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Effects of Surfactants on a PCE Dechlorinating Mixed Culture

by

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A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

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ABSTRACT

Effects of Surfactants on a PCE Dechlorinating Mixed Culture

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Travis M. McGuire

The influence of surfactants on a perchloroethene dechlorinating mixed culture was investigated in laboratory experiments. Seven surfactants, representing each of the surfactant classes, were screened for their effects on the rate and extent of perchloroethene dechlorination. Based on screening results, a nonionic surfactant, Tween 80, and an anionic surfactant, Steol CS-330, were selected for use in further studies. Dechlorination of perchloroethene to dichloroethene, vinyl chloride, and ethene occurred in all Tween 80 amended microcosms with a depressed rate of ethene production as the only adverse effect. Steol CS-330, however, inhibited dechlorination beyond dichloroethene at all surfactant concentrations exceeding 25 mg/L. Attempts to acclimate a culture to the surfactant were unsuccessful. Inhibition of vinyl chloride and ethene production was reversible upon dilution of the surfactant to 10 mg/L or below indicating that surfactant interactions with the enzyme system responsible for reductive dechlorination of dichloroethene was the most likely cause of inhibition.

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As I pause and consider this point in my life and the events that have led to now, I realize that many of my accomplishments are due to the love and support my parents and family

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1. INTRODUCTION

Contamination of aquifers with chlorinated solvents, particularly perchloroethene (PCE) and trichloroethene (TCE), is one of the most prevalent ground water remediation challenges facing environmental engineers (*1,2*). The low aqueous solubilities of these compounds and densities greater than water facilitate the existence of these solvents as dense nonaqueous phase liquids (DNAPL) in aquifers. Dissolution of the chlorinated contaminant from the DNAPL into flowing ground water occurs very slowly under typical aquifer conditions. Attempts to enhance dissolution through pumping have proven ineffective at significantly reducing the source of contamination, which may exist and contaminate ground water for decades or centuries (*3*). Development of other technologies such as enhanced dissolution/mobilization of DNAPL by surfactants (*4-6*) and biologically enhanced removal of chlorinated ethenes from source zones (*7-9*), however, may be viable remediation strategies.

The anaerobic process of biological reductive dechlorination through which PCE or TCE is sequentially reduced to one or more of the dichloroethene (DCE) isomers (*cis*-DCE, *trans*-DCE, and 1,1-DCE), vinyl chloride (VC), and finally to the innocuous product ethene (ETH), has been studied widely (*10-14*). Though the majority of studies focus on the potential for microbial reductive dechlorination to treat the dissolved plume emanating from a DNAPL source zone, more recent laboratory investigations have demonstrated the ability of halorespiring microorganisms to carry out reductive dechlorination proximate to NAPLs (*7-9*). A key challenge in transferring and exploiting dechlorinating microorganisms in DNAPL source zones from the laboratory to the field is obtaining intimate contact between microorganisms, the contaminant, and electron donor.

Hydrogen is known to be a key electron donor supporting the biological reductive dechlorination of chlorinated ethenes. Both direct addition of hydrogen gas and syntrophic hydrogen production via fermentation of organic substrates have been shown to equally support the rate and extent of PCE dechlorination in laboratory studies (*14*). However, flow of hydrogen in an aquifer setting is governed by buoyancy due to the low density and viscosity of hydrogen compared to water. Therefore, hydrogen tends to rise through the aquifer rather than being transported laterally across the formation. This is a major disadvantage when considering enhancement of source zone biological reductive dechlorination since DNAPLs are typically at the base of the aquifer.

Enhanced oil recovery research has shown that dispersing a gas into a surfactant solution, creating surfactant foam, increases the apparent viscosity of the gas and decreases the mobility allowing lateral transport of the gas to occur (*15*). Utilization of hydrogen gas to generate surfactant foam could potentially increase the residual saturation of hydrogen in the aquifer and contact the DNAPL source zone more efficiently. This approach would be greatly beneficial to enhancing electron donor distribution throughout the contaminated zone thereby increasing source zone biological reductive dechlorination. However, for such an approach to be feasible, one must first consider the effects of the surfactants on the microbial process.

Surfactants are amphiphilic molecules containing a both a hydrophilic region and a hydrophobic region. As a result, these compounds are able to alter interfacial properties of aqueous solutions and form micellar pseudophases. Micellar surfactant solutions are capable of increasing the effective aqueous solubility of hydrophobic organic compounds (HOCs) by orders of magnitude (*16,17*). Mass transfer of contaminants to the aqueous

phase often limits biotransformation of many HOCs; therefore, enhanced solubilization of the HOC by a surfactant solution can potentially enhance bioremediation. The effects of surfactants on the biotransformation of HOCs have been studied quite extensively (*18,19*). Studies have demonstrated that surfactants can, in fact, increase rates of contaminant biodegradation through increasing mass transfer to the aqueous phase (*20-23*). However, inhibition of microbial activity has also been observed in the presence of surfactants (*24-26*). Toxicity of the surfactant, brought about by interaction of the surfactant with the cellular membrane or interaction with membrane bound proteins vital to cell function, is the most common explanation for the observed inhibition.

The majority of work relating to the effects of surfactants on biological HOC degradation focuses on aerobic microbial metabolism (*21,22,24,25,27,28*). Though the effects of surfactants on the anaerobic reductive dechlorination of chlorobenzenes have been investigated (*23,29,30*), there is a paucity of information regarding the effects of surfactants on chlorinated ethene reductive dechlorination. Other areas lacking research with respect to effects of surfactants on HOC bioremediation include the effects of ionic surfactants on microorganisms and the effect of surfactant concentrations more representative of those used in surfactant enhanced aquifer remediation (SEAR) on biotransformation processes (*18*). The present study seeks to address each of these issues as part of a comprehensive study aimed at enhancing hydrogen contacting of DNAPL source zones using hydrogen-based surfactant foam in order to enhance chlorinated ethene source zone bioremediation. Another goal of this research is to provide insights into the effects of the more traditional SEAR processes, solubilization and mobilization surfactant floods, on microbial activity, specifically that of dechlorinators.

Solubilization/mobilization floods are capable of dramatically reducing the mass of contaminant in an aquifer; however, a second treatment strategy, such as biological reductive dechlorination, may be required to reduce contaminant concentrations to regulatory acceptance.

A novel part of this research is the investigation of the effects of surfactants on PCE dechlorinating microorganisms. Specifically, the objectives of this research are as follows:

1) Evaluate the effect of representative surfactants from each of the surfactant classes (anionic, cationic, and nonionic) at concentrations representative of use in SEAR on the rate and extent of PCE dechlorination
2) Determine the extent to which surfactants at concentrations representative of use in SEAR are biodegraded by a mixed anaerobic culture
3) Select a nonionic and an anionic surfactant based on results of the initial screening for use in longer-term, more detailed experiments
4) Evaluate the effect of surfactant concentration on rate and extent of PCE dechlorination
5) Evaluate the effects of surfactants on other populations of the mixed anaerobic culture, such as methanogens
6) Describe any observed enhancement of biotransformation by displaying enhanced solubilization or enhanced contacting of microorganism and contaminant
7) Describe any observed inhibition of biotransformation by demonstrating decreased bioavailability due to micellar partitioning, uptake of the surfactant as a preferential substrate, and/or toxicity of the surfactant toward the microorganism(s)

The format of this thesis is as follows: Chapter 2 provides a review of literature relevant to the subjects of anaerobic reductive dechlorination of chlorinated ethenes, surfactant chemistry and mechanisms of SEAR, effects of surfactants on HOC biotransformation, and surfactant biodegradation; Chapter 3 details materials and methods utilized in experiments; the results of experiments are given in Chapter 4; a discussion of experimental results is presented in Chapter 5; the general conclusions drawn from the study are summarized in Chapter 6; and Chapters 7 and 8 provide the engineering significance and recommendations for future work, respectively.

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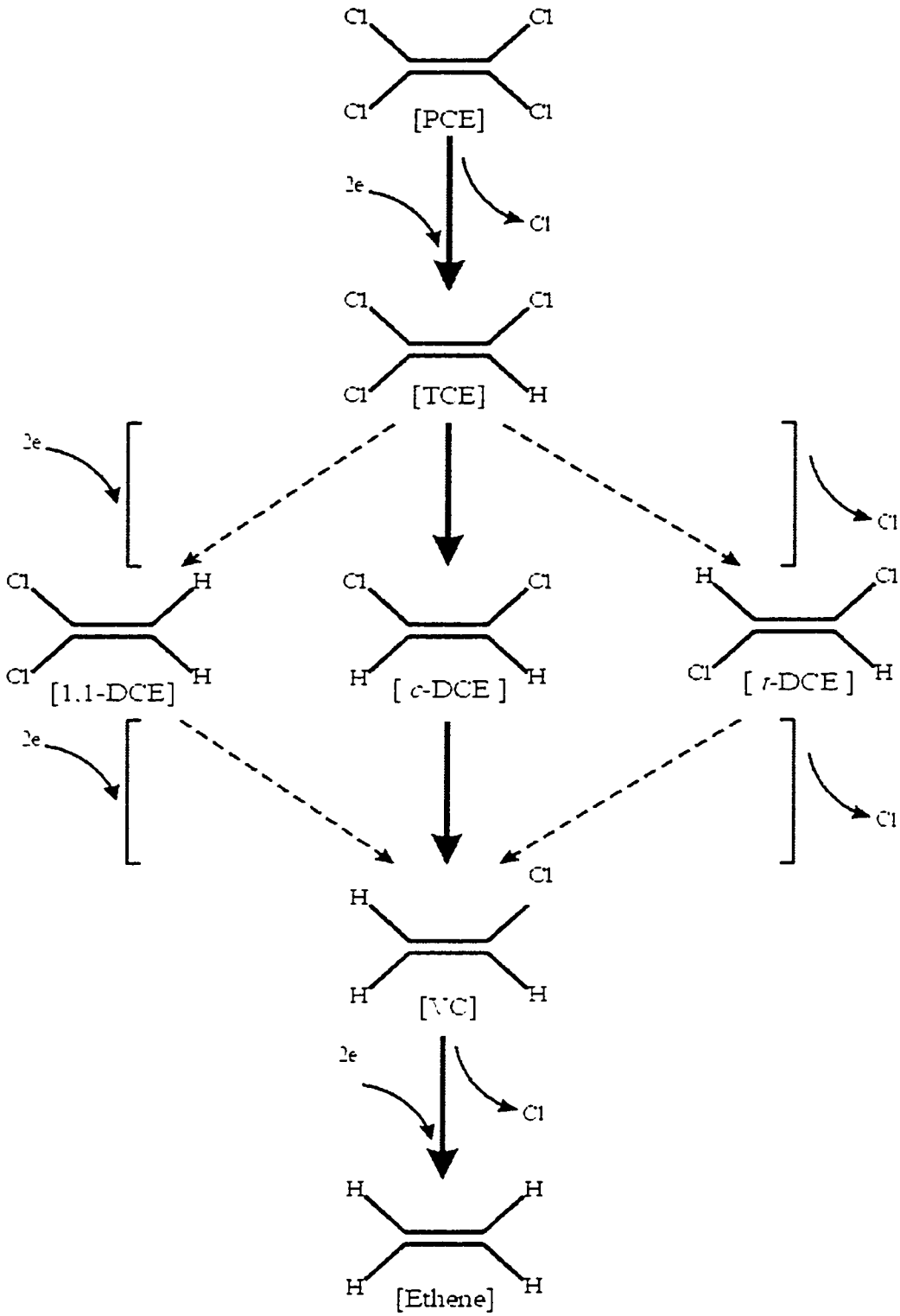
2. Literature Review

The purpose of this chapter is to provide an overview of mechanisms and research concerning the anaerobic biotransformation of chlorinated ethenes and the use of surfactants in enhanced aquifer remediation. Particular attention is given to chlorinated ethene halorespiration and characteristics of microorganisms that carry out this process. A summary of previous experiments characterizing the dechlorinating culture utilized in the present work is also given. The discussion of surfactants begins with an overview of physicochemical properties followed by a brief review of the mechanisms of surfactant enhanced aquifer remediation. Finally, summaries of relevant research concerning the effects of surfactants on the biotransformation of organic compounds and surfactant biodegradation are presented.

2.1 Anaerobic Biotransformation of Chlorinated Ethenes

Microorganisms capable of degrading chlorinated ethenes, which are among the most prevalent ground water contaminants (*1*), have been the focus of much research in recent years, as bioremediation of contaminated aquifers has proven to be a viable remediation option. The anaerobic process in which highly chlorinated ethenes (PCE and TCE) are sequentially reduced to lesser chlorinated species (the DCE isomers and VC) and finally to the innocuous product ethene is known as reductive dechlorination and is depicted in Figure 2.1. The high oxidation state of PCE renders this compound unsusceptible to aerobic biodegradation, however, microbial oxidation of TCE (*2-4*), *cis*-DCE, and VC has been reported (*5-9*). The focus of this review will be the anaerobic process of reductive dechlorination.

Figure 2.1. Reductive Dechlorination Pathway



Biological reductive dechlorination occurs through one of two mechanisms: cometabolism and halorespiration. Cometabolism is the reduction of the chlorinated species by some enzyme or cofactor where the organism does not gain energy from the reaction. Microorganisms that carry out cometabolic reductive dehalogenation of chlorinated ethenes include acetogens (*10*) and methanogens (*11-13*). In contrast to cometabolism, halorespiration is a process in which the chlorinated species serves as the terminal electron acceptor for respiration and growth of the organism is coupled to the reduction of the chlorinated species. Halorespiration typically results in chlorinated species reduction rates several orders of magnitude above those observed for the cometabolic process (*14*). Since microorganisms that carry out halorespiration are the group capable of reductively dehalogenating chlorinated ethenes in timeframes beneficial to aquifer remediation processes, research has focused on this group of organisms.

In 1993, researchers first discovered a microorganism capable of coupling growth to the reduction of PCE (*15*). The bacteria isolated, *Dehalobacter restrictus*, was found to use hydrogen as an electron donor and coupled growth to the reduction of either PCE or TCE to *cis*-DCE. To date, six additional isolates have been shown to carry out halorespiration, *Dehalospirillum multivorans* (*16,17*), Strain TEA (*18*), *Desulfitobacterium* PCE1 (*19*), *Desulfitobacterium* PCE-S (*20*), *Desulfuromonas chloroethenica* (*21,22*), and *Dehalococcoides ethenogenes* strain 195 (*23*). All of the isolates reductively dechlorinate PCE to *cis*-DCE with the exceptions being *Desulfitobacterium* PCE1, which dechlorinates PCE only to TCE, and *Dehalococcoides ethenogenes* strain 195, which is capable of complete dechlorination of PCE to ethene. In

cases where isolation of the dechlorinating microorganism is unsuccessful, the rate and extent of dechlorination are used to determine whether PCE dechlorination is cometabolic or halorespiratory.

Numerous research studies have investigated which electron donors are most capable of supporting reductive dechlorination. An extensive review of dechlorinating cultures and electron donors utilized is found in (*24*). Most of the isolates above, as well as many mixed dechlorinating cultures, have been shown to depend on hydrogen or substrates whose metabolism results in release of hydrogen to support dechlorination. However, some studies have shown that acetate, a substrate whose metabolism does not result in release of hydrogen, is capable of serving as the electron donor in the reductive dechlorination process (*21,22,25-27*).

In situations where hydrogen or syntrophic hydrogen production via fermentation is used to support dechlorination, competition for electron donor between methanogens, acetogens, and dechlorinators may exist. Researchers have examined this issue by comparing the half-velocity coefficients with respect to hydrogen, $K_s(H_2)$, for methanogenic and dechlorinating organisms in mixed cultures (*28,29*). K_s represents the substrate (hydrogen in this case) concentration yielding one-half the maximum specific growth rate according to Monod kinetics of bacterial growth. The K_s value represents the affinity an organism has for a substrate and lower values of K_s represent higher substrate affinities. The $K_s(H_2)$ values for dechlorinating microorganisms were found to be at least an order of magnitude lower than the $K_s(H_2)$ values for methanogens in both experiments. The results from these studies imply that dechlorinators are capable of out-competing methanogens for electron donor, particularly at low hydrogen partial pressures. Studies

conducted where hydrogen was present at higher partial pressures (up to 0.8 atm (*30*)) indicate that dechlorination rates and extents are enhanced over time despite increased electron donor competition from other hydrogenotrophic organisms (*29,30*). In the study by Carr and Hughes (*30*), which compared the effect of equivalent amounts of the electron donors lactate, methanol, and hydrogen on the dechlorination activity of an actively methanogenic mixed culture, it was determined that the rate and extent of dechlorination was the same for all donors. The authors suggested that factors such as cost and delivery of electron donor should determine the donor selected for *in situ* bioremediation processes.

Microorganisms capable of halorespiration have the potential to greatly enhance removal rates of chlorinated ethenes at contaminated sites. For this reason, several studies have been undertaken to characterize the dehalogenase enzymes responsible for halorespiration and to determine the mechanism of the halorespiratory process (*20,31-36*). Results of these studies are detailed in review articles by Wohlfarth and Diekert (*37*), El Fantroussi, *et al.* (*38*), and Middeldorp, *et al.* (*14*). A comparison of reductive dehalogenases characterized for several different pure and enriched cultures is found in Table 2.1. Most of the enzymes isolated to date are capable only of dechlorinating PCE and TCE to *cis*-DCE. All dehalogenase enzymes, with the exception of the PCE reductase in *D. multivorans*, have been determined to be membrane bound (Table 2.1). While the enzyme system in *D. multivorans* was found to be located in the cytoplasm, the enzyme must be associated with the membrane since it is involved in the respiratory process (*31*). Since surfactants are capable of association with membrane bound proteins

Table 2.1. Characteristics of dehalogenase enzymes from various reductive dechlorinating cultures

Organism	Electron Acceptor	Product	Enzyme Location	Cobalt Corrinoid	Other Transition Metal	Reference
D. multivorans	PCE/TCE	*cis*-DCE	Cytoplasm	+	+ (Fe)	Neumann et al. 1996 (31) Miller et al. 1997 (32)
D. restrictus	PCE/TCE	*cis*-DCE	Membrane	+	+ (Fe)	Schumacher et al. 1997 (33)
Desulfitobacterium Strain PCE-S	PCE/TCE	*cis*-DCE	Membrane	+	+ (Fe)	Miller et al. 1998 (20)
Enrichment containing *D. ethenogens*	PCE	TCE	Membrane	+	+ (Fe)	Magnuson et al. 1998 (34)
Enrichment containing *D. ethenogens*	TCE/DCE	VC	Membrane	+	+ (Fe)	Magnuson et al. 1998 (34) Maymo-Gatell et al. 2001 (35)
VC-Enrichment	DCE/VC	VC/ethene	Membrane	–	+ (?)	Rosner et al. 1997 (36)

(*39*), these observations are important, as surfactants may be capable of interfering with the halorespiration process.

As opposed to the process of PCE and TCE dechlorination, little characterization of the organisms and enzymes responsible for DCE and VC dechlorination has been done. The majority of the halorespiring organisms isolated thus far are capable only of dechlorinating PCE to DCE, yet VC and ethene are often observed in the mixed cultures containing the organisms (*15,18,40*). This observation indicates that there is in fact another population of organisms that carry out DCE dechlorination. Only one organism, *Dehalococcoides ethenogenes* 195, has been isolated that is capable of dechlorinating DCE and VC (*23*). Magnuson, *et al.* (*34*) partially purified two enzymes from an enrichment culture containing *Dehalococcoides ethenogenes* strain 195. One enzyme, PCE-RDase, was able to dechlorinate PCE to TCE only. The second enzyme, TCE-RDase, was capable of reducing TCE, the DCE isomers, and VC to ethene, but could not reduce PCE. Reduction of *trans*-DCE and VC was reported to be 2 orders of magnitude slower than reduction rates for TCE, *cis*-DCE, and 1,1-DCE. Each of the enzymes was found to contain Co(I) corrinoid cofactors (Table 2.1). Recent studies carried out on this culture have determined that reduction of VC is in fact not coupled to growth of the organism and depends on the presence of a higher chlorinated ethene (*35*).

Rosner, *et al.* (*36*) studied the reduction of *cis*-DCE and VC to ethene by a VC enriched mixed culture. Cell extracts from the enriched culture were capable only of dehalogenating *cis*-DCE and VC at high rates. TCE was reduced at a very slow rate and PCE was not reduced. Dehalogenation was determined to occur in the membrane fraction of the cell extract indicating that the reduction was a halorespiratory process.

Upon propyl iodide treatment of the cell extract, VC dehalogenation was inhibited and could not be reversed by illumination. This result indicates that a cobalt corrinoid is not involved in the DCE and VC reduction process of this organism. This represents the first non-cobalt containing dehalogenase characterized thus far (Table 2.1). VC dehalogenation was severely inhibited by low levels of potassium cyanide, indicating that a transition metal-containing enzyme is likely involved in the reductive dechlorination process of this organism. The authors concluded that the dehalogenase activity of their VC enriched culture was different than previously reported PCE dehalogenases based on two points. First, a corrinoid was determined to not be involved in the DCE/VC reduction process. Second, they observed VC dechlorination rates approximately 3 orders of magnitude above those observed for reductions mediated by transition metal-containing catalysts, such as vitamin B_{12} and coenzyme F_{430}. Characterization of the biochemical processes of organisms that carry out DCE and VC reductive dechlorination is very important, as many mixed cultures that dechlorinate PCE to ethene have been shown to rely on at least two organisms to carry out the process (*41,42*). While much is known about the enzymes responsible for PCE and TCE dechlorination, very little is known about those that catalyze DCE and VC reduction and as the results of Rosner, *et al.* illustrate, differences in the enzymes involved and mechanisms of the two processes are likely.

In summary, halorespiring microorganisms have the potential to contribute to the remediation of chlorinated ethene contaminated aquifers. While many substrates have been observed to support the reductive dechlorination process, hydrogen has been shown to be a key electron donor in most dechlorinating cultures. Studies have concluded that

dechlorinating microorganisms will not be excluded by methanogens and acetogens due to competition for electron donor, especially at low hydrogen concentrations. Even at high hydrogen partial pressures, dechlorination rate and extent have been observed to enhance over time. Much less is known about the organisms and enzyme systems that carry out DCE and VC reduction than those responsible for PCE and TCE dechlorination. Elucidation of the enzymes and mechanisms responsible for DCE and VC degradation is central to the understanding of the complete dechlorination process.

2.1.1 Characterization of the Rice Dechlorinating Culture

The dechlorinating culture utilized in this study has been partially characterized in studies by Zheng, *et al.* (*41*) and Carr (*30,43*). The inoculum for all cultures in our lab is obtained from an upflow anaerobic sludge blanket (UASB) that has been maintained in the lab for approximately 10 years. The UASB is continuously fed methanol (52 mM) as electron donor, PCE (86 mg/L), and nutrient media (see Section 3.1.2). PCE is consistently converted to VC (~80%, molar) and ethene (~20%, molar), with trace amounts of *cis*- and *trans*-DCE occasionally observed. Sub-cultures from the UASB have been shown to utilize lactate, methanol, and hydrogen as electron donors to support equivalent rates and extents of PCE dechlorination (*30*).

Zheng, *et al.* (*41*) examined the influence of varying hydraulic retention time (HRT) on the rate and extent of PCE dechlorination in continuously fed, complete mixed reactors. Results from these studies indicate that at least two populations of dechlorinating microorganisms are responsible for complete reduction of PCE to ethene. One group of organisms reduces PCE to *cis*-DCE and a second set of organisms transforms *cis*-DCE to VC and ethene. At a short HRT (2.9 days), PCE was only

converted to *cis*-DCE, yet at a longer HRT (11 days), PCE was reduced to VC and ethene. The authors concluded that a slower growth rate for the DCE dechlorinating organisms led to washout of these organisms from the reactor. Further studies showed that the two cultures differed in their response to the methanogenesis inhibitor 2-bromoethanesulfonate (BES). The culture enriched on PCE (2.9 d HRT) was not influenced by BES addition (10 mM), where as the dechlorination activity of the *cis*-DCE enriched culture (11 d HRT) was inhibited by exposure to BES. Similar inhibition of the *cis*-DCE degrading organisms was observed with addition of 2-bromoethanol (BEOH).

Carr (*43*) conducted experiments attempting to isolate the dechlorinating organisms in the UASB. Although isolation was unsuccessful, a purified PCE dechlorinating enrichment culture was obtained and characterized with respect to some nutritional and ecological requirements. The purified culture was capable of PCE dechlorination to TCE and *cis*-DCE when hydrogen, pyruvate, ethanol, glucose, or yeast extract was added as electron donor, acetate was added as the carbon source, and filter-sterilized cell extract from the UASB provided an undefined nutritional requirement. The loss of *cis*-DCE dechlorination was attributed to the addition of BES to the medium, which served to eliminate methanogens from the culture. The dependence of dechlorination activity on filter-sterilized cell extract from the UASB indicated that the dechlorinating organisms rely on other organisms present in the mixed culture for some nutritional supplement. While attempts to isolate the dechlorinating organisms in the Rice mixed culture have been unsuccessful, these microorganisms likely carry out halorespiration based on observed rates and extents of dechlorination similar to those observed in mixed cultures from which halorespiring organisms have been isolated (*44*).

2.2 Surfactant Chemistry and Physical Properties

Surfactants (SURFace ACTive AgeNTs) are molecules characterized by their ability to alter interface properties of fluids and form micellar pseudophases. These characteristics are due to the amphipathic, or dual, nature of the molecules. Surfactants contain a hydrophilic group, typically composed of an ionic species or an ethylene oxide chain, and a hydrophobic group, typically composed of long chain hydrocarbons. Despite the variety in the exact composition of these groups among surfactants, all surfactants share certain physicochemical properties. In recent years, engineers have exploited these physicochemical properties in efforts to enhance remediation of hydrophobic organic compounds in aquifers.

Surfactants are typically classified by the nature of the hydrophilic moiety. The three major surfactants classes are anionic, cationic, and nonionic. A fourth class of surfactants, zwitterionic (containing an anionic and cationic moiety), also exists. An anionic surfactant is one that yields a negative charge in an aqueous solution. This charge is usually due to the presence of a sulfate, sulfonate, or carboxylate group. Conversely, a cationic surfactant yields a positive charge in solution, typically resulting from a quaternary ammonium ion. Nonionic surfactants are those that do not ionize in water. The hydrophilicity of nonionic surfactants is due to the presence of an ethylene oxide (EO) chain or a polyglycoside group. Nonionic surfactants can be further classified by a property known as the hydrophile-lipophile balance (HLB) number. The HLB number represents the affinity of the surfactant toward the aqueous phase, therefore, the

higher the HLB number the more hydrophilic the molecule. HLB numbers are either determined experimentally or can be estimated based on the surfactant structure. To date, much of the research investigating biodegradation of organic compounds in the presence of surfactants has involved the use of nonionic surfactants (see Table 2.2).

The hydrophilic moiety of surfactants makes them soluble in water, while the hydrophobic moiety of the molecule prefers to associate with interfaces in solution. When surfactants are present in an aqueous solution, the surfactant molecules arrange themselves at the air-water interface so that the hydrophilic group is in the water and the hydrophobic group is oriented toward the air. This results in a lowering of the surface tension of the water. A similar phenomenon occurs at other interfaces, such as between aqueous surfactant solutions and hydrocarbons or chlorinated solvents resulting in decreased interfacial tension between the two phases. The concentration of a pure surfactant resulting in the minimum surface tension of the solution is known as the critical micelle concentration (CMC). At this concentration, surfactant monomers aggregate to form clusters of surfactant molecules with their hydrophobic tails associated together and the hydrophilic heads pointing outward into the aqueous solution. This cluster is known as a micelle and is typically thought of as a hydrophobic pseuodophase. Depending on the surfactant and conditions, a micelle may contain from twenty or thirty to hundreds of surfactant molecules, which are in equilibrium with surfactant monomers in solution. The concentration of surfactant monomers in solution remains nearly constant once the CMC is exceeded and any additional surfactant added to the solution results in an increased number of micelles. The CMC for most surfactants lies in the range of approximately 10 mg/L up to 10,000 mg/L and varies with many factors such as

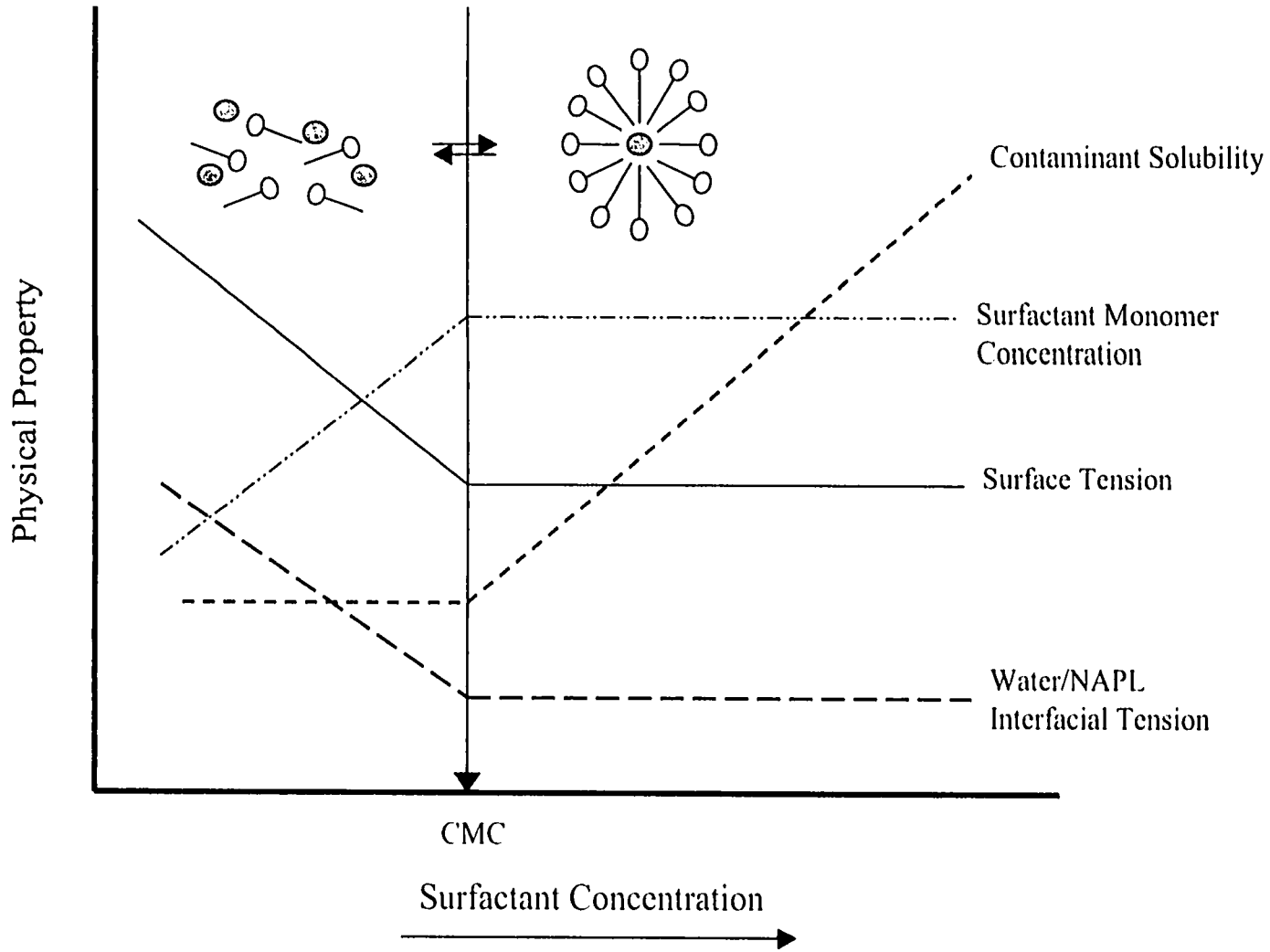
surfactant class, temperature, and ionic strength (*45*). Generally, nonionic surfactants have much lower CMCs than their ionic counterparts. The relationship between surfactant concentration and various physical properties of an aqueous solution is shown in Figure 2.2.

