INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

Université d'Ottawa · University of Ottawa

Soybean Peroxidase Treatment of 2,4-Dichlorophenol

in a Soil Mixture

By

Nicole Driscoll

A Dissertation Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirement for the Degree of

Master's of Applied Science
in Civil Engineering (Environmental)

The Master's program in Civil Engineering is a joint program between Carleton University and the University of Ottawa, which is administrated by the Ottawa-Carleton Institute for Civil Engineering

Department of Civil Engineering
University of Ottawa
Ottawa, ON, Canada
K1N 6N5
May, 2001

ABSTRACT

Soybean peroxidase (SBP) shows potential to remediate water-soil systems, yet relatively little is understood about its reaction in soil environments. While the potential may exist for the use of SBP in soil systems, too many difficulties and unknowns face the research of SBP to soil systems. This study will focus on the use of SBP in soil slurries as a stepping block to future research with soil systems. This study investigates the use of SBP to remediate 2,4-dichlorophenol (2,4-DCP) in wastewater when soil is present in the reactor vessel. Characterization of 2,4-DCP binding to soil (peat moss and silica sand) revealed that the binding was effected by the pH and the mass of the soil. The determination of enzyme kinetic constants and enzyme reactions was not examined in this study.

The application of SBP to reactors with wastewater and soil has the potential to improve or decrease removal, under different reactor conditions, versus wastewater only systems. Operating such that an optimum SBP dose for wastewater treatment is employed removal of 2,4-DCP is higher for wastewater systems without soil, and the removal decreases as the mass of soil increases. Contrary to results at higher doses of SBP, the removal is higher at lower SBP doses for a wide range of pH. At higher SBP doses removal is hindered by the addition of soil, once the SBP dose is reduced below 0.25 units/mL the addition of soil to the reactors improves removal of 2,4-DCP. The removal of 2,4-DCP improved as the SBP concentrations was decreased when soil was present in the reactor.

Assumptions regarding the sorption of the 2,4-DCP to soil allowed conclusions to be made regarding the activity of the SBP. Under best and worst case scenarios,

assumptions of reversible and irreversible sorption of 2,4-DCP, indicated that the SBP activity and removal of 2,4-DCP increased with the addition of soil to the reactor.

It is possible that the addition of soil to the reactor acted as an additive, such as polyethylene glycol, resulting in the improved performance of the SBP through the prevention of enzyme inactivation. The addition of soil to reactors allowed a substantial reduction in enzyme dose of up to 96% of the dose required without the soil to achieve the same removal of 2,4-DCP.

Keywords: soybean peroxidase, 2,4-dichlorophenol, remediation, polyethylene glycol, enzyme inactivation, soil

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to my supervisors, Dr. M. A. Warith and Dr. K. J. Kennedy, who provided insight, guidance, and encouragement throughout the development of this thesis.

I would like to thank Francisco Aposaga for his time and patience in the laboratory. Additional thanks to all my colleagues, who provided excellent advice and laughter, especially K. Alemany, P. Pierce, L. Duguay, P. Gardner, and T. Pham.

A very special thanks to my family and friends, who supported me throughout my studies. And to Nick for his endless encouragement, and support.

TABLE OF CONTENT

ABSTRACT I

ACKNOWLEDGEMENTS III

LIST OF TABLES VII

LIST OF FIGURES VII

ABBREVIATIONS XII

1 INTRODUCTION 1

2 LITERATURE REVIEW 4

2.1 PEROXIDASE ENZYMES 1

2.1.1 Description of Peroxidase Enzymes 1

2.1.2 Kinetics of Peroxidase Enzymes 5

2.1.3 End-Product Polymers 6

2.1.3.1 Advantages and Disadvantages of Enzyme Treatment 7

2.1.3.2 Economic Feasibility of Soybean Peroxidase 8

2.1.3.3 Inactivation of Peroxidase Enzymes 9

2.1.4 Soybean Peroxidase vs. Horseradish Peroxidase 12

2.2 SOIL SYSTEMS 14

2.2.1 Partitioning of Contaminants and Enzymes 14

2.2.2 Fenton's Reagent 16

2.2.2.1 Fenton's Reagent as an Indicator for the Feasibility of Enzymatic Treatment 17

2.2.3 Additional Problems which may be Encountered 19

2.2.4 Promising Indications 20

2.3 RESEARCH CONDITIONS AND RESULTS 22

2.3.1 Substrate 22

2.3.2 Enzyme Dose 25

2.3.3 Hydrogen Peroxide Dose 26

2.3.4 Polyethylene Glycol Dose 28

2.3.5 Type of Reactor 31

2.4 CONCLUSIONS **33**

3 EXPERIMENTAL INVESTIGATION **35**

3.1 MATERIALS AND METHODS **35**

3.1.1 Materials *35*

3.1.2 Experimental Procedure *35*

3.1.3 2,4-DCP Compound Concentrations *38*

3.2 RESULTS AND DISCUSSION **40**

3.2.1 Phase I – The Binding of 2,4DCP with Soil *40*

3.2.1.1 Effect of pH on 2,4-DCP Binding **43**

4.0 Removal of 2,4-Dichlorophenol in Soil Slurries with Soybean Peroxidase **46**

4.1 Influence of pH and SBP Dose 47

4.2 Hydrogen Peroxide 65

4.3 Removal of 2,4-DCP as a Function of Time 68

4.4 Removal of 2,4-DCP as a Function of Temperature and Time 70

4.5 Polyethylene Glycol 72

4.6 Performance of SBP with Higher Soil Concentrations 76

4.7 Implications 80

5.0 Isolating the SBP Activity **82**

5.1 TURNOVERS 89

6.0 CONCLUSIONS **92**

REFERENCES **96**

APPENDIX A 100

APPENDIX B 102

APPENDIX C 103

APPENDIX D 104

LIST OF TABLES

Table 2.1. The effect of humic components on the transformation of 4-chlorophenol by peroxidase (from Park et al., 1999).....21

Table 2.2. The remaining activity of HRP in supernatants as a function of the molecular weight of PEG. (from Nakamoto et al., 1992).....29

