2-Chloroacetophenone as a Probe Compound for Studying Reduction Reactions in Anaerobic Sediments

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2-Chloroacetophenone as a Chemical Probe for Studying Reduction Reactions in Anaerobic Sediments

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Reduction reactions contribute to a significant portion of the chemical transformations that take place in anaerobic sediments. While these processes are known to be strongly linked to microbial activity, little is known about the specific reducing agents responsible for contaminant reduction. One approach to identifying these reductants is to use molecular probes that give reaction products (or kinetics) that are diagnostic for particular reaction mechanisms. A method using 2-chloroacetophenone (2-CAP) has been developed for this purpose. 2-CAP is reduced by electron transfer to form acetophenone (AcPh) and by hydride transfer to form 2-chloro-1-phenylethanol (2-CPE). AcPh can be further reduced by hydride transfer to form 1-phenylethanol (1-PE). Using an appropriate extraction technique and high-performance liquid chromatography (HPLC), 2-CAP and its reduction products can be detected and quantified in anaerobic sediments.

The results of numerous batch experiments consistently showed that 2-CPE formation is favored over AcPh production in sediment collected from a tributary of Rock Creek in Beaverton, Oregon. AcPh reduction to 1-PE was rarely observed and control experiments showed that the kinetics of this reaction are very slow in comparison to 2-CAP reduction. 2-CAP reduction and product formation appear to follow first-order reaction kinetics. However, further analysis revealed that the observed kinetics actually represent the combined effects of adsorption and reduction processes. Detailed kinetic modeling will be necessary to fit both adsorption and reduction data simultaneously.

Model systems allow the 2-CAP probe response to be calibrated in ways that may eventually allow the probe to be used to identify particular reductants. In the presence of NADPH and *Thermoanaerobium brockii* alcohol dehydrogenase, 2-CAP has been shown to react by hydride transfer to form 2-CPE. In the absence of the enzyme, 2-CAP was not reduced. Previous studies in sediments have shown that production of 2-CPE is temperature dependent and distribution of the 2-CPE stereoisomers suggests that the reducing agents are enantioselective. Both phenomenon are indicative of an enzyme-mediated reaction.

To further validate the notion of an enzyme-mediated transformation, the effects of enzyme inhibitors (p-chloromercuribenzoate, 1-formylpiperidine, and pyrazole) on 2-CAP reduction were studied in an NADPH/dehydrogenase model system. The results showed small, but consistent, decreases in the rate of 2-CAP reduction with increasing inhibitor concentration. When applied to sediment, all three inhibitors caused a decrease in both the rate of 2-CPE formation and the amount of 2-CPE formed, but had only minor effects on 2-CAP reduction. In contrast, product inhibition, employing the addition of 2-CPE, successfully inhibited 2-CAP reduction, causing a decrease in the rate of reduction with increasing inhibitor concentration. The results obtained with these inhibitors are further evidence that reduction of 2-CAP in sediment is an enzyme-mediated process.

Additional studies were performed using 2-bromoacetophenone (2-BAP) and 2,2-dichloroacetophenone (2,2-CAP) as complimentary probes to 2-CAP. Both compounds were reduced by hydride and electron transfer in Rock Creek tributary sediment. Preferential production of AcPh from 2-BAP, in a system where hydride activity is apparently high (based on observed 2-CAP reduction trends), may provide evidence for the presence of NAD(P)H which is capable of reducing 2-BAP external to a dehydrogenase enzyme.

CHAPTER 1
INTRODUCTION

One of the outstanding questions regarding the chemistry of environmental contamination degradation is the identity of chemical agents responsible for reduction reactions in anaerobic sediments, soils, and sludges. Until we are able to better understand sediment biogeochemistry and predict rates of contaminant transformations, we are limited in regulatory decision-making regarding contaminant fate and remediation technologies.

Due to the complex nature of sediments, identification of reducing agents has proven challenging. One method that has shown potential for identifying reducing agents is the use of chemical probes that give reaction products (or kinetics) that are diagnostic for particular reduction mechanisms. A variety of chemical probes, including indicator dyes such as sulfonated indigos, tetrazoliums, indophenols, and resazurin have been useful in measuring reduction kinetics in sediment [1, 2]. However, they have provided little insight into the specific reducing agents responsible for transformation. One particular compound that has proven successful in both quantifying reaction rates and providing information about the reducing agents in sediment systems is 2-chloroacetophenone (2-CAP). The fate of 2-CAP in reducing sediments was the focus of this study because it offers well-characterized reaction pathways that can distinguish between hydride and electron transfer pathways in complex media such as sediments.

1.1 Background

1.1.1 Chemical transformations

Sediments are a very complex system, comprised of homogeneous mixtures of particulate organic matter, minerals, and bacteria in an aqueous medium (Figure 1.1). A variety of pollutants, *P*, can be transformed through a number of reactions at or near the sediment surface. These reactions may include reduction by organic matter or mineral surfaces, intracellular transformations, and mediated extracellular transformations. The idea that certain reagents act as mediators to reduce organic substrates by shuttling electrons from an electron donor to a substrate is well established in biochemistry and has been proposed as the basis for redox processes in environmental systems. The substances presumed responsible for mediating reduction reactions in the environment are many of the same substances involved in biochemical reactions, including porphyrins, corrins, flavins, iron-sulfur proteins, and other enzymes or cofactors [3-11]. The initial reduction of these mediators may be a result of microbial metabolism, reaction with mineral surfaces, or other geochemical processes. The sediment model system depicted in Figure 1.1 proposes that mediators of extracellular reduction are generated through microbial metabolism of sediment-associated microorganisms. Once released into the environment, these reducing equivalents are quickly oxidized by way of extracellular reduction of the pollutant. A chemical probe that is diagnostic for particular reaction mechanisms or kinetics can act as the substrate/probe in these environments to provide information about these reducing mediators. In principle, the probe concept is applicable to all of the transformation mechanisms previously mentioned, but it is best suited for investigating mediated extracellular processes where the reactions are not limited by cellular uptake of the substrate.

Not all sediment-associated processes involve transformation of the probe compound. Adsorption often plays a role in substrate disappearance. Cation exchange, hydrophobic effects, and hydrogen bonding are some of the common mechanisms for sorption to organic matter [12]. Often, the substrate is loosely bound to the sediment, allowing for desorption, but it can also be irreversibly bound, in which case the substrate

may not be available for further reaction. Previous studies indicate that while both abiotic reduction and sediment-associated biotransformation processes are favored in systems with high sediment concentrations, they are also inhibited by adsorption of the substrate [13-15]. This study is the first to quantify the role of adsorption on substrate disappearance and subsequent product appearance.

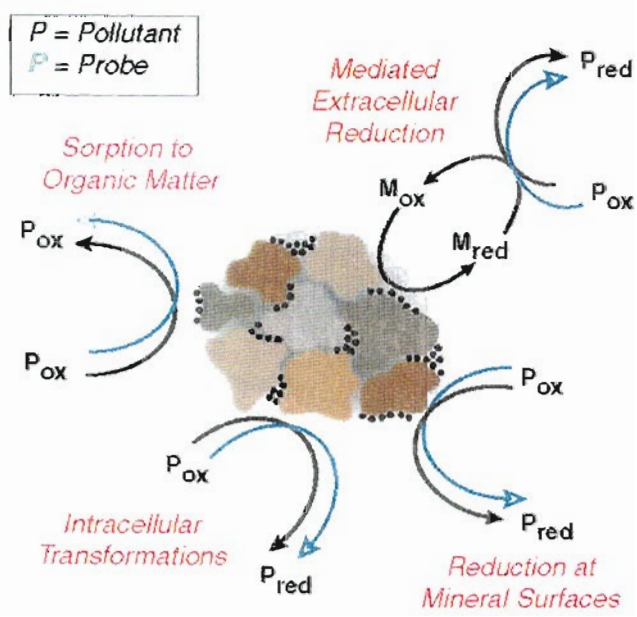

Figure 1.1 Sediment-associated processes for 2-CAP transformation and disappearance in natural environments. While 2-CAP may undergo any of the processes shown here, this study focuses mainly on using the probe compound, *P*, to investigate mechanisms of mediated reduction (*M* represents reducing mediators).

1.1.2 2-CAP probe technique

The 2-CAP probe was first introduced by Tanner and coworkers to study mechanisms of hydride and electron transfer reduction by reduced nicotinamide adenine dinucleotide (NADH) [16]. In these studies, 2-CAP was shown to undergo hydride transfer to form 2-chloro-1-phenylethyl alcohol (2-CPE) and electron transfer to form acetophenone (AcPh), as shown in Figure 1.2. AcPh can subsequently be reduced by hydride transfer to form 1-phenylethanol (1-PE). Since its initial studies in model systems, 2-CAP has been successfully added to sediments as a probe for distinguishing these two reduction pathways in environmental media [17, 18]. Of particular interest is the formation of 2-CPE, which implies hydride transfer in sediment. The apparent role of hydride transfer is significant because no known "abiotic" sources of hydride exist in the environment, implying that the reaction is likely mediated by a biochemical reducing

agent capable of hydride transfer. The most notable source of hydride in biochemical systems is NADH and its phosphate analog, NADPH. The mechanistic selectivity of the 2-CAP probe allows for the identification of such reducing mediators responsible for hydride transfer in anaerobic sediments. In addition, determination of appearance kinetics for the products of both reduction mechanisms may allow for the quantification of reductant concentrations. AcPh formation, unfortunately, is not specific for any particular environmental electron donors.

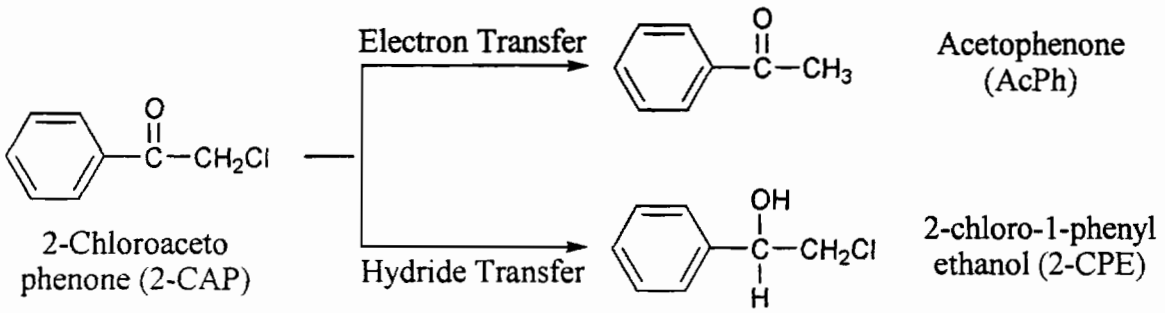

Figure 1.2 2-CAP reduction pathways. AcPh can be further reduced by hydride transfer to give 1-PE (not shown).

1.1.3 Sediment selection

Previous studies indicate that the relative amounts of reduction by electron and hydride transfer vary considerably with sediment source [17]. The sediment source for this study was chosen based on its reactivity, and apparent hydride activity. Of three local water systems tested, sediment from a tributary of Rock Creek in Beaverton, Oregon, showed the greatest production of 2-CPE from 2-CAP [17], making it a desirable sediment system for studying the hydride transfer mechanism. Because 2-CAP is not a specific probe for identifying electron donors, it is more difficult to interpret reduction mechanisms in sediments that primarily produce AcPh. It may be difficult to determine if a given sediment contains adequate hydride activity without testing 2-CAP reduction. However, because hydride is likely linked to microbial activity, it is suspected that sediments with high microbial populations (perhaps those sediments with more organic matter) may be preferable to sediments with lower microbial concentrations for studying hydride transfer reactions.

1.1.4 2-CAP chemistry in model systems

Due to the complexity of environmental media, studying treatments of the 2-CAP probe in well-defined model systems is helpful in understanding 2-CAP reactions in sediment. Tanner and coworkers studied the enzyme-mediated (horse liver alcohol dehydrogenase, HLADH) reduction of α-haloacetophenones (2-CAP) and α,α-dihaloacetophenones by NADH [16]. In the presence of NADH alone, reduction of mono-chlorinated and fluorinated acetophenones was limited, producing only trace quantities of AcPh. With the addition of the enzyme HLADH, both α-haloacetophenones were preferentially reduced by hydride transfer to form 2-halo-1-phenylethanol [16]. Recent studies have suggested that Tanner's NADH/HLADH model system may not be ideal for α-haloacetophenones because the reaction times are slow (2-7 days) and product formation is often less than 50% of the initial substrate concentration. Preliminary studies performed in the Tratnyek lab at OGI with another enzyme model system involving NADPH and the alcohol dehydrogenase from *Thermoanaerobium brockii* (TBADH) give greatly improved kinetics and transformation concentrations for 2-CAP reduction as compared to Tanner's NADH/HLADH model system [19] (Table 1).

Table 1.1 2-CAP reaction in a variety of model systems and percent product formation.

System	*Mechanism*	*Half-life*	*AcPh*	*2-CPE*
Rock Creek				
Smolen et al. [17]	Electron or	4.2 hr	20%	35%
This study	hydride transfer	5.3 hr	5 – 20%	20 – 70%
Goethite/Fe(II)				
Smolen et al. [17]	Electron transfer	2.3 d	100%	None
This study		15 min		
Iron Metal				
Tuck-Lee et al. [20]	Electron transfer	0.1 – 4 hr	100%	None
$NaBH_4$				
Smolen et al. [17]	Hydride transfer		<1%	99%
NADH	No reaction	No reaction		
NADH/HLADH *				
Smolen et al. [17]	Hydride transfer	14 d	None	100%
NADPH/TBADH **				
Nam et al. [19]	Hydride transfer	4 hr	None	100%

*Horse liver alcohol dehydrogenase

***Thermoanaerobium brockii* alcohol dehydrogenase.

Other model systems have been studied simply to validate the two reaction mechanisms of 2-CAP reduction. As expected, in a system containing goethite and Fe(II) (added as $FeCl_2$), 2-CAP was reduced by electron transfer, forming 100% AcPh [17] (Table 1.1). In the presence of a known achiral hydride source, sodium borohydride, 2-CAP was reduced by hydride transfer only, producing a racemic mixture of the 2-CPE enantiomers [17] (Table 1.1).

Model systems have also been useful in predicting the feasibility of secondary reactions (reactions other than reduction by hydride and electron transfer) in sediments. For instance, studying 2-CAP reaction in buffered solutions of varying pH levels has allowed us to predict effects of hydrolysis in environmental systems of known pH (Table 1.1).

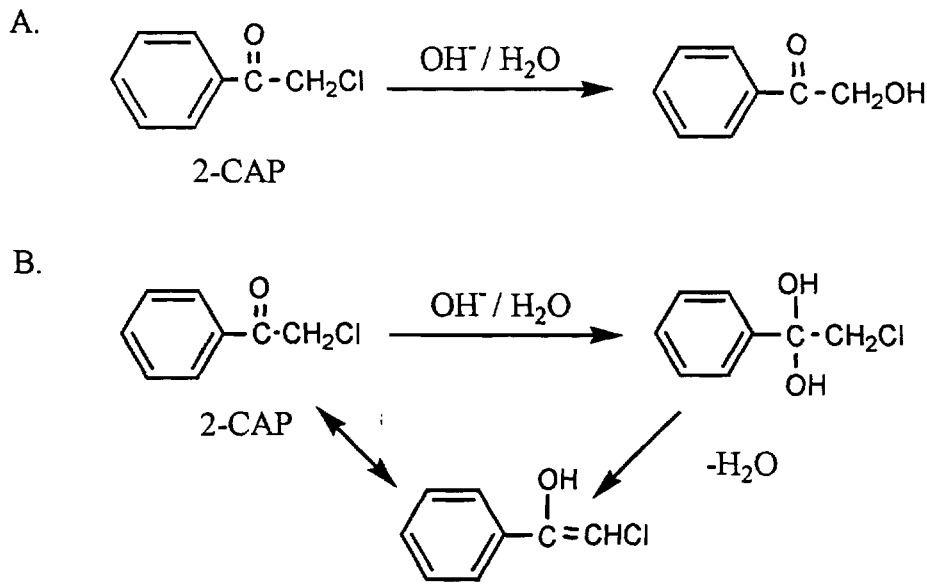

Figure 1.3 Possible routes of hydrolysis for 2-CAP: (A) nucleophilic substitution at C-2 and (B) addition to C-1 to give gem-diol (not stable), followed by elimination of H_2O, and keto-enol tautomerism.

Another set of reactions that could potentially contribute to 2-CAP transformation in sediments involves nucleophilic attack by sulfide (Table 1.1). Smolen et al. observed that mass balance for the 2-CAP probe tended to be poor in sediments that were sulfidogenic (i.e., coastal sediments where sulfate reduction is often the dominant

metabolic regime) [17]. We have hypothesized that this effect could be due to reduction by sulfide species as shown in Figure 1.4. A minor goal of this study was to test this possibility in a model system.

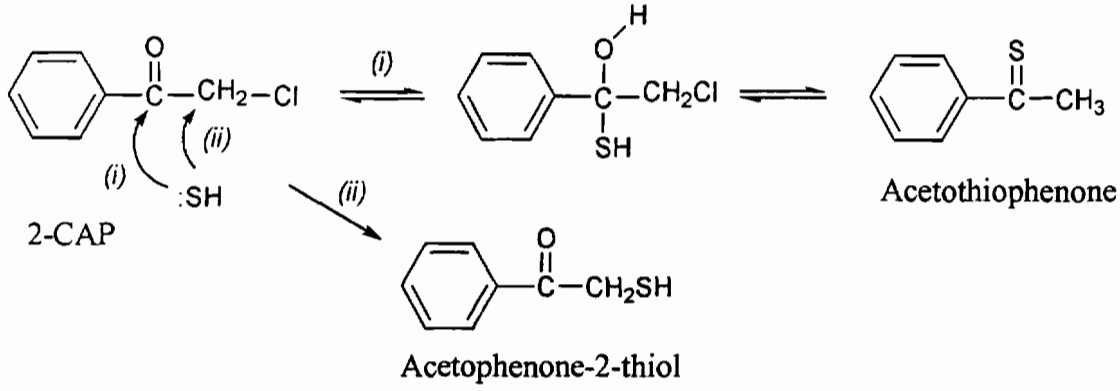

Figure 1.4 Proposed reduction of 2-CAP by sulfide species: (i) attack at C-1 and (ii) attack at C-2.

Another process that was investigated briefly in this study was dehydrohalogenation of 2-CPE. In the environment, dehydrohalogenation of 2-CPE by a Lewis base may contribute to AcPh production (Figure 1.5). This reaction, if it occurs, poses problems in interpreting product formation quantities and kinetics from 2-CAP reduction in sediments. The addition of 2-CPE to a sediment system may or may not provide evidence of dehydrohalogenation, depending on the sediment properties. However, because of the potential complications this reaction could cause, a small portion of this project was dedicated to studying this reaction in Rock Creek tributary sediment

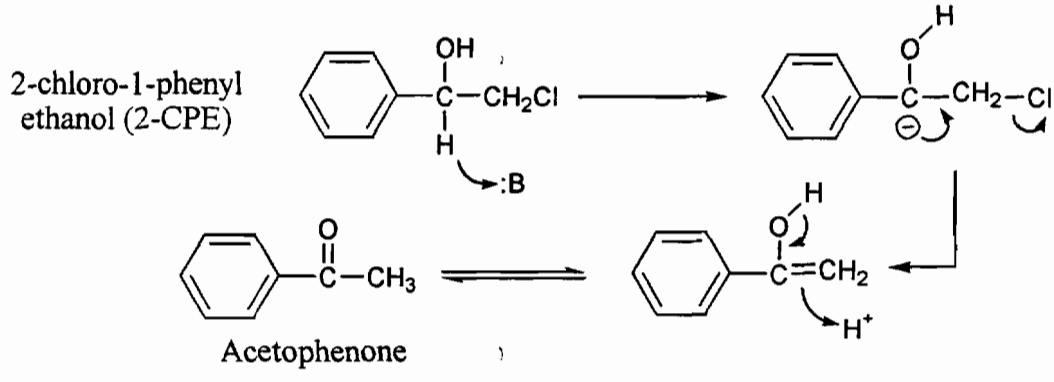

Figure 1.5 Proposed dehydrohalogenation of 2-CPE to give AcPh.

1.1.5 Hydride transfer

Evidence of 2-CAP reduction by hydride transfer in anaerobic sediments has provided a substantial contribution to the identification of reducing agents in the environment. The mechanism of hydride transfer reactions involves the migration of hydride, H^- (hydrogen nucleus accompanied by two electrons), from one atom to another. Hydride transfer to carbonyl compounds, such as 2-CAP, are common redox reactions in biological systems. In such reactions, a hydride is transferred from a cofactor, such as NADH, to the substrate by way of a mediating enzyme, often an alcohol dehydrogenase, to which the cofactor is weakly bound (Figure 1.6) [21].

Sources of environmental hydride are presumably limited to biological agents. The three most common hydride donors in biological processes are NADH, NADPH, and reduced flavin adenine dinucleotide (FADH). While the functions of these cofactors within the cell have been studied exhaustively, their possible roles as extracellular reducing agents in sediment have yet to be determined. Presumably, it is possible to link their reactivity outside the cell to concentrations within the cell, mobility across cell membranes, and availability to substrates once released into the environment.

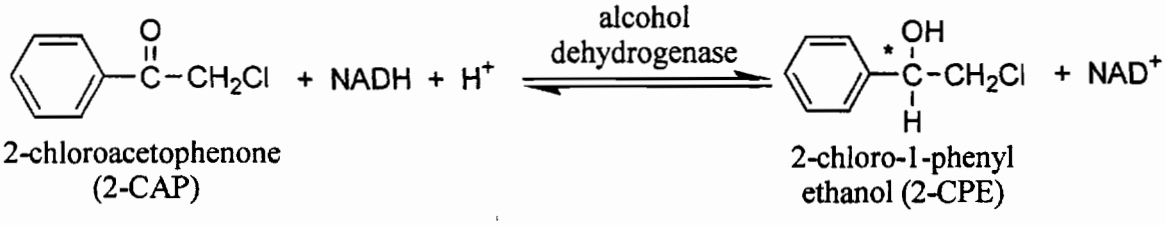

Figure 1.6 2-CAP reduction by NADH in the presence of a dehydrogenase enzyme.

In anaerobic systems, such as flooded soils or river bottoms, NADH is generated by microbes in the process of fermentation. While it is conceivable that NADH or other hydride donors may be released upon cell lysis, with integral functions in biological reaction, their excretion from living, functioning cells is unlikely. Therefore, a more plausible scenario for substrate reduction may involve intracellular reactions or reaction at the cell membrane with reductants that have migrated across the membrane. Both pyridine derivatives, NADH and NADPH, are water-soluble and, therefore, if released

into the environment, would presumably be available for reduction reactions. In contrast, flavin derivatives, such as FADH, do not function as soluble mediators. Instead, they work as prosthetic groups in proteins (i.e., flavoproteins) to which they are tightly bound or even covalently bound [22]. Therefore, if extracellular reduction did occur, FADH would not likely be the source of hydride since it can not function external to the enzyme. In addition to this, unlike, NAD, FAD is tightly bound to the inner mitochondrial membrane structure, making migration out of the cell unlikely.

Studies involving model systems of NADH and NADPH indicate that both compounds may be stable in environmental systems near pH 7 [16, 19]. However, regardless of the potential for these cofactors to be active outside cell wall, model systems also indicate that hydride transfer from either hydride donor is enzyme-mediated, which means that if the reaction were extracellular, both coenzyme and enzyme would have to exist freely outside cell walls.

1.1.6 Dehydrogenase enzymes

Alcohol dehydrogenases are the most likely candidates for mediating hydride transfer in 2-CAP reduction in sediment. Dehydrogenase enzymes are in the class of oxidoreductases, which function in living organisms in processes of hydrogen transfer. They are commonly studied in soil systems and have been detected as freely existing compounds in the environment, originating from bacteria, fungi, plants, and a variety of macroinvertebrates [23-25]. Their activity outside cell walls, however, may not allow for extracellular reactions. Not all enzymatic material released into the environment is stable. In fact, much of it is degraded by other microbes [25]. The stability of those enzymes not degraded may be attributed to the formation of enzyme-clay or enzyme-natural organic matter (NOM) complexes, which aid in protecting the enzymes from microbial proteinases [23, 24, 26]. However, while adsorption to soils and NOM may protect the enzymes from degradation, it may also limit their activity with substrates controlled by the different mechanisms of adsorption, the site of adsorption, the nature of the reacting source, and the properties of the ambient environment [26]. Dehydrogenases

are thought to be inactivated in their extracellular form. This is an idea that has allowed soil scientists to measure microbial activity as a function of dehydrogenase activity within living cells apart from extracellular reactions [27]. Without extracellular dehydrogenase activity, it is difficult to envision an extracellular mechanism of 2-CAP reduction by hydride transfer in sediment systems.

1.1.7 Enzyme-mediated hydride transfer in environmental systems

Whether reduction reactions are intracellular or extracellular, two particular experiments with 2-CAP reduction in sediment systems have provided evidence for enzyme-mediated reactions. Because the reduction of 2-CAP by hydride transfer is stereoselective, giving preferential formation of (R)-(-)-2-CPE or (S)-(+)-2-CPE, it can be used to probe the stereoselectivity of the hydride reduction pathway (Figure 1.7). Smolen et al. [17] used the 2-CAP probe in this way[17]. Because the two faces of the 2-CAP carbonyl group are enantiotopic, the product distribution allowed Smolen to distinguish between attack by a chiral or achiral agent. Chiral reductants (i.e., NADH in the presence of HLADH) are able to differentiate the two faces of the carbonyl group and thus, will give diastereomeric transition states of different energies that are formed in unequal amounts [21]. In contrast, attack by an achiral reductant will give a racemic mixture (50%(R)/50%(S)) of the two products. Because there are no known abiotic sources of achiral hydride in the natural environment, it can be presumed that the production of either enantiomer in excess indicates reduction by a biochemical chiral agent, such as NADH, mediated by a dehydrogenase enzyme.

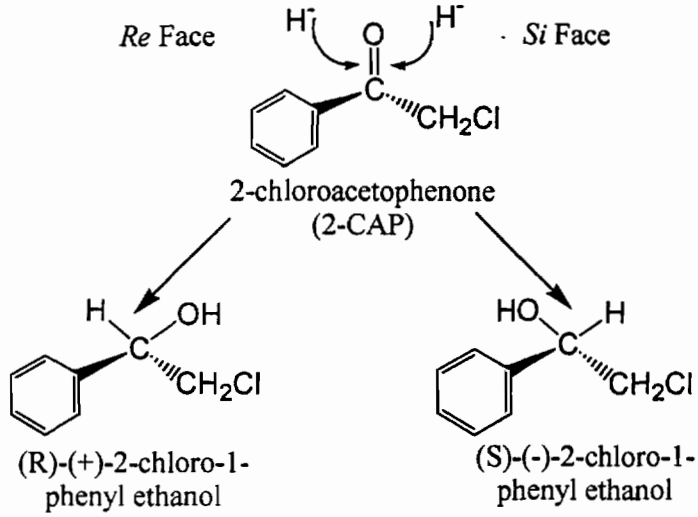

Figure 1.7 Attack by achiral and chiral reductants at the two enatiotopic faces of the 2-CAP carbonyl group produces different enantiomers of 2-CPE.

Further evidence of enzyme-mediated reduction was obtained from studying 2-CAP reduction kinetics as a function of temperature. In this study, Smolen et al. [17] found that while 2-CAP reduction and AcPh formation kinetics increased monotonically with increasing temperature, the formation kinetics of 2-CPE reached an optimum temperature near 45°C (Figure 1.8). While abiotic reactions increase proportionally to temperature increases, biological reactions typically demonstrate a temperature optimum [28]. This suggested that the formation of 2-CPE in sediment was mediated by an enzyme (or another heat-labile agent), while production of AcPh was likely a result of an abiotic reaction.

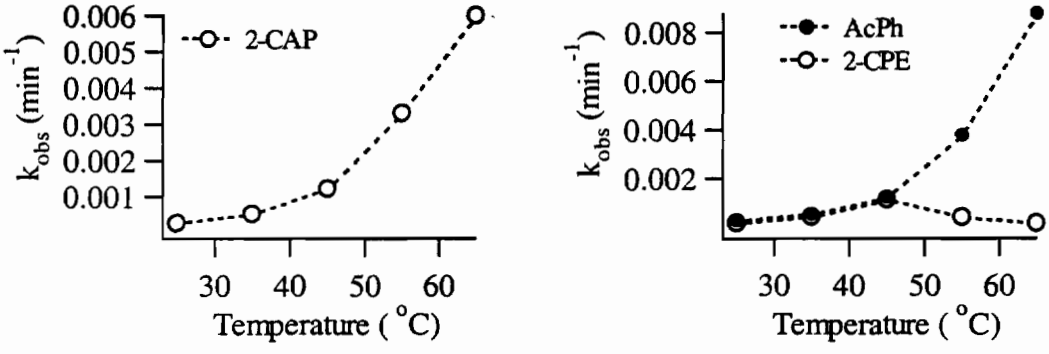

Figure 1.8 Effect of temperature on 2-CAP reduction and AcPh and 2-CPE formation in Rock Creek sediment (from Smolen et al. [17]).

To further test for enzyme activity, Jim Nurmi and Kate Hoffman, in the Tratnyek lab at OGI, initiated new studies involving enzyme inhibitors. A variety of inhibitors of dehydrogenase enzymes including p-chloromercuribenzoate (p-CMB), 1-formylpiperidine (1-FMP), and pyrazole, were studied in NADH/TBADH model systems [19]. All three compounds were successful in inhibiting the hydride transfer mechanism of 2-CAP reduction, with inhibition improving with increasing inhibitor concentration [19]. To relate results obtained in the model system studies to reduction processes in the natural environment, part of our study focused on the implementation of these inhibitors in 2-CAP probe experiments conducted in sediment. In addition to using dehydrogenase-specific inhibitors, 2-CPE was also added to sediment as a secondary mechanism for investigating enzymatic reactions. Generally, enzyme-mediated reactions are reversible and, therefore, with the addition of the product (i.e., 2-CPE), the reaction will occur in the reverse direction, from product to substrate [29]. Therefore, if dehydrogenase enzymes are mediating the hydride transfer reaction, a reversible inhibition (or "product" inhibition) of 2-CAP should be observed in the presence of high 2-CPE concentrations.

A final method for validating conclusions drawn from experiments with 2-CAP is to use other probe compounds that undergo transformations similar to 2-CAP. Tanner et al. [16] studied a variety of mono- and dihaloacetophenones. In their studies with NADH/HLADH model systems, it was observed that 2-bromoacetophenone (2-BAP) and 2-chloroacetophenone were reduced in NADH/HLADH systems by two different mechanisms. While 2-CAP underwent hydride transfer reduction, 2-BAP reacted with NADH alone by a rapid free radical chain process external to the enzyme, producing AcPh. 2,2-dichloroacetophenone (2,2-CAP) was also observed to follow this same reduction mechanism to produce 2-CAP. The addition of these two probe compounds to a sediment system, in which hydride transfer appears to be the favored reduction mechanism, could potentially provide further evidence for the presence of extracellular cofactors, such as NADH.

1.2 Relevance of 2-CAP

As a probe compound, 2-CAP is useful for studying reduction in anaerobic sediments because it offers a well-characterized set of reaction pathways that may allow for the identification of environmental reducing agents. However, the relevance of 2-CAP reduction in sediments extends farther than its use as a probe. As an active ingredient in tear gas and Mace, 2-CAP is also an environmental pollutant. Studies of the disposal of tear gas indicate that substantial quantities of 2-CAP contaminate various regions of the world. Two areas in particular, are the Baltic Sea, where large amounts of war gases were dumped in 1945 and 1948, and the Federal Laboratories Site in Pennsylvania, where 1,700 55-gallon drums of liquid CNS (a form of tear gas) were buried in 1952 [30, 31]. In addition to this, studies have also shown that a variety of industrial and agricultural chemicals, including PCB's and chlorfenvinphos, form 2-CAP analogs upon degradation [32, 33] (Figure 1.9). Studying the reduction of 2-CAP in anaerobic sediments will not only aid in identifying reducing mediators, but may be integral to determining the fate of a number of environmental contaminants, including 2-CAP.

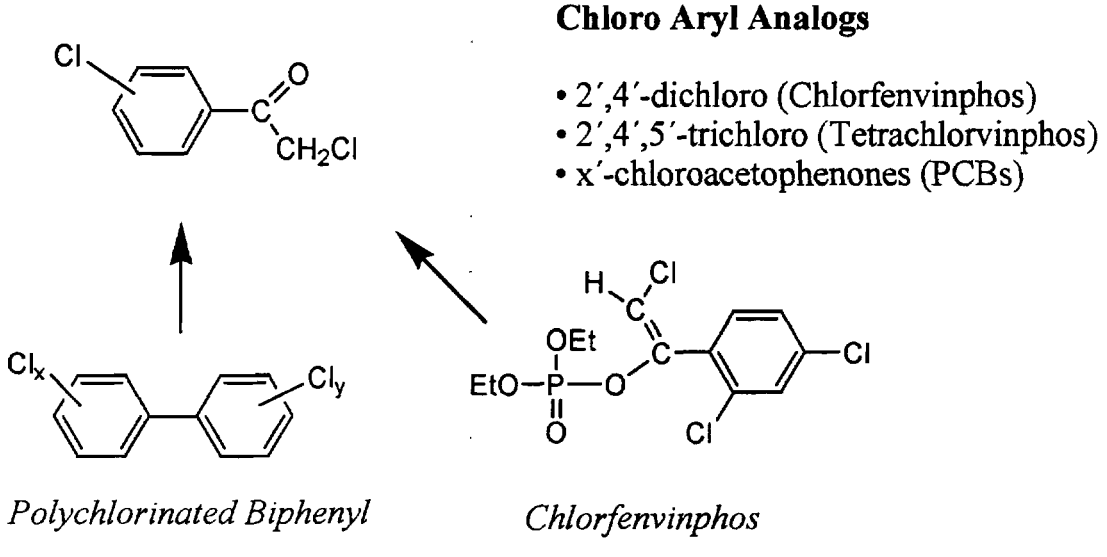

Figure 1.9 Degradation of certain chemical contaminants produces analogs of 2-CAP.

1.3 Objectives of Study

The overall objective of this study was to further develop the 2-CAP probe method for characterizing the reducing properties of anaerobic sediments. The specific goals of this study were as follows:

1. Further validate the 2-CAP probe as a method for identifying specific reductants in sediments.
2. Apply the 2-CAP probe method to learn more about the apparent hydride activity in sediments.
3. Begin to develop a quantitative kinetic model for 2-CAP fate in sediments, so that the probe method can eventually be calibrated for use as a quantitative assay for hydride activity (or other reductants) in sediments.

CHAPTER 2
MATERIALS AND METHODS

2.1 Chemical Reagents

2-Chloroacetophenone (2-CAP), (R)-(-)-2-chloro-1-phenylethyl alcohol ((R)-(-)-2-CPE), (S)-(+)-2-chloro-1-phenylethyl alcohol ((S)-(+)-2-CPE), acetophenone (AcPh), 2-bromoacetophenone (2-BAP), 2,2-dichloroacetophenone (2,2-CAP), 2,2′,4′-trichloroacetophenone (2,2′,4′-CAP), sodium borohydride, 1-formylpiperidine (1-FMP), pyrazole, p-chloromercuribenzoic acid (p-CMB), sodium sulfide nonohydrate, and resazurin were obtained from Aldrich Chemical Company at purities greater than 98% and used as received. (R)-(-)-1-phenylethyl alcohol ((R)-(-)-1-PE) and (S)-(+)-1-phenylethyl alcohol ((S)-(+)-1-PE) were obtained from TCI America at purities of 97% and were used as received. Acetonitrile (HPLC grade) was obtained from Fisher Scientific Company at purity of 99.9%. The reduced forms of nicotinamide adenine dinucleotide (β-NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma Chemical Company at purities of 98%. *Thermoanaerobium brockii* alcohol dehydrogenase (TBADH) was also obtained from Sigma Chemical Company (60% protein). Chemicals Abstracts Service (CAS) numbers for all compounds used are given in Appendix A.