2.3 Mechanisms of Surfactant Enhanced Aquifer Remediation

2.3.1 Solubilization/Mobilization of Non-Aqueous Phase Liquids by Surfactants

The physical-chemical properties of surfactants discussed above allow these chemicals to potentially aid in the remediation of non-aqueous phase liquid (NAPL) contamination in ground water. Surfactant based remediation of NAPL is possible via two mechanisms: solubilization and mobilization. Solubilization occurs as a result of micelle formation. The interior of the micelle acts as a hydrophobic adsorption site capable of interacting with nonpolar organic compounds thereby increasing the effective solubility of the NAPL. Surfactant solutions above the CMC have been shown to increase aqueous concentrations of hydrophobic organic compounds (HOC) orders of magnitude above their aqueous solubilities (*46-52*). The increased mass transfer of NAPL to the aqueous phase allows dissolution of the NAPL to occur in much fewer pore volumes throughput compared to that required for dissolution into flowing ground water alone (i.e., pump-and-treat systems). The amount of HOC capable of being solubilized by a micellar surfactant solution depends on several factors including structure of the surfactant (i.e., HLB number), structure of the HOC (i.e., octanol-water partition coefficient), concentration of the surfactant, temperature, and ionic strength of the solution (*49,53*). Investigations into surfactant-enhanced remediation of NAPLs via

Figure 2.2 Effect of Surfactant Concentration on Various Physical Properties of an Aqueous Solution and Interaction with a Hydrophobic Organic Compound



solubilization have shown that the process is rate-limited rather than at equilibrium under realistic transport conditions (*51,52,54,55*). However, equilibrium partitioning is useful for comparing and selecting potential surfactants.

Mobilization of NAPL occurs as a result of reduction of the interfacial tension between water and NAPL due to the presence of the surfactant. The interfacial tension between the aqueous and NAPL phases must be sufficiently low to overcome capillary forces responsible for trapping NAPL in soil pores for mobilization of the NAPL to occur. The capillary number (N_c), which is a ratio of the viscous forces to capillary forces, is often used to evaluate the effectiveness of surfactant mobilization. The capillary number is defined as follows:

$$N_c = \frac{k\rho g}{\sigma} * \frac{\Delta H}{\Delta l}$$

where k is the intrinsic permeability, ρ is the density of water, g is acceleration of gravity, $\Delta H/\Delta l$ is the hydraulic gradient, and σ is the interfacial tension (IFT) of the water-NAPL interface. IFT values for water-NAPL typically range from 30 to 50 dynes/cm and must be lower than approximately 10^{-3} dynes/cm for maximum mobilization to occur (*45*). Factors that affect the ability of a surfactant solution to reach these ultra low IFT values include the structure of the surfactant, concentration of surfactant, presence of a cosolvent, temperature, and ionic strength of the system (*56*). While mobilization is capable of removing a greater mass of NAPL in a shorter period of time as compared to solubilization, there is concern that mobilization of NAPL may lead to uncontrolled spread of contamination (*57*). This is particularly true for chlorinated ethenes, which are denser than water and may migrate vertically through fractures in the aquifer formation when mobilized by a surfactant flood. More detailed reviews of mechanisms, laboratory

demonstrations, and field demonstrations of surfactant-enhanced aquifer remediation are available (*56,58-61*).

2.3.2 Enhanced Hydrogen Delivery for Reductive Dechlorination by Surfactant Foams

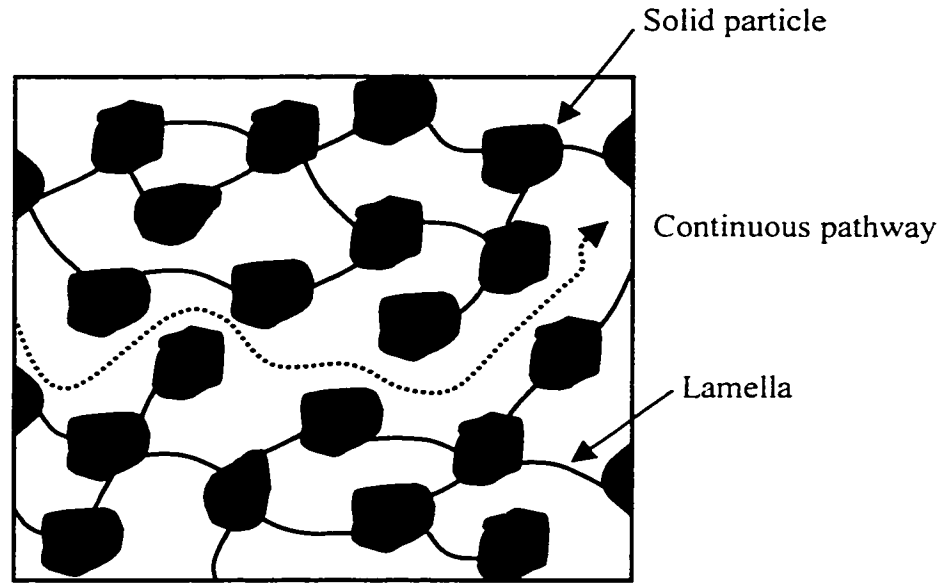
Current research at Rice University is investigating the potential for surfactant foams to enhance hydrogen delivery for biological reductive dechlorination of chlorinated ethene contamination in aquifers. As discussed previously, hydrogen is a key electron donor in the anaerobic reductive dechlorination process. Studies in our lab have indicated that hydrogen driven reductive dechlorination is capable of increasing depletion rates of chlorinated ethenes from a NAPL by 14 to 16 times over dissolution alone (*62,63*). However, achieving such results is dependent on intimate contact among the microorganisms, hydrogen, and the contaminant. For every liter of PCE contaminating an aquifer, a minimum of approximately 960 L of H_2 (1 atm, 25°C) is required for complete dechlorination to ethene. Delivering adequate amounts of hydrogen to DNAPL contaminated zones is a challenge that must be met in order to achieve source zone reductive dechlorination. The use of hydrogen generated surfactant foams is a technology with the potential to efficiently deliver large amounts of hydrogen to DNAPL source zones.

Direct injection of hydrogen gas (biosparging) is one method that has been employed to distribute hydrogen gas in a contaminated aquifer (*64*). Results from this field demonstration showed that hydrogen influenced only a 5-10 ft. radius around the injection point. Since hydrogen gas in less dense and less viscous than water, buoyancy forces dominate viscous forces and the hydrogen gas migrates vertically rather than

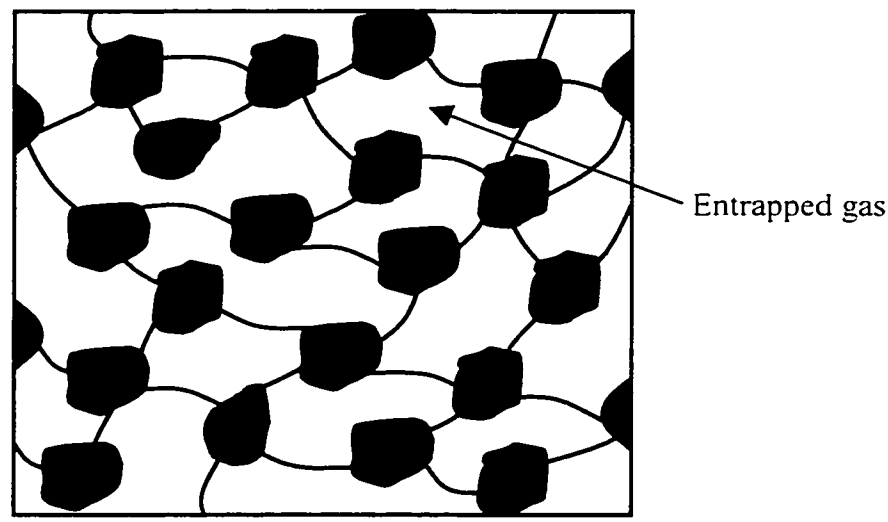
laterally. The use of surfactant foams in enhanced oil recovery research has shown that dispersing a gas as a discontinuous phase with surfactant foam lamella increases the apparent viscosity of the gas and decreases the mobility allowing lateral transport of the gas to occur (*65*). Work in this area has led to increased research into the use of surfactant foams for the remediation of NAPLs in aquifers (*66,67*).

Surfactant foam generation is accomplished by the injection of a surfactant solution into a porous medium followed by the injection of gas. When gas is injected into a surfactant solution, it becomes dispersed into the solution, which is interconnected by thin liquid phase films known as lamellae. In general, the "strength" of foam generated is determined by whether the gas becomes dispersed as a continuous-gas or a discontinuous-gas foam (*68-70*). A continuous-gas foam, or weak foam, exists when pathways for gas flow unblocked by lamellae are present. On the other hand, a discontinuous-gas foam, or strong foam, exists when all pathways for gas flow are blocked by the lamellae. These two situations are illustrated in Figure 2.3. The generation of strong foam would be most beneficial for enhanced hydrogen delivery as it results in larger residual gas saturation. The conditions required for generation of strong foam in porous media have been the focus of much research and depend on factors including flow rate, pressure drop and gradient, permeability, and the length of the system (*71*). Once gas injection ceases, lamellae are no longer propagated and foam production also ceases. Over time, the gas bubbles coalesce releasing the gas phase. This process occurs more slowly for stronger foams than weaker foams. Hence, strong hydrogen-generated foams, which are transported laterally through an aquifer and contact

Figure 2.3. Continuous-Gas (Weak) Foam vs. Discontinuous-Gas (Strong) Foam Modified from Falls et al., 1988 (68)



Continuous-gas (weak) foam



Discontinuous-gas (strong) foam

DNAPL source zone areas, can potentially serve as long-term sources of hydrogen gas available to support anaerobic reductive dechlorination.

2.4 Effects of Surfactants on the Biotransformation of Organic Compounds

The ability of surfactants to alter properties of hydrophobic organic compounds (HOCs) in aqueous solution in ways that may serve to enhance their biodegradation (i.e., increased aqueous concentrations and/or increased interfacial area) has led to considerable research in recent years regarding the influence of surfactants on microbial processes. The presence of surfactants may enhance, as well as inhibit biodegradation of HOCs and complete mechanisms by which surfactants influence microbial activity are not well understood (*72,73*). Table 2.2 summarizes relevant research pertaining to the effects of surfactants on biodegradation of ground water contaminants. As this table shows, the vast majority of research on this subject focuses on aerobic metabolism, particularly that of polycyclic aromatic hydrocarbons (PAHs). Very little work examining the effects of surfactants on anaerobic microorganisms is available. The effect of ionic surfactants on microbial degradation processes is another area lacking research, as pointed out in a review article on the subject (*72*).

An increased aqueous phase contaminant concentration brought about by micellar solubilization is the most common explanation for enhanced rate and/or extent of biodegradation in the presence of surfactants (*74-80*). However, enhanced degradation has been observed without simultaneous enhancement of the substrate's aqueous solubility (*81-83*). In these studies, an increased rate of PAH desorption from soil due to

Table 2.2 Summary of Research Efforts Regarding Effects of Surfactants on Bioremediation of Organic Compounds

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
Guerin and Jones, 1988 (74)	*Mycobacterium* sp. (Aerobic)	Phenanthrene	Tween 20, 40, 60, 80, 85	Nonionic	> CMC	+	Addition of Tween surfactants enhanced phenanthrene degradation due to increased solubilization. Degradation rate increased with decreasing HLB, i.e., decreasing number of EO groups.
Aronstein et al., 1991 (81)	Mixed (Aerobic)	Phenanthrene Biphenyl	Alfonic 810-60 Novel II 1412-56	Nonionic	>,< CMC	See R&C	Low concentrations (~0.04 to 2 x CMC) of two nonionic surfactants were investigated for their potential to enhance desorption and biodegradation of phenanthrene and biphenyl. Both surfactants increased phenanthrene mineralization at concentrations well below the CMC. No effect or decreased mineralization was observed at surfactant concentrations near or above the CMC. Enhancement of phenanthrene degradation at very low surfactant concentrations was attributed to an increased rate of phenanthrene desorption from soil brought about by the surfactant.
Laha and Luthy, 1991 (84)	Mixed PAH degrading (Aerobic)	Phenanthrene	Brij 30 Tergitol NP10 Triton X-100	Nonionic	>,< CMC	>,- <,0	Surfactant concentrations above the CMC inhibited phenanthrene mineralization, however, dilution of the surfactant to below the CMC resulted in resumed phenanthrene degradation. The authors concluded that the observed inhibition was not due to surfactant toxicity or use of the surfactant as a preferential substrate.
Laha and Luthy, 1992 (85)	Mixed PAH degrading (Aerobic)	Phenanthrene	Brij 30,35 Neodol 25-3, 25-9 Tergitol NP10 Triton X-100 Tween 20, 80 CHAPS Octylglucoside	Nonionic	>,< CMC	>,- <,0	Phenanthrene mineralization was inhibited by all surfactants at concentrations above the CMC and resumed upon dilution below the CMC. The inhibition of the surfactants was attributed to a reversible interaction of surfactant micelles with cellular membranes.

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
Bury and Miller, 1993 (75)	*Pseudomonas aeuroginsa* and *Ochrobactrum anthropi* (Aerobic)	n-decane n-tetradecane	Neodol 25-7, 25-9, 25-3	Nonionic	> CMC	+	Surfactant addition increased hydrocarbon degradation rates through an increase in the effective solubility of the hydrocarbon. This study demonstrated that hydrocarbons solubilized in micelles are available for uptake by microorganisms. For the Ochrobactrum sp., significant degradation took place only in the presence of the surfactant.
Tiehm, 1994 (86)	Mixed *Mycobacterium* sp. (Aerobic)	PAHs	SDS Brij 35 Triton X-100 Marlipal 013-90 Genapol X150 Arkopal N300 Sapogenat T300 Pluronic PE6400 Tegropen 5851	Anionic Nonionic	> CMC	See R&C	The anionic surfactant, SDS, was utilized by the culture as a preferential substrate and therefore inhibited PAH degradation. The most lipophilic surfactants studied, Marlipal 013-90 and Triton X-100 having HLB values of 13.3 and 14.6, respectively, inhibited PAH degradation by the pure culture. However, fluorene and pyrene degradation were inhibited in the presence of these surfactants by the mixed culture, while phenanthrene and fluoranthene were degraded in the presence of the surfactants. PAH degradation was enhanced in the presence of the nonionic surfactants with high HLB values (17.0). The authors suggest that surfactant toxicity is related to the lipophilicity of the surfactant.
Volkering et al., 1995 (79)	*Pseudomonas* sp. (Aerobic)	Phenanthrene Naphthalene	Brij 35 Igepal CA-720 Tergitol NPX Triton X-100	Nonionic	> CMC (up to 1%)	+	Biodegradation rates of phenanthrene and naphthalene increased during the dissolution-limited growth phase in the presence of surfactants due to an enhanced PAH dissolution rate. PAH partitioned into micelles was determined to be not readily bioavailable to PAH degrading microorganisms.
Mihelic et al., 1995 (80)	*Pseudomonas* sp. (Aerobic)	Naphthalene	Brij 58 Triton X-100	Nonionic	>,< CMC	>,+ <,0	Increased biodegradation rates of naphthalene were observed in microcosms with a Triton X-100 concentration above the CMC. The specific growth rate of microorganisms in the presence of both surfactants at concentrations above the CMC (up to 12% v/v) was approximately 25% below the growth rate in the absence

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
							of surfactant indicating some inhibition due to the surfactants. A second Pseudomonas sp. examined was inhibited to a much greater extent indicating that the effects of the same surfactant vary among microbial strains.
Rouse et al., 1995 (87)	Mixed (Aerobic)	Naphthalene	DPDS-C6, C10, C12, C16, C22 SDBS	Anionic	>,< CMC	See R&C	SDBS inhibited naphthalene degradation at concentrations equal to and surpassing the CMC. The presence of DPDS surfactants having medium hydrophobic chain lengths of 10 to 16 carbons showed increased oxidation rates over the C6, C22, and surfactant-free controls when the surfactant concentration was greater than the CMC. Experiments investigating sorption of the surfactants onto biomass indicated that biodegradation is likely affected by direct interaction of the surfactant and the microorganism.
Liu et al., 1995 (101)	Mixed (Aerobic)	Naphthalene	Brij 30 Triton X-100	Nonionic	> CMC	0	The objective of this study was to determine whether micellar PAH was available for uptake by PAH degrading organisms. Experiments were performed in the absence of crystalline PAH. Neither surfactant inhibited naphthalene degradation and the authors conclude that micellized PAH is bioavailable.
Tsomides et al., 1995 (88)	Mixed (Aerobic)	Phenanthrene	Alfonic 810-60 Novel II 1412-70 Neodol 25-7, 25-9 Triton X-100, X-114 Tween 60	Nonionic	> CMC	See R&C	All surfactants with the exception of Triton X-100 were found to inhibit phenanthrene biodegradation. The observed inhibition was deemed to be toxicity, however, the potential for the surfactants to serve as a preferential substrate was not explored. In sediment-free microcosms containing Triton X-100 (1% w/v), phenanthrene mineralization lagged that observed in surfactant-free controls. However, overall mineralization was significantly increased in the presence of Triton X-100 after 9 to 21 days. This lag phase was not observed when microcosms were amended with sediments (2-9%), and

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
							phenanthrene degradation was again significantly higher in the presence of Triton X-100. The lack of a lag in degradation in the presence of sediment was attributed to a decrease in surfactant toxicity due to adsorption of the surfactant to the sediments.
Lee et al., 1995 (78)	Indigenous organisms from a contaminated field site (Anaerobic)	Carbon Tetrachloride (CT)	Witconol 2722 Tergitol 15-S-12	Nonionic	> CMC	+	Addition of Tergitol 15-S-12 (0.1%) to microcosms amended with soil (10%) and groundwater (90%) from a field site contaminated with CT DNAPL showed an increase in CT removal over surfactant-free controls. Bottles amended with Witconol 2722 (0.1%) removed slightly less CT than the surfactant-free controls. Reductive dechlorination was presumably supported by biodegradation of the surfactants; however, direct evidence of this assumption was not presented.
Thibault et al., 1996 (76)	*Pseudomonas* spp. (Aerobic)	Pyrene	SDS Witconol SN70 Simple Green Biosolve	Anionic Nonionic	> CMC	See R&C	Witconol SN70 was the most effective of the four surfactants in enhancing desorption of pyrene from sediments and therefore, was the only surfactant used in biodegradation experiments. Under unsaturated soil conditions, addition of Witconol at a concentration exceeding the CMC (0.2% v/v) enhanced mineralization of pyrene 10-20% over the surfactant-free treatment. Under saturated conditions, mineralization was inhibited in the presence of surfactant. The results indicate that surfactant washing of PAH in the unsaturated vadose zone may enhance PAH biodegradation.
Van Hoof and Jafvert, 1996 (82)	2 mixed cultures- 1 high activity, 1 low activity (Anaerobic)	Hexachloro-benzene (HCB)	Brij 30, 35, 58 Tween 80, 85	Nonionic	>,< CMC	See R&C	Rate and extent of HCB dechlorination was reduced in all microcosms amended with Tween surfactants at concentrations above 0.16 x CMC (0.15%) compared to surfactant free controls. For concentrations ranging from 0.0003% to 0.12% Tween, enhanced rate and extent of HCB dechlorination was observed despite no increase in the apparent aqueous phase solubility. Enhancement of

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
							dechlorination in the presence of the Tween surfactants at low concentrations was attributed to possible enhancement of membrane transport of HCB or carbon source through fluidization of the membrane caused by interaction with the sorbitan fatty acid ester. POE alcohol surfactants (Brij) inhibited (> CMC) or had no effect (< CMC) on the rate and extent of HCB degradation. Direct interactions of POE surfactants, particularly the hydrophobic dodecyl chain of Brij 30 and 35, with the bacterial membrane and/or other vital membrane bound proteins was presumed to account for the irreversible lack of HCB dechlorination activity in the presence of these surfactants. All surfactants were said to be degraded by the mixed culture, evidenced by a loss in enhanced HCB solubility and gas accumulation. However, measurements of surfactant concentration by the Picrate method did not support this conclusion.
Tiehm et al., 1997 (77)	Mixed (Aerobic)	PAH	Arkopal N-300 Sapogenat T-300	Nonionic	> CMC	+	Desorption of PAH from contaminated soil was enhanced by surfactant addition in recirculating soil columns. The solubilized PAH was determined to be bioavailable. Inhibition of PAH degradation by the Arkopal surfactant was attributed to rapid degradation of the surfactant resulting in depletion of oxygen. The Sapogenat surfactant was degraded much more slowly and enhanced PAH degradation was observed over the surfactant-free recycle period.
Rothmel et al., 1998 (89)	ENV 435 (Aerobic cometabolism)	TCE	SDS Polystep A-11, A-16 Ninate 411 Stepantan DT-60 Biosoft N-300,	Anionic	>,< CMC	See R&C	Twelve surfactants, ten anionic and two nonionic, were screened for their potential toxicity towards aerobic TCE degrading microorganisms. Toxicity of surfactants at concentrations of 0.025% and 0.1% (> CMC for nonionics, but < CMC for anionics) was determined by comparing cell counts in the presence of surfactants to cell counts in surfactant-free controls after 7 days of

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
			D-40 Petronate L Bioterge AS-40 (AOS) Steol CS-330 Tergitol 15-S-12 Triton X-705	Nonionic			incubation. Survival of TCE degraders was highest for the nonionic surfactants with 70% surviving in the presence of 0.1% Triton X-705 and 39% surviving in 0.1% Tergitol 15-S-12. The anionic surfactants Steol CS-330 and Petronate L were the least toxic of the anionic surfactants with 16% and 26% of the cells surviving in the presence of the these surfactants at 0.1%, respectively. Studies were also performed to examine the effect of Steol CS-330 foam versus Steol CS-330 solution on cell survival. Results from this experiment indicate that cell survival was drastically higher in the presence of the surfactant foam. In this study, surfactant foam was utilized to enhance mobility of TCE DNAPL in sand columns resulting in residual TCE concentrations nontoxic to the TCE degrading strain (< 500 μg/g). Surfactant foam was also used to enhance delivery of the microorganisms into the sand columns demonstrating the potential for the use of surfactant foam in bioaugmentation of contaminated aquifers.
Yeh et al., 1998 (90)	Mixed (Anaerobic)	HCB	SDS Brij 30, 35 Witconol SN-70, 90, 120 Tween 20, 21, 40, 60, 61, 65, 80, 81, 85	Anionic Nonionic	> CMC	See R&C	Surfactants were screened for potential toxicity and biodegradability by a methanogenic consortia enriched on HCB. At a surfactant concentration of 200 mg/L, all non-Tween surfactants inhibited the extent of methane production. The Tween surfactants did not inhibit extent of methane production over a period exceeding 80 days. The rate of methane production was slowest for microcosms amended with Tween 20, 21, and 80 and fastest in bottles amended with Tween 40, 60, and 85. Attempts to correlate methane production with various surfactant properties such as HLB number, carbon number, and number of EO groups were unsuccessful and it was concluded that a combination of fatty acid group and number of EO groups is responsible for the observed effects on methane produced. Experiments investigating

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
							the biodegradability of the surfactants showed that the Tween surfactants were slowly biodegraded, while the non-Tween surfactants were not.
Yeh et al., 1999 (91)	Mixed (Anaerobic)	HCB	Tween 20, 21, 40, 60, 61, 65, 80, 81, 85	Nonionic	>,< CMC	See R&C	No adverse effects on rate or extent of HCB dechlorination were observed for surfactants at 10 mg/L (< CMC) in either culture. At surfactants concentration of 200 mg/L (>CMC), all surfactants slowed dechlorination, but did not affect the extent as all HCB was degraded after 35 days (degraded after 12 days in surfactant-free control). At surfactant concentrations of 1000 mg/L (~100 x CMC), only bottles amended with Tween 61 and 65 transformed all HCB present. Lack of dechlorination in the presence of other Tween surfactants was attributed to some form of toxicity or inhibition due to the surfactant rather than a decrease in bioavailability due to micellar partitioning. Overall, the authors conclude that surfactants with similar properties, even within a homologous series, can have largely differing impacts on biological processes, such as methanogenesis and reductive dechlorination.
Macur and Inskeep, 1999 (83)	Mixed (Aerobic)	Phenanthrene Hexadecane	Witconol SN70	Nonionic	>,< CMC	>,- <,+	Effects of surfactant addition at concentrations ranging from 0 to ~3 x CMC on the degradation of phenanthrene and hexadecane in soil was studied via batch and column experiments. Degradation rates of phenanthrene and hexadecane were increased above that of controls at surfactant concentrations below the CMC. However, degradation rates were reduced at surfactant concentrations exceeding the CMC. Enhanced rates of contaminant degradation at concentrations below the cmc were possibly due to enhanced contact between the cell, the soil, and the NAPL resulting in increased mass transfer rates and/or increased contaminant uptake due to membrane fluidity. Inhibition of contaminant

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
							degradation at surfactant concentration exceeding the CMC was attributed to decreased bioavailability due to micellar solubilization or influences of sorbed phase surfactant that decreased cell adhesion and hydrocarbon mass transfer across interfaces. Degradation of the surfactant was evidenced by increased CO_2 evolution at surfactant concentrations of ~0.5 x CMC and above without concomitant decrease in hydrocarbon concentration.
Colores et al., 2000 (92)	Mixed (Aerobic)	Phenanthrene Hexadecane	Witconol SN70	Nonionic	>,< CMC	>,- <,0	Witconol SN70 was added to soil microcosms at concentrations directly above and below the CMC (13 mg/g). At concentrations below the CMC (2 mg/g) degradation of phenanthrene and hexadecane was unaffected. However, at a concentration near the CMC (10 mg/g), phenanthrene was not degraded and hexadecane degradation was delayed. Mineralization of both contaminants was inhibited at a surfactant concentration exceeding the CMC (40 mg/g). PCR amplification of the 16S rRNA gene indicated that *Rhodococcus* and *Nocardia* sp. dominated surfactant-free microcosms. Upon addition of supra-CMC surfactant concentrations, the dominant species in microcosms shifted to *Pseudomonas* and *Alcaligenes*. The authors determined that the *Pseudomonas* sp. was capable of degrading hexadecane while the *Alcaligenes* sp. can degrade both hexadecane and Witconol SN70. This study indicates that surfactant addition above the CMC may result in population changes of a mixed hydrocarbon degrading culture.
Cort and Bielefeldt, 2000 (93)	*Sphingomonas chloro-phenolicium* sp. (Aerobic)	Pentachloro-phenol (PCP)	CTAB SDS Triton X-100 Tergitol	Cationic Anionic Nonionic	>,< CMC	See R&C	The cationic surfactant, CTAB, inhibited PCP degradation at all concentrations above and below the CMC. The anionic surfactant, SDS, was found to be inhibitory at concentrations exceeding 50 mg/L (~0.025 x

Author(s)	Culture(s)	Contaminant(s)	Surfactant(s)	Type	Conc.	Effect[a]	Results and Conclusions
			NP-10				CMC). Only in the presence of the nonionic surfactants was PCP degradation observed at surfactant concentrations exceeding the CMC. At 16-20 x CMC (1,000-3,000 mg/L), however, even the nonionic surfactants showed inhibition of PCP degradation. PCP degradation was enhanced at nonionic surfactant concentrations near their CMCs. The authors suggested that toxicity of the cationic and anionic surfactants was due to the interaction of the ionic charge with the microorganisms. The inhibition of PCP degradation in the presence of the nonionic surfactants at high concentrations was speculated to be decreased bioavailability of PCP due to micellar solubilization. The authors concluded that observed enhancements of PCP mineralization at low nonionic surfactant concentrations (≤ CMC) was possibly due to beneficial fluidization of the cell membrane.
Yeh and Pavlostathis, 2001 (100)	Mixed (Anaerobic)	HCB	Tween 60, 61, 65	Nonionic	> CMC	0	The three Tween surfactants served as the sole carbon source to support methanogenesis and reductive dechlorination of HCB. The carbon source for three HCB dechlorinating cultures was changed from glucose and methanol to surfactant only (100 – 400 mg/L) over a period of one year. Rate, extent, and pathway of HCB dechlorination were the same when Tween served as the carbon source as when glucose and methanol were utilized. This study shows that fermentation of the Tween surfactants by a mixed anaerobic culture can sustain reductive dechlorination of HCB.