Table 2.3. Modes of soybean peroxidase and hydrogen peroxide addition (from Alemany (2000)).....32

Table 2.4. Results for different modes of enzyme and hydrogen peroxide addition (from Alemany (2000)).....33

Table 3.1. Experimental conditions for phase I and II.....39

Table 3.2. K values, standard error and confidence intervals for k values derived from isotherms.....45

Table A.1. Preparation of tris (hydroxymethyl) aminomethane (tris) buffer.....100

Table A.2. Preparation of citrate-phosphate buffer.....101

LIST OF FIGURES

Figure 2.1 The catalytic cycle for soybean peroxidase (adapted from Buchanan et al., 1996; Flock et al., 1999) 6

Figure 2.2 The catalytic cycle for soybean peroxidase (adapted from Buchanan et al., 1996; Flock et al., 1999) 10

Figure 2.3 Thermal denaturing of soybean, horseradish, and C. *cinereus* Peroxidases. (Arnao et al., 1990).....13

Figure 2.4. Carbon content with respect to depth of soil for a highly organic soil.....16

Figure 2.5. Phenol, a benzene ring with attached hydroxyl group – OH 23

Figure 2.6. H-bonding between phenols and organic material or clay. (from Boyd, 1982) 24

Figure 2.7. 2,4 dichlorophenol 24

Figure 2.8. Removal efficiency as a function of peroxidase dose. (from Klibanov, 1983) 26

Figure 2.9. Phenol removal as a function of hydrogen peroxide. 1000ppm Phenol, 40g soybean seed-hulls (from Flock et al., 1999) 27

Figure 2.10. Phenol removal efficiency as a function of PEG dose for a pH of 6.0, with 10 mg/L HRP and 10 g/L phenol, conducted at room temperature, and 5 mmol of hydrogen peroxide was added at 0.175 mmol/min (from Nakamoto et al., 1992).....30

Figure 3.1 Sorption of 2,4-DCP to soil with time.....36

Figure 3.2 Reactor bottles.....37

Figure 3.3 Tumbler.....38

Figure 3.4 Adsorption of 2,4 DCP to reactor bottles, cap, or seal.....42

Figure 3.5a. Sorption isotherm for 2,4-DCP at pH 3.....44

Figure 3.5b. Sorption isotherm for 2,4-DCP at pH 6.....44

Figure 3.5c. Sorption isotherm for 2,4-DCP at pH 8.....45

Figure 4.1. TR 2,4-DCP as a function of silica sand concentration 48

Figure 4.2a. $SR_{2,4\text{-DCP}}$ for various pH levels and soil concentrations. 49

Figure 4.2b. $TR_{2,4\text{-DCP}}$ as a function of pH levels and soil concentration.....49

Figure 4.3a. $SR_{2,4\text{-DCP}}$ for varying soil concentrations for doses of SBP ranging from 0.25 to 2 units/mL.....53

Figure 4.3b. $TR_{2,4\text{-DCP}}$ for varying soil concentrations.....53

Figure 4.4a. $SR_{2,4\text{-DCP}}$ versus SBP dose for varying soil concentrations. 55

Figure 4.4b. $TR_{2,4\text{-DCP}}$ versus SBP dose for varying soil concentrations. 55

Figure 4.5a. $SR_{2,4\text{-DCP}}$ versus soil for doses of SBP ranging from 0.001 to 0.1 units/mL.....56

Figure 4.5b. $TR_{2,4\text{-DCP}}$ versus soil for doses of SBP between 0.001 and 0.1 units/mL 56

Figure 4.6a. $SR_{2,4\text{-DCP}}$ versus SBP dose for 0, 25, and 50 g/L of soil. 60

Figure 4.6b. $TR_{2,4\text{-DCP}}$ versus enzyme dose for 0, 25, and 50 g/L of soil. 60

Figure 4.7a. $SR_{2,4\text{-DCP}}$ for pH 3 to 9.....61

Figure 4.7b. $TR_{2,4\text{-DCP}}$ versus pH for various soil concentrations. 61

Figure 4.8a Percentage of 100 mg/L 2,4-DCP remaining versus SBP concentration for various concentrations of PEG8000, at pH 6.2. (from Alemany, 2000).....62

Figure 4.8b. Soluble 2,4-DCP remaining versus soil concentration.....62

Figure 4.9a. $SR_{2,4\text{-DCP}}$ for 0 g/L soil for various SBP doses.....64

Figure 4.9b. $SR_{2,4\text{-DCP}}$ for 12.5 g/L soil for various SBP doses.....64

Figure 4.9c. $SR_{2,4\text{-DCP}}$ for 25.0 g/L soil for various SBP doses.....64

Figure 4.9d. $SR_{2,4\text{-DCP}}$ for 37.5 g/L soil for various SBP doses.....64

Figure 4.9e. $SR_{2,4\text{-DCP}}$ for 50.0 g/L soil for various SBP doses.....64

Figure 4.10a. The EC80 as a function of soil concentration.....66

Figure 4.10b. The EC50 as a function of soil concentration.....66

Figure 4.11. $SR_{2,4\text{-DCP}}$ as a function soil concentration for various hydrogen peroxide dose. 67

Figure 4.12. $SR_{2,4\text{-DCP}}$ as a function of soil concentration for various hydrogen peroxide dose. 67

Figure 4.13a. The percent remaining of 100 mg/L 2,4-DCP with time after activation of 1 unit/mL SBP at 22°C with 0.613 mM H_2O_2 at pH 6.2. (from Alemany, 2000) 69

Figure 4.13b. The percent soluble 2,4-DCP remaining as a function of time at 22°C with soil. 69

Figure 4.14b. The percent remaining of 100 mg/L 2,4-DCP with time after the activation of 1 unit/mL SBP with 0.613 mM H_2O_2 at 4°C, and pH 6. (from Alemany, 2000) 71

Figure 4.14b. The percent soluble 2,4-DCP remaining as a function of time at 4°C with soil in the reactor. 71

Figure 4.15. $SR_{2,4\text{-DCP}}$ from supernatant at pH 6 for various additions of PEG.....74

