively than methanogenesis.

Generally speaking, the added activated carbon is able to adsorb a substantial part of PCE and, thus, lower its influence on the biomass and reduce its inhibitory effect on the bioprocess.

5.8. Biological activated carbon process

Biological activated carbon process is a combined adsorption and biodegradation process. Adsorption and biodegradation are two of the most important removal mechanisms of the process. However, the interaction between these two removal mechanisms has not been fully understood. Collectively, biodegradation and adsorption occur simultaneously and competitively. In addition, these two mechanisms may be functioning synergistically. The preferential adsorption of toxic or inhibitory substances can enhance biological activity. Similarly, biological activity may regenerate the adsorption capability of the carbon and greatly extend the period of performance.

Removal mechanisms:

In this study, adsorption was initially a faster process than dechlorination. The major added PCE, which may cause biotoxicity, was preferentially adsorbed onto carbon before significant dechlorination occurred. Carbon adsorption has obviously caused a lower PCE concentration in the liquid phase. However, the sequential

reductive dechlorination of PCE was still observed. About 36 to 70% of initial added PCE was recovered as ethylene at the end of the test, while all remaining PCE was transformed to V.C. in each different initial PCE dose sample. During the period of experiment, the sequential adsorption, desorption and biodegradation seemed to be the major mechanisms for PCE dechlorination occurred in BAC process. Prior to degradation by the sludge cultures, the major added PCE in BAC experimental bottles was adsorbed on carbon. This may be due to a much faster adsorption rate on carbon. As degradation occurred, desorption of adsorbed PCE from carbon may be caused by the reversed concentration gradient. The decrease in PCE and appearance of less chlorinated ethylenes is indicative of microbial transformation.

Complete dechlorination:

Feasibility of the BAC process for complete dechlorination of PCE was successfully demonstrated at all initial PCE doses. The initial concentration of PCE achieved for complete reductive dechlorination is much higher than reported previously in the literature. At the highest PCE concentration of 813 mg/L, PCE still could be totally dechlorinated and a significant amount (189 μmoles) of PCE was recovered as ethylene. Most of PCE in each bottle was dechlorinated and terminated at V.C. This is very possibly due to that the right environmental conditions were not maintained continuously. Although we still do not know the exact nature of the right environment required for complete dechlorination, the possible factors might include temperature, inorganic trace elements, carbon sources (electron donors), and electron acceptors. To achieve optimum performance in BAC and bioregeneration systems, these environmental factors should be further studied and optimized.

Carbon performance:

During the operation of BAC, difficult-to-degrade or nondegradable organics should accumulate on the carbon. This would be the major factor responsible for the decrease in adsorption capacity gradually. The adsorbed organics which are degradable will be desorbed into liquid phase and then degraded when their concentrations are dropped below equilibrium. In biological activated carbon process, part of the added virgin PCE may undergo biodegradation before it is adsorbed on carbon, while most of added PCE is expected to be adsorbed first and then desorbed to be degraded. Typically, there has no attempt to distinguish between these two in this study.

Activated carbon adsorption is prominent in its attenuation of high transient peak influent PCE concentrations. The distinctive feature of the addition of activated carbon to the biological treatment observed in the BAC processes is, at least, to improve stability to shock loads and toxic upsets. However, it is not clear if biodegradation rates in the combined biological degradation and carbon adsorption system have been improved. A major disadvantage of the use of activated carbon is the costs associated with the replacement or regeneration of the spent carbon. Commonly, regeneration of spent carbon can be more economical than replacement with virgin carbon. Among many regeneration methods (including solvent regeneration, steam stripping, thermal regeneration, etc.), microbial regeneration of spent carbon might be the most advantageous if the adsorbate is biodegradable (Sigurdson and Robinson, 1978).

However, very few work has been concentrated on the development of biological regeneration as an alternative to traditional methods of carbon regeneration.

Effectiveness of BAC process:

This study focused on the effectiveness of granular activated carbon on the improvement of microbial tolerance of high initial PCE dosage during the dechlorination of PCE. In biological activated carbon process, carbon rapidly reduced PCE concentration in the liquid phase and increased methane production compared to the amount produced from the system without carbon addition. The high adsorption efficiency and probably low desorption and subsequent biodegradation of PCE and its dechlorinated products have enabled the improvement of biological system in the tolerance of high PCE loading during the dechlorination processes.

The success of PCE dechlorination in the BAC process is significant for utilizing existing anaerobic digestors for the treatment of PCE contamination. It is a less expensive way because adding on carbon adsorption columns to existing biological treatment facilities needs large capital expenditures and increased operation costs (Sublette, et al., 1982)

5.9. Offline biological regeneration

Offline biological regeneration is another way to combine biological treatment with carbon adsorption. In such a process, sorbed substrate is supposed to desorb from carbon and undergo biodegradation in bulk liquid. Biological activity is utilized to regenerate activated carbon. The major purpose of this study is to examine the feasibility of the offline biological regeneration process for PCE laden carbon. The regeneration of spent, PCE-bearing granular activated carbon by anaerobic digested sludge cultures was achieved in all test samples. The PCE adsorbed on carbon could be completely detoxified to non-toxic ethylene in acclimated sludge cultures. The nature of dechlorination products is exactly same as that observed in the sludge system without carbon.

Regeneration mechanisms:

Numerous studies have shown that adsorbed substrates on carbon can be degraded biologically. It is feasible to regenerate the spent carbon with a biological culture. However, if microorganisms can directly utilize an adsorbed substrate is still not exactly understood yet. In this study, PCE dechlorination in OBR system seemed more likely to be a two-step reaction, that is, adsorbed PCE was desorbed from carbon first and then degraded by sludge cultures in the liquid phase. Desorption continuously occurred until all of the PCE, adsorbed and unadsorbed, was degraded.

Bioregeneration efficiency:

Usually, bioregeneration is defined as the renewal of the adsorptive capacity of activated carbon by the microbial activity that is capable of metabolizing the adsorbed compounds subsequent to their desorption from the carbon (Sublette, et al., 1982) and is recognized by the increase in adsorptive capacity. However, many other mechanisms can also achieve the removal of material adsorbed on carbon and hence increase adsorptive capacity. To recognize the occurrence of bioregeneration in this study, PCE-dechlorinated products were used as the indicator. Once these compounds appear in the liquid phase, a certain amount of adsorbed PCE would have been removed from the carbon and biologically degraded. In addition, ethylene, the end product of PCE dechlorination, was found to be the best one for evaluating bioregeneration efficiency because it adsorbs neither on carbon nor on sludge materials. Total ethylene production based on the analysis of gas samples would be relatively more accurate when it is used for the evaluation of the regenerated portion of the carbon's adsorptive capacity.

At the end of the experiment, the percentages of PCE recovered as ethylene production for each initial dose ranged from 22.5 to 72.0% (see Table 20). However, to correctly estimate the bioregeneration efficiency of carbon, any dechlorination product which has not been adsorbed on the carbon and stay in the liquid and sludge solid phases should be accounted. Therefore, the ethylene production plus the total mass of VC found in both liquid and sludge phases is used as an indicator for the evaluation of bioregeneration efficiency because only VC and ethylene were found at the end of the test. Based on the experimental results, a high efficiency of bioregeneration of PCE laden carbon was achieved in all test samples. The total regeneration efficiency for each

PCE dose ranges from 68 to 100%. It obviously depends upon the initial amount of adsorbed PCE. In this study, the effect of various PCE loading on bioregeneration efficiency at different time points was also investigated. The results showed that bioregeneration efficiency is also dependent of regeneration time. Besides, a small part of work was conducted as a function of the carbon dosage. The amount of activated carbon added was also demonstrated to inversely affect bioregeneration. This may be due to a slower desorption of chemicals resulting from the introduction of too much carbon.

Generally speaking, bioregeneration efficiency will be highly influenced by the environmental conditions. Such environmental factors as temperature, trace nutrients, electron donors, electron acceptors, spent carbon adsorbate loading, carbon type, ratio of carbon mass to biomass, total biomass, and retention time can be modified to optimize the environment for bioregeneration (Baker and Herson, 1994; Sigurdson and Robinson, 1978; Goeddertz, Matsumoto, and Weber, 1988).

Reproducibility:

Bioregeneration observed from another regeneration trial using Hyperion sludge obtained on different date is quite similar. The reproducible bioregeneration may be due to the reproducibility of PCE reductive dechlorination which has been previously observed in anaerobic digested sludge.

Equilibrium between carbon and bulk liquid:

During bioregeneration, it was found that the isotherm constants for virgin carbon in deionized water were not applicable to the spent carbon being regenerated in the sludge system. A change in carbon adsorption characteristics may have occurred during the regeneration process because the possible bacterial growth around the carbon particle and the relative thick solid materials of digested sludge were expected to decrease the carbon adsorption and desorption ability.

Biodegradability:

From the analysis of liquid samples, it was observed that TCE, cis-DCE, V.C. and ethylene appeared sequentially and then vanished in the same order except ethylene which was gradually accumulated during the bioregeneration process. However, 100% of recovery of ethylene from adsorbed PCE was never achieved among test samples. V.C. was the major accumulated dechlorination product. This indicated that each compound's biodegradability will highly affect the efficiency of bioregeneration. For more biodegradable compound, the loss of effective adsorption site may be less during each cycle of a multiple adsorption and bioregeneration.

Loss of adsorptive capacity:

Because of the difficulty of removal of the adherent sludge materials to the carbon, the equilibrium adsorptive capacity of the regenerated carbon was not experimentally tested. However, after three runs, the initial equilibrium PCE concentration in the liquid phase of the sample used for preliminary BAC test was still

close to the value of the first run. This suggested that there is no significant loss occurred for the PCE equilibrium adsorption capacity of carbons that were regenerated biologically three times. In Sigurdson and Robinson's study, it was also observed that no appreciable change in the biologically regenerated carbon adsorption capacity after the first regeneration. The loss (about 53% of the fresh carbon adsorption capacity observed in their experiment) occurred during the first regeneration is probably due to the limit of the physical desorption process, i.e. adsorbate might be no longer desorbed from carbon once the amount of adsorbed compound on carbon is below a certain level.

Based on a study of adsorbate distribution in the pores of carbon (Jiang and Huang,1982; cited in Zhang's paper, 1991), the strength of adsorption was observed to be partly dependent upon the ratio of the pore diameter to the adsorbate diameter. When the ratio is less than three, it is very difficult for solvent regeneration because the adsorbate is tightly adsorbed in pores. The another reason for the incomplete desorption of adsorbate may be due to the existence of a threshold concentration of the adsorbate in the liquid for the microbial degradation. Liquid concentration lower than the threshold value will not be achievable during bioregeneration. In addition, reduction in the adsorptive capacity of regenerated carbon may result from microbial fouling of the pore openings or irreversible adsorption of metabolites during the bioregeneration (Hutchinson and Robinson, 1990b).

If any intermediate metabolite produced from the metabolism of treated compounds were irreversibly adsorbable or reversibly adsorbable but

nonbiodegradable, it would eventually cause a significant loss in adsorption capacity of biologically regenerated carbon. In this study, PCE was sequentially converted to TCE, cis-DCE, V.C. and eventually ended at ethylene without any other intermediate metabolite. Therefore, such kind of regeneration loss may not be encountered in PCE-bearing carbon bioregeneration process.

Rate-limiting step:

For free suspended microorganisms in the liquid phase, the available substrate is those dissolved in the bulk aqueous phase. The biodegradation of PCE in liquid phase will disturb the equilibrium of PCE between carbon and aqueous phases and result in the desorption of PCE from carbon. In such a case, if the biodegradation rate is much greater than the rate of PCE desorption, bioregeneration of carbon will be governed by the rate of PCE desorption and the equilibrium of PCE between carbon and the bulk aqueous solution will never be reached. Otherwise, adsorbed-PCE on carbon is always equilibrating with the PCE concentration in the liquid phase and biodegradation will limit the bioregeneration rate. In bioregeneration test, we found that PCE desorption from carbon is more likely to be the limiting reaction when compared to biodegradation of PCE in pure sludge. Actually, these two reactions are all possible to be the rate-limiting step during the bioregeneration. For example, in Goeddertz's study (1988) desorption was observed to limit regeneration rate, while biodegradation was reported to be the limiting step in Sigurdson's research (1978). It can be altered by many factors, such as the nature of adsorbate and carbon, biodegradability of adsorbate and characteristic of microorganisms, etc.

Bioregenerability:

In bioregeneration process, PCE will desorb from the carbon until a specified final equilibrium is reached. The specified equilibrium has to be determined based on the biological regenerability of carbon. The microorganisms used in the regeneration process will highly influence the regenerability. Generally speaking, biological regeneration in anaerobic digested sludge can produce a significant degree of carbon regeneration. It makes bioregeneration a promising method to regenerate the spent, PCE-bearing carbon. However, to obtain a conclusive result, a long-term cyclical adsorption-bioregeneration study is essential.

The high bioregenerability achieved in all OBR test bottles demonstrates not only the feasibility of the offline biological regeneration process for PCE laden carbon, but also the concept of a three-stage treatment process consisting of adsorption of PCE by activated carbon followed by desorption and anaerobic dechlorination for the reclamation or treatment of groundwater contaminated with PCE and/or other less chlorinated ethylenes. The process will use activated carbon to adsorb PCE from liquid phase (contaminated groundwater), instead of allowing it to directly enter the biological treatment system. Then, the activated carbon will be regenerated in an offline biological regeneration system. PCE will be detoxified completely in the bioregeneration system. Using this scheme, deoxygenation of the PCE contaminated groundwater would be un-necessary and 100% of the removal of PCE from the contaminated groundwater can be achieved without any secondary pollution. Also, higher concentration of PCE can be obtained which will probably result in higher treatment efficiencies in the biological

system. The use of activated carbon column should be more reliable than direct biological treatment for the remediation of polluted groundwater.

6. CONCLUSIONS

The widespread contamination of groundwater and surface water with PCE and TCE is a serious problem facing the industrialized world today. Although many regulatory strategies have been adopted in an attempt to reduce the use of PCE and TCE, this contamination is still likely to occur in the future because of their versatility as industrial organic solvents. To remediate such environmental contamination and keep the continued value of PCE and TCE as industrial organic solvents, a technology capable of detoxifying these chlorinated compounds is needed.

Among several techniques used previously, biological remediation processes are most potentially suited for completely treating large volumes of contaminated groundwaters and industrial wastewaters without generating toxic end products. Many previous studies have demonstrated that PCE and TCE are susceptible to reductive biodegradation under a variety of anaerobic environments. However, little is known about the feasibility of the exploitation of microorganisms in anaerobic digested sludge (the most reducing environment) to treat such contamination. In view of this consideration, the present research concentrates on the study of complete detoxification of chlorinated ethylenes in anaerobic sludge and the feasibility of using existing wastewater treatment plants to the reclame or treat of groundwater contaminated with PCE and TCE.

Reductive dechlorination was demonstrated to be the primary mechanism involved in the biological transformation of six chlorinated ethylenes under

methanogenic conditions in all test anaerobic sludges. In this process, chlorine atoms were sequentially removed and replaced with hydrogen.

Complete dechlorination of six chlorinated ethylenes (including PCE, TCE, 3 isomers of DCE and VC) to non-toxic ethylene was demonstrated in fresh anaerobic Hyperion digested sludge. Reductive dechlorination of PCE was even proved to be a general characteristic of anaerobic digested sludges obtained from different wastewater treatment plants. All of these compounds were transformed under methanogenic conditions. PCE and TCE, the higher chlorinated ethylenes, more readily undergo reductive dechlorination. The lower chlorinated ethylenes are less reactive to dechlorination under methanogenic conditions. In fresh digested sludge, dechlorination of TCE and VC can not occur until PCE is entirely dechlorinated. However, in "old" sludge, all these compounds can be dechlorinated simultaneously. This indicates that it is not necessarily the presence of higher chlorinated compounds that inhibits the dechlorination of lower chlorinated compounds. Furthermore, it may be due to decreasing methanogenic activity allowing the lower chlorinated compounds to successfully compete for electrons in the "old" sludge.

The competition for electrons between methanogenesis and reductive dechlorination may exist in the digested sludge incubated with chlorinated compounds. A slower and partial dechlorination of PCE is observed from the sludge sample with higher methanogenic activity resulting from a higher amount of methanol added. The partial inhibition and retardation of PCE dechlorination is also observed with the sludge sample under mixing condition which causes a higher amount of methane production. This suggests that reductive dechlorination is related to methanogenesis in digested

sludge and, for each chlorinated ethylene, the reductive dechlorination requires different methanogenic conditions.

Complete dechlorination of PCE in a biological activated carbon process can be achieved at much higher initial PCE concentration (813 mg/l without accounting for volatilization and sorption). The added activated carbon protects dechlorinating bacteria from inhibitory substances and improves the biological system's tolerance of high PCE loading.

The feasibility of bioregeneration of PCE-laden carbon is demonstrated in digested sludge. The total regeneration efficiency is inversely proportional to the amount of PCE initially added under the experimental conditions. The high bioregenerability also demonstrates that the concept of a three-stage treatment process consisting of adsorption of PCE by activated carbon followed by desorption and anaerobic dechlorination is a promising method for the reclamation or treatment of groundwater contaminated with PCE and/or other less chlorinated ethylenes.

A summary of the most important findings in this research is as follows:

1. Reductive dechlorination of PCE is a general characteristic of anaerobic digested sludges. PCE-dechlorination ability was demonstrated in all the tested anaerobic cultures, including seven fresh digested sludges (obtained from Hyperion, Terminal Island, Alvarado, Valencia, JWPCP, Chino Basin (RP1) and (RP2) Wastewater Treatment Plants), pond sediment, a mixture of pond sediment and Hyperion sludge, and methanol-enrichment cultures. The dechlorination ability varied in the extent of dechlorination of PCE depending upon the culture tested.

While complete dechlorination of PCE to ethylene was found in most of sludge culture samples and concentrated methanol-enrichment cultures, partial dechlorination of PCE to cis-DCE was observed in Chino Basin (RP1) digested sludge and pond sediment.

2. An improved PCE-dechlorination ability was achieved in both methanol-enrichment culture and Chino Basin (RP2) sludge culture by the renewal of nutrient medium and concentrated cell mass. Without the replacement of nutrient medium, PCE-dechlorination ability of the fresh Hyperion sludge cultures could not be sustained.

3. An apparent lack of specificity for the reductive dechlorination of chlorinated ethylenes was observed in the fresh anaerobic Hyperion sludge. All six chlorinated ethylenes (including PCE, TCE, 3 isomers of DCE, and VC) were biodegraded via reductive dechlorination. Among these six chlorinated ethylenes, PCE is most readily dechlorinated completely to ethylene, while trans-DCE and VC showed most resistance to reductive dechlorination.

4. Complete dechlorination, resulting in the formation of ethylene (an environmentally harmless metabolite), was demonstrated under methanogenic conditions for all chlorinated ethylenes. The same dechlorination pattern was observed from dechlorinations of all six chlorinated ethylenes. The observed dechlorination pattern is summarized in Fig. 49. In the complete dechlorination process, the dechlorination of VC to ethylene was observed as a rate limiting step. VC was the major end product of reductive dechlorination of chlorinated ethylenes.

5. The presence of higher chlorinated compounds will not inhibit dechlorination of the less chlorinated compounds. The inhibition of dechlorination of the less

chlorinated ethylenes may be due to the increasing methanogenic activity in the fresh digested sludge. In old sludge, dechlorination of PCE, TCE, cis-DCE and VC occurred concurrently.

6. Of the three possible isomers of DCE, cis-DCE is the most significant intermediate from dechlorination of PCE and TCE. The other two isomers are only produced insignificantly in most cases.

7. The lower LC_{50} values obtained from PCE, TCE, and cis-DCE correlate with the chemicals that appear to be dechlorinated easier by the Hyperion sludge cultures. It appears to indicate that the reductive dechlorination and methanogenesis are in competition with each other for limited electrons.

8. For most chlorinated ethylenes (except trans-DCE which showed no significant dechlorination at all concentrations), the dechlorination rate of each compound was observed to be proportional to their concentration, up to an upper limit .

9. In semi-continuous operation, a total of 30 to 40% of PCE could be recovered as ethylene. Similar results were also obtained from dechlorinations of other chlorinated ethylenes. With the replacement of nutrient medium and addition of methanol, 49 μmoles per bottle (81 mg/L) repetitive doses of PCE were dechlorinated within 4 days, for 5 months of semi-continuous operation.

10. There seems to be an inverse relationship between reductive dechlorination and methanogenic reaction. Over the concentration of 24.6 mM of methanol, both of the extent and the rate of PCE dechlorination were significantly inhibited by methanogenesis. Under such a condition, PCE was only partially dechlorinated to cis-DCE, at a slower rate. A competition for electrons between reductive dechlorination and methanogenesis may exist.

11. PCE dechlorination was retarded by mixing. Mixing is more favorable for methanogenesis. The use of CO_2 as an electron acceptor by methanogens also significantly inhibited reductive dechlorination under mixing conditions.

12. The complete dechlorination of PCE to ethylene at high PCE concentration makes reductive dechlorination in anaerobic digested sludge an attractive method for removal of PCE in bioremediation processes.

13. The amount of added activated carbon will influence the effectiveness of carbon addition on the improvement of complete PCE dechlorination in the carbon-sludge system. Too much carbon added would retard complete dechlorination.

14. In the biological activated carbon process, the carbon adsorption provided significant protection against inhibitory factors to complete dechlorination. The overall complete dechlorinating capacity observed in both BAC and OBR systems exceeded the ability of the pure sludge system alone.

15. In the biological activated carbon process, the total ethylene production increased with the amount of PCE added if the incubation time was long enough. However, an inverse relationship between methane production and initial PCE dose was demonstrated. The more PCE added, the more electrons were diverted to PCE for reductive dechlorination.

16. Bioregeneration of PCE-laden carbon was demonstrated in batch serum bottles with acclimated digested sludge. A high bioregeneration efficiency of the adsorptive capacity of activated carbon was achieved in this study. The concept of the treatment process, consisting of activated carbon adsorption of PCE followed by desorption and biological treatment system for the remediation of groundwater contaminated with PCE and/or other less chlorinated ethylenes, was proved. The offline biological regeneration process is appropriate for the use in this case.

17. Further research is needed for completely understanding the bioregeneration process.

Appendix A

Experimental Data

Appendix A

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Appendix A

List of Tables

Fig. A-1 PCE-dechlorinating ability and formation of intermediates in a batch culture of fresh anaerobic Hyperion sludge after successive additions of PCE and 25 μmoles of methanol.

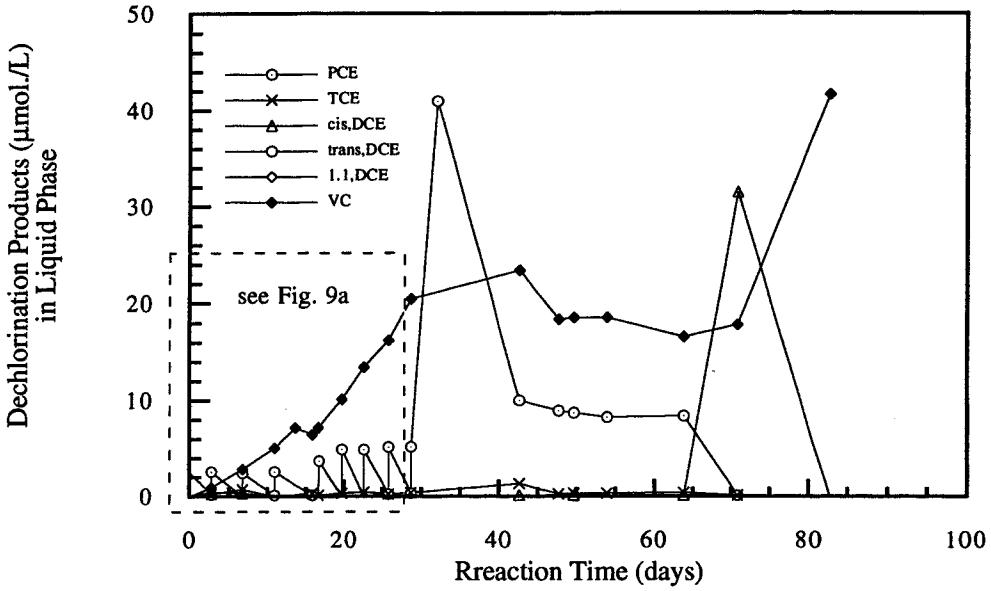


Fig. A-1a PCE-dechlorinating ability and formation of intermediates in a batch culture of fresh anaerobic Hyperion sludge after successive additions of PCE and 25 μmoles of methanol.

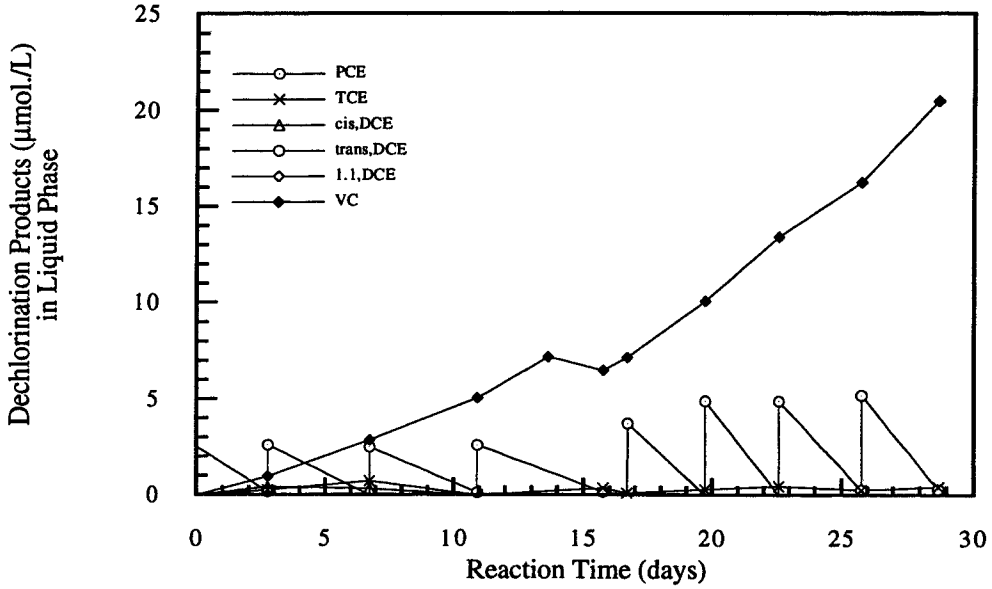


Fig. A-2 PCE-dechlorinating ability and formation of intermediates in a batch culture of fresh anaerobic Hyperion sludge after successive additions of PCE and 2.5 μmoles of methanol.