Due to their low water solubility, 2-CAP, AcPh, 2-CPE, 1-PE, 2-BAP, 2,2-CAP, 2,2′,4′-CAP, and pyrazole stock solutions were prepared in 50% aqueous methanol. p-CMB stock solution was prepared in 50% methanol/50% acetonitrile. Sonication was used to accelerate dissolution of p-CMB.

2.2 Sediment Collection and Preparation

Sediment was collected from a tributary of Rock Creek in Beaverton, Oregon (site location and sediment characterization are given in Appendix B). A core sampler was used to obtain anaerobic sediment samples. A single sediment sample was collected from the same location in the deepest part of the creek for each experiment. In general, a 2:1 ratio by volume of water to sediment was obtained. Samples were mixed to form a homogeneous slurry and sieved (1 mm) to remove plant debris. This was done in the laboratory under constant flow of argon to minimize sediment exposure to atmospheric oxygen. 100-mL aliquots of the homogeneous slurry were transferred to 110-mL serum bottles. The headspace was purged with argon before each bottles was crimp-sealed with butyl rubber septa. In addition, one 100-mL aliquot of slurry per experiment was oven-dried at 50°C and weighed to determine the dry mass of the sediment. Sediment samples that were not used for experimentation on the day they were collected were stored at 4°C in sealed core sampling tubes.

In one series of experiments, two other areas in the Rock Creek tributary were sampled to determine the effects of varying collection location on 2-CAP reduction. Both of these sediment samples were collected in Mason jars (jars were submerged in the water and used to scoop sediment samples) from relatively shallow parts of the stream. Again, an approximate 2:1 ratio of water to sediment was obtained. The first of these sampling locations was near the rocky banks adjacent to the center of the creek where sediment sampling was generally performed. The second sample was collected about 3 meters upstream in a marshy area adjoining the creek. Both sediment samples were prepared for experimentation in the same manner as core samples.

Coastal sediment samples were obtained from Anna Farrenkopf of OGI who collected sediment from two sampling locations within the Columbia River estuary (Tansy Point and Lewis & Clark stations). Both samples were collected in May, 1999

prior to the spring freshet. Sampling was done at Tansy Point during slack tide and at Lewis & Clark approximately 15 min after low tide. Both samples were shoveled into buckets with approximately 45 cm of standing water over top. The sediment was prepared for experimentation in the same manner as described for Rock Creek tributary sediment. Because all sediment samples were mixed prior to use, the method used for sediment collection should have no affect on 2-CAP reduction results.

2.3 Experimental Design

Well-mixed anaerobic sediment samples were spiked with 0.4 mL of 25 mM 2-CAP to give a final concentration of approximately 100 μM (depending on the amount of sediment in solution). Immediately following 2-CAP addition, the samples were gently turned end-over-end 2–3 times for mixing. Using a 1 mL sterile syringe (with 23 gauge needle), a 0.15-mL aliquot was pulled from the top 2 mm of slurry, where the amount of sediment was lowest due to settling. The aliquot was filtered through a 0.45 μm Nalgene 4-mm nylon syringe filter. The sediment slurries were continuously mixed on a rotating disk at an 85° angle from the bench top with bottles rotating so that the slurry followed a circular path around the sides of the bottle. The bottles were rotated at a speed of 13 rpm with a radius of 18 mm. Samples were collected at regular intervals over the course of 2–3 days (experiments usually ran until 2-CAP was undetectable), with more frequent sampling during the first few hours of the experiment.

2.4 Analytical Methods

2-CAP reduction and 2-CPE and AcPh formation were measured using high-performance liquid chromatography (HPLC). 100 μL of the filtered sample was injected into a fixed volume sample loop (20 μL) on a Rainin HPLC. Samples were analyzed

with an Alltech Econosil C-18 column (5 μm, 250 x 4.6 mm). Flow rate and eluent concentrations were kept constant at 1.0 mL/min and 55% acetonitrile, respectively. Compound elution was monitored at 210 nm using a UV-visible detector. Most compounds were identified and concentrations were quantified by comparison with known standards. Identification of some compounds required gas chromatograph/mass spectrometer (GC/MS) analysis because standards were not available for comparison.

2.5 Extraction Procedure

At the completion of an experiment, a simple extraction method using acetonitrile was employed to remove 2-CAP, 2-CPE, and AcPh adsorbed to the sediment. Sediment slurries were centrifuged for 10 min to separate sediment from water. The water was removed and weighed to determine its volume. Acetonitrile was then added to the sediment in the same volume as water removed. The samples were vortex-mixed (and usually sonicated) to resuspend the pellet and create a homogeneous mixture. Mixing was allowed to continue for 24 hours before samples were analyzed for adsorbed concentrations. The method employed for this procedure was the same as that described for measuring concentrations in the sediment slurry prior to extraction.

In one series of experiments, supercritical fluid extraction (SFE) and soxhlet extraction were used as comparison methods to determine the effectiveness of the acetonitrile procedure. The sediment slurry from a 2-CAP reduction experiment was divided in half and centrifuged. The water from both samples was removed and its volume was measured. The sediment in one sample was used for acetonitrile extraction as detailed above, while the second sample was further divided into two equal parts (determined by mass) and packaged in glass filter paper for SFE and soxhlet extraction. CO_2 was chosen as the supercritical fluid for SFE based on its availability. SFE was run for 30 min., during which, the extracted compounds were collected (in 20 mL of acetonitrile). The soxhlet extraction was run for 24 hours using acetonitrile as the extracting solvent. Analysis for all three methods was done using HPLC as described for

measuring concentration in the sediment slurry. Dilution effects were taken into account in calculating the concentrations extracted in SFE and soxhlet experiments.

Although extractions were usually performed only at the end of an experiment, a number of reduction experiments were run in which acetonitrile extraction was performed at each time point. For these experiments, a 0.2-mL aliquot of slurry was withdrawn at each time point (in addition to the 0.1-mL aliquot withdrawn for the control data) and mixed with 0.2 mL of acetonitrile. This mixture was subsequently filtered and the filtrate was analyzed with HPLC. The concentrations of extracted compounds were determined based on measured concentrations (using HPLC) and the calculated dilution factor. Adsorbed concentrations were determined by subtracting control data (concentration in solution) from extraction data (concentrations in solution and extracted from the sediment) at each time point.

2.6 Experimental Protocols

2.6.1 Mass balance and extraction recovery

Two types of "mass balances" were calculated for all 2-CAP reduction experiments: i) C_s, which is the concentration of 2-CAP and all transformation products in solution, and ii) C_f, which is a final mass balance equal to C_s plus the adsorbed concentrations of 2-CAP and all transformation products recovered with acetonitrile extraction (C_e). C_s is equal to the total amount of 2-CAP, 2-CPE, and AcPh (n_{tot}, measured in moles) per unit volume water (V_w) in the slurry. C_s was calculated at each time point in reduction experiments using eq. 2.1. Because, the same value of V_w applies to all terms in eq. 2.1, the concentrations, as measured by HPLC, are simply additive.

$$C_s = \frac{n_{tot}}{V_w} = \frac{n_{2CAP}}{V_w} + \frac{n_{2CPE}}{V_w} + \frac{n_{AcPh}}{V_w} \qquad \textbf{(eq. 2.1)}$$

The total extracted concentration (C_e) was determined in the same manner as C_s, with the exception that C_e is the amount of substances recovered (n_{tot}) per unit volume of extraction solvent (V_e). C_e was generally determined only upon completion of the

experiment, however, on three occasions, acetonitrile extraction was performed at each time point.

The final "mass balance" (C_f) is equal to the total 2-CAP, 2-CPE, and AcPh concentrations in solution (C_s) plus those concentrations extracted from the sediment (C_e) per unit volume water in the sediment slurry (V_w). C_f was calculated at the end of each experiment using eq. 2.2.

$$C_f = \frac{n_{tot}}{V_w} + \frac{n_{tot}}{V_e} = \frac{C_s \times V_w}{V_w} + \frac{C_e \times V_e}{V_w} \qquad \textbf{(eq. 2.2)}$$

In this case, V_w in the two terms on the right side of eq. 2.2 refers to two different phases. However, since the value of $V_e = V_w$ (by design), eq. 2.2 simplifies to eq. 2.3.

$$C_f = C_s + C_e \qquad \textbf{(eq. 2.3)}$$

Because V_w was equal to V_e in all 2-CAP experiments, the final mass balance was calculated by simply adding the total 2-CAP and product concentrations in solution to the concentrations of those compounds extracted from the sediment.

The total unrecovered concentration prior to extraction was determined by subtracting the final concentrations in solution (C_s) from the initial slurry concentration (C_0). (C_0 is the "true" initial concentration calculated from the nominal concentration of the spike, corrected for dilution only.) The percent of the initial concentration that was unrecovered was calculated using eq. 2.4. The percent of the initial concentration unrecovered after extraction was determined in the same manner with the replacement of C_s with C_f (eq. 2.5). The percent unrecovered is approximately equal to the percent adsorbed.

$$\%\ \text{unrecovered before extraction} = [(C_0 - C_s)/C_0] \times 100 \qquad \textbf{(eq. 2.4)}$$

$$\%\ \text{unrecovered after extraction} = [(C_0 - C_f)/C_0] \times 100 \qquad \textbf{(eq. 2.5)}$$

2.6.2 Determination of particle density and initial 2-CAP concentration

Particle density (D_p) is the dry mass of particles per unit volume of particles (i.e., excluding pore space) [12]. The average solid particle density of sediment in the slurries

collected from Rock Creek tributary was determined by adding a known dry mass of sediment (m_p) to a 100-mL volumetric flask and gravimetrically measuring the amount of water (m_w) needed to fill to a convenient volume (V_T) of 100 mL. Assuming the density of water is equal to 1 g/mL, the measured mass of water (m_w, in grams) is equal to the volume of water (V_w, in mL). After the volume of water was determined, it was subtracted from the total volume of 100 mL to give the volume occupied by the dry sediment (V_p), as shown in eq. 2.6.

$$V_p = V_T - V_w \qquad \textbf{(eq. 2.6)}$$

Particle density was then determined by dividing the mass of dry sediment by the volume it occupied (eq. 2.7). This procedure was performed three times in order to determine an average particle density of sediment from Rock Creek tributary.

$$D_p = \frac{m_p}{V_p} \qquad \textbf{(eq. 2.7)}$$

For all experiments, the particle volume in the slurry (V_p) was determined with eq. 2.7 where the variable between experiments was the amount of sediment (m_p). After the particle volume was determined, it was used to calculate the slurry water volume (eq. 2.6). The water volume was, in turn, used to calculate the initial 2-CAP concentration in solution (C_0) as shown in eq. 2.8.

$$C_0 = \frac{C_{stock} \times V_{stock}}{V_{stock} + V_w} \qquad \textbf{(eq. 2.8)}$$

The "nominal" initial 2-CAP concentration, C_0, is the starting concentration at time = 0 min ($*t_0$), calculated from eq. 2.8 $*t_0$ is the time point immediately following substrate addition and prior to mixing.

2.6.3 *Adsorption of 2-CAP and reduction products*

100-mL aliquots of slurry were air-dried to render them inactive for experiments run to determine the kinetics of 2-CAP, 2-CPE, and AcPh sorption to sediment. Large aggregates of dried sediment were broken up with a glass rod before the sediment was added to a 110-mL serum bottle. Creek water was autoclaved three times at 121°C for 20 min over a course of 5 days and filtered (0.2 μm) prior to use. The sediment slurry was reconstituted with the addition of the autoclaved water for a final slurry volume of 100 mL. The slurry was then allowed to mix for 1 hour prior to 2-CAP addition.

2.6.4 *Kinetics of reduction and adsorption*

All 2-CAP disappearance data, as well as 2-CPE and AcPh appearance data, were fit to first-order kinetics. Because the initial 2-CAP loss due to adsorption often complicated the kinetics of 2-CAP reduction, the first few data points (where disappearance was dominated by adsorption) were separated from 2-CAP reduction data and omitted in reduction kinetics calculations. The amount of data that were omitted varied from one experiment to another, depending on the extent of sorption. In general, however, the reduction and sorption data could be separated between the first 20–60 min of the experiment, commonly near a 2-CAP concentration of 60 μM.

Two methods of calculating rates of 2-CAP disappearance were used. The first method involved a linear fit of ln [2CAP] versus time, in which the slope of the line was equal to the rate of 2-CAP reduction (k_{obs}). The second method involved a non-linear fit of the raw 2-CAP concentration data versus time with eq. 2.9, where the parameters, C_0 (nominal concentration at $*t_0$) and k_{obs} were allowed to vary. The two methods generally produced comparable k_{obs} values, so the linear fit was only used for plotting data so that the variation in 2-CAP disappearance rates could be easily seen.

$$C = C_0 \cdot e^{-k_{obs} \cdot t} \qquad \textbf{(eq. 2.9)}$$

2-CPE and AcPh appearance kinetics were determined by fitting the appearance curves (concentration versus time) with eq. 2.10. The theoretical maximum concentration reached (C_∞) at t = ∞ was also determined by eq. 2.10. Initial guesses were made for C_∞ based on the observed data. Again, C_∞ and k were used as fitting parameters that were allowed to vary.

$$C = (C_\infty)(1 - e^{k_{obs} \cdot t}) \qquad \textbf{(eq. 2.10)}$$

To account for the effects of the amount of sediment on 2-CAP reduction rate, k_{obs} was normalized to the dry mass of sediment per unit mass of water (ρ) to give k_{rho} (eq. 2.11 and 2.12). Because the density of water is approximately 1 g/mL, ρ may also be thought of in terms of mass per unit volume (m_p/V_w).

$$\rho = m_s\,(\text{g}) \,/\, m_w\,(\text{g}) \qquad \textbf{(eq. 2.11)}$$

$$k_{rho}\ (\text{min}^{-1}) = k_{obs}\,(\text{min}^{-1}) \,/\, \rho \qquad \textbf{(eq. 2.12)}$$

2.6.5 Reduction of 2-CAP by sulfide species

The proposed reactions of 2-CAP reduction by sulfide species were studied by observing 2-CAP transformation in an anoxic buffered solution of sodium sulfide. In this experiment, 1 mL of 100 mM sodium sulfide solution and 0.08 mL of 25 mM 2-CAP solution were added to 3.92 mL (V_T = 5 mL) potassium phosphate buffer at pH 8, for final concentrations of 20 mM NaS and 400 µM 2-CAP. All solutions were prepared in an anaerobic chamber to prevent sulfur precipitation. Three data points were collected over a period of 1 hour and analyzed by HPLC. Product identification was performed using mass spectrometry by Clint Church of OGI.

2.6.6 Hydrolysis of 2-CAP

Six buffered solutions of varying pH were prepared to determine the effect of pH on 2-CAP reduction. The following buffers were used: 50 mM potassium biphthalate (pH 3), 50 mM potassium phosphate (pH 7 and 8), Trizma® HCl and base (pH 9), and

sodium carbonate (pH 10 and 11). 80 µL of 25 mM 2-CAP was added to 4.02 mL buffer solution for a total volume of 5 mL and final 2-CAP concentration of 400 µM. Samples were continuously mixed on a Fisher Scientific hematology mixer. 2-CAP and reaction products were analyzed using HPLC. Product identification was performed by Clint Church of OGI using mass spectrometry.

2.6.7 Dehydrohalogenation of 2-CAP and reduction of AcPh in sediment

The same procedure for measuring 2-CAP reduction in sediments was used to determine 2-CPE and AcPh reduction. The initial concentration of AcPh was lowered from 100 µM to 50 µM in order to observe the very slow reduction to 1-PE. Reduction of both (R)-(-)-2-CPE and (S)-(+)-2-CPE enantiomers were studied, with starting concentrations varying from 100 µM to 1 mM. Both AcPh and 2-CPE reduction experiments were allowed to continue for more than one week.

2.6.8 2-CAP reduction in sonicated sediment

Sonication of sediment was employed for the purpose of lysing cells as a method of increasing extracellular hydride activity. In this experiment, 100 mL of sediment slurry was sonicated with a Branson Sonifier (Model 250) for 15 min prior to 2-CAP addition. The temperature of the slurry increased with sonication, but was kept below 40°C. Effects of sediment sonication were determined by comparing 2-CAP reduction in sonicated sediment to an unsonicated sediment.

2.6.9 2-CAP reduction in the presence of added NAD(P)H in sediment

NADH and NADPH were added to sediment slurries as potential stimulators of the hydride transfer reduction pathway. The volume of sediment slurry used for this experiment was lowered from 100 mL to 50 mL. 50-mL aliquots of sediment slurry filled three 50-mL serum bottles. 0.2 mL of 25 mM 2-CAP was added to each bottle for a final concentration of approximately 100 µM. NAD(P)H (1 mL, 3 mM) was added to achieve final concentrations of 60 µM (concentration used in Nam's NADPH/TBADH

model system) [19]. 2-CAP reduction was measured using HPLC. NAD(P)H peaks were too large to integrate and, thus, were not analyzed. Effects of NAD(P)H addition were determined by comparison with the control sample.

2.6.10 2-CAP reduction in the presence of enzyme inhibitors

2-CPE, p-chloromercuribenzoate (p-CMB), 1-formylpiperidine (1-FMP), and pyrazole were used to inhibit 2-CAP reduction in sediment. Effects of inhibition were determined by comparison with a control for each experiment. All inhibition experiments were run in a similar manner, generally varying only the inhibitor concentration. 2-CPE concentrations of 100 μM, 1 mM, and 5 mM were used for product inhibition of 2-CAP reduction. Due to solubility problems with p-CMB, the final concentration in the sediment slurry was unknown, but was approximately 100 μM. 1-FMP concentrations were varied from 30 μM to 60 mM. Pyrazole concentrations ranged from 2 mM to 20 mM. In final experiments using 1-FMP and pyrazole (15 Sept. 99), inhibitors were added to the slurry and allowed to mix on a rotator for 1 hour prior to 2-CAP addition.

2.6.11 Reduction of other probe compounds

2-BAP, 2,2-CAP, and 2,2′,4′-CAP reduction was studied following the same procedure used for 2-CAP reduction. Reduction of the three compounds in 40 mM sodium borohydride allowed for the identification of products of hydride transfer (i.e., 2-bromo-1-phenylethyl alcohol (2-BPE), 2,2-dichloro-1-phenylethyl alcohol (2,2-CPE), and 2,2′,4′-trichloro-1-phenylethyl alcohol (2,2′,4′-CPE)).

Resazurin was used as an indicator dye to compare reactivity in sediment collected from three different locations within Rock Creek tributary (method from Tratnyek et al. [2]). Resazurin experiments were carried out in an anaerobic chamber to prevent oxidation of the indicator dye. 10-mL aliquots of sediment slurry filled eight 10-mL test tubes. One 10-mL aliquot was dried and weighed to determine the dry mass of the sediment. 75 μL of 10 mM resazurin was added to the first test tube. The slurry was

slowly mixed by hand in the test tube for a measured period of time and then immediately emptied into a 10 mL syringe containing glass wool. The slurry was then filtered through a Whatman® cellulose acetate syringe filter (0.2 μm pore size, 25 mm diameter) into a cuvette. This procedure was repeated for the remaining test tubes, varying mixing time for each run. Cuvettes were capped and removed from the anaerobic chamber for analysis. Wavelength scans were run using a Perkin Elmer (Lambda 20) UV/VIS spectrometer, measuring absorbance from 300-800 nm. The maximum absorbance (λ_{max}) for resazurin was at 600 nm, λ_{max} for the reduction product, resorufin, was at 570 nm. The natural log of absorbance values at 600 nm was plotted versus mixing time and fit to first-order kinetics to determine the rate of reduction.

CHAPTER 3
RESULTS

3.1 Primary Processes

3.1.1 2-CAP reduction in sediment

The primary results of a typical 2-CAP reduction experiment are shown in Figure 3.1. 2-CAP was reduced to 2-CPE and AcPh in all cases using sediment collected from Rock Creek tributary. 2-CPE was the major product in all experiments, indicating that hydride transfer is an important reduction pathway in this sediment. Mass balance (sum of 2-CAP, 2-CPE, and AcPh concentrations in solution) varied greatly over the many times the experiment was performed, ranging from 10% of the initial 2-CAP concentration to nearly 100%.

3.1.2 Adsorption of 2-CAP, 2-CPE, and AcPh to sediment

Dried and reconstituted sediment was used as a non-reaction control for studying adsorption. When 2-CAP was added to dried/reconstituted sediments, its concentration decreased with no apparent product formation. The disappearance of 2-CAP, presumably due to sorption, was greatest during the first 1–5 min, with a large drop from the $*t_0$ (nominal concentration calculated for t = 0 min) concentration to the first collected data point, t_1 (collected at t = 1 min). After this initial loss, adsorption apparently continued at a moderate rate over the next 30 min of the experiment. After 150–300 min, a plateau was reached, at which point 2-CAP disappearance was minimal (Figure 3.2). Adsorption concentrations at the plateau region varied widely among experiments. After a period of approximately 2880 min (48 hr), the products of 2-CAP reduction were observed, indicating that reducing conditions had been restored (presumably due to the regrowth of bacterial populations). The majority of adsorption experiments were not allowed to reach this point. The adsorption of 2-CPE and AcPh generally followed the same trend with no observed product formation.

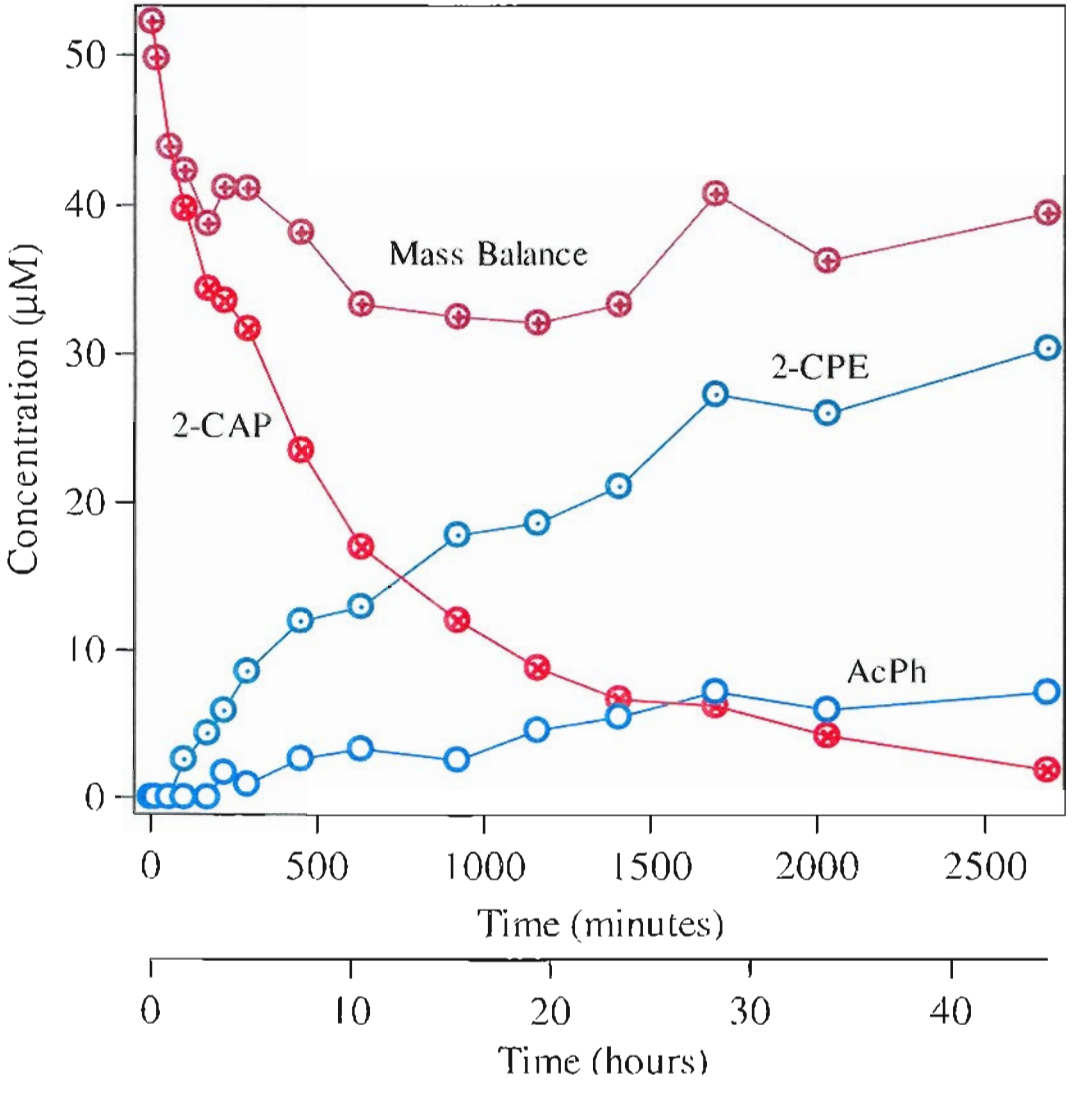

Figure 3.1 Representative example of 2-CAP reduction in an anaerobic sediment slurry, showing formation of 2-CPE and AcPh. This experiment was run on 16 April 99 with sediment collected from a tributary of Rock Creek in Beaverton, Oregon. The dry mass of the sediment was 10.5 g and the initial 2-CAP concentration was 106 µM. For all experiments, upon addition of 2-CAP, the slurry was mixed gently and a sample aliquot was collected for analysis. The sample was then placed on a rotator for continuous mixing. The first measured data point was at t = 1 min (t_1). The nominal starting concentration of 106.0 µM, calculated for $^{*}t_0$, is not plotted.

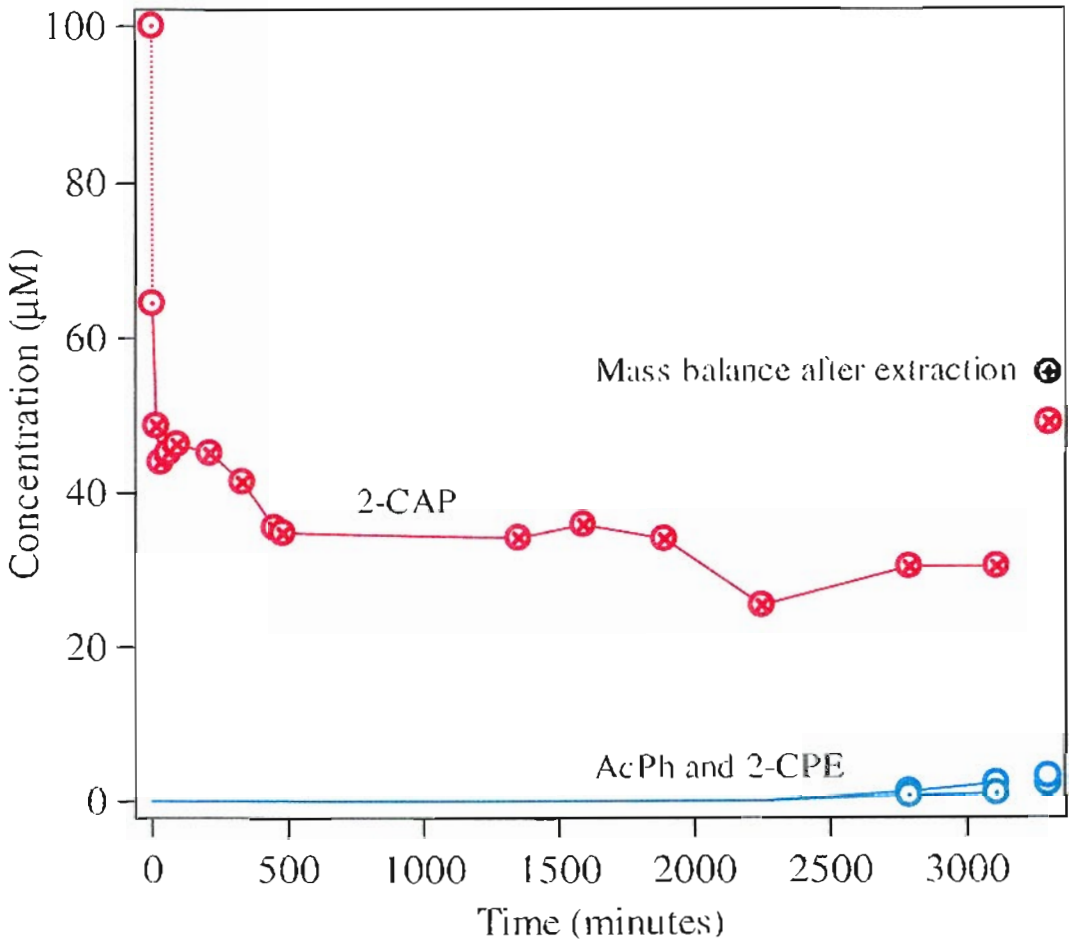

Figure 3.2 Adsorption of 2-CAP in a slurry of dried and reconstituted sediment (12 July 99). The first data point represents the nominal starting concentration, $^{*}t_0$, and the dotted line represents the drop in concentration from $^{*}t_0$ to t_1. After a period of approximately 48 hours (2880 min), formation of 2-CPE and AcPh was observed, indicating that reducing conditions had been restored. The final data points represent total concentrations after acetonitrile extraction, calculated with eq. 2.2. The amount of sediment used in this experiment was 14.1 g and the initial 2-CAP concentration was 106.7 µM. Experimental data is summarized in Appendix D.

Sorption experiments were run on numerous occasions, varying experimental run time, sediment amount, and initial 2-CAP, 2-CPE, and AcPh concentrations. Varying the initial concentrations from 100 to 400 μM appeared to affect both the rate and amount of adsorption of each compound (Figure 3.3). However, normalizing the data to the concentration at t_1 revealed a trend in the magnitude to which each compound adsorbed (Figure 3.4). In this case, 2-CPE sorbed to the least extent in both 100 μM and 400 μM solutions, while 2-CAP sorbed to the greatest extent. However, in general, all three compounds showed comparable amounts of sorption.

A comparison of adsorption observed in adsorption and reduction experiments indicated a similar trend in adsorption effects on initial 2-CAP disappearance. The rapid initial loss in 2-CAP from $*t_0$ to t_1 was observed in all 2-CAP reduction and adsorption experiments, as was a relatively high rate of loss over the next 30–60 min of the reaction (Figure 3.5). The kinetics of these two "adsorption" steps were determined for two reduction experiments run on 8 July and 9 August 1999 (Figure 3.6). The large number of data points collected in these two experiments during the initial stage of the reaction allowed for an easy separation and kinetic fitting of both adsorption steps. Both steps were fit to first-order kinetics. In all other experiments, adsorption effects were simply removed from the disappearance data in order to fit reduction kinetics. Due to insufficient numbers of data points, adsorption kinetics were generally not determined.

3.1.3 Extraction of 2-CAP, 2-CPE, and AcPh from sediment

The results of acetonitrile extraction of 2-CAP, 2-CPE, and AcPh, performed at the end of each experiment, varied greatly from one experiment to another, with recovery ranging from 1 to 100% of sorbed concentrations. There was no indication that this variation in extraction efficiency was due to temporal effects or the amount of sediment in the slurry (Figures 3.7 - 3.11 and Appendix D). The acetonitrile extraction method was consistently effective in recovering concentrations adsorbed at each time point for several experiments (Figure 3.12). In each of these experiments, it was observed that the amount of 2-CAP adsorbed decreased as the concentration in solution dropped, while the amount of 2-CPE adsorbed increased with 2-CPE formation. In experiments run on 16 June and 8 July 1999, the final mass balance after extraction was 100%.

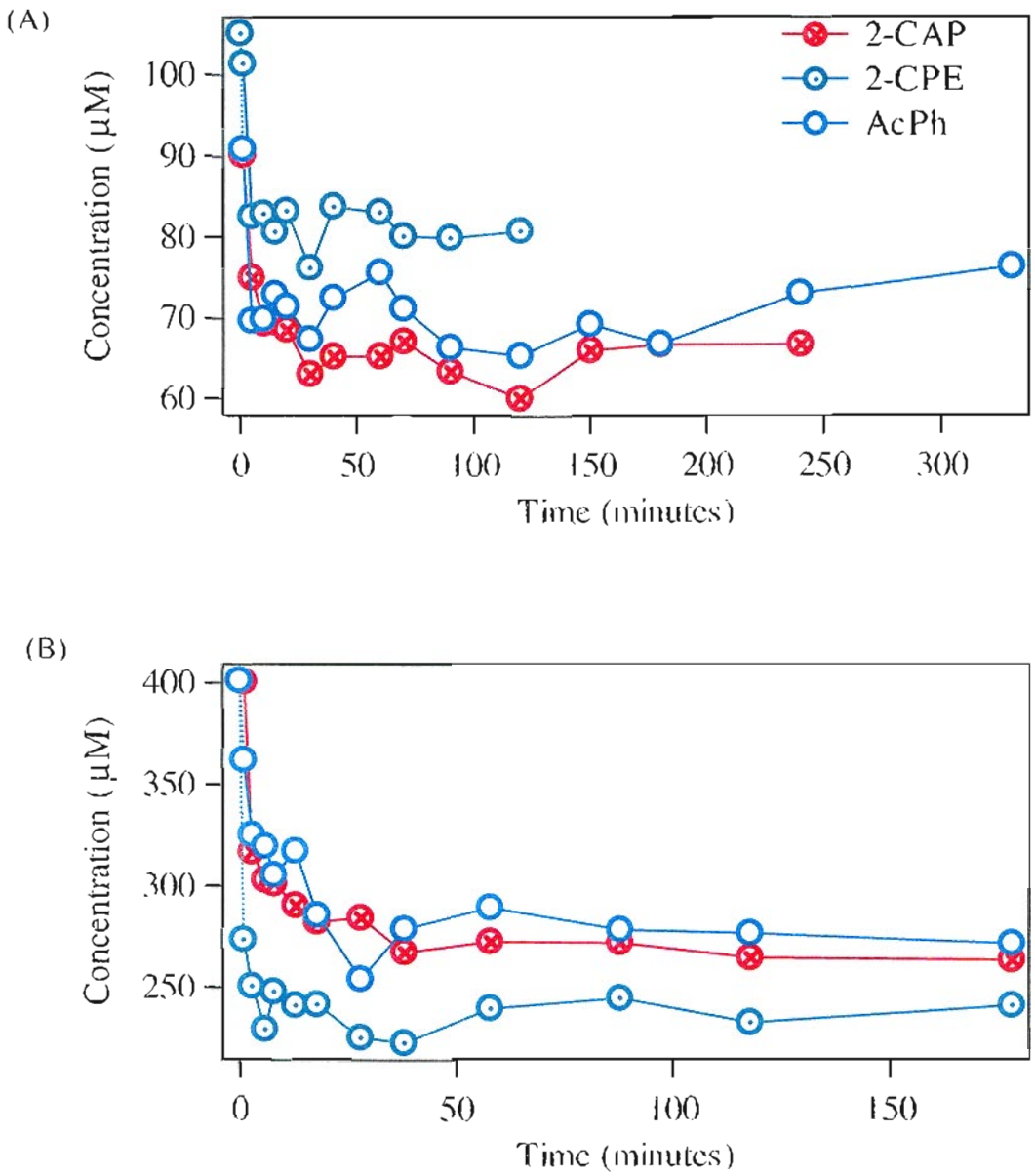

Figure 3.3 Sorption of 2-CAP, 2-CPE, and AcPh in slurries of dried and reconstituted sediment at two different initial concentrations: (A) 100 µM and (B) 400 µM. Dotted lines represent the drop from $^*t_0$ to t_1. This experiment was performed 22 September 99 with 10.2 g of sediment. Experimental 2-CAP data is summarized in Appendix D. (Experiment includes Figures 3.3 and 3.4).