Figure 4.16. $SR_{2,4\text{-DCP}}$ as a function of soil at pH 8 for various additions of PEG.....75

Figure 4.17a. $SR_{2,4\text{-DCP}}$ as a function of soil concentration.....77

Figure 4.17b. $TR_{2,4\text{-DCP}}$ for various concentrations of soil. 77

Figure 5.1a. Removal of 2,4-DCP for various pH and soil concentrations assuming reversible sorption of 2,4-DCP.....83

Figure 5.1b. Removal of 2,4-DCP for various pH and soil concentrations assuming irreversible sorption of 2,4-DCP.....83

Figure 5.2a. Removal of 2,4-DCP for various pH and SBP concentrations assuming reversible sorption of 2,4-DCP.....85

Figure 5.2b. Removal of 2,4-DCP for various pH and SBP concentrations assuming irreversible sorption of 2,4-DCP.....85

Figure 5.3a. Removal of 2,4-DCP versus SBP concentrations assuming reversible sorption of 2,4-DCP.....86

Figure 5.3b. Removal of 2,4-DCP versus SBP concentrations assuming irreversible sorption of 2,4-DCP.....86

Figure 5.4a. Removal of 2,4-DCP versus low SBP concentrations assuming reversible sorption of 2,4-DCP.....88

Figure 5.4b. Removal of 2,4-DCP versus low SBP concentrations assuming reversible sorption of 2,4-DCP.....88

ABBREVIATIONS

a	Langmuir constants
α	Variable
b	Langmuir constants
β	Variable
C_e	Concentration of effluent (mg/L)
C_s	Concentration of substrate sorbed onto the soil (mg/mg)
C_{sb}	Concentration of 2,4-DCP removed by sorbing (mg/L)
C_i	Initial concentration of phenol (mg/L)
CSTR	Continuously stirred tank reactor
EC	Enzyme concentration (units/mL)
EC_{SBP}	Soybean peroxidase concentration (units/mL)
EC_{min}	Enzyme concentration required to achieve 95% removal of target phenol (units/mL)
ECX	Enzyme concentration required to meet X% removal of target phenol (units/mL)
FR	Fenton's reagent
HPLC	High pressure liquid chromatograph
HRP	Horseradish peroxidase
HRT	Hydraulic retention time
H_2O_{2max}	Hydrogen peroxide dose resulting in maximum activity (mM)
k	Proportionality constant
O*	Free radical
OH*	Hydroxy radical

PEG	Polyethylene glycol
PEG_{min}	Polyethylene glycol dose required to achieve minimum of 95% phenol removal (g/L)
PEG1000	Polyethylene glycol with a molecular weight of 1000 g/mol
PEG3350	Polyethylene glycol with a molecular weight of 3350 g/mol
PEG8000	Polyethylene glycol with a molecular weight of 8000 g/mol
Q	Mass of sorbate per mass of sorbent
S	Substrate
S*	Substrate radical
SBP	Soybean peroxidase
SBPi	Intermediate form of soybean peroxidase
SBPii	Intermediate form of soybean peroxidase
SBPiii	Inactive form of soybean peroxidase
$SR_{2,4\text{-DCP}}$	Percent soluble removal of 2,4-DCP
$TR_{2,4\text{-DCP}}$	Percent total removal of 2,4-DCP
$TRM_{2,4\text{-DCP}}$	Percent total remediation of 2,4-DCP
2,4-DCP	2,4-Dichlorophenol

Chapter 1

Introduction

In the past, various peroxidase enzymes from horseradish (HRP), *Coprinus macrorhizus*, and *Arthromyces ramosus* have been shown to be effective in the treatment of chlorinated phenols in aqueous solution (Klibanov et al., 1981; Nakamoto and Machida, 1992; McEldoon et al., 1996; Dec and Bollag, 1994a; Buchanan and Nicell, 1996). Currently the use of enzymes to remediate chlorinated phenols has focused on remediation of wastewater systems. More recently soybean peroxidase (SBP) has been found to be an effective enzyme in the treatment of chlorinated phenols in aqueous solution (McEldoon et al., 1995; McEldoon et al., 1996; Nicell and Wright, 1998; Wu et al., 1998; Alemany, 2000). Despite the very effective nature of peroxidase enzymes and their advantages over conventional treatment, very few applications outside of aqueous systems have been investigated.

An alternate application is the addition of enzymes to soil systems, either small concentrations of soil in wastewater, soil slurries, or *in situ* remediation. Remediation of contaminated soil is an $ 8 billion industry in North America (Icpet, 2001), hence this application of enzymes should not be ignored. However, little is known about the soil-enzyme interaction with the addition of soil to wastewater reactors. Some of the potential problems which face the use of enzymes to remediate soil systems follow. Firstly, remediation efforts may be hindered by the sorption of contaminants, hydrogen peroxide and/or enzymes to the soil. Secondly, excess hydrogen peroxide may be required to overcome sorption, or degradation due to interactions with organics in the soil, which may result in the accelerated inactivation of SBP. Thirdly the potential toxicity of the

end product polymer is of concern. The toxicity of the end product polymer is a product of the reaction between the toxic parent compound and the free radicals produced by the peroxidase. Finally, the high cost of enzyme treatment is problematic, although costs may be reduced if optimum design and application conditions are employed (Alemany, 2000).

Despite these potential problems, promising research with humic acid has shown that enzyme remediation may be possible in the presence of organic compounds, with either enhanced, similar or reduced transformation (Park et al., 1999). The application of SBP for remediation of actual soil systems is not understood. The potential for the remediation of soil systems with SBP is enormous. Chlorinated phenolic compounds are important toxic compounds which have widespread use in many industrial applications. They have potential to contaminate wastewater, soil, and groundwater. SBP has shown an ability to remediate wastewater under a large range of conditions and substrate. Its presence causes the reaction to proceed very quickly. If SBP can maintain these characteristics in soil systems, the use of SBP for treatment of contaminated soil systems becomes a viable option. Applications of enzyme to soil systems which may be explored are the enzymatic remediation of groundwater, soil slurries, ex situ, and in situ remediation.