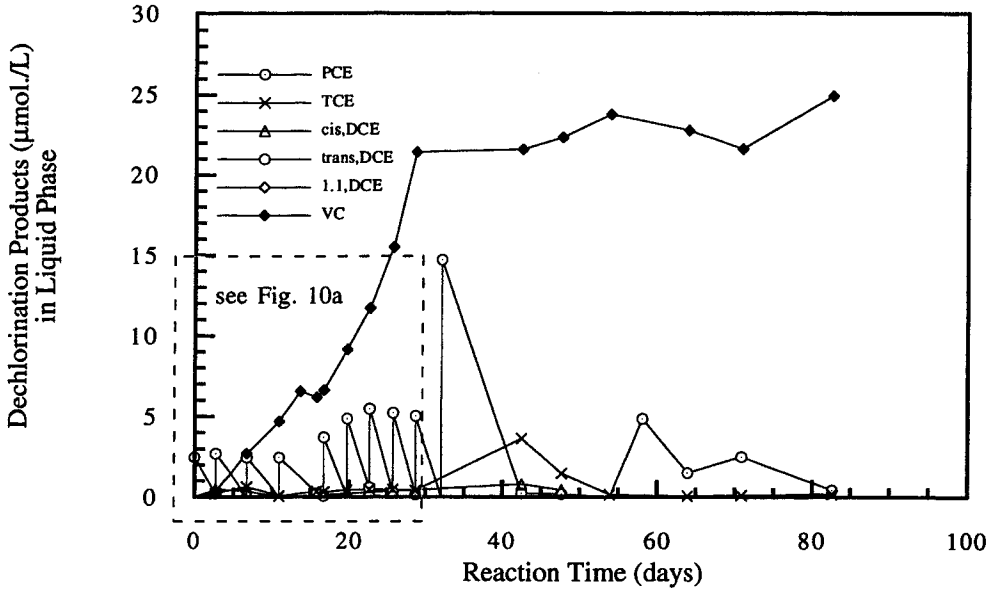


Fig. A-2a PCE-dechlorinating ability and formation of intermediates in a batch culture of fresh anaerobic Hyperion sludge after successive additions of PCE and 2.5 μmoles of methanol.

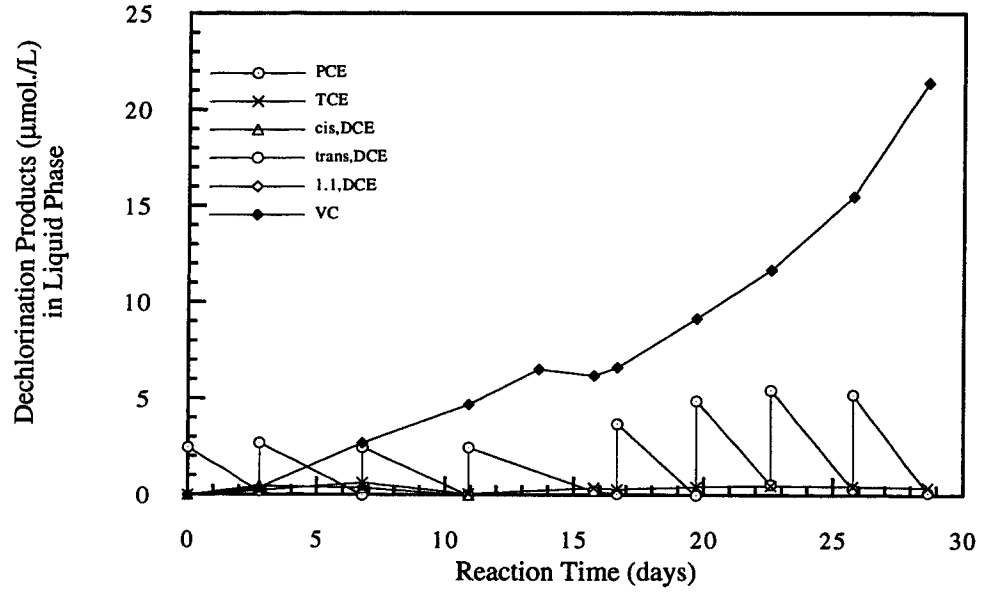


Fig. A-3 **PCE-dechlorinating ability and formation of intermediates in anaerobic pond sediment after successive additions of PCE and 25 μmoles of methanol.**

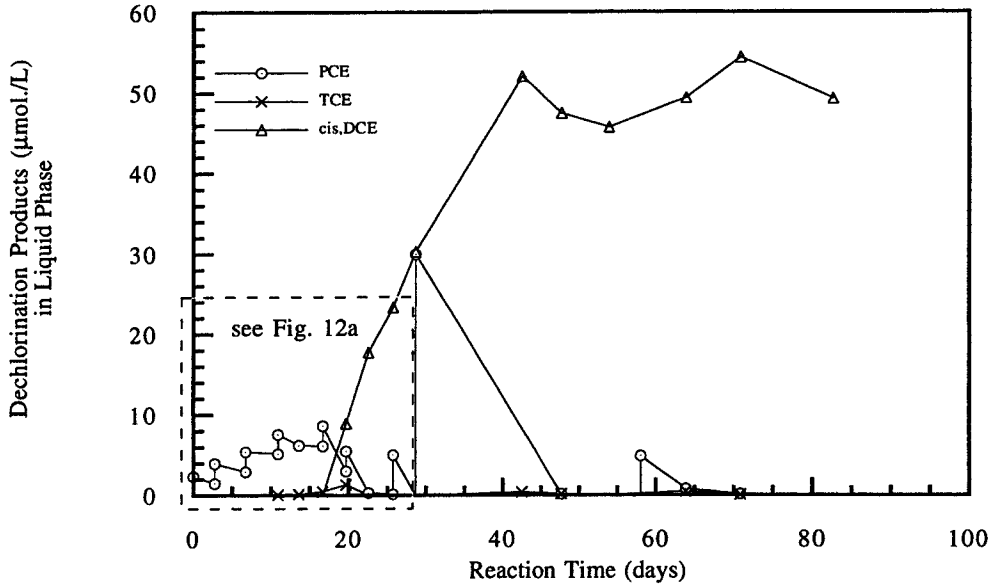


Fig. A-3a **PCE-dechlorinating ability and formation of intermediates in anaerobic pond sediment after successive additions of PCE and 25 μmoles of methanol.**

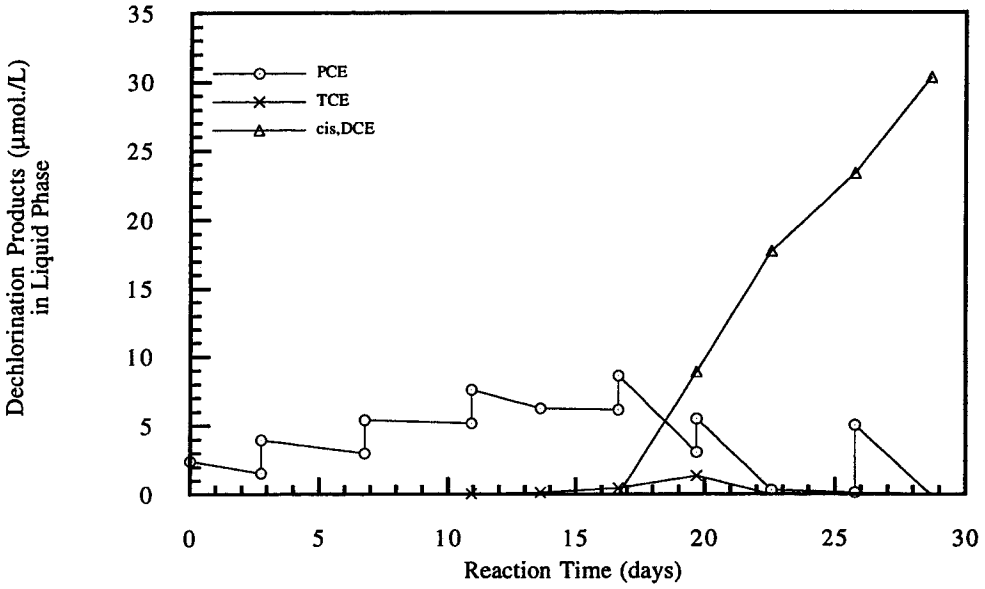


Fig. A-4 **PCE-dechlorinating ability and formation of intermediates in anaerobic pond sediment after successive additions of PCE and 2.5 μmoles of methanol.**

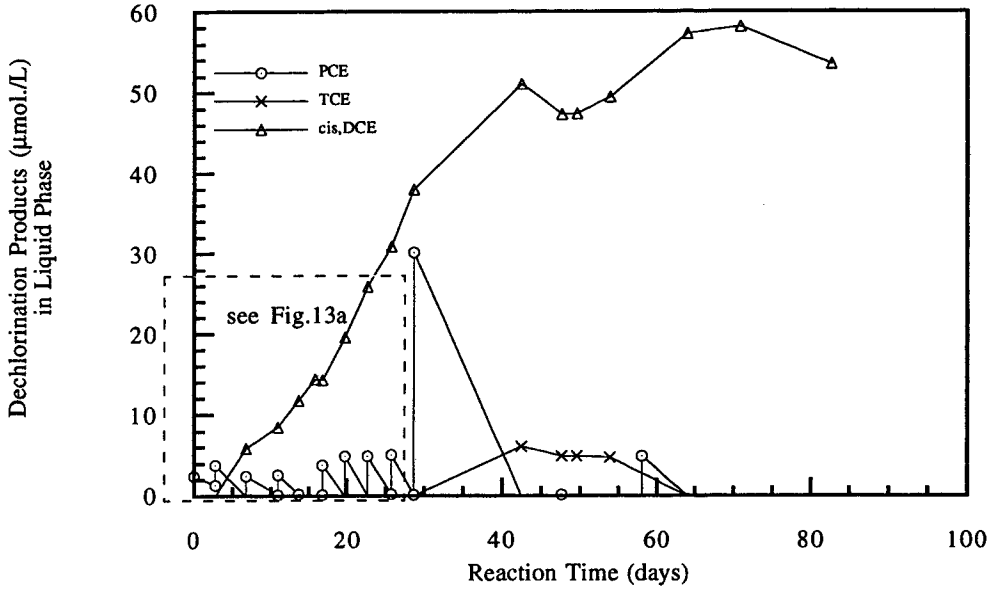


Fig A-4a **PCE-dechlorinating ability and formation of intermediates in anaerobic pond sediment after successive additions of PCE and 2.5 μmoles of methanol.**

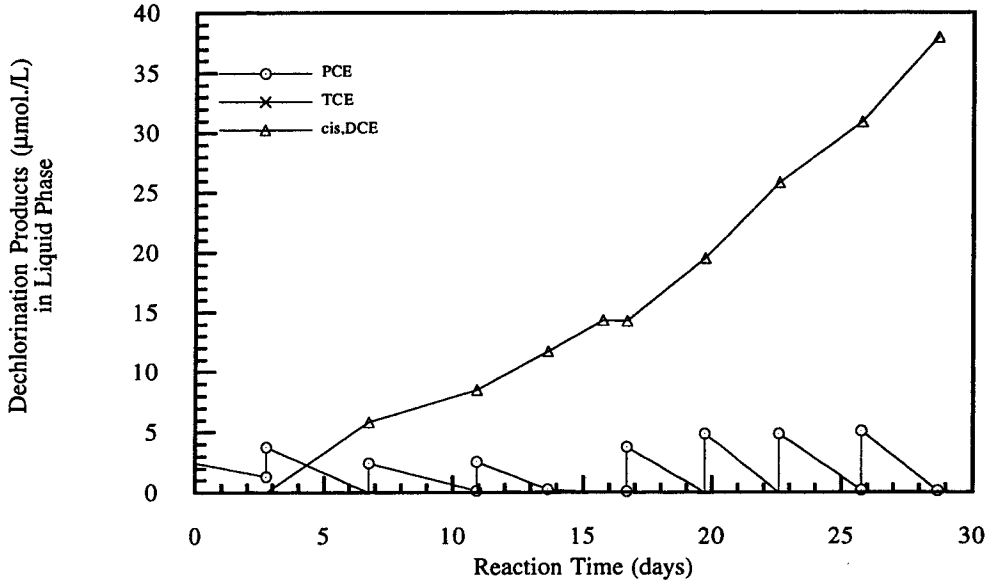


Fig. A-5 PCE-dechlorinating ability and formation of intermediates in a mixture culture (1:1) of fresh anaerobic Hyperion sludge and anaerobic pond sediment after successive additions of PCE and 25 μmoles of methanol.

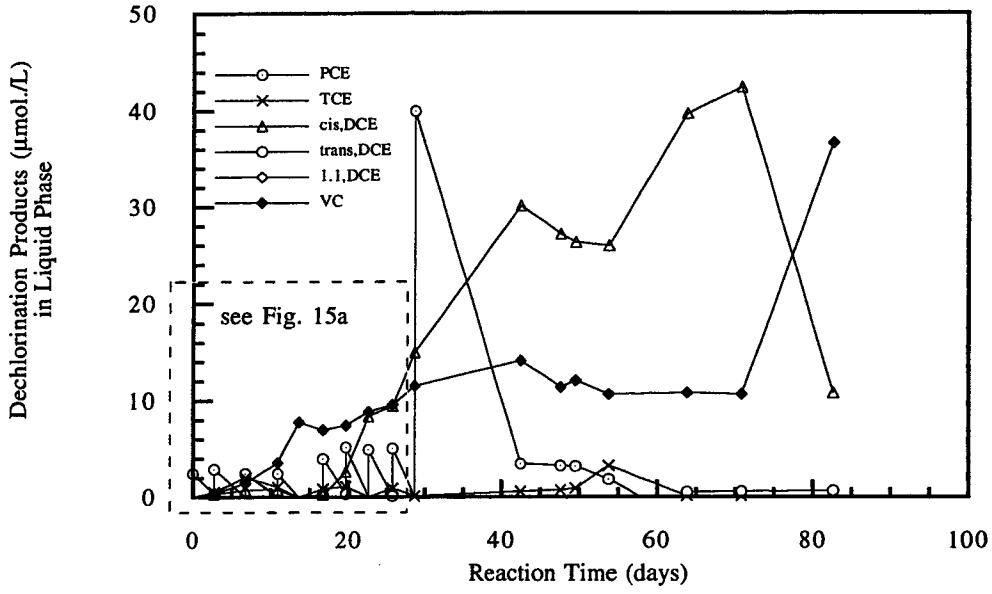


Fig. A-5a PCE-dechlorinating ability and formation of intermediates in a mixture culture (1:1) of fresh anaerobic Hyperion sludge and anaerobic pond sediment after successive additions of PCE and 25 μmoles of methanol.

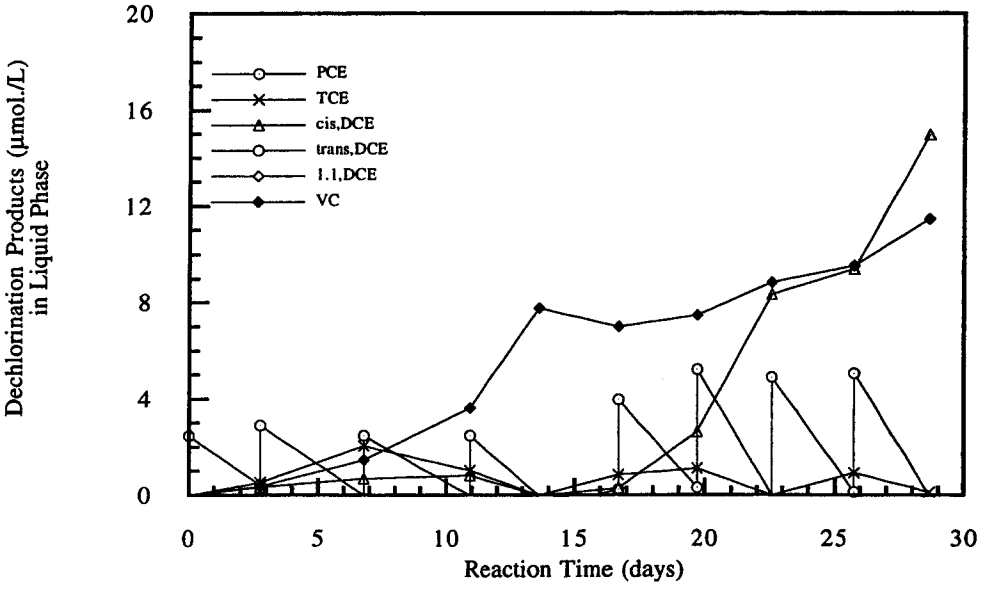


Fig. A-6 PCE-dechlorinating ability and formation of intermediates in a mixture culture (1:1) of fresh anaerobic Hyperion sludge and anaerobic pond sediment after successive additions of PCE and 2.5 μmoles of methanol.

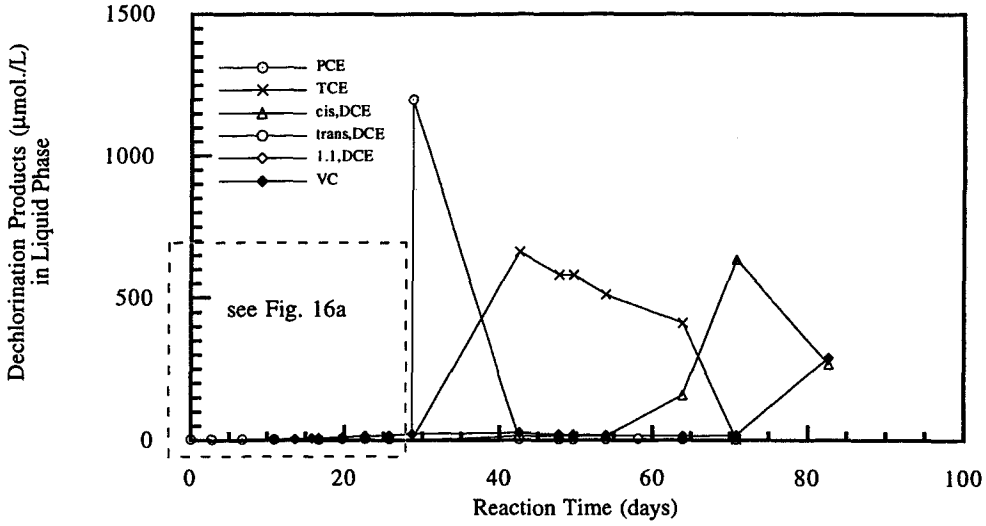
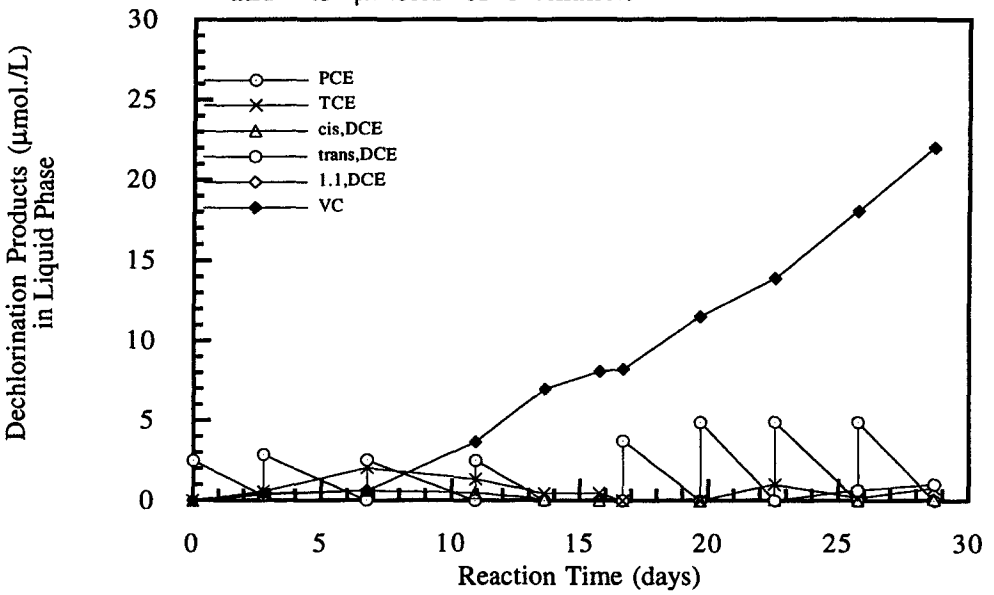


Fig. A-6a PCE-dechlorinating ability and formation of intermediates in a mixture culture (1:1) of fresh anaerobic Hyperion sludge and anaerobic pond sediment after successive additions of PCE and 2.5 μmoles of methanol.



Note: The major product from PCE dechlorination was VC. These results appear to indicate that the Hyperion sludge culture is predominately responsible for PCE dechlorination when both are present.

Fig. A-7 PCE-dechlorination ability and formation of intermediates in a batch culture of fresh anaerobic Chino Basin (RP2) sludge I.

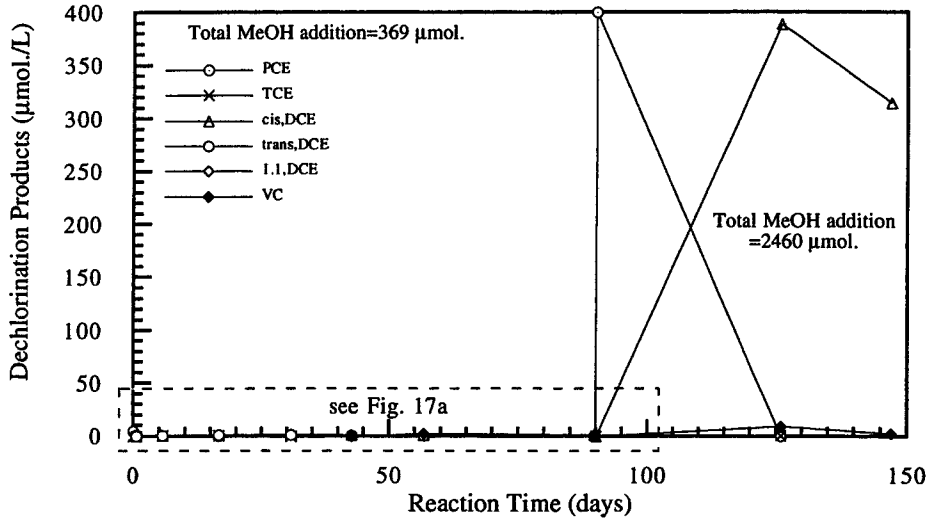
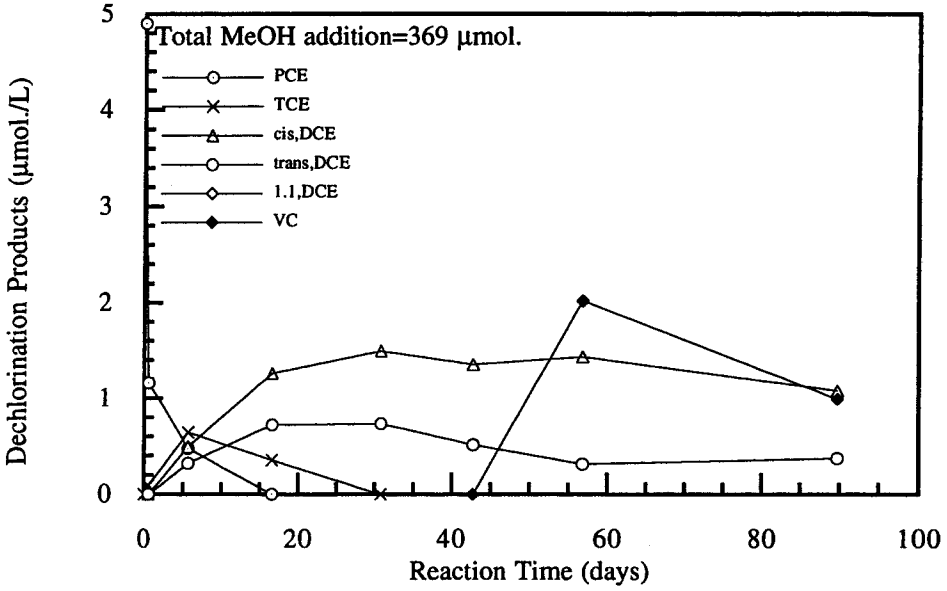


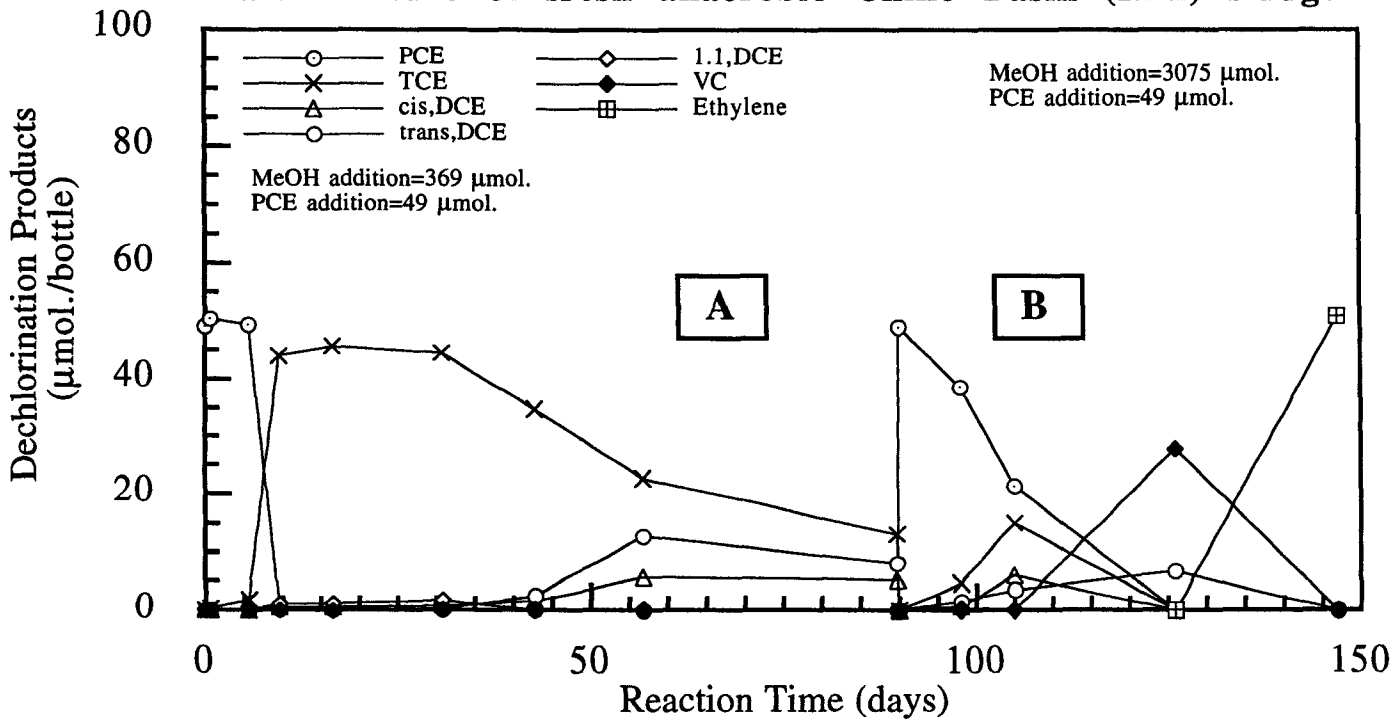
Fig. A-7a PCE-dechlorination ability and formation of intermediates in a batch culture of fresh anaerobic Chino Basin (RP2) sludge I.



Note: 1. Only initial 0.6 µmoles of PCE was added in the first 90 days.

2. Data obtained after 90 days indicates PCE dechlorination and formation of intermediates with initial addition of 49 µmoles of PCE, increased concentration of methanol, and fresh medium replacement.

Fig. A-8 PCE-dechlorination ability and formation of intermediates in a batch culture of fresh anaerobic Chino Basin (RP2) sludge I.



Note: 1. 49 µmoles of PCE was added at the beginning of both stages (A & B).

2. Data obtained after 90 days indicates PCE dechlorination and formation of intermediates with initial addition of 49 µmoles of PCE, increased concentration of methanol, and fresh medium replacement.

Fig. A-9 PCE-dechlorination ability and formation of intermediates in a batch culture of fresh anaerobic Chino Basin (RP2) sludge II.