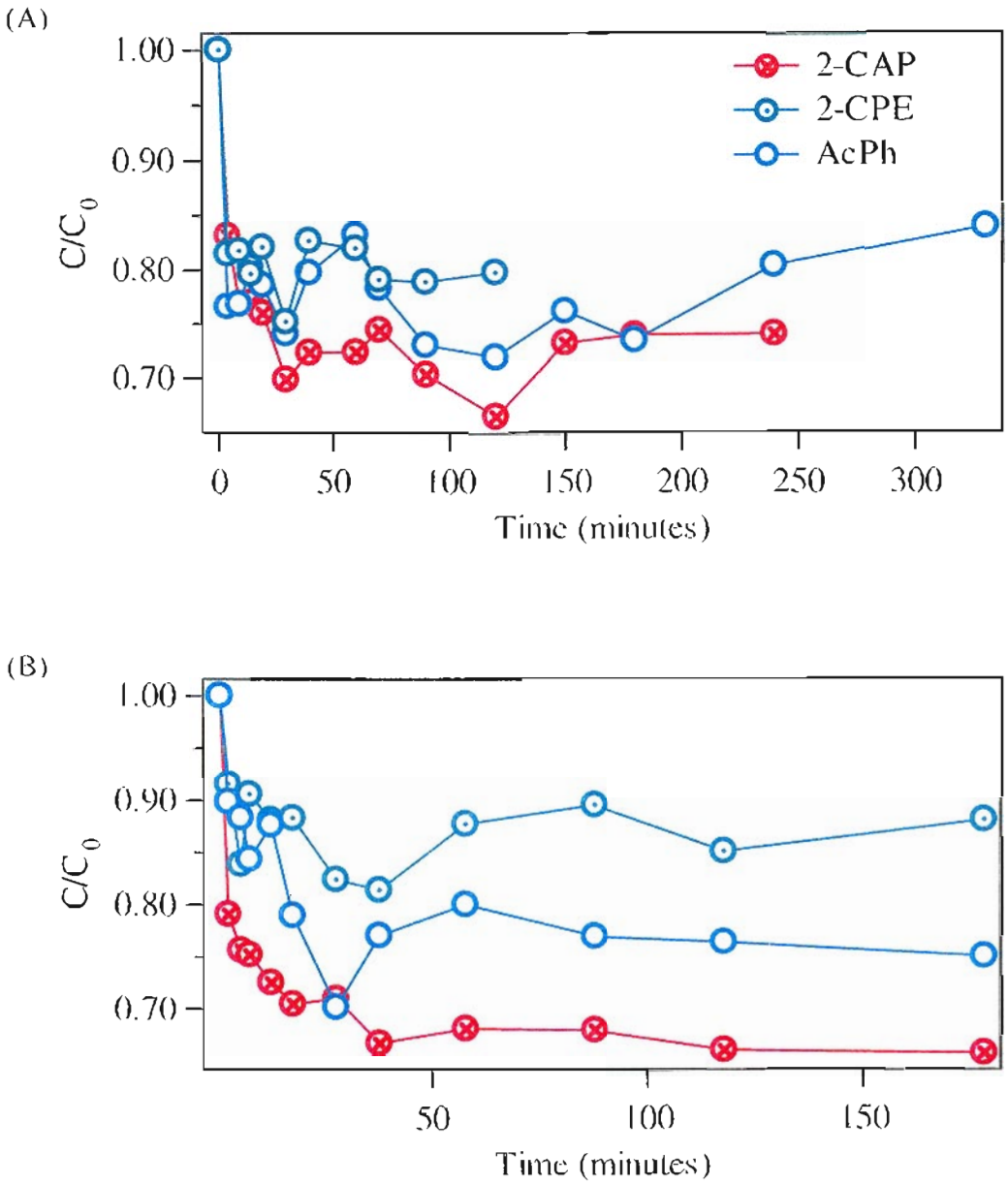

Figure 3.4 Concentration of 2-CAP, 2-CPE, and AcPh normalized to their respective t_1 concentrations in order to compare the degree to which each compound was adsorbed. (Data are derived from those in Figure 3.3). Initial concentrations were (A) 100 µM and (B) 400 µM. Experimental 2-CAP data is summarized in Appendix D. (Experiment includes Figures 3.3 and 3.4)

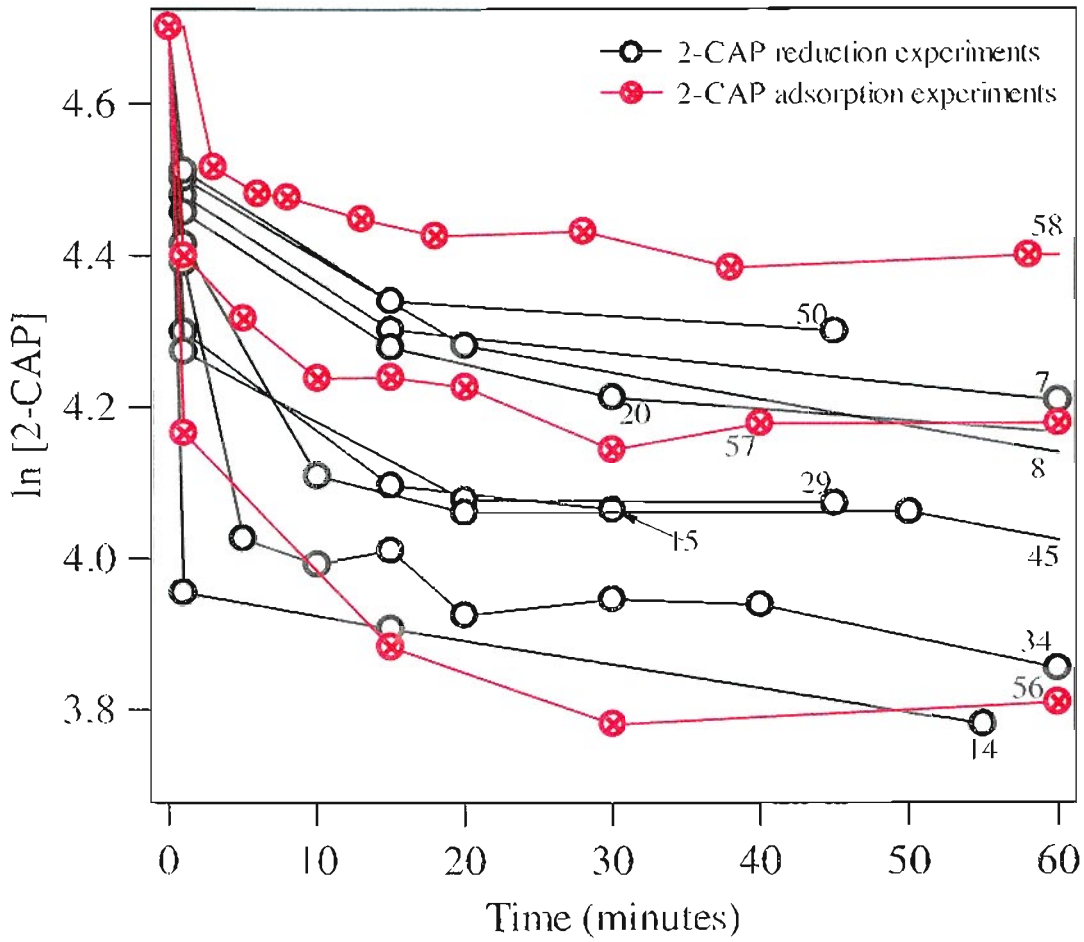

Figure 3.5 A comparison of 2-CAP reduction experiments and sorption experiments. Both reduction and adsorption experiments show a similar pattern of adsorption, with rapid initial disappearance, followed by much slower disappearance of 2-CAP. The adsorption steps could generally be divided at t_1, with rapid adsorption occurring from $^*t_0$ to t_1 and the subsequent slower adsorption step occurring from t_1 to approximately 60 min. Experiments can be identified by their respective labels, which can be found in Appendices D and E.

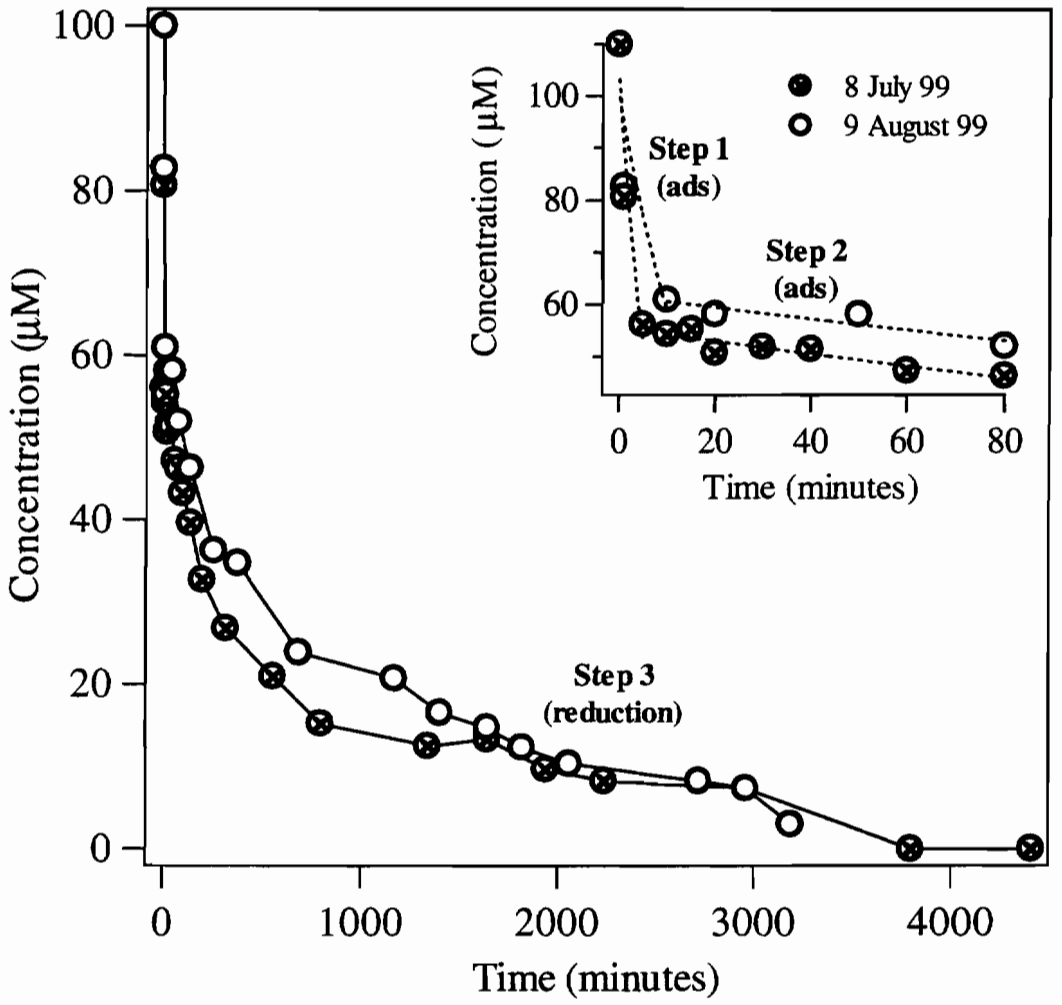

Figure 3.6 Adsorption data from two 2-CAP reduction experiments (in Figure 3.5). The initial loss of 2-CAP was divided into two sorption steps, which were fit to separate first order kinetics (inset). Both adsorption steps are significantly faster than 2-CAP reduction (the gradual decrease after 100 min) and therefore, can easily be separated from the reduction curve. All reduction and adsorption data were fit to first-order kinetics (reduction data fit not shown). k_{obs} for adsorption and reduction steps: (8 July 99) adsorption (step 1) = 1.34 x 10^{-1} min^{-1}, adsorption (step 2) = 2.41 x 10^{-3} min^{-1}, reduction (step 3) = 8.07 x 10^{-4} min^{-1}; (9 August 99) adsorption (step 1) = 5.25 x 10^{-2} min^{-1}, adsorption (step 2) = 1.93 x 10^{-3} min^{-1}, reduction (step 3) = 9.33 x 10^{-4} min^{-1}. 13.1 g of sediment was used in the July 8 experiment and 12.0 g was used in the August 9 experiment. Experimental data and fitting coefficients for reduction kinetics are summarized in Appendices D and E.

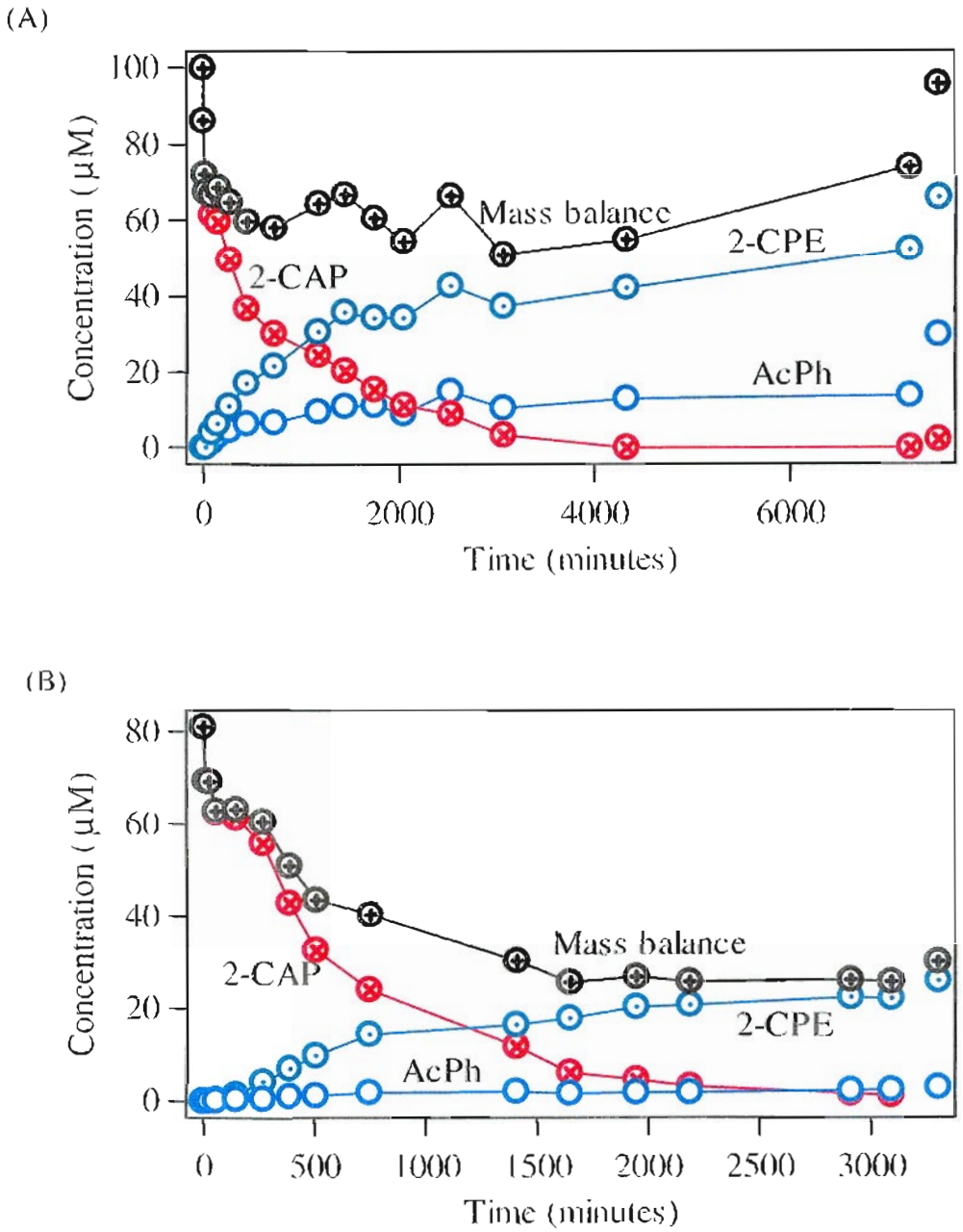

Figure 3.7 Extraction of sorbed 2-CAP, 2-CPE, and AcPh from the sediment with acetonitrile. The acetonitrile extraction method used for the experiment run 6 May 99 with 12.7 g dry sediment recovered nearly 100% of adsorbed concentrations, bringing the final mass balance to nearly 100% (A). The same procedure used 30 August 99 with 14.2 g dry sediment recovered only 2% of adsorbed (or unaccounted for) concentrations for a final mass balance below 40% (B). Experimental data and fitting coefficients are summarized in Appendices D and E. (Experiment includes Figures 3.7-3.13).

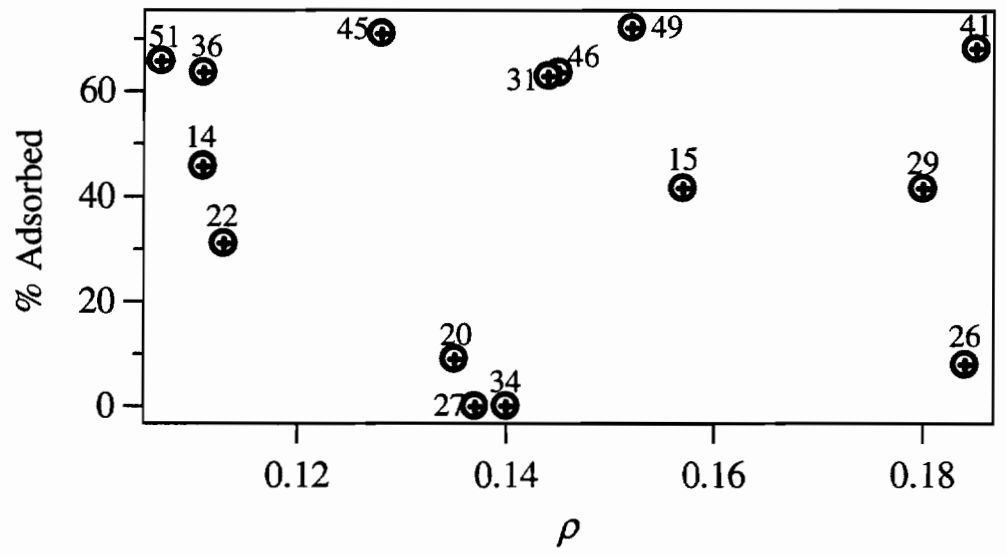

Figure 3.8 Effects of sediment amount on total 2-CAP, 2-CPE, and AcPh adsorption. Numbered labels identify each experiment and can be found in Appendices D and E. (Experiment includes Figures 3.7-3.13).

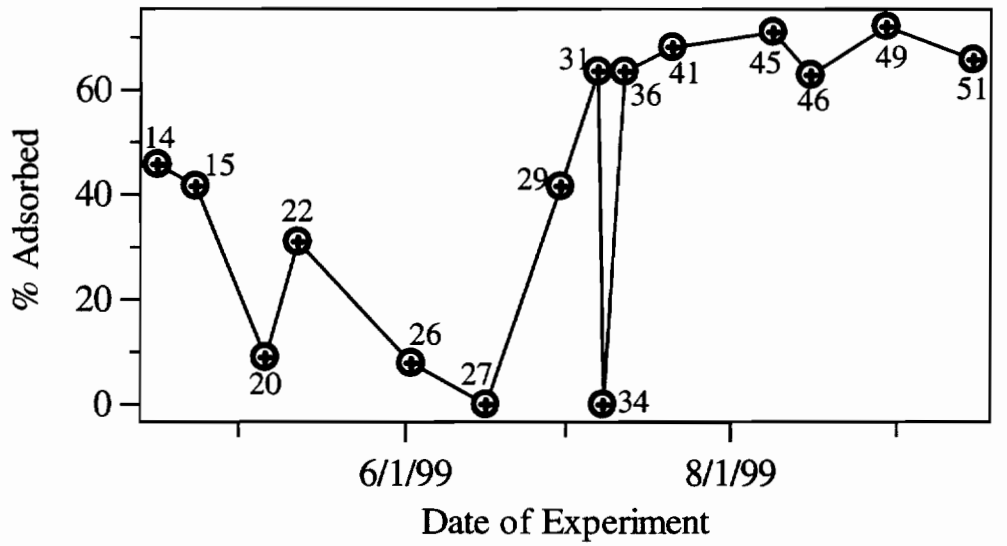

Figure 3.9 Seasonal effects on 2-CAP, 2-CPE, and AcPh adsorption. Numbered labels identify each experiment and can be found in Appendices D and E. (Experiment includes Figures 3.7-3.13).

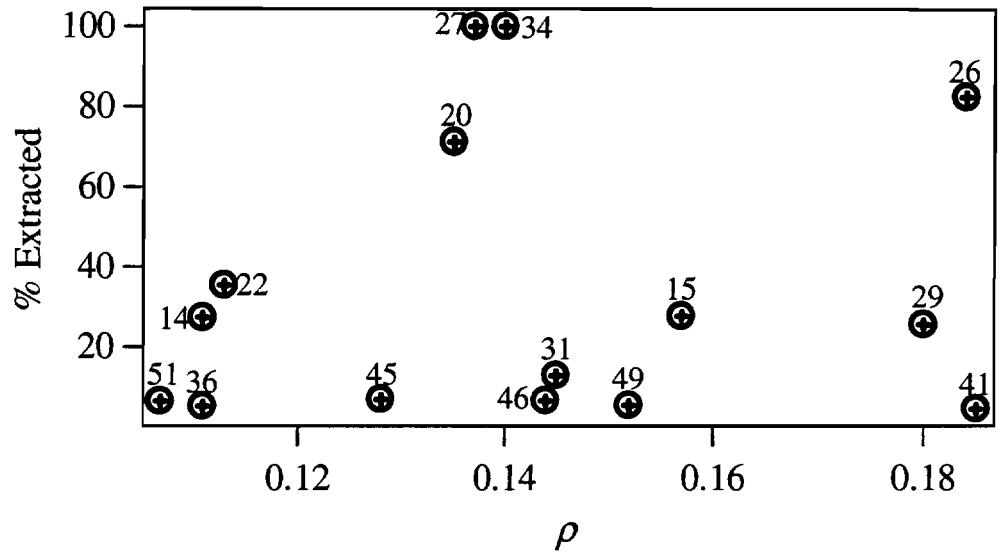

Figure 3.10 Effects of the amount of sediment on acetonitrile recovery of sorbed compounds. Numbered labels identify each experiment and can be found in Appendices D and E. (Experiment includes Figures 3.7-3.13)

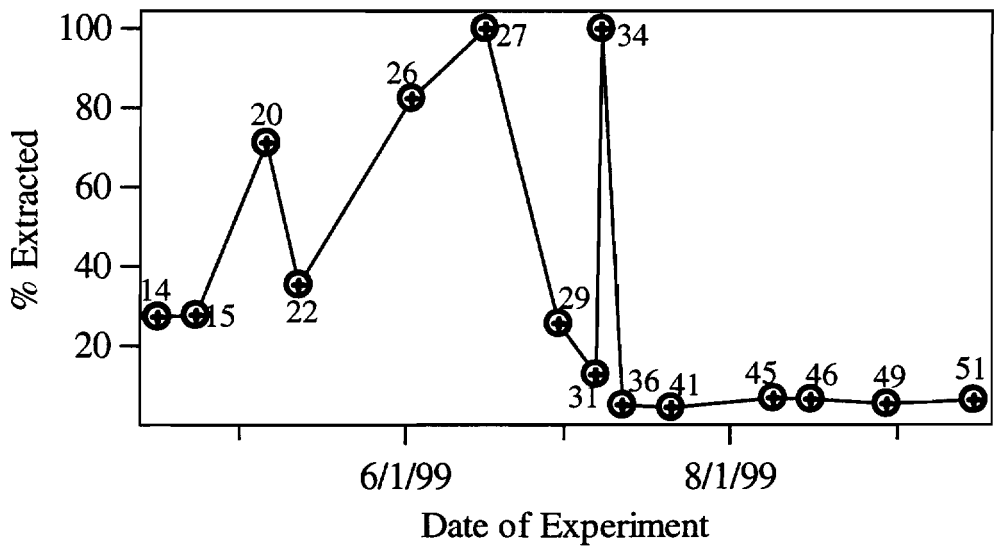

Figure 3.11 Seasonal effects on acetonitrile extraction of sorbed compounds. Numbered labels identify each experiment and can be found in Appendices D and E. (Experiment includes Figures 3.7-3.13).

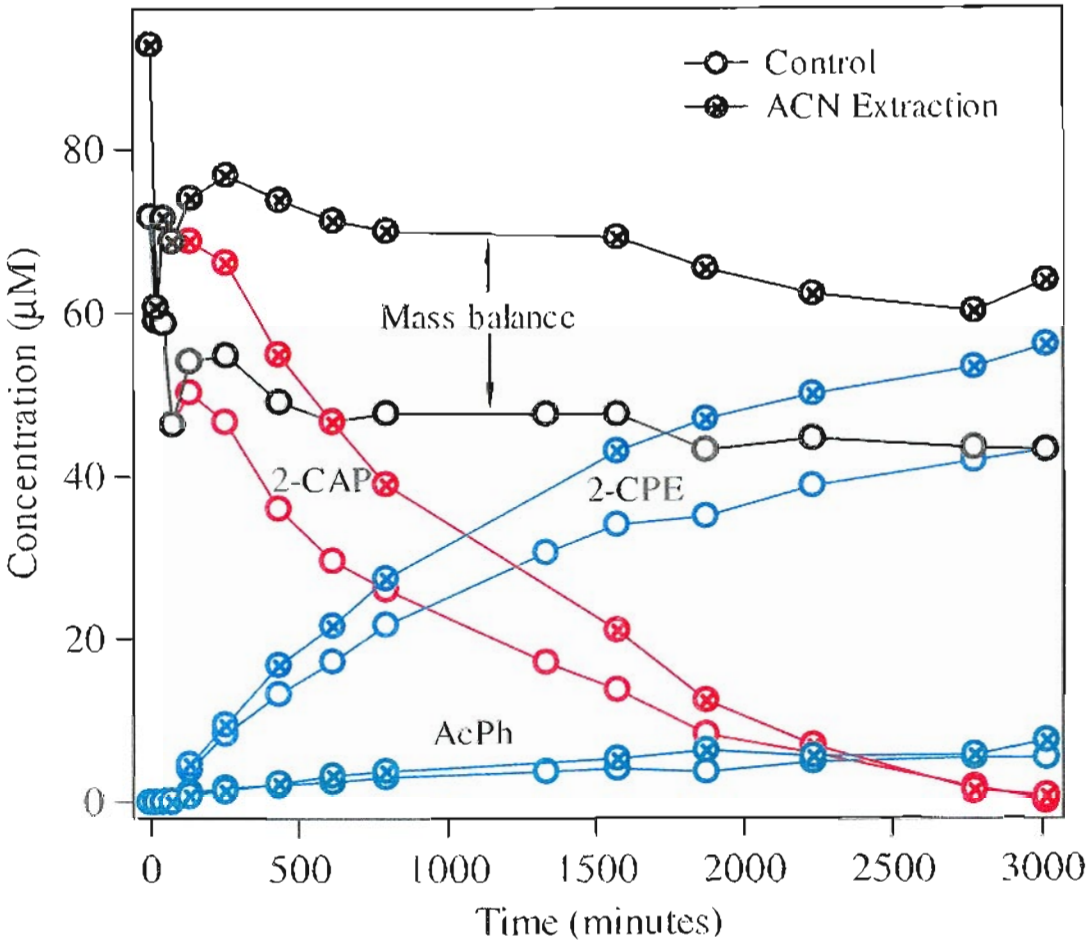

Figure 3.12 Acetonitrile was used to extract 2-CAP, 2-CPE, and AcPh from the sediment at each time point in order to determine how sorption affects 2-CAP loss and product appearance throughout the experiment. This study was performed on 30 June 99 with 16.6 g sediment. The initial 2-CAP concentration was 109.2 µM; therefore, the final mass balance was less than 60%. In similar experiments (16 June 99 and 8 July 99), 100% of the adsorbed compounds were extracted upon completion of the experiment with acetonitrile extraction of the entire slurry. In this experiment, a final extraction of the slurry was not performed. Experimental data and fitting coefficients are summarized in Appendices D and E. (Experiment includes Figures 3.7-3.13).

In a comparison experiment of three extraction methods, 30% of the total sorbed concentration was recovered with supercritical fluid extraction (SFE), while nearly 100% was recovered using soxhlet extraction. The acetonitrile extraction procedure used in most of this study fell between the two other methods for efficiency, recovering approximately 50% of the total sorbed concentration (Figure 3.13).

3.2 Environmental Effects

3.2.1 Seasonal survey of 2-CAP reduction in Rock Creek sediment

A seasonal survey of 2-CAP reduction in sediment collected from Rock Creek tributary was implemented into this study to determine how seasonal variations affect 2-CAP reduction (Figure 3.14). While a few experiments were run specifically for this purpose, the majority of the data were obtained from the control samples of various experiments run over the course of six months. In all experiments, the disappearance of 2-CAP was fit to first-order kinetics and the k_{obs} (eq. 2.9) values obtained fell within a range of 0.4×10^{-3} to 1.4×10^{-3} min^{-1}. A comparison plot of the rate of reaction versus the collection date indicated no seasonal trend (Figure 3.15). Because the amount of sediment varied somewhat from one experiment to another, it was deemed necessary to take these effects into consideration by normalizing k_{obs} to ρ (eq. 2.11 and 2.12). While this did impact the temporal variation in k_{obs}, "seasonal" trends still could not be distinguished (Figure 3.15).

3.2.2 Effects of varying sampling location on 2-CAP and resazurin reduction

To determine differences in sediment reactivity within Rock Creek, 2-CAP and resazurin reduction were measured in samples collected from three different locations. Due to an inadequate amount of sediment, the rate of 2-CAP reduction could not be measured for any of the three samples, and thus, comparisons between the location sites

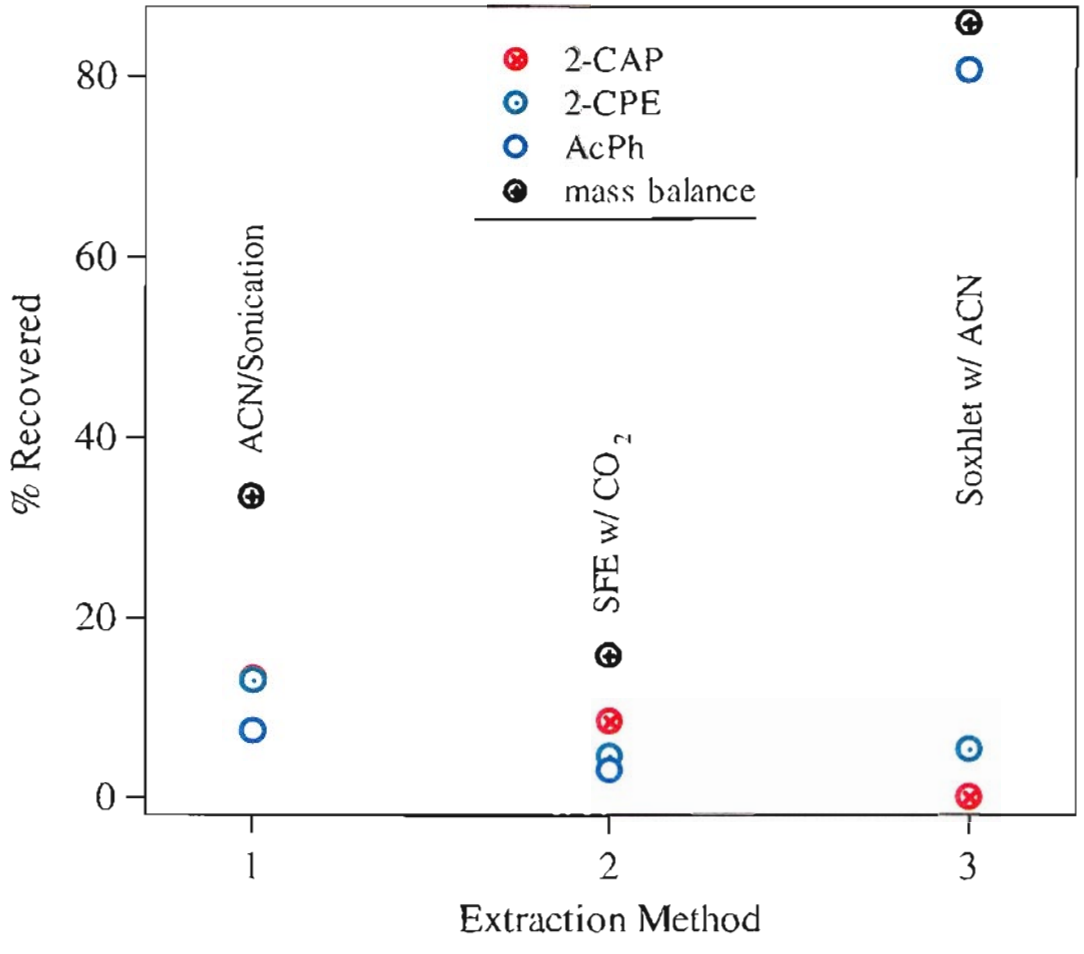

Figure 3.13 Comparison of three extraction methods performed on 2-CAP adsorption experiment (22 September 99) as a means for determining the efficiency of the acetonitrile extraction procedure used throughout this study. Soxhlet extraction proved most successful, recovering more than 30 µM of sorbed compounds, and thus, bringing mass balance to 100% of the initial 2-CAP concentration. The amount of sediment used in this experiment was 10.2 g. Experimental data is summarized in Appendix D. (Experiment includes Figures 3.7-3.13).

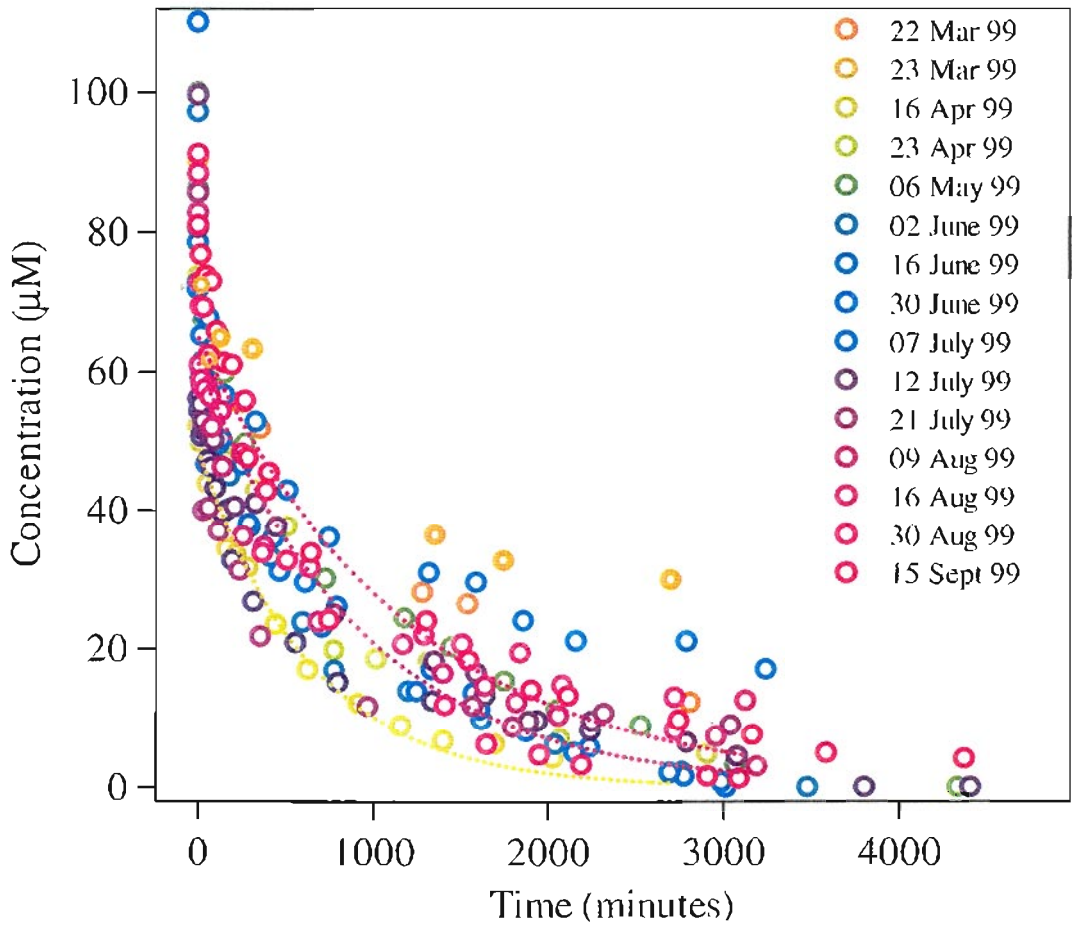

Figure 3.14 Summary of all data for a seasonal survey for 2-CAP reduction in sediment collected in Rock Creek tributary. For illustration, the reduction portions of three experimental data curves were fit to first-order kinetics (represented as dotted lines). All experimental data and fitting coefficients are summarized in Appendices D and E. (Experiment includes Figures 3.14 and 3.15).