This following research focused on the treatment of 2,4-DCP contaminated water with SBP in the presence of soil. Experiments with SBP treatment for the removal of 2,4-DCP with and without soil present will be investigated to determine if the potential exists for the use of SBP to remediate wastewater contaminated with 2,4-dichlorophenol (2,4-DCP) in the presence of soil.

Layout of the Thesis

Chapter 2 presents a literature review discussing the application of SBP to wastewater contaminated with 2,4-DCP, as well as potential problems that may be encountered with the use of SBP in soil systems. Optimum conditions are discussed, as well as the potential impact of soil on these variables.

Materials and methods are presented in chapter 3. The remainder of chapter 3 is investigates the sorption of 2,4-DCP to soil. Chapter 4 presents the results of the experiments involving the application of SBP to water contaminated with 2,4-DCP with soil present. The removal of 2,4-DCP with SBP is examined under various conditions. This section takes a "black box" approach to the removal of 2,4-DCP by focusing on the removal of 2,4-DCP from the supernatant and not specifically the enzymatic removal from the reactor. Chapter 5 attempts to investigate the enzymatic removal by making assumptions about the fate of the end product. The final mass remaining in the reactor is calculated using the assumptions of both reversible and irreversible conditions to draw conclusions regarding the activity of the SBP.

Chapter 6 presents the overall conclusions and recommendations for future research.

Chapter 2

Literature Review

2.1 Peroxidase Enzymes

2.1.1 Description of Peroxidase Enzymes

Peroxidases are heme proteins found in many living things such as plants, mold, bacteria and microorganisms (Dunford and Stillman, 1976). Enzymes are either simple (composed solely of amino acids), or conjugated (consisting of amino acid and a prosthetic group, a non-amino acid component). The catalytic abilities of enzymes are located in a relatively small section of the enzyme called the active site. The active site is where the enzymatic treatment of the contaminant occurs. Part of the active site is the binding site, where the enzyme and the contaminant are physically bonded to one another. A second part of the active site is the catalytic site where the enzyme reacts with the contaminant.

Peroxidase is an enzyme that is activated in the presence of hydrogen peroxide. The activated peroxidase catalyzes the oxidation of many organic and inorganic substances. The selectivity of an enzyme to oxidize various substrates is a function of the protein matrix structure surrounding the prosthetic group (Banci, 1997) and the shape of the active site.

2.1.2 Kinetics of Peroxidase Enzymes

The heme found in the enzyme consists of iron ions, located in the prosthetic group, with an oxidative state of +3. The catalytic oxidation of compounds is a three-step process. The first step is the oxidation of the ferric enzyme (heme[Fe(III)$_{(PX)}$) to water and Compound I. Compound I is a protein which contains an oxyferryl (Fe(IV)=0) center and an organic cation radical. In the next step, Compound I reacts with a molecule of substrate (S) to oxidize the substrate molecule to a substrate radical (S*) and Compound II is formed. Compound II then reacts with another substrate molecule to become reduced to the original ferric enzyme (heme[Fe(III)$_{(PX)}$), and the substrate molecule is oxidized to a substrate radical. The catalytic cycle is given by equations [1] to [3] (Banci, 1997) and shown in figure 2.1.

$$\text{heme[Fe(III)]}_{(PX)} + H_2O_2 \rightarrow \text{heme[0=Fe(IV) - R}^{+}\text{*]}_{(\text{comp I})} + H_2O \qquad [2.1]$$

$$\text{heme[0=Fe(IV)-R}^{+}\text{*]}_{(\text{comp I})} + S \rightarrow \text{heme[0=Fe(IV)]}_{(\text{comp II})} + S^* \qquad [2.2]$$

$$\text{heme[0=Fe(IV)]}_{(\text{comp II})} + S \rightarrow \text{heme[Fe(III)]}_{(PX)} + S^* \qquad [2.3]$$

The substrate radicals then form insoluble polymers, as a result of their low solubility, which settle from the solution. These polymers may be removed via filtration or sediment collection.

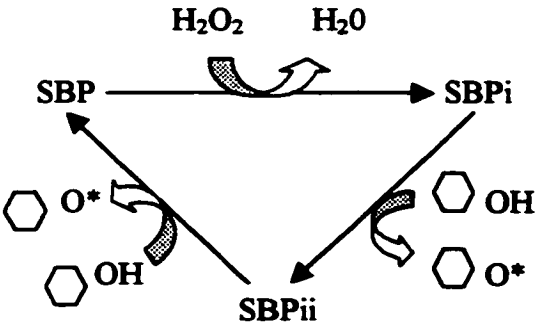


Figure 2.1 The catalytic cycle for soybean peroxidase (adapted from Buchanan and Nicell, 1996; Flock et al., 1999)

2.1.3 End-Product Polymers

The feasibility of contaminant removal from both wastewater and soil through enzyme use depends on the end-products of the reaction. Ideally the procedure should destroy the toxin as opposed to altering its phase (Aitken, 1993). The basic characteristics which should be examined to determine the feasibility for use both in aqueous and soil environments is the toxicity, partitioning, and ultimate biodegradability of the reaction products (Aitken, 1994).

The toxicity of the products of the reaction is dependent on both the parent compound and the enzyme used to treat them. Some of the parent compounds tested had reduced toxicity, while the toxicity of others increased regardless of the enzyme (Heck et al., 1992). Although the end product polymers of soybean peroxidase have not been examined, it seems more than likely that SBP will not be an enzyme that universally reduces the toxicity from that of the target parent compound.

This increase in toxicity poses little problem for batch reactors where the insoluble polymer is easily removed from solution and the sediment can be properly disposed. But in natural systems increased toxicity in the form of insoluble sediment

is unacceptable and caution must be exercised such that the best enzyme is used and toxicity is reduced. For contaminants where no enzyme has been found to reduce toxicity, this treatment may not be an option.