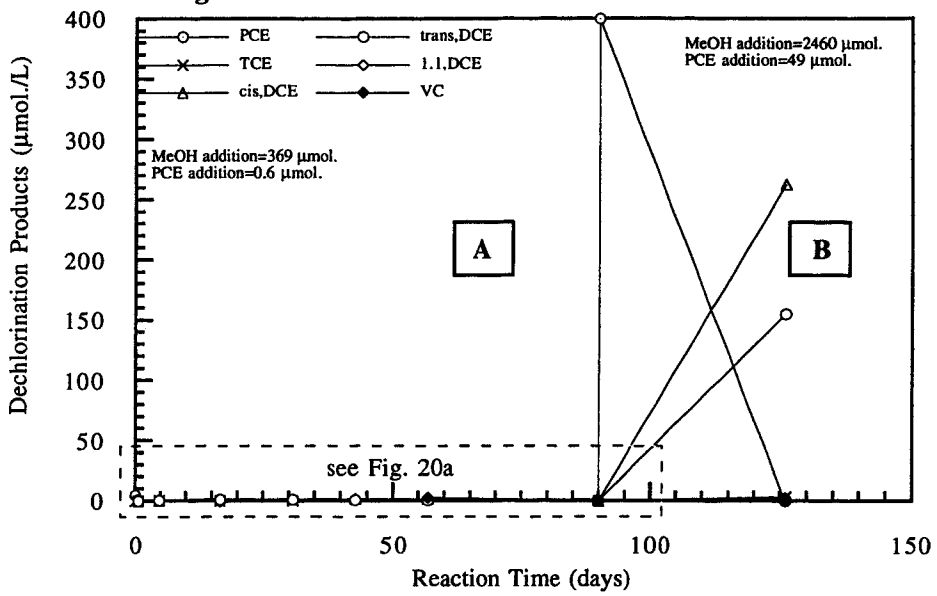
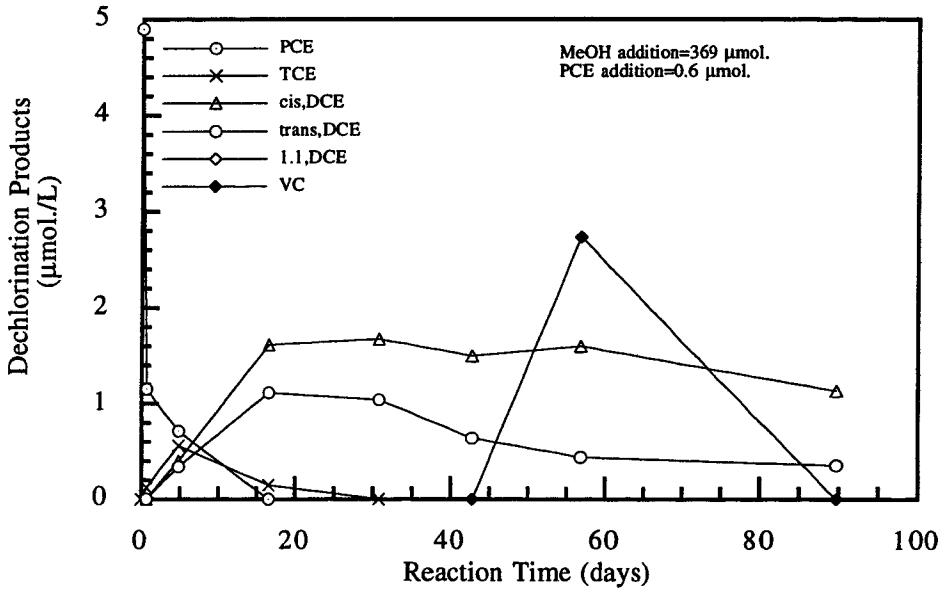


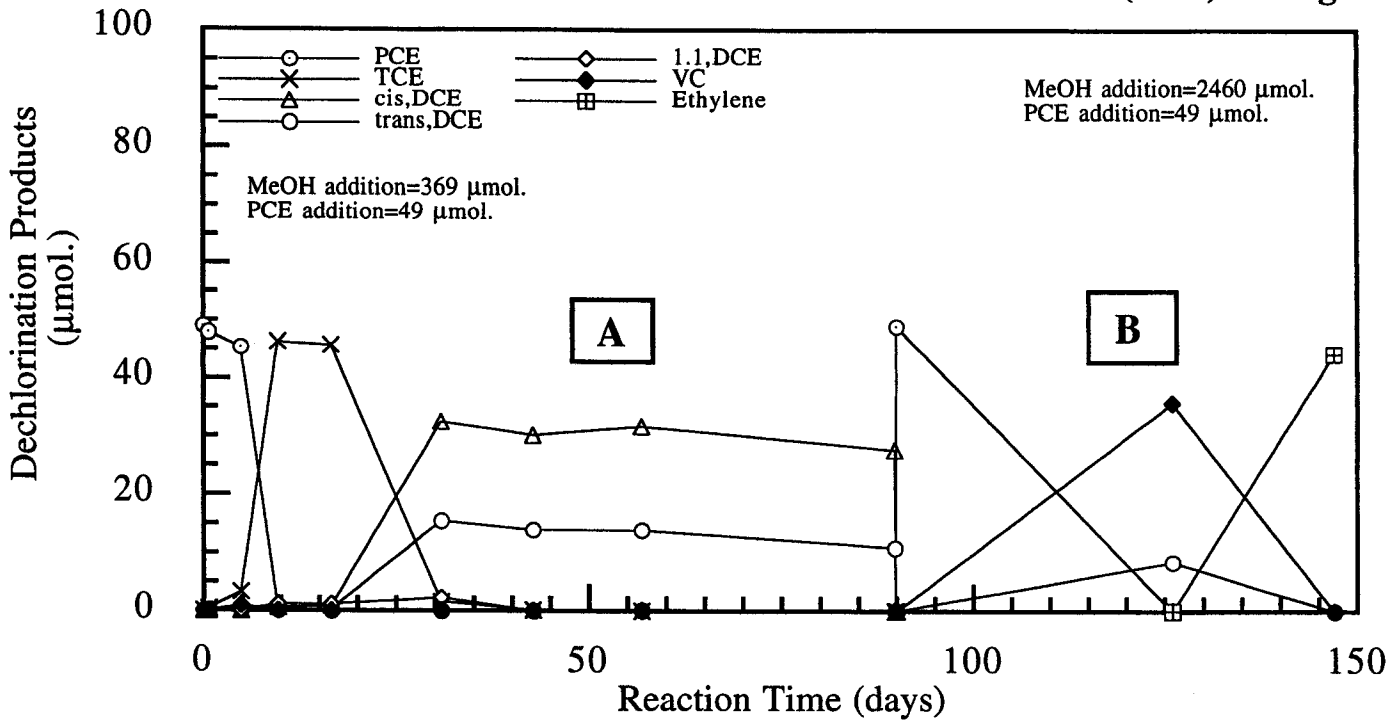
Fig. A-9a PCE-dechlorination ability and formation of intermediates in a batch culture of fresh anaerobic Chino Basin (RP2) sludge II.



Note: 1. Only initial 0.6 µmoles of PCE was added in the first 90 days.

2. Data obtained after 90 days indicates PCE dechlorination and formation of intermediates with initial addition of 49 µmoles of PCE, increased concentration of methanol, and fresh medium replacement.

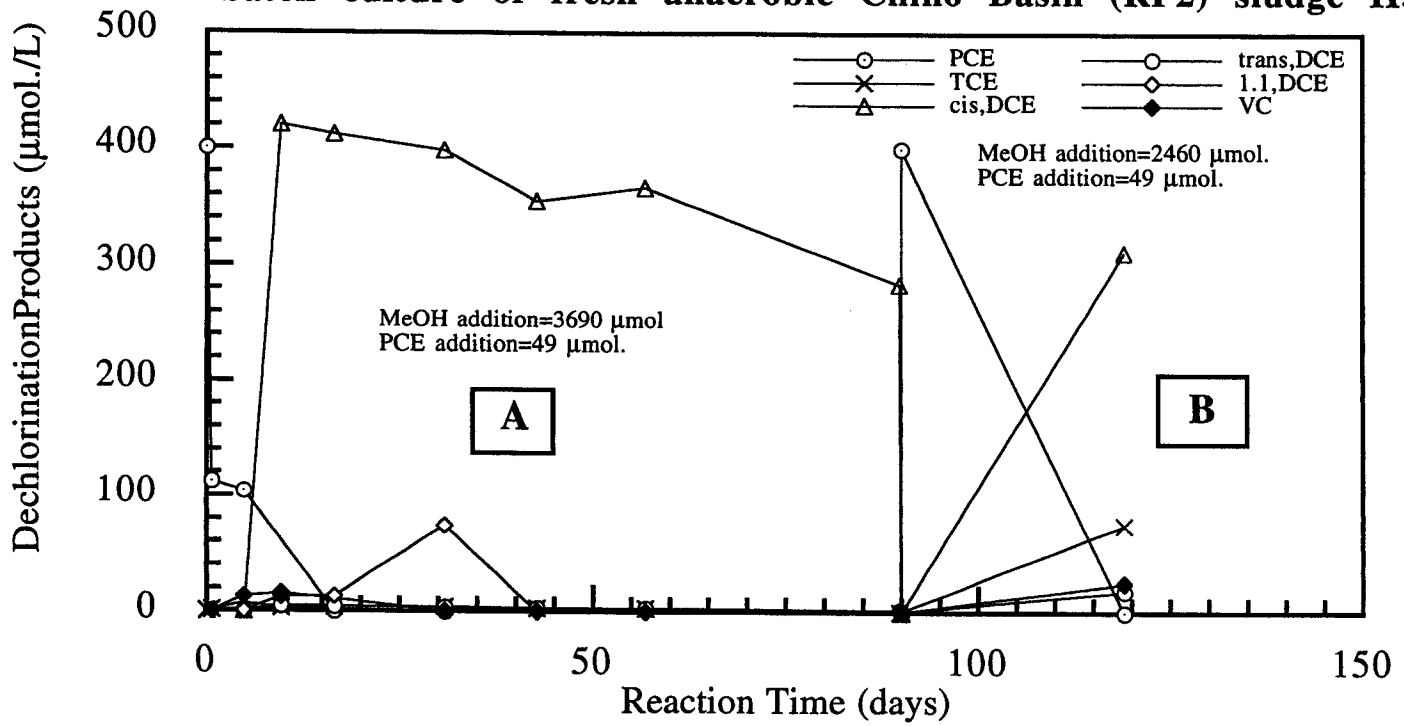
Fig. A-10 PCE-dechlorination ability and formation of intermediates in a batch culture of fresh anaerobic Chino Basin (RP2) sludge II.



Note: 1. 49 µmoles of PCE was added at the beginning of both stages (A & B).

2. Data obtained after 90 days indicates PCE dechlorination and formation of intermediates with initial addition of 49 µmoles of PCE, increased concentration of methanol, and fresh medium replacement.

Fig. A-11 PCE-dechlorination ability and formation of intermediates in a batch culture of fresh anaerobic Chino Basin (RP2) sludge II.



Note: 1. 49 µmoles of PCE was added at the beginning of both stages (A & B).

2. Data obtained after 90 days indicates PCE dechlorination and formation of intermediates with initial addition of 49 µmoles of PCE, decreased concentration of methanol, and fresh medium replacement.

Fig. A-12 Anaerobic Hyperion sludge ability to degrade PCE.

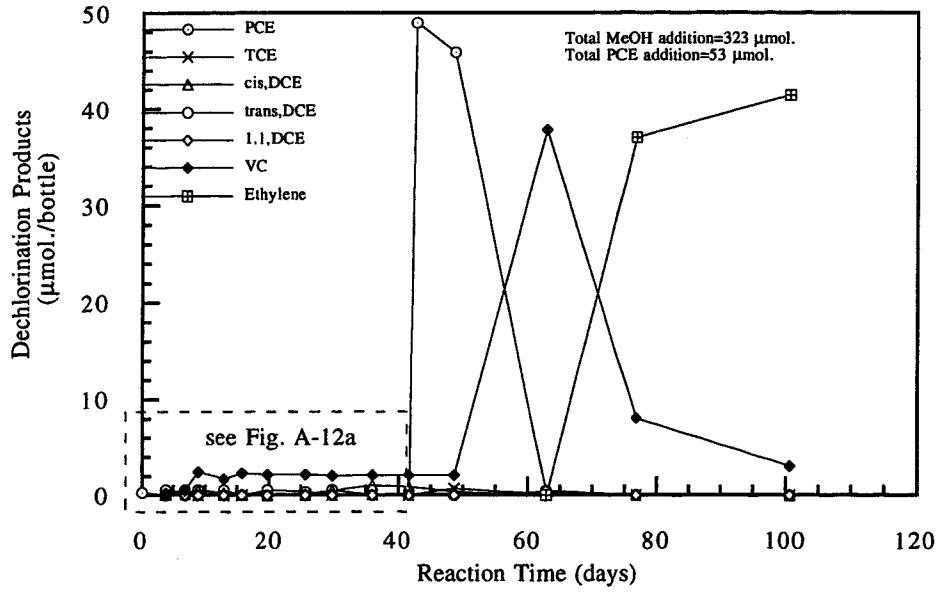
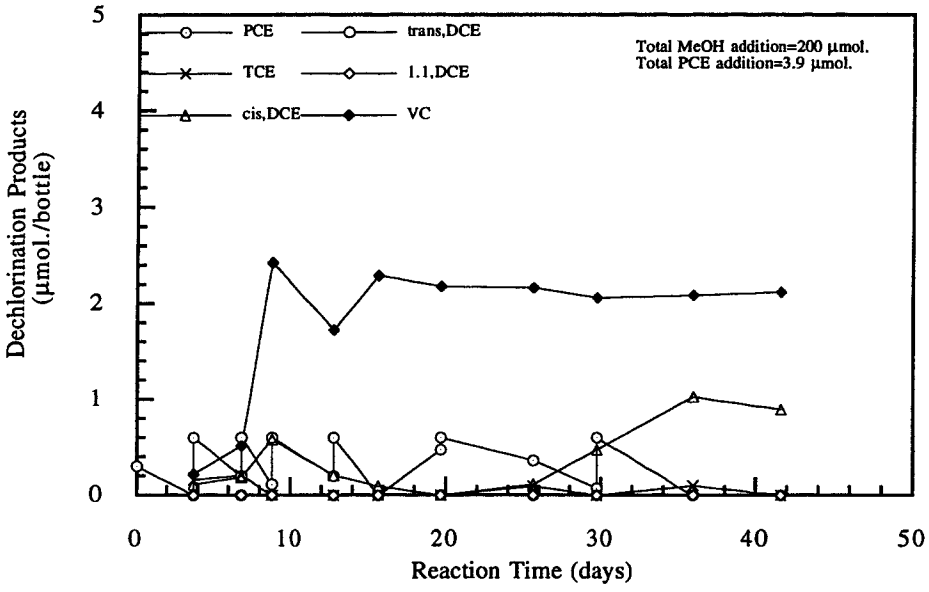


Fig. A-12a Anaerobic Hyperion sludge ability to degrade PCE.



Note: 1. At the first 45 days, low PCE concentration was tested.
2. After 45 days, high PCE concentration was tested.

Fig. A-13 Anaerobic Hyperion sludge ability to degrade TCE.

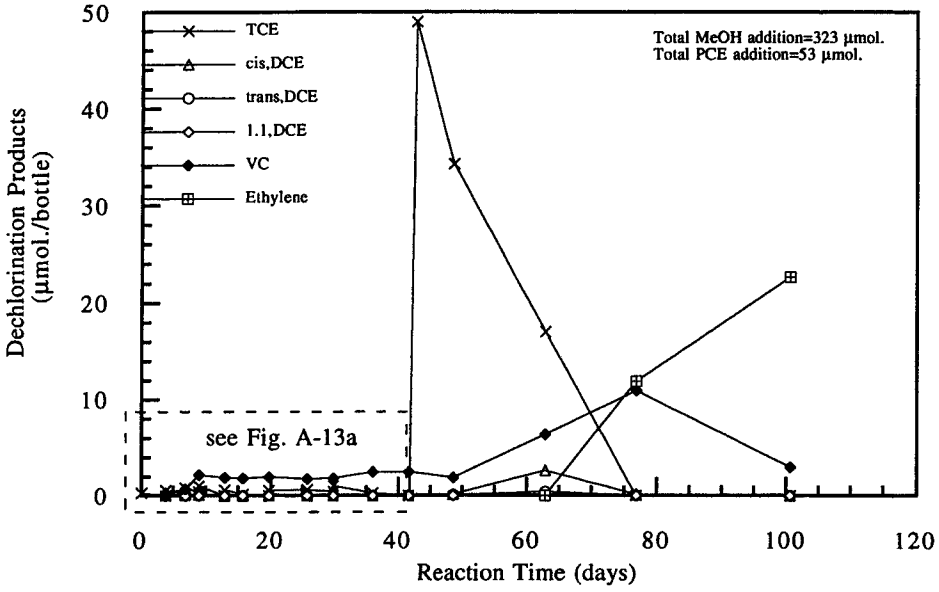
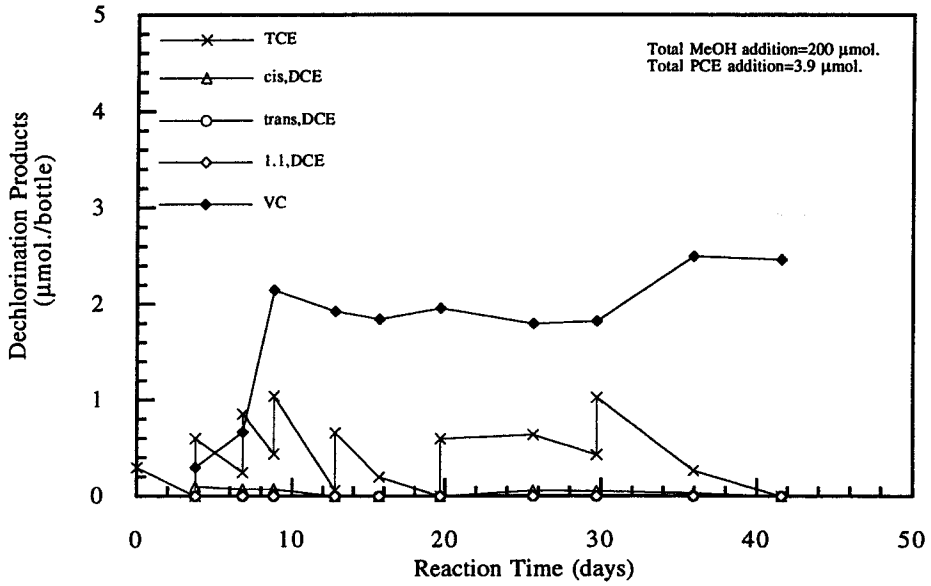


Fig. A-13a Anaerobic Hyperion sludge ability to degrade TCE.



Note: 1. At the first 45 days, low TCE concentration was tested.
2. After 45 days, high TCE concentration was tested.

Fig. A-14 Anaerobic Hyperion sludge ability to degrade cis-DCE.

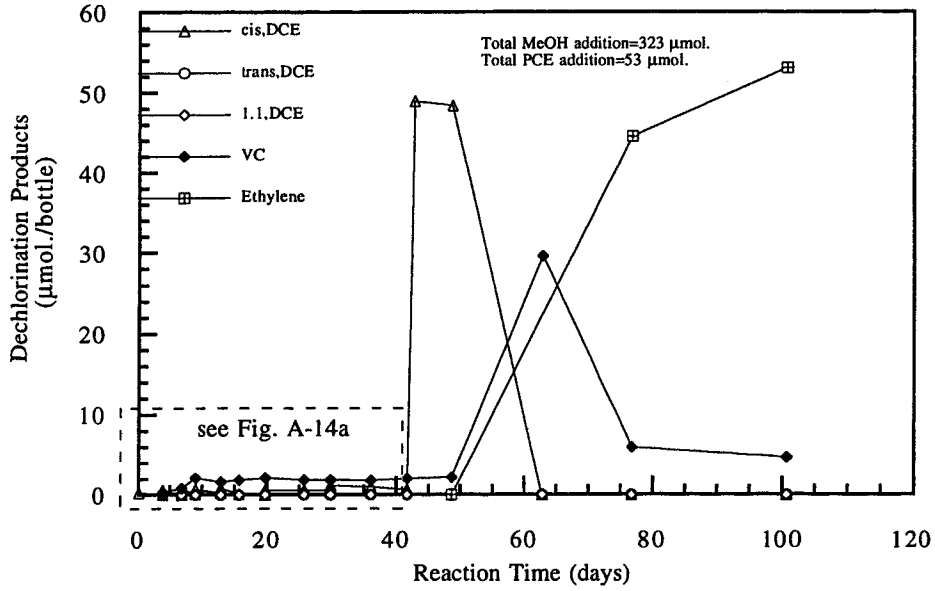
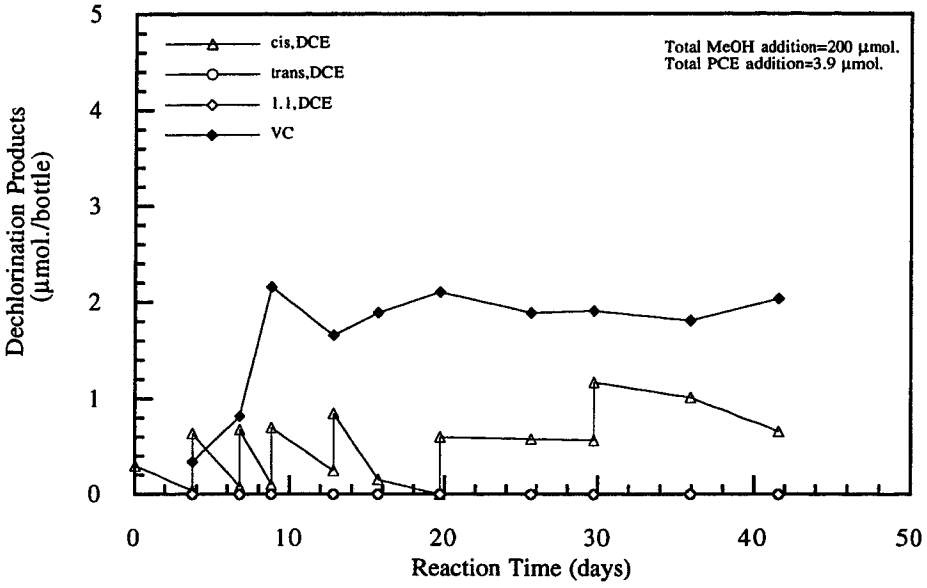


Fig. A-14a Anaerobic Hyperion sludge ability to degrade cis-DCE.



Note: 1. At the first 45 days, low cis,DCE concentration was tested.
2. After 45 days, high cis,DCE concentration was tested.

Fig. A-15 Anaerobic Hyperion sludge ability to degrade trans-DCE.

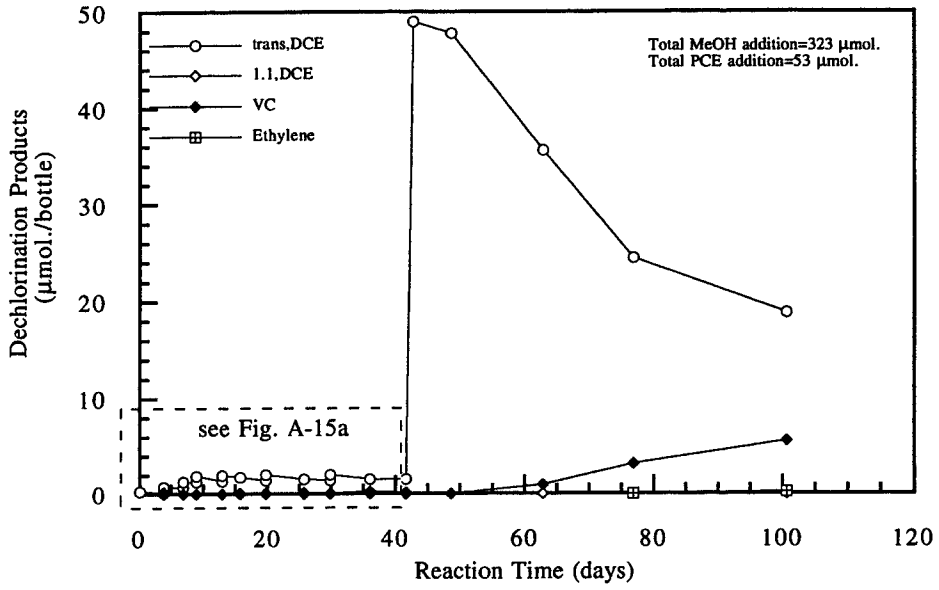
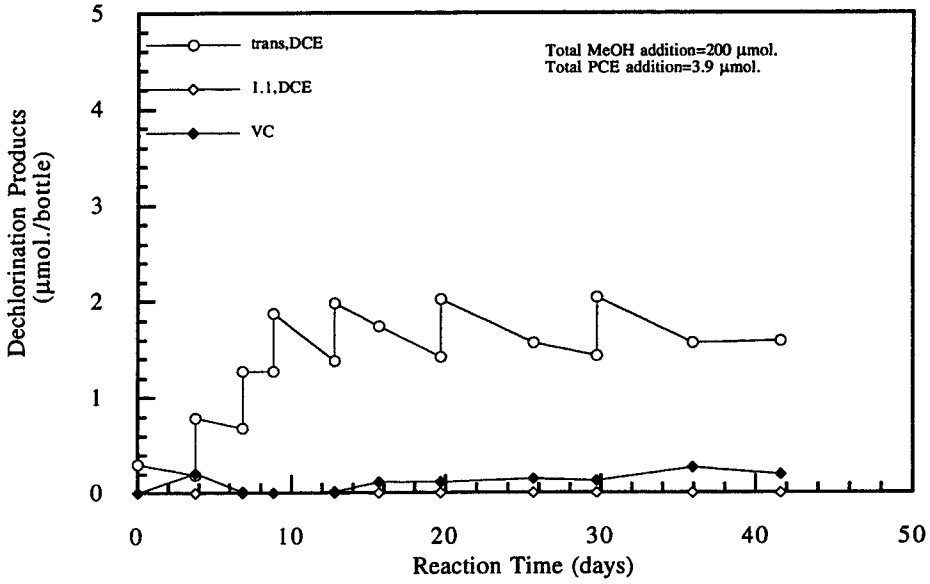


Fig. A-15a Anaerobic Hyperion sludge ability to degrade trans-DCE.



Note: 1. At the first 45 days, low trans,DCE concentration was tested.
2. After 45 days, high trans,DCE concentration was tested.

Fig. A-16 Anaerobic Hyperion sludge ability to degrade 1.1-DCE.