[a] >/< = Concentration of surfactant was greater than or less than the CMC; 0 = No effect on biotransformation; – = Inhibited biotransformation; + = Enhanced biotransformation; See R&C = See Results and Conclusions for effect

low levels of surfactant (*81*) or interactions of the surfactant with cellular membranes resulting in increased substrate uptake (*82,83*) explained the enhancement.

Inhibition of microbial activity, ranging from decreased rates and/or extents of biodegradation to complete inhibition of activity, in the presence of surfactants is also a common observation (*83-93*). The inhibiting activity of surfactants is usually accounted for via one or a combination of three mechanisms: decreased bioavailability of the contaminant due to micellar solubilization or decreased sites of microorganism attachment due to adsorption of the surfactant to the contaminant (*83,84*), uptake of the surfactant as a preferential substrate over the contaminant (*77,86,92*), or toxicity of the surfactant to the microorganism (*82,85-89,91*). Elucidating the exact mechanism of inhibition, however, is often difficult or impossible, especially when investigating surfactant effects on a mixed culture. As evident from Table 2.2, toxicity of the surfactant, especially at higher concentrations, is the most common explanation for inhibition of biodegradation given in the literature.

Surfactants interact with microorganisms in two ways, each capable of resulting in toxicity (*39,73*). First, surfactants may interact with the lipid structure of the cellular membrane resulting in membrane fluidization. This fluidization of the membrane can lead to loss of cellular material to the exterior of the cell. Second, surfactants may react with membrane bound proteins that are vital to cell function. The charged groups of the ionic surfactants are capable of interacting with positively charged amino groups and negatively charged carboxylate groups of proteins, as well as with metals contained in enzymes. The hydrophobic moiety of surfactants is also capable of associating with the

hydrophobic portions of proteins. In either case, these interactions can lead to reversible or irreversible inactivation of protein function (*39*).

Some general trends relating to the toxicity of surfactants toward microorganisms do exist. Among the classes of surfactants, cationics are typically most toxic, followed by anionics, with the nonionics being most "benign" to microorganisms. Researchers have determined that antibacterial activity increases for anionic surfactants in the presence of divalent metals (*94*) and at low pH (*39,73*). The mechanism for the enhanced toxicity appears to be increased interaction of anionic surfactants with the cellular membrane. Although the cell membrane carries a net negative charge at neutral pH, the presence of divalent cations or a lowered pH reduces the net negative charge of the bacteria and repulsive forces between the anionic surfactant and the microorganism are lessened. Surfactants have been shown to generally be more toxic towards gram-positive bacteria rather than gram-negative due to different cell envelope structures of the two (*39,72*). Within homologous series of surfactants, increased toxicity is usually observed with increasing carbon number of the hydrophobe. In the case of ethoxylated surfactants, toxicity generally decreases with increasing length of the ethoxide chain (*39*). These trends, however, are not ubiquitous in research evaluating the effects of homologous series of surfactants on contaminant biodegradation. Rouse *et al.* (*87*) found that among a series of diphenyl oxide disulfonate (DPDS) surfactants with hydrocarbon chains ranging from C_6 through C_{22}, microcosms containing the medium length carbon chain (C_{10} to C_{16}) surfactants showed increased naphthalene oxidation rates over those containing the C_6 and C_{22} hydrocarbon chain surfactants. Yeh *et al.* (*90,91*) examined the effects of nine Tween (polyoxyethylene sorbitan fatty acid ester) surfactants on

methanogenesis and reductive dechlorination of hexachlorobenzene (HCB) in a mixed anaerobic culture. Attempts to correlate methane production with various surfactant parameters such as number of ethoxide groups, number of carbons in the fatty acid chain, and HLB number were unsuccessful. HCB dechlorination patterns among the various Tween amended microcosms also showed a lack of correlation to surfactant properties. The authors concluded that the observed results were due to some combination of hydrocarbon chain length and degree of ethoxylation of the surfactant and that surfactants within a homologous series can have widely varying impacts on biological processes such as methane production and reductive dechlorination.

2.5 Surfactant Biodegradation

Mass production of synthetic surfactants began in the 1950's and their appearance in wastewaters, treated sewage, and receiving streams was nearly immediate due to incomplete biodegradation of these compounds. The exposure of surface-active compounds to the environment, however, is not limited to the time since commercialization of these chemicals. Many bacteria are known to secrete biosurfactants that often possess structures and physical-chemical properties similar to synthetic surfactants, therefore the prevalence of microbial enzyme systems that transform commercial surfactants is not surprising. Surfactants, while inhibiting the activity of certain microorganisms, are capable of being utilized by some microorganisms as a source of carbon and energy.

Degradation of surfactants by microorganisms is typically facilitated under aerobic conditions rather than anaerobically and may occur to different degrees in either

situation (*39*). Surfactant biodegradation resulting in the loss of the surface-active properties of the molecule is termed primary biodegradation. This extent of biodegradation can also be defined by the lack of an analytical response to a method aimed at detecting the undegraded surfactant. Ultimate biodegradation is characterized by complete mineralization of the surfactant to carbon dioxide, water, and applicable inorganic endproducts.

Surfactant biodegradability is typically researched under conditions representative of wastewater treatment operations (*39*), implying low concentrations of surfactant (~10 mg/L) and an aerobic environment. Most surfactants are readily degraded under these conditions as evidenced by an extensive review of the subject (*39*). These studies, however, may not be useful in determining the fate of a surfactant employed for the remediation of chlorinated ethene contamination since this process requires higher surfactant concentrations and occurs in an anaerobic environment. Few studies examining surfactant biodegradation at high concentrations and/or under anaerobic conditions exist.

Zhang *et al.* (*95*) examined the aerobic biodegradation of four surfactants at concentrations exceeding their CMC values by a mixed culture. The two anionic surfactants screened, sodium dodecyl sulfate (SDS) and sodium dodecylbenzene sulfonate (SDBS), yielded differing results with respect to biodegradation. SDS at a concentration of 0.25% (w/v) was 80% primarily biodegraded and 40% ultimately biodegraded, while SDBS was not biodegraded over the three-week period of the experiment. The authors suggest that the presence of the aromatic ring in SDBS renders the compound resistant to biodegradation by their culture. Two nonionic surfactants,

Tergitol 15-S-12 and Witconol SN-90, each showed both primary and ultimate biodegradation, but to a lesser extent than SDS. The authors hypothesized that an observed reduction in biodegradation from sub- to supra-CMC concentrations may be due to decreased bioavailability of surfactants in micellar form.

Two bacterial strains capable of utilizing the nonionic surfactants Brij 30 ($C_{12}EO_4H$), Brij 35 ($C_{12}EO_{23}H$), or Brij 58 ($C_{16}EO_{20}H$) at concentrations of 1,000 mg/L as the sole carbon and energy source were isolated from anaerobic sludge enrichment cultures (*96*). The linear alkyl ethoxylates (LAE) were completely fermented to methane and carbon dioxide with fatty acids (acetate, propionate, laurate, and palmitate) forming as intermediates. These results suggest that fermentation of surfactants may be capable of supporting other microbial processes, such as reductive dechlorination. Huber *et al.* (*97*) studied the mechanism of anaerobic LAE biodegradation further. They determined the initial reaction of anaerobic biodegradation is cleavage of the terminal EO group resulting in the release of acetaldehyde. The EO chain is progressively shortened in this manner until the hydrophobe is reached. This mechanism is in contrast to the aerobic pathway in which cleavage of the ether bond between the hydrophobe and the EO chain is the initial attack.

Federle and Schwab (*98*) investigated the biodegradation of cationic, anionic, and nonionic surfactants in anaerobic microcosms containing sediments from a laundromat wastewater pond that received high concentrations of surfactants. The cationic surfactant, stearyltrimethyl ammonium chloride, and the anionic surfactant, sodium tridecylbenzene sulfonate, were not mineralized after 87 days of incubation. The nonionic surfactant, a mixture of $C_{12}EO_9OH$ (70%) and $C_{12}EO_8OH$ (30%), was

mineralized approximately 23.5 – 39.5% to methane and CO_2 by the pond sediment bacteria. Specific inhibitor studies showed that degradation of the nonionic surfactant depended on the activity of both methanogenic and sulfate reducing organisms.

Salanitro and Diaz (*99*) examined the biodegradation of low levels (5 – 50 mg/L) of anionic and nonionic surfactants using anaerobic municipal digester solids. They found that all surfactants were primarily degraded (89 – 99%) by the anaerobic culture after a period of 40 to 50 days. Ultimate biodegradation was found to be higher (60 – 85% of the theoretical CH_4 recovered) for the linear alcohol sulfates (C_{12-14}- and $C_{14-15}OSO_3^-$) and the linear alcohol ethoxylates ($C_{9-11}EO_8OH$) than for the branched alkyl phenol ethoxylate (32 – 43% of the theoretical CH_4 recovered) over the 40 to 50 days of the experiment.

Yeh and Pavlostathis (*100*) recently demonstrated the use of Tween surfactants as the sole source of carbon to support methanogenesis and reductive dechlorination of hexachlorobenzene (HCB) in mixed anaerobic cultures. Over a period of one year, the cultures were acclimated to growth on Tween 60, 61, or 65 at concentrations of 100 – 400 mg/L. Fermentation of the Tween surfactants was able to sustain rate and extent of HCB reductive dechlorination compared to the pre-surfactant period during which dechlorination was supported by the fermentation of glucose and methanol. Interestingly, volatile fatty acids (VFA) were not detected during the period when Tween served as the carbon source indicating that the shortening of the hydrophobic carbon chain into short chain VFA was rate-limiting and that any VFA formed was immediately converted to methane.

Evidence of surfactant biodegradation under anaerobic conditions exists. However, extrapolating results of these studies, typically carried out at low surfactant concentrations, to determine the fate of surfactants in aquifer remediation processes may be unreliable. Higher surfactant concentrations typical of use in aquifer remediation may have inhibiting effects on certain organisms or require much longer acclimation periods before their biodegradation commences. The nonionic surfactants are typically the most readily biodegradable of the three surfactant classes and fermentation of these compounds may be capable of supporting growth of other organisms, such as halorespirators. Cationic and anionic surfactants are the most resistant to biodegradation, with the cationics exhibiting more resistance, or perhaps toxicity, than anionics.

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3. Materials and Methods

3.1 Materials

3.1.1 Chemicals

The following chemicals were obtained in liquid form: tetrachloroethene (PCE) (99+%, Acros), trichloroethene (TCE) (99.5%, Acros), *cis*-dichloroethene (*cis*- DCE) (97%, Acros), ethanol (200 proof, Pharmco), chloroform (99.9%, Fisher Scientific), 1,2-dichloroethane (99.9%, Fisher Scientific). The following chemicals were obtained in solid form: methylene blue chloride (Acros) and tetrabromophenolphthalein ethyl ester potassium salt (Sigma-Aldrich). Gaseous chemicals obtained from Trigas included vinyl chloride (VC) (8%, balance N_2) and hydrogen (80%, balance CO_2). Ethene (ETH) (99.5%) and methane (99%) were obtained from Scott Specialty Gases.

3.1.2 Nutrient Medium

Reagent-grade chemicals and deionized water were used in the preparation of nutrient medium. The medium consisted of the following: 400 mg/L NH_4Cl, 400 mg/L KCl, 400 mg/L $MgCl_2 \cdot 6H_2O$, 140 mg/L KH_2PO_4, 25 mg/L $CaCl_2 \cdot 2H_2O$, 10 mg/L $(NaPO_3)_{13}$, 2.5 mg/L KI, 2.5 mg/L $CoCl_2 \cdot 6H_2O$, 0.5 mg/L $ZnCl_2$, 0.5 mg/L $MnCl_2 \cdot 4H_2O$, 0.5 mg/L H_3BO_3, 0.5 mg/L $NiCl_2 \cdot 6H_2O$, 0.5 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg/L NH_4VO_3, 200 mg/L yeast extract, and $NaHCO_3$ as needed to buffer the systems (ranging in concentration from 2 g/L to 3 g/L).

3.1.3 Surfactants

The following anionic surfactants were obtained in liquid solution: Steol CS-330 (28% active) purchased from Stepan Company (Northfield, IL), Aerosol MA 80-I (80% active) supplied by Cytec (West Paterson, NJ), AOS 14-16 (39% active) supplied by

Shell Chemical Company (Houston, TX). Steol CS-330 is an alkyl ethoxy sulfate containing a C_{12} hydrophobe and is ethoxylated to an average of 3 EO groups. Aerosol MA 80-I is the trade name for sodium dihexylsulfosuccinate. AOS 14-16 is a mixture of alpha olefin sulfonate and hydroxyalkane sulfonate. The numbers 14-16 indicate the average number of carbon atoms in the hydrophobic carbon chain.

The following nonionic surfactants were obtained in liquid form: Neodol 25-7 (100% active) supplied by Shell Chemical Company (Houston, TX), Tween 80 (100% active) purchased from Sigma Chemical Company, and APG (50% active) obtained as a gift from the lab of Dr. Clarence Miller (Rice University, Houston, TX). Neodol 25-7 represents a linear alcohol ethoxylate surfactant and is commonly used in household detergents. The number 25 indicates that the average number of carbons in the hydrophobic carbon chain is 12 to 15, while the number 7 represents the average number of ethoxide groups. Tween 80 is representative of the ethoxylate sorbitan ester surfactant class. This particular surfactant contains an average of 20 ethoxide groups and an oleic fatty acid group. Tween 80 is a food grade surfactant and has common uses in food and pharmaceutical products. The APG surfactant represents an alky polyglycoside and is a mixture of $C_{10\text{-}16}$ D-glucopyranoside (16.7%) and $C_{8\text{-}10}$ D-glucose (33.3%).

The cationic surfactant, hexadecyltrimethylammonium bromide (C_{16}TAB), was obtained in solid form from Lancaster Synthesis (Pelham, NJ). This surfactant is used in fabric softening products as well as personal care products. A summary of physical and chemical properties of all surfactants used is listed in Table 3.1. All surfactants were used as received without purification.

Table 3.1 Properties of Surfactants Utilized in Experiments

Surfactant	Chemical Class	Chemical Formula[a]	Avg. MW g/mole	CMC[b] mg/L (mM)	COD[c] g O_2/L
Neodol 25-7	Nonionic	$CH_3(CH_2)_{11\text{-}14}O(EO)_7H$	515	9 (0.02)	19.8 ± 0. 2[d]
APG	Nonionic	See section 3.1.3	404	45 (0.11)	ND[e]
Tween 80	Nonionic	$C_{18}S_6(EO)_{20}$	1308	13 (0.01)	15.6 ± 0.2
Aerosol MA 80-I	Anionic	$[(C_2H_5)_2CHCH_2COOCH]_2SO_3Na$	388	7100 (18.3)	15.7 ± 0.5
AOS 14-16	Anionic	$CH_3(CH_2)_{10\text{-}12}CH{=}CHCH_2SO_3Na$	315	1200 (3.81)	16.6 ± 0.4
Steol CS-330	Anionic	$CH_3(CH_2)_{11}(EO)_3OSO_3Na$	422	449 (1.07)	16.5 ± 0.2
C_{16} TAB	Cationic	$CH_3(CH_2)_{15}(CH_3)_3NH_4Br$	364	361 (0.99)	ND

[a] EO = ethylene oxide, S_6 = sorbitan ring
[b] CMC, critical micelle concentration (provided by manufacturer)
[c] COD, chemical oxygen demand
[d] Concentrations for 1% (w/w) solution
[e] ND, not determined

3.2 Analytical Methods

3.2.1 Chromatography Methods

Gas chromatography was used to determine the aqueous and gas phase concentrations of all chlorinated ethenes, ethene, and methane. In experiments where the predominant dechlorination products were vinyl chloride and ethene or surfactant concentrations were below the CMC, the following headspace analysis was used to determine total concentrations of all chlorinated ethenes, ethene, and methane. Headspace samples (100 μL) were directly injected onto a GC (Hewlett-Packard 5890) equipped with a flame ionization detector (FID) and a packed column (6 ft x 1/8 in. OD) containing 60/80 Carbopack B/1% SP-1000 (Supelco). The operating parameters were as follows: 40 °C, hold 2 min., ramp at 20 °C/min. to 150 °C, ramp at 10 °C/min. to 200 °C, hold 10 min. The detector temperature was set at 275 °C and the injection port at 200 °C. The flow rates for the gases were: helium (12 mL/min.), hydrogen (40 mL/min.), zero air (460 mL/min.). Standards were prepared by adding *cis*-DCE, TCE, and PCE dissolved in methanol, and VC, ETH, and methane gases, all at known volumes, to a serum bottle (70 mL) containing deionized water (40 mL). This method was also used to quantify the gas phase concentrations of PCE, TCE, and *cis*-DCE in experiments where the surfactant concentration exceeded the CMC.

Since PCE, TCE, and *cis*-DCE partition into the hydrophobic core of surfactant micelles, a second gas chromatography method was developed to quantify aqueous phase concentrations of these species when surfactant concentrations exceeded the CMC. The method approximates EPA method 502.2 and involves the use of a purge-and-trap apparatus coupled with a gas chromatograph. Aqueous samples (10 μL) were diluted in

40 mL deionized water (4000:1 dilution factor) contained in 40 mL screw top vials sealed with screw caps and Teflon-lined silicone rubber septa. The 4000:1 dilution factor was necessary to prevent surfactant foaming in the purge chamber of the purge-and-trap apparatus. The purge-and-trap apparatus (OI Analytical Model 4560) was equipped with the following: 51 place autosampler (OI Analytical Model 4551), 5 mL purge chamber, infrared heater, OI No. 9 Trap containing silica gel, Tenax resin, and activated charcoal. The operating parameters of the purge-and-trap apparatus were as follows: 40 mL/min. purge rate, 11 minute purge time, 80 °C sparge temperature, 185 °C desorb temperature, 2 minute desorb time, 100 °C transfer line temperature, 185 °C bake temperature, and 12 minute bake time. The GC (Hewlett-Packard 6890) was equipped with a flame ionization detector (FID) and a Hewlett Packard VOCOL capillary column (105 m x 0.53 mm x 3 mm) and was operated using Hewlett Packard Chem Station software. The operating parameters were as follows: 40 °C, hold 5 min., ramp at 10 °C/min. to 150 °C, ramp at 30 °C/min. to 200 °C, hold 5 min. The detector temperature was set at 250 °C and the injection port at 250 °C. The flow rates for the gases were: helium (10 mL/min.), hydrogen (30 mL/min.), zero air (400 mL/min.).

The high dilution factor (4000:1) necessary to prevent surfactant foam carry over from the purge chamber into the trap resulted in detection limits higher than desired. The detection limit was highest for PCE at approximately 12 mg/L, which corresponds to 3 μg/L in the diluted sample. In order to quantify chlorinated ethenes at concentrations below the detection limit at this dilution factor, a standard addition (5 μL) of PCE, TCE, and *cis*-DCE dissolved in methanol was added at concentrations of 60 mg/L, 48 mg/L, and 35 mg/L, respectively. The value for PCE corresponds to a final concentration of 7.5

μg/L in the diluted sample, 2.5 times the detection limit. Standards were prepared by adding PCE, TCE, and *cis*-DCE dissolved in methanol to a serum bottle (70 mL) containing deionized water (40 mL). The 4000:1 dilution was then carried out and the standard addition solution was added to the vial. With each set of samples or standards analyzed, three vials containing only deionized water and the standard addition solution were analyzed. These vials corresponded to a concentration of 0 mg/L analyte in the original sample and the mean response obtained for each analyte represented the y-intercept of the calibration curve for each respective compound.

3.2.2 COD Measurements

Chemical oxygen demand (COD) of each surfactant was measured for a surfactant concentration of 1.0% (w/w). The COD of each of surfactant was determined in order to calculate the theoretical amount of methane that would be produced if all of the surfactant were degraded to methane and CO_2. Based on stoichiometry, 0.35 L of methane is produced per g of COD degraded.

Samples (250 μL) of the 1.0% surfactant solution were diluted in volumetric flasks (25 mL) containing deionized water. The flasks were shaken thoroughly, then 2.5 mL of the dilute surfactant solution was pipetted into Bioscience, Inc. 20-900 mg/L range COD vials. The COD vial containing the surfactant sample was shaken vigorously then placed in a COD heat block preheated to 150 °C. The vials were allowed to react in the COD heat block for a period of 2 hours after which they were removed and allowed to cool to room temperature for approximately 1 hour. The COD vials were analyzed via a Hach DR/2000 spectrophotometer set to 600 nm (Method 435). The spectrophotometer

was blanked with a COD vial containing deionized water prior to analyzing samples and the COD was reported in mg/L.

3.2.3 Surfactant Measurements

Aqueous phase anionic surfactant concentrations were measured via a colorimetric method modified from the Methylene Blue Active Substances (MBAS) method from *Standard Methods for the Examination of Water and Wastewater* (*1*). The method involves the formation of an ion pair between the anionic moiety of the surfactant (sulfate, sulfonate, or sulfosuccinate group in this case) and the methylene blue cation. This colored complex is then extracted into an organic solvent and the absorbance of the extract is measured.

Samples (10 μL) containing anionic surfactant were added to separatory funnels (250 mL) containing deionized water (50 mL), methylene blue reagent (15 mL), and chloroform (10 mL). A few drops of hydrogen peroxide (30%) were added to oxidize any sulfides present in the sample thereby eliminating their interference. The separatory funnel was gently swirled for approximately one minute then was allowed to settle 5 minutes for phase separation. An initial aliquot was withdrawn into a cuvette (1 cm) and discarded. Two additional aliquots were withdrawn and their absorbance was measured using a Turner SP-830 spectrophotometer at 652 nm. The average absorbance of the two aliquots was compared to a standard calibration curve, obtained using sodium dodecylsulfate (SDS) (98%, Aldrich) as the reference anionic surfactant, to determine the concentration of anionic surfactant in the sample.

Nonionic surfactants in the aqueous phase were measured using a colorimetric method described by Toei et al. (*2*). In this method, the ethylene oxide chain of the

nonionic surfactant reacts with potassium ions forming a colored complex that is extractable as an ion-pair with tetrabromophenolphthalein ethyl ester (TBPE) in an organic solvent.

Samples (5 μL) containing nonionic surfactant were added to test tubes (25 mL) containing deionized water (3.6 mL), 2 M KCl solution (2.0 mL), and 1.5×10^{-3} M tetrabromophenolphthalein ethyl ester potassium salt (TBPE-K) solution (0.7 mL). The pH was then buffered (to approximately 7.8) by adding a solution (0.7 mL) consisting of 1M KH_2PO_4 mixed with 1M K_2HPO_4. Finally, 1,2-dichloroethane (5 mL) was added as the extracting solvent. The test tubes were stoppered and shaken for 15 minutes then 5 minutes were allowed for phase separation after which two aliquots were withdrawn via pipet and placed in cuvettes (1 cm). The absorbance of each aliquot was measured at 609 nm using a Turner SP-830 spectrophotometer. Nonionic surfactant concentrations in the samples were determined by comparison to standard calibration curves generated from the surfactant being measured.

3.3 Cultures

3.3.1 Mixed Dechlorinating Culture 1

The suspended growth culture used for the microcosm dechlorination experiments and the surfactant biodegradation experiment was maintained as a fill-and-draw bioreactor in a glass bottle (2.3 L, 1,400 mL liquid, 900 mL headspace) modified with a sidearm sampling port. The bottle was sealed with an open-top screw cap containing a stainless steel plug and a Viton O-ring. A stainless steel tube extended to the bottom of the reactor and was used to exchange medium. Twice a week, an aliquot of culture (120

mL) was removed and replaced with fresh nutrient medium (hydraulic retention time of 40.8 days). Following withdrawal of culture and addition of nutrients, the culture was purged with H_2/CO_2 gas to remove dechlorination products. Electron donor (H_2/CO_2 gas) was added daily as needed via a syringe. Neat PCE (10 – 40 μL) was also added daily resulting in a final aqueous concentration of 7.9 mg/L – 31.7 mg/L. The PCE added was consistently dechlorinated to VC (70% - 97%, molar), ETH (2% - 15%), and DCE (1% - 15%) over the period of each fill-and-draw cycle.

3.3.2 Mixed Dechlorinating Culture 2

The suspended growth culture used as inoculum for the Steol CS-330 acclimation experiment was maintained as a fill-and-draw bioreactor in a glass bottle (1.1 L, 750 mL liquid, 350 mL headspace) modified with a sidearm sampling port. The bottle was sealed with a heavy rubber stopper and glass tubing extending through the stopper was used to exchange medium. Every 4 days, an aliquot of culture (60 mL) was removed and replaced with fresh nutrient medium (hydraulic retention time of 50.0 days). The hydraulic retention time in this reactor was approximately 10 days longer than that of Culture 1 in order to enhance the population of the slower growing DCE and VC dechlorinating microorganisms. Following withdrawal of culture and addition of nutrients, the culture was purged with H_2/CO_2 gas to remove dechlorination products. Electron donor (H_2/CO_2 gas) was added daily as needed via a syringe. Neat PCE (10 μL) was also added daily resulting in a final aqueous concentration of 16.4 mg/L. The PCE added was consistently dechlorinated to VC (38% - 56%, molar), ETH (44% - 62%), and trace amounts of DCE over the period of each fill-and-draw cycle. As evident from the

extent of PCE dechlorination, the longer hydraulic retention time was successful in enhancing the population of microorganisms that reduce DCE to VC and ETH.

3.4 Dechlorination Experiments

3.4.1 Microcosm Experiments

Experiments investigating the effects of surfactants on the dechlorination activity of the mixed anaerobic culture were carried out in serum bottles (12 mL for short-term experiments and 70 mL for long-term experiments) with a headspace-to-liquid ratio of approximately 0.73. The serum bottles were sealed with Teflon lined butyl rubber septa and aluminum crimp caps. Oxygen was evacuated from the systems by sparging with N_2 gas (ultra high purity) at 10 psi for about 10 minutes. The bottle was then inoculated with culture followed by sparging with H_2/CO_2 gas at 10 psi for approximately 10 minutes to remove any dissolved chlorinated ethenes, ethene, and methane remaining in the seed culture and also provide electron donor. Electron donor was replenished daily as needed via syringe. Surfactant was added immediately following the H_2/CO_2 sparge, with the exception of surfactant-free controls, followed by the addition of neat PCE. PCE was added at a liquid concentration of 40 mg/L (neglecting micellar partitioning), unless stated otherwise. Bottles were inverted and mixed continuously with magnetic stir bars on a stir plate. The effect of the surfactants on the rate and extent of PCE degradation was evaluated by sampling for remaining PCE and production of lesser-chlorinated degradation products over a period of time. Methane production and H_2/CO_2 consumption was also monitored as indicators of the effects of surfactants on other microorganisms in the mixed culture.

3.4.2 Steol CS-330 Acclimation Culture Experiment

A bottle (620 mL) modified with a sidearm sampling port and sealed with an open-top screw cap containing a stainless steel plug, a Viton O-ring, and a three-way valve was sparged with N_2 gas at 10 psi for about 30 minutes to remove oxygen from the system. Inoculum (300 mL) from Culture 2 was added to the bottle along with fresh nutrient medium (70 mL). Culture 2 was utilized to seed this experiment due to the greater extent of VC and ETH production by this culture. Next, the bottle was sparged with H_2/CO_2 gas at 5 psi for approximately 15 minutes to supply electron donor and remove any chlorinated species, ethene, and methane remaining from the inoculum. Electron donor was replenished daily as needed via syringe. The culture was maintained as a fill-and-draw, suspended growth bioreactor. Every fourth day, an aliquot of culture (36 mL) was removed and replaced with fresh nutrient medium containing a mass of Steol CS-330 surfactant required to attain the desired surfactant concentration in the culture. Following withdrawal of culture and addition of nutrients and surfactant, the headspace of the culture was purged with H_2/CO_2 gas to remove dechlorination products. The culture contained no surfactant the first 4 days of operation, hence this period served as the surfactant-free reference state. The initial surfactant addition (day 4) resulted in a concentration of 1 mg/L. After 4 days (day 8), the Steol CS-330 concentration was increased to 5 mg/L, this procedure continued with surfactant being increased to 10 mg/L (day 12), 25 mg/L (day 16), and 50 mg/L (day 20). Due to a drastic change in the dechlorination pattern at a surfactant concentration of 50 mg/L, this concentration was maintained for an additional 4 days before increasing the surfactant to 100 mg/L (day 28). The surfactant concentration was held at 100 mg/L through the remainder of the

experiment, or 8 additional days. At the end of the experiment (day 36), surfactant concentration was measured via the MBAS method and the culture was sacrificed for denaturing gradient gel electrophoresis (DGGE) analysis. Neat PCE (6 μL) was added daily, with the exception of day 21, to the culture resulting an aqueous phase concentration of 17.7 mg/L. The headspace of the culture was sampled daily for PCE, dechlorination metabolites, and methane.