At this point it remains inconclusive as to the characteristics of the polymer formed when soybean peroxidase is used as the enzyme. This may create problems for remediation of soil and soil slurries as the insoluble polymers could be virtually impossible to separate and/or remove. Therefore the feasibility of enzyme treatment for contaminated soil is dependent on the toxicity of the polymer formed. For other systems such as streams, lakes, and rivers, additional problems may be encountered due to the addition of these polymers (both suspended and settled) that may be detrimental to aquatic life.

2.1.3.1 Advantages and Disadvantages of Enzyme Treatment

There are many advantages to using enzymes over conventional treatment methods. As well a wide range of operating conditions are possible with remediation occurring at extreme temperature, pH, salinity levels, and high or low concentrations. The advantages over microbial treatment are numerous: remediation of water which is toxic to microorganisms; no concern for shock loading; no delays as a result of biomass acclimatization; and no generation of biomass (Nicell et al., 1992).

Conventional treatment available for phenol removal has been solvent extraction, microbial degradation, adsorption on activated carbon, and chemical oxidation. Disadvantages included high cost, toxic by-products, operation only in small concentration ranges, and limited removal abilities (Klibanov et al., 1983).

There are two main disadvantages to enzyme treatment: the high cost of the enzyme required to treat the contaminant, and the uncertainty of the toxicity of the end product polymer. The use of enzymes may not be feasible when there is a variety of pollutants present and their reactants vary in toxicity. Continued research is required to determine the best enzyme for each type of contaminant.

2.1.3.2 Economic Feasibility of Soybean Peroxidase

Despite the promising results peroxidases have shown in the laboratory, the costs of these enzymes for use in larger scale projects have been prohibitive. Much of the expense is a result of the high cost of the enzyme itself. An alternative to purchasing enzymes from chemical suppliers is to purée the original form of the enzyme (i.e. horseradish), although costs still exceed conventional treatment methods, (due to the high cost of raw horseradish). The advantage of SBP is that the enzyme is found in the seed coat, which is a by-product of the soybean industry and can be utilized without reducing the value of the soybean's oil and meat. Since there is currently no market for this by-product it is difficult to estimate an associated cost, but it could be assumed to be very inexpensive, as it is currently considered to be refuse. Recent research indicates the use of crude soybean hulls to be extremely efficient (Flock et al., 1999).

A reduction in cost could also be achieved by increasing the peroxidase activity in the seed coat, increasing the enzyme catalytic lifetime, or the number of turnovers. The raw soybean seed coat may contain zero to five percent peroxidase by dry weight, although genetically engineered soybean plants have been shown to contain much higher levels of the peroxidase enzyme (Goetz, 1996). Increasing the catalytic lifetime can be

accomplished by minimizing adverse operating conditions that result in the inactivation of the enzyme. Through research, optimum conditions for the enzyme can be determined. (Trombly, 1995)

2.1.3.3 Inactivation of Peroxidase Enzymes

There are several mechanisms by which peroxidase enzymes become inactivated. As a result of inactivation of the enzyme, larger quantities of the enzyme are required which dramatically increase the cost of treatment. The mechanisms for inactivation include thermal, production of compound III with excess hydrogen peroxide or polymerization of the enzyme.

There are various conditions for which enzymatic activity is decreased. The ideal temperature range varies depending on the thermal stability of each peroxidase. At temperatures above this range inactivation may occur rapidly.

Nicell et al. (1993) found that the number of turnovers the enzyme exhibited was higher when the reaction occurred at lower temperatures. They hypothesized that the high temperature is not inactiving the enzyme, but that at lower temperatures the reaction proceeds at a slower rate. This results in a lower concentration of substrate radicals, thus reducing the probability of free radicals binding to the active site and rendering the enzyme inactive.

Enzyme inactivation may result from the presence of excess hydrogen peroxide. If excess H_2O_2 is available in the solution then Compound II may react with the excess H_2O_2 to form Compound III, an inactive verdohaemoprotein (Arnao et al., 1990) rather than returning to the original form of the enzyme (figure 2.2). Although Compound III is

inactive, it may spontaneously decompose back to the original form of the enzyme. However this spontaneous return to the enzyme is very slow and the overall catalytic ability of the enzyme is hampered (Arnao et al., 1990). Hence the amount of catalyst is reduced and the required quantity and cost of the enzyme is increased for the removal of the same contaminant as would be removed if the optimum amount of H_2O_2 was used. It is important that this optimum be known as too little hydrogen peroxide will limit the reaction, and too much will inactivate the peroxidase. Optimization of this and other variables increases the economic feasibility of enzymatic treatment.

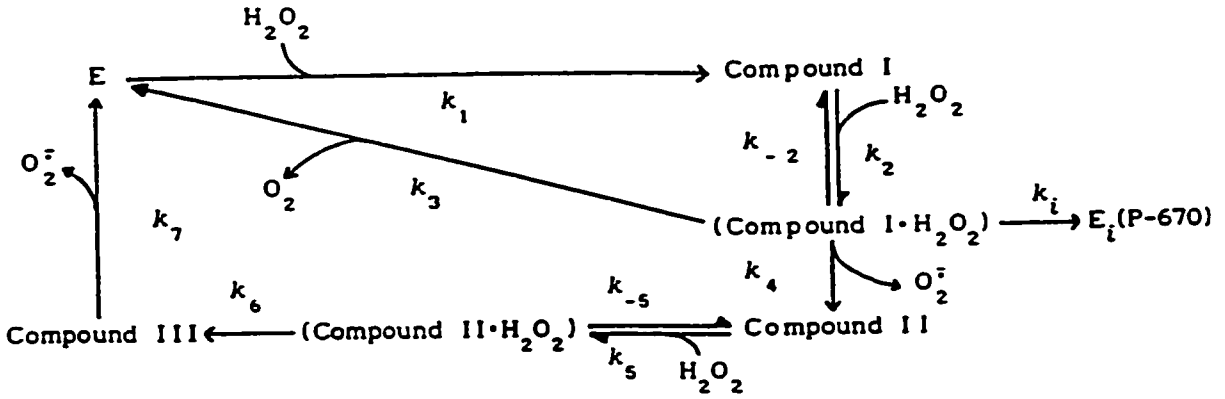


Figure 2.2 The catalytic cycle and inactivation of Compound II to Compound III. (from Arnao et al., 1990)

Mechanism-based inactivation occurs when peroxidase enzyme becomes inactivated as a result of becoming entrapped in the insoluble polymer product or the binding of the phenoxy radical to the active site as a result of the reaction. Thus there is no active site for the substrate to enter and be consumed. Several studies compared the peroxidase activity in the solution to that in the supernatant after centrifuging. The activity was found to be higher in solution than in the supernatant alone (Nakamoto and Machida, 1992; Wu et al., 1998). This indicated that during initial entrapment of the

enzyme it still retains some enzymatic activity, but as polymerization continues the active site becomes increasingly difficult to access and inactivation occurs (Nakamoto and Machida, 1992).