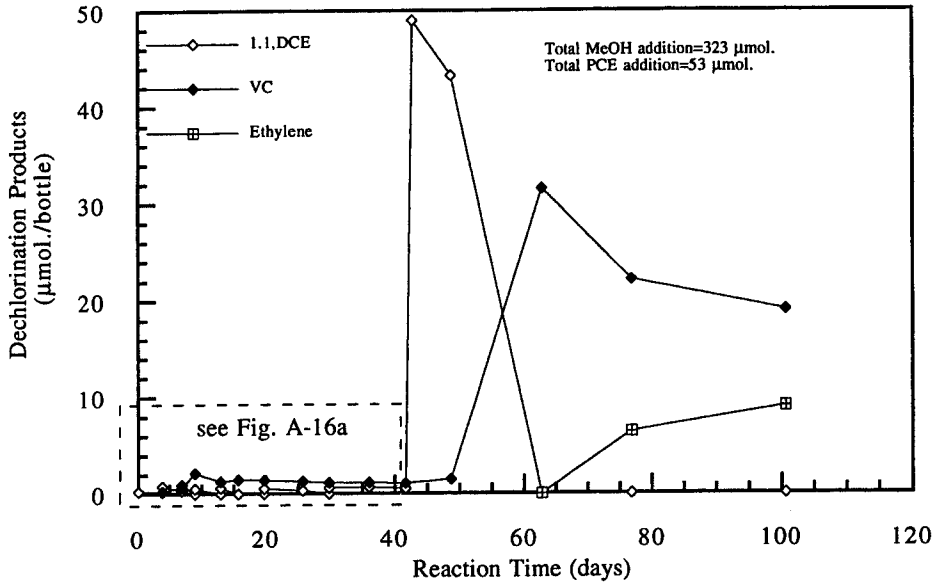
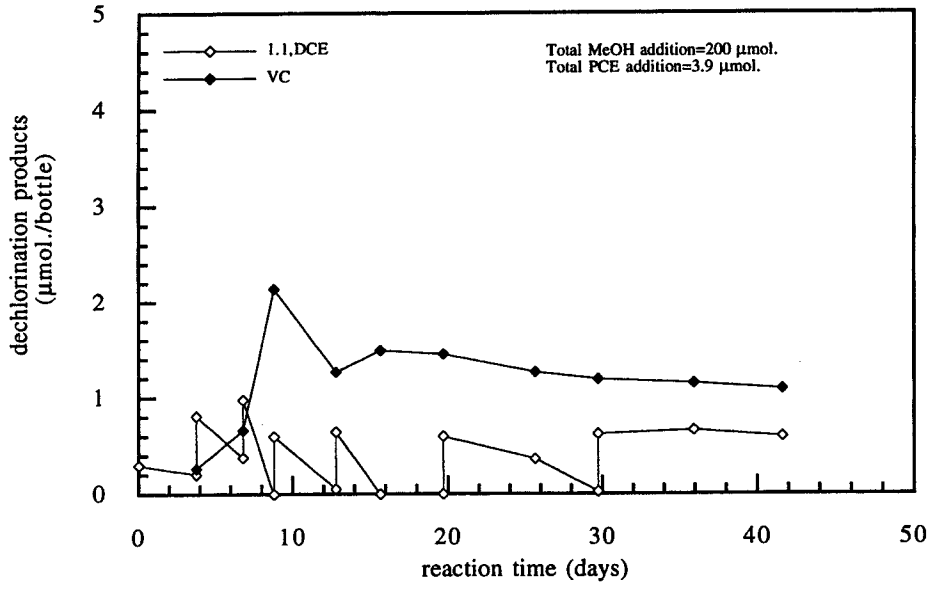


Fig. A-16a Anaerobic Hyperion sludge ability to degrade 1.1-DCE.



Note: 1. At the first 45 days, low 1.1,DCE concentration was tested.
2. After 45 days, high 1.1,DCE concentration was tested.

Fig. A-17 Anaerobic Hyperion sludge ability to degrade VC.

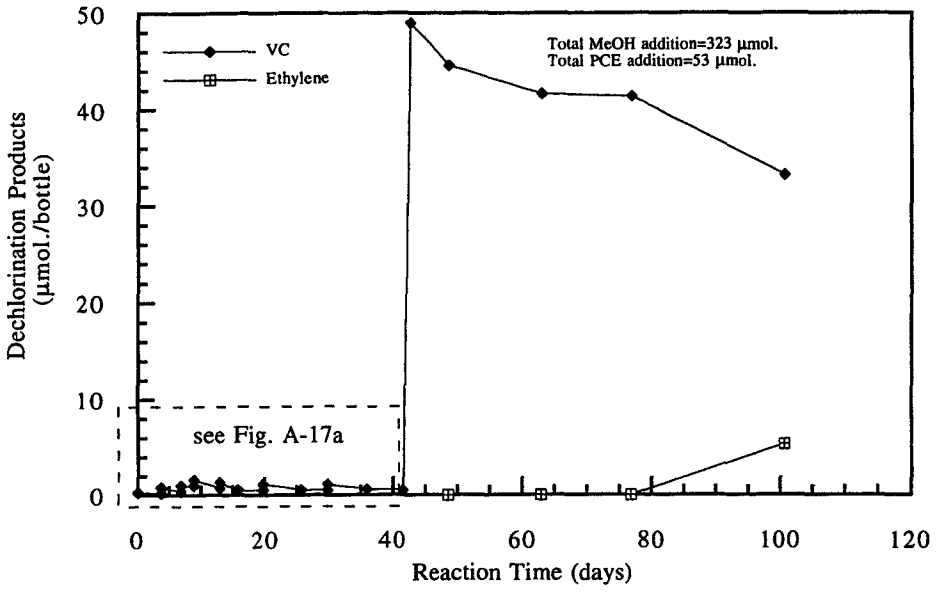
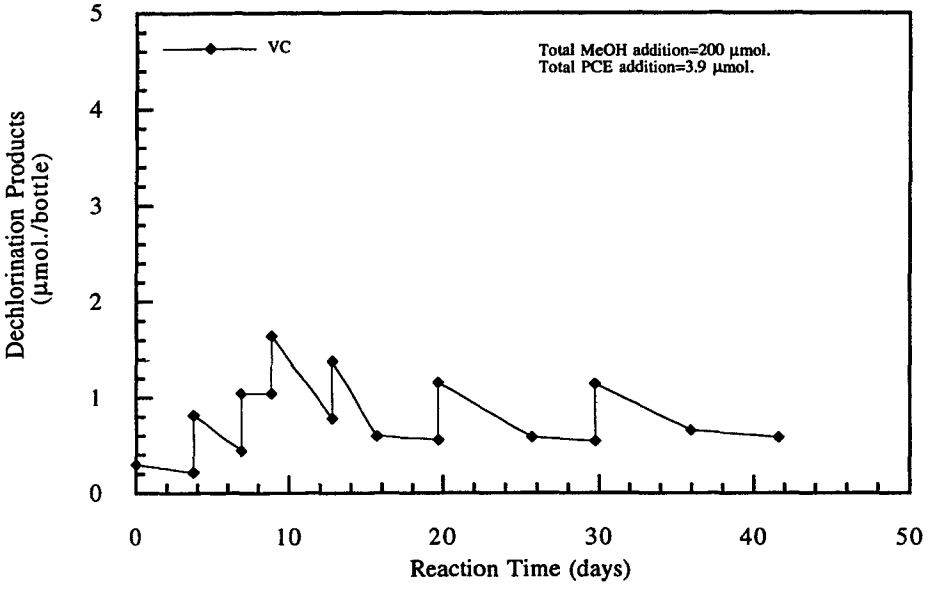
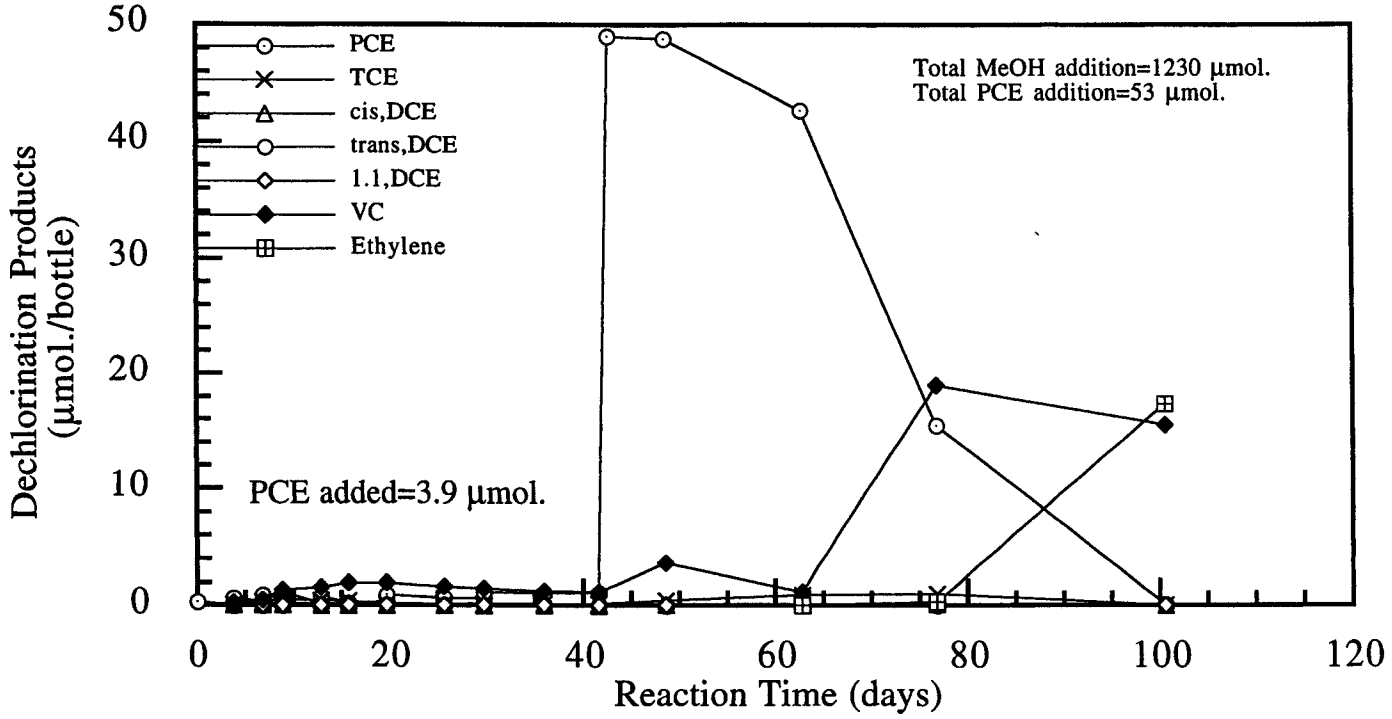


Fig. A-17a Anaerobic Hyperion sludge ability to degrade VC.



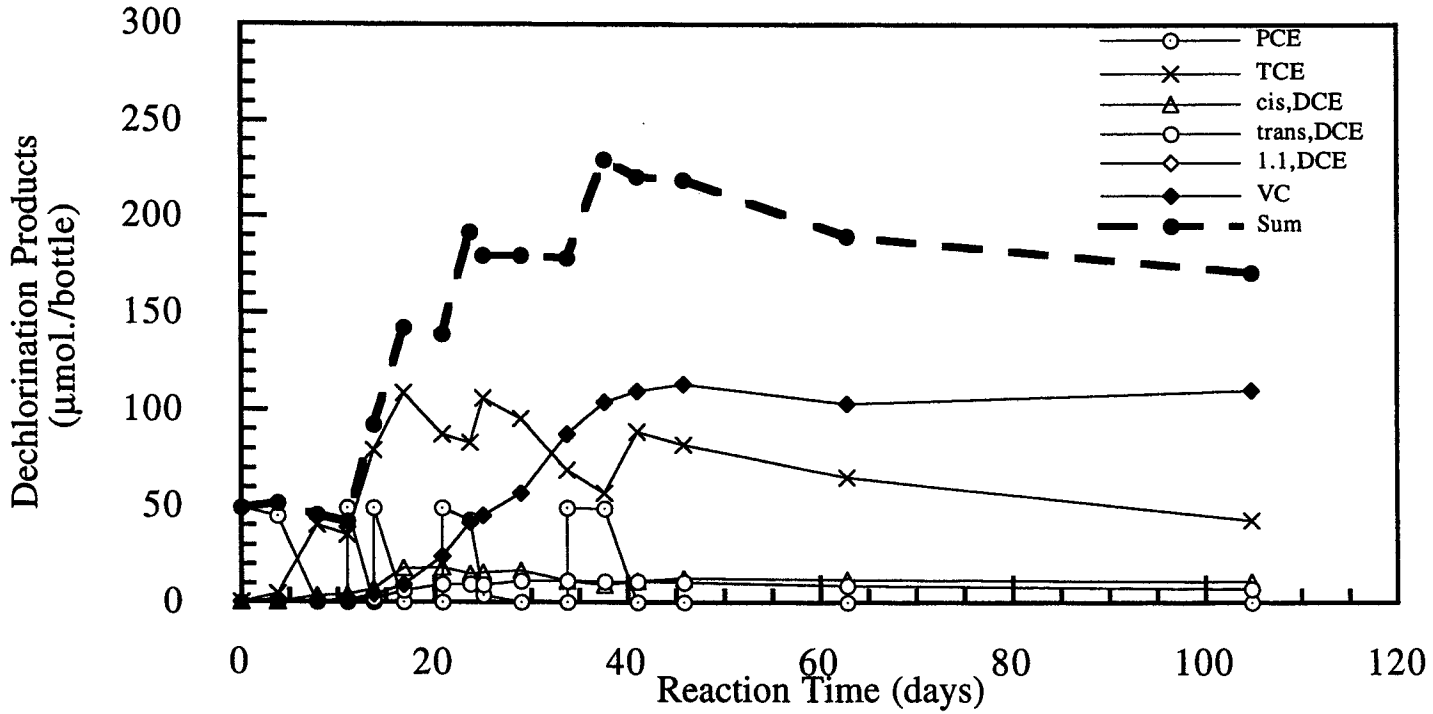
Note: 1. At the first 45 days, low VC concentration was tested.
2. After 45 days, high VC concentration was tested.

Fig. A-18 Anaerobic Hyperion sludge ability to degrade PCE at high methanol concentration.



Note: Compared to the results shown in Fig. A-12, the higher methanol concentration does not improve the PCE dechlorination and ethylene production.

Fig. A-19 Decrease in PCE-dechlorination ability without replacement of nutrient medium.



Notice that when nutrient media is not replenished, major end products accumulated are TCE and lower than expected amounts of VC. Accumulation of TCE that is usually converted completely to VC demonstrates that some inhibitions may be due to nutrient deprivation.

Fig. A-20 Reductive dechlorination progression of PCE at a low PCE concentration in a fresh anaerobic Hyperion sludge.

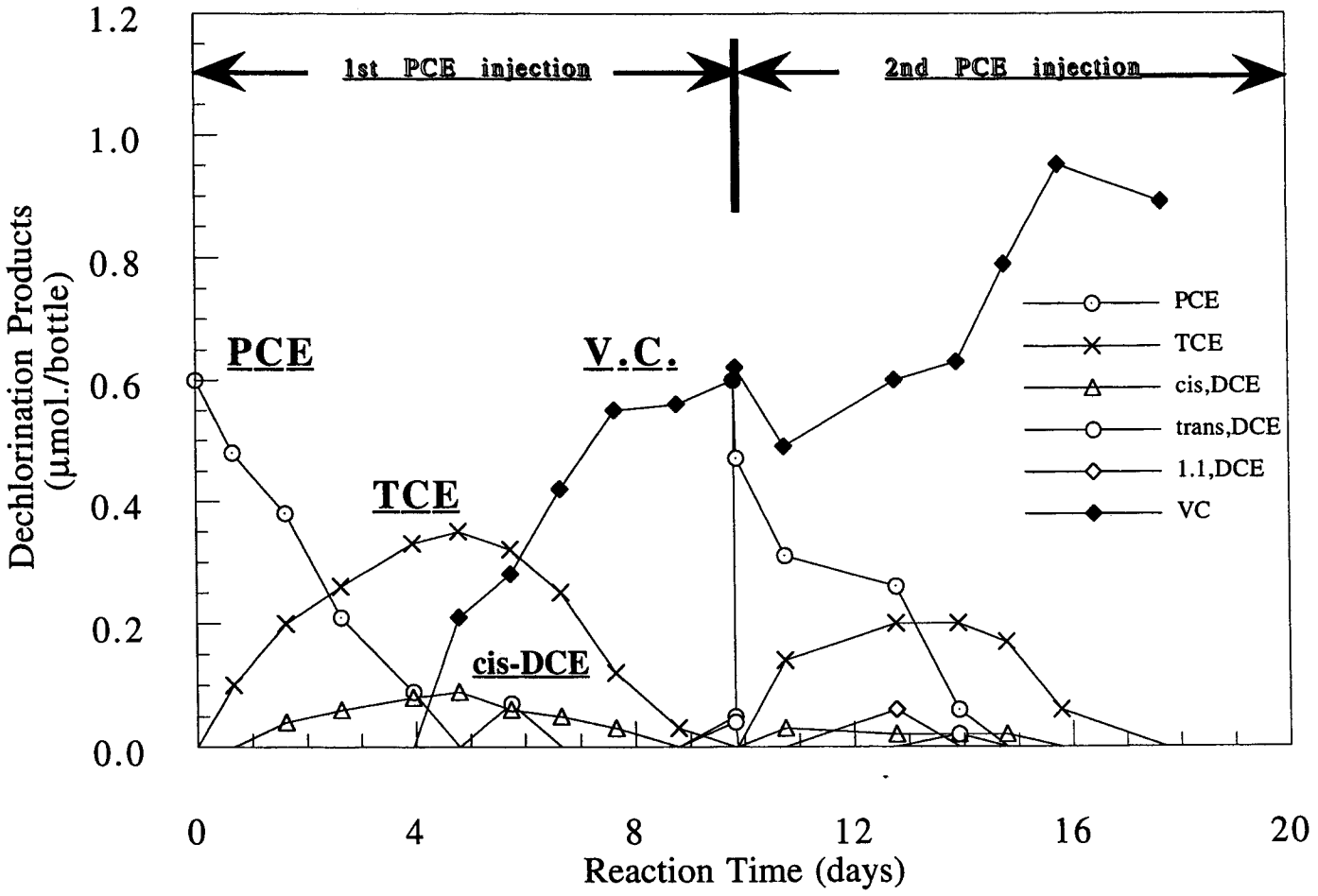


Fig. A-21 Long term dechlorination of PCE and formation of intermediates in a semi-continuous operation serum bottle.

(There was no methanol added from day 0 to 60.)

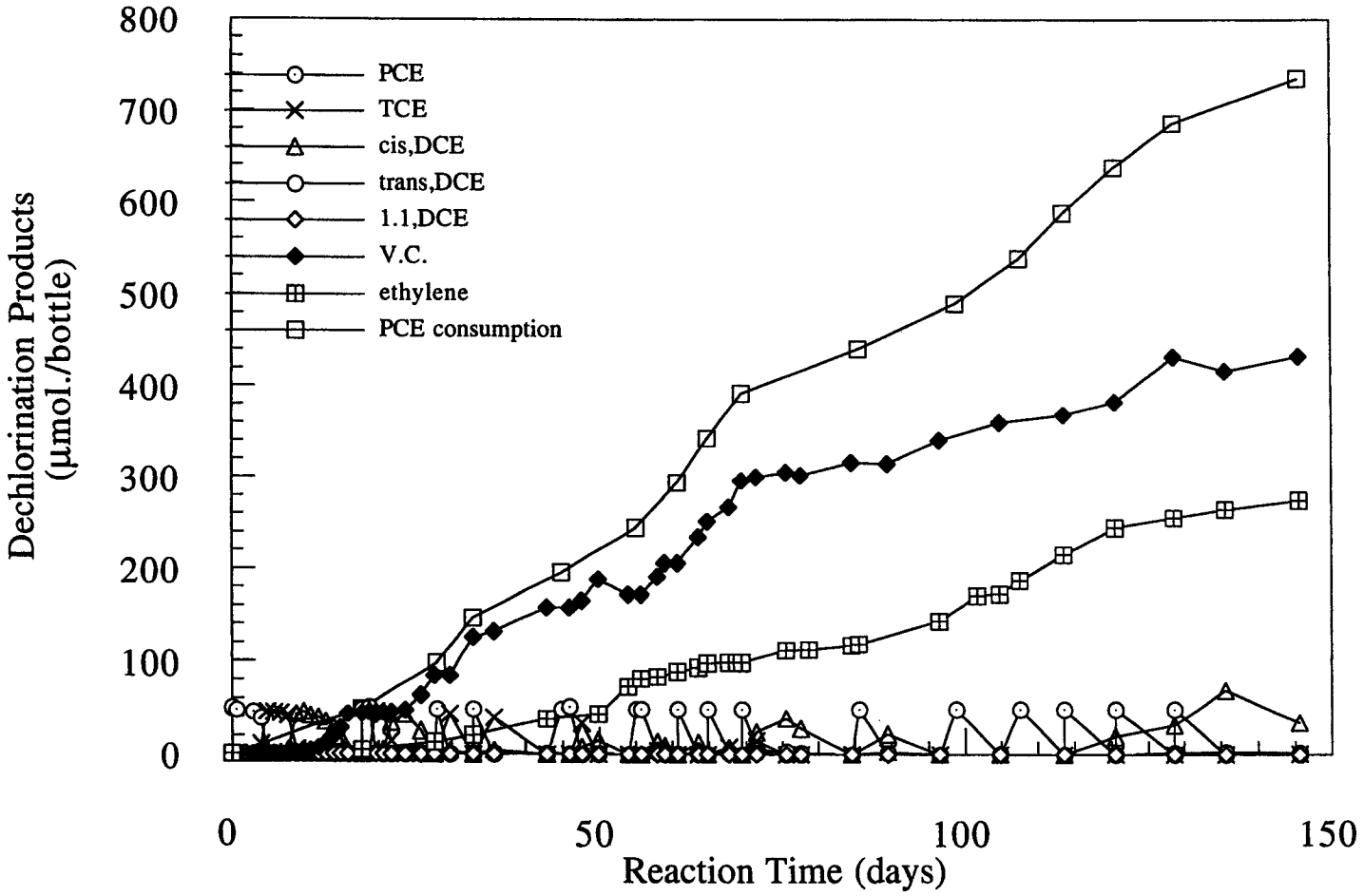


Fig. A-22 Long term dechlorination of TCE and formation of intermediates in a semi-continuous operation serum bottle.

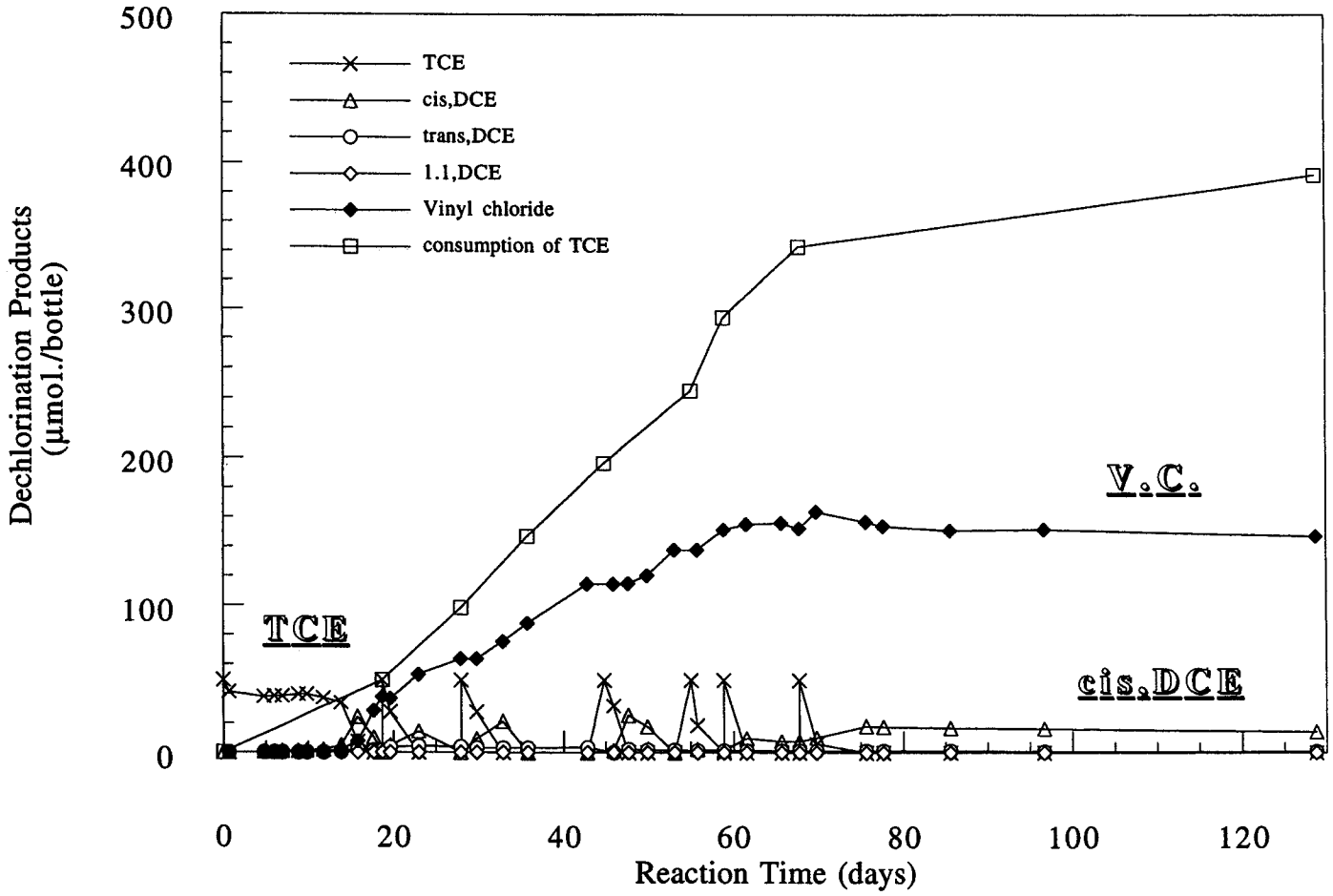


Fig. A-23 Long term dechlorination of cis-DCE and formation of intermediates in a semi-continuous operation serum bottle.