On days 24, 28, and 36, an aliquot (4 mL) from the withdrawal was placed in a serum bottle (70 mL) containing fresh nutrient medium (36 mL) that had been evacuated of oxygen with N_2 gas and sealed with Teflon lined butyl rubber septa and aluminum crimp caps. This resulted in dilution of the surfactant from a concentration adversely affecting the dechlorination process to a concentration below the lowest-observed-adverse-effect level (LOAEL). Specifically, on days 24 and 28 the Steol CS-330 concentration was reduced from 50 mg/L to 5 mg/L and on day 36 the concentration was lowered from 100 mg/L to 10 mg/L. Electron donor was supplied to the bottles via sparging with H_2/CO_2 gas and was replenished via syringe as needed. Neat PCE (0.3 μL) was added to the bottles resulting in an aqueous phase concentration of 8 mg/L. Bottles were inverted and mixed continuously with magnetic stir bars on a stir plate. The headspace of the bottles was sampled weekly for remaining PCE and production of lesser-chlorinated degradation products.

3.5 Surfactant Biodegradation Experiment

The biodegradation of the surfactants by the mixed anaerobic culture was examined via batch microcosm experiments. Seed culture (20 mL) was added to serum

bottles (38 mL) sealed with Teflon lined butyl rubber septa and aluminum crimp caps. The bottles were sparged with N_2 gas (ultra high purity) to remove oxygen prior to inoculation. The bottles were then sparged with H_2 gas (ultra high purity), which acted as electron donor and removed any dissolved phase methane remaining in the seed culture. Electron donor (H_2 gas) was replenished periodically as needed via syringe. Surfactant (1.0%, w/w acitve) was added gravimetrically to the serum bottles as the sole externally added carbon source, with the exception of a surfactant-free reference bottle that contained no externally added source of carbon. Bottles were inverted and mixed continuously with magnetic stir bars on a stir plate. Initial and final surfactant concentrations were measured via the MBAS method for anionic surfactants and the TBPE-K method for nonionics. Methane production relative to the surfactant-free reference and to the maximum theoretical amount based on COD measurements was also monitored over 70 days as an indicator of surfactant biodegradation.

3.5 References

(1) Greenberg, A. E.; Clesceri, L. S.; Eaton, A. D., Eds. *Standard Methods for the Examination of Water and Wastewater*; 18 ed. American Public Health Association: Washington, D.C., 1992.

(2) Toei, K.; Motomizu, S.; Umano, T. 1982. Extractive spectrophotometric determination of non-ionic surfactants in water. *Talanta* 29:103-106.

4. RESULTS

4.1 Surfactant Screening: Effect on PCE Dechlorination Activity

Representative surfactants from each of the surfactant classes, including three nonionic surfactants, three anionic surfactants, and one cationic surfactant, were screened for their potential effects on dechlorination activity in order to select an appropriate surfactant(s) for use in further studies. The results of this experiment, which investigated the short-term effect of surfactants at a concentration of 1.0% (w/w, active) on the rate and extent of PCE reduction by the mixed anaerobic dechlorinating culture, are presented in Table 4.1. In 2-day incubations, PCE was dechlorinated to nondetectable levels in all microcosms with the exception of those amended with the anionic surfactant Aerosol MA80-I (87.1%, molar PCE remaining) and the cationic surfactant CTAB (91.2%, molar PCE remaining). The extent of PCE transformation varied widely among the surfactants screened. The nonionic surfactant, Tween 80, had very little impact on transformation of PCE to the lesser-chlorinated species. After 2 days, PCE was reduced in the Tween containing microcosm to DCE (1.8%, molar), VC (91.4%, molar), and ETH (6.9%, molar). This was the only microcosm exhibiting substantial dechlorination past DCE except the surfactant-free control, in which PCE was reduced predominantly to VC (81.3%, molar) and ETH (18.1%, molar). The nonionic surfactant, Neodol 25-7, showed nearly complete dechlorination of PCE to DCE (94.0%, molar). PCE was dechlorinated to TCE and DCE in microcosms amended with the nonionic surfactant APG and the anionic surfactants AOS 14-16 and Steol CS-330. Though the microcosm containing Steol CS-330 showed a greater extent of PCE dechlorination (76.6% TCE, 23.2% DCE) than the microcosms containing APG (88.8% TCE, 11.0% DCE) and AOS 14-16 (96.5%

Table 4.1 Screening of Surfactants for Effect on Rate and Extent of PCE Dechlorination ($[PCE]_{aq}$ = 40 mg/L; t = 2d)

	PCE and Dechlorination Metabolites Recovered (%, molar)				
Surfactant	PCE	TCE	DCE	VC	ETH
None – Control	0.0	0.0	0.6	81.3	18.1
Tween 80	0.0	0.0	1.8	91.4	6.9
Neodol 25-7	0.0	5.9	94.0	0.1	0.0
Steol CS-330	0.0	76.6	23.2	0.1	0.0
APG	0.0	88.8	11.0	0.2	0.0
AOS 14-16	0.0	96.5	2.9	0.1	0.6
Aerosol MA80-I	87.1	12.5	0.0	0.3	0.1
CTAB	91.2	0.4	8.5	0.0	0.0

TCE, 2.9% DCE). Based on the screening experiments investigating the effect of surfactants on the rate and extent of PCE reduction and experiments investigating foaming potential of the surfactants by other research groups, the nonionic surfactant Tween 80 and the anionic surfactant Steol CS-330 was selected for further investigation.

4.2 Surfactant Screening: Biotransformation of Surfactants by the Mixed Culture

Surfactants, at a concentration of 1.0% (w/w, active), were evaluated for their biodegradability by the mixed culture. Since surfactant biodegradation may occur to different extents (i.e., primary biodegradation versus ultimate biodegradation, see Section 2.5), two indicators of surfactant breakdown were used. First, direct measures of surfactant concentration were made after 70 days of incubation in microcosms where the surfactant was added as the sole source of carbon. The second indicator utilized was production of methane by the surfactant-amended microcosms. The amount of methane produced by these microcosms was compared to a surfactant-free control that contained no externally supplied source of carbon. Any methane production above that of the reference was attributed to complete metabolism of the surfactants. Methane production was also compared to the theoretical amount of methane that could be produced from complete reduction of the surfactant, which is calculated based on COD measurements of the surfactant solutions.

As evident from Table 4.2, direct measurements of surfactant made after 70 days of incubation indicate that Tween 80 is the most biodegraded surfactant screened by the mixed anaerobic culture. The remaining surfactants exhibited only slight removal by the

Table 4.2. Direct Measures of Surfactant and Methane Production as Indicators of Surfactant Biodegradation by a Mixed Anaerobic Culture ([Surfactant] = 1.0% w/w, active; t = 70 d)

Surfactant	Initial Surfactant Conc. (%)[a]	Final Surfactant Conc. (%)[a]	Percent Degraded	COD (g/L)	Theoretical CH_4 (mL)[c]	Actual CH_4 (mL)	Percent Converted
None – Reference	NA[b]	NA	NA	0.6	4	1.20	28.7
AOS 14-16	0.93	0.86	7.2	16.6	120	2.85	2.4
Steol CS-330	1.02	0.94	7.6	16.5	120	1.94	1.6
Aerosol MA80-I	0.95	0.96	-1.2	15.7	110	2.80	2.5
Neodol 25-7	1.00	0.99	1.2	19.8	140	2.97	2.1
Tween 80	1.05	0.67	36.9	15.6	110	2.03	1.8

[a] Anionic surfactants measured by MBAS; Nonionic surfactants measured by TBPE-K
[b] NA = Not Applicable
[c] Theoretical CH_4 based on 0.35L CH_4 produced per g COD

mixed culture. Table 4.2 also illustrates the fraction of methane produced relative to the theoretical maximum amount based on COD measurements. Clearly, very little of the original surfactant added is completely degraded to methane. However, Figure 4.1 does demonstrate that methane production in all surfactant-amended microcosms is enhanced over the surfactant-free reference after 70 days of incubation.

4.3 Effect of Tween 80 Concentration on PCE Dechlorination

The effect of Tween 80 at concentrations of 1.0% (w/w, active), 0.5% (w/w, active), and 0.1% (w/w, active) on the rate and extent of PCE dechlorination over a period of 15 days was investigated. Results of this experiment appear in Figures 4.2a – 4.2d. PCE was reduced nearly completely to VC after one day in all Tween amended microcosms and the surfactant-free control microcosm. Subsequent reduction of VC and production of ETH occurred slower in all Tween 80 amended microcosms compared to the surfactant-free control.

4.4 Effect of Steol CS-330 Concentration on PCE Dechlorination

4.4.1 Short-Term/High PCE Concentration Experiment

The effect of Steol CS-330 concentration was investigated in microcosms amended with Steol CS-330 at concentrations of 1.0% (w/w, active), 0.5% (w/w, active), and 0.1% (w/w, active) and PCE at 150 mg/L (aqueous phase, neglecting micellar partitioning). Results from this experiment appear in Table 4.3. In all systems, the PCE was completely transformed after only 2 days of incubation. In these short-term

Figure 4.1. Surfactant Biodegradation: Methane Production Relative to Surfactant-free Reference as an Indicator

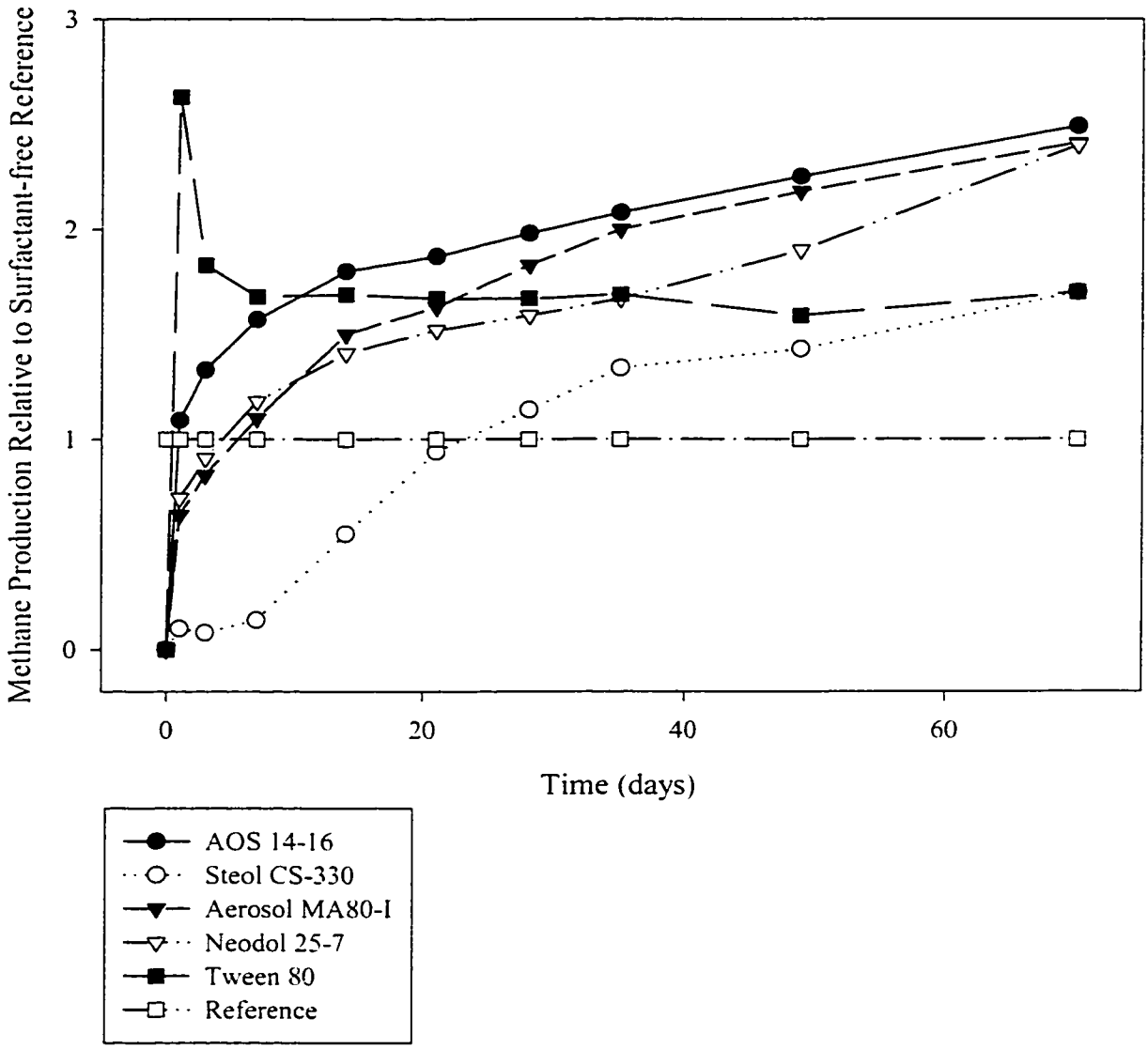


Figure 4.2a. Effect of Tween 80 Concentration on Rate and Extent of PCE Dechlorination - Surfactant-free Control

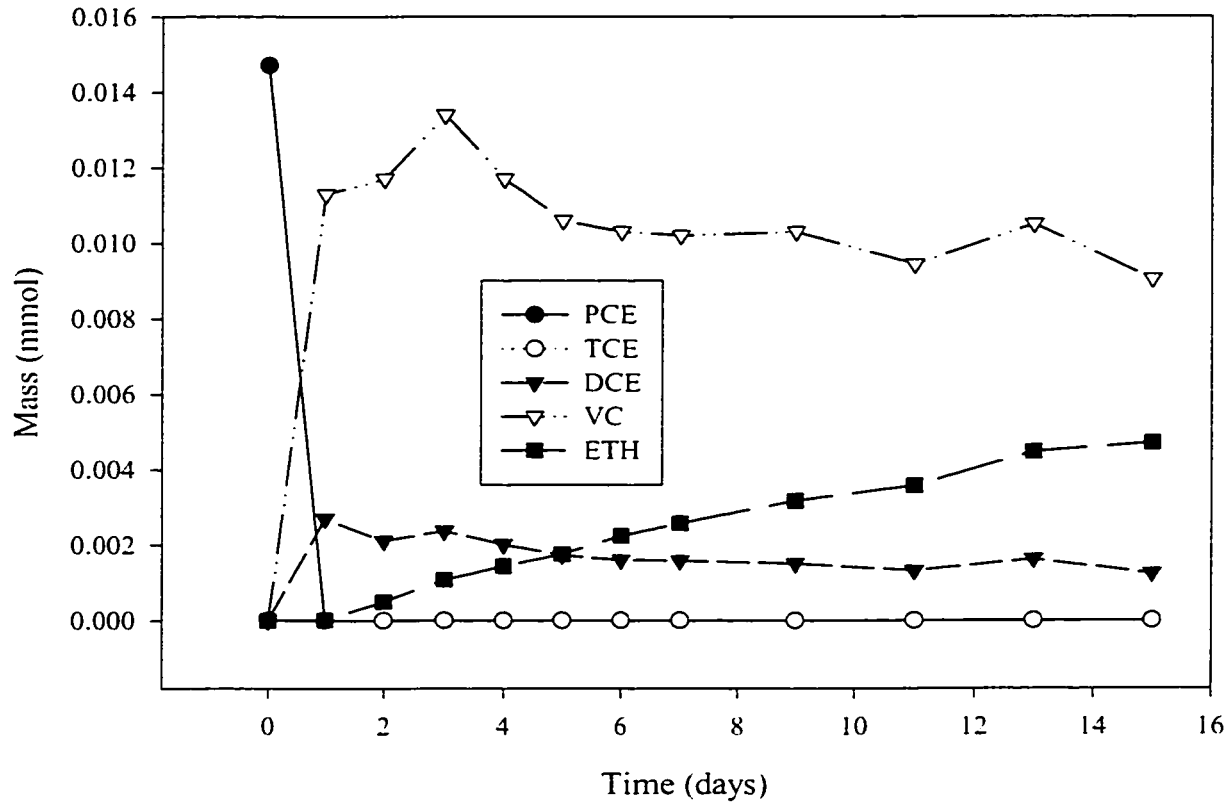


Figure 4.2b. Effect of Tween 80 Concentration on Rate and Extent of PCE Dechlorination - 0.1% Tween 80

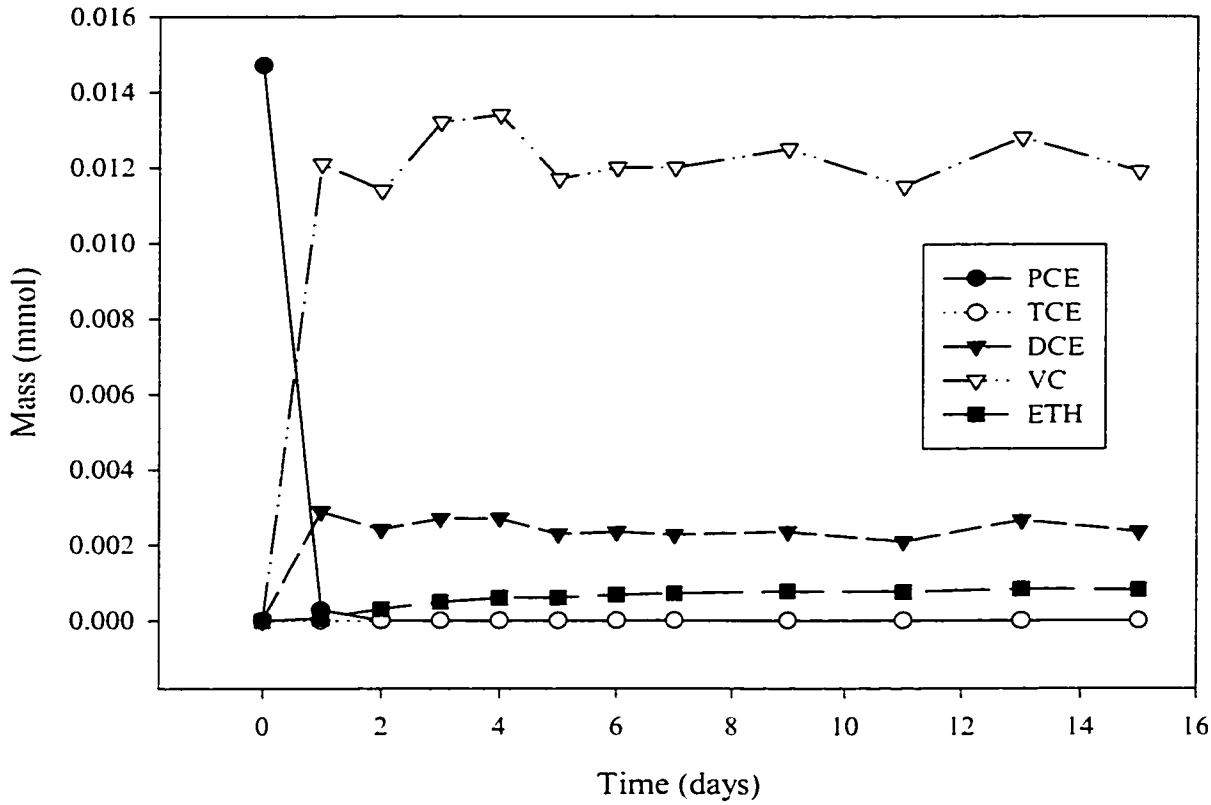


Figure 4.2c. Effect of Tween 80 Concentration on Rate and Extent of PCE Dechlorination - 0.5% Tween 80

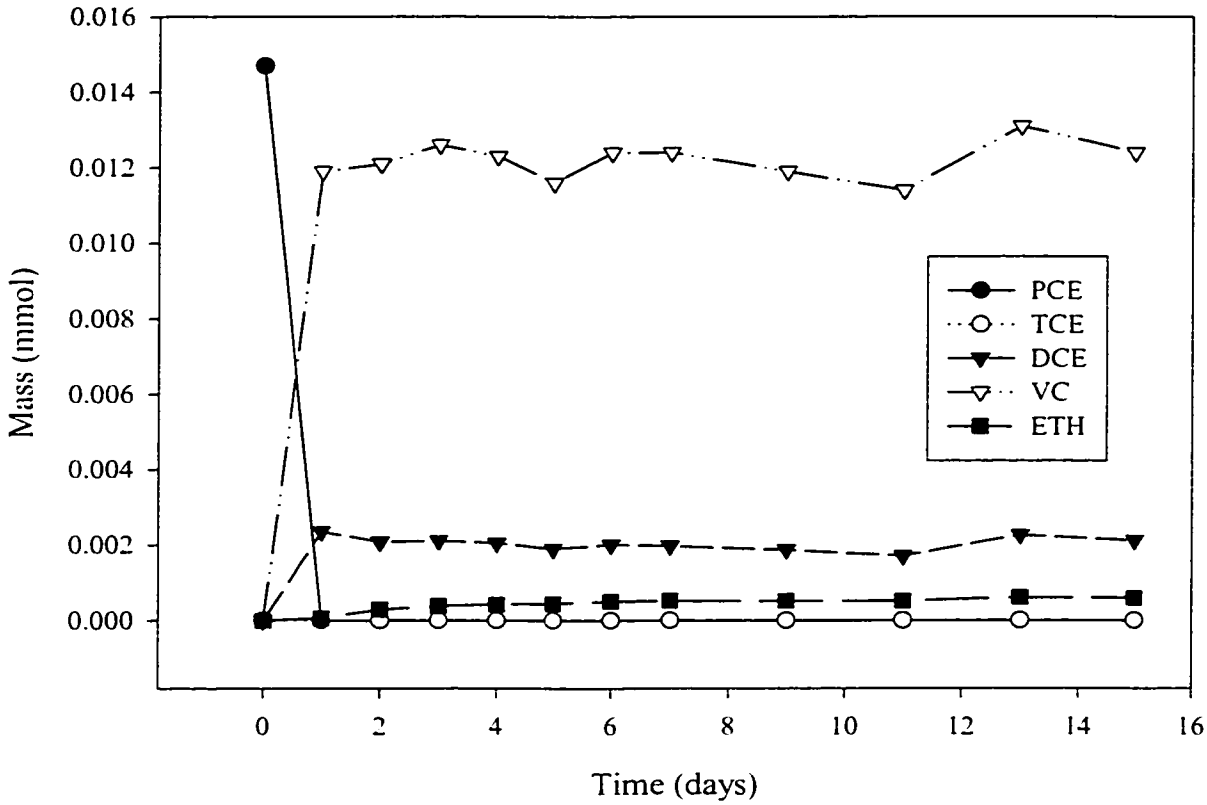


Figure 4.2d. Effect of Tween 80 Concentration on Rate and Extent of PCE Dechlorination - 1% Tween 80

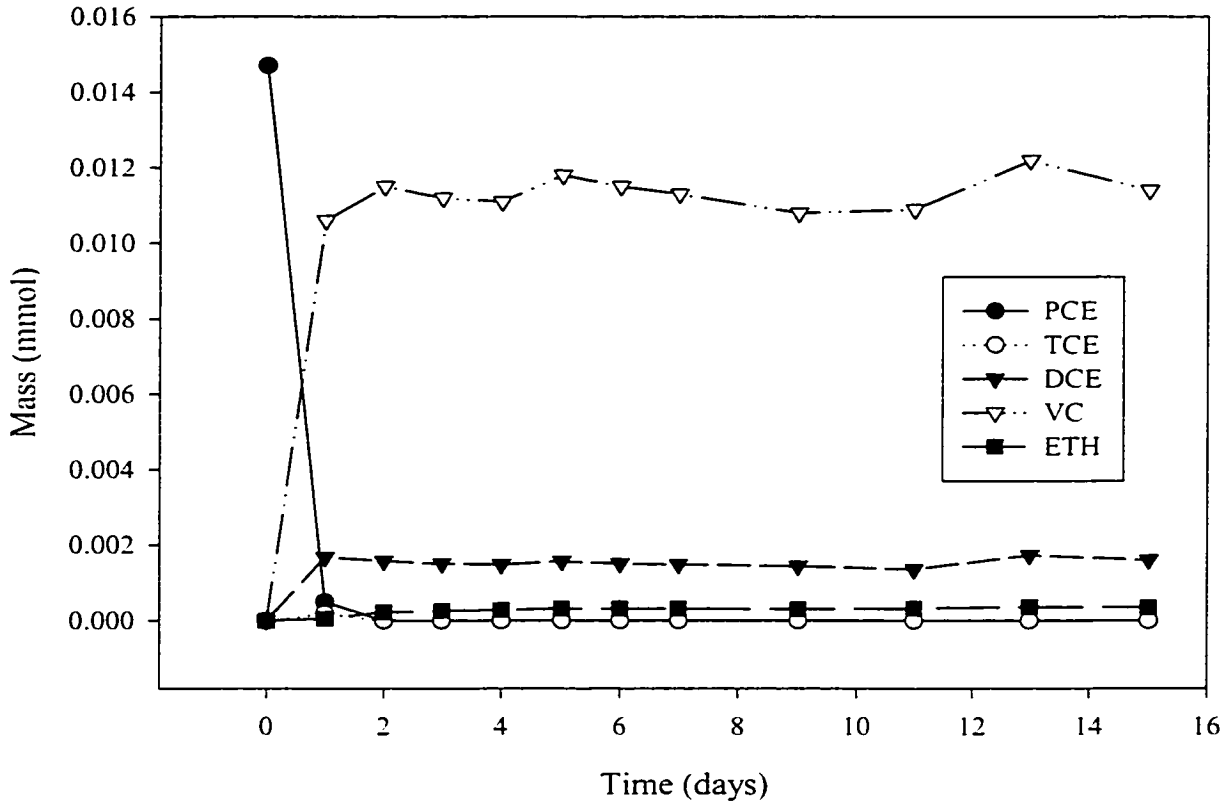


Table 4.3 Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination ([PCE]aq = 150 mg/L; t = 2 d)

Steol CS-330 Concentration (%)	PCE and Dechlorination Metabolites Recovered (%, molar)				
	PCE	TCE	DCE	VC	ETH
0.0	0.0	0.0	95.7	4.3	0.0
0.1	0.0	5.5	93.6	0.9	0.0
0.5	0.0	63.8	35.2	1.0	0.0
1.0	0.0	83.0	15.6	1.3	0.0

experiments, a relationship existed between the extent of PCE dechlorination and the concentration of Steol CS-330 added to the microcosm. After 2 days, the PCE in the microcosm containing 1.0% Steol CS-330 was dechlorinated to TCE (85%, molar) and DCE (15%, molar), which is consistent with results from the screening experiment. However, in the microcosm containing 0.5% Steol CS-330, the PCE was reduced to approximately 65% TCE (molar) and 35% DCE (molar). Finally, the PCE in the microcosm containing only 0.1% Steol CS-330 was transformed to about 5% TCE (molar) and 95% DCE (molar). Interestingly, the PCE in the surfactant free-control was dechlorinated to 95% DCE and 5% VC, indicating that the high PCE concentration may contribute to decreased activity of the organisms that reduce DCE to VC and ethene (at least in a 2-day study).