This type of polymerization inactivation can be limited by the addition of proteins or hydrophilic synthetic polymers. Addition of a polymer also has the ability to allow for a reduction of enzyme dose. An additive such as polyethylene glycol is a high molecular weight compound and provides protection from mechanism based inactivation of the enzyme. The best additive was found to be polyethylene glycol (PEG), in particular a PEG+ with a molecular weight exceeding 1000 g/mol (Nakamoto and Machida, 1992). PEG with higher molecular weights did not drastically increase the peroxidase activity (5% increase in removal was seen between a PEG with a molecular weight of 1000 and 7500 g/mol) (Nakamoto and Machida, 1992). However a recent study has shown more dramatic increases in peroxidase activity with higher molecular weight PEG (Alemany, 2000). Alemany (2000) found that the removal of 2,4-DCP with PEG3350 was 23% to 65% higher than control experiments without PEG. With PEG8000 the removal of 2,4-DCP was 65% to 84% higher than control experiments without PEG. At low molecular weights (less than 1000 g/mol) it was found that activity of the enzyme was not well preserved (Nakamoto and Machida, 1992; Wu et al., 1998) or the activity of the peroxidase (for molecular weight of 300 g/mol) decreased (Wu et al., 1998). The addition of PEG has been shown to reduce the required enzyme dose up to 1/200 of the original dose required to achieve the same removal of chlorophenol (Nakamoto and Machida, 1992). The addition of PEG and resulting reduction in enzyme dose may result in reducing the cost to 0.5% of that required without PEG.

2.1.4 Soybean Peroxidase vs. Horseradish Peroxidase

In the past numerous studies have been performed which have shown that HRP has the ability to oxidize a large number of aromatic compounds, exhibiting upto 99% removal over a very short contact time (Klibanov et al., 1983; Wu et al., 1993; Nicell et al., 1993). More recently studies have examined the use of SBP as an alternative to HRP due to its superior stability.

Soybean peroxidase is much more stable than horseradish peroxidase in the presence of high levels of H_2O_2 (Wright and Nicell, 1999), and at pH levels below 3, which seems to be the result of soybean peroxidase having a stronger bond with its heme group (McEldoon et al., 1996). Results have shown that SBP retained over 50% of its activity at a pH of 3.0, while HRP was completely inactivated. Additional experiments with SBP and HRP examined the oxidation of veratryl alcohol. While SBP treatment was optimal at pH 2.4, HRP was inactivated very quickly as an effect of the acidic environment it was in (McEldoon et al., 1996). The pH range for HRP has shown excellent contaminant removal between a pH of 6 to 9 and reduced removal between 4 to 6 and 9 to 10 (Nicell et al., 1992; Bewtra et al., 1995) with optimum removal at a pH of 8 (Bewtra et al., 1995). At pH levels below 2 and above 11 no catalytic ability or removal occurred with HRP (Nicell et al., 1992), it is possible that these pH levels lead to the loss of the prosthetic group containing the enzyme (McEldoon et al., 1995). Alemany (2000) reported a minimum of 80% 2,4-DCP removal between pH 2.5 and 9.2 with a 1 unit/mL dose of SBP.

SBP has also been found to be more thermally stable, allowing reactions to occur at higher temperatures without experiencing thermal inactivation. For HRP the ideal temperature range is 5°C to 35°C, with rapid inactivation occurring above 45°C (Nicell et al., 1992; Bewtra et al., 1995). SBP essentially exhibits no inactivation at temperatures below 75°C, while HRP began to experience inactivation at 65°C (figure 2.3) (McEldoon et al., 1996). Additionally, SBP remains thermally stable at high temperatures even when high temperatures are combined with acidic conditions (i.e., 60°C at pH of 2.4) (McEldoon et al., 1995).

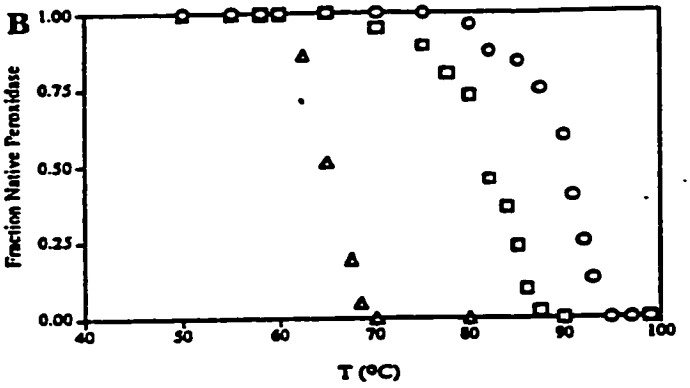


Figure 2.3 Thermal denaturing of soybean (o), horseradish (□), and C. *cinereus* (Δ) Peroxidases. 0.1mg/mL SBP in 25 mM Tris-HCL buffer (pH 8) with 1mM $CaCl_2$. (McEldoon et al., 1996)

The advantage of HRP is that it appears to oxidize phenols at a rate an order of magnitude faster than SBP (Wright and Nicell, 1999). While the HRP reaction may reach completion quicker than SBP, SBP reactions are still very fast. Alemany (2000) reported 99.6% removal of 100 mg/L 2,4-DCP, with a dose of 1 unit/mL SBP, occurred

within one minute at 22°C. At 4°C the reaction took twenty minutes to remove 95% of 100 mg/L 2,4-DCP, and 180 minutes to reach 99.6% removal of 2,4-DCP.