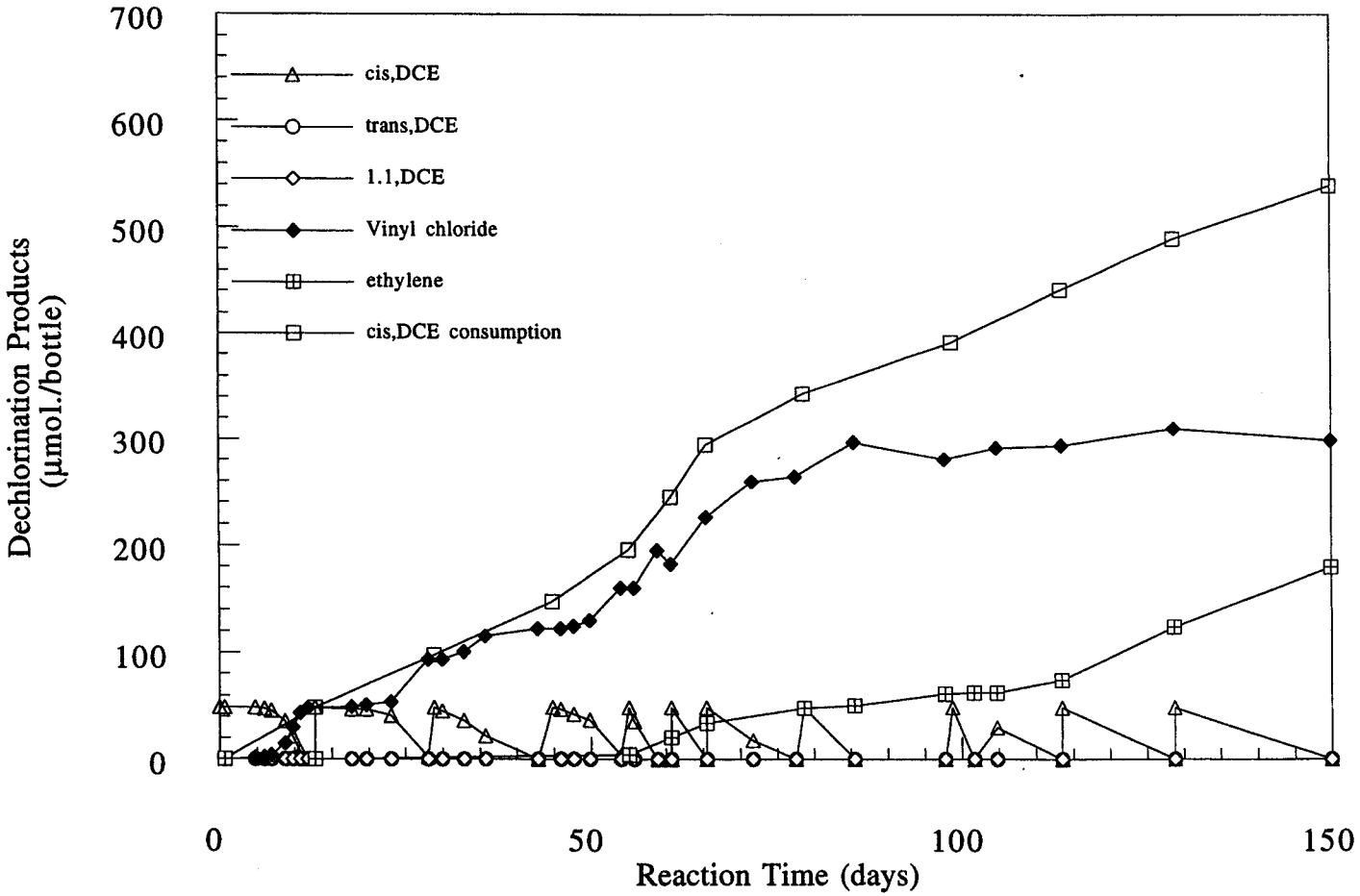


Fig. A-24 Long term dechlorination of 1.1-DCE and formation of intermediates in a semi-continuous operation serum bottle.

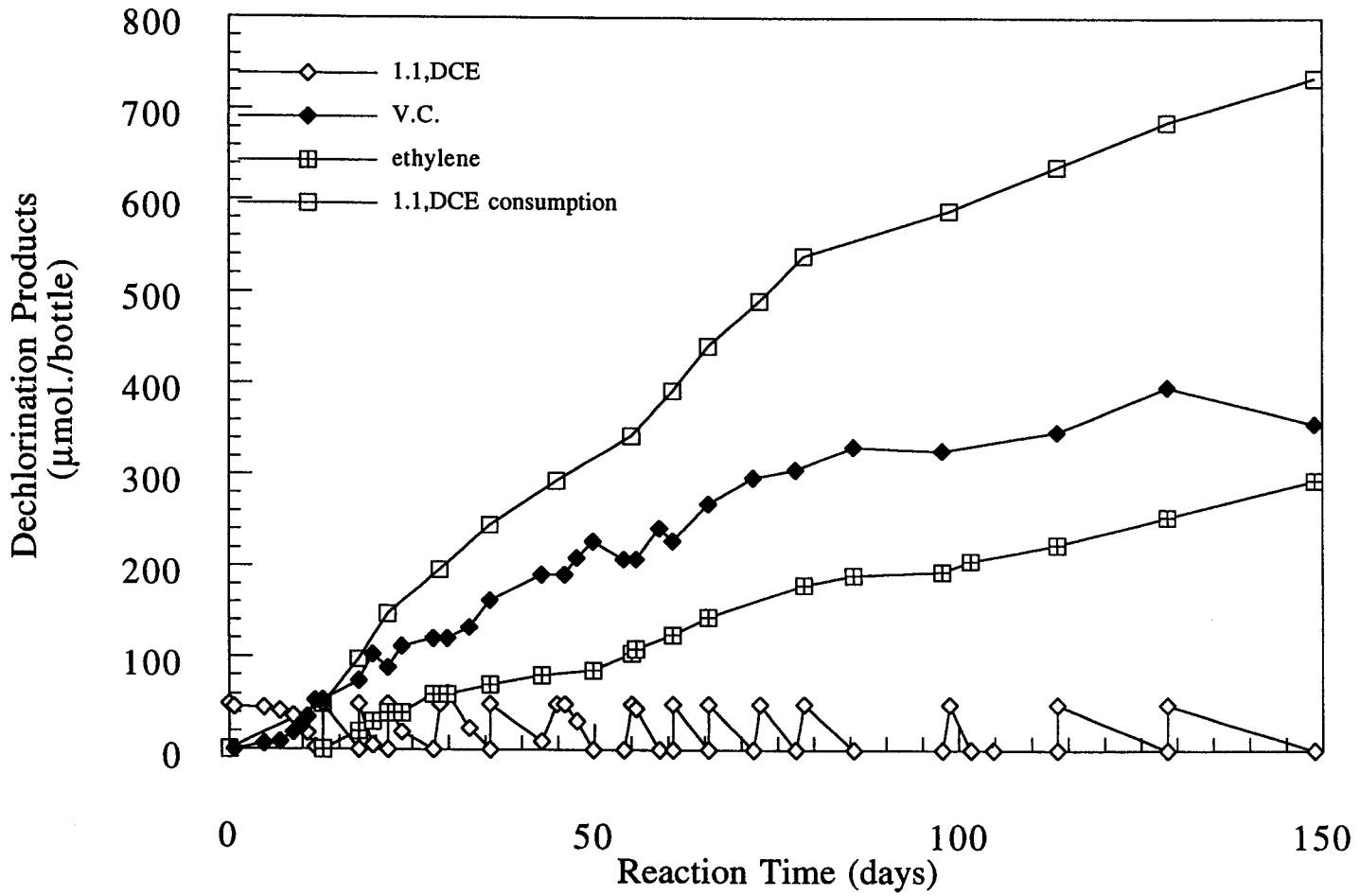


Fig. A-25 Dechlorination of PCE and formation of intermediates in a batch culture of fresh anaerobic Hyperion sludge.

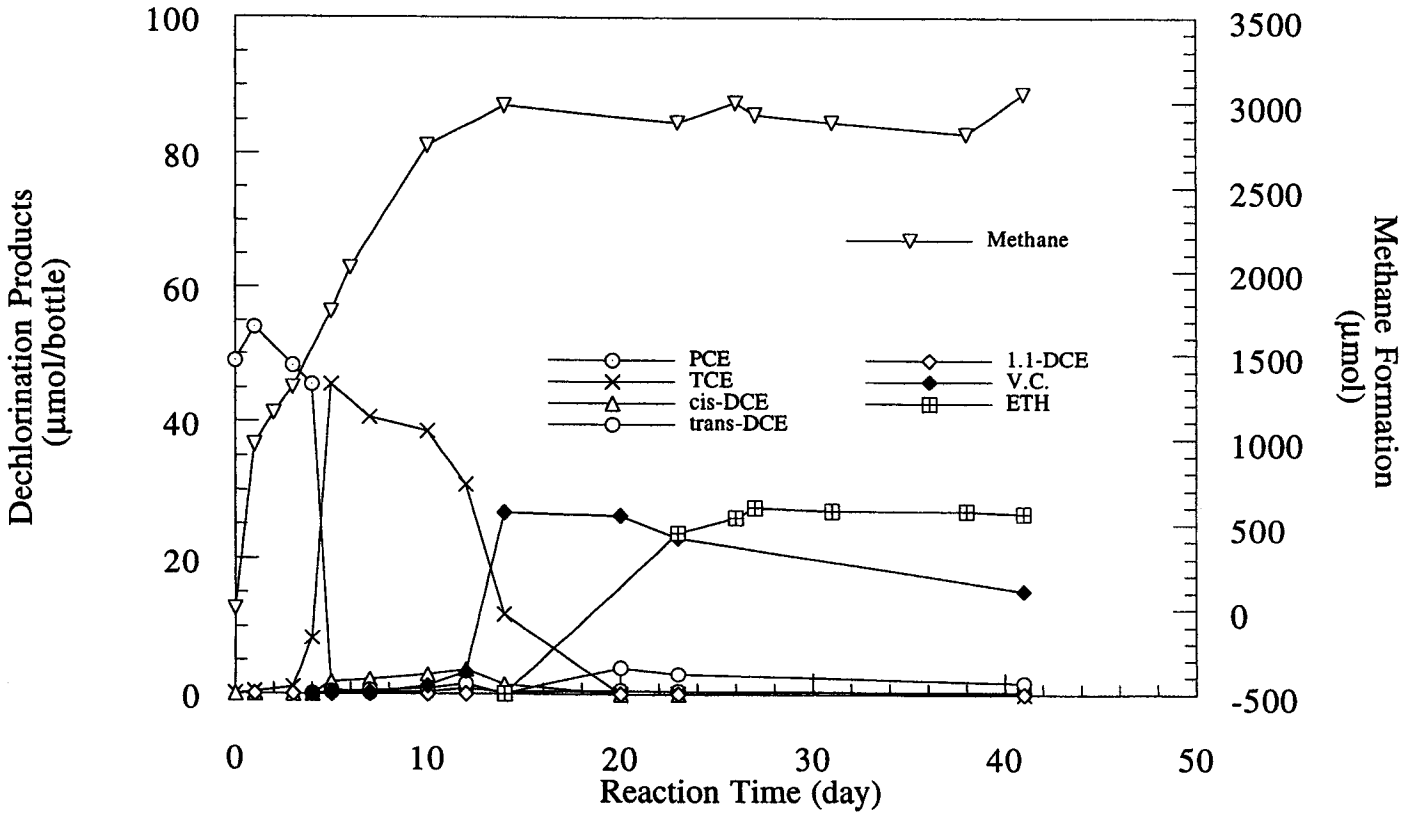
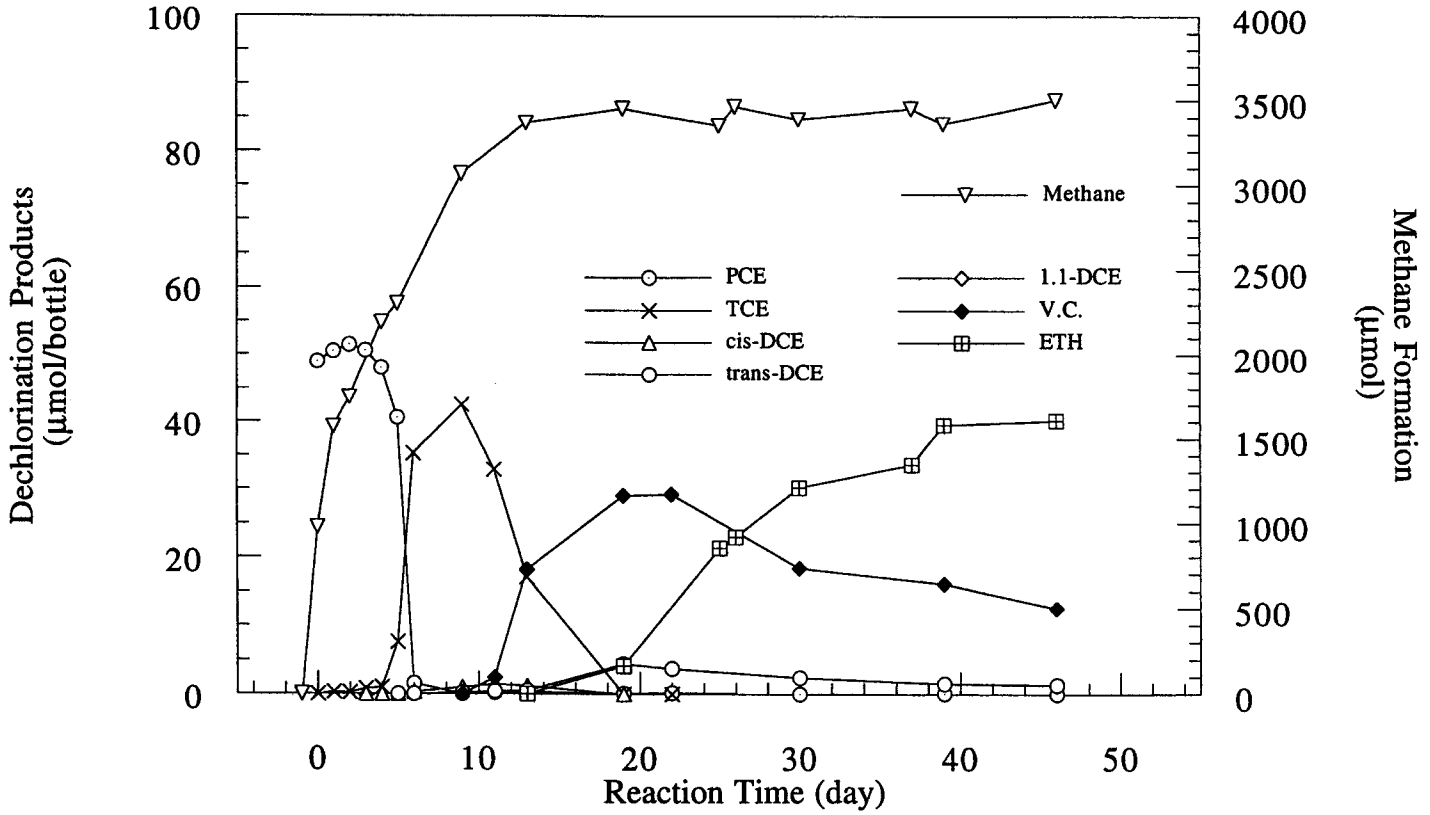


Fig. A-26 Dechlorination of PCE and formation of intermediates in a batch culture of one-day old* anaerobic Hyperion sludge.



Note: One-day old sludge means that the sludge culture was incubated for one day before PCE injection.

Fig. A-27 Dechlorination of PCE and formation of intermediates in a batch culture of two-day old anaerobic Hyperion sludge

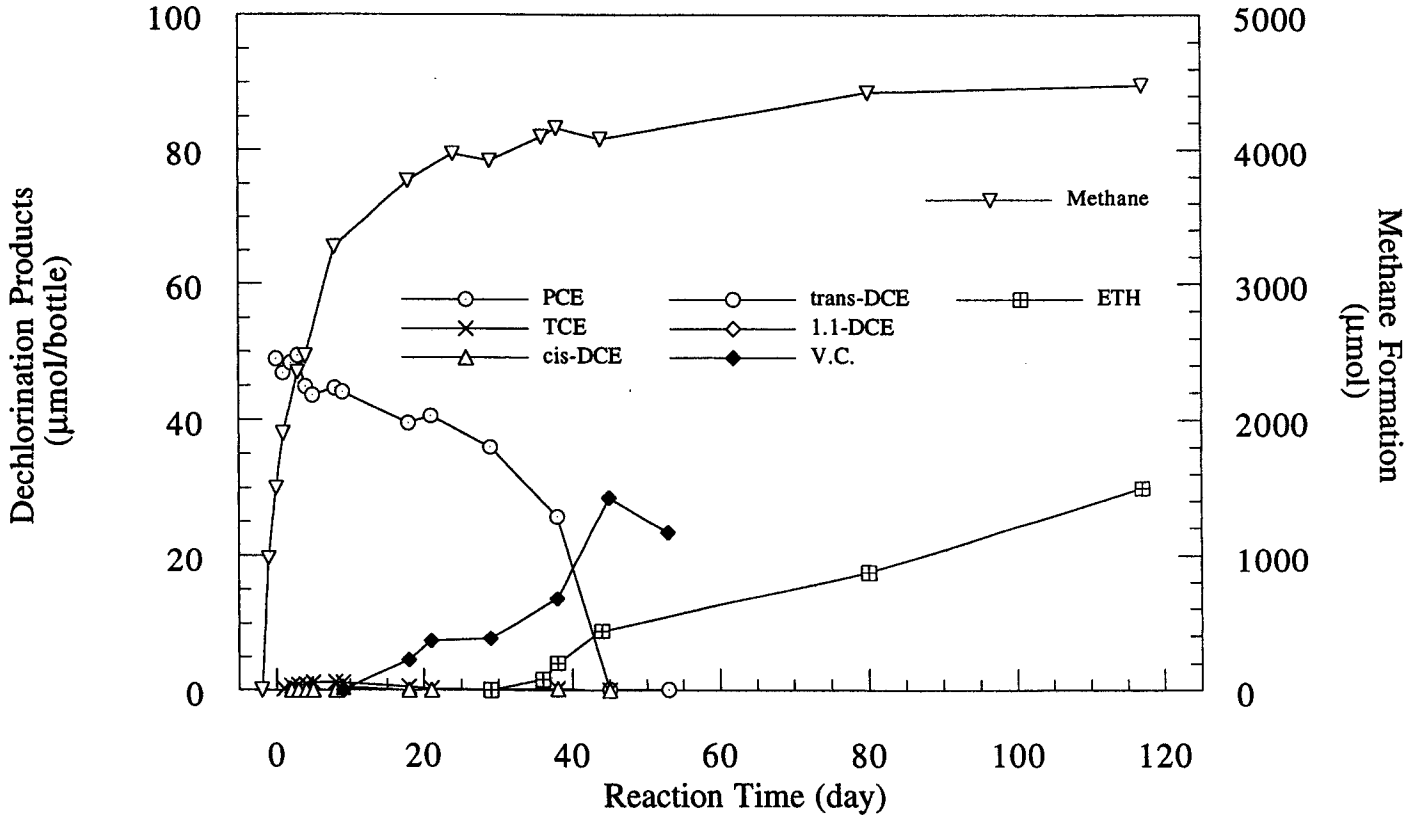


Fig. A-28 Dechlorination of PCE and formation of intermediates in a batch culture of three-day old anaerobic Hyperion sludge.

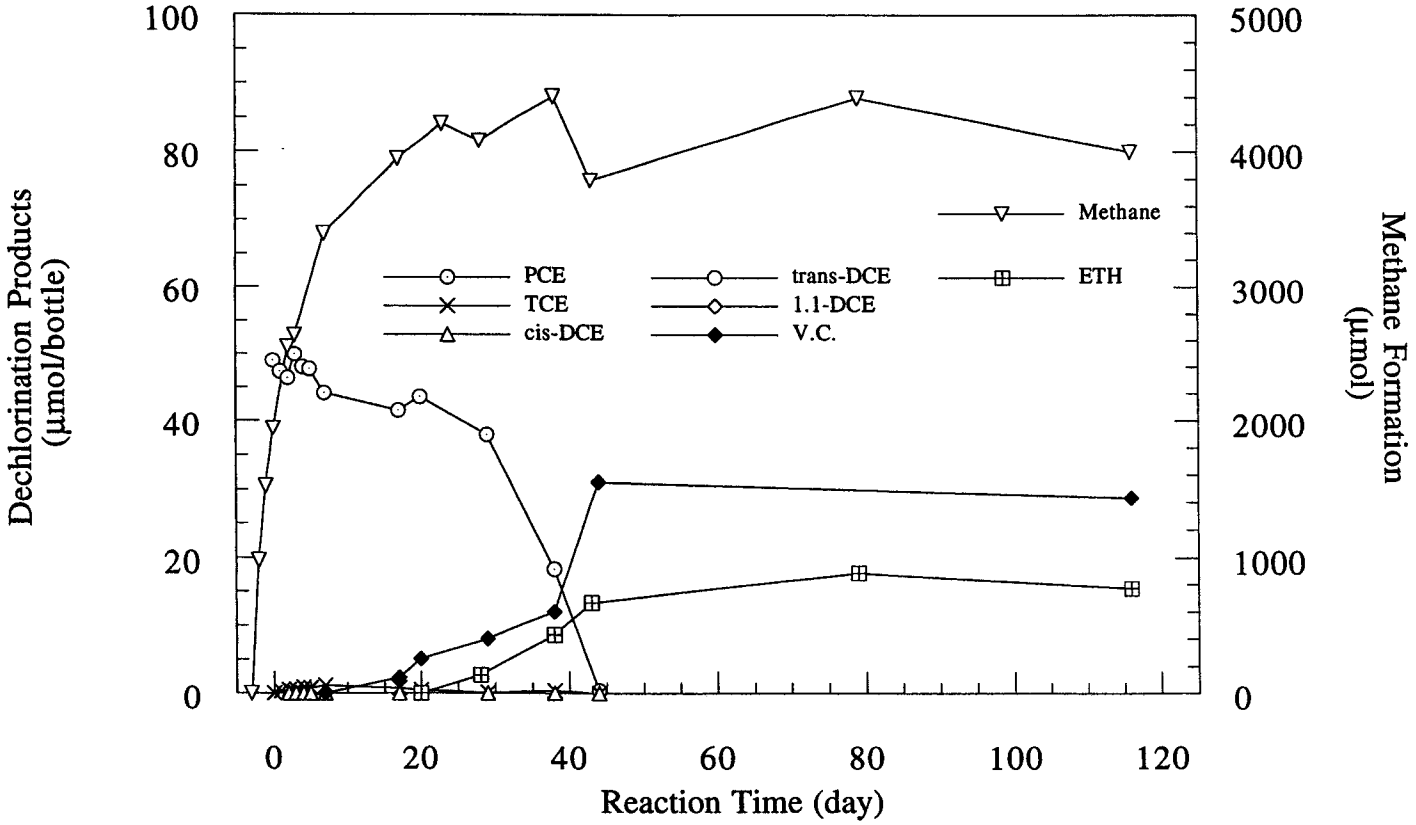


Fig. A-29 Dechlorination of PCE and formation of intermediates in a batch culture of four-day old anaerobic Hyperion sludge.

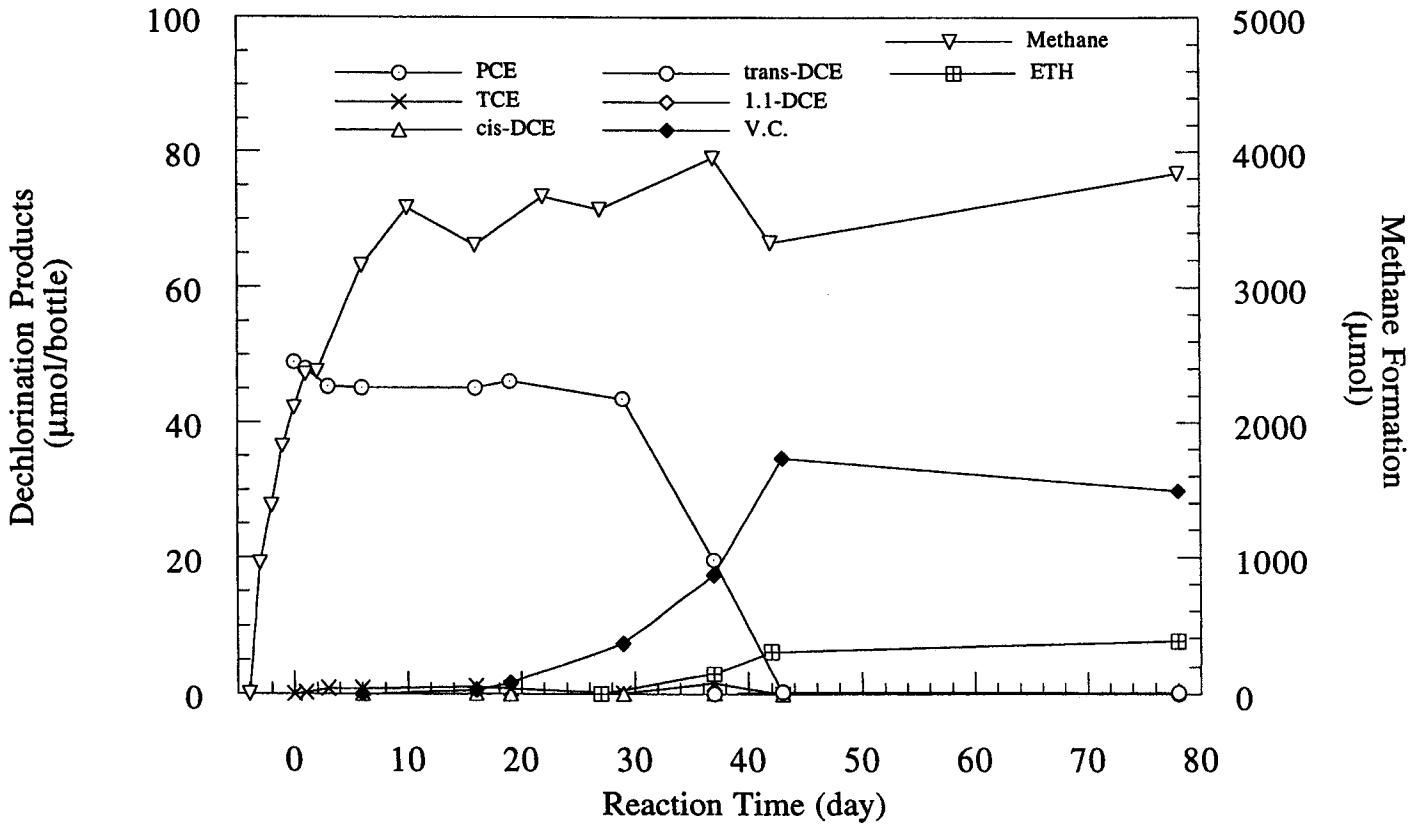


Fig. A-30 Dechlorination of PCE and formation of intermediates in a batch culture of 12-day old anaerobic Hyperion sludge.

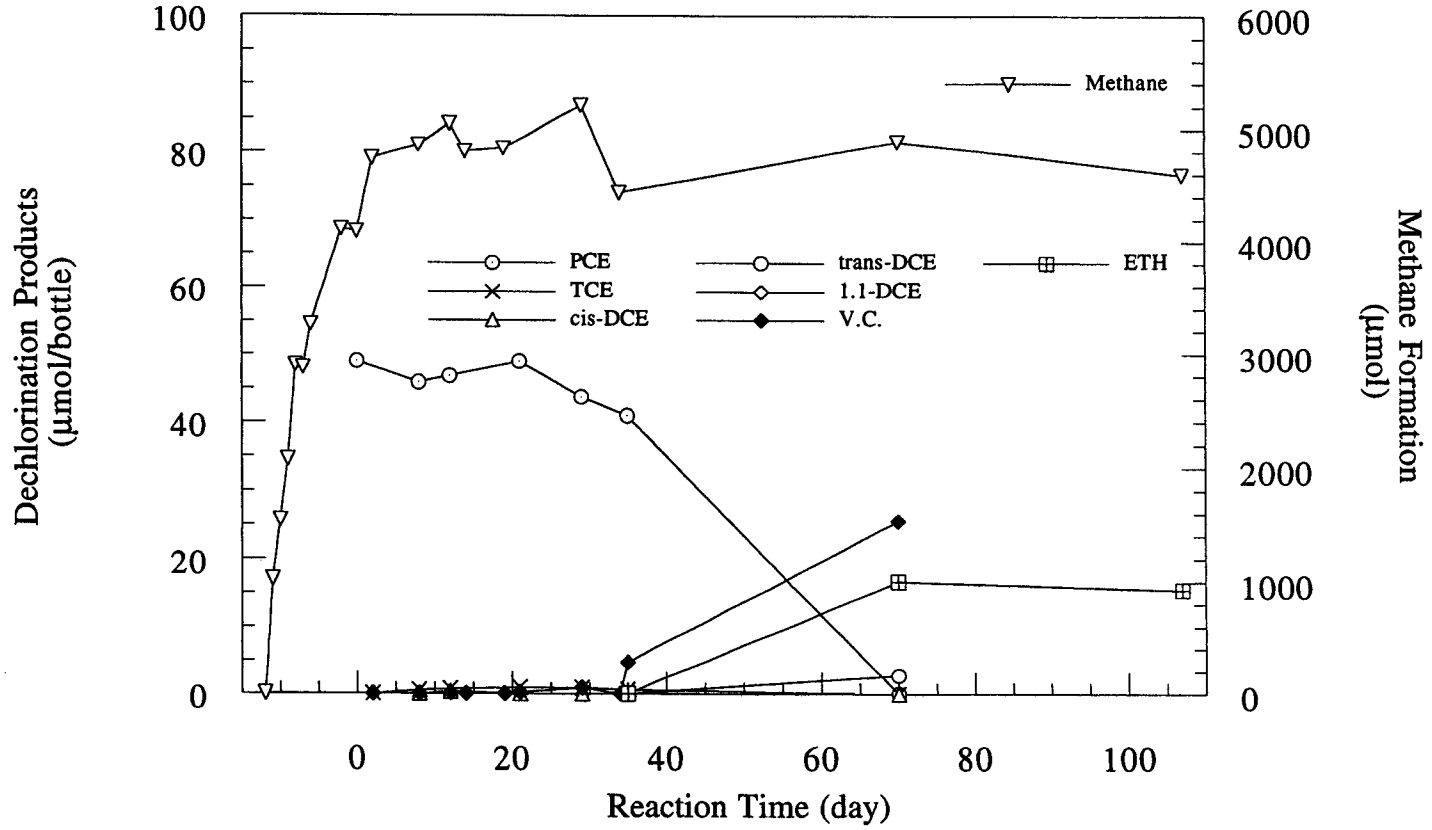


Fig. A-31 Dechlorination of PCE and formation of intermediates in a batch culture of 14-day old anaerobic Hyperion sludge.