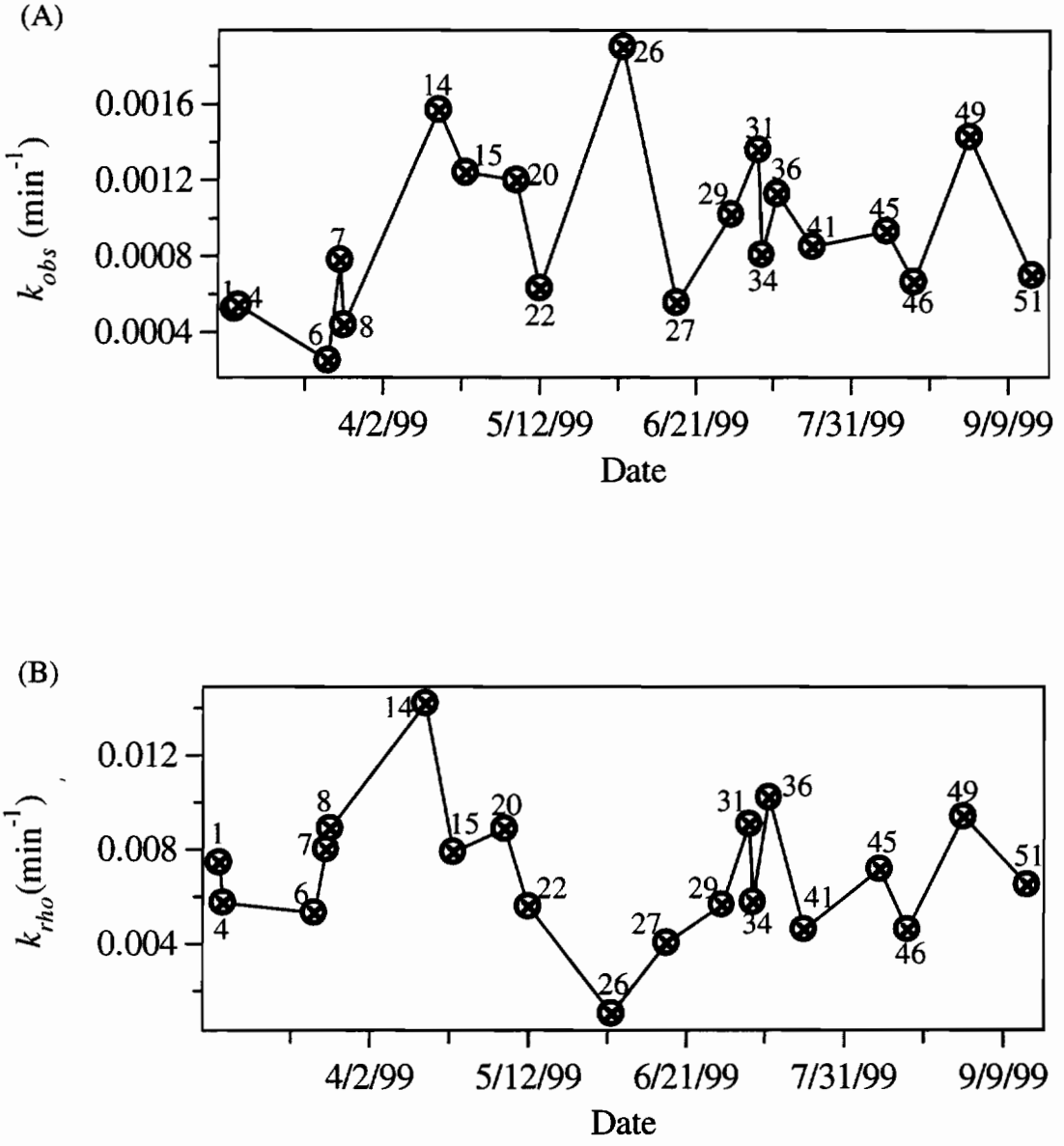

Figure 3.15 Seasonal effects on the rate of 2-CAP reduction in sediment collected from Rock Creek tributary. (A) plots 2-CAP reduction rate constant, k_{obs}, against the date of the experiment to determine if seasonal effects can be observed. Normalizing 2-CAP k_{obs} to the amount of sediment produces less variance with regards to season (B), however, neither plot indicates the presence of a seasonal trend. (Experiment includes Figures 3.14 and 3.15).

could not be made (Figure 3.16). In contrast, differences in the rate of resazurin reduction were easy to identify. In this experiment, sample 3, which was collected near the rocky banks of the creek, appeared to be the most reducing (gave the largest k_{rho}), while sample 2, collected from an adjacent marshy area, appeared least reactive (Figure 3.17). The rate of resazurin reduction was determined from a linear fit of ln[resazurin] versus time data because the number of data points collected for sample 3 was not enough to obtain a reliable non-linear fit. Because all three samples had varying amounts of sediment, kinetics comparisons were made based on k_{rho} values.

3.2.3 2-CAP reduction in coastal sediments

To compare reduction mechanisms in freshwater and coastal sediments, 2-CAP reduction was studied in two coastal sediments, collected from sites within the Columbia River estuary. In both coastal sediments, 2-CPE and AcPh were formed from 2-CAP reduction (Figures 3.18 and 3.19). Similar to reduction in Rock Creek, 2-CPE was the major product in both coastal sediments. Apparent differences between the two coastal sediments were observed in studying the effects of adsorption and reduction on 2-CAP disappearance as well as subsequent product formation (reaction rates are summarized in Appendix E). k_{obs} values for 2-CAP reduction, as well as 2-CPE and AcPh formation, were greater in the sample collected from Tansy Point, however, the amount of sediment used was also higher in this sample, as was the amount of products formed. When normalized to ρ, the 2-CAP rate constants for both samples became roughly equal.

3.2.4 Effects of storing sediment on 2-CAP reduction

To determine the effects of sediment storage time on reduction processes, 2-CAP reduction was measured on six occasions over the course of 2.5 months in sediment samples that had been refrigerated for varying amounts of time prior to 2-CAP addition. In this experiment, the rate of 2-CAP reduction increased from samples stored for 1 to 24 days, but then dropped again by 71 days of storage (Figure 3.20). The production of 2-CPE appeared to decline with increasing storage time, with

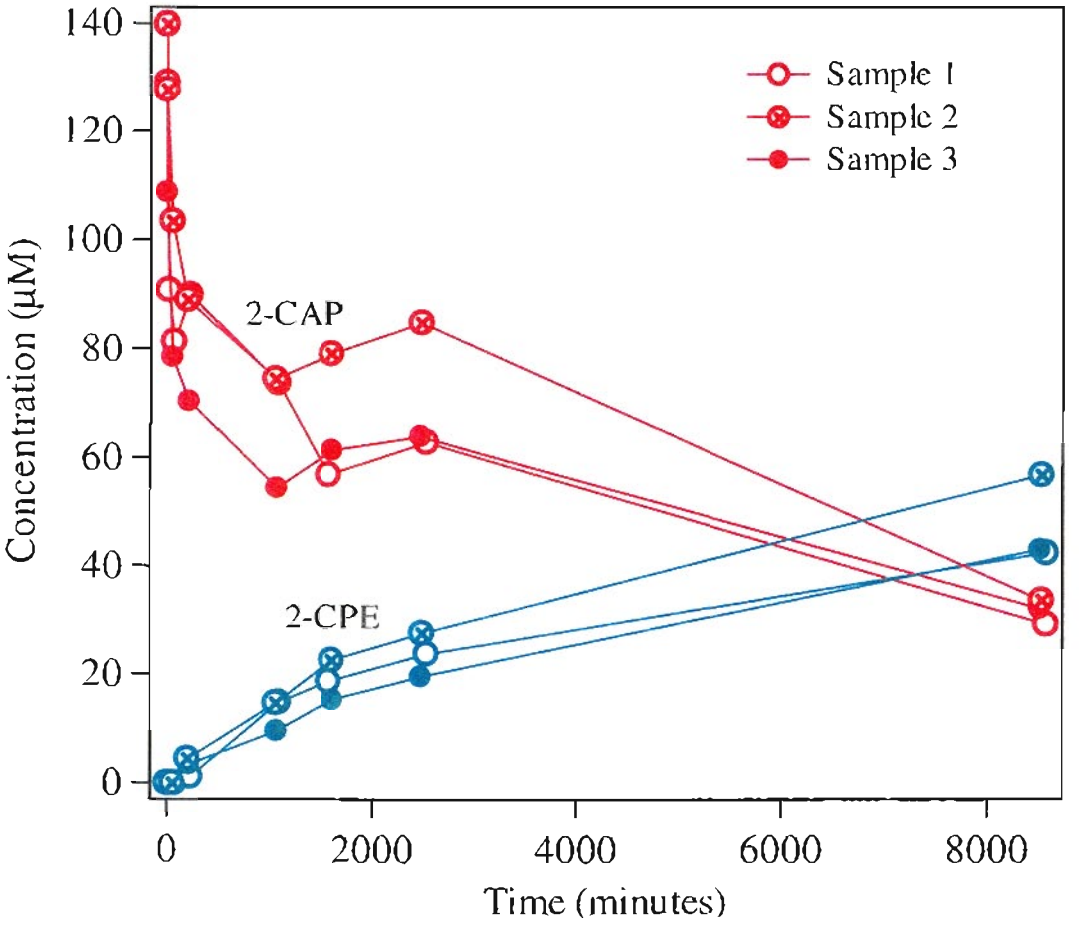

Figure 3.16 Effect of varying sampling location within Rock Creek tributary on 2-CAP reduction. Sediment samples were collected from the center of the creek (sample 1), a marshy area adjacent to the creek (sample 2), and near the rocky banks (sample 3) on 23 February 99. The amount of sediment in each sample slurry varied from 3.0 g in samples 2 and 3 to 6.8 g in sample 1. Subsequent experiments were performed with larger amounts of sediment in order to obtain a greater degree of reduction. (Experiment includes Figures 3.16 and 3.17).

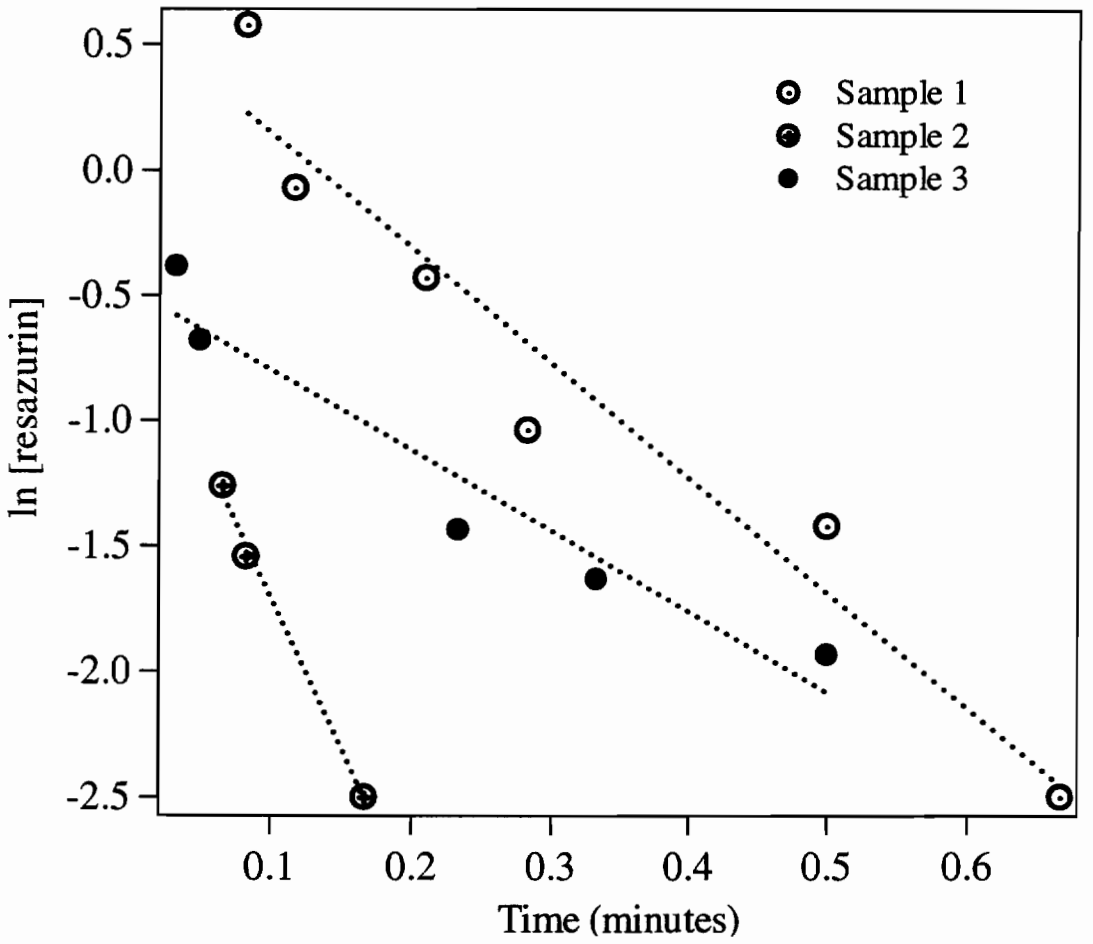

Figure 3.17 Resazurin reduction in sediments collected from three different locations within Rock Creek tributary. Samples: (1) 0.6 g sediment, 1.25 s^{-1} (k_{rho}); (2) 1.2 g sediment, 0.42 s^{-1} (k_{rho}); (3) 0.9 g sediment, 2.05 s^{-1} (k_{rho}). Refer to Figure 3.16 for collection location. (Experiment includes Figures 3.16 and 3.17).

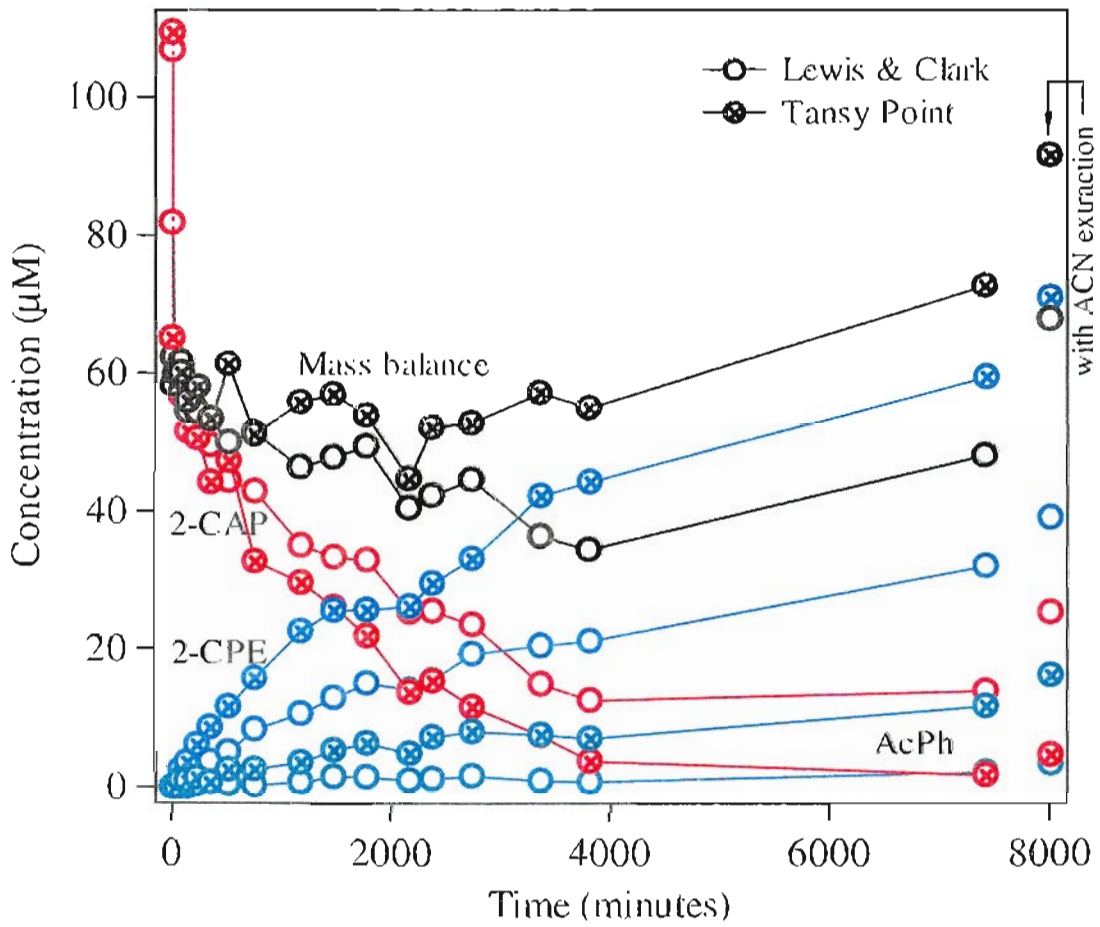

Figure 3.18 Reduction of 2-CAP in coastal sediments collected from two sampling stations in the Columbia River estuary (4 May 99). In general, results are similar to 2-CAP reduction observed in Rock Creek sediments. The amount of sediment used was 12 g (Lewis & Clark) and 16 g (Tansy Point). Points at 8000 min represent final concentrations after acetonitrile extraction. Experimental data is summarized in Appendix D. (Experiment includes Figures 3.18 and 3.19).

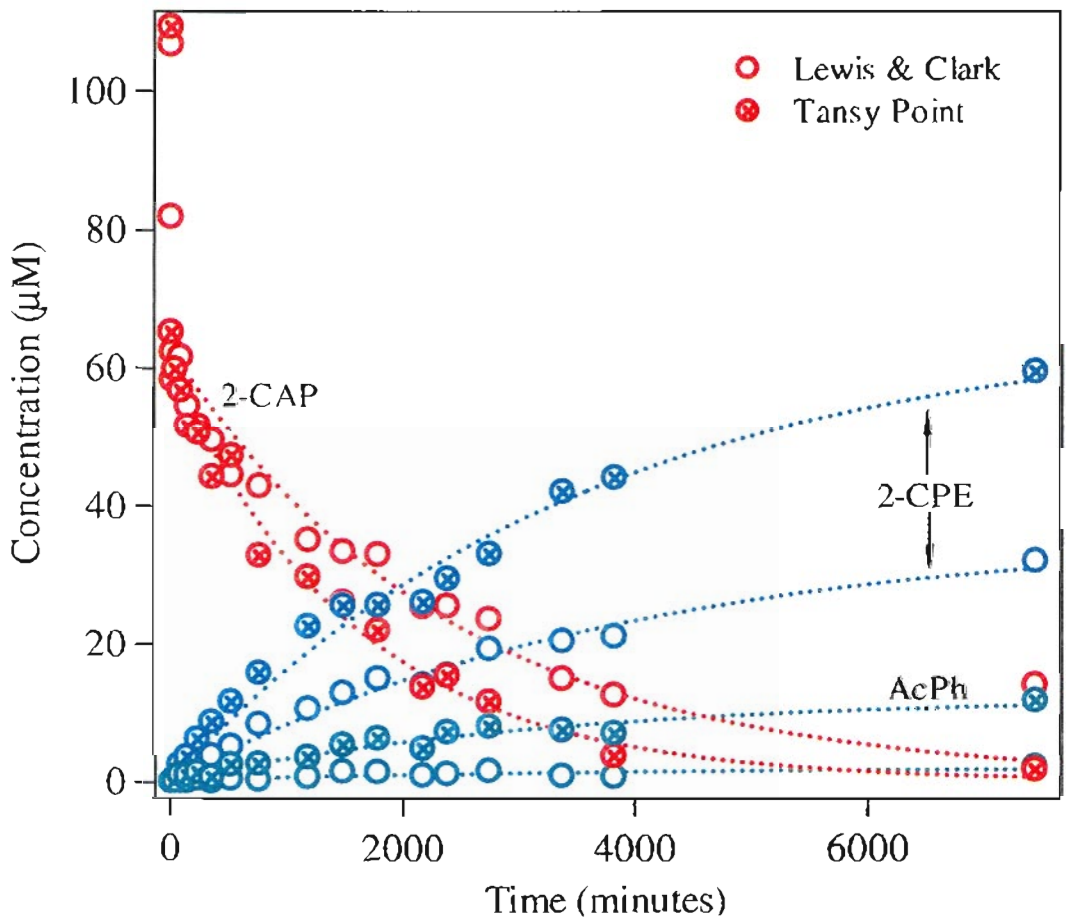

Figure 3.19 Reduction of 2-CAP and formation of 2-CPE and AcPh in coastal sediments fit to first-order kinetics. Fitting coefficients are summarized in Appendix E. (Experiment includes Figures 3.18 and 3.19).

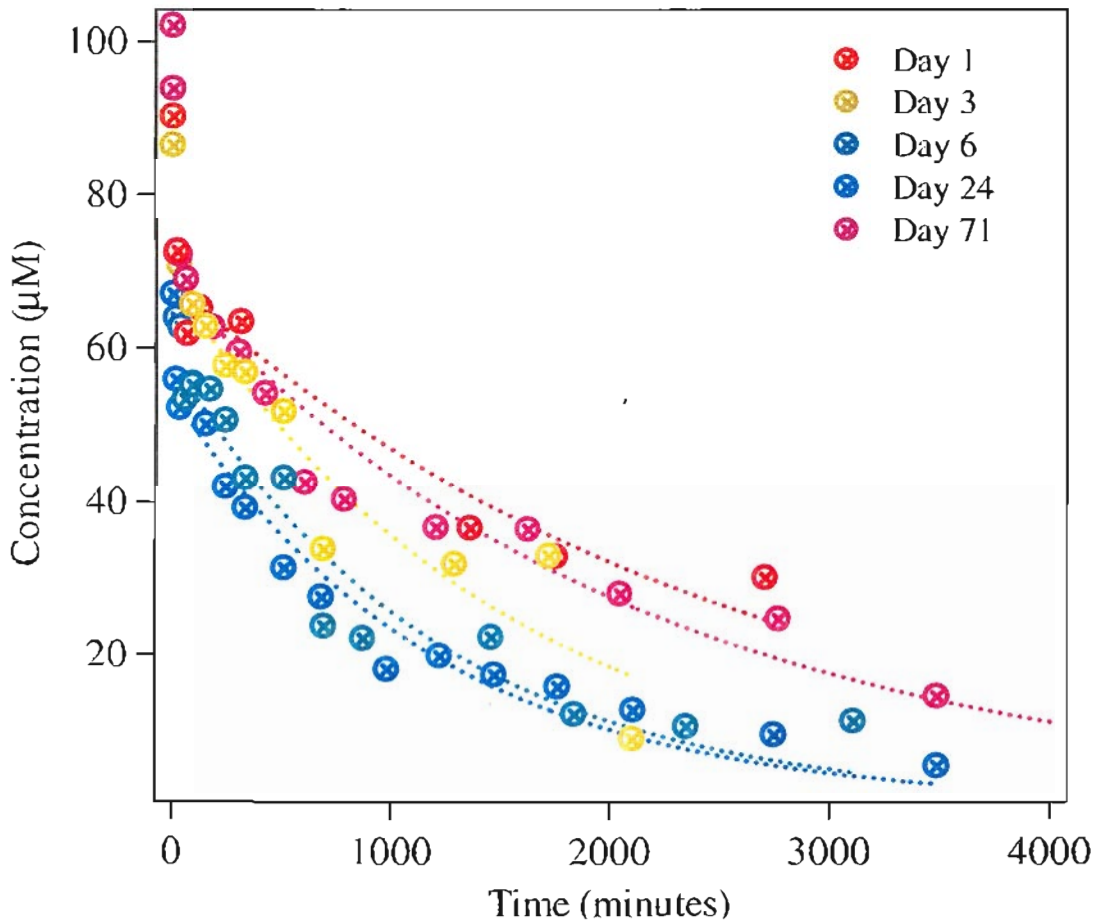

Figure 3.20 Effects of storage time on 2-CAP reduction. This experiment was begun on 23 March 99. All samples had an initial 2-CAP concentration of 101.9 µM and sediment mass of 5 g. Excluding initial adsorption, 2-CAP reduction data was fit to first-order kinetics. All experimental data and fitting coefficients are summarized in Appendices D and E. (Experiment includes Figures 3.20 and 3.21).

the exception of the sample stored for 3 days, which had the greatest formation rate (Figure 3.21). A small effect of storage time was also evident from the AcPh appearance data, however, because its production was minimal, the effect appeared negligible in comparison to changes in 2-CPE and 2-CAP data.

3.2.5 Effects of varying the amount of sediment on 2-CAP reduction

To determine the effects of sediment quantity on 2-CAP reduction, one sediment sample was divided into four parts, containing unequal amounts of sediment, to prepare slurries with ρ values ranging from 0.11 to 0.25. In this experiment, both k_{obs} for 2-CAP reduction and 2-CPE formation increased with ρ (Figures 3.22-3.25). In general, AcPh formation also increased with increasing sediment quantity, however, its production was minimal in comparison to 2-CPE. A second comparison, thought possible to reveal an effect caused by varying the amount of sediment, was between 2-CAP reduction and ρ over the seasonal time frame of this study (Figures 3.26 and 3.27). When this comparison was made, however, no apparent correlation between the two was observed.

3.3 Secondary Processes

3.3.1 Effects of sulfide species on 2-CAP reduction

To investigate whether 2-CAP might be reduced by HS^- (or H_2S) in sulfidogenic sediments, a model system of buffered sodium sulfide (pH 8) was incorporated into this study to investigate potential 2-CAP transformations. In this system, the 2-CAP concentration decreased rapidly with the formation of a product of similar retention time. We were unable to identify this product using HPLC and GC/MS. Products with retention times greater than 2-CAP were not observed using HPLC because the sample run time was minimized in order to complete the experiment within the time frame needed for measuring 2-CAP disappearance with time.

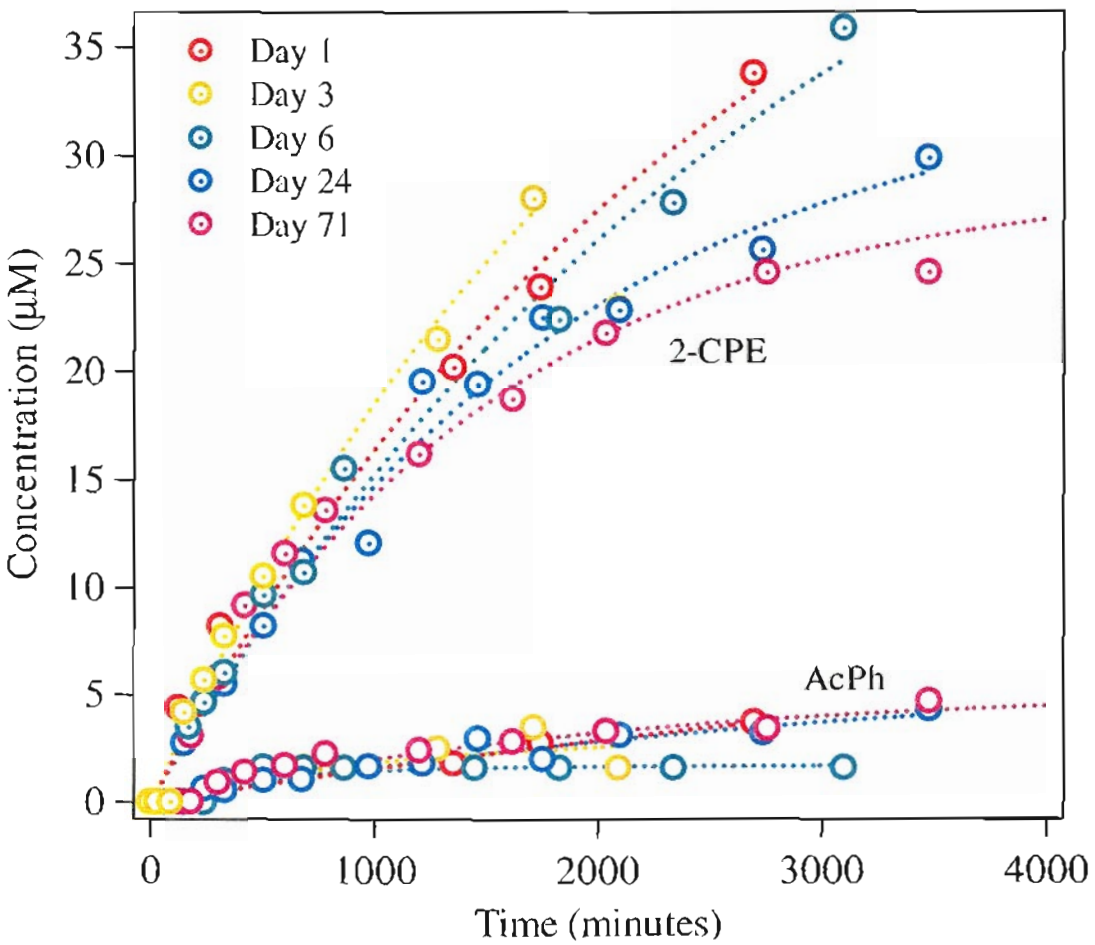

Figure 3.21 Effects of storage time on 2-CPE and AcPh formation. Both 2-CPE and AcPh data were fit to first-order kinetics without excluding any data. Fitting coefficients are summarized in Appendix E. (Experiment includes Figures 3.20 and 3.21).

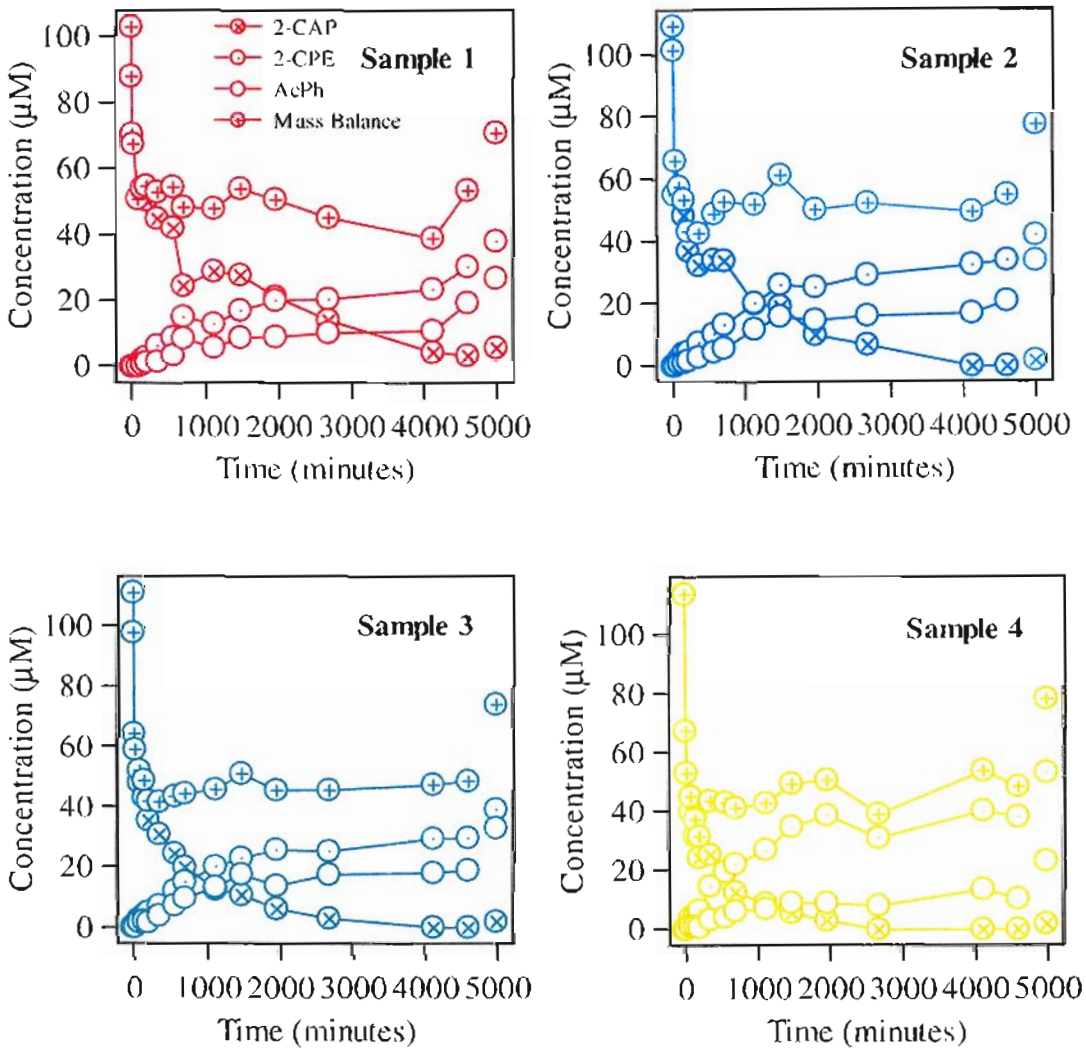

Figure 3.22 Effects of varying the amount of sediment on 2-CAP reduction (12 May 99). Points at 5000 min represent final concentrations after acetonitrile extraction. Sample 1: 10.7 g sediment, 103 µM (C_0); Sample 2: 15.6 g sediment, 109.2 µM (C_0); Sample 3: 18.8 g sediment, 111 µM (C_0); Sample 4: 22.3 g sediment, 113 µM (C_0). Experimental data is summarized in Appendix D. (Experiment includes Figures 3.22-3.25).

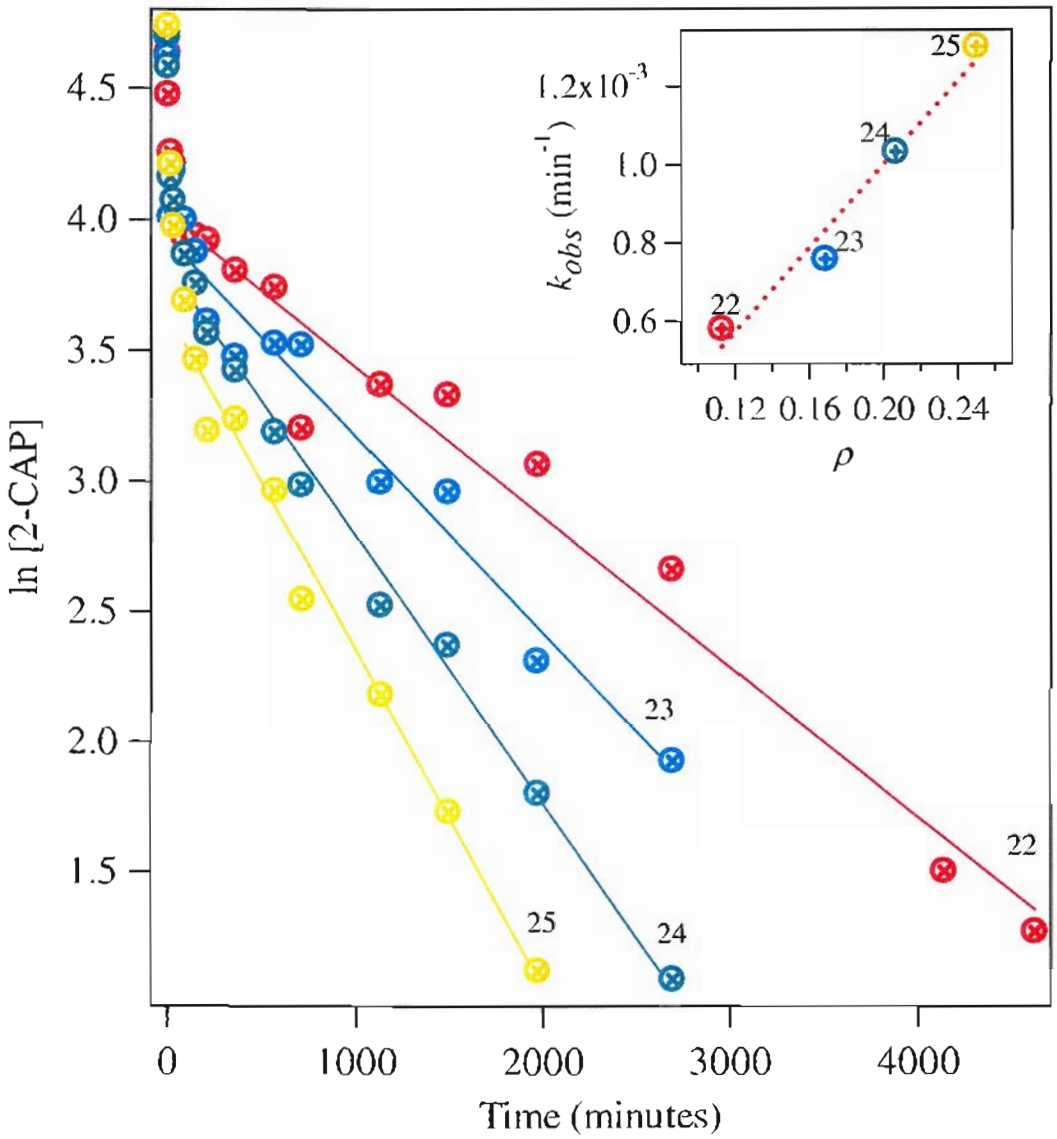

Figure 3.23 Effect of varying the amount of sediment on 2-CAP reduction. The inset plot shows the correlation between the rate of 2-CAP reduction (k_{obs}) and the amount of sediment. The slope of the fitted k_{obs} vs. ρ data is equal to 5.36 x 10^{-3}. Numbered labels represent the experiment. Fitting coefficients summarized in Appendix E are based on non-linear kinetic fits (not represented in this figure). (Experiment includes Figures 3.22-3.25).