4.4.2 Long-Term Experiments

Microcosms were prepared with Steol CS-330 at 0%, 0.1%, 0.5%, and 1.0% concentrations and PCE at an aqueous concentration of 40 mg/L. Dechlorination activity was monitored over a period of 15 days. Figures 4.3a – 4.3d present the results from this experiment. All microcosms showed complete reduction of PCE after only 1 day of incubation. After 15 days, the surfactant-free control microcosm (Figure 4.3a) dechlorinated PCE to VC (40%, molar) and ETH (60%, molar). The microcosm containing the lowest surfactant concentration (Figure 4.3b) showed little TCE accumulation over the first 2 days of the experiment, after which the entire mass of initial PCE was converted to DCE. In the microcosm with 0.5% surfactant (Figure 4.3c), however, the PCE is rapidly degraded to entirely TCE, which remains for approximately 3 days before being rapidly degraded to DCE. This same trend was evident for the

Figure 4.3a. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination - Surfactant-free Control

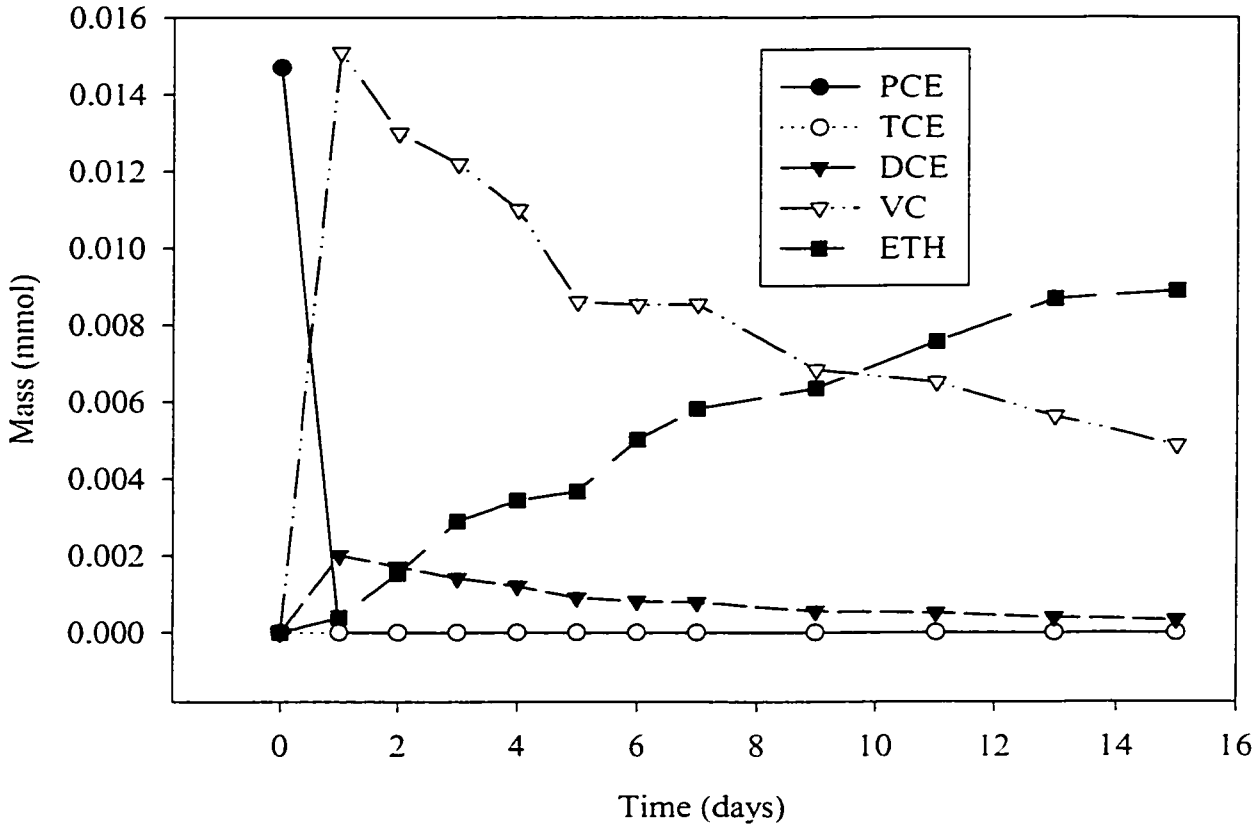


Figure 4.3b. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination - 0.1% Steol CS-330

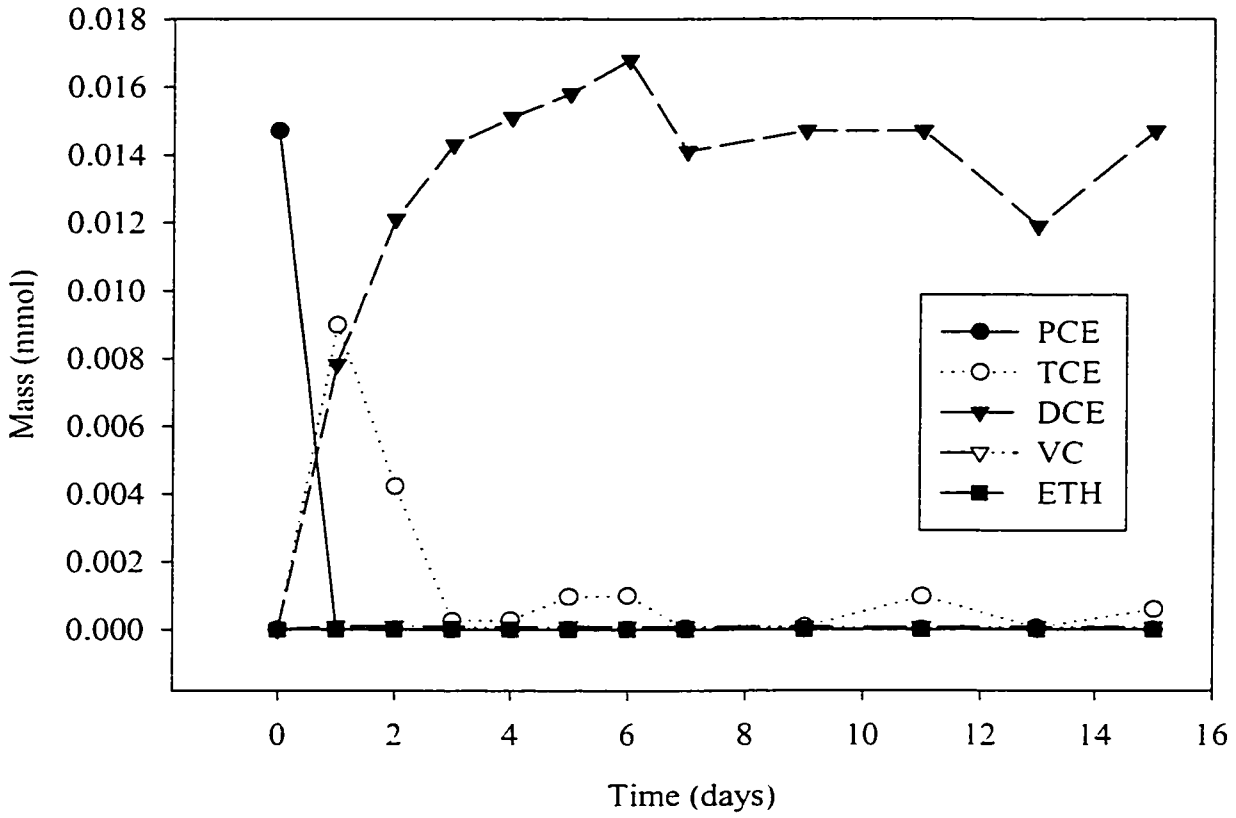


Figure 4.3c. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination - 0.5% Steol CS-330

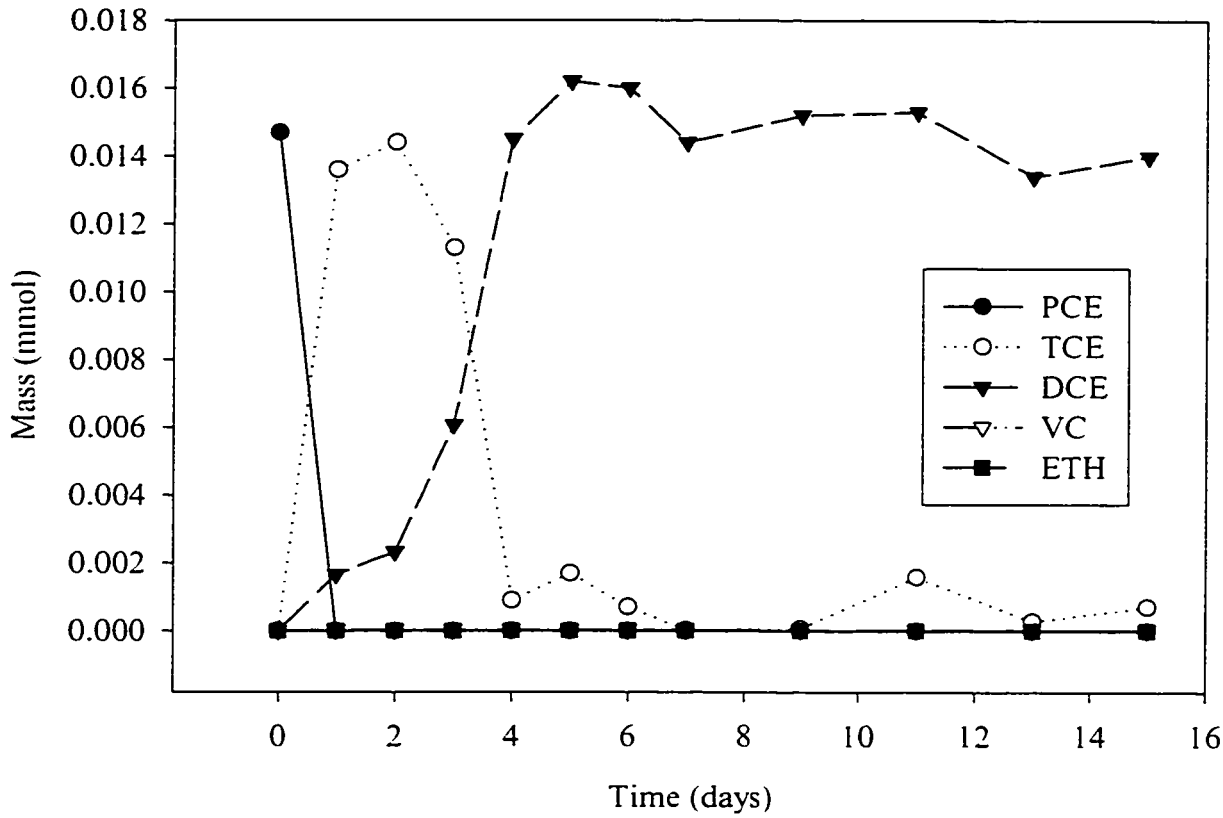
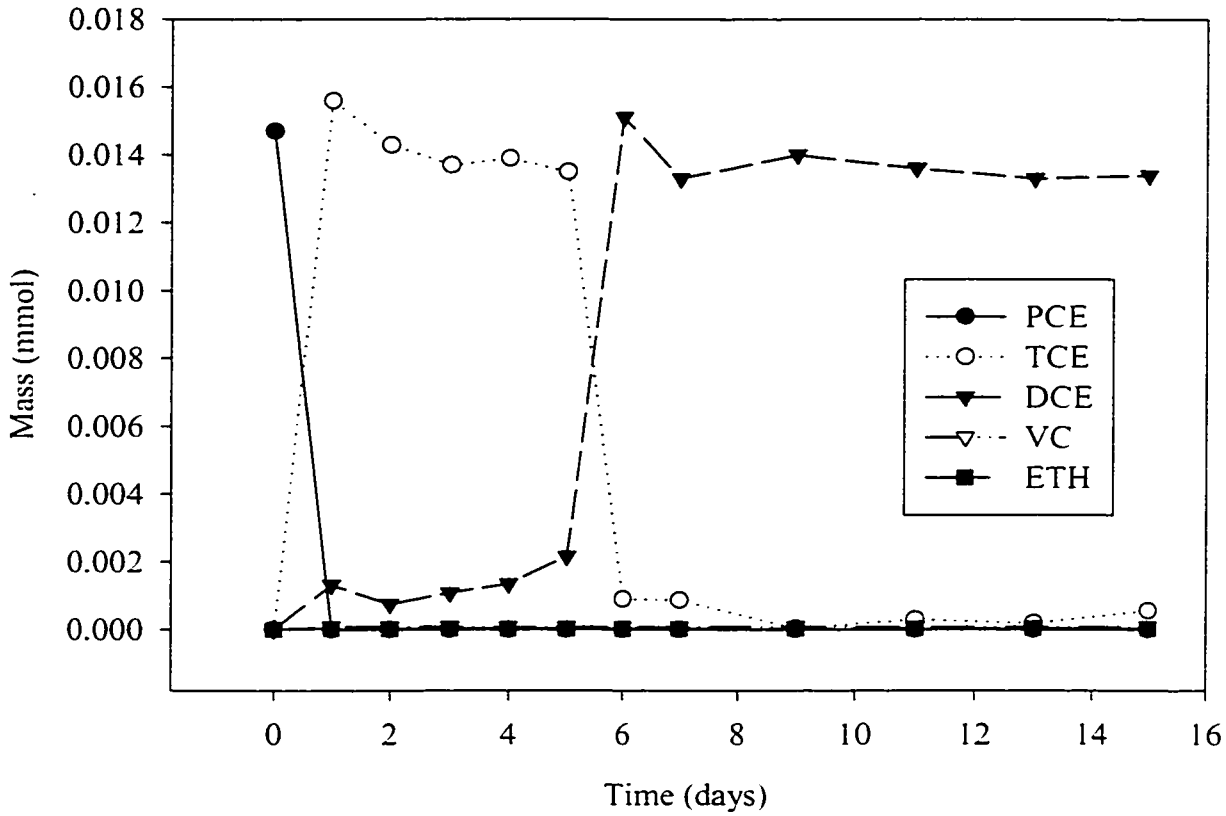


Figure 4.3d. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination - 1% Steol CS-330



microcosm containing 1.0% surfactant (Figure 4.3d), however the TCE lingered for approximately one week before being rapidly dechlorinated to DCE. In all surfactant-containing microcosms, the organisms responsible for DCE degradation appear to be inhibited.

Experiments with Steol CS-330 at 0.5% and 1.0% were repeated with the intention of determining whether additional PCE added after the apparent acclimation period could be rapidly degraded to DCE without TCE accumulation. In these experiments, once the TCE acclimation period occurred and all TCE was reduced to DCE, the headspaces of the microcosms were sparged to remove most dechlorination products and the microcosms were respiked with PCE at 40 mg/L. Results from this experiment are shown in Figure 4.4a (0.5% Steol CS-330) and Figure 4.4b (1.0% Steol CS-330). Both microcosms showed acclimation periods similar to those observed in the previous experiment. The second addition of PCE was rapidly dechlorinated entirely to DCE in both microcosms without accumulation of TCE.

4.5 Effect of Steol CS-330 on *cis*-DCE Dechlorination

The effect of surfactant concentration on *cis*-DCE dechlorination was investigated as in previous experiments except the microcosms were fed *cis*-DCE rather than PCE and the experimental period extended to 35 days. In this experiment, *cis*-DCE (23 mg/L) was fed to microcosms containing Steol CS-330 at 0%, 0.1%, 0.5%, and 1.0% (w/w, active) concentrations and dechlorination activity was monitored periodically. Results from this experiment are presented in Figure 4.5a (surfactant-free control), Figure 4.5b (0.1% Steol CS-330), Figure 4.5c (0.5% Steol CS-330), and Figure 4.5d (1.0% Steol CS-330). As

Figure 4.4a. Acclimation of PCE Dechlorination to DCE at High Steol CS-330 Concentrations - 0.5% Steol CS-330

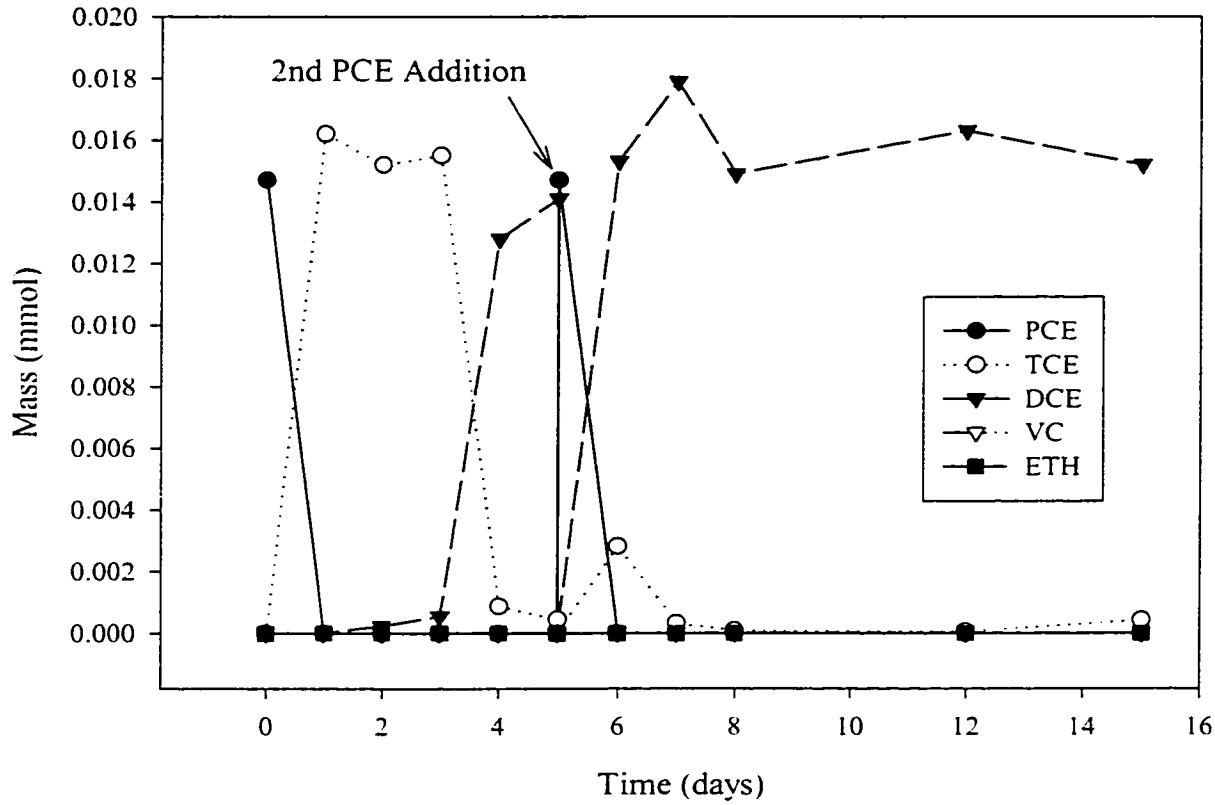


Figure 4.4b. Acclimation of PCE Dechlorination to DCE at High Steol CS-330 Concentrations - 1% CS-330

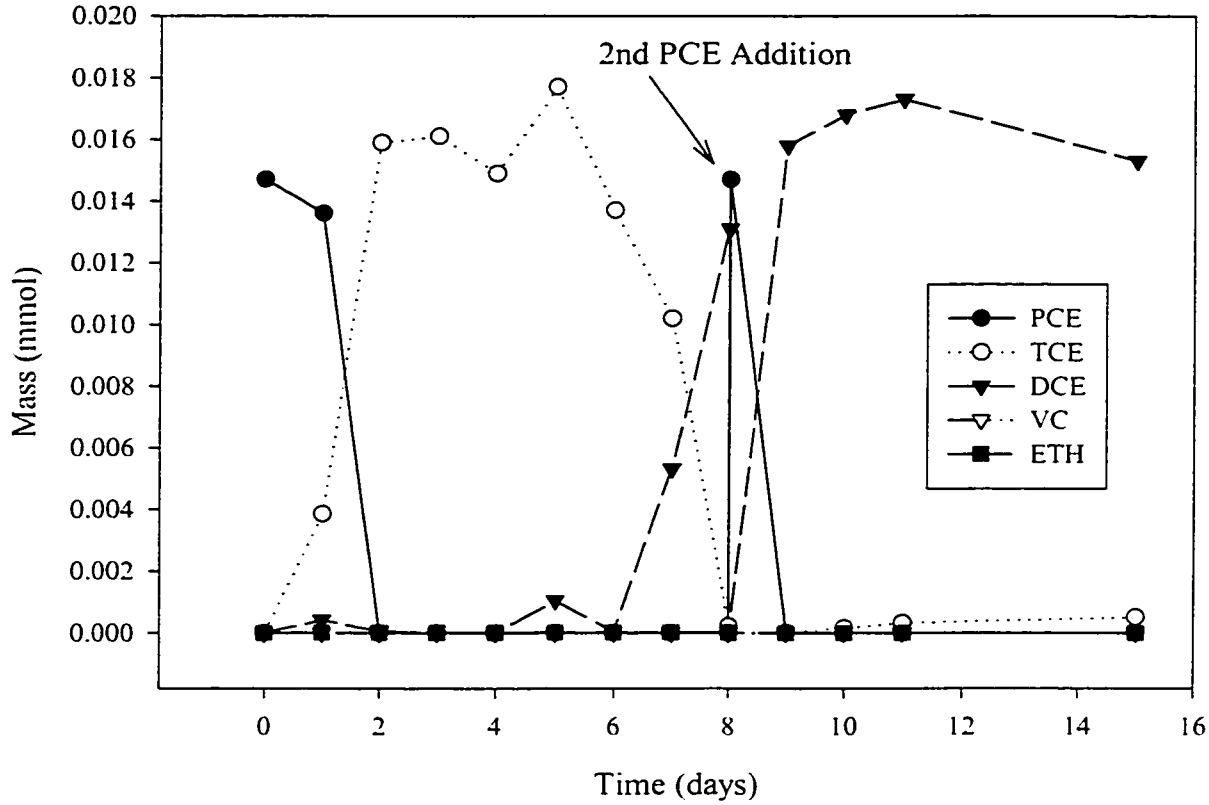


Figure 4.5a. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of *cis*-DCE Dechlorination - Surfactant-free Control

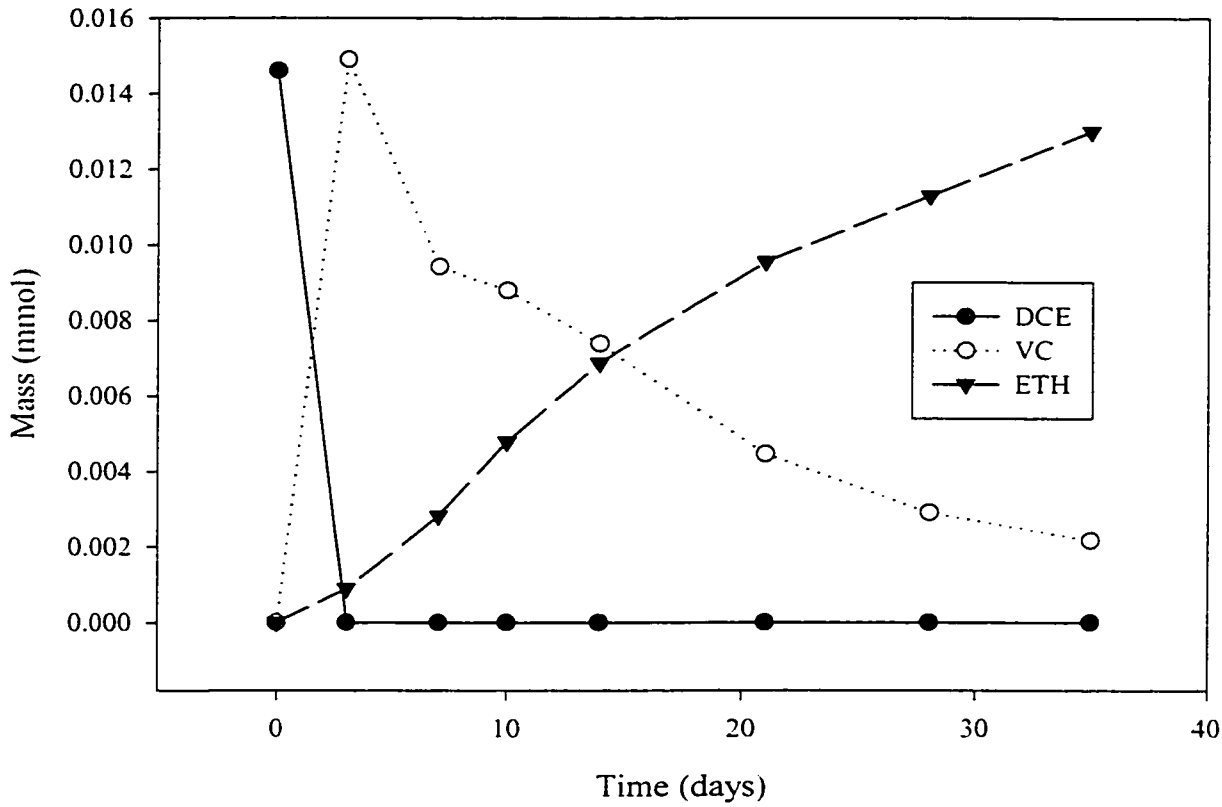


Figure 4.5b. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of *cis*-DCE Dechlorination - 0.1% Steol CS-330

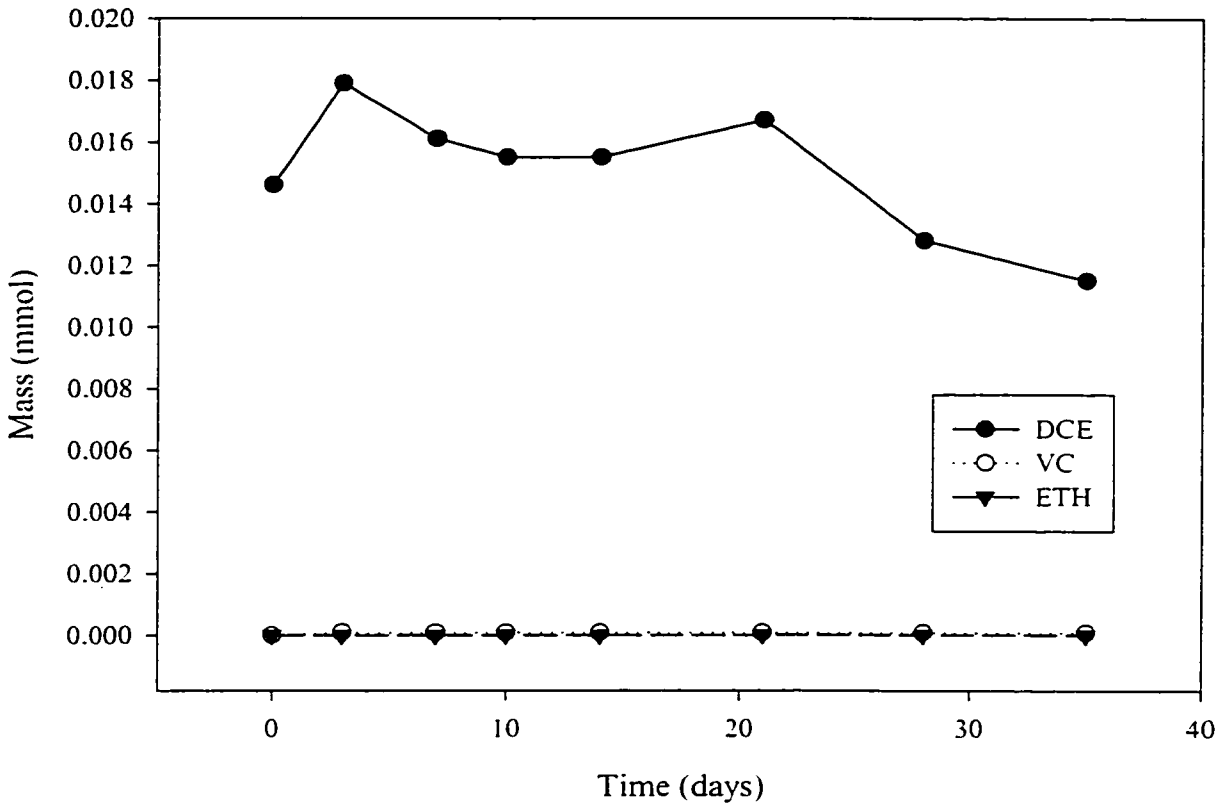


Figure 4.5c. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of *cis*-DCE Dechlorination - 0.5% Steol CS-330

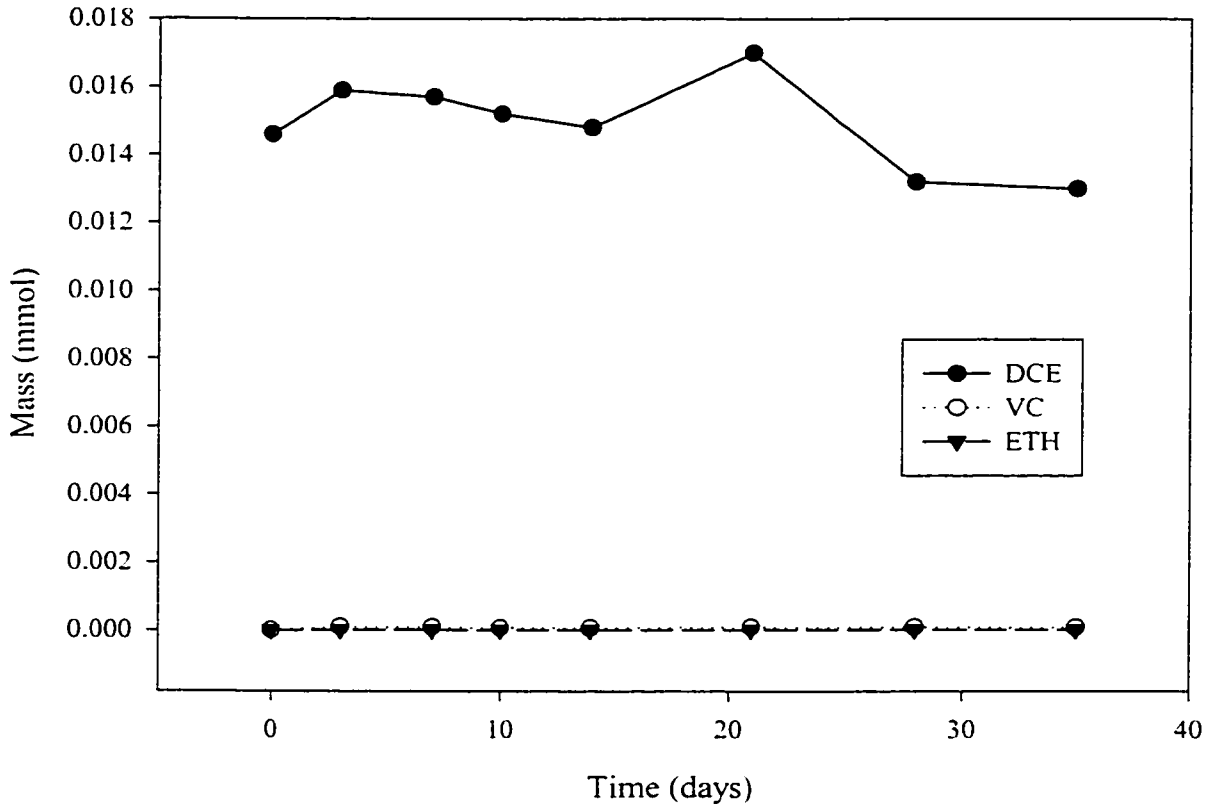
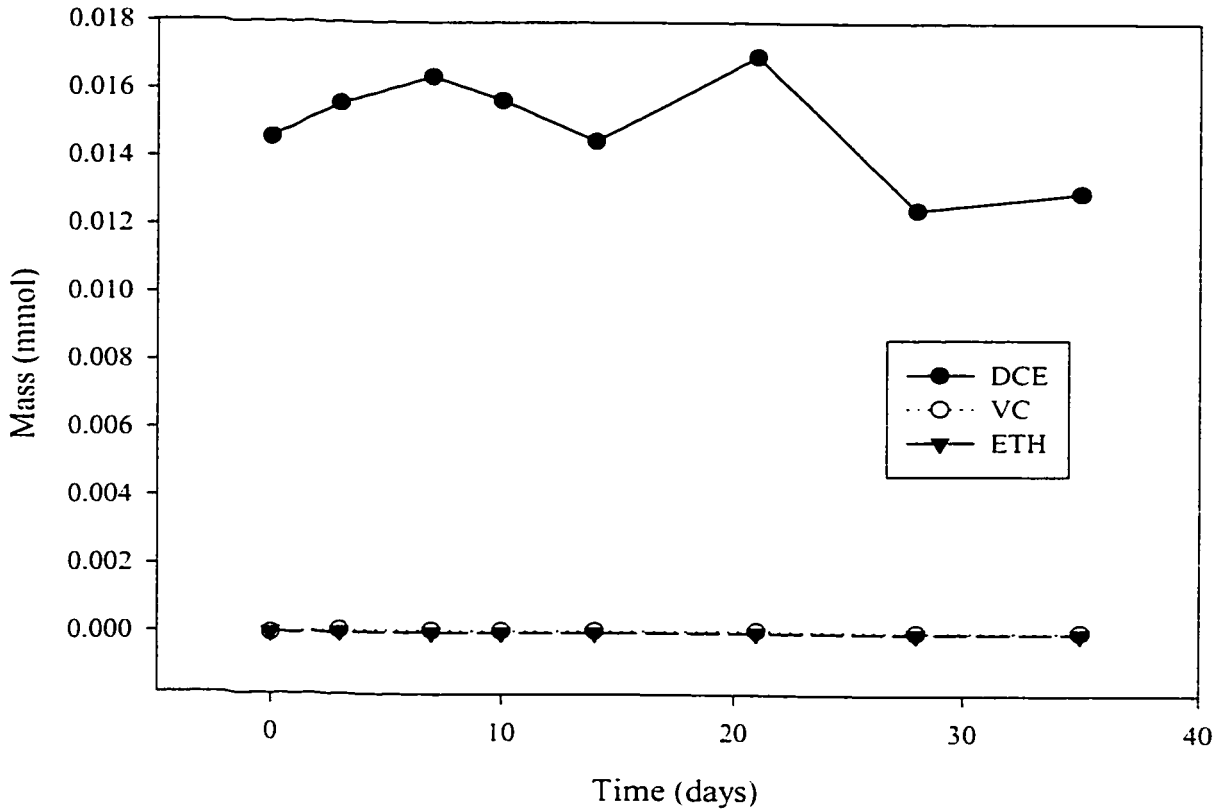


Figure 4.5d. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of *cis*-DCE Dechlorination - 1% Steol CS-330



evident from these figures, the DCE was not reduced in any surfactant-amended microcosms over the 35-day period while nearly complete reduction to ETH was observed in the surfactant-free control microcosm.