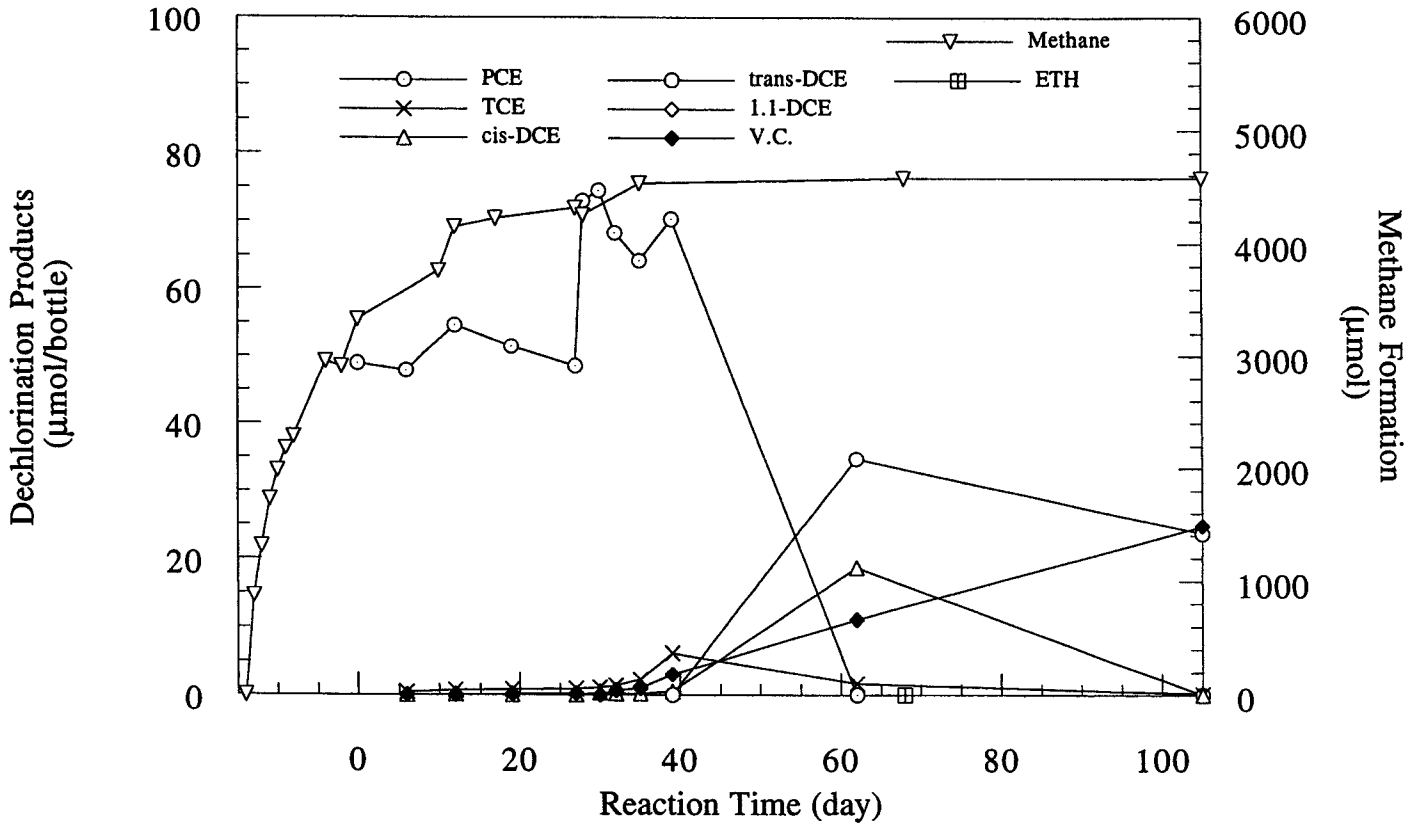


Fig. A-32 Dechlorination of PCE and formation of intermediates in a batch culture of 20-day old anaerobic Hyperion sludge.

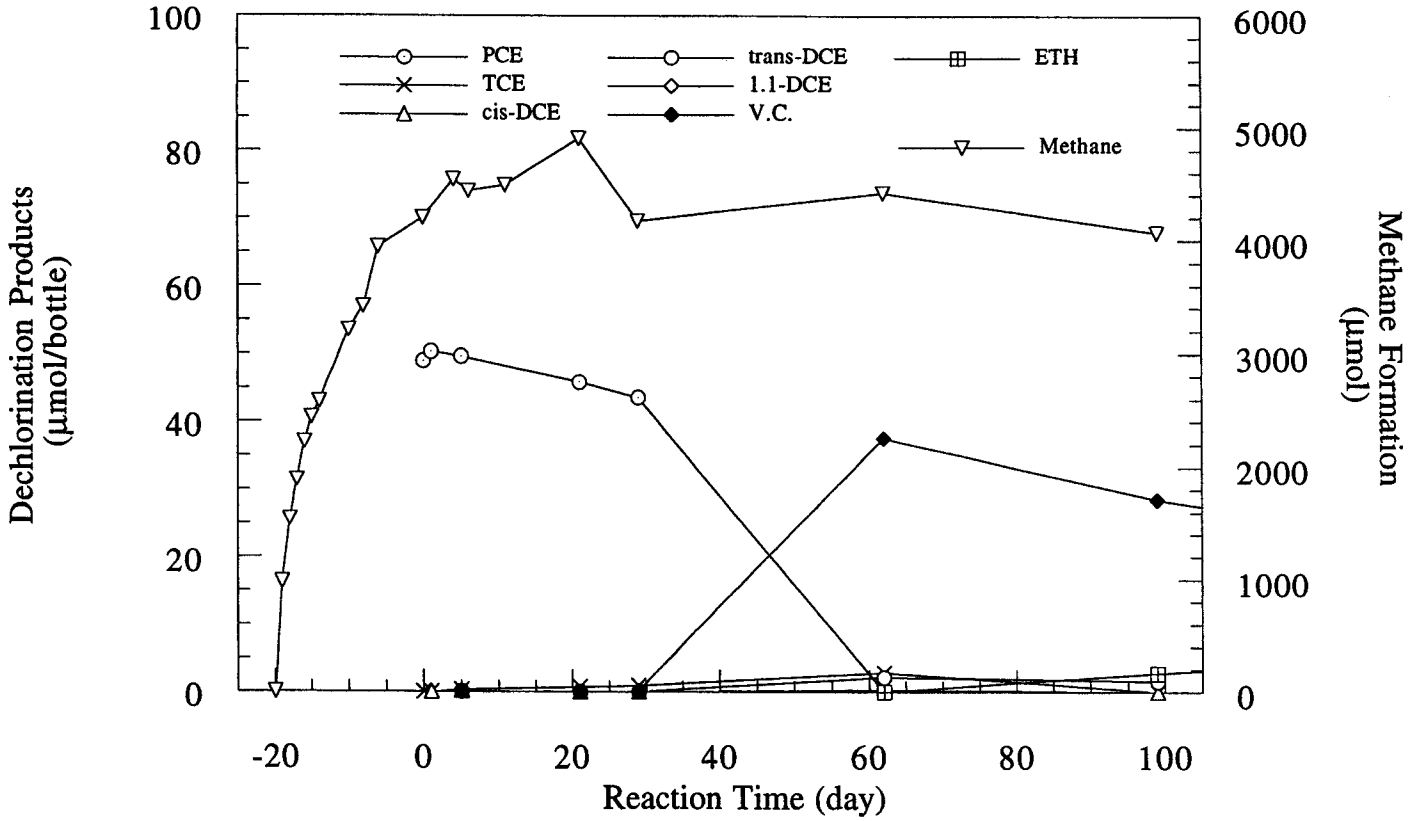


Fig. A-33 Dechlorination of PCE and formation of intermediates in a batch culture of 21-day old anaerobic Hyperion sludge.

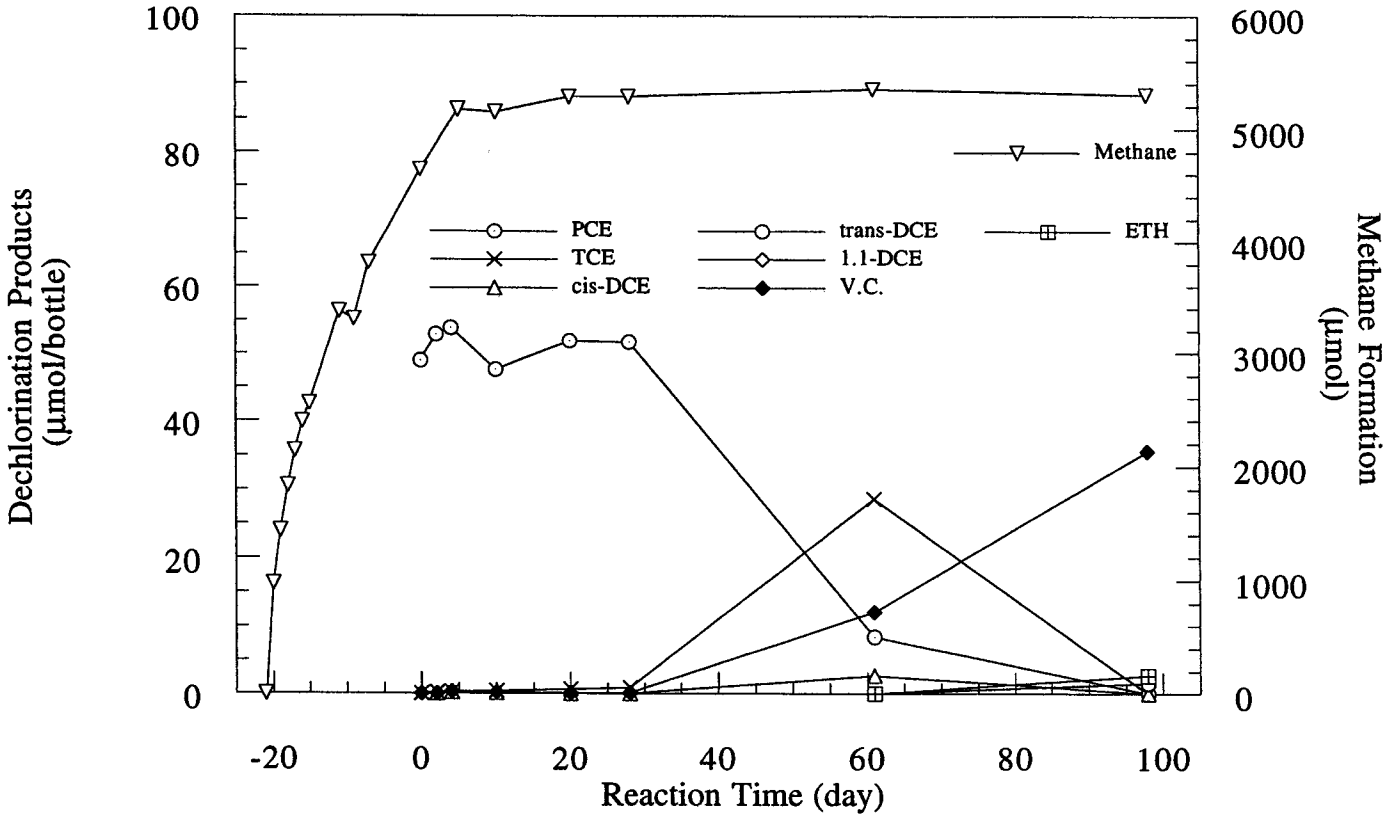
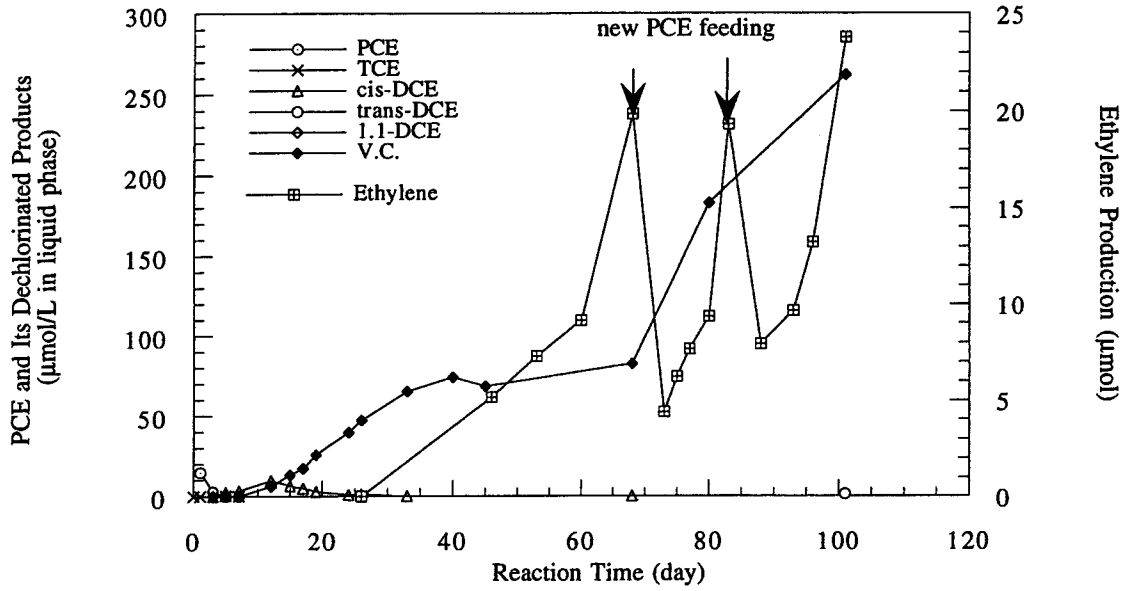


Fig. A-34 Initial PCE dechlorination in the sludge culture with GAC addition.



Initial PCE dechlorination in the sludge culture without GAC addition.

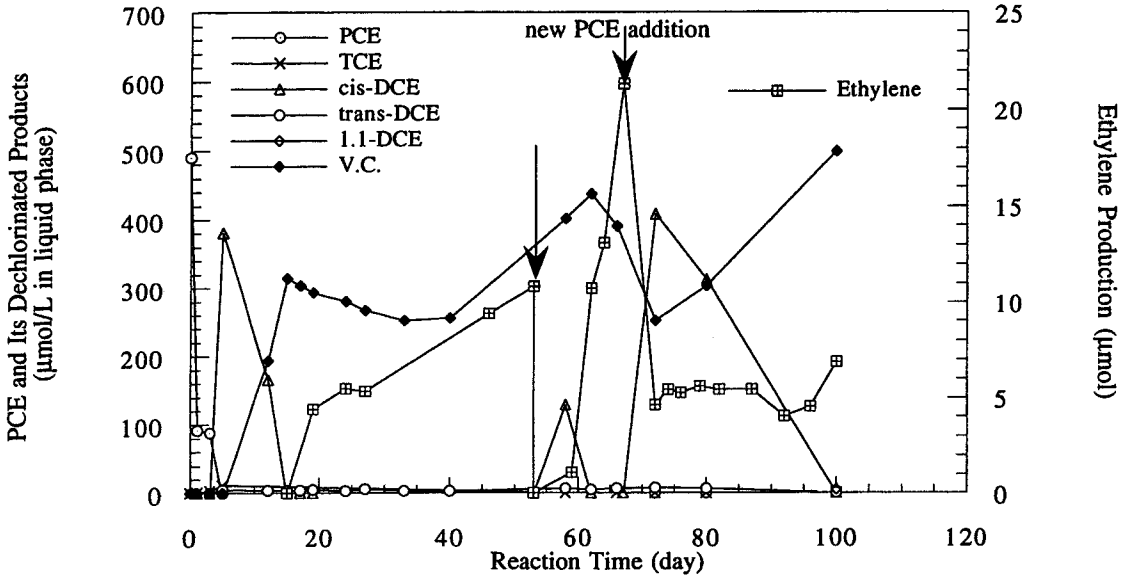
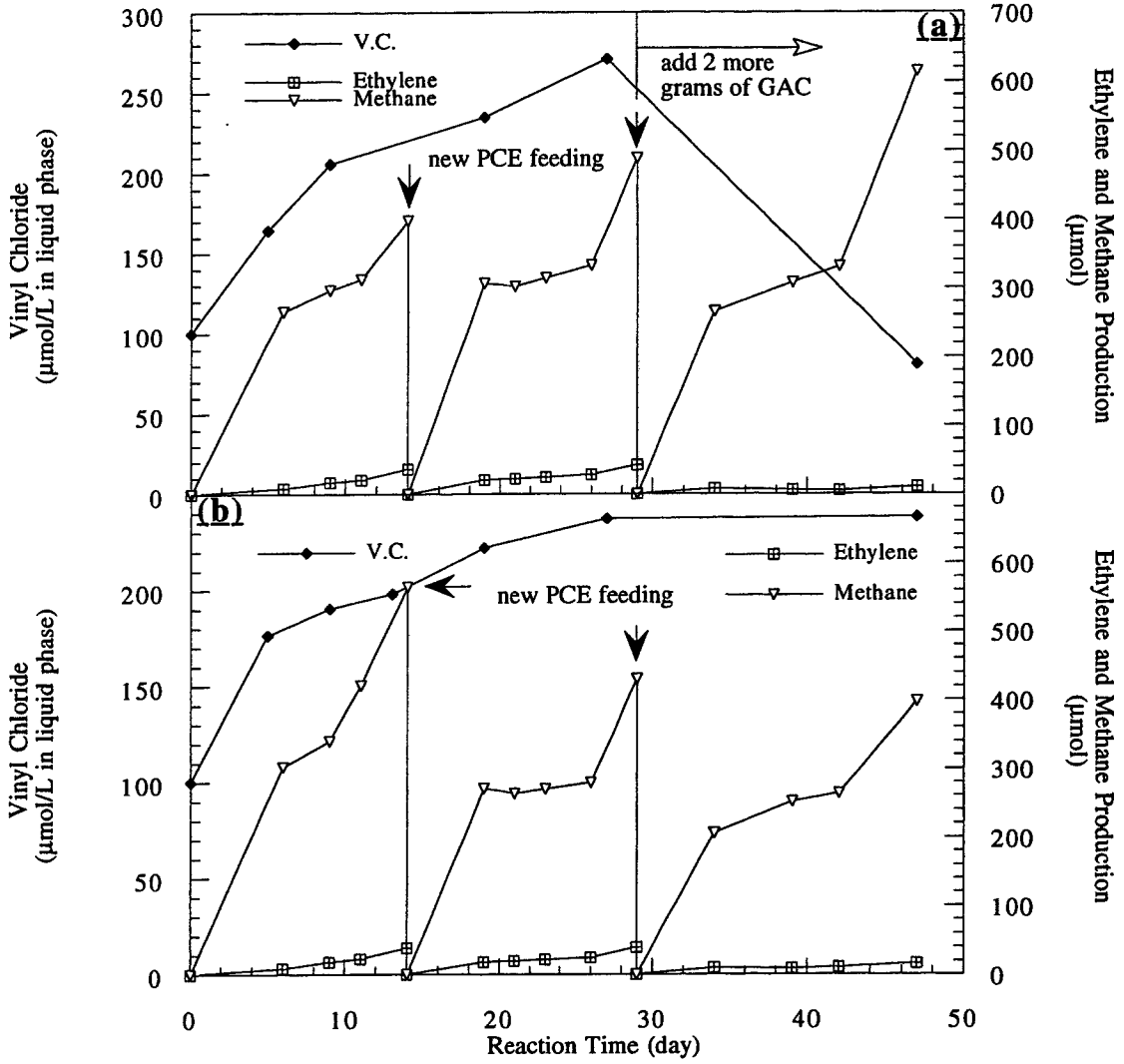


Fig. A-35 The effect of different amounts of carbon addition on PCEdechlorination and methane production.



Note: a). Culture with 2 grams of GAC initially, and add 2 more grams of GAC after 30 days.

b). Culture with 2 grams of GAC throughout the experiment.

Fig. A-36 The effect of activated carbon on PCE dechlorination.

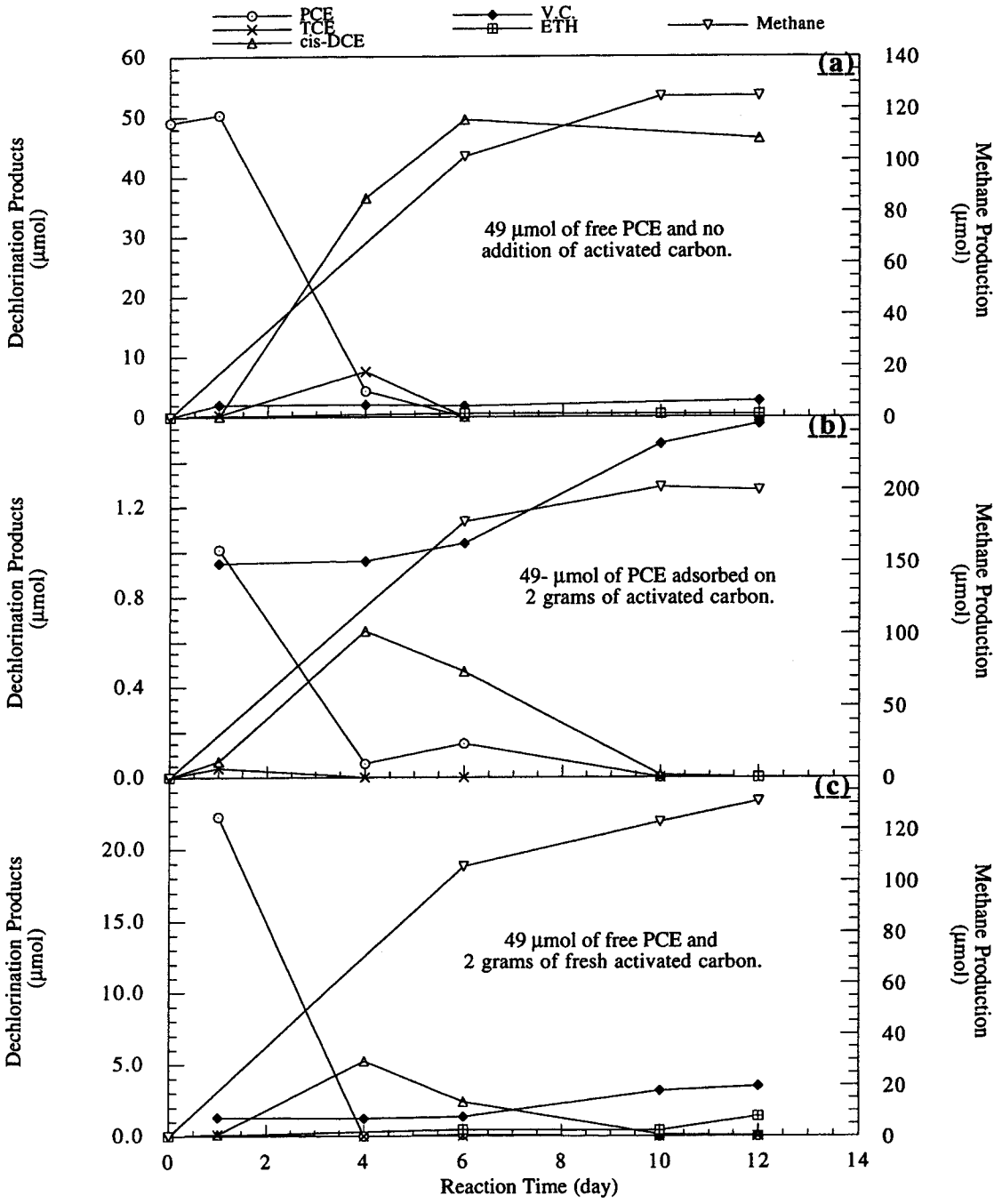


Fig. A-37 **The effect of different amounts of carbon addition on PCE dechlorination.**

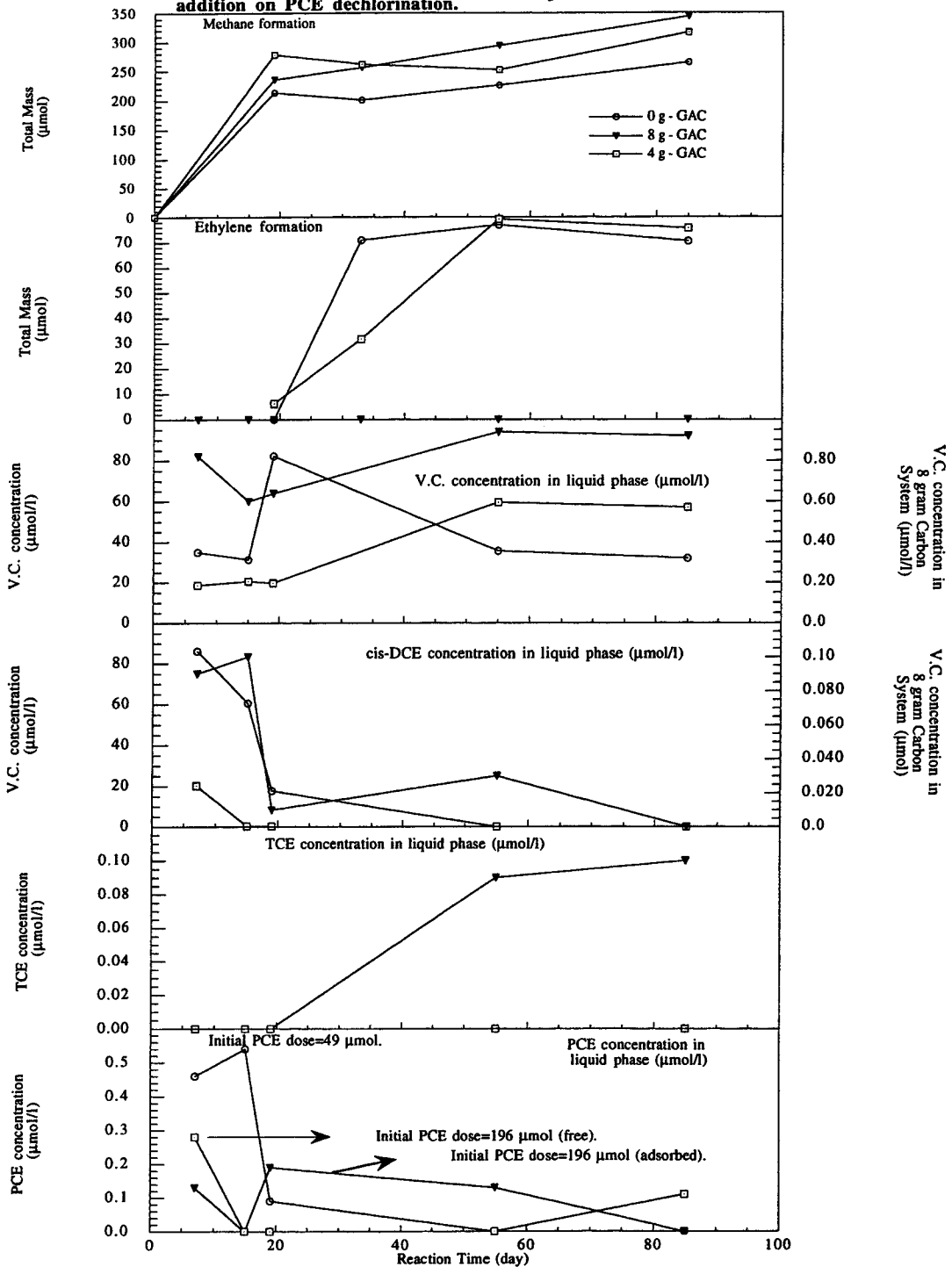


Fig. A-38 PCE dechlorination in a biological activated carbon system with acclimated anaerobic digested sludge. (Initial free PCE dose = 49μmol)