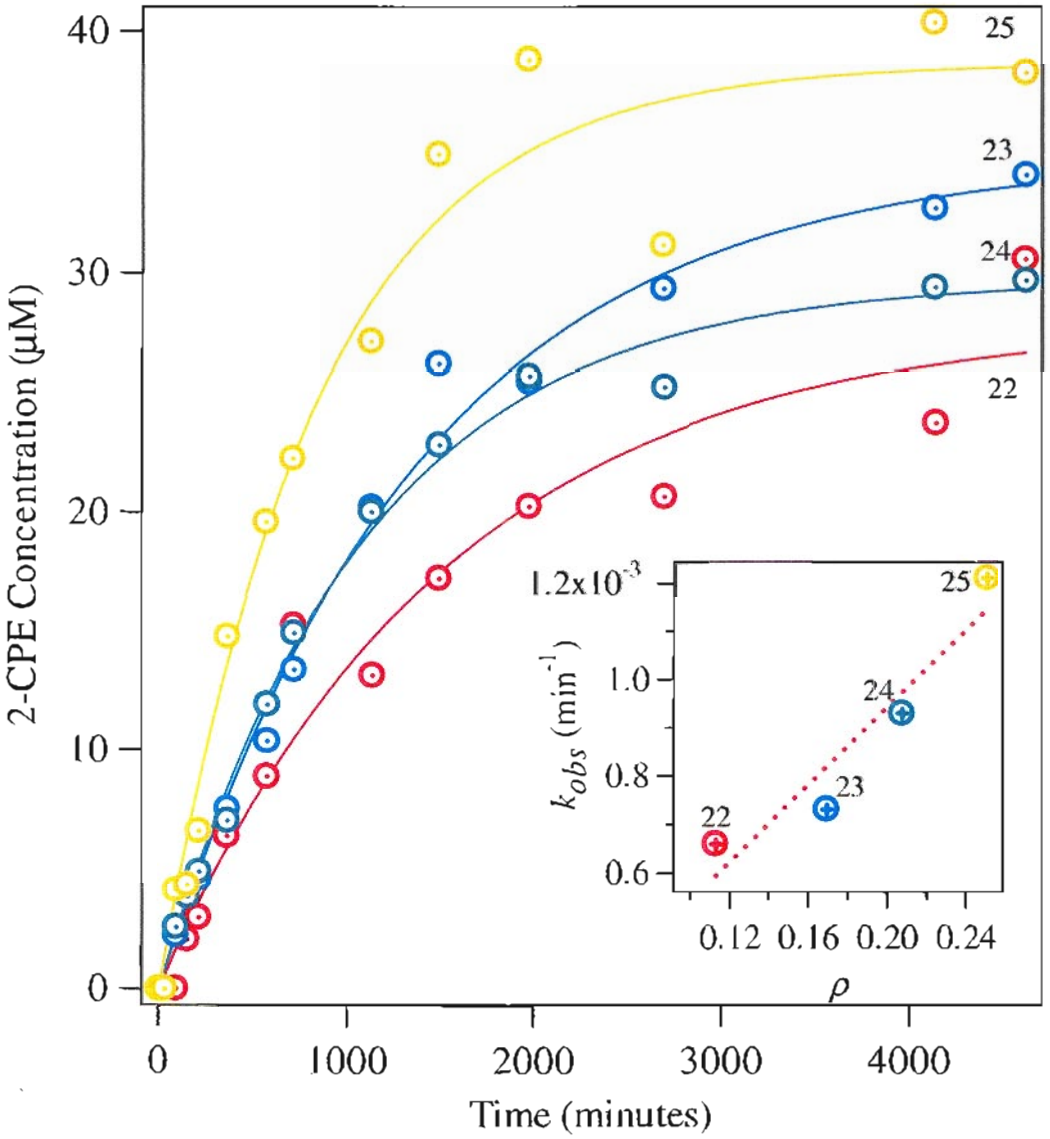

Figure 3.24 Effects of varying sediment amount on 2-CPE production. The inset plot represents the correlation between the rate of 2-CPE formation (k_{obs}) and the amount of sediment. The slope of the fitted k_{obs} vs. ρ data is equal to 4.03 x 10^{-3}. Numbered labels represent the experiment. Fitting coefficients are summarized in Appendix E. (Experiment includes Figures 3.22-3.25).

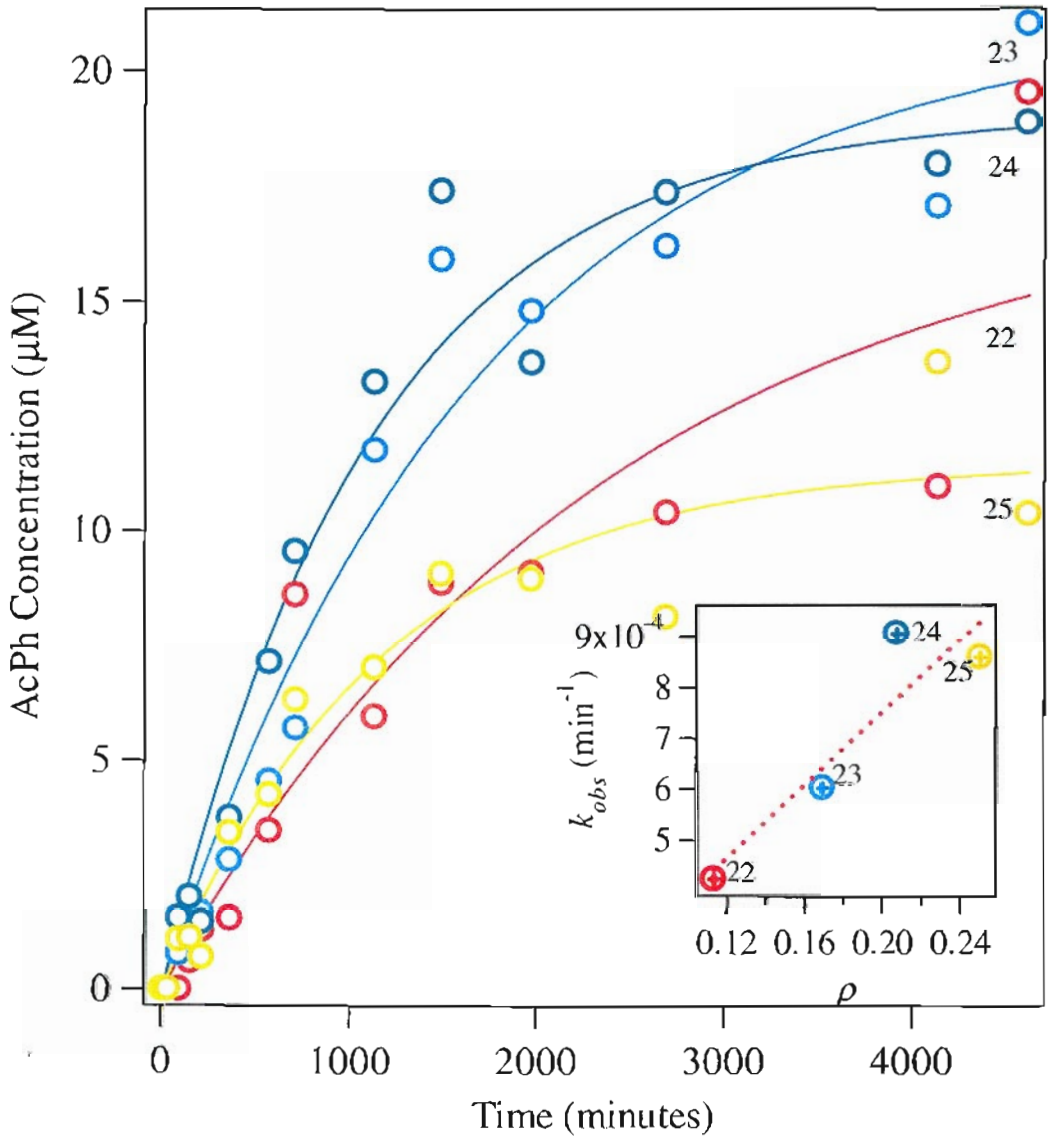

Figure 3.25 Effects of the amount of sediment on AcPh formation. The slope of the fitted k_{obs} vs. ρ data is equal to 3.60 x 10^{-3}. Numbered labels represent the experiment. Fitting coefficients are summarized in Appendix E. (Experiment includes Figures 3.22-3.25).

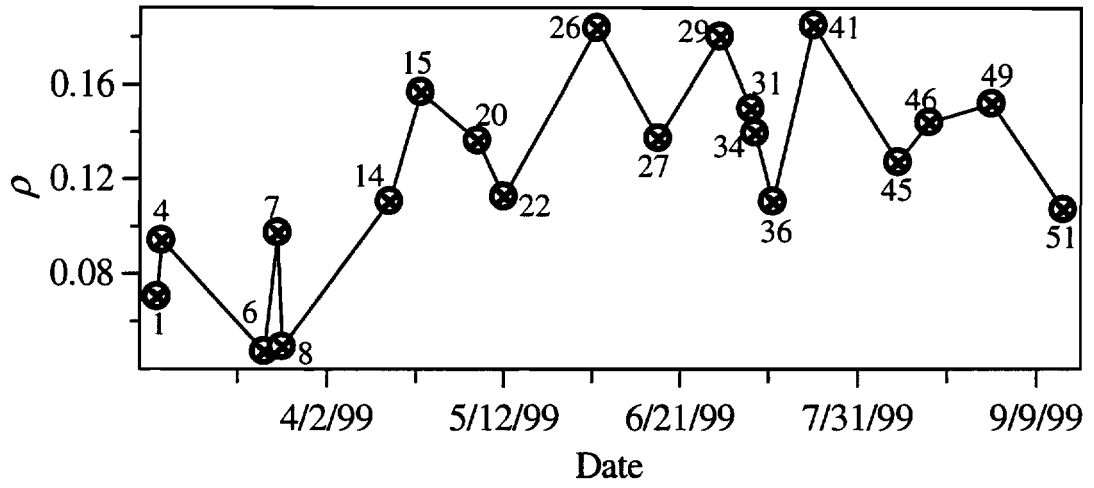

Figure 3.26 Variation in ρ used in experiments throughout this study.

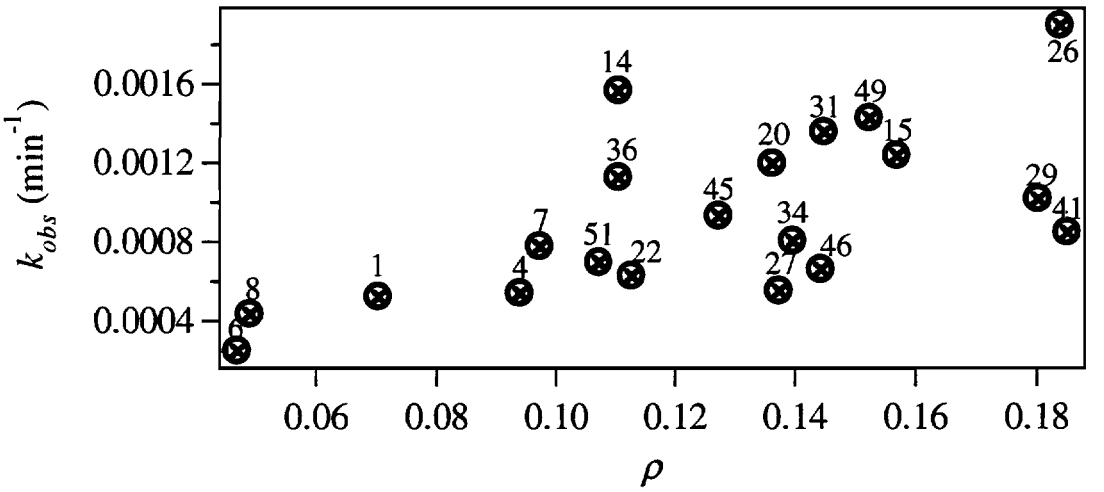

Figure 3.27 Effects of varying ρ on 2-CAP reduction kinetics.

3.3.2 Effects of pH on 2-CAP transformations

Because 2-CAP could be subject to hydrolysis, a model system employing buffered solutions was studied to determine the potential for this reaction between pH 3 and 11. After several days of mixing, no reaction was observed in pH 3 and pH 7 solutions. However, transformation was seen in solutions at pH 8-11, with the rate of reaction increasing with higher pH (Figures 3.28-3.32). The products formed were the same at each pH, with the exception of pH 10, which showed a product peak at 17 min. This product may have been formed at pH 11, however, because experimental run time was limited to 15 min, it was not observed. The product peaks observed could neither be identified nor quantified using HPLC without known standards for the expected hydrolysis products. Mass spectrometry was also unsuccessful in identifying the products formed.

3.3.3 Transformation of 2-CPE and AcPh in sediment

The transformation of AcPh and 2-CPE in sediments could potentially complicate the interpretation of product formation kinetics in 2-CAP reduction experiments (Figures 1.2 and 1.5). To test for this possibility, AcPh and 2-CPE were added to sediment slurries to investigate transformation mechanisms and determine the potential for reaction in comparison to 2-CAP reduction. While AcPh was observed to undergo reduction to form 1-phenylethyl alcohol (1-PE), the reaction was slow in comparison to 2-CAP reduction (Figure 3.33). However, mass balance of the two compounds, even after extraction, was generally poor. In numerous experiments, initial 2-CPE disappearance was observed with no reaction product formation. However, in one final study, in which the experiment was allowed to continue past one week, a small amount of AcPh formation was observed (Figure 3.34). Like AcPh reduction, 2-CPE dehydrohalogenation was much slower than 2-CAP reduction.

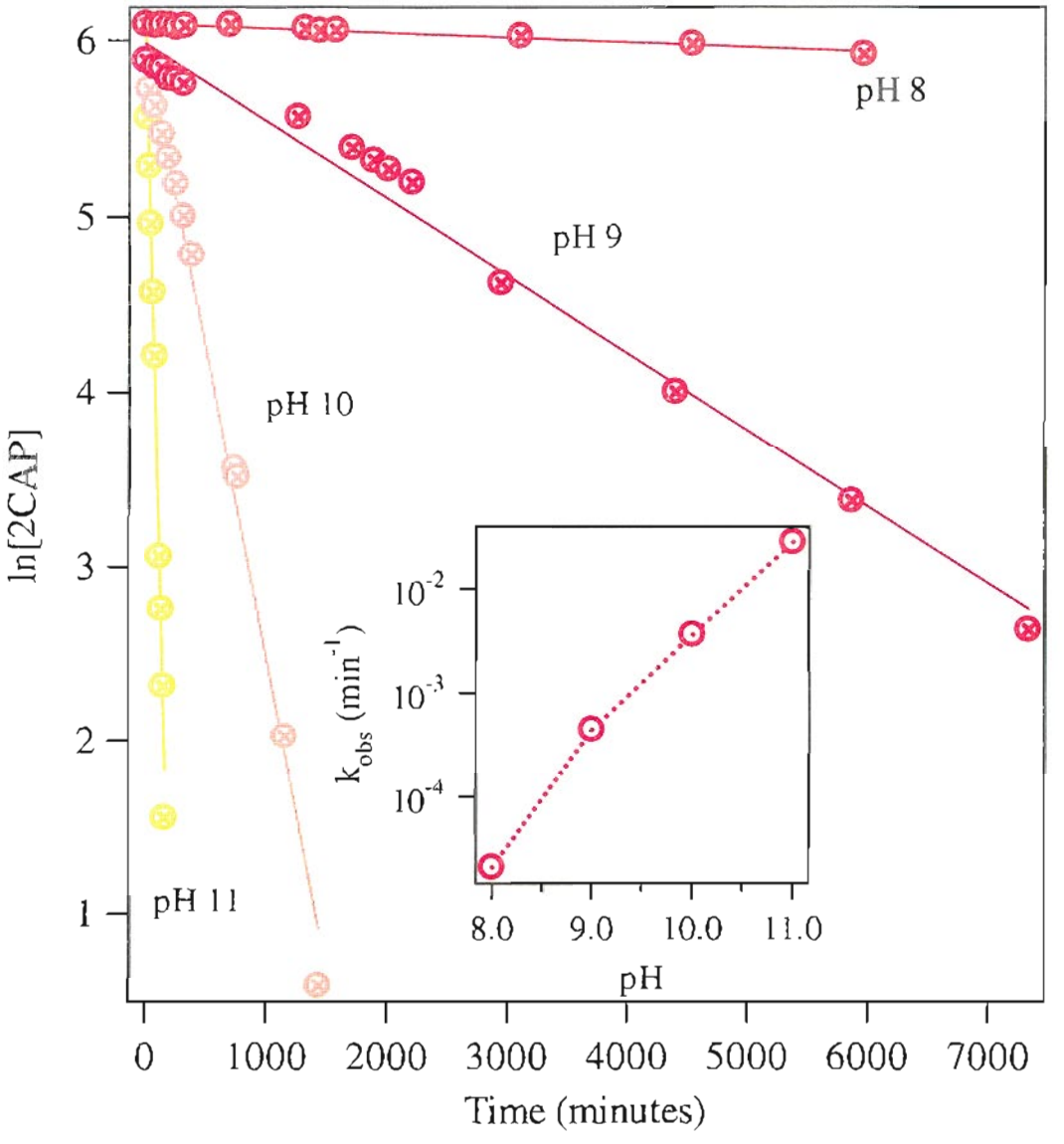

Figure 3.28 Effects of pH 8-11 on 2-CAP in buffer solutions. The inset plot represents the relationship between the rate of 2-CAP disappearance (k_{obs}) and pH. (Experiment includes Figures 3.28-3.32).

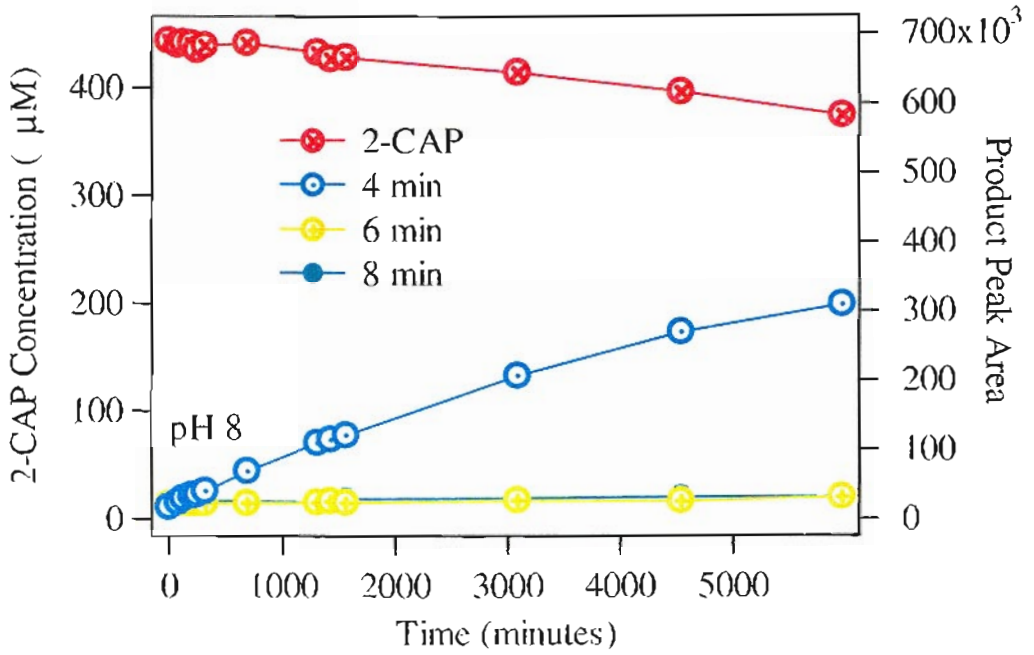

Figure 3.29 Hydrolysis of 2-CAP in buffered solution pH 8. Unknown products are represented by their retention time. (Experiment includes Figures 3.28-3.32).

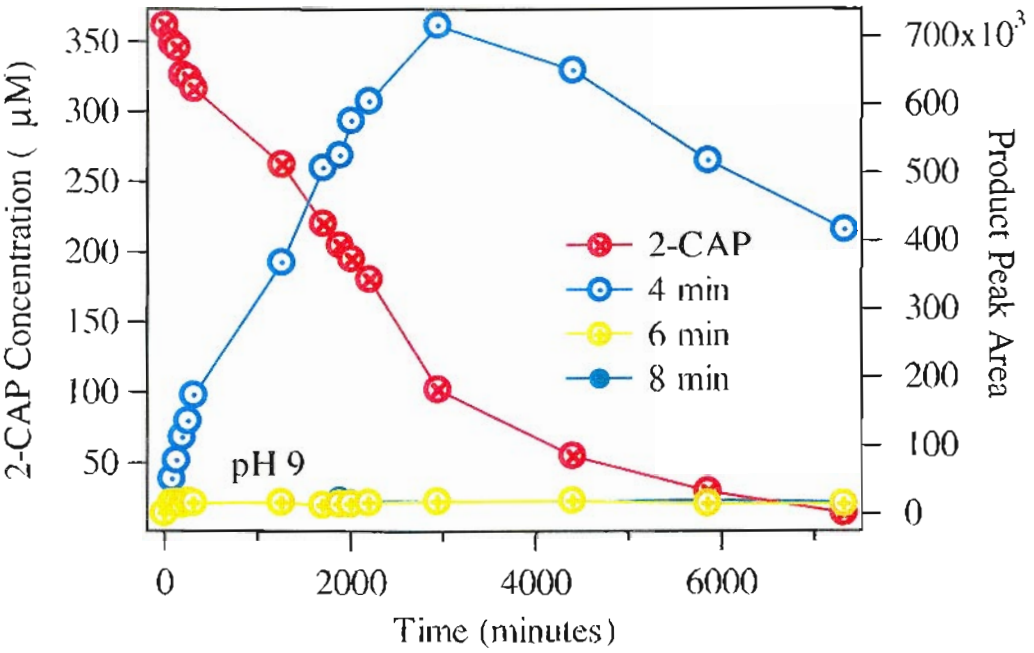

Figure 3.30 Hydrolysis of 2-CAP in buffered solution pH 9. Unknown products are represented by their retention time. (Experiment includes Figures 3.28-3.32).

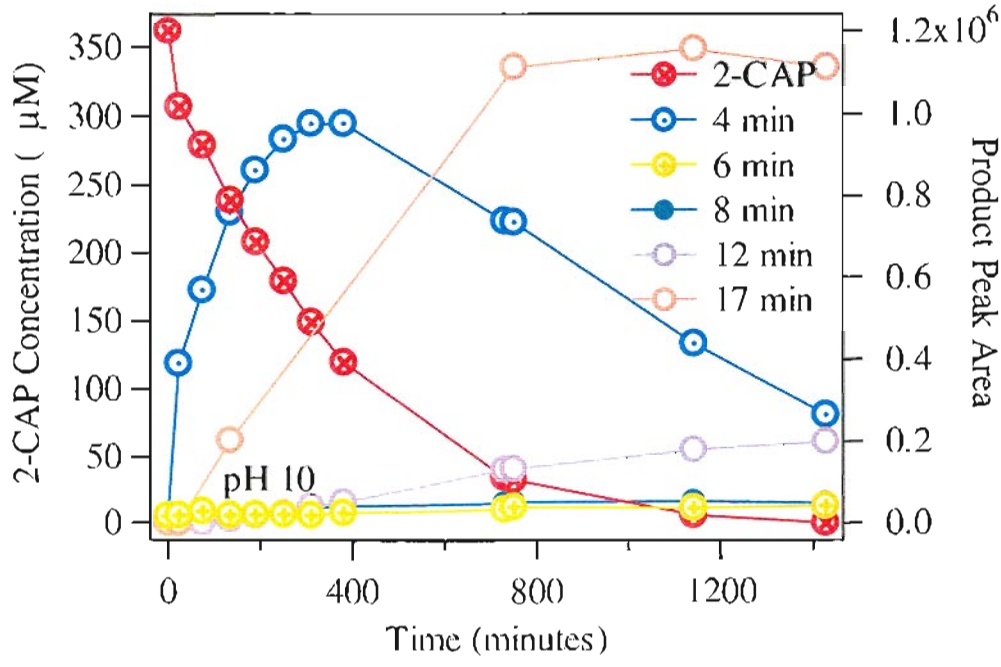

Figure 3.31 Hydrolysis of 2-CAP in buffered solution pH 10. Unknown products are represented by their retention time. (Experiment includes Figures 3.28-3.32)

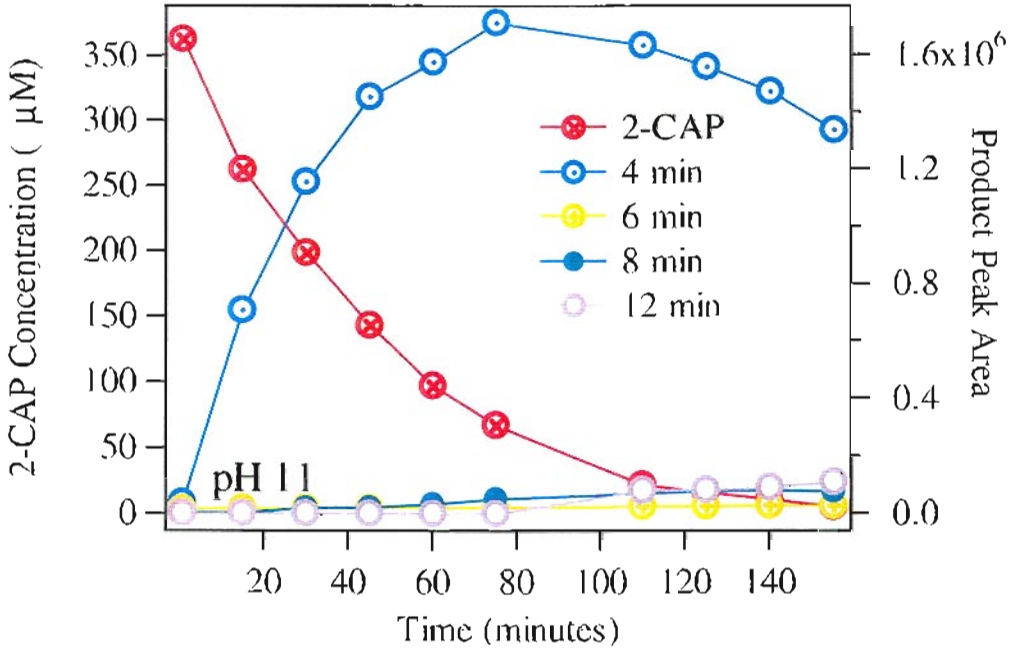

Figure 3.32 Hydrolysis of 2-CAP in buffered solution pH 11. Unknown products are represented by their retention time. (Experiment includes Figures 3.28-3.32).

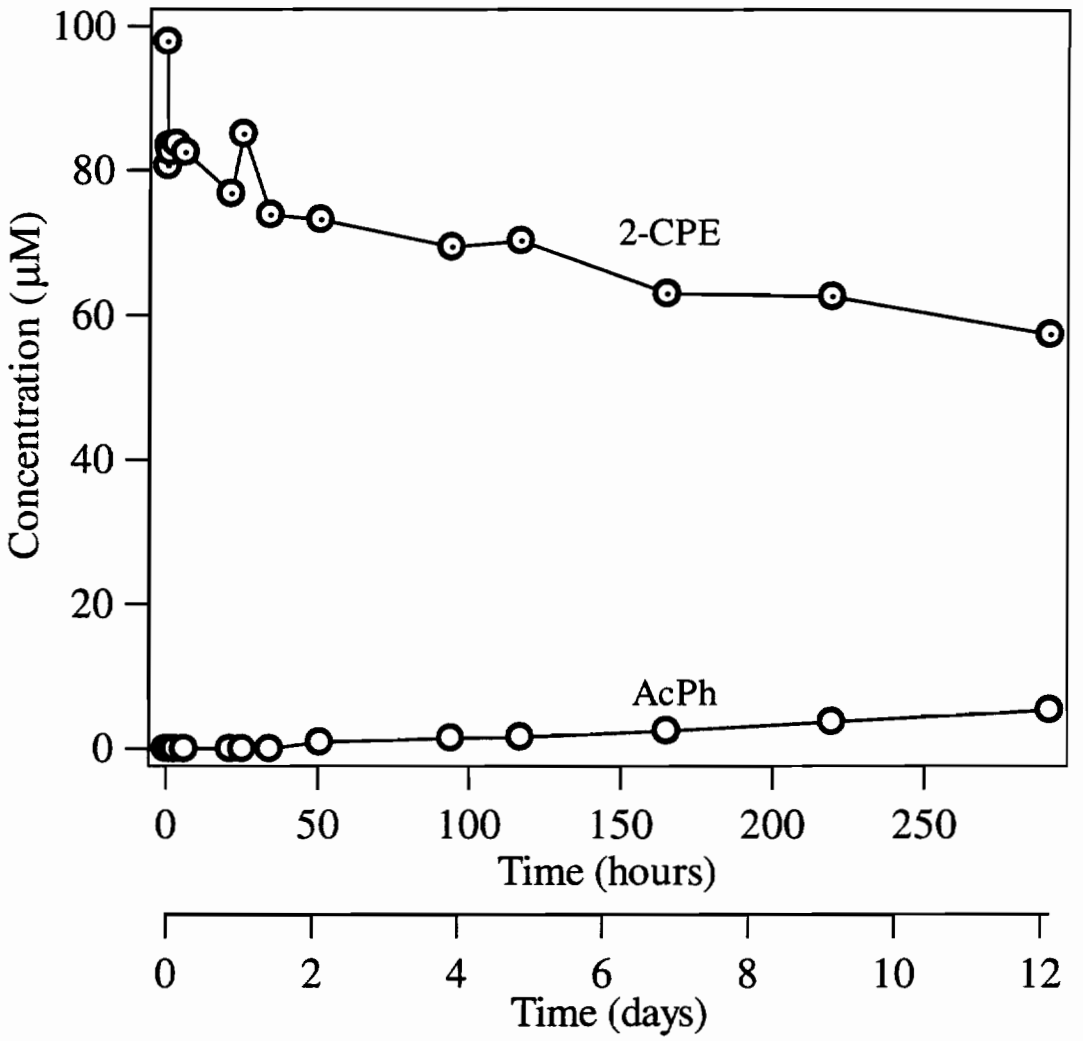

Figure 3.33 Dehydrohalogenation of (R)-(-)-2-CPE in sediment (2 December 99). The initial concentration was approximately 100 μM and the dry mass of sediment was 7.2 g.

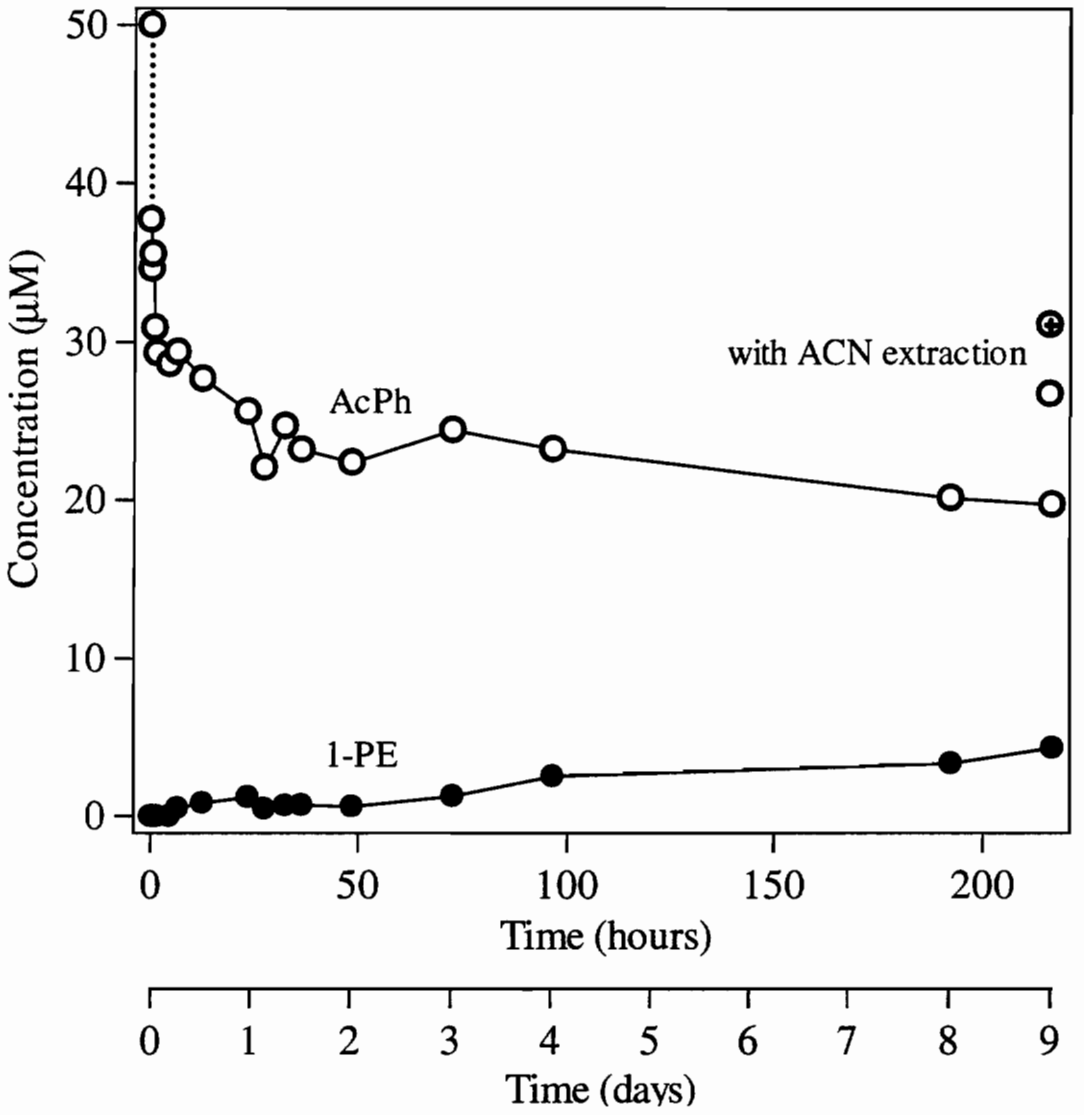

Figure 3.34 AcPh reduction to 1-PE in sediment (30 August 99). The initial AcPh concentration was approximately 50 μM and the dry mass of sediment was 14.0 g.

3.4 Treatment Effects

3.4.1 Effects of sonication and addition of NAD(P)H on 2-CAP reduction

A variety of treatments were tested to determine their influence on 2-CAP reduction in hopes of furthering the identification of reductants responsible for hydride transfer. Sonication of the sediment slurry was performed to break cell membranes in order to release potential reductants, such as NAD(P)H and dehydrogenase enzymes. However, this treatment negatively influenced 2-CAP transformation, lowering the rate of reduction and product formation (summarized in Appendix E), while increasing the initial concentration lost to sorption from approximately 20 μM in the control to 50 μM in the sonicated sample (Figures 3.35 and 3.36). The apparent increase in adsorption was likely a result of the increased sediment surface area due to sonication. The sonicated sediment sample was visually more turbid than the control.