Overall the advantage of SBP as a result of its stability under extreme temperature, pH, and high hydrogen peroxide concentrations may lie in its ability to remediate sites whose conditions do not fall in the range of optimum conditions for alternative peroxidases or treatment procedures. This may lead to the development of a remediation tool with commercial and environmental applications (McEldoon et al., 1996).

2.2 Soil Systems

2.2.1 Partitioning of Contaminants and Enzymes

An aqueous solution when exposed to solid particles becomes a system of two phases. It is important to determine if the contaminants and enzymes within the solution remains in the aqueous phase or if they sorb to the solids. This is referred to as the partitioning of the substance. Partitioning coefficients can be determined by examining the distribution of the substance between the solid and aqueous phase.

Three classifications of sorption are: electrostatic, chemical, and physical. Electrostatic sorption occurs as a result of opposite charges on the compound and the solid. Chemical sorption is very characteristic of chemical bonds. Physical sorption is the bonding that results from hydrogen bonding and Van der Waals forces. Sorption is a function of the properties of the solid, and the properties of the compound in the aqueous phase (the liquid phase may also alter the sorption). The solid or sorbent properties are influenced by its hydrophobicity, and specific surface area. Hydrophobic, or nonpolar

sorbents, tend to sorb compounds much more readily than polar sorbents. Additionally, increased surface area provides more sorption sites for contaminants. Hydrophobic compounds or sorbates in solution prefer to sorb to the solid phase.

Chloronated phenols are strongly or irreversibly bonded to both organic and inorganic solids (Isaacson and Frink, 1984). For reversible bonds, the strength of the bond increases with time. One mechanism that may be responsible for the strengthening of the bonds is migration of contaminants into micropores (Watts, 1998).

The composition of the sorbent may vary considerably from silica to clay minerals. Higher organic content of solids increases the sorption of contaminants to solid particles (Isaacson and Frink, 1984). The clay content of a soil can also affect the sorption, as clay has an extremely high specific surface area and is negatively charged which effects the transport of chemicals. (Watts, 1998)

Since the source of organic carbon is at the surface of the soil layer, the organic carbon decreases exponentially with respect to depth (figure 2.4), therefore the sorption to the solid phase will decrease with depth if the organic carbon is the primary sorbent.

The importance of sorption in remediation processes is two-fold. Firstly, the sorption of contaminants to solid particles influences the transport or migration of the contaminant or enzyme. It is important that the enzyme is able to penetrate to the contamination area. Additionally it is preferable that the contaminant does not migrate creating larger volumes of soil requiring remediation. Secondly, once the contaminant has sorbed to the soil its treatability by biochemical or physicochemical may be reduced. Consequently sorption impacts the design of remediation strategies (Watts, 1998).

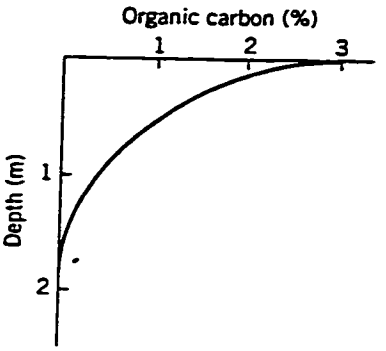


Figure 2.4. Carbon content with respect to depth of soil for a highly organic soil. (Watts, 1998)

The outcome of the addition of enzymes to soils is unknown, although it is hypothesized that enzymes may sorb to the soil (Aitken, 1993), resulting in an inability to distribute the enzymes throughout the contaminated soil. Additionally there is concern over the inactivation of enzymes once sorbed to the soil (Park et al., 1999). The organic components have the potential to compete with the phenol for the enzyme's active site, which may permanently block the active site and render the enzyme inactive (Park et al., 1999). Aitken (1993) postulated that despite the obstacles of enzymatic remediation of contaminated soil, this method may surpass microbial treatment as enzymes may have increased access to micropores and voids where microorganisms do not. Extensive research for each enzyme and soil environment would need to be undertaken to determine the feasibility for enzyme treatment at each contaminated site.

2.2.2 Fenton's Reagent

Fenton's reagent has been found to oxidize various contaminants in the aqueous phase and more recently in soil or soil slurries (Ravikumar and Gurol, 1994; Marten and

Williams, 1995; Watts et al., 1993). Fenton first used the mixture of hydrogen peroxide and ferrous salts in 1894. The process is quite similar to peroxidase treatment and involves the generation of hydroxyl radicals, OH*, through the degradation of H_2O_2 by an iron (II) catalyst (equation 2.4). Approximately 2 to 10 moles of hydrogen peroxide are required to degrade 1 mole of substrate (Watts et al., 1993).

$$H_2O_2 + Fe^{2+} \rightarrow OH^* + OH^- + Fe^{3+} \qquad [2.4]$$

The hydroxyl radical then reacts with the organic contaminant to degrade it. This process is quite similar to enzyme treatment and the problems encountered with Fenton's reagent may indicate potential problems or obstacles for enzyme use in the presence of soil.

2.2.2.1 Fenton's Reagent as an Indicator for the Feasibility of Enzymatic Treatment

The use of Fenton's reagent for remediation of chlorophenols in a two phase system of sand and water has shown some encouraging results (Ravikumar and Gurol, 1994; Watts et al., 1993). When phenol was added to soil and treated with iron and high concentration of H_2O_2 (Fenton's reagent) pentachlorophenol (PCP) was reduced to less than 5 mg PCP/kg soil from 250 mg PCP/kg soil in 24 hours (Watts et al., 1993). Unfortunately a high concentration of H_2O_2 (693 moles H_2O_2 consumed/mole PCP degraded) was necessary for treatment of a 10% mixture of goethite in silica sand contaminated with PCP. The higher concentrations of H_2O_2 were required as lower concentrations did not oxidize the particulate and sorbed contaminants. It was hypothesized that the higher concentrations of H_2O_2 were necessary to generate high

concentrations of hydroxyl radicals, which are then able to actively oxidize the particulate and soluble matter (Watts et al., 1993). Indications are that the requirement of a high concentration of H_2O_2 and hydroxyl radicals will promote the inactivation of the SBP.