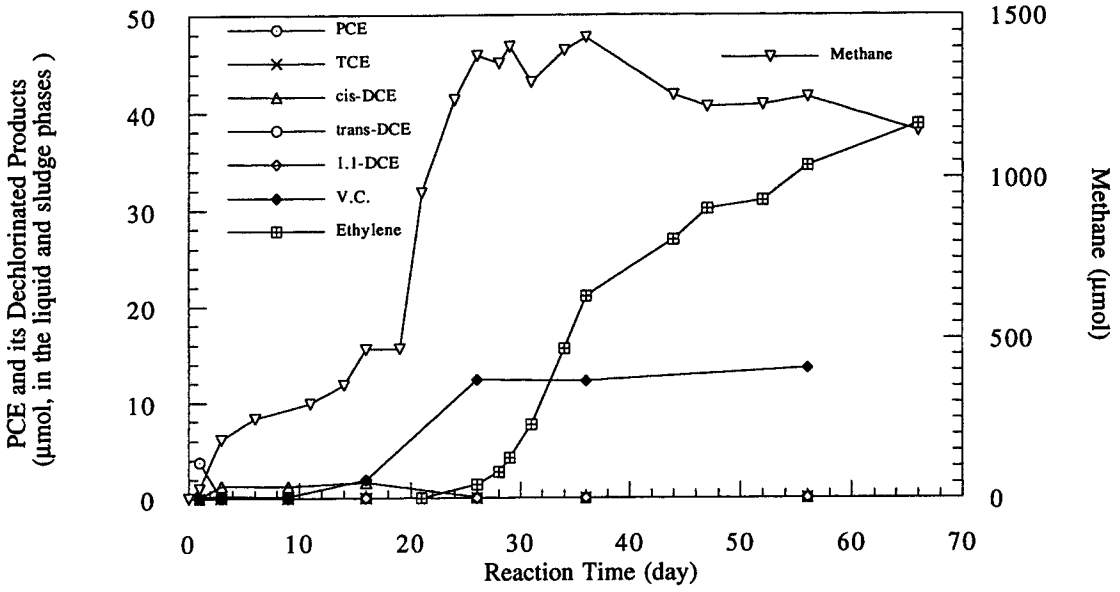


Fig. A-44 Biological regeneration of PCE laden carbon. (Adsorbed PCE = 49μmol)

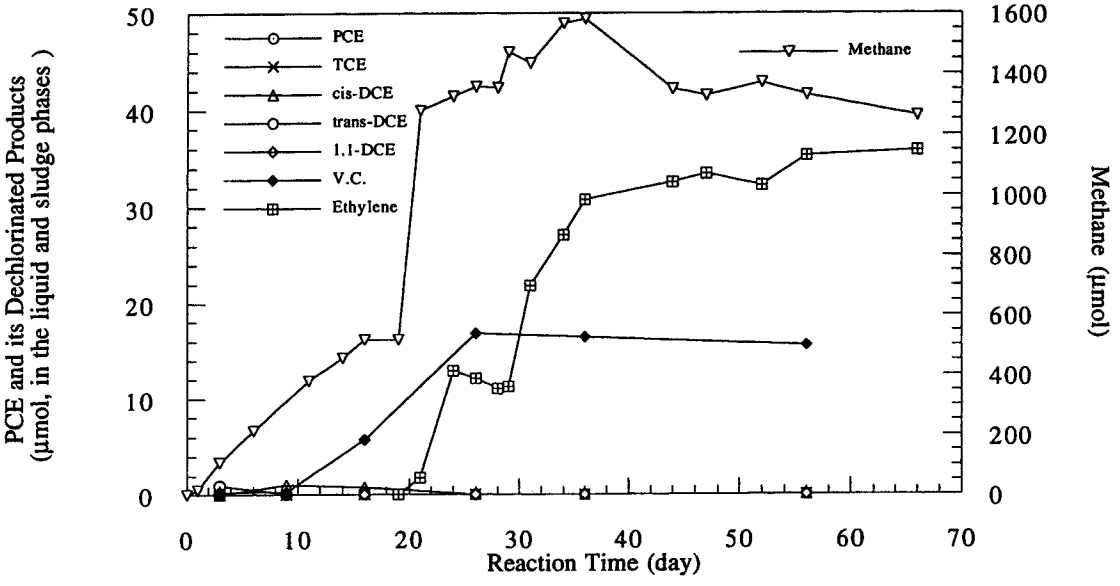


Fig. A-39 PCE dechlorination in a biological activated carbon system with acclimated anaerobic digested sludge. (Initial free PCE dose = 98μmol)

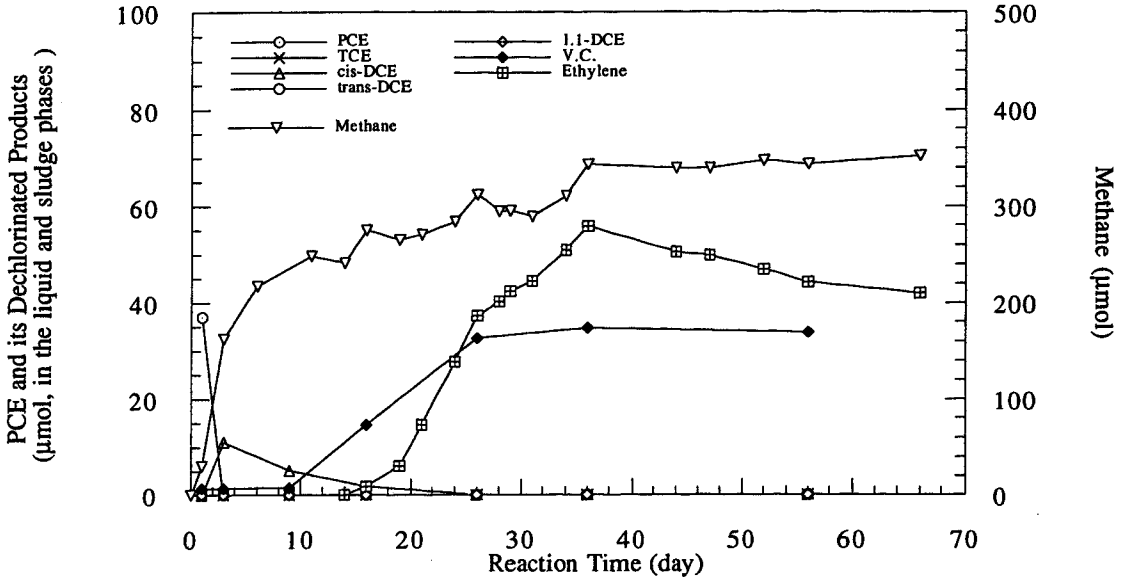


Fig. A-45 Biological regeneration of PCE laden carbon. (Adsorbed PCE = 98μmol)

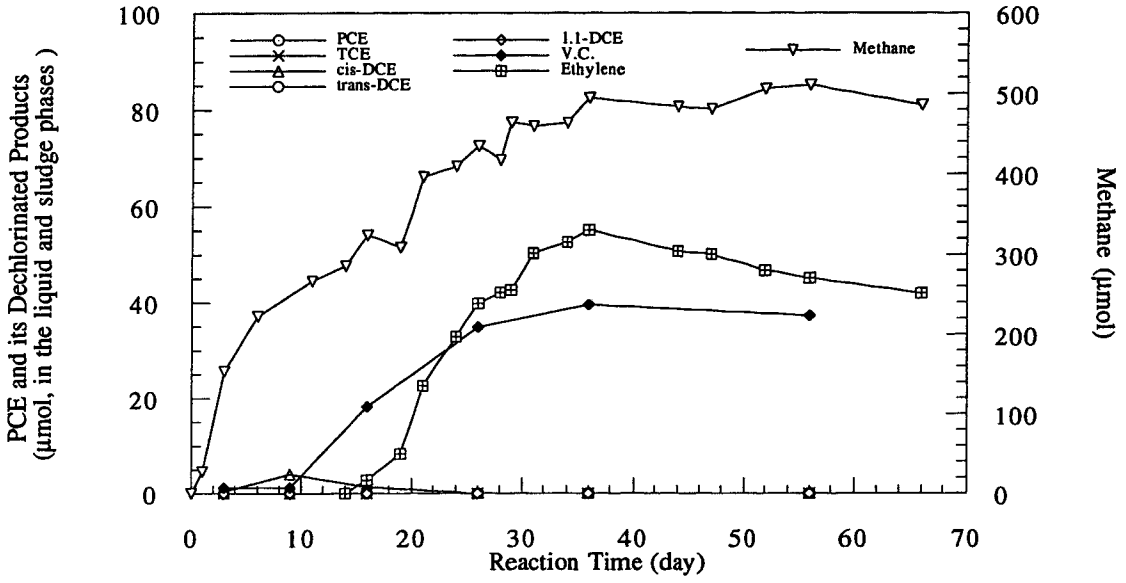


Fig. A-40 PCE dechlorination in a biological activated carbon system with acclimated anaerobic digested sludge (Initial free PCE dose = 196μmol)

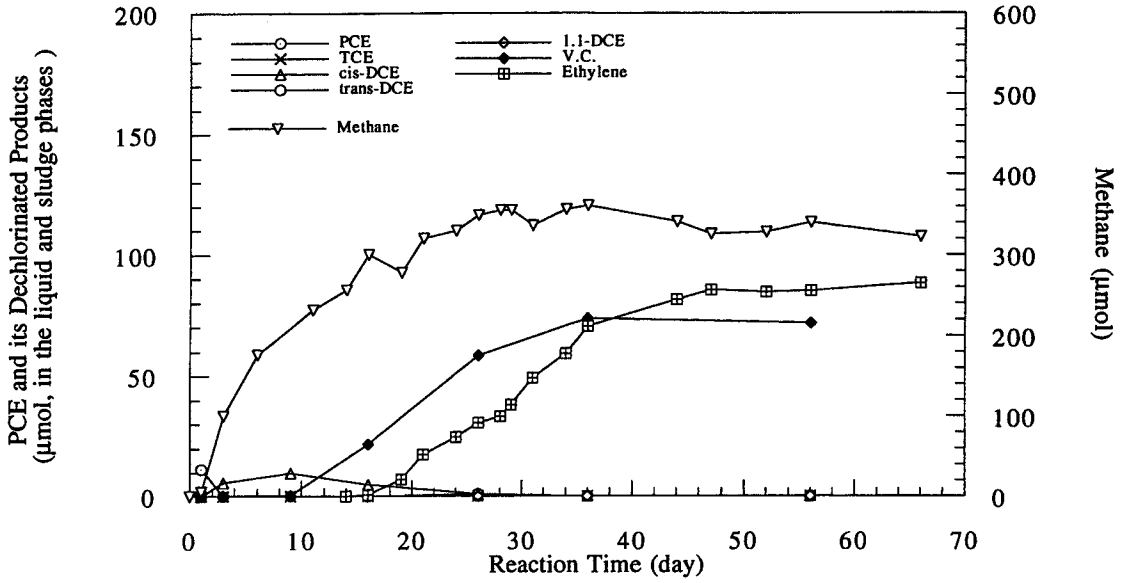


Fig. A-46 Biological regeneration of PCE laden carbon. (Adsorbed PCE = 196μmol)

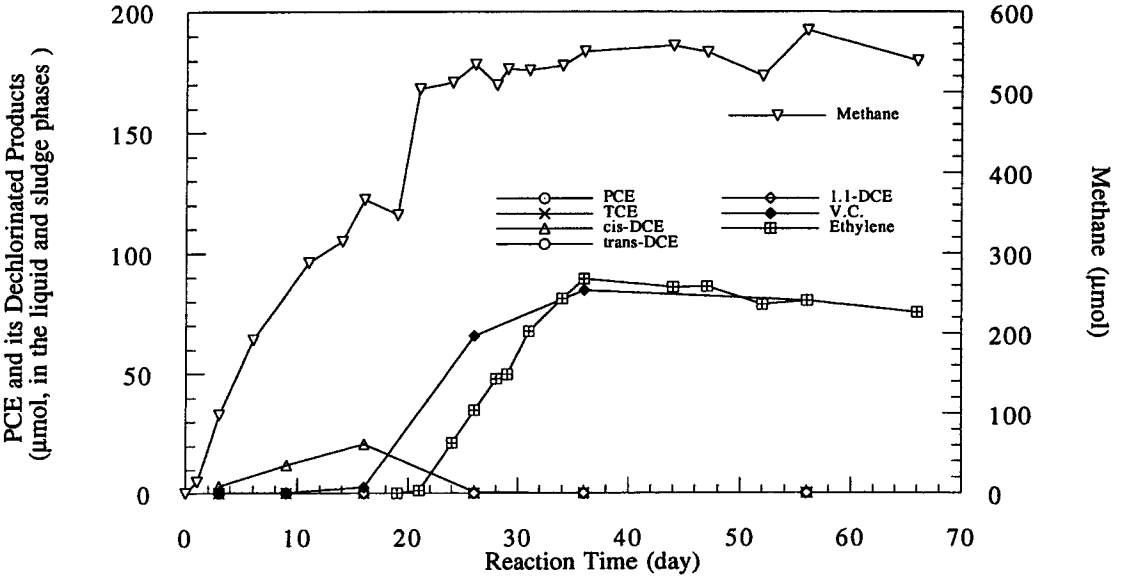


Fig. A-41 PCE dechlorination in a biological activated carbon system with acclimated anaerobic digested sludge. (Initial free PCE dose = 294μmol)

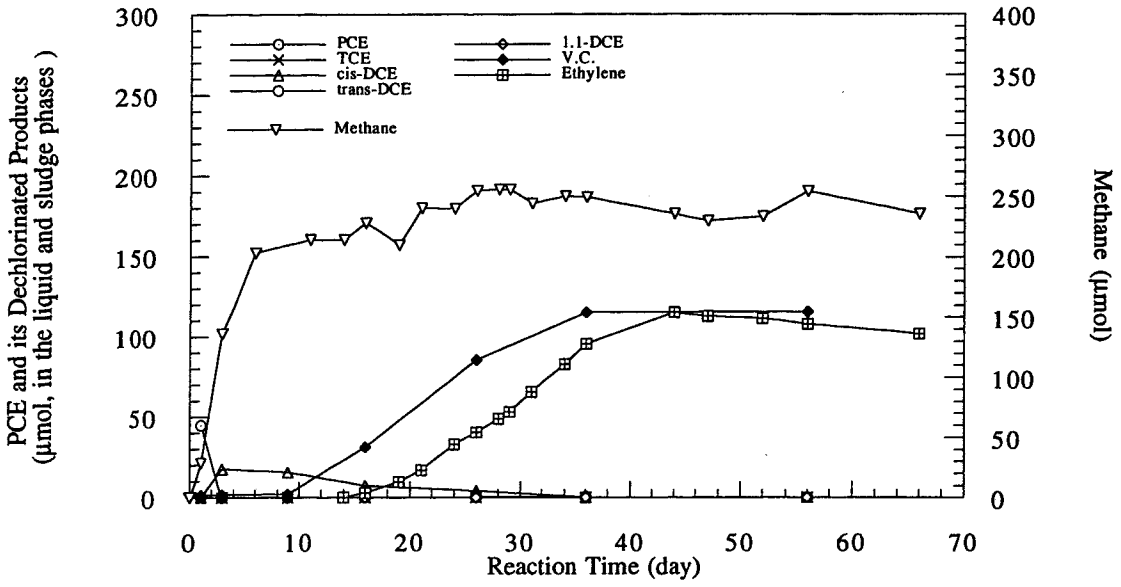


Fig. A-47 Biological regeneration of PCE laden carbon. (Adsorbed PCE = 294μmol)

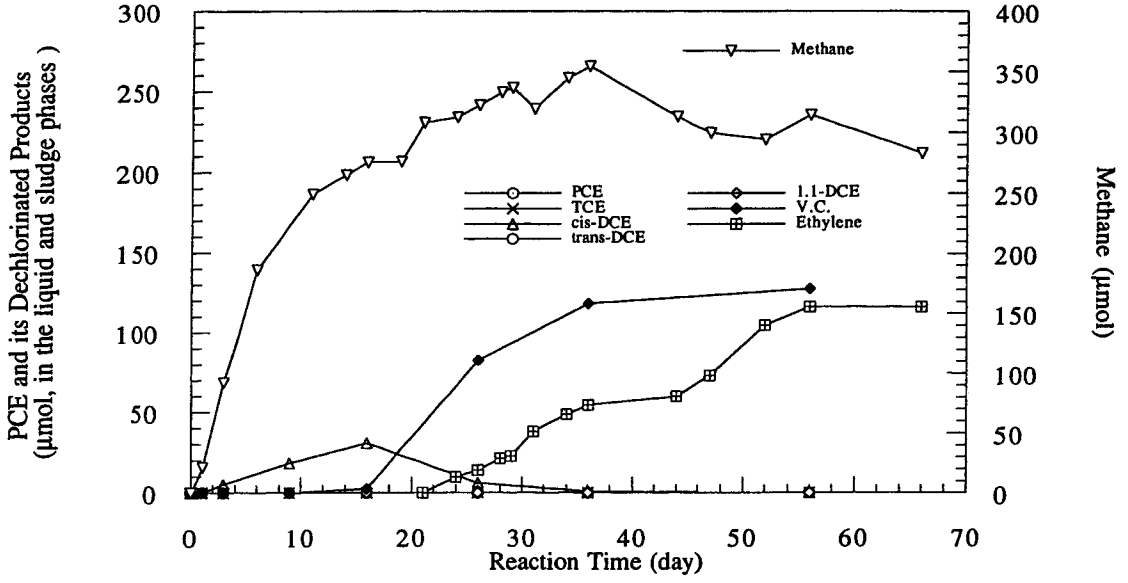


Fig. A-42 **PCE dechlorination in a biological activated carbon system with acclimated anaerobic digested sludge. (Initial free PCE dose = 392μmol)**

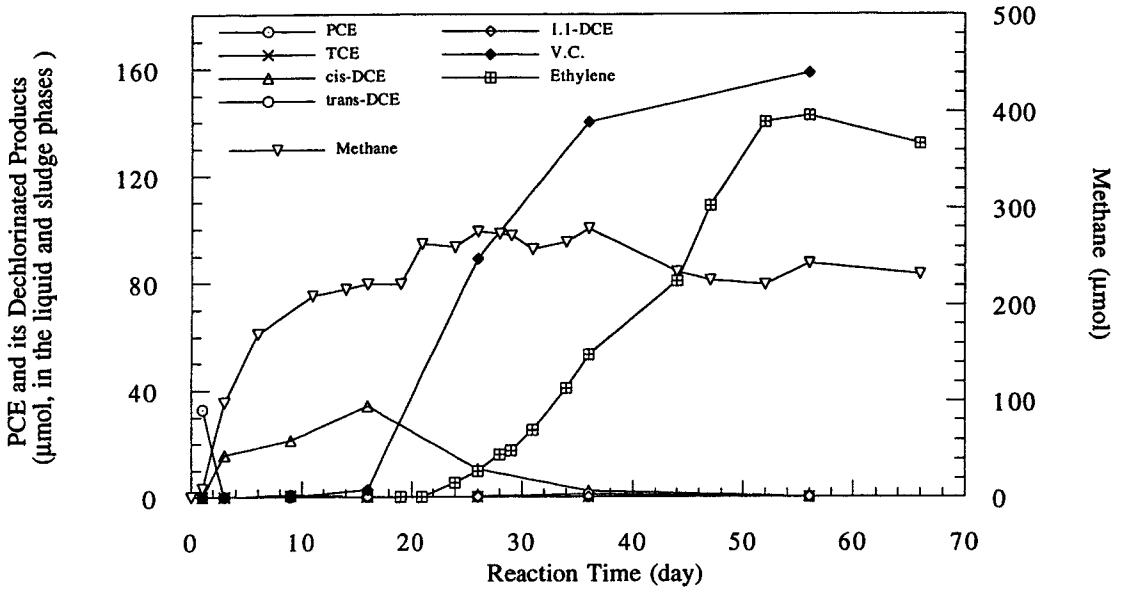


Fig. A-48 **Biological regeneration of PCE laden carbon. (Adsorbed PCE = 392μmol)**

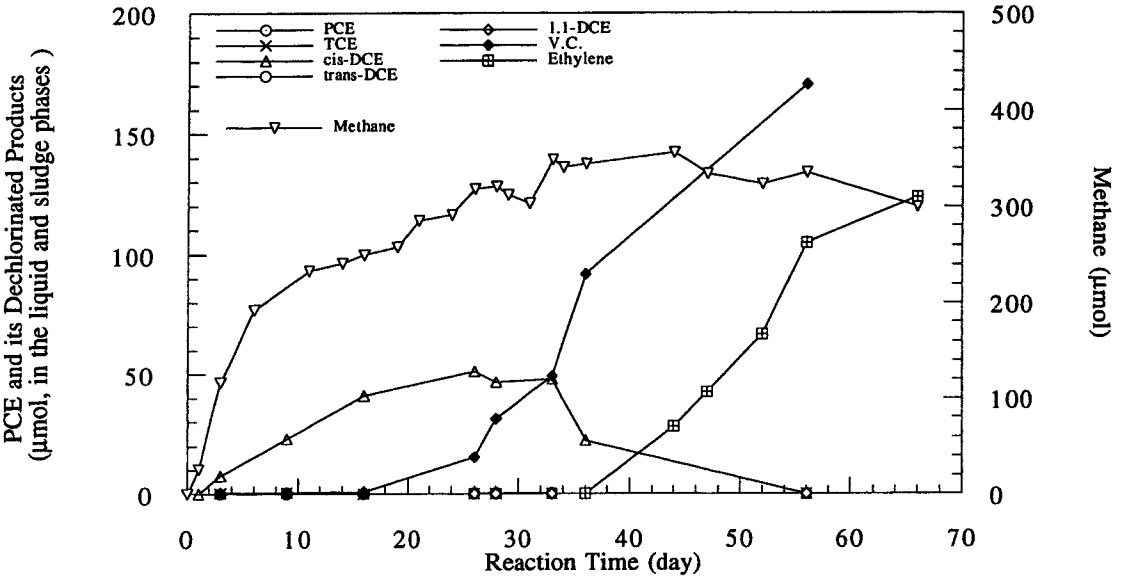


Fig. A-43 PCE dechlorination in a biological activated carbon system with acclimated anaerobic digested sludge. (Initial free PCE dose = 490μmol)

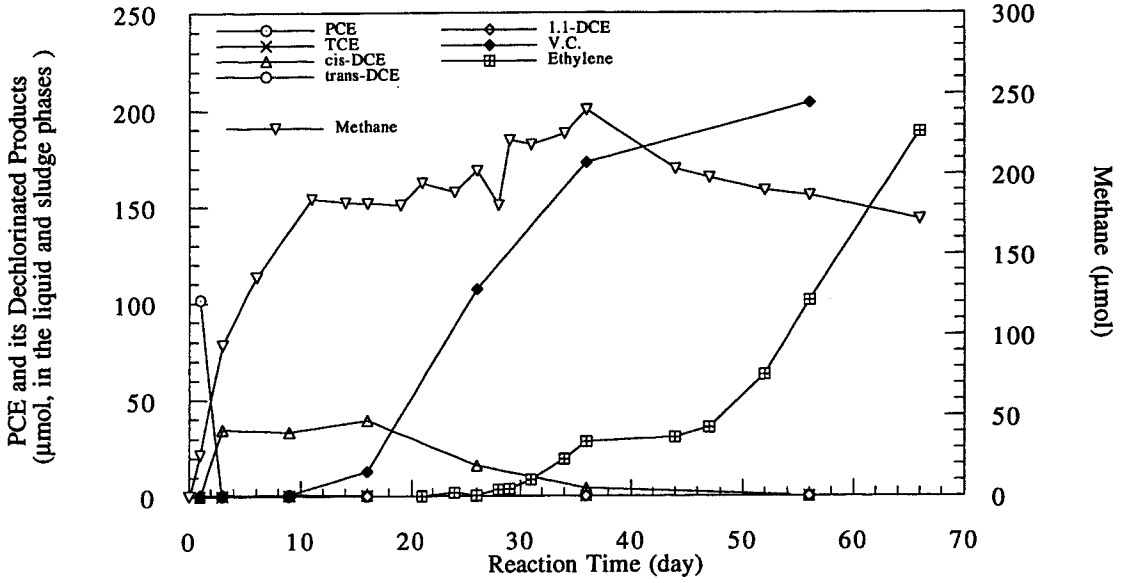


Fig. A-49 Biological regeneration of PCE laden carbon. (Adsorbed PCE = 490μmol)