To test for the presence of extracellular dehydrogenase enzymes, NADH and NADPH were added to sediment samples. If dehydrogenases were available and the reaction rates were limited by availability of the cofactor, it was thought that the addition of these cofactors would stimulate 2-CAP reduction. k_{obs} for 2-CAP reduction was slightly lowered in the presence of NADH and slightly raised in the presence of NADPH as summarized in Appendix E and shown in Figures 3.37 and 3.38. While NADH slowed reduction kinetics, 2-CPE production was increased in its presence from 27 μM in the control to 35 μM in the NADH sample. 2-CPE appearance kinetics and C_∞ values for NADPH and control samples were nearly identical. Mass balance was good for all three samples, falling just below 80% of the initial concentration. The largest effect of NAD(P)H addition was on the initial amount of 2-CAP adsorption. While the NADH-containing sample showed negligible sorption of 2-CAP at t_1, approximately 25% and 60% of the initial 2-CAP concentration was lost to sorption in the control and NADPH samples, respectively.

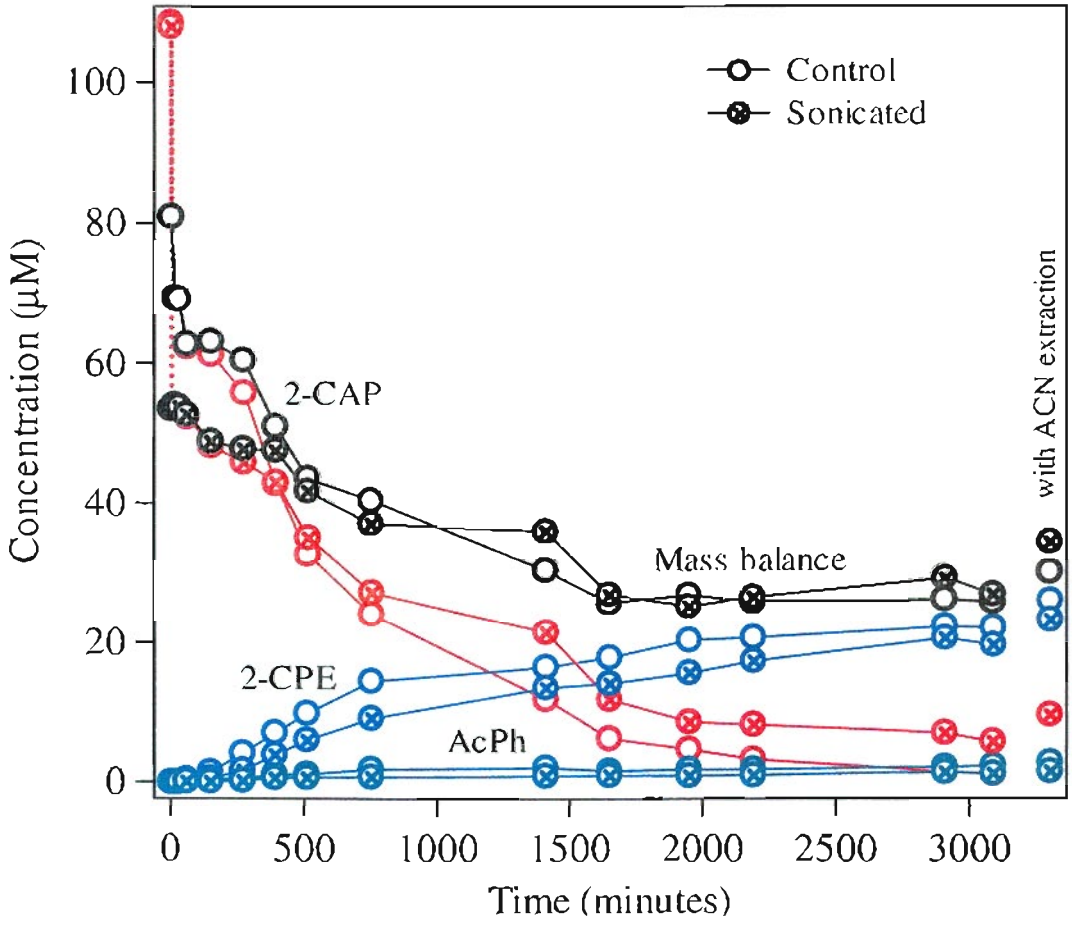

Figure 3.35 Effects of sonicating sediment on 2-CAP reduction (30 August 99). The final data points represent concentrations after acetonitrile extraction. The amount of sediment used for this experiment was 14.2 g. Experimental data is summarized in Appendix D. (Experiment includes Figures 3.35 and 3.36).

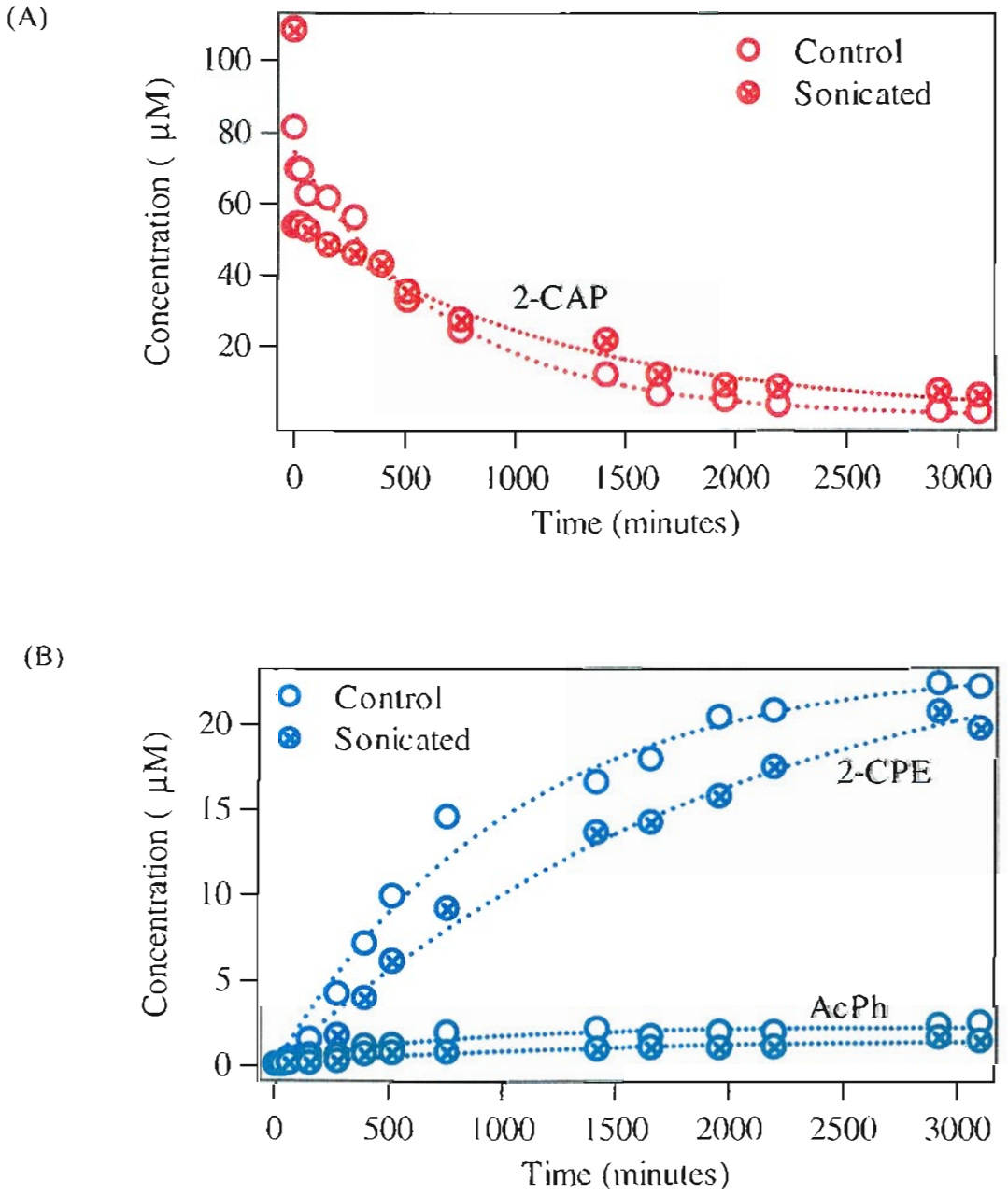

Figure 3.36 Effects of sonicating sediment on 2-CAP reduction (A) and product formation (B) kinetics. All data is fit to first-order kinetics. Fitting coefficients are summarized in Appendix E. (Experiment includes Figures 3.35 and 3.36).

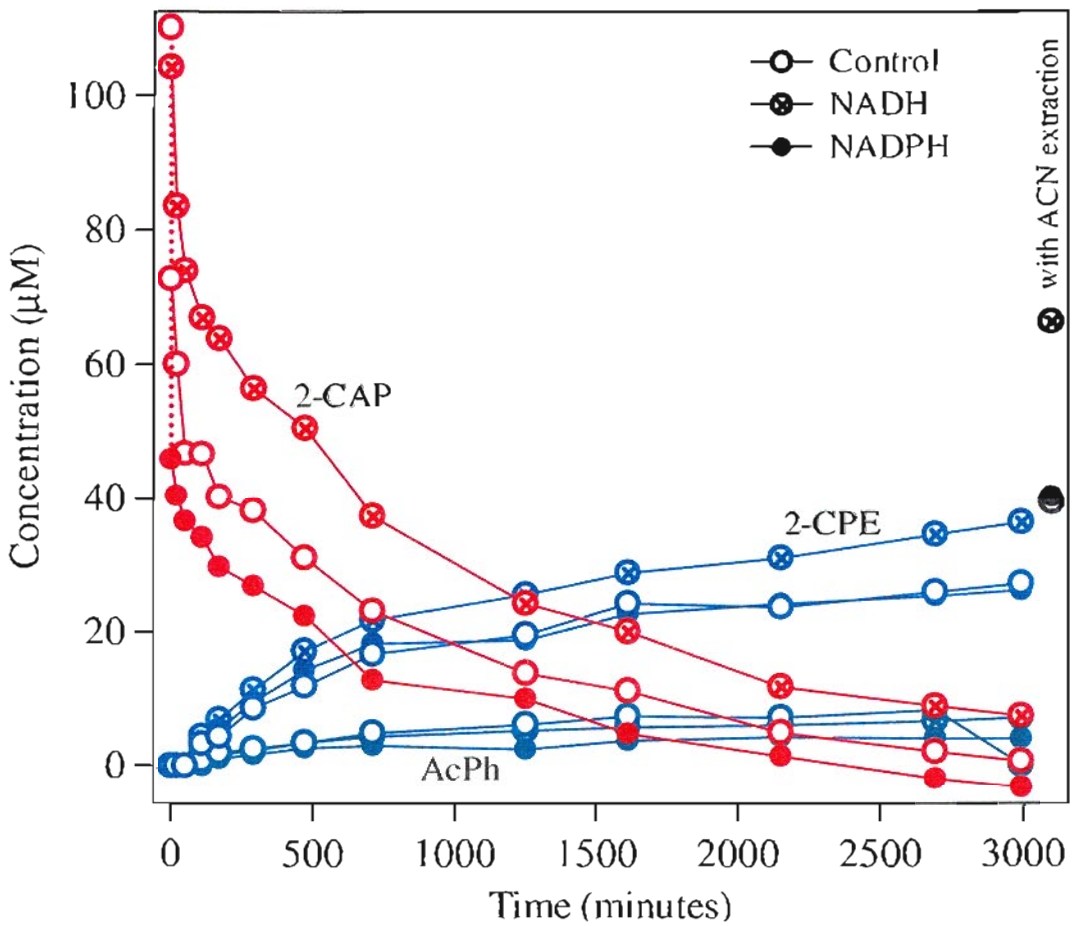

Figure 3.37 Effects of NAD(P)H on 2-CAP reduction in sediment (7 July 99). The initial NAD(P)H concentrations were 60 µM and the dry mass of sediment in each slurry was approximately 7 g. Experimental data is summarized in Appendix D. (Experiment includes Figures 3.37 and 3.38).

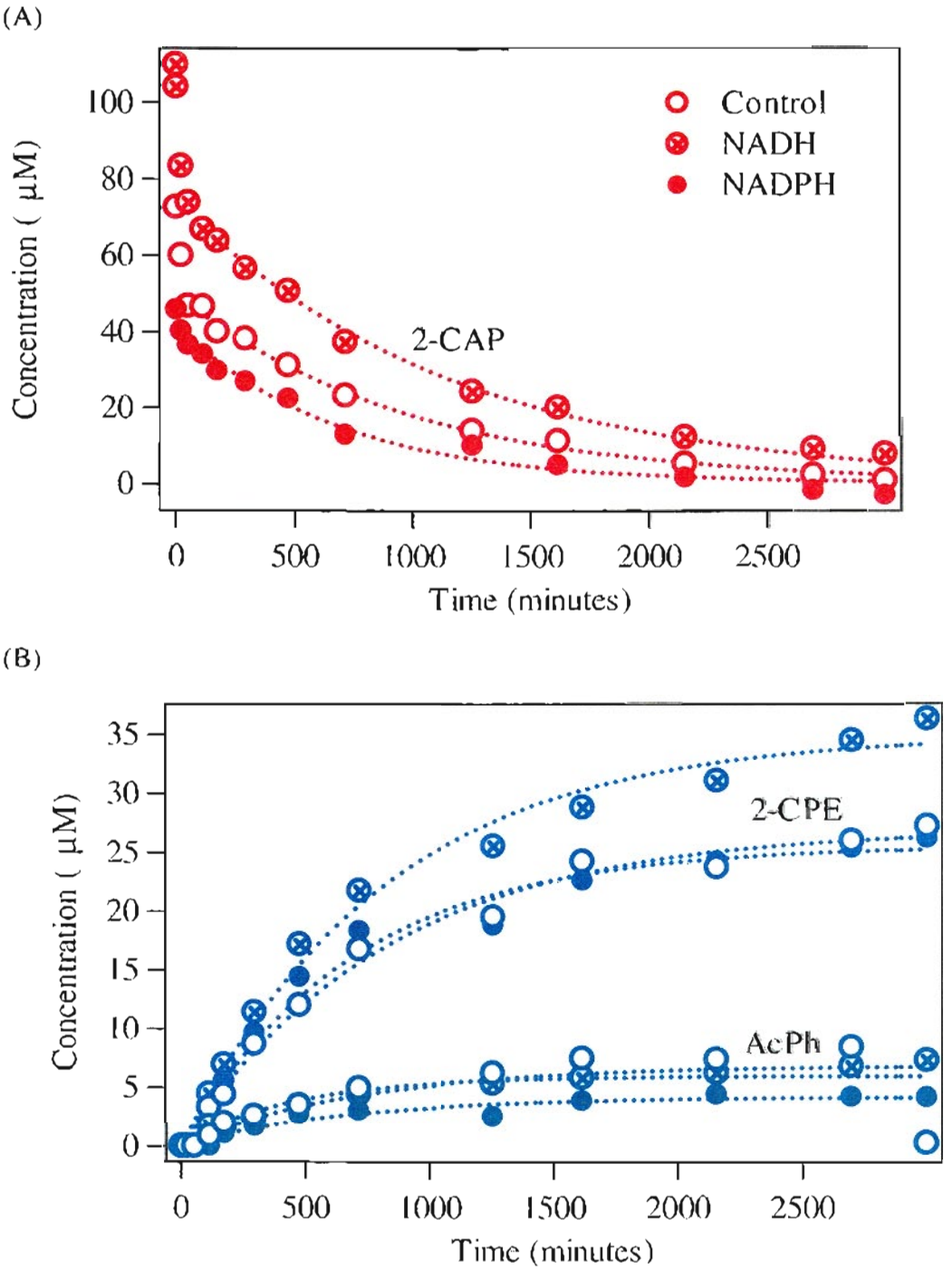

Figure 3.38 Effects of NAD(P)H on 2-CAP reduction (A) and product formation (B) kinetics. Fitting coefficients are summarized in Appendix E. (Experiment includes Figures 3.37 and 3.38).

3.4.2 Effects of addition of enzyme inhibitors on 2-CAP reduction

As a method of verifying enzyme activity in the sediments, a variety of inhibitors were added to determine their effects on 2-CAP reduction. In the presence of 100 μM p-CMB, inhibition of 2-CAP reduction via hydride transfer was apparent in the 2-CPE data, while no significant difference was observed in the disappearance of 2-CAP (Figure 3.39). While the rate of 2-CPE formation decreased in the presence of p-CMB, the largest effect of inhibition was on the amount of 2-CPE formed. With inhibition, 20 μM less 2-CPE was produced. However, mass balances varied from 98% in the control sample to 62% in the sample containing p-CMB.

While inhibition was less apparent in the presence of 1-FMP and pyrazole, effects were similar to those observed in the p-CMB experiment (Figures 3.40 and 3.41). For both 1-FMP and pyrazole experiments, the rate of 2-CAP disappearance decreased slightly with increasing inhibitor concentration (summarized in Appendix E). 2-CPE formation concentrations, as well as rates of formation, decreased with rising inhibitor concentration (summarized in Appendix E). A very poor mass balance of the compounds was observed in both of these experiments with only a few percent of the adsorbed concentration recovered with extraction. Due to interference with product peaks, 60 mM 1-FMP and 10 mM pyrazole were the maximum concentrations used for this experiment.

A second type of inhibitor treatment employed was product inhibition. To investigate this, 2-CPE was added as the inhibitor and 2-CAP reduction was measured. In the presence of 100 μM, 1 mM, and 5 mM 2-CPE, product inhibition apparently occurred (Figure 3.42), with the rate of 2-CAP reduction decreasing with increasing concentrations of 2-CPE as summarized in Appendix E and shown in Figure 3.43. Because so much 2-CPE was added at the beginning of the experiment, the formation of 2-CPE via 2-CAP reduction could not be determined using HPLC. A significant effect in AcPh formation was seen, however. AcPh production increased as the inhibitor concentrations were raised. Due to interference with 2-CPE peaks in 1 mM and 5 mM samples, AcPh concentrations could not be measured from $*t_0$ to t = 1500 min.

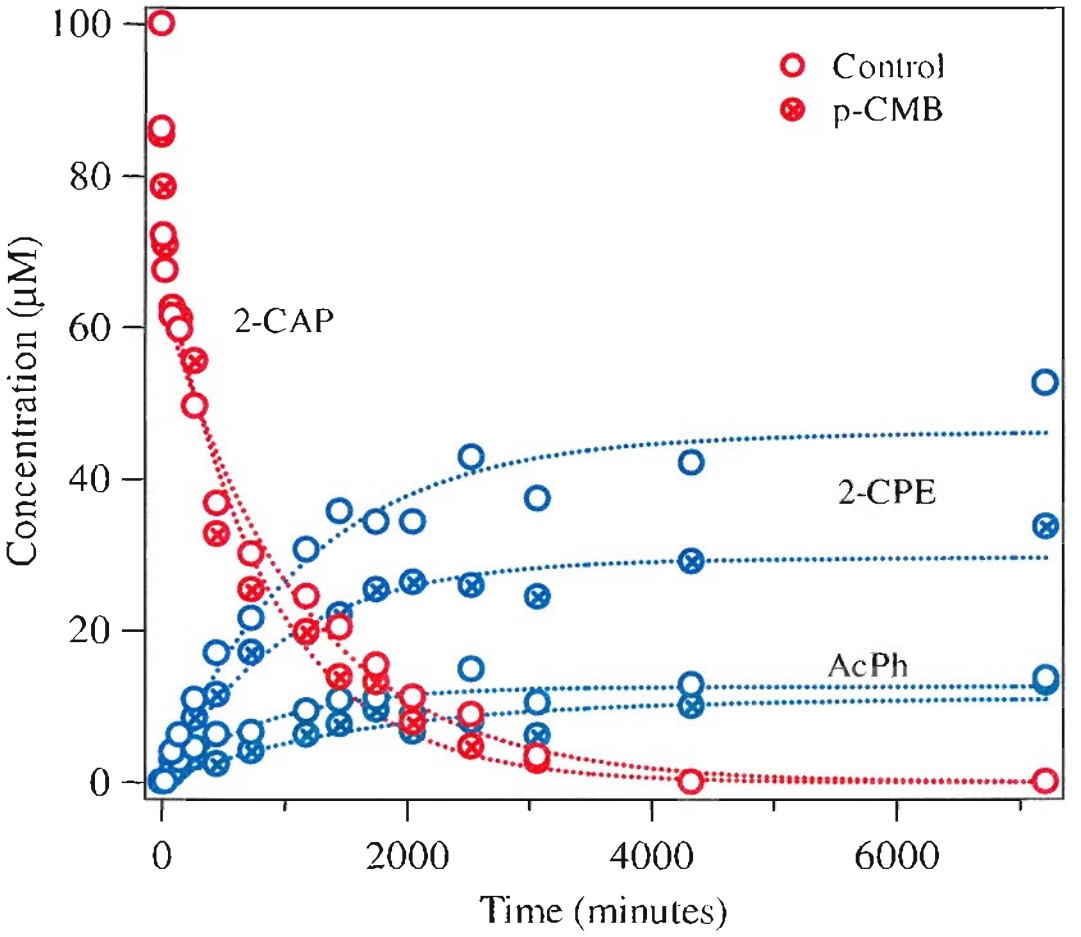

Figure 3.39 Effects of inhibition of 2-CAP reduction in sediment using p-CMB as the inhibitor compound (6 May 99). The inhibitor concentration was approximately 100 µM and the mass of dry sediment was 13 g. Experimental data and fitting coefficients are summarized in Appendices D and E.

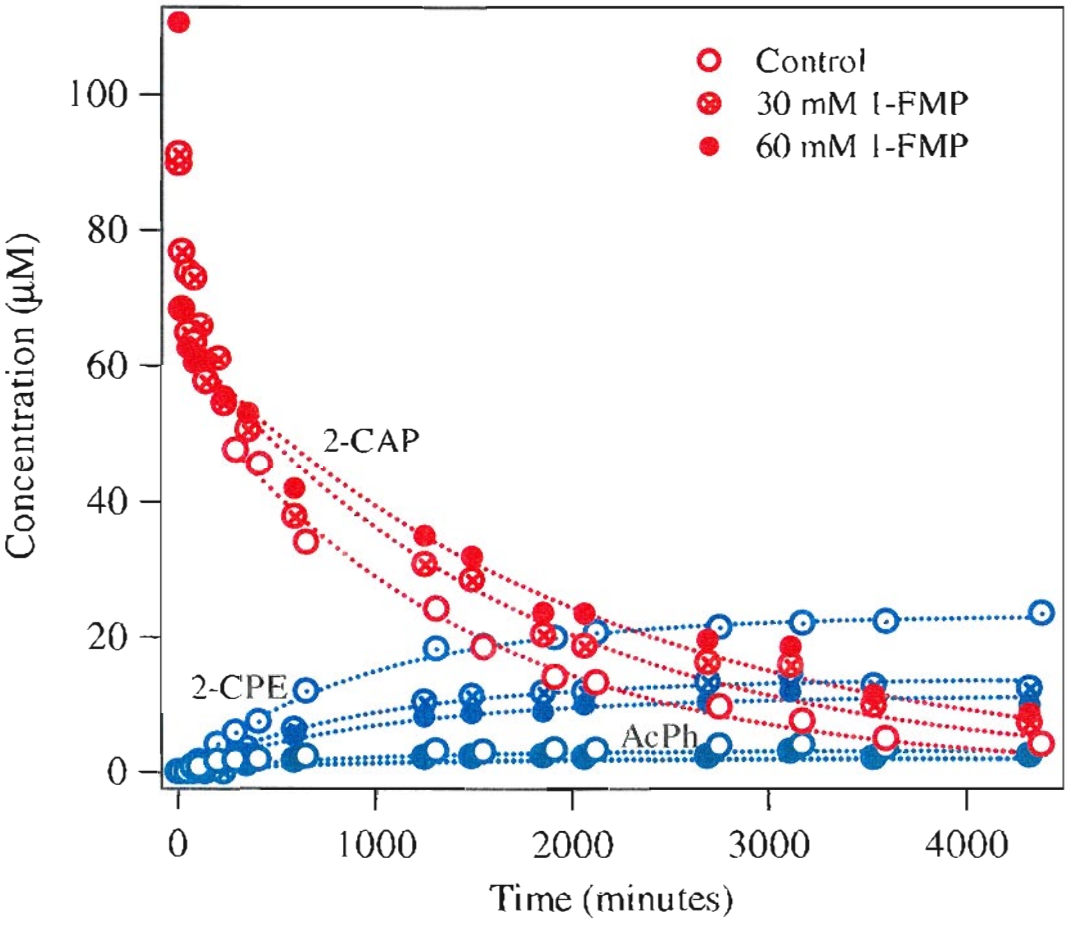

Figure 3.40 Effects of inhibition of 2-CAP reduction in sediment using 1-FMP as an inhibitor (15 September 99). 2-CAP reduction data and product formation data were fit to first-order kinetics. The dry mass of sediment was 10 g. Experimental data and fitting coefficients are summarized in Appendices D and E.

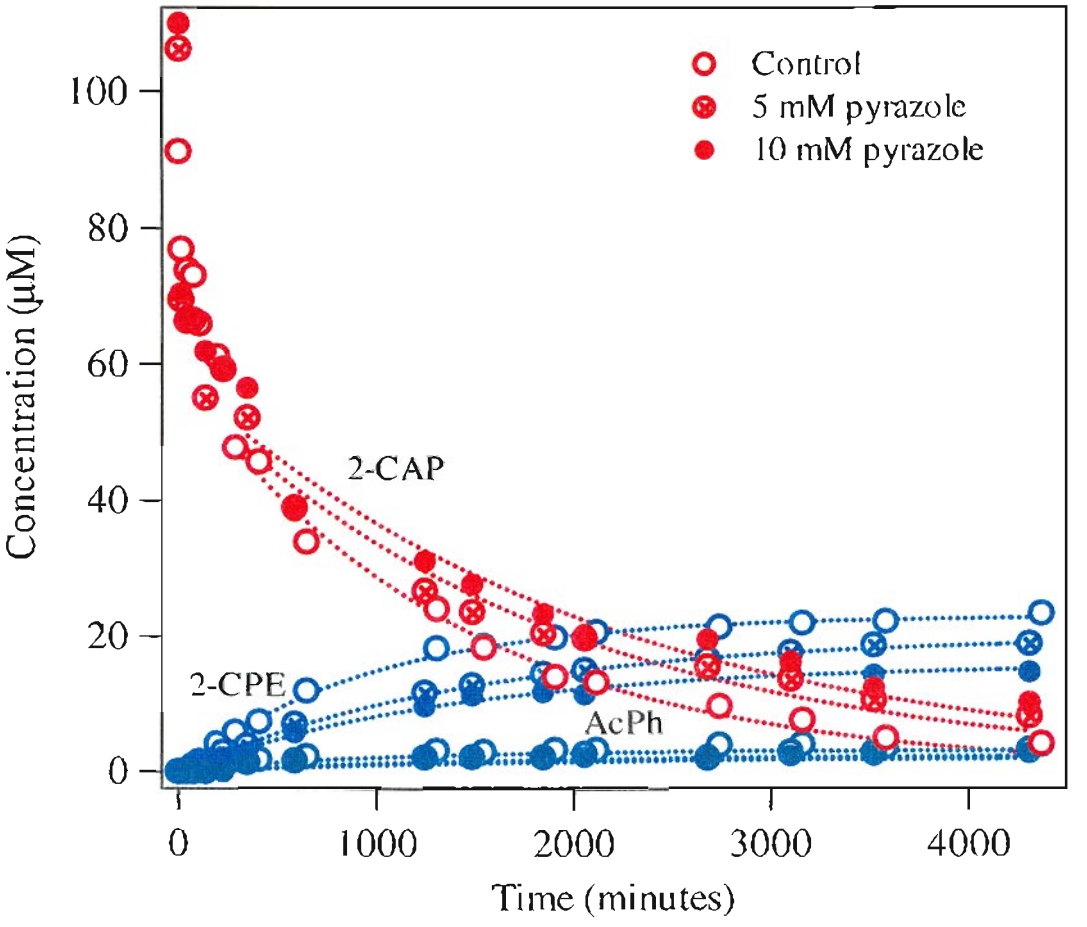

Figure 3.41 Effects of inhibition of 2-CAP reduction in sediment using pyrazole as an inhibitor (15 September 99). 2-CAP reduction data and product formation data were fit to first-order kinetics. The dry mass of sediment was 10 g. Experimental data and fitting coefficients are summarized in Appendices D and E.

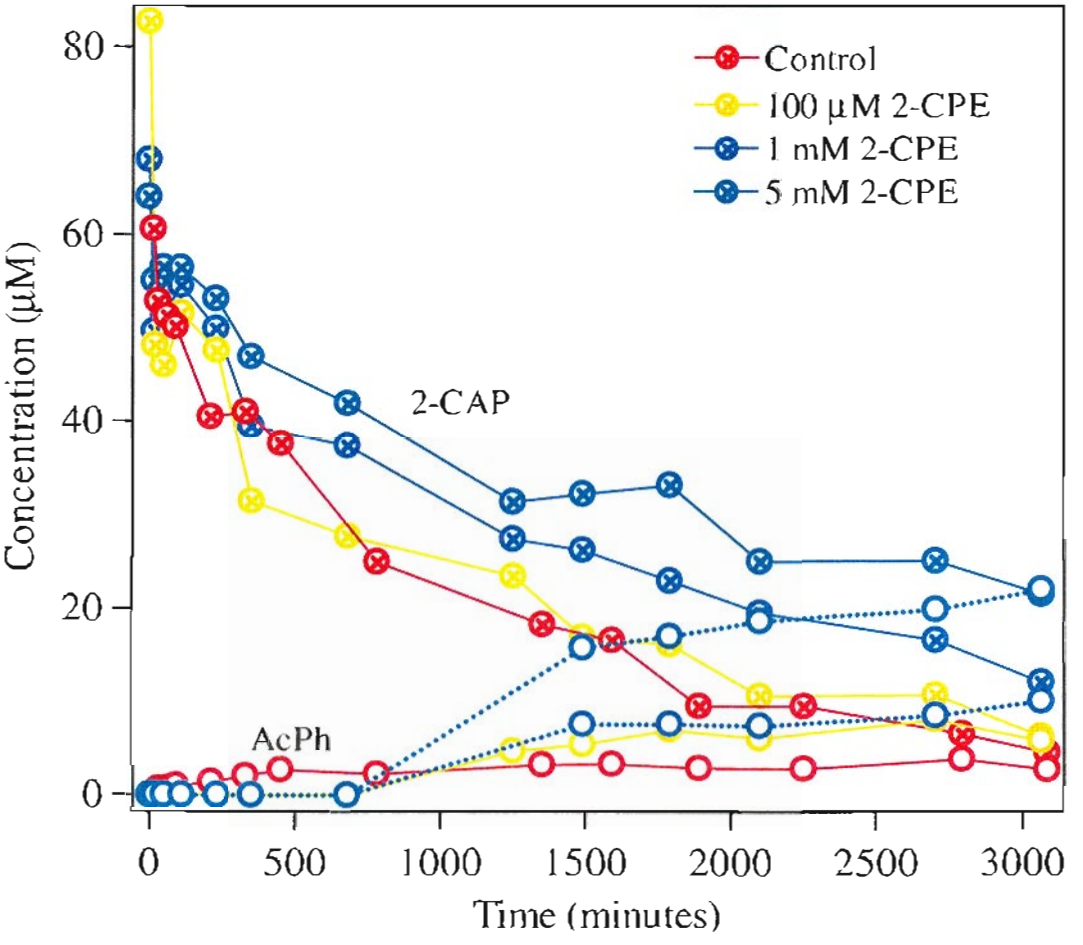

Figure 3.42 Effects of product inhibition (2-CPE) on 2-CAP reduction in sediment. The control experiment was run on 12 July 99 with 10.4 g of sediment; the inhibition experiments were run on 20 July 99 with 15.2 g sediment. Due to peak interference with 2-CAP, AcPh concentrations could not be measured before 1500 min for the 1 mM and 5 mM samples. Experimental data is summarized in Appendix D. (Experiment includes Figures 3.42 and 3.43).

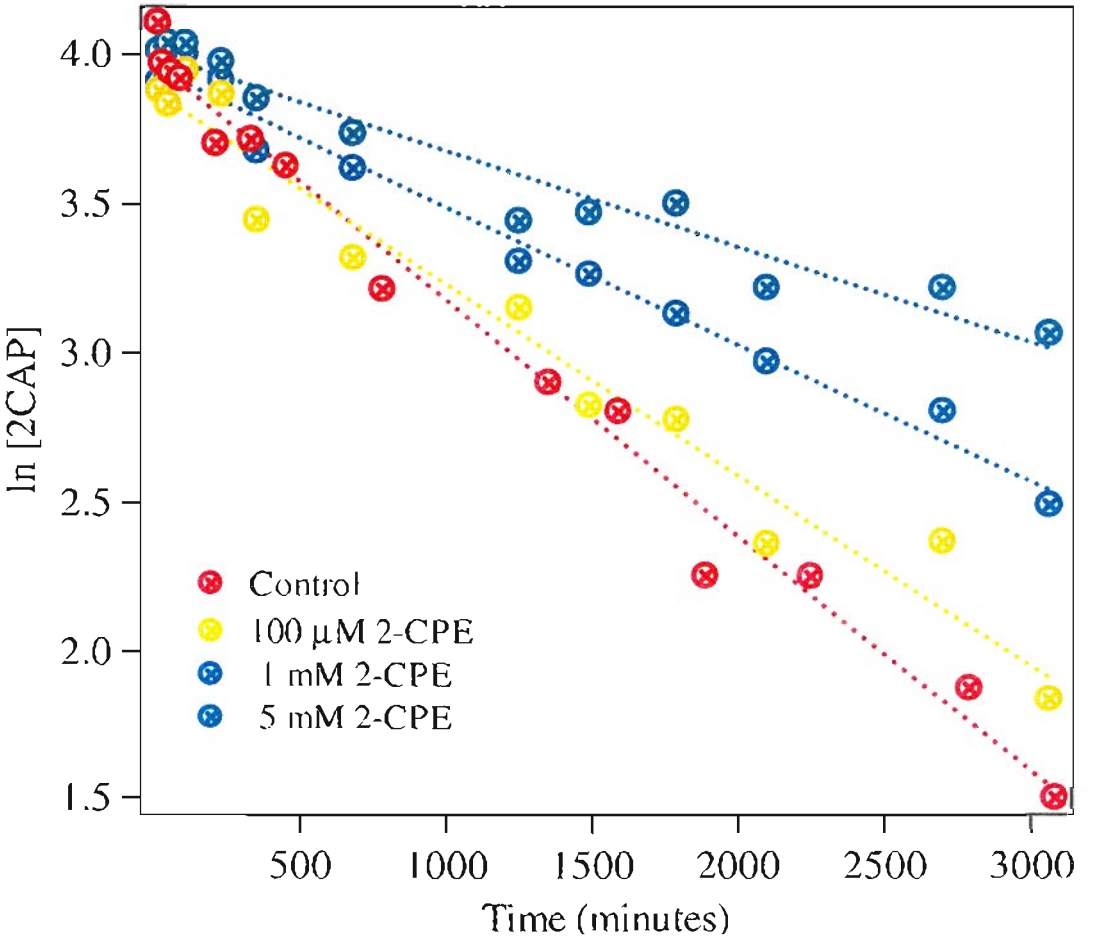

Figure 3.43 Effects of product inhibition on 2-CAP reduction kinetics in sediment. Fitting coefficients summarized in Appendix E are based on non-linear first-order kinetic fits to the data (not represented in this figure). (Experiment includes Figures 3.42 and 3.43).

3.5 Other Probe Compounds

3.5.1 2-Bromoacetophenone (2-BAP)

Preliminary experiments were done with alternative probe compounds that have structures similar to 2-CAP. 2-BAP was reduced in sediment to form AcPh, 2-bromo-1-phenylethanol (2-BPE), and an unknown compound (with retention time = 10.9 min) that could not be identified using HPLC (Figure 3.44). The electron transfer reaction, forming AcPh, appeared to be the favored reduction mechanism. However, product formation was minimal, amounting to less than 10% of the initial 2-BAP concentration. Even after extraction with acetonitrile, mass balance in this experiment was poor, recovering just over 10% of the initial 2-BAP concentration. However, concentrations could not be quantified for either 2-BPE or the unknown product.