A series of microcosms were amended with 0.5% Steol CS-330 and increasing amounts of the following nutrients: $ZnCl_2$, $MnCl_2$, $CoCl_2$, $NiCl_2$, Na_2MoO_4, KI, and NH_4VO_3. Results of this experiment are given in Table 4.4. After 4 days of incubation, the surfactant-free control microcosm dechlorinated 40 mg/L PCE to DCE (92.0%, molar), VC (7.9%, molar), and only a trace amount of ETH. The extent of dechlorination in this control is considerably less than observed for controls in other experiments indicating that the activity of DCE and VC reducing organisms was low to begin with in this experiment. The bottles containing metal nutrients at 1× and 2× the normal concentration each showed nearly complete dechlorination of PCE to DCE. As the metal nutrient concentration increased to 4× and 10× the normal concentration, however, PCE was reduced only to TCE.

Steol CS-330 is commercially available in an aqueous solution containing approximately 28% active surfactant. To avoid possible degradation of surfactant in the aqueous solution, formaldehyde is added to the solution as a preservative. The concentration of formaldehyde is typically about 600 mg/L in the commercial solution. An experiment investigating the effect of the preservative on the reductive dechlorination of PCE was performed. Also investigated, was the addition of a more pure surfactant solution, Steol CS-460. Steol CS-460 is the identical surfactant to Steol CS-330 except it is available at 60% active material and does not contain preservative. The results of these screening experiments are shown in Table 4.5. After 7 days of incubation, the surfactant-

Table 4.4. Effect of increasing trace metal nutrient (Zn, Mn, Co, Ni, Mo, K, V) concentration on extent of PCE dechlorination in the presence of Steol CS-330 ($[PCE]_{aq}$ = 40 mg/L; t = 4 d)

		PCE and Dechlorination Metabolites Recovered (%, molar)				
CS-330 Conc. (%)	Trace Metal Nutrients Relative to Normal Conc.	PCE	TCE	DCE	VC	ETH
0.0	1	0.0	0.0	92.0	7.9	0.1
0.5	1	0.0	4.4	95.5	0.1	0.0
0.5	2	0.0	10.9	89.1	0.0	0.0
0.5	4	0.0	99.0	0.9	0.1	0.1
0.5	10	0.0	98.3	1.4	0.1	0.2

Table 4.5 Effect of Steol CS-330 Preservative (Formaldehyde) and Steol CS-330 without Preservative (Steol CS-460) on PCE Dechlorination ($[PCE]_{aq}$ = 40 mg/L; t = 7 d)

	PCE and Dechlorination Metabolites Recovered (%, molar)				
Vial	PCE	TCE	DCE	VC	ETH
Control	0.0	0.0	0.4	79.3	20.3
CS-330	0.0	2.9	95.7	1.3	0.1
Formaldehyde	0.0	0.0	0.3	85.1	14.6
CS-460	0.0	1.7	95.0	3.1	0.2

free, preservative-free control dechlorinated 40 mg/L PCE to VC (79.3%, molar) and ETH (20.3%, molar). The microcosm containing 0.5% Steol CS-330 exhibited similar dechlorination to that observed in previous experiments with this surfactant as nearly all PCE was transformed to DCE. Formaldehyde was added to a surfactant-free microcosm at approximately 20 mg/L, which is the corresponding concentration in a 1.0% active solution of Steol CS-330. As shown in Table 4.5, the preservative had no effect on the normal dechlorination pattern as the PCE added was reduced to VC (85.1%, molar) and ETH (14.6%, molar). Finally, the microcosm amended with 0.5% Steol CS-460 displayed a similar extent of dechlorination to the microcosm containing Steol CS-330.

A series of microcosms were prepared where the surfactant concentration was serially diluted from 0.1% (1,000 mg/L) to 0.0001% (1 mg/L). After 7 days, the microcosms were sampled to determine dechlorination products present. Results of this experiment are displayed in Table 4.6. As evident from this table, the threshold concentration of Steol CS-330 that inhibits the DCE dechlorinating organisms lies in the range of 10 to 100 mg/L.

4.6 Long-term Acclimation to Steol CS-330

An experiment was conducted to determine if the microorganisms responsible for dechlorination beyond DCE could be acclimated to Steol CS-330 by incrementally increasing the surfactant concentration over time. In effort to determine if acclimation is possible, surfactant was initially added at a concentration (1 mg/L) that was known to not inhibit VC and ETH production. Every 4 days, the surfactant concentration was increased until VC and ETH production ceased.

Table 4.6. Determination of Steol CS-330 Concentration Inhibiting DCE Dechlorinating Microorganisms

CS-330 Conc.	PCE and Dechlorination Metabolites Observed[a]				
% (mg/L)	PCE	TCE	DCE	VC	ETH
0.1 (1,000)	–	+	+	–	–
0.01 (100)	–	+	+	–	–
0.001 (10)	–	–	+	+	+
0.0001 (1)	–	–	+	+	+
0.0	–	–	+	+	+

[a] + = Observed; – = Not observed

Results of this experiment are shown in Figures 4.6a and 4.6b and a summary appears in Table 4.7. The culture contained no surfactant for the first 4 days of the experiment; this period served as the surfactant-free reference. Over the reference period, PCE (16 mg/L/day) was dechlorinated to VC (54.5%, molar) and ETH (45.3%). On day 4 of the experiment, an aliquot of culture (37 mL) was withdrawn and replaced with nutrient medium and Steol CS-330 at a mass sufficient to attain an active surfactant concentration of 1 mg/L. This routine was maintained and the surfactant concentration was increased to 5 mg/L, 10 mg/L, 25 mg/L, and 50 mg/L over 16 days. The extent of PCE dechlorination over the period of each fill-and-draw cycle actually increased over the surfactant-free reference period with each surfactant addition up to 10 mg/L. The greatest extent of PCE reduction occurred over the 10 mg/L surfactant period with a total of 64 mg/L PCE (added over 4 days) being transformed to 41.1% VC and 58.7% ETH. Upon increasing the surfactant concentration to 25 mg/L, ETH production decreased severely as after 4 days VC represented 82.7% of the mass recovered with the balance being comprised of ETH (16.7%) and DCE (< 1%). The next surfactant step, to 50 mg/L, resulted in complete loss of VC and ETH production. In fact, some accumulation of TCE occurred during this period as PCE was dechlorinated in this period to DCE (56.1%) and TCE (40.9%). The surfactant concentration was maintained at 50 mg/L for 4 additional days to allow for possible acclimation. During the next 4 days, each PCE addition was completely dechlorinated to DCE. Finally, the surfactant concentration in the acclimation culture was increased to 100 mg/L. The complete reduction of each PCE addition to DCE continued over the following 8 days with no production of VC or ETH. The concentration of Steol CS-330 measured on day 36 of the experiment was 98.6 mg/L.

Figure 4.6a. Effect of Incremental Increases in Steol CS-330 Concentration on Rate and Exten of PCE Dechlorination by a Mixed Anaerobic Culture - Daily PCE Addition and Metabolite Production

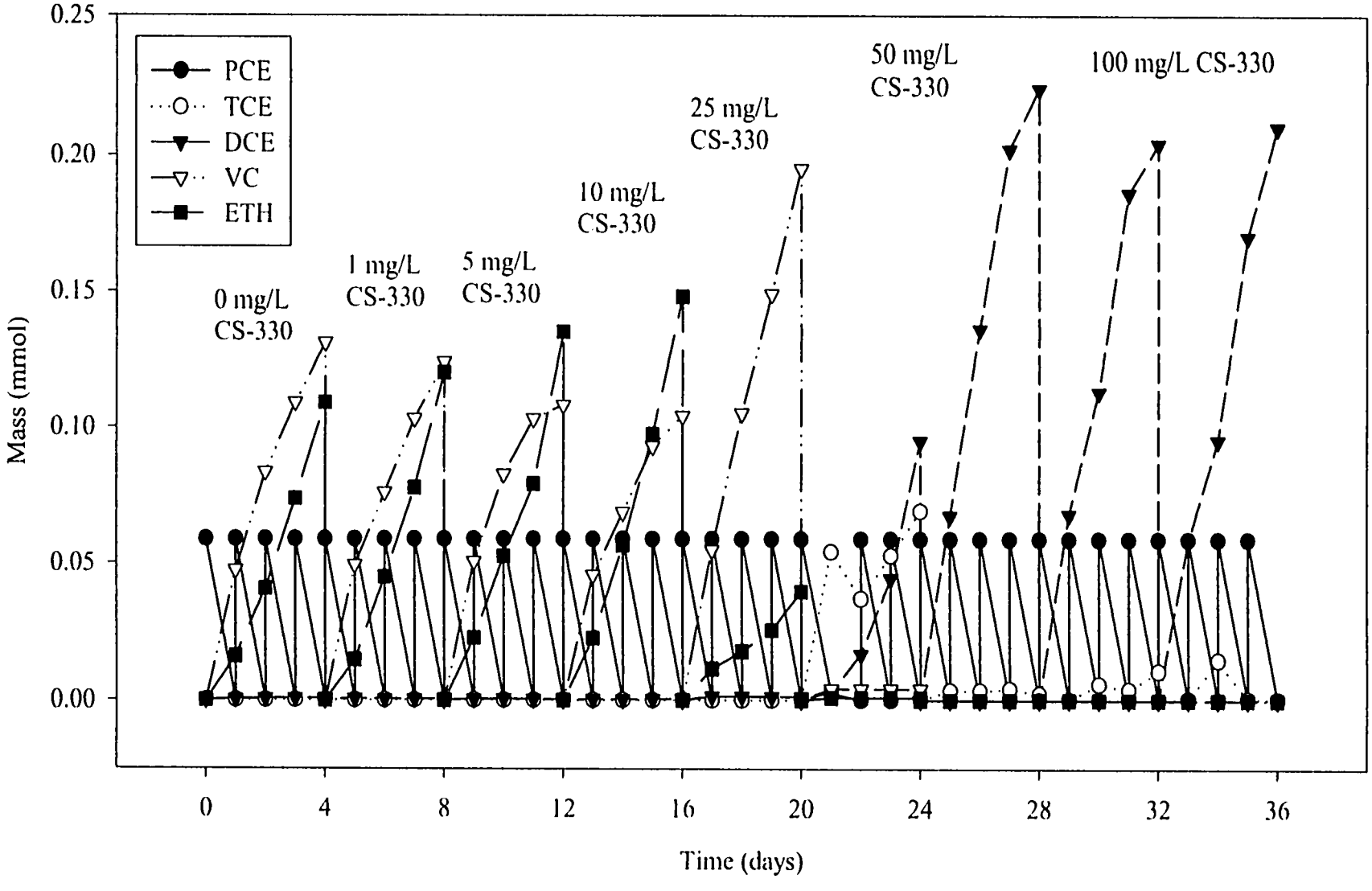


Figure 4.6b. Effect of Incremental Increases in Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination by a Mixed Anaerobic Culture - Cumulative PCE Addition and Metabolite Production

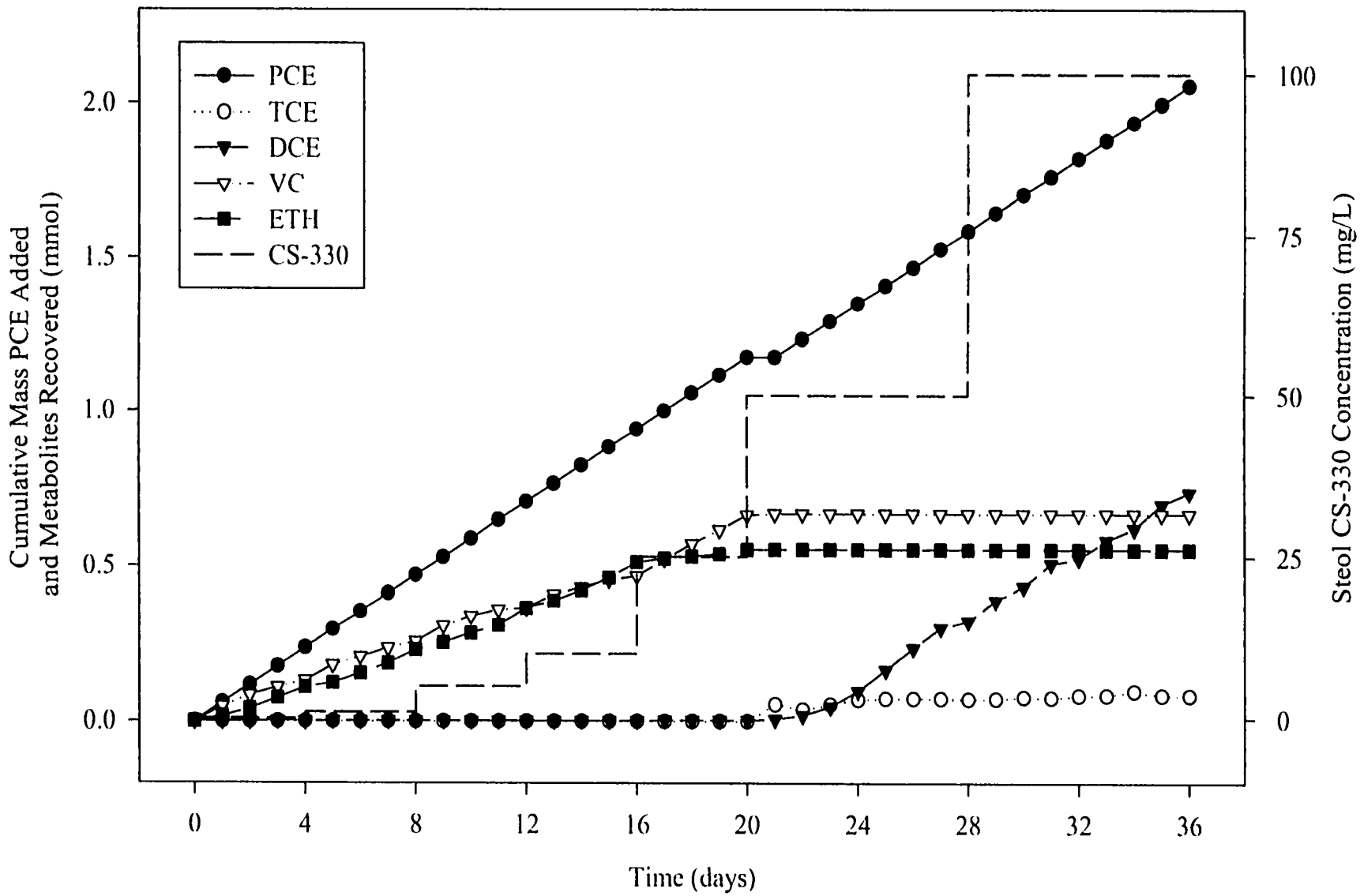


Table 4.7. Summary of Steol CS-330 Acclimation Culture Results at the End of Each Fill-and-Draw Cycle

Steol CS-330 Acclimation Culture		End Products Recovered (%, molar)				
Time (days)	CS-330 Conc. (mg/L)	PCE	TCE	DCE	VC	ETH
4	0	0.0	0.0	0.2	54.5	45.3
8	1	0.0	0.0	0.1	50.6	49.2
12	5	0.0	0.0	0.1	44.3	55.6
16	10	0.0	0.0	0.1	41.1	58.7
20	25	0.0	0.0	0.5	82.7	16.7
24	50	0.0	40.9	56.1	2.5	0.5
28	50	0.0	1.1	98.9	0.0	0.0
32	100	0.0	5.0	95.0	0.0	0.0
36	100	0.0	0.1	99.9	0.0	0.0

Microcosms seeded with the Steol CS-330 acclimation culture were assembled on days 24, 28, and 36. The surfactant in microcosms began on days 24 and 28 was diluted from 50 mg/L to 5 mg/L through a 1:10 dilution of the Steol CS-330 acclimation culture in fresh nutrient medium. Steol CS-330 in the microcosm began on day 36 was diluted from 100 mg/L to 10 mg/L through the same dilution. Microcosms were spiked with PCE and the extent of reduction in each bottle was monitored weekly. Results of this experiment are shown in Table 4.8. After one week, the PCE added to the day-24 microcosm was reduced to TCE (20.7%, molar), DCE (56.2%), VC (23.0%), and ETH (0.2%). After 3 weeks, however, the dominant dechlorination product was VC (83.2%) with some DCE (10.4%) remaining and ETH (6.3%) production evident. Similar trends were observed in the day-28 and day-36 microcosms except much less TCE was observed in the initial sample taken after 7 days of incubation. VC was also the dominant dechlorination product in each of these microcosms after 21 days of incubation, with ETH also being produced and some DCE remaining.

4.7 Effect of Surfactants Similar in Structure to Steol CS-330 on Extent of PCE Dechlorination

Surfactants with molecular structures similar to Steol CS-330 were examined for their effects on the extent of PCE dechlorination. First a nonionic surfactant, Neodol 25-7, containing a C_{12-15} group as the hydrophobe and an average of 7 EO groups as the hydrophile, was screened. This surfactant has a similar hydrophobic group to Steol CS-330 (C_{12}) and also contains EO groups, as does Steol CS-330 (average EO groups = 3), however lacks the sulfate group present in Steol CS-330. SDS, containing a C_{12}

Table 4.8. Extent of PCE Dechlorination in Microcosms Developed from the Steol CS-330 Acclimation Culture with Surfactant Concentrations Diluted below the Threshold Concentration (25 mg/L) for VC and ETH Production

Steol CS-330 Acclimation Culture		Microcosms		PCE and Dechlorination Metabolites Recovered (%, molar)				
Time (d)	CS-330 Conc. (mg/L)	Diluted CS-330 Conc. (mg/L)	Sample Time (d)	PCE	TCE	DCE	VC	ETH
24	50	5	7	0.0	20.7	56.2	23.0	0.2
			14	0.0	0.0	12.8	84.3	2.9
			21	0.0	0.0	10.4	83.2	6.3
28	50	5	7	0.0	1.6	46.0	52.4	0.0
			14	0.0	0.0	13.2	85.2	1.6
			21	0.0	0.0	12.3	84.0	3.7
36	100	10	7	0.0	0.0	94.8	5.2	0.0
			14	0.0	0.0	76.2	23.8	0.0
			21	0.0	0.0	8.9	89.7	1.4

hydrocarbon chain and a sulfate head group, was the other surfactant investigated. The lack of the 3 EO groups in SDS is the only difference in this surfactant compared to Steol CS-330.

Microcosms were amended with Neodol 25-7 or SDS at concentrations of 0.1%, 0.5%, and 1.0% (w/w) and PCE dechlorination was monitored after 3 and 7 days of incubation and compared to the results obtained for Steol CS-330 at the same concentrations and time. Results of this experiment are shown in Table 4.9. SDS completely inhibited the dechlorination process at all surfactant concentrations examined, as PCE was the only chlorinated ethene detected throughout the experiment. The nonionic surfactant, Neodol 25-7, limited the extent of PCE dechlorination, as DCE remained the dominant product at all surfactant concentrations. The observed extent of dechlorination in the Neodol 25-7 amended microcosm is very similar to that of Steol CS-330 after 7 days of incubation. However, conversion of PCE to DCE in the Neodol 25-7 microcosms at high surfactant concentrations does not appear to require the acclimation period that is necessary for PCE reduction to DCE at higher concentrations of Steol CS-330.

4.8 Comparison of Effects of Steol CS-330 versus Tween 80 on Methane Production and Hydrogen Consumption by the Mixed Anaerobic Culture

Plots of methane production observed in microcosms containing the surfactants at concentrations of 0.1%, 0.5%, and 1.0% (w/w, active) over the 15-day interval of the PCE dechlorination experiments are shown in Figures 4.7a (Tween 80) and Figure 4.7b (Steol CS-330). As evident from these figures, Steol CS-330 inhibited methane

Table 4.9. Comparison of Rate and Extent of PCE Dechlorination in Microcosms Amended with Surfactants Similar in Structure to Steol CS-330 ($[PCE]_{aq}$ = 40 mg/L)

		Dechlorination Products (%, t = 3d)					Dechlorination Products (%, t = 7d)				
Surfactant	Conc. (%)	PCE	TCE	DCE	VC	ETH	PCE	TCE	DCE	VC	ETH
SDS	1.0	100	0	0	0	0	100	0	0	0	0
	0.5	100	0	0	0	0	100	0	0	0	0
	0.1	100	0	0	0	0	100	0	0	0	0
Steol CS-330	1.0	0	91.9	7.3	0.5	0.2	0	6.1	93.1	0.5	0.2
	0.5	0	64.9	34.8	0.2	0.1	0	0.2	99.5	0.2	0.1
	0.1	0	1.8	97.6	0.6	0	0	0.3	99.1	0.6	0
Neodol 25-7	1.0	0	3.7	96.3	0	0	0	2.6	97.3	0	0.1
	0.5	0	1.8	98.1	0	0.1	0	1.5	98.4	0	0.1
	0.1	0	0.3	99.7	0	0.1	0	0.2	99.7	0	0.1

Figure 4.7a. Effect of Tween 80 Concentration on Methane Production by the Mixed Anaerobic Culture

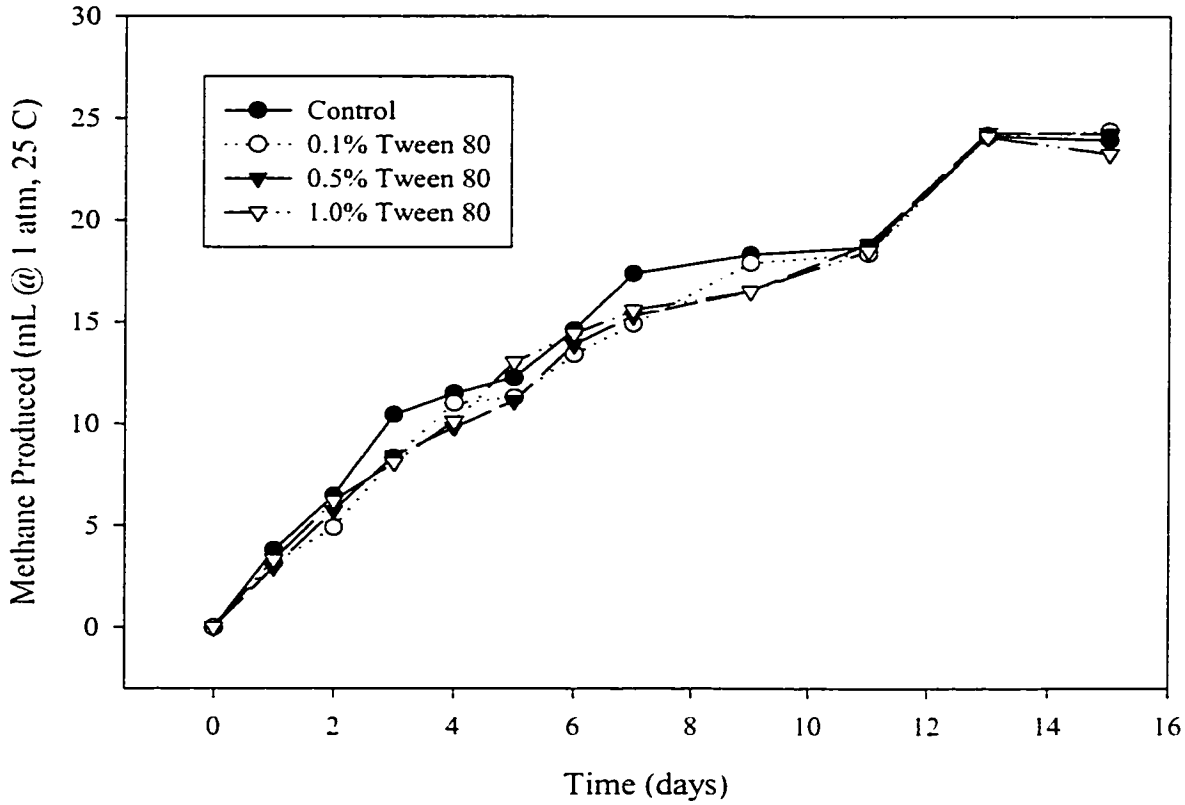
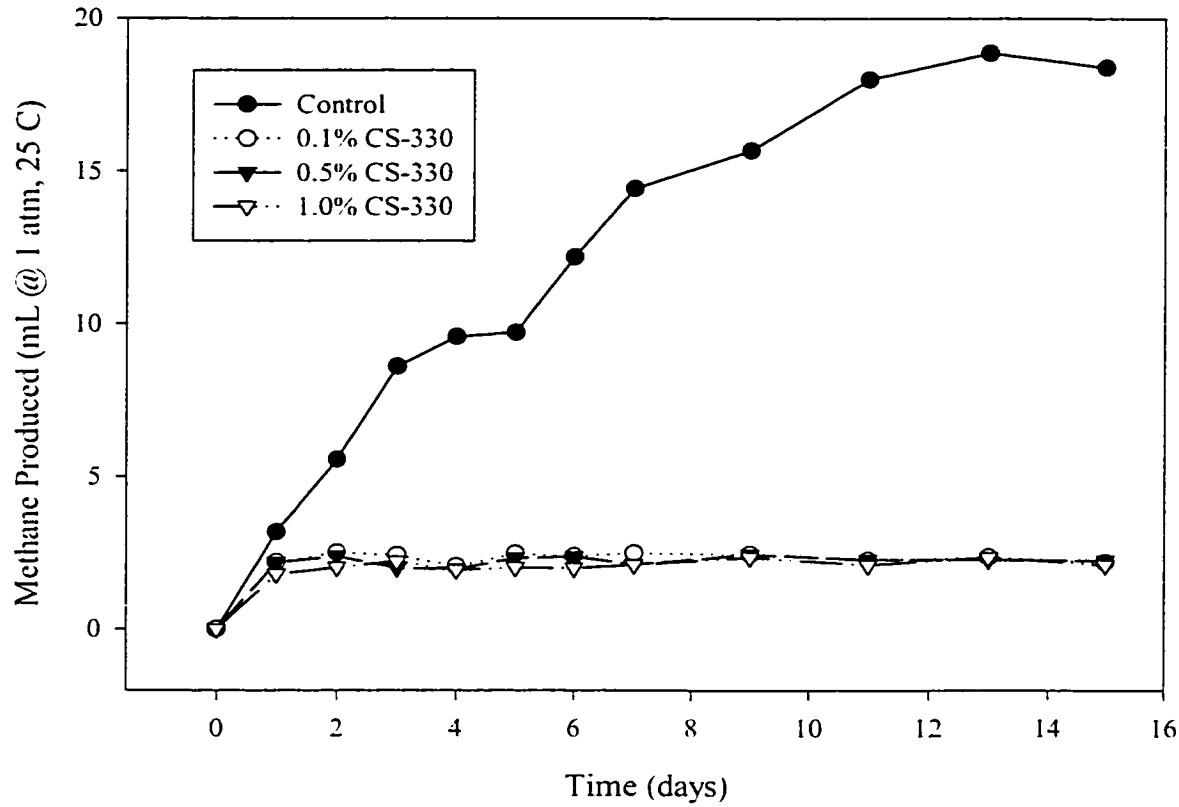


Figure 4.7b. Effect of Steol CS-330 Concentration on Methane Production by the Mixed Anaerobic Culture



production at all concentrations over the 15-day period. In contrast, Tween 80 apparently has no inhibitory effect on methanogens as methane produced in all surfactant-amended microcosms was nearly identical to that observed in the surfactant-free control.