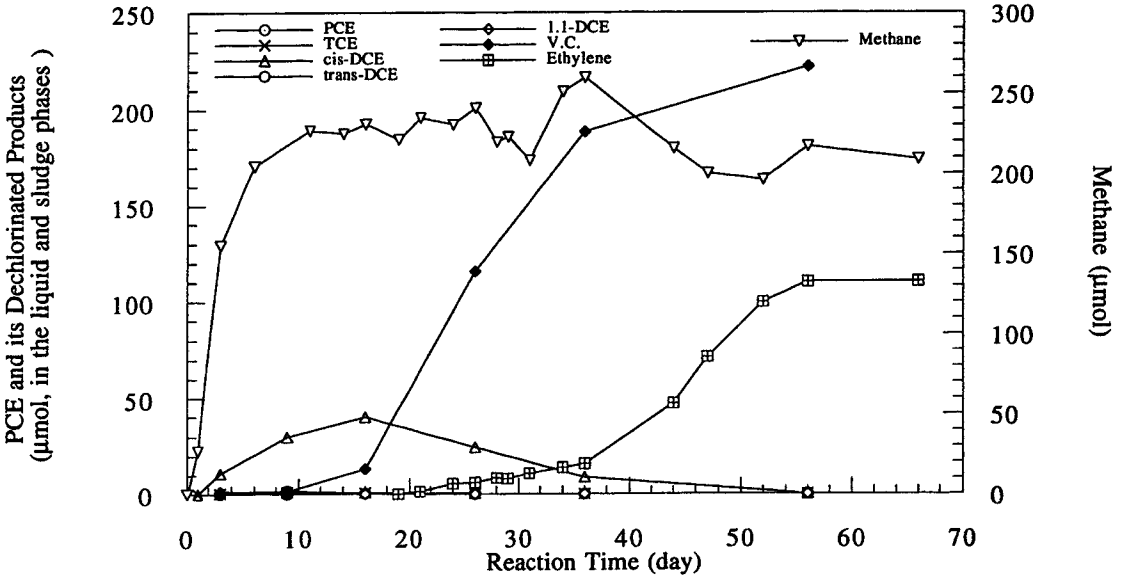


Fig. A-50 **A comparison of anaerobic PCE dechlorinating systems with adsorbed or free PCE doses. (PCE dose = 49 μmol)**

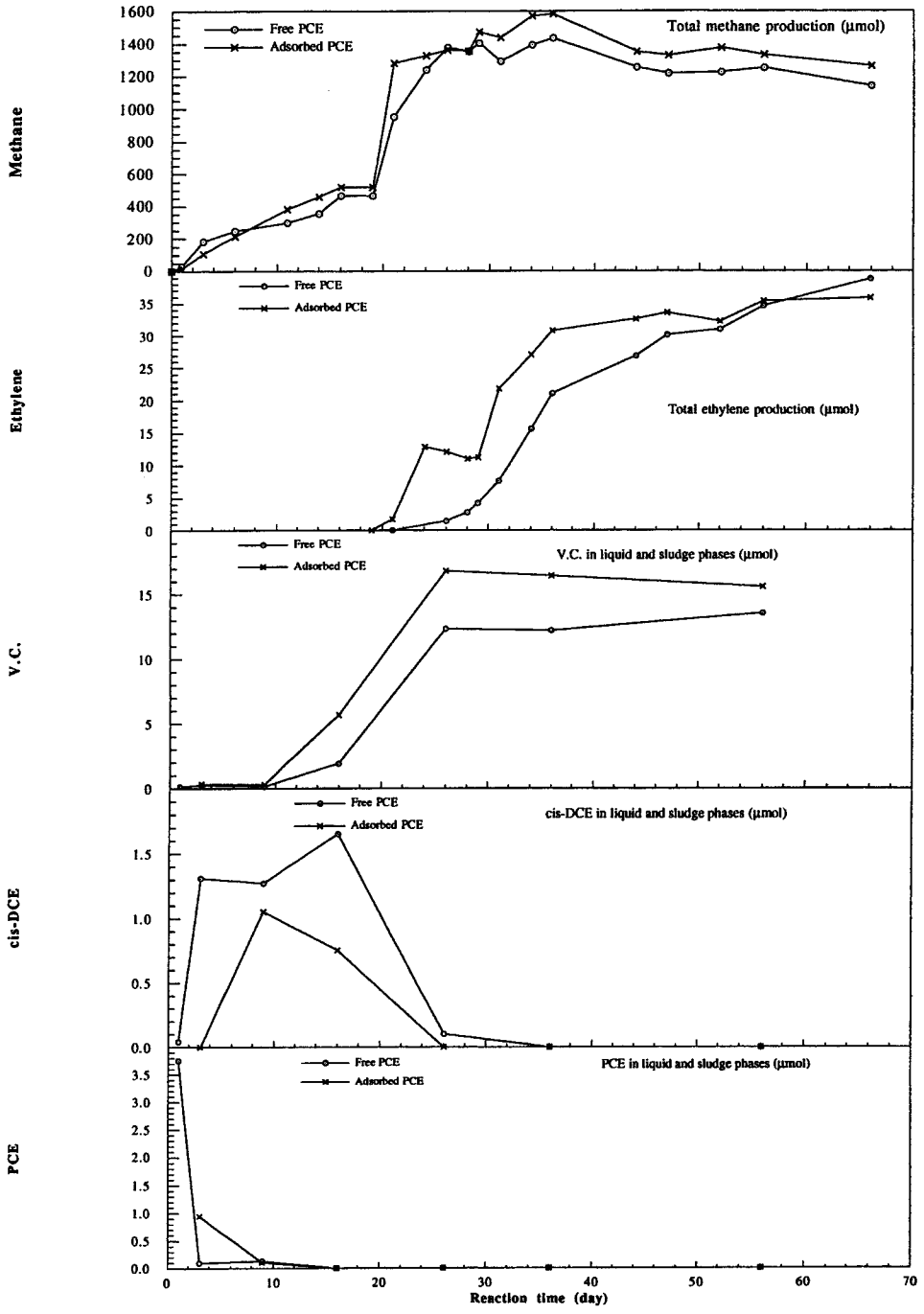


Fig. A-51 **A comparison of anaerobic PCE dechlorinating systems with adsorbed or free PCE doses. (PCE dose = 98 μmol)**

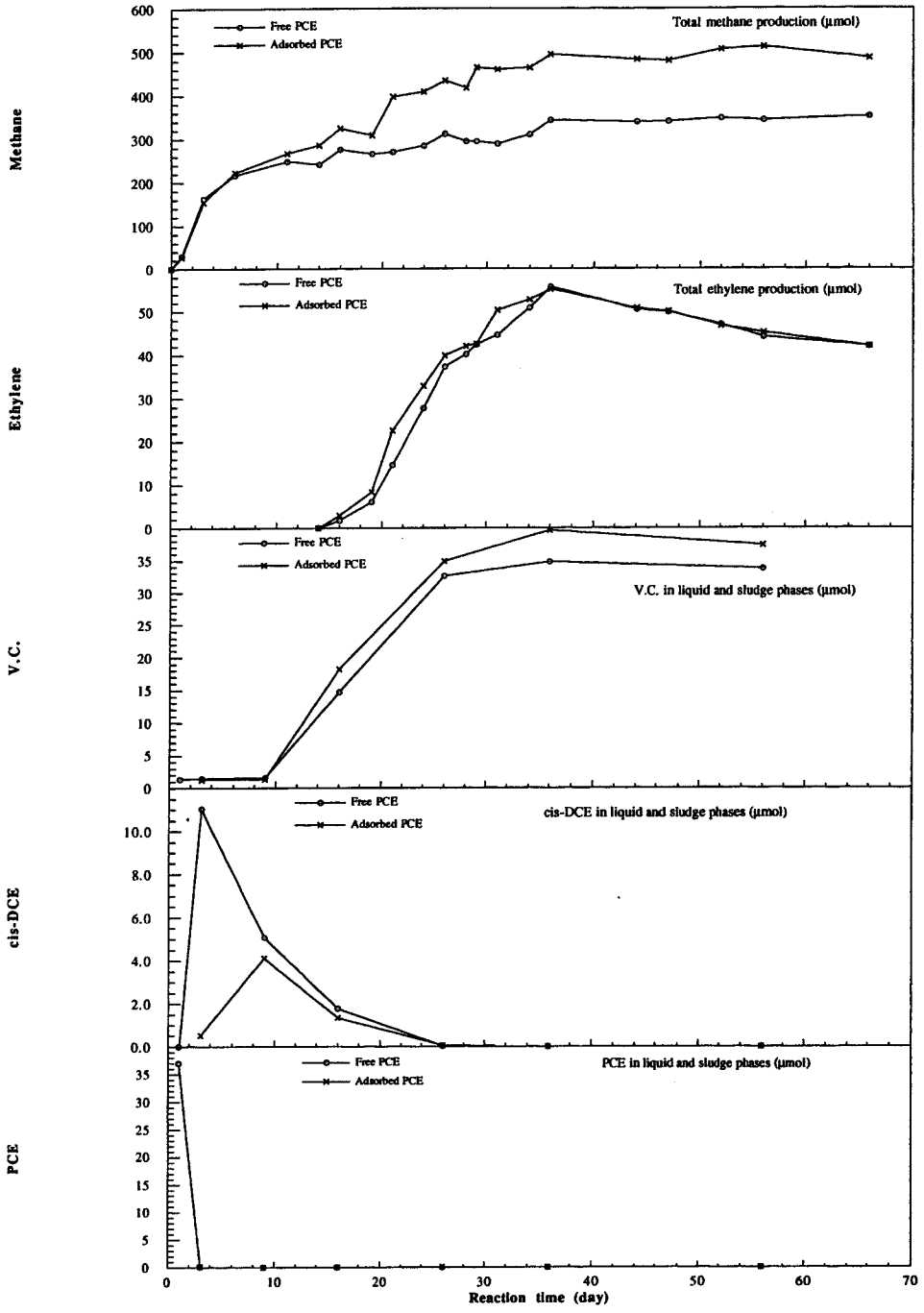


Fig. A-52 A comparison of anaerobic PCE dechlorinating systems with adsorbed or free PCE doses. (PCE dose = 196 μmol)

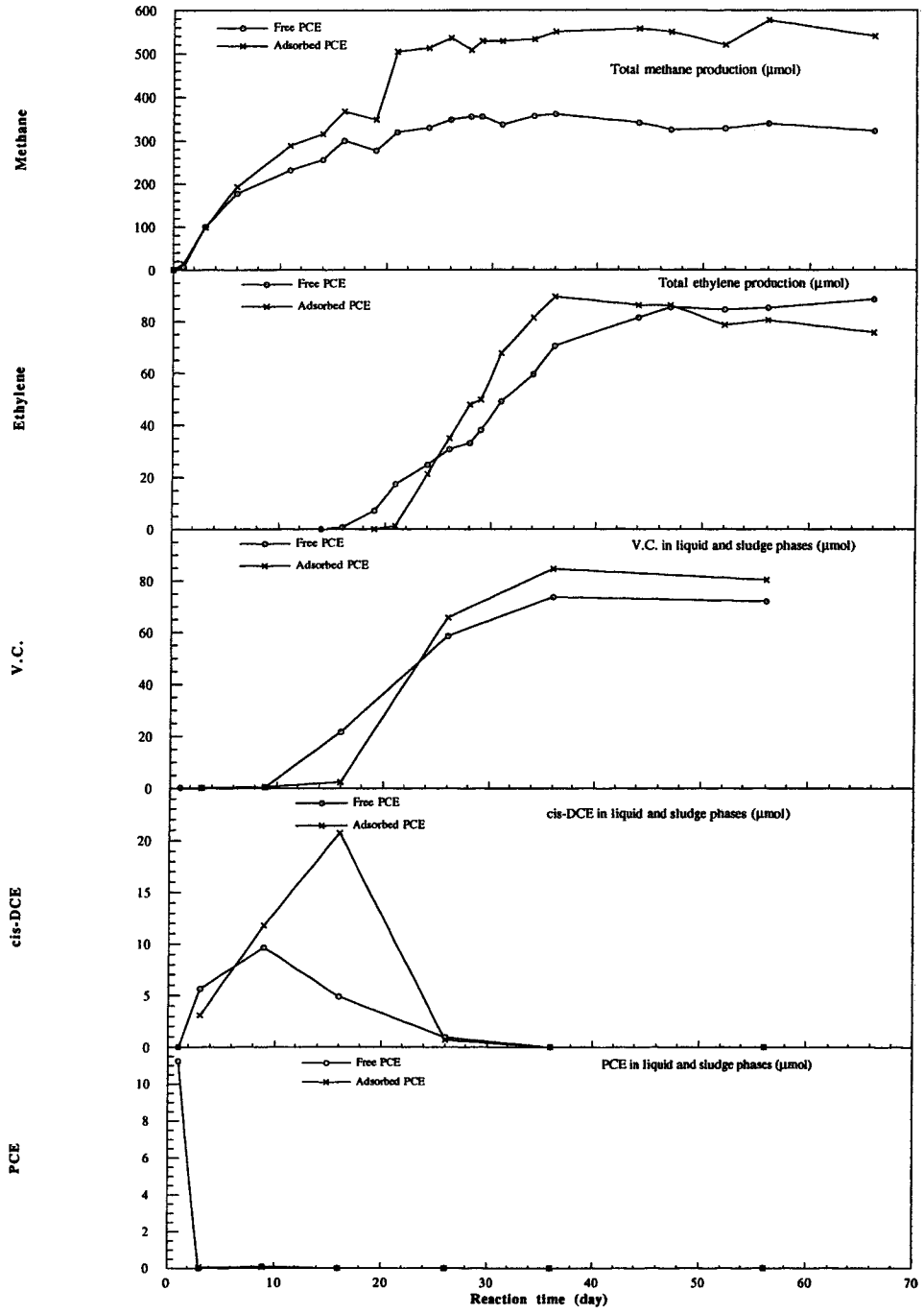


Fig. A-53 A comparison of anaerobic PCE dechlorinating systems with adsorbed or free PCE doses. (PCE dose = 294 μmol)

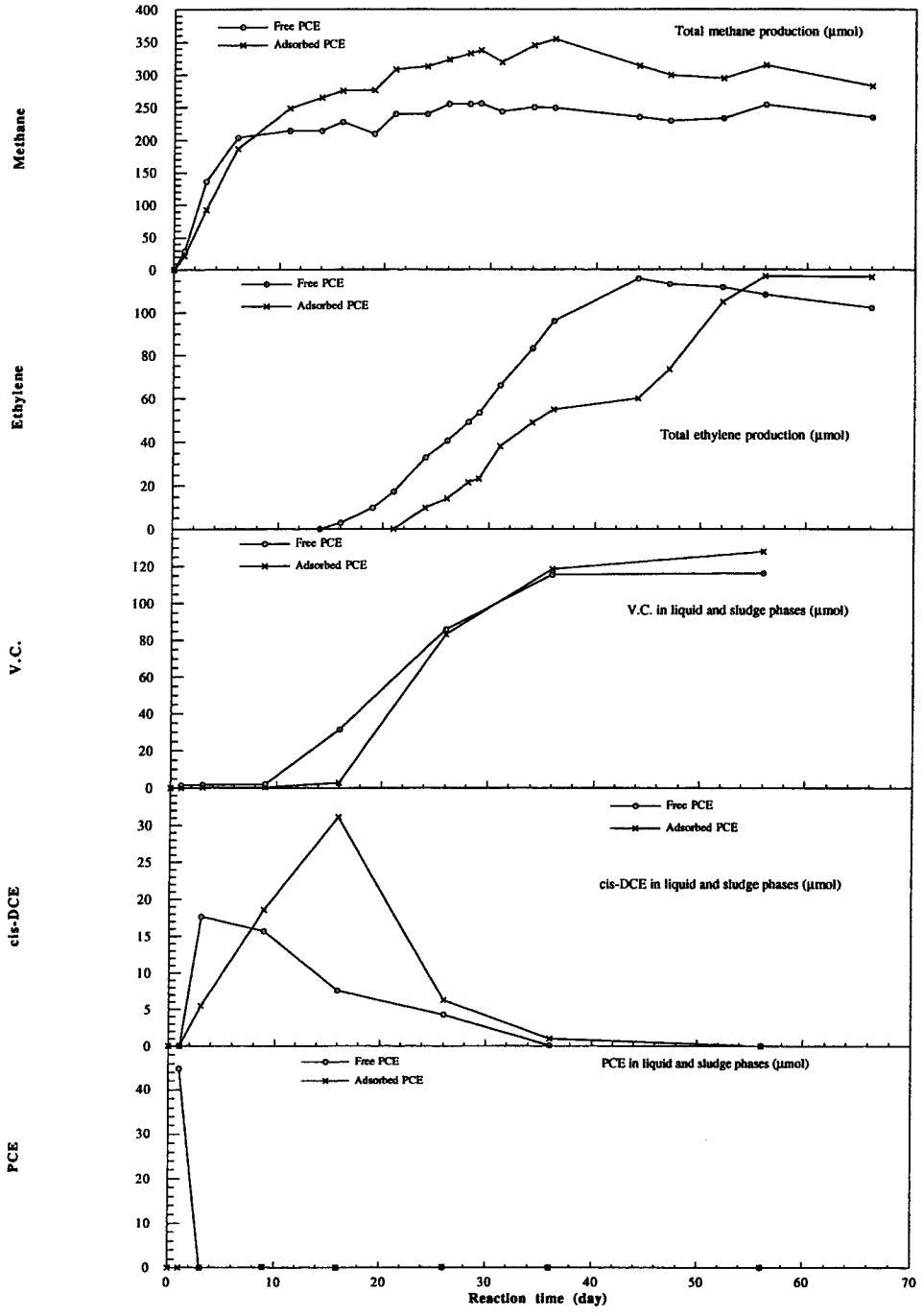


Fig. A-54 **A comparison of anaerobic PCE dechlorinating systems with adsorbed or free PCE doses. (PCE dose = 392 μmol)**

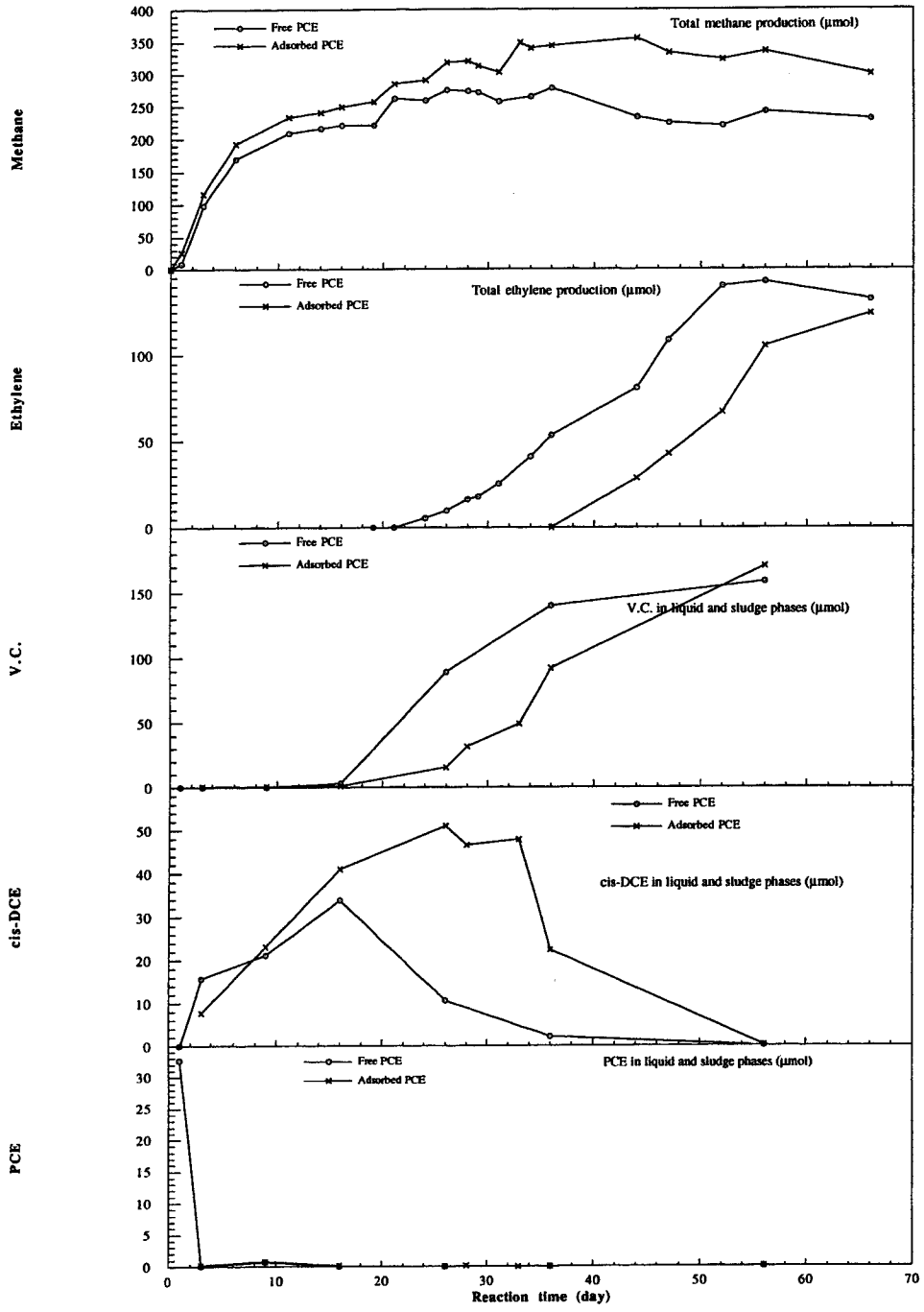


Fig. A-55 **A comparison of anaerobic PCE dechlorinating systems with adsorbed or free PCE doses. (PCE dose = 490 μmol)**

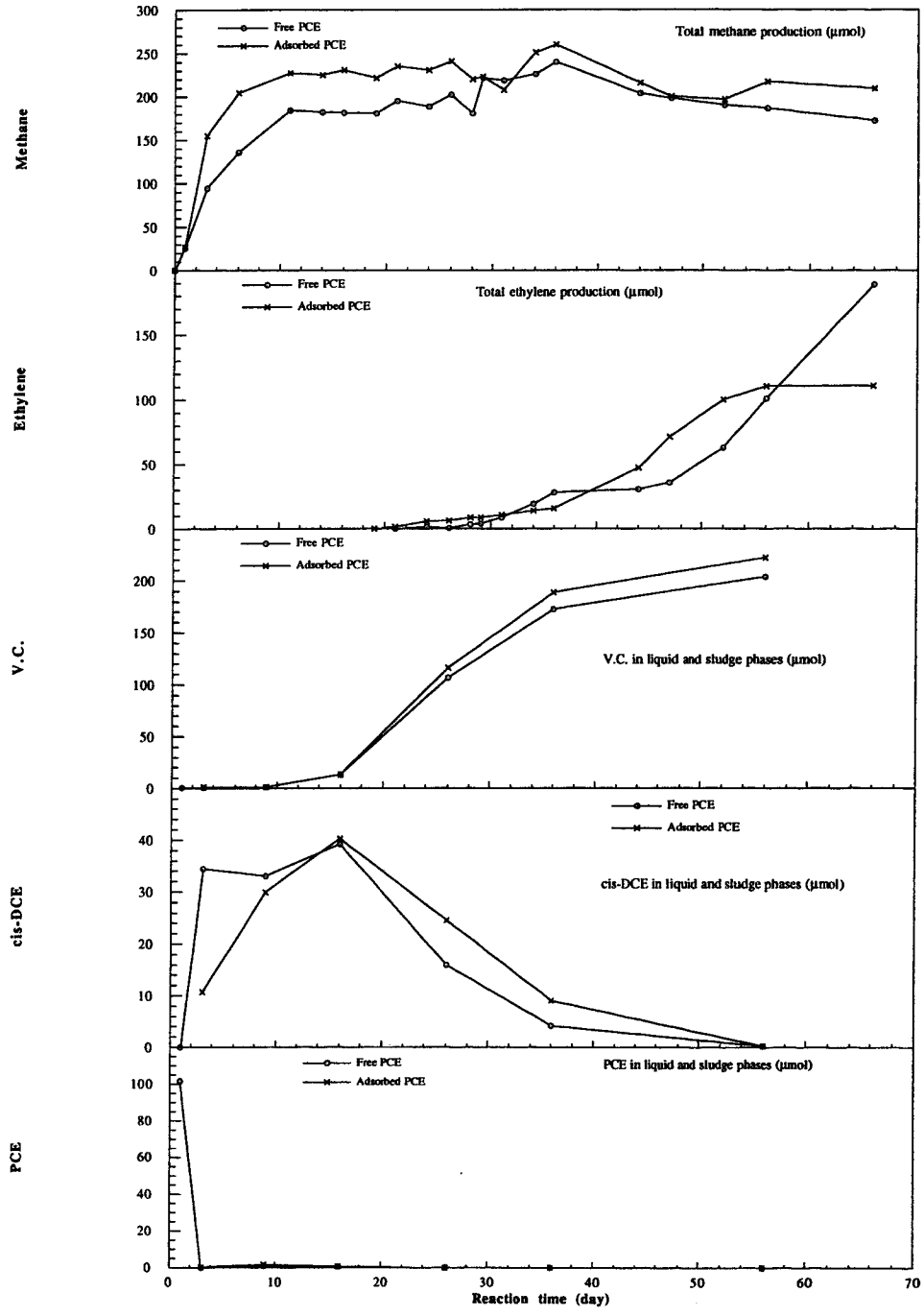


Table A-1. Feeding Summary for the experiment of the MeOH and media effect on PCE dechlorination.

	0 MeOH			5 MeOH			20 Medium			50 MeOH			100 MeOH			200 MeOH		
Time	N	M	P	N	M	P	N	M	P	N	M	P	N	M	P	N	M	P
(day)	(mL)	(μL)	(μL)	(mL)	(μL)	(μL)	(mL)	(μL)	(μL)	(mL)	(μL)	(μL)	(mL)	(μL)	(μL)	(mL)	(μL)	(μL)
13	5	0	5	5	5	5	20	5	5	5	50	5	5	100	5	5	200	5
18	5	0	5	5	5	5	20	5	5	5	50	5	5	100	5	5	200	5
22	5	0	5	5	5	5	20	5	5	5	50	5	5	100	5	5	200	5
26	5	0	5	5	5	5	20	5	5	5	50	5	5	100	5	5	200	5
32	5	0	5	5	5	5	20	5	5	5	50	5	5	100	5	5	200	5
38	5	0	5	5	5	5	20	5	5	5	50	5	5			5		
44	5	0	5	5	5	5	20	5	5	5	50	5	5	100	5	5	200	5
49	5	0	5	5	5	5	20	5	5	5	50	5	5	100		5	200	
55	5	0	5	5	5	5	20	5	5	5	50	5	5			5		
61	5	0	5	5	5	5	20	5	5	5	50	5	5			5		
67	5	0	5	5	5	5	20	5	5	5	50	5	5			5		
73	5	0	5	5	5	5	20	5	5	5	50	5	5	5	5	5	5	5
80	2.5	0	5	2.5	5	5	2.5	5	5	2.5	50	5						
87	2.5	0	5	2.5	5	5	10	5		2.5	50		5	5		5	5	
93	2.5	0	5	2.5	0	5												
99	2.5	0	5	2.5	0	5				2.5	50	5	2.5	100	5	2.5	200	5
105	5	0	5	5	0	5	20	0	5									
115	5	0	5	5	0	5	20	0	5									
122	5	0	5	5	0	5	10	0	5									
133	5	0	5	5	0	5	10	0	5									
147	5	0	5	5	0	5	10	0	5									
161	5	0	5	5	0	5	10	0	7.5									
201	5	0	5	5	5	5	5	5	5									
Total	105	0	115	105	75	115	338	75	103	67.5	750	70	67.5	810	40	67.5	1610	40
Total																		
(μmol)		0	1127		1845	1127		1845	1005		18450	686		19926	392		39606	392

Note: N = Nutrient medium; M = Methanol; P = PCE.

Table A-2.
Complete dechlorinating ability of acclimated anaerobic Hyperion sludge

Time (day)	PCE (μmol)	V.C. (μmol)	ETH (μmol)	Methane (μmol)
Sample 1				
0	49.00	27.01		
14	0.00	22.09	56.69	33.11
Sample 2				
0	49.00	28.24		
14	0.00	20.54	55.46	29.33
Sample 3				
0	49.00	27.96		
14	0.00	21.76	56.02	34.52

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