3.5.2 2,2-Dichloroacetophenone (2,2-CAP)

2,2-CAP was reduced via hydride transfer, forming 2,2-dichloro-1-phenylethanol (2,2-CPE) and via electron transfer, forming 2-CAP (Figure 3.45). 2-CAP was, in turn, reduced to 2-CPE and AcPh. Hydride transfer was clearly the favored reduction pathway for 2,2-CAP, accounting for the formation of 40 μM 2,2-CPE. In contrast, electron transfer was the dominant mechanism for reducing 2-CAP, producing more AcPh than 2-CPE. Without a standard for comparison, 2,2-CPE could not be quantified.

3.5.3 2,2′,4′-Trichloroacetophenone (2,2′,4′-CAP)

While 2,2′,4′-CAP was readily transformed in the sediment system, the reaction products, unfortunately, could neither be identified nor quantified using HPLC. Three products were observed with retention times of 18.2 min, 22.5 min, and 24.6 min (Figure 3.46).

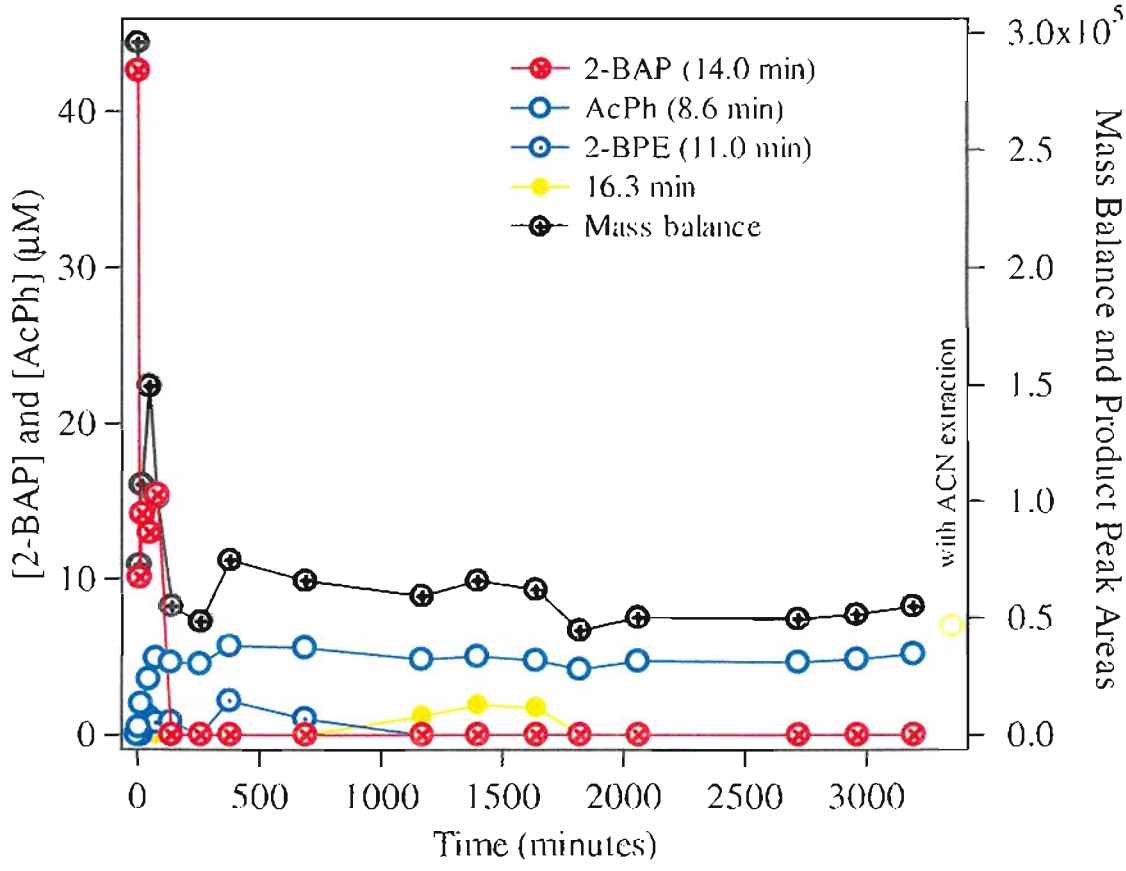

Figure 3.44 Reduction of 2-bromoacetophenone (2-BAP) in sediment (9 August 99). One product could not be identified using HPLC and neither it nor 2-BPE could be quantified because standards were not available for comparison. The initial 2-BAP concentration was approximately 100 μM and the dry mass of sediment was 12 g. The final ACN extraction point represents the amount of AcPh recovered from the sediment. (Experiment includes Figures 3.44-3.46).

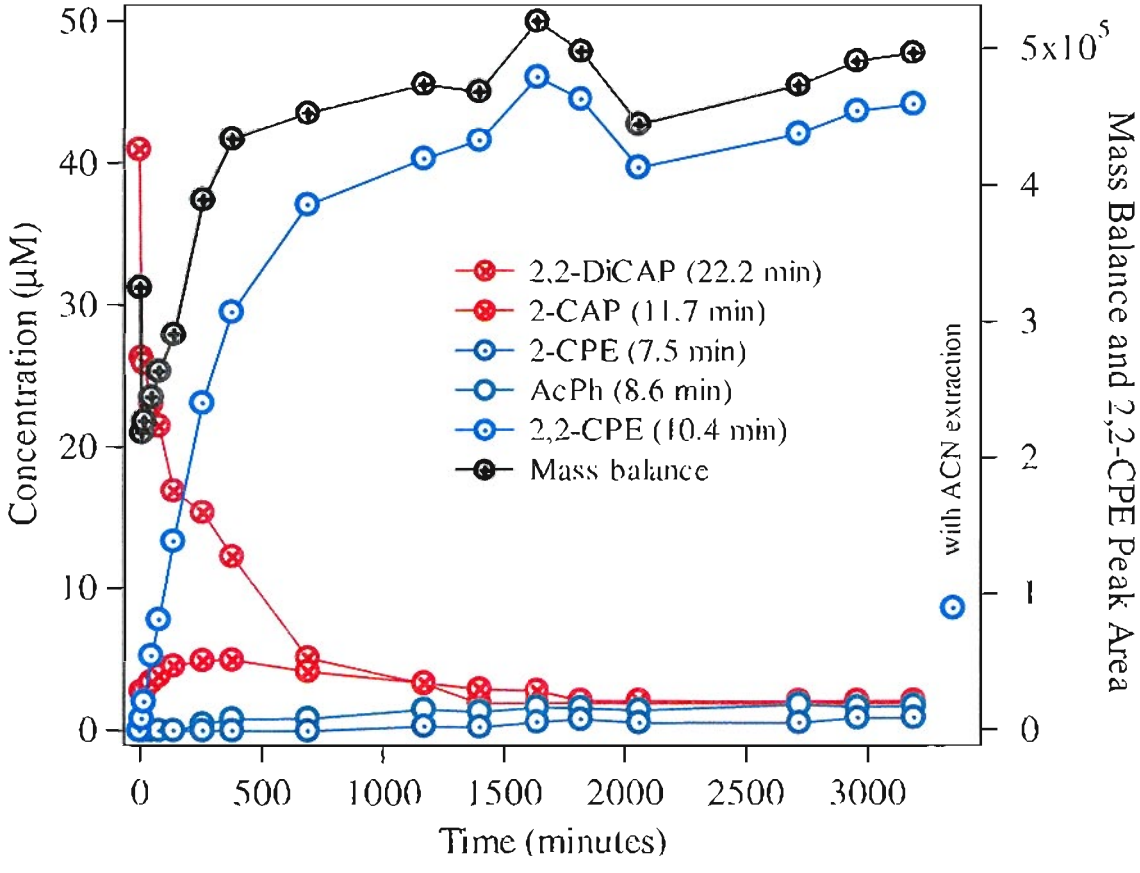

Figure 3.45 Reduction of 2,2-chloractophenone (2,2-CAP) in sediment (9 August 99). While all products were identified, a standard was not available for quantifying 2,2-CPE concentrations. The initial 2,2-CAP concentration was approximately 100 µM and the dry mass of sediment was 12 g. (Experiment includes Figures 3.44-3.46).

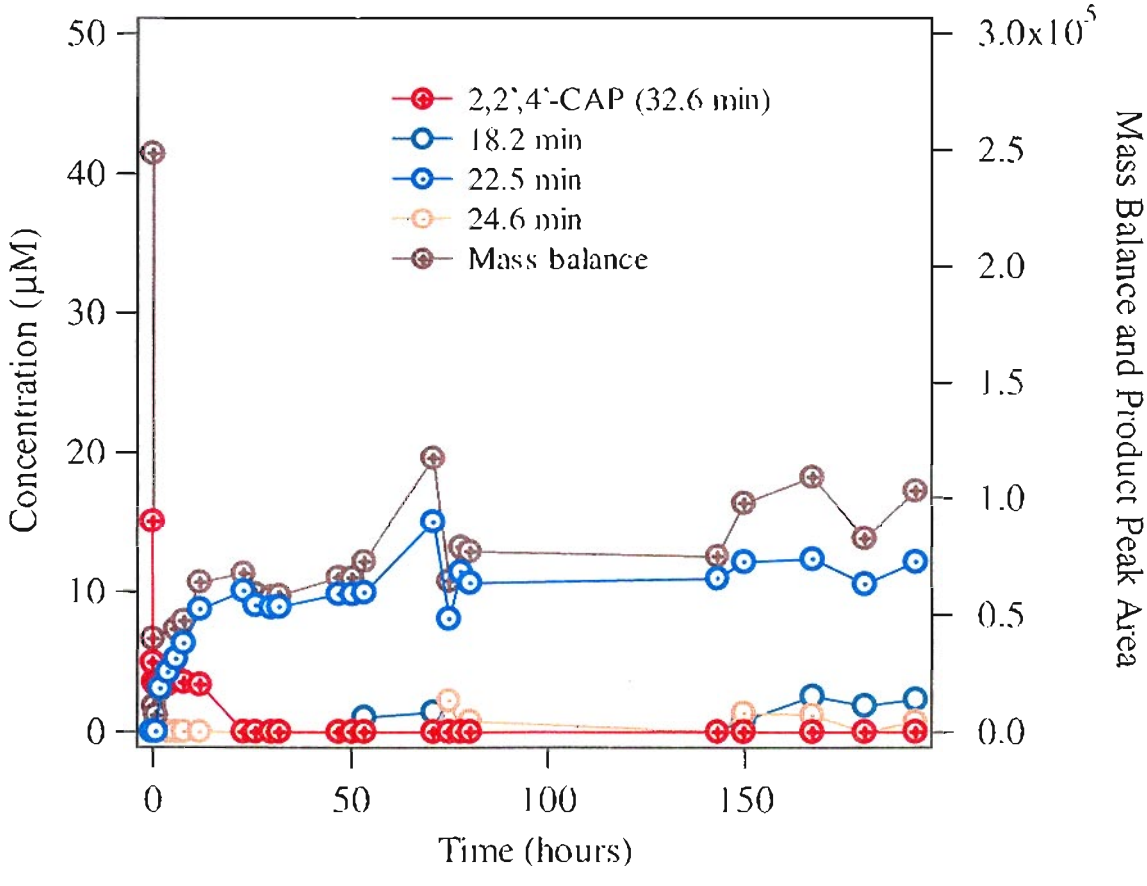

Figure 3.46 Reduction of 2,2′,4′-trichloroacetophenone (2,2′,4′-CAP) in sediment (9 August 99). None of the reduction products could be identified using HPLC or quantified without known standards. The initial 2,2′,4′-CAP concentration was approximately 100 µM and the dry mass of sediment was 12 g. (Experiment includes Figures 3.44-3.46).

CHAPTER 4
DISCUSSION

4.1 Primary Processes

4.1.1 2-CAP reduction and adsorption processes

Two primary processes—reduction and adsorption—were the focus of this study of 2-CAP fate in sediment systems. With proper HPLC and extraction methods, it was possible to quantify the kinetics of reduction by hydride and electron transfer as well as adsorption of 2-CAP and its reduction products to sediments.

2-CAP reduction in sediment from both Rock Creek and the Columbia River estuary consistently produced more 2-CPE than AcPh, suggesting that the hydride transfer mechanism is the dominant route of 2-CAP reduction in these systems. However, since the adsorbed 2-CAP and products were not fully recovered or quantified in all experiments, it is not possible to be completely sure whether hydride or electron transfer was predominant.

In order to determine the degree to which each reduction product could be adsorbed in any given 2-CAP experiment, adsorption of both 2-CPE and AcPh was studied in dried and reconstituted sediment slurries (dried to create non-reducing conditions). Unfortunately, the results of these experiments did not show a significant difference between the two compounds. In fact, all three compounds (2-CAP included) showed similar degrees of adsorption, although, results did indicate that the amount of adsorption could be quite variable from one experiment to the next (as seen in Figures 3.3 and 3.4). The cause for variation in adsorption effects between experiments is unknown, but may be a result of the amount of fine-grain organic or mineral matter in the slurry, which could have varied from one sample to the next.

4.1.2 Reduction and adsorption kinetics

From the kinetics of 2-CAP reduction, the significance of adsorption effects on 2-CAP loss became apparent. Using dried and reconstituted sediment for measuring adsorption of 2-CAP proved very useful in understanding the trends observed in initial 2-CAP reduction data in active sediments. The strong similarity between the initial data of the 2-CAP adsorption curve (from experiments with dried and reconstituted sediment) and the reduction curve (from experiments with active sediment) is a good indication that sorption was the dominant process during the first 30–80 min of all experiments and was responsible for the observed 2-CAP disappearance during this time period (Figure 3.5). Separating 2-CAP disappearance curves into two steps: (i) fast adsorption (<80 min) and (ii) relatively slow reduction (>80 min), makes it possible to remove data dominated by adsorption effects and fit reduction data to first-order kinetics (Figure 3.6). There were very few experiments in which adsorption did not appear to be significant and, thus, this method of fitting first-order kinetics to reduction data only was used routinely throughout this study.

After separating 2-CAP adsorption and reaction data in each experiment, it was also possible to determine the rate of sorption by fitting the initial data to a first-order kinetic model. During this analysis, it was discovered that it was not possible to fit all sorption data to one first-order decay, beginning with the nominal starting concentration ($*t_0$) of each experiment. However, the data could be divided into two sorption steps, which fit first-order kinetics separately (Figure 3.6). It is not clear what is responsible for this bimodal adsorption kinetics, however, it is likely a result of differences in sediment structure and composition (i.e., faster sorption to dissolved or colloidal organic matter in the first step versus slower sorption to sediment aggregates in the second step). Although both adsorption steps were generally observed in reduction experiments, adsorption kinetics could not be determined in most experiments due to inadequate numbers of data points.

4.1.3 Reduction of compounds sorbed to sediment

The similarity in initial 2-CAP disappearance trends between adsorption and reduction experiments indicated that the initial 2-CAP disappearance in active sediment was likely due to adsorption. However, it was the successful extraction of sorbed compounds (for approximately 100% mass balance) at each time point in reduction experiments that provided proof that adsorption is, indeed, responsible for the initial decrease in 2-CAP concentrations (Figure 3.12). These experiments consistently produced a trend in which the observed amount of sorbed 2-CAP decreased with time, while the amount of sorbed 2-CPE increased with time, causing the total sorbed concentration to remain roughly constant. This phenomenon is particularly interesting when compared to the results of adsorption experiments (Figure 3.2). The plateau in the solution concentration data that was routinely observed in adsorption experiments suggested that adsorbed concentrations ceased to change following the initial sorption steps. Whether this was due to an equilibrium reached between solution and sorbed concentrations or saturation of adsorption sites is unclear, since, both effects would presumably give a similar plateau effect. Nonetheless, this trend is consistent with that observed in the extraction experiment, in which the total adsorbed concentration remained constant.

Perhaps the most important unresolved question with respect to these data is whether 2-CAP reduction occurred at the sorption sites to produce 2-CPE, which then remained adsorbed, or whether 2-CAP was desorbed and reduced in solution to produce 2-CPE, which, subsequently, was adsorbed to the sediment. Either scenario is conceivable and it is difficult to say which would be more likely. An argument could be made for desorption over reaction at sorption sites because transformation of sorbed compounds, in general, is less favorable than reaction in solution [13-15]. Also, in support of an equilibrium of sorbed and soluble compounds, desorption would be favored in this system as 2-CAP concentration in solution drops.

4.1.4 Extraction of 2-CAP, 2-CPE, and AcPh from sediment

In general, it appears that adsorption, and not transformation, is responsible for the lack of mass balance in some experiments. Unfortunately, the extraction technique applied in the majority of experiments (extraction with acetonitrile at the completion of experiment) did not always account for the decrease in total 2-CAP concentration. While the same acetonitrile extraction method was used throughout this study, its effectiveness varied greatly from one experiment to the next, ranging from 1-100% recovery of adsorbed concentrations. Without measuring other properties of the sediment (e.g., % organic matter of each sample collected), it is difficult to explain such variability in the efficiency of the extraction method. In the method comparison experiment, soxhlet extraction appeared to be much more effective than the acetonitrile method (Figure 3.13). However, soxhlet extraction is very time consuming and may not give unbiased results unless all samples for one experiment could be extracted simultaneously (in order to quench reduction at the same time in each sample). This would require the set-up of at least 3–5 soxhlet systems. SFE was the least effective of the extraction methods that were tried, however, solvents other than CO_2 might have given better results.

4.2 Environmental Effects

In order to understand variations in 2-CAP reduction kinetics from one experiment to another, a number of environmental effects were considered, including seasonal variations, sampling location, amount of sediment, and storage time (Figures 3.14–3.27). Although the results were difficult to interpret due to a limited characterization of the system, the four environmental variables studied effected 2-CAP reduction kinetics to varying degrees. While some environmental effects appeared more significant than others, none could solely account for the variation in reduction kinetics observed between experiments. It is possible that environmental variables that were not measured, such as percent organic carbon or microbial activity, had even stronger effects

on 2-CAP reduction. However, it is also possible that there was no dominant environmental variable and that the variability observed in 2-CAP reduction from one experiment to another was the combined effect of multiple variables.

4.2.1 Seasonal survey of 2-CAP reduction

The tributary of Rock Creek undergoes a variety of changes throughout the season, especially with regard to flow. While the creek flows rapidly during the winter months, the creek becomes stagnant during the dry summer months. With associated changes in biogeochemistry, one might expect to observe seasonal trends in 2-CAP disappearance. In this study, however, no temporal trend in either k_{obs} or k_{rho} for 2-CAP reduction was observed (Figure 3.15). A number of factors may have been responsible for this lack of seasonal relationship. First, the time period studied (February through September) may have been too short to adequately reveal "seasonal" trends. Second, the sampling period may have been of adequate length, but frequency of sampling may not have been. Third, variability due to sampling at different depths or within different microbial communities may have caused "seasonal" trends to be obscured. Because there are numerous environmental factors that may affect 2-CAP reduction, it conceivable that no "seasonal" trend, per se, would exist, but rather, a complex variation in 2-CAP reduction over a season as a result of changing environmental conditions.

4.2.2 Sampling location and storage time

Because microbial communities and populations as well as sediment composition were expected to vary in different locations of the creek, the effects of sampling location on both 2-CAP and resazurin reduction were studied. As expected, reduction kinetics did vary in sediments collected from different locations (Figures 3.16 and 3.17). Unfortunately, in this particular experiment, this effect was not observed with the addition of 2-CAP because not enough sediment was used (to give measurable reduction). Effects were observed, however, in resazurin reduction experiments, in which k_{rho} values varied greatly from one sample to another.

The second part of the location variable was to compare freshwater sediment to coastal sediment. Smolen et al. [17] reported that AcPh was the dominant product of 2-CAP reduction in coastal sediments, while 2-CPE was the major product in freshwater systems. Two coastal sediments were collected from two locations within the Columbia River estuary for this study. Neither sediment showed the phenomenon previously reported, but instead, provided 2-CAP reduction data that was very similar to that obtained in Rock Creek sediment (Figures 3.18 and 3.19). It appeared in this experiment that, at least during the time of collection, the coastal sediments shared similar reducing properties with Rock Creek sediment. However, because estuarine sediments may change greatly during tidal cycles (with respect to microbial populations, salinity, etc.), it is likely that a variety of results would have been obtained if a more detailed study of the coastal site had been made.

Both coastal sediments used in this study and the sample used by Smolen et al. came from the Columbia River estuary. Due to seasonal changes and location variability, the difference in 2-CAP reduction observed was initially attributed to differences in sediment properties. However, studies on the effects of sediment storage time indicate that shipping the sediment from Oregon to Georgia (to Smolen) may also have had a influence on her results. In this study, the storage time variable was studied to determine whether or not it was possible to use a sediment sample for more than one experiment over the course of many days or weeks and expect the reducing properties to remain constant throughout that time period. The storage time did appear to significantly affect both 2-CAP reduction and product formation, indicating that the redox properties of the sediment changed during storage (Figures 3.20 and 3.21). Because there is no practical way to prevent or correct for changes in sediment redox properties, storage for any amount of time is not recommended.

4.2.3 Amount of sediment

Of the four environmental effects tested, storage time had shown the greatest influence on 2-CAP reduction. However, since the samples used in most of this study were collected fresh for each experiment, storage time could not explain the differences in 2-CAP reduction and product formation observed in this project. While the amount of sediment in the slurry was also known to greatly influence 2-CAP reduction, the relationship between 2-CAP reduction kinetics and ρ (dry mass of sediment per mass of water) had not been determined. Therefore, an experiment was devised in which 2-CAP reduction was studied with varying amounts of sediment. As expected, both 2-CAP reduction and product formation increased with increasing ρ (Figures 3.22-3.25). This result was likely due to the increased reductive capacity (or amount of reductants) that result from increased surface area or microbial populations that are inherent to greater sediment quantities. While the effects of the amount of sediment on 2-CAP reduction were easily observed in this one experiment, on a "seasonal" scale, the amount of sediment alone could not entirely account for changes in the rate of 2-CAP reduction, as seen in Figure 3.27. This indicates that it was not necessarily the mass of sediment that was used, but more precisely, the amount of sediment-associated reductants that determined 2-CAP reduction kinetics. The likelihood that sediment characteristics (i.e., % organic matter, microbial populations, etc.) were not the same for each experiment in this study is great, even though the sediment was collected from the same location each time. The variability in the sediment composition would be quite difficult to determine without routinely running a large suite of sediment characterization experiments. Note that the 2-CAP experiment was a sediment characterization itself and, perhaps, once we understand how various environmental factors affect 2-CAP reduction, we will be able to use the reactivity of 2-CAP to explain other environmental phenomenon or experimental results.

4.3 Secondary Processes

4.3.1 Reduction by sulfide species

A number of possible secondary reactions were studied to determine their potential for occurring in anaerobic sediments. The first process, reduction of 2-CAP by sulfide species, was expected primarily in coastal sediments and was studied as a follow-up to the results of Smolen et al. [17], where they obtained poor mass balance in sediment collected from the Columbia River estuary. It was initially proposed that the formation of undetected transformation products, possibly due to reduction by sulfide species, was responsible for the unexplained loss in 2-CAP concentration. Therefore, in this study, a buffered system (pH 8) of sodium sulfide and 2-CAP was set up to determine the feasibility of this reaction (Figure 1.4). In this model system, 2-CAP was transformed rapidly, but we had trouble identifying any of the products. A product peak was observed, but it had a very similar retention time to 2-CAP, suggesting that if this reaction had occurred in the sediment used by Smolen, the product would likely have been observed. This result does not imply that reaction with sulfide does not occur in coastal sediments, but that it has not been detected using our methods.

4.3.2 Hydrolysis

Another secondary process of concern was hydrolysis. Due to likelihood for hydrolysis of 2-CAP (Figure 1.3), a model system was set up in buffered solutions to study effects of pH 3 through 11. The results of this study showed that hydrolysis of 2-CAP occurs in systems with a pH of 8 or higher (Figures 3.28-32) and, therefore, would likely have more impact on marine systems where pH values approaching 8 are common. However, because the reaction observed in the control study was quite slow at both pH 8 and 9, it is possible that 2-CAP reduction experiments in sediment could be conducted within an adequate time period so that the effects of hydrolysis on 2-CAP disappearance would be negligible in comparison to 2-CAP reduction via electron and hydride transfer.

4.3.3 AcPh and 2-CPE transformations

Two final secondary processes considered in this study involved transformation of AcPh and 2-CPE in anaerobic sediments. In order to accurately interpret product formation kinetics from 2-CAP reduction, it was necessary to determine whether further transformation of these products occurred subsequent to their formation. To address this issue, AcPh and 2-CPE were added to sediment slurries to observe their reaction over time. While the most significant decrease in concentration was due to adsorption, a small amount of transformation was also observed for both compounds (Figures 3.33 and 3.34). As expected, AcPh underwent reduction via hydride transfer to form 1-PE. Because this compound can be observed and quantified using HPLC, its production should not pose any significant problems in measuring AcPh formation upon 2-CAP reduction, although, it will complicate the interpretation of AcPh formation kinetics. However, reduction of AcPh by hydride transfer appears to be much slower than 2-CAP reduction by this same mechanism. Therefore, we would expect production of 1-PE upon AcPh reduction to be negligible in comparison to both AcPh and 2-CPE formation from 2-CAP. In this study, 1-PE formation was rarely observed and, therefore, did not pose any problem in measuring AcPh formation upon 2-CAP reduction.

Two experiments gave no indication that 2-CPE underwent dehydrohalogenation to form AcPh. However, one experiment, allowed to run past one week, did provide evidence that this reaction can occur in the sediment collected from Rock Creek tributary (Figures 1.5 and 3.33). The formation of AcPh from 2-CPE could greatly complicate the analysis of 2-CAP reduction and product formation studies, but, as the model system showed, this reaction is too slow to have a notable effect on AcPh formation in 2-CAP studies carried out in under four or five days. This conclusion, however, will only hold in general if the conditions favorable to this reaction did not vary greatly in sediment samples collected throughout this study. Because measurements of 2-CPE transformation were not routinely made, the effects of dehydrohalogenation were based on the limited number of 2-CPE transformation studies run. All of these studies indicated that 2-CPE dehydrohalogenation should have no visible effect on the amount of AcPh formed in 2-CAP reduction studies.

4.4 Treatment Effects

4.4.1 Addition of NAD(P)H

A number of treatment effects were used in this study to investigate the role of dehydrogenase enzymes and cofactors. The first set of treatments, which included the addition of NAD(P)H and sonication of the sediment slurry, were initially set up with the intent of stimulating the hydride transfer reaction. Unfortunately, neither treatment showed any indication of increased hydride transfer. However, both experiments provided useful information that was not anticipated. In the first treatment, to test for dehydrogenase activity in sediment, coenzymes capable of hydride transfer in the presence of an alcohol dehydrogenase (NAD(P)H) were added to sediment to stimulate 2-CAP reduction. The results of this experiment showed no evidence of an increased reduction rate (Figures 3.35 and 3.36). In fact, k_{obs} was slightly smaller in the presence of added NADH (as summarized in Appendix E). What this experiment did show was that the effects of adsorption varied between three samples that were presumed to be identical with regard to the amount of sediment. It is unclear why the addition of 60 μM NAD(P)H would affect adsorption of 2-CAP, which suggests that this effect may be simply a consequence of sample–to–sample variability. While no experiments were performed explicitly to test reproducibility, a number of experiments unexpectedly provided evidence of sample-to-sample reproducibility. Figures 4.1 through 4.3 demonstrate the reproducibility in 2-CAP reduction, product formation, and adsorption effects between samples for three different experiments prepared and analyzed in parallel. In all three experiments, treatment effects had been used unsuccessfully to inhibit 2-CAP reduction. Due to inadequate inhibitor concentrations, no effects on 2-CAP reduction were observed. Instead, all three experiments showed that the primary processes (adsorption, reduction, and product formation) were nearly identical with regard to rate and quantity for all samples within each experiment (summarized in Appendix E).

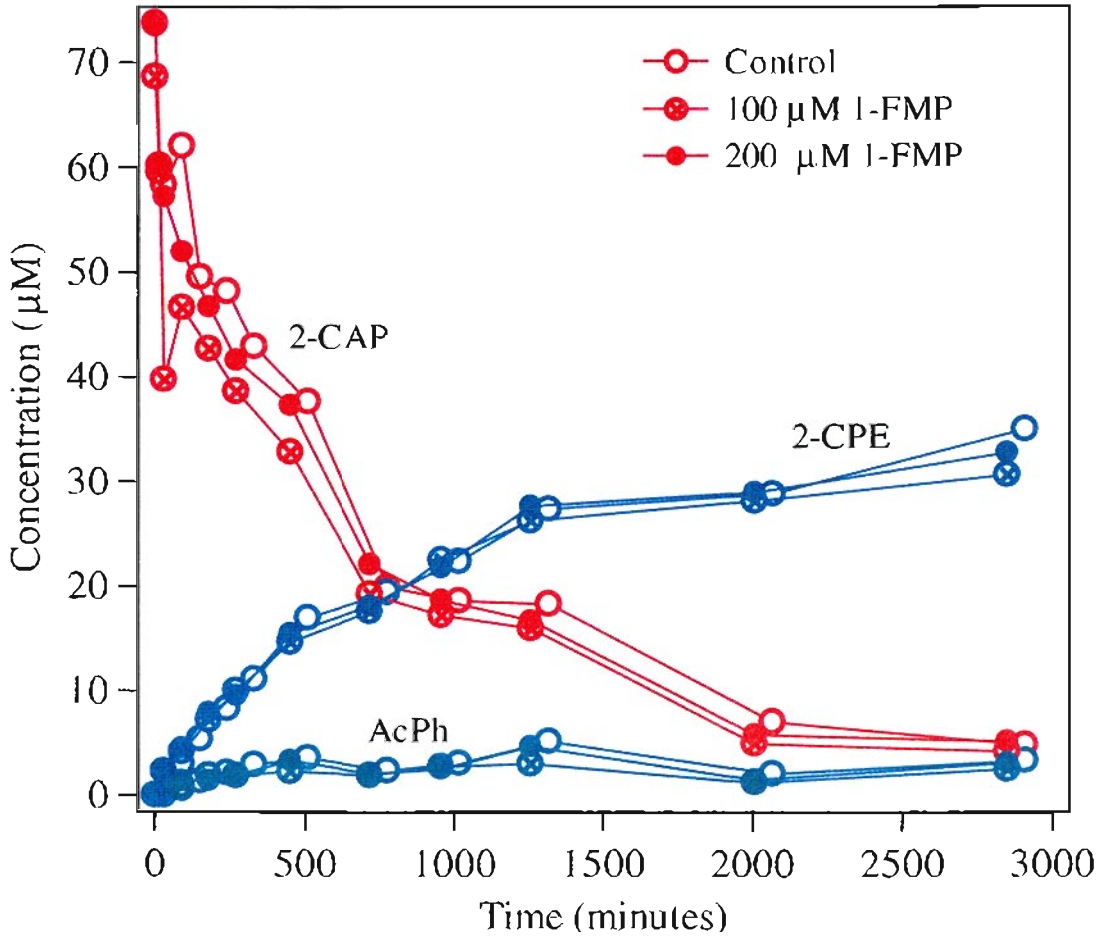

Figure 4.1 Representative plot of data reproducibility (23 April 99). The mass of dry sediment was 14.6 g. Experimental data and fitting coefficients are summarized in Appendices D and E. (Experiment includes Figures 4.1-4.3).

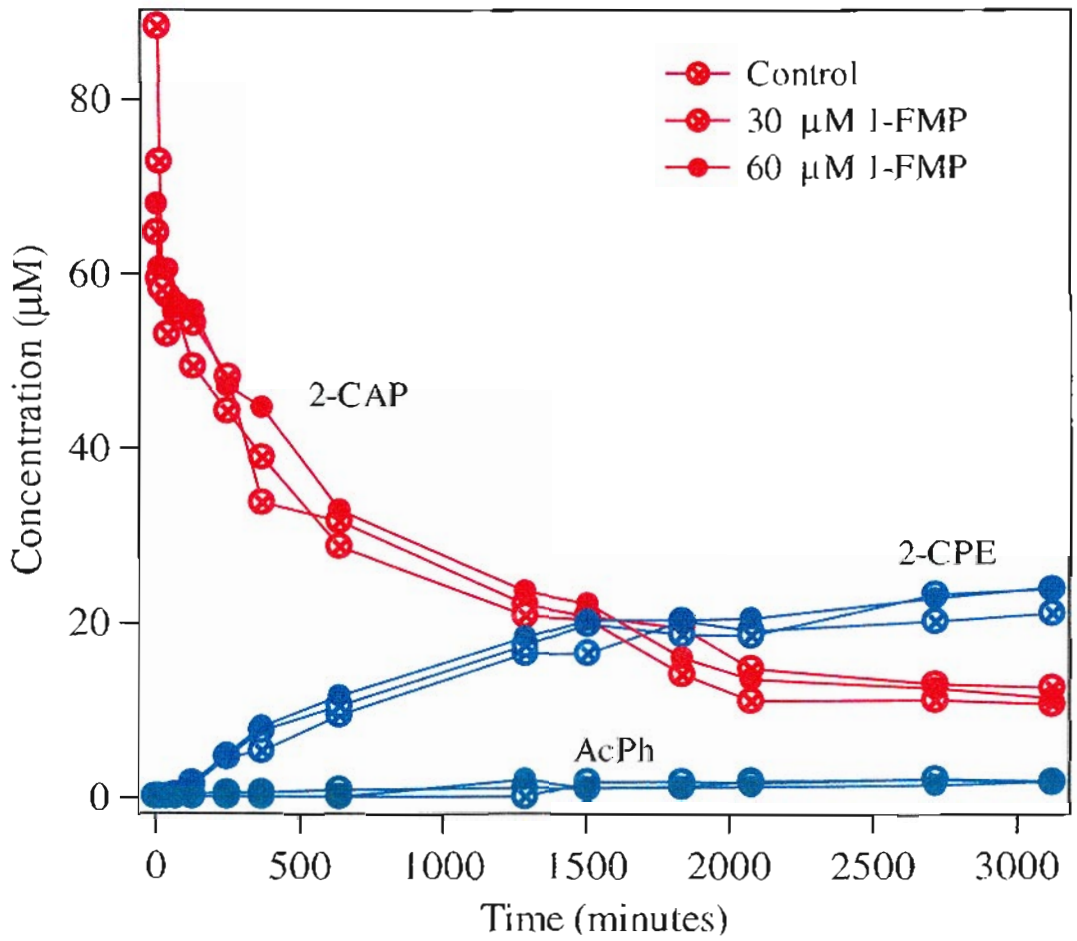

Figure 4.2 Representative plot of data reproducibility (16 August 99). The mass of dry sediment was 13.5 g. Experimental data and fitting coefficients are summarized in Appendices D and E. (Experiment includes Figures 4.1-4.3).