An additional indicator of the effects of the two surfactants on the overall microbial community is the consumption of electron donor by the cultures. Plots of cumulative hydrogen/CO_2 (80:20% v/v) consumption by microcosms amended with 0.1%, 0.5%, and 1.0% (w/w) of the surfactants over the 15-day interval of the PCE dechlorination experiments are given in Figure 4.8a (Tween 80) and Figure 4.8b (Steol CS-330). Again, the presence of Tween 80 has no effect on hydrogen consumption by the mixed culture, while some inhibition is exhibited in the presence of Steol CS-330. Hydrogen consumption lagged approximately 5 days in Steol CS-330 amended microcosms before considerable hydrogen consumption was observed.

Figure 4.8a. Effect of Tween 80 Concentration on Hydgrogen/CO_2 Gas Consumption by the Mixed Anaerobic Culture

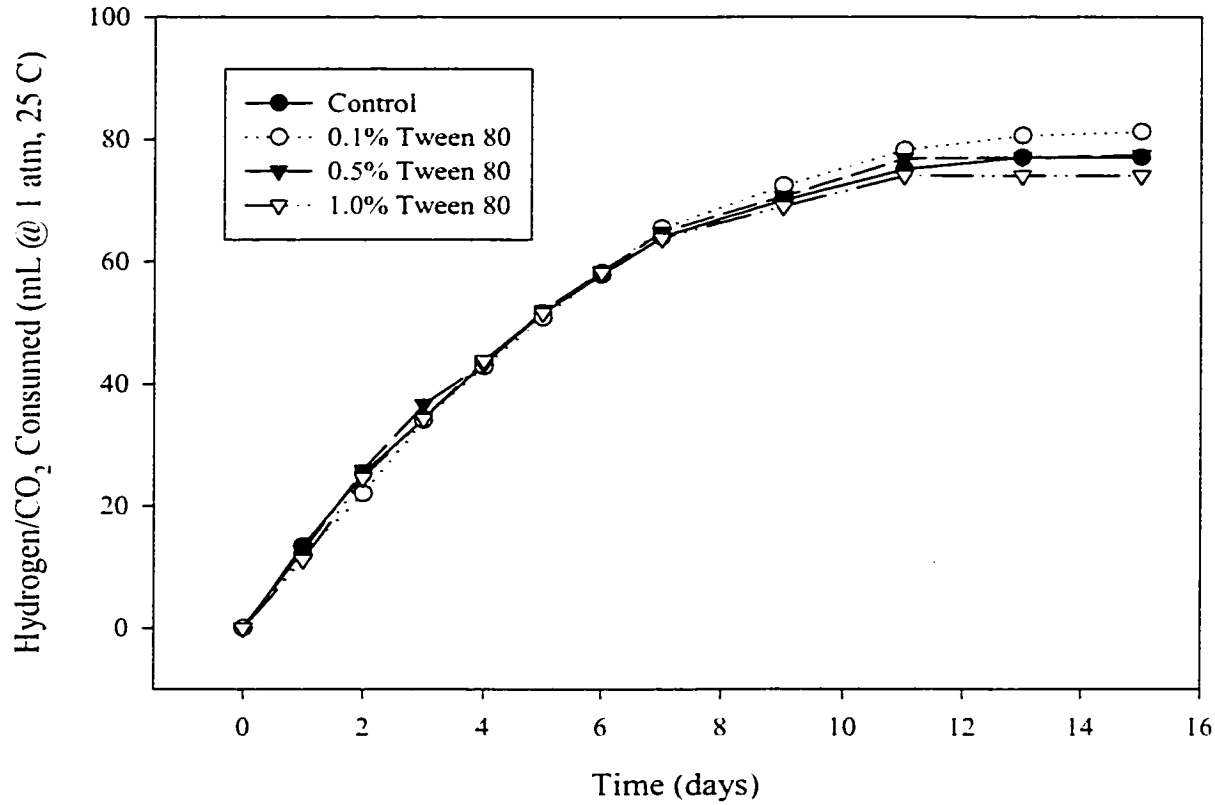
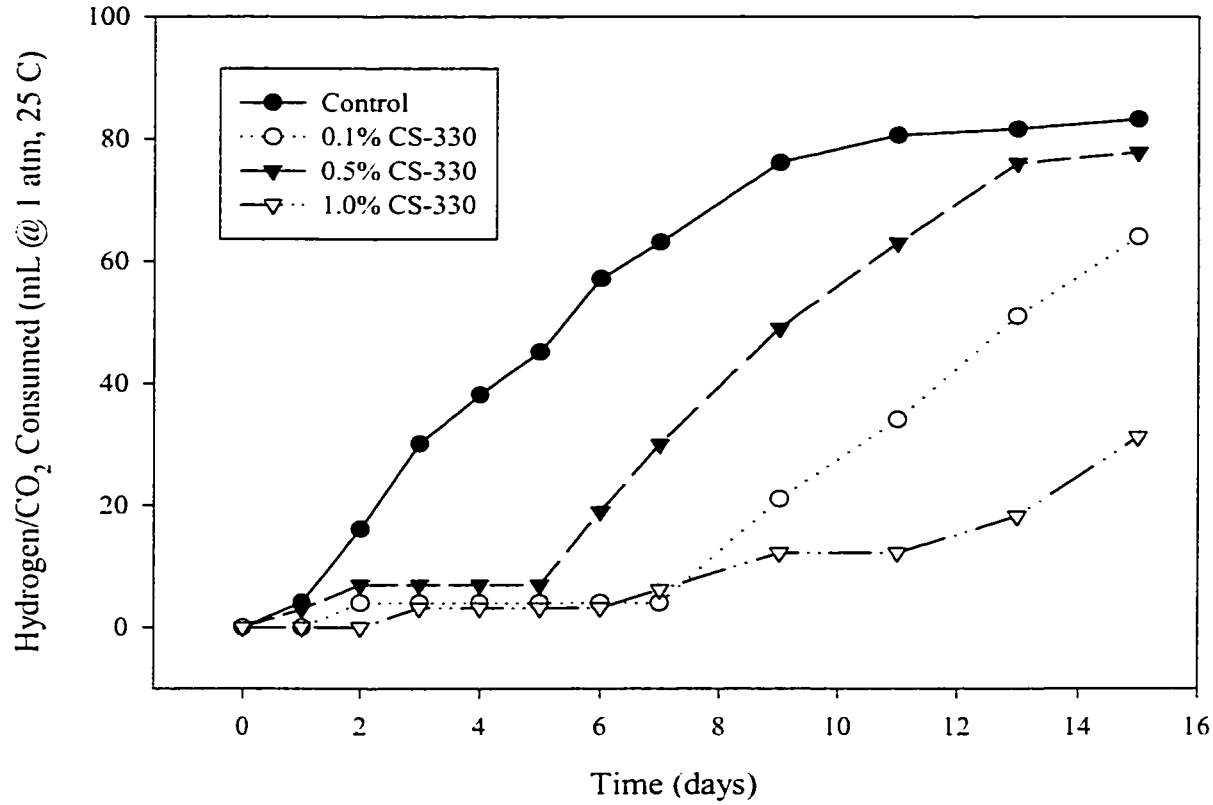


Figure 4.8b. Effect of Steol CS-330 Concentration on Hydrogen/CO_2 Gas Consumption by the Mixed Anaerobic Culture



5. Discussion

The objective of this research was to investigate the effects of surfactants on the dechlorination activity of a PCE enriched mixed anaerobic culture for potential use in SEAR. Surfactants have the potential to enhance remediation of chlorinated ethene contaminated ground water through enhanced solubilization/mobilization and also to enhance electron donor delivery via hydrogen-generated foams. While the latter process is directly associated with biological reductive dechlorination, solubilization/mobilization floods are designed to physically remove the bulk of contaminant mass and biological reductive dechlorination could potentially serve as a tool to "polish off" remaining contamination. In either case, the importance of a comprehensive study of the effects of surfactants on reductive dechlorinating microorganisms is evident.

Seven surfactants, representing each of the surfactant classes, were screened in a 2-day study for their effect on the rate and extent of PCE dechlorination. A surfactant concentration of 1.0% (w/w, active) was selected based on the observation that many SEAR processes utilize similar surfactant concentrations. The cationic surfactant, CTAB, severely inhibited reduction of PCE. This result was not unexpected, however, as cationic surfactants often result in microbial toxicity (*1*). Aerosol MA80-I, an anionic surfactant, also exhibited an adverse effect on PCE reduction. All other surfactants screened did not affect the rate of PCE reduction as all PCE was transformed after 2 days of incubation. However, the extent to which the PCE was reduced varied widely among microcosms amended with the remaining surfactants. The nonionic surfactant, Tween 80, had little effect on extent of PCE dechlorination as the dominant products after 2 days were VC and ETH, similar to the surfactant-free control. Of the remaining anionic

surfactants, Steol CS-330 appeared to be less inhibitory than AOS 14-16, although each exhibited TCE and DCE as the dominant products of PCE dechlorination. Thus Tween 80 and Steol CS-330 were selected for further investigation.

Surfactants were also screened for their potential biodegradation by the mixed anaerobic culture. Again, the concentration of surfactant was 1.0%. The objective of this experiment was to determine whether degradation of surfactants was occurring and to determine the extent to which the mixed anaerobic culture would rapidly acclimate to the high surfactant concentrations. Since surfactant biodegradation occurs to varying extents (*1*) and a comprehensive method of determining biodegradation of a variety of surfactants did not exist, two indicators of surfactant biodegradation were utilized. First, direct measures of surfactant were made initially and after 70 days of incubation, in microcosms amended with surfactant as the sole carbon source. Methane (CH_4) production by surfactant-amended microcosms was also measured and compared to a surfactant-free microcosm and to theoretical CH_4 production based on COD measurements. Based on surfactant measurements, only the Tween surfactant exhibited substantial degradation over the experimental period. Other researchers have also observed Tween degradation at similar concentrations by a mixed anaerobic culture (*2-5*). However, CH_4 produced in all surfactant-containing microcosms accounted for only 2 – 3 % of the amount theoretically resulting from complete degradation of the surfactants to CH_4. These results indicate that the surfactants are not likely to be degraded to a large extent over the experimental periods of the present study. That is not to say that the surfactants would not eventually be degraded given much longer periods for microbes to acclimate to the presence of surfactants. The high surfactant concentration may have contributed to

decreased microbial activity evidenced by the lack of Neodol 25-7 degradation, a surfactant from a class that is known to biodegrade under mixed, anaerobic conditions at low concentrations (*6-8*).

Anionic surfactants and nonionic surfactants are each commonly utilized in SEAR research and field demonstrations (*9-16*). One surfactant from each of these classes was selected, based on results from the short-term PCE dechlorination screening experiment, for use in further investigations. The effect of Tween 80 (nonionic) and Steol CS-330 (anionic) at various concentrations (0.1%, 0.5%, and 1.0% w/w) on rate and extent of PCE dechlorination over a period of 15 days was investigated. Tween 80 exhibited no effect on the conversion of PCE to VC; however, further reduction of VC to ETH occurred slower in all Tween 80 amended microcosms compared to the surfactant-free control. The cause for the decreased rate of ETH production in Tween 80 amended microcosms was not investigated further. Yeh *et al.* (*4*) investigated the effects of a series of Tween surfactants on the reductive dechlorination activity of a mixed culture enriched on hexachlorobenzene (HCB). HCB dechlorination was found to vary among microcosms amended with the Tween surfactants and Tween 80 exhibited greater microbial inhibition than some of the other surfactants in the series. Thus other surfactants within the Tween series could possibly have an even lesser effect on complete PCE dechlorination than Tween 80.

The effect of Steol CS-330 concentration was also investigated in a 15-day experiment. Although PCE was reduced in all microcosms after 1 day, TCE accumulation was observed to depend on Steol CS-330 concentration. The length of time TCE was detected as the dominant dechlorination product also depended on surfactant

concentration. A potential reason for this observation is the bioavailability of TCE in surfactant micelles. Since all surfactant concentrations examined are above the CMC for Steol CS-330 (0.05% w/w), the increasing surfactant concentration directly relates to an increasing number of micelles present. Furthermore, studies investigating the bioavailability of non-chlorinated HOCs partitioned into micelles have concluded a fraction of the solubilized contaminant may not be directly available for microbial uptake (*17,18*). However, further incubation of the microcosms resulted in rapid reduction of TCE after a lag phase with concomitant production of DCE. This event took place after 3 days in the microcosm amended with 0.5% Steol CS-330 and after 6 days in the microcosm containing 1.0% of the surfactant. The rapid reduction of TCE after several days of incubation suggests that the TCE in a micellar Steol CS-330 solution is bioavailable, but the microorganisms responsible for degrading TCE to DCE require a period of acclimation to the presence of the surfactant before reduction commences. Contaminant biodegradation in the presence of surfactants, particularly at similar surfactant concentrations, has been observed to undergo a lag period in other studies as well (*2,4,19*).

To test the hypothesis that dechlorination of PCE to DCE requires an acclimation period at high Steol CS-330 concentrations, the experiment was repeated with surfactant concentrations of 0.5% and 1.0%. Results from this experiment were similar to those observed in the previous experiment as PCE was reduced to TCE and eventually to DCE over a period of 3 days (0.5% Steol CS-330) and 7 days (1.0% Steol CS-330). A second addition of PCE to the microcosms, following the apparent acclimation period, was rapidly dechlorinated to DCE without TCE accumulation. This observation confirmed

the requirement of an acclimation period for conversion of PCE to DCE at the highest surfactant concentrations.

In all previous experiments utilizing Steol CS-330, dechlorination past DCE was inhibited indicating that the group of microorganisms responsible for reduction of DCE to VC and ETH are negatively affected by the surfactant. A series of experiments were carried out to explore possible mechanisms of this observed inhibition. An experiment similar to the dechlorination experiments described above was performed, with the exception being DCE was fed to the microcosms rather than PCE. Also, the experiment was extended for a longer period to determine if the organisms that transform DCE require an acclimation period similar to that observed for TCE reduction beyond the timeframe of previous experiments. No dechlorination occurred in surfactant-amended microcosms over the 35-day period while the DCE in the surfactant-free control was reduced almost entirely to ETH. This indicates that DCE dechlorinating microorganisms are affected by high initial surfactant concentrations in a manner that cannot be overcome by prolonged exposure to the surfactant.

Anionic surfactants are capable of association with metal ions in solution, particularly multivalent metal ions. Trace amounts of certain metal ions serve as nutrients required to support microbial function and are often incorporated into enzyme systems. Therefore, surfactant addition to microbial medium may result in surfactant chelation of metal ions rendering the metals unavailable for uptake by the microorganisms. An experiment was performed to determine if surfactant chelation of nutrient metals was negatively impacting the extent of PCE dechlorination. Results from this study indicate that decreased bioavailability of metal nutrients due to surfactant

chelation is not limiting the extent of PCE dechlorination. In fact, these results show that increasing the metal concentration actually enhances the negative effect of the anionic surfactant on the extent of PCE dechlorination. This result is consistent with observations from other studies (*20*) where increased microbial toxicity of anionic surfactants was related to increased cations in solution.

The preservative (formaldehyde) and impurities in commercially available Steol CS-330 were also excluded as the cause of DCE dechlorination inhibition. Dechlorination in a formaldehyde amended microcosm proceeded with similar rate and extent of dechlorination as observed in the surfactant-free control. Steol CS-460, an identical surfactant to Steol CS-330 that is commercially available at a higher active material concentration, inhibited dechlorination beyond DCE similarly to inhibition observed with Steol CS-330. Results of these experiments suggest that the loss of DCE degrading activity is directly related to interactions of the surfactant with the microorganisms that dechlorinate DCE.

Since previous experiments demonstrate that interactions of Steol CS-330 with dechlorinating microorganisms are the cause of the observed adverse effect on extent of PCE reduction, an experiment was carried out to determine an approximate concentration at which the surfactant is inhibitory to the DCE degrading microorganisms. Serial dilutions of Steol CS-330 in microcosms containing the PCE dechlorinating culture, which had no previous exposure to the surfactant, resulted in loss of VC and ETH production at a surfactant concentration between 10 and 100 mg/L.

From the previous experiment, an unacclimated culture was shown to cease VC and ETH production at a Steol CS-330 concentration between 10 and 100 mg/L. It was

hypothesized that adding large amounts of surfactant at one time to a culture with no previous exposure to the surfactant inhibited the microorganisms in such a manner that an opportunity for acclimation to the surfactant by the organisms was not observed. Therefore, a culture was developed in which Steol CS-330 concentration was increased periodically. After 16 days of the experiment, at which the surfactant concentration had reached 10 mg/L, the extent of PCE dechlorination over the fill-and-draw cycle actually increased over the initial surfactant-free cycle. The slight enhancement of PCE dechlorination observed with each surfactant addition up to 10 mg/L was not likely due to the presence of the surfactant, although researchers have observed enhanced PAH degradation rates at extremely low surfactant concentrations (*21*). A dramatic decrease in ETH production was observed upon increase of Steol CS-330 to 25 mg/L, and a further increase to 50 mg/L resulted in complete loss of VC and ETH production. The initial PCE addition at a surfactant concentration of 50 mg/L was dechlorinated almost entirely to TCE; in fact, a very small amount of PCE was detected. Therefore, the PCE addition on day 21 was omitted. Subsequent PCE additions during this fill-and-draw cycle resulted in increased concentrations of DCE and decreasing amounts of TCE. By day 25, PCE was completely reduced to only DCE and this trend continued throughout the remainder of the experiment. Thus, an acclimation period for PCE reduction to DCE was evident even at a very low surfactant concentration. Conversely, the organisms responsible for reduction of DCE to VC and ETH exhibited no acclimation to the surfactant. In fact, the dramatic loss of VC and ETH production upon Steol CS-330 concentration to 50 mg/L indicates that the surfactant is likely interfering with the enzyme system responsible for DCE dehalogenation. The surfactant concentration

measured at the end of the experiment verified that preferential degradation of the surfactant was not occurring.

Surfactant interactions with proteins, especially membrane bound enzymes such as those involved in respiratory processes, are a known mechanism of surfactant toxicity toward microorganisms (*22*). In some cases, enzymes have been shown to regain activity upon removal of the surfactant-enzyme complex (*1*). Therefore, an experiment was performed to determine whether the observed inhibition of DCE reducing microorganisms in the preceding experiment was reversible upon dilution of the surfactant to a concentration that had been shown to have no adverse effect on complete PCE dechlorination. On days 24, 28, and 36, aliquots from the Steol CS-330 acclimation culture were diluted (1:10) into fresh nutrient medium, spiked with PCE, and dechlorination products were monitored weekly. Results from this experiment demonstrate that production of VC and ETH is regained upon dilution of the surfactant below the threshold concentration. This evidence supports the hypothesis that interaction of Steol CS-330 monomers with the dehalogenase enzyme system of the population of microorganisms responsible for DCE and VC reduction is likely the cause for the observed inhibition.

In attempt to better understand the mechanism of surfactant interaction with dechlorinating microorganisms, a final experiment investigated the effects of surfactants with similar molecular structures to Steol CS-330 on the extent of PCE dechlorination. The goal of screening these surfactants was to provide insights as to whether the hydrophobe or the hydrophile was adversely interacting with the microorganisms. The influence of an ethoxylated nonionic surfactant, Neodol 25-7, containing a similar

hydrophobe (C_{12-15}), and an anionic surfactant, SDS, void of ethoxy groups, but containing a C_{12} hydrophobe and a sulfate hydrophile, on PCE dechlorination was screened and compared to Steol CS-330. Over a period of 7 days, SDS was found to completely inhibit the PCE reductive dechlorination process, as PCE was the only chlorinated ethene detected. In microcosms amended with Neodol 25-7, however, PCE was almost entirely reduced to DCE after 3 days, which remained the dominant dechlorination product through the 7-day period.

These observations further the understanding of Steol CS-330 interactions with dechlorinating microorganisms. First, the anionic sulfate group is demonstrated to not be responsible for inhibition of DCE reduction, as Neodol 25-7, a nonionic surfactant, exhibited a very similar dechlorination extent to Steol CS-330. However, the microorganisms that dechlorinate TCE to DCE do not appear to require a similar acclimation period in the presence of Neodol 25-7 that is necessary in the presence of Steol CS-330. This result is likely accounted for by the fact that Neodol 25-7 contains an average of four more EO groups compared to Steol CS-330, and microbial toxicity trends among similar surfactants imply that an increasing number of EO groups relates to decreasing toxicity (*1*). A similar argument can be made to explain the observed toxicity of SDS compared to Steol CS-330. The only structural difference in the two surfactants is the presence of an EO chain in the hydrophilic portion of Steol CS-330. The results suggest that hydrophobic interactions with the microorganisms may be contributing to inhibition to a greater degree than hydrophilic interactions. Previous observations of loss of DCE degradation by this culture in the presence of tridecane support this supposition (*23,24*). The presence of EO groups possibly adds increased distribution of

electronegativity and steric hindrance to the molecule, which could allow for decreased interaction of the molecule with the bacterial membrane and/or membrane bound proteins.

Several trends relating surfactant properties to microbial inhibition were observed and may be useful in selecting potential surfactants for use in SEAR processes that also implement reductive dechlorination. The most evident of surfactant properties appearing to affect PCE transformation is the presence and number of EO groups in the surfactant molecule. SDS ($C_{12}SO_4^-$) completely inhibited PCE reduction, while Steol CS-330 ($C_{12}EO_3SO_4^-$) had no effect on the initial PCE reduction step and a lag in PCE reduction to DCE was overcome by a short acclimation period. Increasing the degree of ethoxylation to an average of 7 EO groups (Neodol 25-7, $C_{12\text{-}15}EO_7H$) effected rapid reduction of PCE to DCE without need for acclimation. Moreover, the surfactant containing the most EO groups, Tween 80, which has on average of 20 EO groups distributed throughout the hydrophilic portion of the molecule, was the only surfactant screened exhibiting dechlorination beyond DCE and VC.

The CMC of the surfactants is another property that may aid in selection of potential surfactants. Among the anionic surfactants screened, the degree of inhibition was found to correlate to the CMC of the surfactants. Aerosol MA80-I nearly completely inhibited PCE dechlorination. This surfactant has the highest CMC (18.3 mM) of the anionic surfactants screened. The other anionic surfactants investigated, AOS 14-16 (CMC = 3.81 mM) and Steol CS-330 (CMC = 1.07 mM), each showed no effect on reduction of PCE to TCE in the 2-day study, and Steol CS-330 exhibited a slightly greater extent of DCE production. Since the CMC values for nonionic surfactants are

generally orders of magnitude below those for anionic surfactants, comparison of this property between surfactant classes is less useful. Also, the small variation in typical CMC values of nonionic surfactants suggests that a similar correlation to that observed for the anionics may not exist for nonionics. These observations suggest that the presence and number of EO groups and CMC values, especially for anionic surfactants, may be useful in predicting potential surfactant effects on PCE reductive dechlorination. However, further studies screening a wider variety of surfactants are warranted to confirm these observations. Researchers have shown that relations between surfactant properties and microbial toxicity likely exist, but quantification of the correlations is difficult, especially when utilizing a mixed culture (*3*). Considering that any *in situ* implementation consists of diverse microbial populations, screening surfactants for adverse effects on contaminant biotransformation should always be conducted.

The inhibiting activity of surfactants toward microbial degradation processes is usually accounted for via one or a combination of three mechanisms: decreased bioavailability of the contaminant due to micellar solubilization (*25*), uptake of the surfactant as a preferential substrate over the contaminant (*26,27*), or interaction of the surfactant with vital components of the microorganism (*19,28,29*). Experiments investigating the effect of Steol CS-330 concentration demonstrated that both PCE and TCE in a micellar solution are rapidly degraded indicating that decreased bioavailability does not limit dechlorination. Since PCE and TCE are each more hydrophobic than DCE, one can assume that DCE partitions less to surfactant micelles and therefore is also bioavailable. Preferential degradation of the surfactant was excluded based on results of the surfactant biodegradation study and the Steol CS-330 acclimation culture experiment.

The only remaining explanation for the observed effects on the reductive dechlorination process is surfactant-microorganism interaction. Several studies verified this was in fact the most likely mechanism of inhibition.

Previous work in our laboratory has demonstrated that the mixed culture utilized in this study contains at least two populations of reductive dechlorinating microorganisms (*30*). One population reduces PCE to TCE and DCE, while a second population reduces DCE to VC and ETH. Similar patterns of the complete dechlorination process have been observed by other research groups (*31*), and characterization of the cultures demonstrated that the reductive dechlorination process carried out by a culture enriched on VC and DCE differed from the reductive dechlorination of PCE and TCE. The disparate responses of these two populations to the presence of Steol CS-330 illustrate that differences in the reductive dechlorination process of the microorganisms that dechlorinate PCE and those that dechlorinate DCE exist in our culture as well.

In summary, two surfactants, with potentially different uses in SEAR, were investigated for their effects on the reductive dechlorination of PCE. The surfactants exhibited very different effects both on dechlorination and other processes such as methanogenesis. The nonionic surfactant, Tween 80, appears to be a surfactant with potential utilization in DNAPL remediation via solubilization/mobilization with reductive dechlorination serving as a second-step treatment option. The anionic surfactant, Steol CS-330, reversibly inhibited dechlorination beyond DCE in this study. Further studies, such as investigation of surfactant interactions with other dechlorinating cultures and the effects of foam on the dechlorination process, are warranted before implementation of a

Steol CS-330 hydrogen-based foam for the enhancement of hydrogen delivery to DNAPL source zones.

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6. CONCLUSIONS

The following conclusions are made based on investigations into the effects of surfactants on the reductive dechlorination of PCE by a mixed culture of microorganisms:

- All surfactants influenced the rate and/or extent of PCE dechlorination by the mixed culture. The cationic surfactant, CTAB, and the anionic surfactant, Aerosol MA80-I, each nearly completely inhibited PCE reduction. The rate of PCE transformation was not affected by the presence of the other surfactants. However, dechlorination beyond the extent of DCE was inhibited by all surfactants with the exception of Tween 80.
- Chlorinated ethenes in micellar surfactant solutions are available for utilization by dechlorinating microorganisms. However, TCE accumulation occurred under some circumstances and required an acclimation period before rapid reduction proceeded.
- Surfactant concentration was determined to affect the rate and extent of dechlorination by the mixed culture. ETH production increased slightly with decreasing Tween 80 concentration from 1.0% to 0.1%, however, all concentrations of Tween 80 decreased the production of ETH below that in surfactant-free controls. Dechlorination patterns also varied with concentration in the presence of Steol CS-330. At high surfactant concentrations (0.5 – 1.0%), an acclimation period of a few days was required for rapid reduction of PCE to DCE. The threshold concentration of Steol CS-330 toward DCE dechlorinating microorganisms was determined to be approximately 25 mg/L. Attempts to acclimate a culture to the surfactant were unsuccessful. Inhibition of DCE dechlorination was determined to be reversible upon dilution of the surfactant below the threshold concentration.

- Steol CS-330 and Tween 80 demonstrated differing effects not only on dechlorinators, but also on other populations in the mixed culture, such as methanogens. Steol CS-330 nearly completely inhibited methanogenesis at concentrations of 0.1 to 1.0%, while Tween 80 had no effect on methane production at the same concentrations.
- The only surfactant exhibiting degradation by the mixed anaerobic culture was Tween 80. However, based on methane production, only a small fraction of Tween 80 and the other surfactants were completely degraded to methane using stoichiometry and COD measurements of the surfactants.

The following statements are made based on observations resulting from investigations into the effects of surfactants on reductive dechlorination of PCE by a mixed culture and warrant further research to obtain more conclusive evidence:

- Interaction of Steol CS-330 with DCE dechlorinating microorganisms is the most likely cause of the observed inhibition of these organisms. The exact mechanism of inhibition has not been proven; however, evidence suggests that interactions of the surfactant with the enzyme system of DCE dehalogenation are likely.
- The degree of ethoxylation of surfactants was found to relate to adverse effects on the PCE dechlorination process, as increasing EO number resulted in decreased inhibition.
- Differences in the reductive dechlorination processes of PCE dechlorinating microorganisms and DCE dechlorinating microorganisms in the mixed anaerobic culture exist.

7. Engineering Significance

The use of surfactants to enhance the remediation of DNAPL source zones is an emerging technology that has received increased attention in laboratory studies and field demonstrations over the past decade. The primary mechanism by which surfactants enhance NAPL remediation is via solubilization and/or mobilization due to micelle formation and reduced interfacial tensions. However, even the most properly designed surfactant flood is unlikely to remove all contamination present. Therefore, a second treatment option may be required to reduce contaminant concentrations below drinking water standards. In the case of chlorinated ethene DNAPLs, which the EPA reports are present at the majority of NPL sites (*1*), microbial reductive dechlorination represents a potential option as a final step in the treatment scheme. For such a process to be successful however, the surfactant must minimally impact the microorganisms involved in the dechlorination process. The results obtained in this study demonstrate that Tween 80, or at least the Tween series of surfactants, warrant further examination for use in such a treatment strategy.