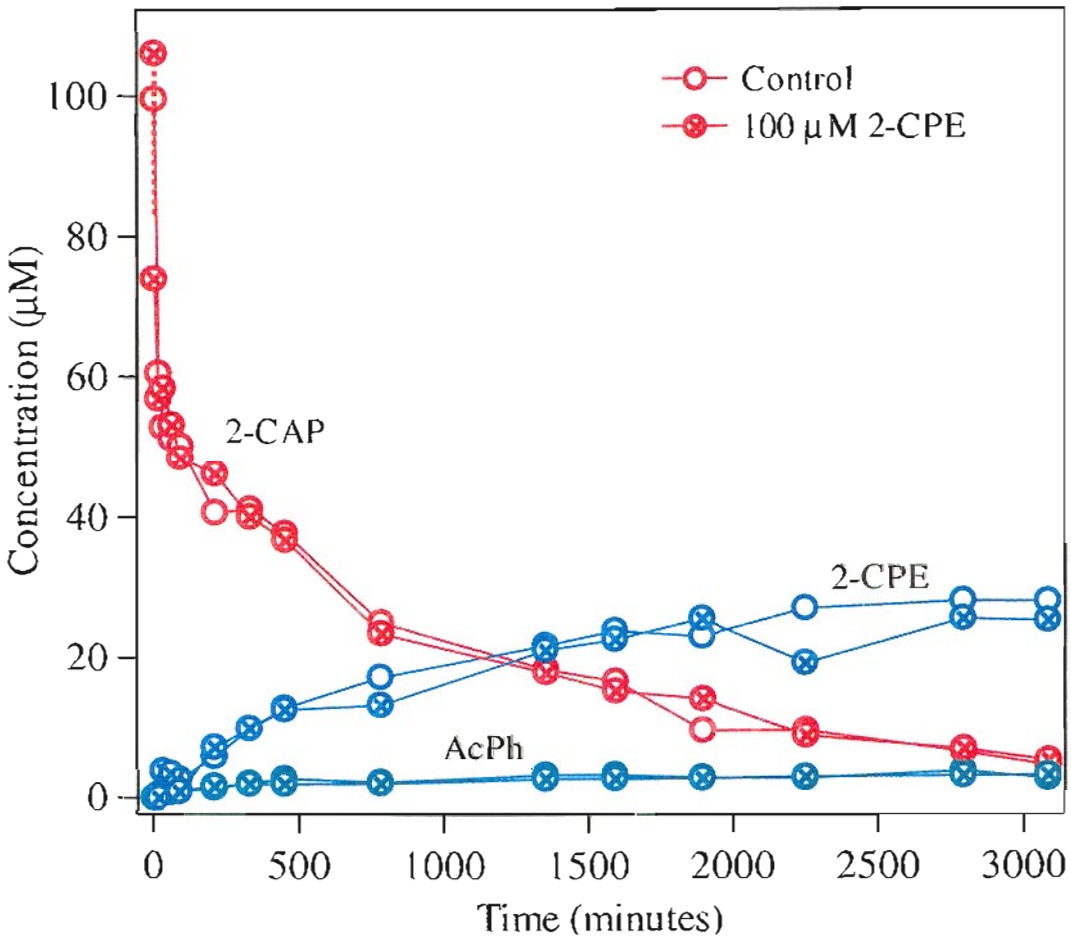

Figure 4.3 Representative plot of data reproducibility (12 July 99). The mass of dry sediment was 10.5 g. Experimental data and fitting coefficients are summarized in Appendices D and E. (Experiment includes Figures 4.1-4.3).

4.4.2 Sonication of sediment

The second treatment used in this study for stimulating 2-CAP reduction involved sediment sonication. The idea behind this experiment was that sonication could lyse cell walls, releasing, without denaturing, the dehydrogenase enzymes needed for hydride transfer. Interestingly, the rate of 2-CAP reduction was not increased, but instead, decreased in the sonicated sediment (as summarized in Appendix E and shown in Figures 3.37 and 3.38). It is not entirely clear what caused the drop in 2-CAP reduction rate, but a number of possibilities can be hypothesized. First, the break-up of sediment aggregates upon sonication could have greatly changed the adsorptive properties of the sediment, which could potentially have had a substantial influence on observed 2-CAP reduction in solution. Another possibility is that the enzymes released upon cell lysis were inactivated in the sediment slurry, thus, 2-CAP reduction was slower as a result of a decrease in reducing agents. If this were true, the experiment would provide further evidence for inactivity of extracellular dehydrogenases [27]. Unfortunately, there is no independent evidence with which to decide between these possibilities that cell lysis occurred upon sonication or that any enzymes were released into solution.

4.4.3 Addition of dehydrogenase inhibitors

Another set of treatment effects used to study dehydrogenase activity in sediments involved a number of enzyme inhibitors. In NADPH/TBADH model systems, recently studied by Nurmi and Hoffman [19], 1-FMP, p-CMB, and pyrazole were observed to inhibit the hydride transfer pathway of 2-CAP reduction. In these model systems, evidence of inhibition was observed in results showing a decrease in 2-CAP reduction rate with increased inhibitor concentrations. In this study, these same inhibitors were applied to Rock Creek tributary sediment (Figures 3.39–3.43). While the effects were not as strong as in those observed in the model system, all three additives gave effects consistent with inhibition of dehydrogenase activity. In general, inhibition effects were more noticeable on the formation of 2-CPE (rate of formation and quantity), with little effect on observed 2-CAP reduction and AcPh formation (summarized in Appendix E).

The best evidence for an enzyme-mediated reaction was obtained from the product inhibition studies. The most noticeable effects of inhibition of 2-CAP reduction were observed with the addition of 2-CPE. In this experiment, the rate of 2-CAP reduction decreased with increasing 2-CPE concentration as summarized in Appendix E and shown in Figures 3.42 and 3.43. Interestingly, a substantial change in the production of AcPh was also observed upon adding and increasing the concentration of 2-CPE. The increased production of AcPh that was seen with increasing 2-CPE concentration was not expected, nor was its source identified. However, it is possible that 2-CPE underwent dehydrohalogenation to form AcPh and with increased concentrations of 2-CPE in solution, the effects of dehydrogenation became visible and quite substantial.

4.5 Other Probe Compounds

Three probe compounds with similar structures to 2-CAP were used in this study to further explore reduction by hydride transfer in sediments. Tanner reported that unlike 2-CAP, which underwent hydride transfer in the presence of NADH/HLADH, 2-BAP and 2,2-CAP were reduced by NADH, without catalysis, to form AcPh and 2-CAP, respectively [16]. These two probe compounds were used in Rock Creek tributary sediment to determine how they would react in an environmental system where reduction by hydride transfer appears to be the dominant reduction mechanism (as evident in 2-CAP reduction data). Tanner did not study the reduction of 2,2′,4′-CAP in his model system, but it, too, should have potential for being reduced by both electron and hydride transfer. The interest in using 2,2′,4′-CAP was to determine how the ring substituents affect reduction.

In sediment, 2-BAP was reduced by both electron and hydride transfer to form AcPh and 2-bromo-1-phenylethanol (2-BPE), respectively (Figures 3.44). If we accept that hydride transfer is the dominant reduction pathway of 2-CAP in the Rock Creek sediment (based on observed 2-CAP reduction trends), it may be reasonable to predict

that 2-BAP would also undergo rapid hydride transfer to form 2-BPE. This was not the case, as AcPh appeared to be the dominant reduction product. Although AcPh formation accounted for less than 10% of the initial 2-BAP concentration, its production may have interesting implications. Tanner et al. [16] observed that 2-BAP, in the presence of NADH and a dehydrogenase enzyme, formed AcPh via radical chain reduction by NADH alone. This presumably reflects the relative ease of hydrogenolysis of the C-Br bond over the C-Cl bond. It is unclear what this formation of AcPh in sediment indicates, whether it is the result of reduction by NADH or other electron donators cannot be determined from this experiment. However, if more AcPh had been formed in this study, it may have been possible to conclude that NADH was the likely reductant for this reaction since hydride activity is generally high in this sediment.

Tanner's model system of 2,2-CAP in the presence of NADH and alcohol dehydrogenase also showed preferential reduction of 2,2-CAP to 2-CAP by NADH alone [16]. However, when 2,2-CAP was added to sediment from Rock Creek tributary, it was preferentially reduced via hydride transfer to form 2,2-dichloro-1-phenylethyl alcohol (2,2-CPE). While 2,2-CAP continued to rise in concentration, the small amount of 2-CAP formed was further reduced to 2-CPE and AcPh (Figure 3.45). This was the only experiment done in this study where 2-CAP reduction via electron transfer appeared to be favored over hydride transfer. While neither AcPh nor 2-CPE was produced in large quantities, the greater production of AcPh may have been a result of the limited availability of hydride sources, due to high levels of reduction of 2,2-CAP by this same mechanism. The 2,2-CAP probe is very appealing because it allows for the measurement of both 2,2-CAP and 2-CAP reduction by hydride and electron transfer in sediments.

The final probe compound used in this study was 2,2′,4′-CAP. Although it was transformed in the sediment slurry, its reaction products could not be identified using HPLC, making it difficult to determine how the ring substituents may have affected reduction by hydride and electron transfer (Figure 3.46). Future experiments will employ the use of mass spectrometry for product identification.

CHAPTER 5
CONCLUSIONS

2-CAP was predominantly reduced by hydride transfer to form 2-CPE in sediments collected from Rock Creek tributary and two sites within the Columbia River estuary. Adsorption played a significant role in 2-CAP disappearance and product appearance and was responsible for the initial decrease in 2-CAP concentration in all reduction studies. While acetonitrile extraction successfully recovered adsorbed compounds in some experiments, this method was not consistently effective.

2-CAP reduction and product formation appear to be first-order. However, further analysis revealed that the observed kinetics represent the combined effects of adsorption and reduction. While adsorption effects could be eliminated in the initial 2-CAP reduction data by omitting data dominated by adsorption from kinetic fits, detailed kinetic modeling is necessary for fitting all adsorption and reduction data simultaneously. The kinetics of this complex system are currently being modeled in the Tratnyek lab by Joel Bandstra.

Three environmental factors, including storage of sediment, amount of sediment, and temporal changes in the creek, influenced the primary process of 2-CAP reduction. While no single environmental variable studied appeared to be predominantly responsible for the variations in k_{obs} observed between experiments, the most pronounced environmental effect observed was with respect to the amount of sediment. To account for this effect, 2-CAP k_{obs} values were normalized to ρ in all experiments.

Control studies showed that 2-CAP can of undergo hydrolysis and reduction by sulfide species in model systems of buffered solution. However, there was no evidence of these reactions in sediment. Both 2-CPE and AcPh were further transformed in sediments by dehydrohalogenation and reduction by hydride, respectively. The rates of

both reactions were slow in comparison to 2-CAP reduction and, therefore, did not affect the product formation kinetics determined in 2-CAP reduction studies.

Sonication of sediment significantly affected 2-CAP reduction and product formation, primarily by changing the amount of sorption to the sediment. Although sonication was performed with the intent of lysing cells as a mechanism of increasing the extracellular concentration of reducing agents, it is unknown whether cell lysis actually occurred or whether reducing species in solution increased in concentration. The addition of NAD(P)H to sediment was also shown to primarily affect the initial 2-CAP concentration lost to adsorption. Variations observed in 2-CAP reduction and product formation rates in the presence of NAD(P)H are presumably results of the variation in initial adsorption. p-CMB, 1-FMP, pyrazole, and 2-CPE inhibited 2-CAP reduction to varying degrees. While p-CMB, 1-FMP, and pyrazole had the most noticeable effect on product formation, 2-CPE had an apparent effect on 2-CAP reduction. The addition of 2-CPE to sediment also caused an increase in AcPh formation. It is unclear whether this increased AcPh production was a result of 2-CAP reduction or dehydrohalogenation of 2-CPE.

2,2-CAP was reduced by both hydride and electron transfer to give 2,2-CPE and 2-CAP, respectively. Hydride transfer was the dominant reduction pathway in this experiment. 2-BAP was also reduced in sediment by both hydride and electron transfer to give 2-BPE and AcPh, respectively. AcPh appeared to be the dominant product formed, however, a large percent of the initial 2-BAP concentration could not be accounted for. Because NADH is capable of reducing 2-BAP to AcPh, the production of AcPh in an environment where hydride activity is apparently high suggests the presence of NADH.

While it is yet to be determined what specific reagents are responsible for 2-CAP reduction in sediment, this research has provided ample evidence supporting biological mediators. Smolen et al. [17] provided the initial work in this area with temperature and enantioselective 2-CAP reduction studies. The inhibition of 2-CAP reduction by enzyme inhibitors, as well as, the results of 2-BAP and 2,2-CAP reduction in sediment observed in this study further substantiates this claim.

APPENDIX A

Chemicals used and their Chemicals Abstracts Service (CAS) registry numbers.

Chemical Name	CAS #
Acetonitrile (ACN)	75-05-8
Acetophenone (AcPh)	98-86-2
Alcohol dehydrogenase, from *Thermoanaerobium brockii* (TBADH)	9028-12-0
2-Bromoacetophenone (2-BAP)	70-11-1
2-Chloroacetophenone (2-CAP)	532-27-4
4-Chloromercuribenzoic acid (p-CMB)	59-85-8
(S)-(+)-2-Chloro-1-phenylethyl alcohol ((S)-(+)-2-CPE)	56751-12-3
(R)-(-)-2-Chloro-1-phenylethyl alcohol ((R)-(-)-2-CPE)	70111-05-6
2,2-Dichloroacetophenone (2,2-CAP)	2648-61-5
1-Formylpiperidine (1-FMP)	2591-86-8
β-NADH, disodium salt	606-68-8
β-NADPH, tetrasodium salt	2646-71-1
(S)-(+)-1-Phenylethyl alcohol ((S)-(+)-1-PE)	1445-91-6
(R)-(-)-1-Phenylethyl alcohol ((R)-(-)-1-PE)	1517-69-7
Potassium biphthalate	877-24-7
Potassium phosphate, monobasic	7778-77-6
Pyrazole	288-13-1
Resazurin	62758-13-8
Sodium borohydride	16940-66-2
Sodium carbonate	497-19-8
Sodium sulfide, nonahydrate	1313-84-4
2,2′,4′-Trichloroacetophenone (2,2′,4′-CAP)	4252-78-2
Trizma® base	77-86-1
Trizma® hydrochloride	1185-53-1

APPENDIX B

Summary of sediment and site characterization.

Rock Creek is a tributary of the Tualatin River, which drains a 711 sq. mile watershed in northeast Oregon. The sediment used for this study was collected from a small tributary of Rock Creek (Figure E.1). The tributary runs through a newly developed business area near the Oregon Graduate Institute in Beaverton, Oregon. At the sampling location, the creek is approximately 3.5 m wide and 1 m deep at its center. Both of these parameters, as well as the flow vary with season. The biota in the area include various species of deciduous and coniferous trees, blackberry bushes, ducks, and an active beaver. The beaver dam is located just downstream from the sampling area. A seasonal field survey was begun in the summer of 1998 to measure basic water quality parameters. The sediment has yet to be characterized in terms of microbial populations or percent organic matter. However, mineral content in the sediment has been measured by the Soil Analytical Lab at Montana State University with the aid of Jeff Darland of OGI using a 50/50 lithium metaborate/tetraborate mixed fusion or mineral content (data shown below). According to the Washington County Soil Survey (Figure B.1), the soil in the surrounding area is likely a Dayton (15) or Willamette silt loam (44) [34]. The sediment is a silty-clay.

Table B.1 Mineral content for sediment samples collected in Rock Creek tributary: (1) near rocky banks, (2,3) center of creek.

Sample	m_p (g)	Ca (mg/g)	Cu (mg/g)	Fe (mg/g)	K (mg/g)	Mg (mg/g)	Mn (mg/g)	Na (mg/g)	P (mg/g)	Zn (mg/g)	Cr (mg/g)	Al (mg/g)
Blank	0	0.090	0.028	0.230	0.600	0.030	0.016	0.570	—	0.020	0.040	—
1	0.0560	13.286	0.105	51.000	14.464	7.375	1.057	13.824	1.125	0.268	0.179	65.714
2	0.0518	12.606	0.116	49.633	14.865	7.278	1.093	14.575	1.236	0.251	0.193	67.761
3	0.0577	11.924	0.080	48.908	14.731	7.002	1.345	13.865	1.404	0.225	0.173	65.858

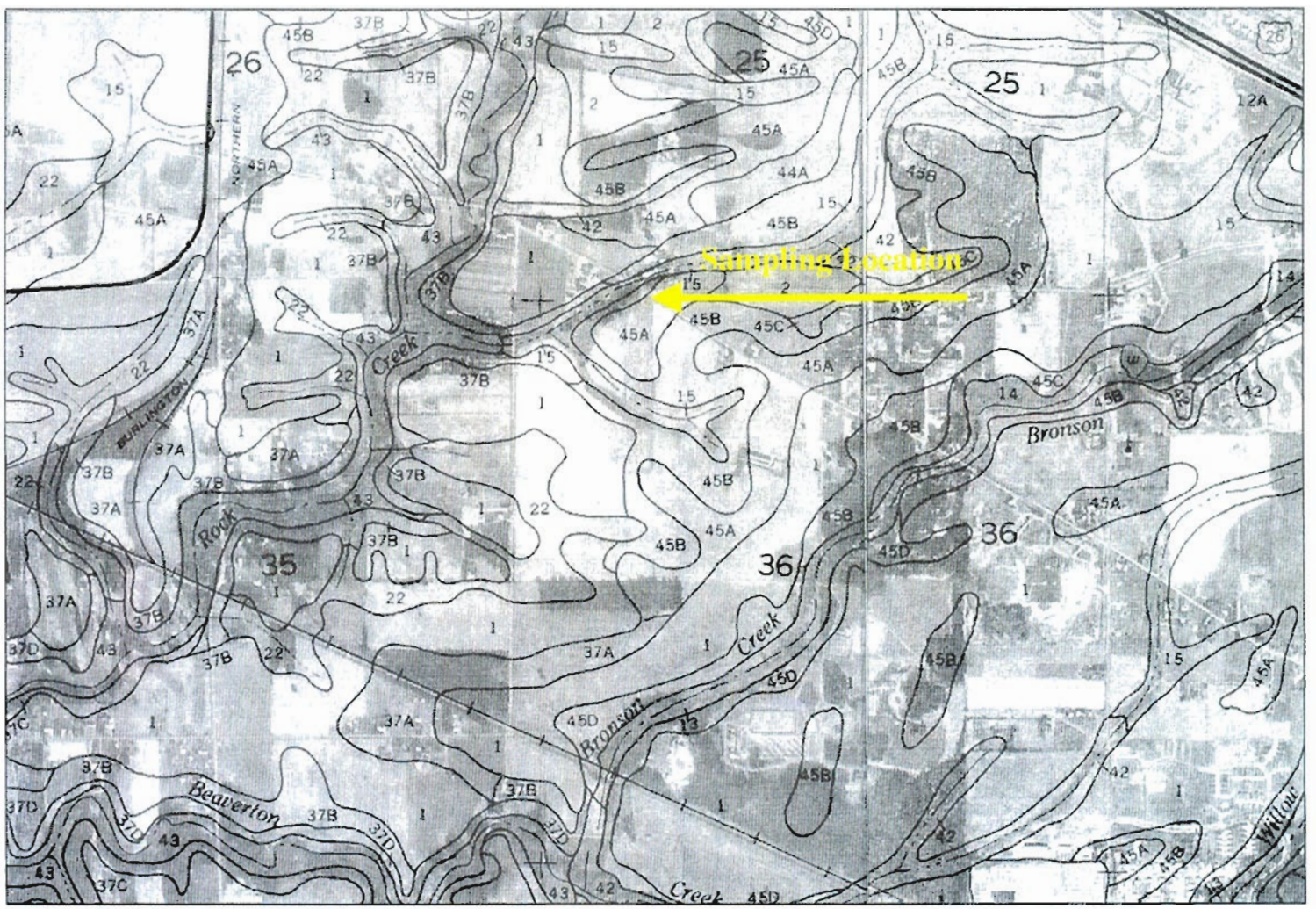

Figure B.1 Soil Survey of area surrounding sampling region (taken from the Washington County Soil Survey) [34].

APPENDIX C

List of symbols.

λ_{max}	Wavelength maximum absorbance
k_{obs}	Psuedo first-order rate constant for 2-CAP disappearance or product formation (min^{-1})
k_{rho}	First-order rate constant (k_{obs}) normalized to ρ (min^{-1})
ρ	Dry mass of sediment per unit mass of water (g/g)
V_p	Unit volume occupied by sediment particles not including pore space (mL)
V_T	Total volume of sediment slurry (mL)
V_w	Volume of water in sediment slurry, calculated from $V_T - V_p$ (mL); also equal to m_w (g)
V_e	Volume of extraction solvent (mL)
V_{stock}	Volume of 2-CAP stock solution (mL)
n_{tot}	Total amount of 2-CAP, 2-CPE, and AcPh (moles)
C_0	Nominal concentration calculated at t = 0 min (μM)
C_s	Concentration of 2-CAP and/or products in solution (μM)
C_e	Concentration of 2-CAP and/or products extracted from sediment (μM)
C_f	Final concentration after extraction, calculated from $C_s + C_e$ (μM)
C_{stock}	Concentration of 2-CAP stock solution (μM)
C_∞	Theoretical maximum concentration (at t = ∞) of 2-CPE and AcPh, calculated by fitting first-order appearance kinetics (μM)
m_p	Dry mass of sediment in slurry (g)
D_p	Particle density: weight of solid particles per unit volume of particles not including pore space (g/cm^3)
$*t_0$	Time when reagents are added to slurry (0 min), prior to initial mixing
t_1	Time at which the first sample is extracted from the slurry (1 min); after initial mixing

APPENDIX D

Summary of experimental data, including initial 2-CAP concentration, dry mass of sediment, final concentration of all compounds in solution, % adsorption, and % extraction of all compounds.

	Experiment	2-CAP C_0 (μM)	m_p (g)	*Before Extraction*		*After Extraction*		
				Mass Balance in soln. (μM)	% Adsorbed (μM)	Mass Balance in soln. (μM)	% Adsorbed (μM)	% Extracted (μM)
1	23 Feb 99 Location Sample 1	102.8	6.8	71.89	30.07	—	—	—
2	23 Feb 99 Location Sample 2	101.0	3.0	90.60	10.30	—	—	—
3	23 Feb 99 Location Sample 3	101.0	2.9	75.47	25.28	—	—	—
4	24 Feb 99 Control	104.1	9.0	50.59	51.36	—	—	—
5	24 Feb 99 NADH/NADPH	104.1	9.1	49.33	52.57	—	—	—
6	19 Mar 99 Field Survey	101.8	4.6	60.25	40.82	—	—	—
7	22 Mar 99 Field Survey	104.2	9.3	47.66	54.26	—	—	—
8	23 Mar 99 Storage Day 1	101.9	4.8	67.36	33.90	—	—	—
9	26 Mar 99 Storage Day 3	101.9	4.8	33.36	67.26	—	—	—
10	29 Mar 99 Storage Day 6	101.9	4.8	46.87	54.00	—	—	—
11	16 Apr 99 Storage Time	103.0	4.8	39.41	61.74	47.97	53.43	13.46
12	02 Jun 99 Storage Time	103.0	4.8	58.51	43.19	73.15	28.98	32.90
13	25 Mar 99 Field Survey	102.2	5.4	70.70	29.38			
14	16 Apr 99 Field Survey	106.0	10.5	39.40	62.83	57.57	45.69	27.28
15	23 Apr 99 Control	108.0	14.4	45.82	57.57	63.04	41.63	27.69
16	23 Apr 99 100 μM 1-FMP	108.0	14.6	51.86	51.98	66.75	38.19	26.53
17	23 Apr 99 200 μM 1-FMP	108.0	14.4	51.67	52.16	65.21	39.62	24.04
18	04 May 99 Lewis & Clark	106.7	11.9	48.09	54.93	67.68	36.57	33.42
19	04 May 99 Tansy Point	109.2	15.6	72.64	33.48	91.68	16.04	52.09
20	04 May 99 Control	107.5	12.7	73.92	31.24	97.85	8.98	71.25

	Experiment *Continued*	2-CAP C_0 (μM)	m_p (g)	*Before Extraction* Mass Balance in soln. (μM)	*Before Extraction* % Adsorbed (μM)	*After Extraction* Mass Balance in soln. (μM)	*After Extraction* % Adsorbed (μM)	*After Extraction* % Extracted (μM)
21	06 May 99 p-CMB	107.5	12.8	46.65	56.60	62.67	41.70	26.33
22	12 May 99 Sample 1	103.0	10.7	53.57	47.99	71.05	31.02	35.36
23	12 May 99 Sample 2	109.2	15.6	55.00	49.63	77.67	28.87	41.83
24	12 May 99 Sample 3	111.0	18.8	48.47	56.33	74.02	33.32	40.85
25	12 May 99 Sample 4	113.0	22.3	48.64	56.96	78.43	30.59	46.30
26	02 June 99 Field Survey	110.0	16.9	61.05	44.5	101.38	7.84	82.38
27	16 June 99 Control	107.5	12.9	63.55	40.88	111.00	0	100.00
29	30 June 99 Control	109.2	16.6	48.29	55.78	63.87	41.51	25.58
31	07 July 99 Control	108.0	7.0	29.22	72.84	39.38	63.54	12.77
32	07 July 99 NADH	108.0	6.9	51.20	52.59	66.44	38.48	26.83
33	07 July 99 NADPH	108.0	6.9	30.33	71.92	43.31	59.90	16.71
34	08 July 99 Control	107.5	13.1	66.88	37.79	113.19	0	100.00
36	12 July 99 Control	106.0	10.5	35.14	66.85	38.68	63.51	5.00
37	12 July 99 100 μM 2-CPE	106.7	10.4	107.69	49.54	127.49	40.26	18.73
38	20 July 99 100 μM 2-CPE	108.7	15.2	102.98	52.63	121.78	43.98	16.44
39	20 July 99 1 mM 2-CPE	——	15.2	——	——	——	——	——
40	20 July 99 5 mM 2-CPE	——	15.2	——	——	——	——	——
41	21 July 99 Control	110.0	17.0	31.36	71.25	35.04	68.15	4.35
42	21 July 99 2 mM pyrazole	110.0	17.0	31.45	71.41	36.75	66.59	6.75
43	21 July 99 10 mM pyrazole	110.0	17.0	29.93	72.79	36.28	67.02	7.93
45	09 Aug 99 Control	106.7	12.0	25.44	76.16	30.91	71.03	6.74
	09 Aug 99 2-BAP	106.7	12.0	5.14	95.18	6.90	93.53	1.73
46	16 Aug 99 Control	107.5	13.5	35.32	67.14	39.95	62.84	6.40
47	16 Aug 99 30 μM 1-FMP	107.5	13.5	36.37	66.17	39.83	62.95	4.87

	Experiment *Continued*	2-CAP C_0 (μM)	m_p (g)	*Before Extraction* Mass Balance in soln. (μM)	*Before Extraction* % Adsorbed (μM)	*After Extraction* Mass Balance in soln. (μM)	*After Extraction* % Adsorbed (μM)	*After Extraction* % Extracted (μM)
48	16 Aug 99 60 μM 1-FMP	107.5	13.5	37.30	65.30	41.47	61.42	5.94
49	30 Aug 99 Control	108.0	14.2	25.79	76.12	30.10	72.13	5.24
50	30 Aug 99 Sonicated	108.0	14.2	26.87	75.12	34.47	68.08	9.37
51	15 Sept 99 Control	105.0	10.2	31.42	70.08	35.98	65.73	6.21
52	15 Sept 99 30 mM 1-FMP	105.0	10.2	22.16	78.90	30.05	71.38	9.53
53	15 Sept 99 60 mM 1-FMP	105.0	10.2	21.04	79.96	30.83	70.64	11.66
54	15 Sept 99 5 mM pyrazole	105.0	10.2	30.40	71.05	37.35	64.43	9.32
55	15 Sept 99 10 mM pyrazole	105.0	10.2	27.63	73.69	34.44	67.20	8.81
	Adsorption Experiments							
56	12 July 99 2-CAP	106.7	14.1	33.73	68.39	54.47	48.95	39.71
57	22 Sept 99 100 μM 2CAP	105.0	10.2	66.72	36.43	79.55	24.24	50.41
58	22 Sept 99 400 μM 2CAP	416.9	10.2	263.4	36.82	——	——	——

APPENDIX E

Summary of experimental conditions and results, including rate constants of 2-CAP reduction and 2-CPE and AcPh formation (determined by first-order non-linear fit to concentration versus time), predicted final 2-CPE and AcPh concentrations, dry mass of sediment, volume of water in 100 mL slurry, ρ, and 2-CAP disappearance rate constant normalized to ρ.

	Experiment	2-CAP k_{obs}^{1} ($\times 10^{-3}$)	2-CAP k_{rho}^{1} ($\times 10^{-2}$)	2-CPE k_{obs}^{1} ($\times 10^{-3}$)	2-CPE C_{∞}^{2}	AcPh k_{obs}^{1} ($\times 10^{-3}$)	AcPh C_{∞}^{2}	m_p (g)	ρ (g/V) ($\times 10^{-1}$)	V_w (mL)
1	23 Feb 99 Location Sample 1	0.524	0.746	0.321	45.3	——	——	6.8	0.703	96.8
2	23 Feb 99 Location Sample 2	0.168	0.552	0.242	64.9	——	——	3.0	0.304	98.6
3	23 Feb 99 Location Sample 3	0.211	0.718	0.197	53.0	——	——	2.9	0.294	98.6
4	24 Feb 99 Control	0.542	0.576	0.761	39.0	——	——	9.0	0.940	95.7
5	24 Feb 99 NADH/NADPH	0.345	0.363	0.596	43.4	——	——	9.1	0.951	95.7
6	19 Mar 99 Field Survey	0.250	0.532	0.662	36.2	1.360	4.06	4.6	0.470	97.8
7	22 Mar 99 Field Survey	0.779	0.801	0.987	35.2	0.727	4.37	9.3	0.973	95.6
8	23 Mar 99 Storage Day 1	0.437	0.890	0.394	50.3	0.764	10.46	4.8	0.491	97.7
9	26 Mar 99 Storage Day 3	0.680	1.380	0.888	30.9	0.848	3.20	4.8	0.491	97.7
10	29 Mar 99 Storage Day 6	0.903	1.840	0.469	47.9	2.020	1.72	4.8	0.491	97.7
11	16 Apr 99 Storage Day 24	0.932	1.980	0.571	33.9	0.307	6.22	4.6	0.470	97.8
12	02 Jun 99 Storage Day 71	0.399	0.830	0.862	26.2	0.484	5.24	4.7	0.481	97.8
13	25 Mar 99 Field Survey	0.799	1.440	0.430	69.7	0.349	6.56	5.4	0.554	97.4
14	16 Apr 99 Field Survey	1.570	1.420	0.820	33.3	0.569	9.49	10.5	1.110	95.0
15	23 Apr 99 Control	1.240	0.790	1.110	34.6	4.090	3.41	14.6	1.570	93.0
16	23 Apr 99 100 µM 1-FMP	1.260	0.815	1.370	30.8	6.210	2.36	14.4	1.550	93.1
17	23 Apr 99 200 µM 1-FMP	1.280	0.828	1.280	32.7	5.270	2.92	14.4	1.550	93.1
18	04 May 99 Lewis & Clark	0.412	0.327	0.266	27.5	0.408	1.86	11.9	1.260	94.3
19	04 May 99 Tansy Point	0.620	0.368	0.288	57.4	0.325	12.16	15.6	1.690	92.6
20	06 May 99 Control	1.200	0.888	1.010	46.1	1.20	12.63	12.7	1.350	94.0
21	06 May 99 100 µM p-CMB	1.500	1.100	0.615	29.6	0.618	11.06	12.8	1.360	93.9
22	12 May 99 Sample 1	0.630	0.589	0.659	23.8	0.420	17.17	10.7	1.130	94.9
23	12 May 99 Sample 2	0.886	0.526	0.731	34.4	0.599	21.12	15.6	1.690	92.6
24	12 May 99 Sample 3	1.190	0.576	0.929	29.4	0.906	19.00	18.8	2.070	91.0

	Experiment *Continued*	2-CAP k_{obs}[1]	2-CAP k_{rho}[1]	2-CPE k_{obs}[1]	2-CPE C_∞[2]	AcPh k_{obs}[1]	AcPh C_∞[2]	m_p (g)	ρ (g/V)	V_w (mL)
25	12 May 99 Sample 4	0.162	0.649	1.210	42.1	0.857	11.50	22.3	2.500	89.4
26	02 June 99 Field Survey	1.900	1.030	1.420	46.1	1.810	13.94	16.9	1.840	92.0
27	16 June 99 Control	0.555	0.404	0.798	51.4	0.675	5.55	12.9	1.370	93.9
28	16 June 99 ACN extracted	0.357	0.260	0.851	63.2	0.596	9.90	12.9	1.370	93.9
29	30 June 99 Control	1.100	0.610	0.727	48.4	0.934	5.32	16.6	1.800	92.1
30	30 June 99 ACN extracted	9.160	5.080	0.682	64.0	0.887	7.01	16.6	1.800	92.1
31	07 July 99 Control	1.030	0.907	1.220	27.0	2.310	5.91	7.0	1.500	*46.7
32	07 July 99 NADH	0.862	0.838	1.240	35.0	1.440	6.80	6.9	1.480	*46.7
33	07 July 99 NADPH	1.480	0.986	1.460	25.6	1.550	4.15	6.9	1.480	*46.7
34	08 July 99 Control	0.807	0.579	0.394	51.1	3.60	2.61	13.1	1.400	93.8
35	08 July 99 ACN extracted	0.731	0.523	0.544	49.9	1.220	3.68	13.1	1.400	93.8
36	12 July 99 Control	1.130	1.020	1.120	28.3	3.290	3.07	10.5	1.110	95.0
37	12 July 99 100 µM 2-CPE	0.962	0.879	1.350	24.8	3.380	2.85	10.4	1.090	95.0
38	20 July 99 100 µM 2-CPE	0.683	0.417	——	——	0.351	11.50	15.2	1.640	92.8
39	20 July 99 1 mM 2-CPE	0.474	0.289	——	——	0.121	33.40	15.2	1.640	92.8
40	20 July 99 5 mM 2-CPE	0.345	0.211	——	——	0.121	75.30	15.2	1.640	92.8
41	21 July 99 Control	0.850	0.460	0.512	23.5	0.390	4.12	17.0	1.850	91.9
42	21 July 99 2 mM pyrazole	0.491	0.265	1.240	14.9	0.529	3.10	17.0	1.850	91.9
43	21 July 99 10 mM pyrazole	0.506	0.274	0.193	32.0	0.122	5.58	17.0	1.850	91.9
44	21 July 99 20 mM pyrazole	0.602	0.325	0.151	23.3	0.100	7.19	17.0	1.850	91.9
45	09 Aug 99 Other Probes	0.933	0.729	0.126	20.5	2.460	2.90	12.0	1.280	94.1
46	16 Aug 99 Control	0.664	0.460	0.854	23.2	0.518	2.43	13.5	1.440	93.6
47	16 Aug 99 30 µM 1-FMP	0.752	0.521	0.839	25.2	0.163	5.37	13.5	1.440	93.6
48	16 Aug 99 60 µM 1-FMP	0.696	0.482	0.968	24.9	0.388	2.54	13.5	1.440	93.6
49	30 Aug 99 Control	1.430	0.939	0.967	23.5	1.560	2.16	14.2	1.520	93.2
50	30 Aug 99 Sonicated	0.814	0.534	0.475	26.6	0.829	1.42	14.2	1.520	93.2
51	15 Sept 99 Control	0.698	0.651	1.060	23.1	1.140	3.28	10.2	1.070	95.1
52	15 Sept 99 30 mM 1-FMP	0.577	0.538	1.040	13.8	1.030	2.07	10.2	1.070	95.1
53	15 Sept 99 60 mM 1-FMP	0.482	0.450	0.967	11.3	1.140	2.14	10.2	1.070	95.1
54	15 Sept 99 5 mM pyrazole	0.519	0.484	0.685	20.9	0.789	2.44	10.2	1.070	95.1
55	15 Sept 99 10 mM pyrazole	0.462	0.431	0.739	15.9	0.447	2.13	10.2	1.070	95.1

*Volume in 50 mL sediment slurry, [1]in min^{-1}, [2]in µM.

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BIOGRAPHICAL SKETCH

I was born December 2, 1974 in Mayville, North Dakota. I began my academic career at Mayville State University in 1993 and graduated with honors in 1997 with a B.S. in Chemistry. During my four years of college, I served as President of the Student Senate, Biology Club, and Alpha Phi Sigma National Scholastic Honor Society. I was also a member of the North Dakota Student Association and Young Democrats and was selected as a delegate to the 1996 state and national Democratic conventions. I received numerous Mayville State scholarships as well as a NASA Science Foundation Scholarship and American Institute of Chemists Foundation Award. In 1996, I was selected to *Who's Who Among American Universities and Colleges.*

Immediately following undergraduate school, I moved to Oregon to attend the Oregon Graduate Institute, where I received a research assistantship and tuition scholarship. During my time at OGI, I served as Student Council President and as a member of the Educational Policy Committee. I will graduate in 2000 with a degree in Environmental Science and Engineering. The following is a list of my undergraduate and graduate publications:

1. Thomasson, K. A.; Ouporov, I. G.; Baumgartner, T.; Czlapinski, J.; Kaldor, T., Northrup, S. H. Free energy of nonspecific binding of cro repressor protein to DNA. *J. Phys. Chem. B.* 1997, 101, 9127-9136.

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