Another surfactant-enhanced remediation mechanism currently under investigation is the use of hydrogen-generated surfactant foams to enhance electron donor delivery to DNAPL source zones for utilization by halorespiring microorganisms. Currently, hydrogen biosparging and production of hydrogen via fermentation of organic substrates are the predominant mechanisms for delivery of electron donor. However, biosparging has a small zone of influence in source areas and the fermentation method produces low levels of hydrogen that may not be sufficient to support extensive dechlorination in source zones. The use of surfactant foams represents an innovative

technology that is capable of increasing the residual saturation of hydrogen in an aquifer, as well as enhancing contact of DNAPL source zones since foams are transported laterally through porous media. The studies presented herein demonstrate that the surfactants with optimal foaming properties, such as Steol CS-330, may not be the most benign to the microbial reductive dechlorination process. Further research in this area is warranted to obtain the required combination of a good foaming surfactant having minimal impact on dechlorinating microorganisms.

Economic analyses of the first SEAR process discussed above have been provided in other sources (*2,3*), and implementation of a reductive dechlorination "polishing" step could potentially add a lesser financial and/or operative burden compared to some other alternatives. This would be especially true if a slowly degradable surfactant, such as a Tween, was fermented to support reductive dechlorination. An analysis of the latter SEAR process would be premature given the early stages of research involving hydrogen-generated foams. However, given that this process is aimed at treatment of the source zone rather than the dissolved plume, a higher initial cost may outweigh the costs associated with operation and maintenance of lengthy plume treatment strategies such as pump-and-treat.

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8. Further Research Recommendations

The effect of surfactants on PCE dechlorinating microorganisms is an area that has received little attention. Therefore, opportunities for further research on the topic abound. The varying effects on the PCE dechlorination process exhibited by the surfactants suggest that careful selection and screening of potential surfactants for use in laboratory studies or field demonstrations is very important. The capability to predict surfactant toxicity based on surfactant properties such as CMC, degree of ethoxylation, bulk of the hydrophile, length of the hydrophobe, etc., would prove valuable in such selection and screening.

The nonionic surfactant Tween 80 showed the most potential among the surfactants screened as a candidate for use in a SEAR process in which solubilization/mobilization via surfactant removes the bulk of contaminant and reductive dechlorination is used as a tool to remove residual contamination. Other studies have shown that Tween 80 is not always the most benign of this surfactant series towards microorganisms and therefore, an investigation into other Tween surfactants on PCE dechlorination would be beneficial. Also, the Tween surfactant was shown to biodegrade in the presence of the mixed culture and it is possible that fermentation of the surfactant could be used to support the reductive dechlorination of PCE.

Surfactants that are capable of producing strong foam, such as Steol CS-330, have the potential to greatly enhance contacting and storage of hydrogen in DNAPL source zones. However, aqueous Steol CS-330 solutions were found to inhibit the complete dechlorination of PCE to ETH at a concentration of 50 mg/L. This concentration is likely too low to produce consistently strong foam under field conditions. A study to verify that

Steol CS-330 foam has the same effect as an aqueous solution on dechlorination is warranted. A nonionic surfactant that is also a good foamer, APG, was also found to inhibit the dechlorination process at a concentration of 1.0%. However, since nonionic surfactants are generally more benign to microorganisms, the threshold concentration of APG toward DCE dechlorinating microorganisms should be explored, as it may be significantly greater than for Steol CS-330.

The differing effect of Steol CS-330 on the microorganisms that reduce PCE versus those that reduce DCE suggests that the two processes occur dissimilarly. This result was not expected and illustrates the need for better characterization of the microbes that carry out reductive dechlorination. This is especially true for the population of microorganisms that reduce DCE and VC.

9.0 APPENDIX – EXPERIMENTAL DATA

Figure 4.1. Surfactant Biodegradation: Methane Production Relative to Surfactant-free Reference as Indicator

Time (d)	Reference	AOS 14-16	Steol CS-330	Aerosol MA80-I	Neodol 25-7	Tween 80
0	1	0.00	0.00	0.00	0.00	0.00
1	1	1.09	0.10	0.64	0.72	2.63
3	1	1.33	0.08	0.83	0.91	1.83
7	1	1.57	0.14	1.10	1.18	1.68
14	1	1.80	0.55	1.50	1.41	1.69
21	1	1.87	0.94	1.63	1.52	1.67
28	1	1.98	1.14	1.83	1.59	1.67
35	1	2.08	1.34	2.00	1.67	1.69
49	1	2.25	1.43	2.18	1.90	1.59
70	1	2.49	1.70	2.41	2.40	1.70

Figure 4.2a. Effect of Tween 80 Concentration on Rate and Extent of PCE Dechlorination – Surfactant-free Control

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	0.00E+00	0.00E+00	2.70E-03	1.13E-02	1.15E-05
2	0.00E+00	0.00E+00	2.11E-03	1.17E-02	4.98E-04
3	0.00E+00	0.00E+00	2.37E-03	1.34E-02	1.08E-03
4	0.00E+00	0.00E+00	2.01E-03	1.17E-02	1.44E-03
5	0.00E+00	0.00E+00	1.73E-03	1.06E-02	1.76E-03
6	0.00E+00	0.00E+00	1.60E-03	1.03E-02	2.24E-03
7	0.00E+00	0.00E+00	1.58E-03	1.02E-02	2.57E-03
9	0.00E+00	0.00E+00	1.51E-03	1.03E-02	3.17E-03
11	0.00E+00	0.00E+00	1.32E-03	9.44E-03	3.57E-03
13	0.00E+00	0.00E+00	1.62E-03	1.05E-02	4.47E-03
15	0.00E+00	0.00E+00	1.24E-03	9.04E-03	4.70E-03

Figure 4.2b. Effect of Tween 80 Concentration on Rate and Extent of PCE Dechlorination – 0.1% Tween 80

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	2.83E-04	0.00E+00	2.89E-03	1.21E-02	7.00E-05
2	0.00E+00	0.00E+00	2.41E-03	1.14E-02	3.03E-04
3	0.00E+00	0.00E+00	2.70E-03	1.32E-02	4.93E-04
4	0.00E+00	0.00E+00	2.71E-03	1.34E-02	6.05E-04
5	0.00E+00	0.00E+00	2.29E-03	1.17E-02	6.09E-04
6	0.00E+00	0.00E+00	2.34E-03	1.20E-02	6.79E-04
7	0.00E+00	0.00E+00	2.27E-03	1.20E-02	7.14E-04
9	0.00E+00	0.00E+00	2.35E-03	1.25E-02	7.78E-04
11	0.00E+00	0.00E+00	2.09E-03	1.15E-02	7.54E-04
13	0.00E+00	0.00E+00	2.66E-03	1.28E-02	8.35E-04
15	0.00E+00	0.00E+00	2.35E-03	1.19E-02	8.16E-04

Figure 4.2c. Effect of Tween 80 Concentration on Rate and Extent of PCE Dechlorination – 0.5% Tween 80

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	0.00E+00	0.00E+00	2.35E-03	1.19E-02	6.37E-05
2	0.00E+00	0.00E+00	2.09E-03	1.21E-02	2.92E-04
3	0.00E+00	0.00E+00	2.11E-03	1.26E-02	3.85E-04
4	0.00E+00	0.00E+00	2.05E-03	1.23E-02	4.24E-04
5	0.00E+00	0.00E+00	1.90E-03	1.16E-02	4.45E-04
6	0.00E+00	0.00E+00	2.01E-03	1.24E-02	5.05E-04
7	0.00E+00	0.00E+00	1.97E-03	1.24E-02	5.20E-04
9	0.00E+00	0.00E+00	1.87E-03	1.19E-02	5.15E-04
11	0.00E+00	0.00E+00	1.71E-03	1.14E-02	5.19E-04
13	0.00E+00	0.00E+00	2.26E-03	1.31E-02	6.08E-04
15	0.00E+00	0.00E+00	2.12E-03	1.24E-02	5.93E-04

Figure 4.2d. Effect of Tween 80 Concentration on Rate and Extent of PCE Dechlorination – 1.0% Tween 80

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	4.96E-04	1.85E-04	1.68E-03	1.06E-02	5.22E-05
2	0.00E+00	0.00E+00	1.58E-03	1.15E-02	2.28E-04
3	0.00E+00	0.00E+00	1.51E-03	1.12E-02	2.63E-04
4	0.00E+00	0.00E+00	1.49E-03	1.11E-02	2.81E-04
5	0.00E+00	0.00E+00	1.57E-03	1.18E-02	3.19E-04
6	0.00E+00	0.00E+00	1.51E-03	1.15E-02	3.19E-04
7	0.00E+00	0.00E+00	1.49E-03	1.13E-02	3.17E-04
9	0.00E+00	0.00E+00	1.44E-03	1.08E-02	3.10E-04
11	0.00E+00	0.00E+00	1.36E-03	1.09E-02	3.22E-04
13	0.00E+00	0.00E+00	1.73E-03	1.22E-02	3.66E-04
15	0.00E+00	0.00E+00	1.60E-03	1.14E-02	3.50E-04

Figure 4.3a. Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination – Surfactant-free Control

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	0.00E+00	0.00E+00	2.01E-03	1.51E-02	3.90E-04
2	0.00E+00	0.00E+00	1.72E-03	1.30E-02	1.54E-03
3	0.00E+00	0.00E+00	1.42E-03	1.22E-02	2.90E-03
4	0.00E+00	0.00E+00	1.21E-03	1.10E-02	3.44E-03
5	0.00E+00	0.00E+00	9.12E-04	8.61E-03	3.68E-03
6	0.00E+00	0.00E+00	8.14E-04	8.54E-03	5.02E-03
7	0.00E+00	0.00E+00	7.89E-04	8.55E-03	5.83E-03
9	0.00E+00	0.00E+00	5.58E-04	6.84E-03	6.36E-03
11	0.00E+00	0.00E+00	5.13E-04	6.51E-03	7.57E-03
13	0.00E+00	0.00E+00	3.96E-04	5.63E-03	8.69E-03
15	0.00E+00	0.00E+00	3.32E-04	4.87E-03	8.90E-03

Figure 4.3b. Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination – 0.1% Steol CS-330

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	0.00E+00	8.98E-03	7.82E-03	9.22E-05	2.56E-06
2	0.00E+00	4.22E-03	1.21E-02	9.60E-05	2.72E-06
3	0.00E+00	2.59E-04	1.43E-02	9.14E-05	3.01E-06
4	0.00E+00	2.78E-04	1.51E-02	7.70E-05	2.50E-06
5	0.00E+00	9.67E-04	1.58E-02	8.80E-05	2.94E-06
6	0.00E+00	9.98E-04	1.68E-02	8.52E-05	3.46E-06
7	0.00E+00	3.88E-05	1.41E-02	8.88E-05	3.93E-06
9	0.00E+00	8.82E-05	1.47E-02	8.63E-05	5.08E-06
11	0.00E+00	9.72E-04	1.47E-02	8.18E-05	5.68E-06
13	0.00E+00	5.56E-05	1.19E-02	8.53E-05	7.72E-06
15	0.00E+00	6.00E-04	1.47E-02	8.14E-05	8.16E-06

Figure 4.3c. Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination – 0.5% Steol CS-330

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	0.00E+00	1.36E-02	1.66E-03	3.59E-05	1.56E-05
2	0.00E+00	1.44E-02	2.33E-03	3.28E-05	1.55E-05
3	0.00E+00	1.13E-02	6.08E-03	2.98E-05	1.31E-05
4	0.00E+00	9.02E-04	1.45E-02	3.05E-05	1.27E-05
5	0.00E+00	1.70E-03	1.62E-02	3.64E-05	1.51E-05
6	0.00E+00	7.09E-04	1.60E-02	3.31E-05	1.60E-05
7	0.00E+00	2.81E-05	1.44E-02	3.00E-05	1.47E-05
9	0.00E+00	7.42E-05	1.52E-02	3.36E-05	1.78E-05
11	0.00E+00	1.59E-03	1.53E-02	3.08E-05	1.74E-05
13	0.00E+00	2.93E-04	1.34E-02	3.07E-05	1.85E-05
15	0.00E+00	7.15E-04	1.40E-02	3.19E-05	1.91E-05

Figure 4.3d. Effect of Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination – 1.0% Steol CS-330

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	0.00E+00	1.56E-02	1.32E-03	8.51E-05	2.95E-05
2	0.00E+00	1.43E-02	7.56E-04	7.93E-05	2.63E-05
3	0.00E+00	1.37E-02	1.09E-03	7.96E-05	2.81E-05
4	0.00E+00	1.39E-02	1.35E-03	7.12E-05	2.42E-05
5	0.00E+00	1.35E-02	2.16E-03	7.28E-05	2.55E-05
6	0.00E+00	8.94E-04	1.51E-02	7.34E-05	2.54E-05
7	0.00E+00	8.75E-04	1.33E-02	7.47E-05	2.73E-05
9	0.00E+00	3.84E-05	1.40E-02	8.55E-05	3.13E-05
11	0.00E+00	2.96E-04	1.36E-02	7.42E-05	2.93E-05
13	0.00E+00	1.87E-04	1.33E-02	8.15E-05	3.38E-05
15	0.00E+00	5.61E-04	1.34E-02	7.66E-05	3.12E-05

Figure 4.4a. Acclimation of PCE Dechlorination to DCE at High Steol CS-330 Concentrations – 0.5% Steol CS-330

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	0.00E+00	1.62E-02	1.17E-05	0.00E+00	7.89E-06
2	0.00E+00	1.52E-02	2.33E-04	0.00E+00	7.28E-06
3	0.00E+00	1.55E-02	5.54E-04	0.00E+00	8.58E-06
4	0.00E+00	8.78E-04	1.28E-02	0.00E+00	8.27E-06
5	0.00E+00	4.54E-04	1.41E-02	0.00E+00	9.84E-06
5.0001	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
6	0.00E+00	2.81E-03	1.53E-02	0.00E+00	0.00E+00
7	0.00E+00	3.24E-04	1.79E-02	0.00E+00	0.00E+00
8	0.00E+00	9.33E-05	1.49E-02	0.00E+00	0.00E+00
12	0.00E+00	5.46E-05	1.63E-02	0.00E+00	3.41E-06
15	0.00E+00	4.25E-04	1.52E-02	0.00E+00	3.26E-06

Figure 4.4b. Acclimation of PCE Dechlorination to DCE at High Steol CS-330 Concentrations – 1.0% Steol CS-330

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	1.36E-02	3.85E-03	4.16E-04	0.00E+00	1.59E-05
2	0.00E+00	1.59E-02	6.04E-05	0.00E+00	1.52E-05
3	0.00E+00	1.61E-02	3.44E-06	0.00E+00	1.68E-05
4	0.00E+00	1.49E-02	3.45E-06	0.00E+00	1.65E-05
5	0.00E+00	1.77E-02	1.05E-03	0.00E+00	2.00E-05
6	0.00E+00	1.37E-02	3.57E-05	0.00E+00	1.94E-05
7	0.00E+00	1.02E-02	5.31E-03	0.00E+00	1.91E-05
8	0.00E+00	2.22E-04	1.31E-02	0.00E+00	1.89E-05
8.001	1.47E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
9	0.00E+00	1.73E-05	1.58E-02	0.00E+00	0.00E+00
10	0.00E+00	1.53E-04	1.68E-02	0.00E+00	0.00E+00
11	0.00E+00	3.28E-04	1.73E-02	0.00E+00	2.26E-06
15	0.00E+00	5.07E-04	1.53E-02	0.00E+00	3.71E-06

Figure 4.5a. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of cis-DCE Dechlorination – Surfactant-free Control

	DCE and Dechlorination Metabolites (mmol)		
Time (d)	DCE	VC	ETH
0	1.46E-02	0.00E+00	0.00E+00
3	0.00E+00	1.49E-02	8.91E-04
7	0.00E+00	9.41E-03	2.82E-03
10	0.00E+00	8.79E-03	4.79E-03
14	0.00E+00	7.38E-03	6.87E-03
21	0.00E+00	4.46E-03	9.55E-03
28	0.00E+00	2.90E-03	1.13E-02
35	0.00E+00	2.17E-03	1.30E-02

Figure 4.5b. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of cis-DCE Dechlorination – 0.1% Steol CS-330

	DCE and Dechlorination Metabolites (mmol)		
Time (d)	DCE	VC	ETH
0	1.46E-02	0.00E+00	0.00E+00
3	1.79E-02	9.04E-05	0.00E+00
7	1.61E-02	7.94E-05	0.00E+00
10	1.55E-02	8.13E-05	2.21E-06
14	1.55E-02	7.77E-05	0.00E+00
21	1.67E-02	8.67E-05	2.85E-06
28	1.28E-02	8.61E-05	3.03E-06
35	1.15E-02	7.99E-05	2.97E-06

Figure 4.5c. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of cis-DCE Dechlorination – 0.5% Steol CS-330

	DCE and Dechlorination Metabolites (mmol)		
Time (d)	DCE	VC	ETH
0	1.46E-02	0.00E+00	0.00E+00
3	1.59E-02	1.02E-04	1.16E-05
7	1.57E-02	9.00E-05	1.04E-05
10	1.52E-02	6.77E-05	8.14E-06
14	1.48E-02	6.99E-05	6.75E-06
21	1.70E-02	9.38E-05	1.15E-05
28	1.32E-02	9.92E-05	1.22E-05
35	1.30E-02	8.92E-05	1.15E-05

Figure 4.5d. Long-term Effect of Steol CS-330 Concentration on Rate and Extent of cis-DCE Dechlorination – 1.0% Steol CS-330

	DCE and Dechlorination Metabolites (mmol)		
Time (d)	DCE	VC	ETH
0	1.46E-02	0.00E+00	0.00E+00
3	1.56E-02	8.82E-05	2.45E-05
7	1.64E-02	8.58E-05	2.38E-05
10	1.57E-02	7.35E-05	1.98E-05
14	1.45E-02	6.98E-05	1.97E-05
21	1.70E-02	8.67E-05	2.40E-05
28	1.25E-02	4.81E-05	3.78E-06
35	1.30E-02	5.04E-05	3.77E-06

Figure 4.6a. Effect of Incremental Increases in Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination by a Mixed Anaerobic Culture – Daily PCE Addition and Metabolite Production

	PCE and Dechlorination Metabolites (mmol)				
Time (d)	PCE	TCE	DCE	VC	ETH
0	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	0.00E+00	0.00E+00	2.57E-04	4.73E-02	1.58E-02
1	5.87E-02	0.00E+00	2.57E-04	4.73E-02	1.58E-02
2	0.00E+00	0.00E+00	4.73E-04	8.32E-02	4.04E-02
2	5.87E-02	0.00E+00	4.73E-04	8.32E-02	4.04E-02
3	0.00E+00	0.00E+00	5.56E-04	1.09E-01	7.36E-02
3	5.87E-02	0.00E+00	5.56E-04	1.09E-01	7.36E-02
4	0.00E+00	0.00E+00	4.28E-04	1.31E-01	1.09E-01
4	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
5	0.00E+00	0.00E+00	4.46E-04	4.93E-02	1.46E-02
5	5.87E-02	0.00E+00	4.46E-04	4.93E-02	1.46E-02
6	0.00E+00	0.00E+00	3.24E-04	7.58E-02	4.48E-02
6	5.87E-02	0.00E+00	3.24E-04	7.58E-02	4.48E-02
7	0.00E+00	0.00E+00	3.40E-04	1.03E-01	7.75E-02
7	5.87E-02	0.00E+00	3.40E-04	1.03E-01	7.75E-02
8	0.00E+00	0.00E+00	3.52E-04	1.24E-01	1.20E-01
8	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
9	0.00E+00	0.00E+00	2.42E-04	5.05E-02	2.25E-02
9	5.87E-02	0.00E+00	2.42E-04	5.05E-02	2.25E-02
10	0.00E+00	0.00E+00	4.70E-04	8.25E-02	5.25E-02
10	5.87E-02	0.00E+00	4.70E-04	8.25E-02	5.25E-02
11	0.00E+00	0.00E+00	2.64E-04	1.03E-01	7.89E-02
11	5.87E-02	0.00E+00	2.64E-04	1.03E-01	7.89E-02
12	0.00E+00	0.00E+00	2.72E-04	1.08E-01	1.35E-01
12	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
13	0.00E+00	0.00E+00	2.44E-04	4.55E-02	2.24E-02
13	5.87E-02	0.00E+00	2.44E-04	4.55E-02	2.24E-02
14	0.00E+00	0.00E+00	1.85E-04	6.86E-02	5.65E-02
14	5.87E-02	0.00E+00	1.85E-04	6.86E-02	5.65E-02
15	0.00E+00	0.00E+00	3.24E-04	9.28E-02	9.73E-02
15	5.87E-02	0.00E+00	3.24E-04	9.28E-02	9.73E-02
16	0.00E+00	0.00E+00	3.71E-04	1.04E-01	1.48E-01
16	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
17	0.00E+00	0.00E+00	1.34E-03	5.49E-02	1.14E-02
17	5.87E-02	0.00E+00	1.34E-03	5.49E-02	1.14E-02
18	0.00E+00	0.00E+00	1.32E-03	1.05E-01	1.76E-02
18	5.87E-02	0.00E+00	1.32E-03	1.05E-01	1.76E-02
19	0.00E+00	0.00E+00	1.16E-03	1.49E-01	2.53E-02
19	5.87E-02	0.00E+00	1.16E-03	1.49E-01	2.53E-02
20	0.00E+00	0.00E+00	1.22E-03	1.95E-01	3.95E-02

20	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
21	2.50E-03	5.42E-02	3.22E-03	4.18E-03	9.01E-04
21	2.50E-03	5.42E-02	3.22E-03	4.18E-03	9.01E-04
22	0.00E+00	3.69E-02	1.66E-02	4.18E-03	9.01E-04
22	5.87E-02	3.69E-02	1.66E-02	4.18E-03	9.01E-04
23	0.00E+00	5.28E-02	4.42E-02	4.18E-03	9.01E-04
23	5.87E-02	5.28E-02	4.42E-02	4.18E-03	9.01E-04
24	0.00E+00	6.91E-02	9.48E-02	4.18E-03	9.01E-04
24	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
25	0.00E+00	3.56E-03	6.73E-02	0.00E+00	0.00E+00
25	5.87E-02	3.56E-03	6.73E-02	0.00E+00	0.00E+00
26	0.00E+00	3.56E-03	1.36E-01	0.00E+00	0.00E+00
26	5.87E-02	3.56E-03	1.36E-01	0.00E+00	0.00E+00
27	0.00E+00	4.19E-03	2.02E-01	0.00E+00	0.00E+00
27	5.87E-02	4.19E-03	2.02E-01	0.00E+00	0.00E+00
28	0.00E+00	2.42E-03	2.24E-01	0.00E+00	0.00E+00
28	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
29	0.00E+00	1.66E-04	6.78E-02	0.00E+00	0.00E+00
29	5.87E-02	1.66E-04	6.78E-02	0.00E+00	0.00E+00
30	0.00E+00	5.90E-03	1.13E-01	0.00E+00	0.00E+00
30	5.87E-02	5.90E-03	1.13E-01	0.00E+00	0.00E+00
31	0.00E+00	3.93E-03	1.86E-01	0.00E+00	0.00E+00
31	5.87E-02	3.93E-03	1.86E-01	0.00E+00	0.00E+00
32	0.00E+00	1.06E-02	2.04E-01	0.00E+00	0.00E+00
32	5.87E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
33	0.00E+00	4.69E-04	5.78E-02	0.00E+00	0.00E+00
33	5.87E-02	4.69E-04	5.78E-02	0.00E+00	0.00E+00
34	0.00E+00	1.47E-02	9.51E-02	0.00E+00	0.00E+00
34	5.87E-02	1.47E-02	9.51E-02	0.00E+00	0.00E+00
35	0.00E+00	5.38E-04	1.70E-01	0.00E+00	0.00E+00
35	5.87E-02	5.38E-04	1.70E-01	0.00E+00	0.00E+00
36	0.00E+00	3.08E-04	2.10E-01	0.00E+00	0.00E+00

Figure 4.6b. Effect of Incremental Increases in Steol CS-330 Concentration on Rate and Extent of PCE Dechlorination by a Mixed Anaerobic Culture – Cumulative PCE Addition and Metabolite Production

	CS-330	PCE and Dechlorination Metabolites (mmol)				
Time (d)	(mg/L)	PCE	TCE	DCE	VC	ETH
0		0.000	0.000	0.000	0.000	0.000
1		0.059	0.000	0.000	0.047	0.016
2		0.117	0.000	0.000	0.083	0.040
3		0.176	0.000	0.001	0.109	0.074
4	1	0.235	0.000	0.000	0.131	0.109
5		0.294	0.000	0.001	0.180	0.123
6		0.352	0.000	0.001	0.207	0.154
7		0.411	0.000	0.001	0.234	0.186
8	5	0.470	0.000	0.001	0.255	0.229
9		0.528	0.000	0.001	0.305	0.252
10		0.587	0.000	0.001	0.337	0.282
11		0.646	0.000	0.001	0.358	0.308
12	10	0.704	0.000	0.001	0.363	0.364
13		0.763	0.000	0.001	0.408	0.387
14		0.822	0.000	0.001	0.431	0.421
15		0.881	0.000	0.001	0.455	0.462
16	25	0.939	0.000	0.001	0.466	0.512
17		0.998	0.000	0.003	0.521	0.524
18		1.057	0.000	0.003	0.571	0.530
19		1.115	0.000	0.003	0.615	0.538
20	50	1.174	0.000	0.003	0.661	0.552
21		1.174	0.054	0.006	0.665	0.553
22		1.233	0.037	0.019	0.665	0.553
23		1.291	0.053	0.047	0.665	0.553
24		1.350	0.069	0.097	0.665	0.553
25		1.409	0.073	0.165	0.665	0.553
26		1.468	0.073	0.234	0.665	0.553
27		1.526	0.073	0.299	0.665	0.553
28	100	1.585	0.071	0.321	0.665	0.553
29		1.644	0.072	0.389	0.665	0.553
30		1.702	0.077	0.434	0.665	0.553
31		1.761	0.075	0.508	0.665	0.553
32		1.820	0.082	0.525	0.665	0.553
33		1.878	0.083	0.583	0.665	0.553
34		1.937	0.097	0.620	0.665	0.553
35		1.996	0.083	0.696	0.665	0.553
36		2.055	0.082	0.735	0.665	0.553

Figure 4.7a. Effect of Tween 80 Concentration on Methane Production by the Mixed Anaerobic Culture

	Methane Produced (mL @ 1 atm, 25 C)			
Time (d)	Control	0.1% Tween	0.5% Tween	1% Tween
0	0.00	0.00	0.00	0.00
1	3.79	3.16	2.96	3.33
2	6.47	4.91	5.73	6.21
3	10.45	8.33	8.42	8.10
4	11.50	11.02	9.82	10.12
5	12.26	11.30	11.14	13.04
6	14.62	13.41	13.90	14.43
7	17.37	14.91	15.31	15.58
9	18.31	17.92	16.53	16.53
11	18.69	18.37	18.85	18.51
13	24.14	24.18	24.30	24.11
15	23.93	24.38	24.23	23.24

Figure 4.7b. Effect of Steol CS-330 Concentration on Methane Production by the Mixed Anaerobic Culture

	Methane Produced (mL @ 1 atm, 25 C)			
Time (d)	Control	0.1% CS-330	0.5% CS-330	1% CS-330
0	0.00	0.00	0.00	0.00
1	3.18	2.19	2.18	1.80
2	5.55	2.51	2.39	2.02
3	8.60	2.43	2.00	2.23
4	9.58	2.06	1.96	1.94
5	9.72	2.47	2.33	2.02
6	12.19	2.39	2.37	2.00
7	14.43	2.49	2.12	2.11
9	15.68	2.46	2.45	2.36
11	18.00	2.27	2.28	2.11
13	18.86	2.36	2.27	2.35
15	18.39	2.19	2.26	2.11

Figure 4.8a. Effect of Tween 80 Concentration on H_2/CO_2 Gas Consumption by the Mixed Anaerobic Culture

	H_2/CO_2 Consumed (mL @ 1 atm, 25 C)			
Time (d)	Control	0.1% Tween	0.5% Tween	1% Tween
0	0	0	0	0
1	13.4	12	12.6	11.2
2	25.2	22	25.8	24.6
3	34.2	34	36.6	34.2
4	43.2	42.8	43.2	43.8
5	51.6	50.8	52	51.6
6	57.8	58.2	58.4	58.2
7	64	65.4	64.8	63.8
9	70	72.4	70.6	69
11	75	78.2	76.8	74
13	77	80.6	77	74
15	77	81.2	77.4	74

Figure 4.8b. Effect of Steol CS-330 Concentration on H_2/CO_2 Gas Consumption by the Mixed Anaerobic Culture

	H_2/CO_2 Consumed (mL @ 1 atm, 25 C)			
Time (d)	Control	0.1% CS-330	0.5% CS-330	1% CS-330
0	0	0	0	0
1	4.1	0	3	0
2	16.1	4	7	0
3	30.1	4	7	3.2
4	38.1	4	7	3.2
5	45.1	4	7	3.2
6	57.1	4	19	3.2
7	63.1	4	30	6.2
9	76.1	21	49	12.2
11	80.6	34	63	12.2
13	81.6	51	76	18.2
15	83.2	64	77.8	31.2