Kakarla and Watts (1997) examined the relationship between the stability of H_2O_2 and the depth of soil in a column. The instability of H_2O_2 is a result of interactions with organic and inorganic reactants, which lead to the decomposition of hydrogen peroxide. Kakarla and Watts (1997) showed that H_2O_2 without the addition of a stabilizer was consumed within the top 2 to 4 cm of soil. When the H_2O_2 was accompanied by a stabilizer ($KHPO_4$ showed the best stabilization results) the H_2O_2 was detectable at three times the depth the unstabilized H_2O_2 reached. The depth to which H_2O_2 was detectable was also increased for cases where the soil had a high permeability and low iron content. This study used a silt loam with an average iron concentration of 35.2 mg/g soil.

Ravikumar and Gurol (1994), investigated the degradation of H_2O_2 with increasing depth using silica sand and glass beads. Silica sand with a low iron content of 0.8 mg/g showed that 40% of the hydrogen peroxide remained at a depth of 90 cm, and there was no H_2O_2 degradation in the columns containing only glass beads. When naturally occurring iron was present in silica sand or glass beads were spiked with ferrous salts, then the decomposition of H_2O_2 was a function of the concentration of ferrous salt. The application of H_2O_2 to soil systems may result in Fenton's reagent if there is iron available in the soil (Ravikumar and Gurol, 1994).

The inability for H_2O_2 to penetrate deeply into the soil as a result of permeability and iron content indicates that in-situ remediation may prove to be difficult. Since

hydrogen peroxide is required for peroxidase treatment, remediation of chlorophenol contaminated soil may not be feasible or the feasibility may be directly related to the soil characteristics such as the permeability and iron content. It appears that the peroxidase enzyme and the naturally occurring iron in soil may compete for the hydrogen peroxide. Therefore in a two phase system of soil and water, peroxidase reactions and Fenton's reaction may be occurring simultaneously and competing for H_2O_2.

Even if all indications for enzyme treatment in soil or aqueous solution in the presence of soil were promising, it may be difficult to apply lab results to the field. Most research has indeed indicated that Fenton's reagent shows promise for remediation, but these results are based on the contaminant being exposed to soil for a relatively short duration compared to field conditions. Since the strength of bonds between soil and phenols increase with time, it appears that removal of phenol contamination in the field may be increasingly difficult as a result of increased contact time.

2.2.3 Additional Problems which may be Encountered

The pH of the soil may affect the pH of the reactor, indicating that manipulation of pH may be necessary to keep operating at optimum conditions. As for temperature, the optimum range is not likely to be exceeded as soil is usually quite cool, but may be substantially below the ideal temperature. Nicell et al. (1992), report significant reductions in contaminant removal as the temperature decreases below 65°C. Alemany (2000) documented a 180-fold increase in time required to achieve 99.6% removal of 2,4-DCP with a temperature drop from 22 to 4°C. In-situ remediation of soil with enzymes

may be difficult for contaminants near the surface during the colder months, when the ground may be frozen, or throughout the year in regions of permafrost.

2.2.4 Promising Indications

Recent research has examined the behavior of transformation of phenols in the presence of humic components (humic acids) and peroxidase enzymes (Dec and Bollag, 1994b; Park et al., 1999). This research was conducted on reactors containing humic acid, peroxidase enzymes, and phenols. Humic components can be used as indicators for the behavior of organic matter and humic material found in soil. Results from the study indicate that the humic components have the ability to enhance, diminish or unaffect the transformation of phenols (Table 2.1) (Park et al., 1999). The mechanism governing this change in transformation occurs after the initial production of free radicals. The free radicals then rather than sorbing with each other may bind covalently to the humic component (Bollag, 1992; Park et al., 1999). It is interesting to note the humic components listed in table 2.1. The enhanced transformations generally appear to be limited to larger molecules, while the smaller particles tend to exhibit reduced transformation. The higher molecular weight organic components have characteristics similar to PEG. The enhanced transformations for larger molecules may be the result of the humic components acting as PEG.

Table 2.1. The affect of humic components on the transformation of 4-chlorophenol by peroxidase (from Park et al., 1999)

Humic constituent	Molecular structure	Transformation of 4-chlorophenol (%)
None (control)		32.3 ± 2.5
	Enhanced Transformation	
Syringaldehyde	2(OCH_3), 4(CHO), 6(OCH_3)	82.0 ± 3.1
Ferulic acid	2(OCH_3), 4(COOH-CH=CH-)	71.8 ± 8.7
Guaiacol	2(OCH_3)	56.1 ± 0.9
Vanillic acid	2(OCH_3), 4(COOH)	62.8 ± 3.8
2,6-dimethoxyphenol	2(OCH_3), 6(OCH_3)	73.0 ± 4.9
Vanillin	2(OCH_3), 4(CHO)	74.2 ± 6.6
Phloroglucinol	3(OH), 5(OH)	47.9 ± 2.9
	No Effect on Transformation	
4-hydroxybenzoic acid	4(COOH)	36.5 ± 2.2
Salicylic acid	2(COOH)	34.8 ± 2.1
Syringic acid	2(OCH_3), 4(COOH), 6(OCH_3)	32.0 ± 2.1
Protoctechuic acid	2(OH), 4(COOH)	30.1 ± 7.3
Caffeic acid	2(OH), 4(COOH-CH=CH-)	29.8 ± 0.3
	Reduced Transformation	
Catechol	2(OH)	11.1 ± 0.7
Gallic acid	2(OH), 4(COOH), 6(OH)	16.3 ± 1.7
Hydroquinone	4(OH)	21.9 ± 0.8
4-methooxyphenol	4(OCH_3)	7.2 ± 1.2

With the sorption of contaminants, there is a decrease in the interaction of the contaminant with the surrounding environment, as well as immobilizing the toxin and reducing the potential for leaching into the groundwater and surrounding aquatic systems (Bollag, 1992). Once the contaminant has bonded to the humic component, then the contaminant may be slowly released into the environment at levels which should not be considered dangerous as they are mineralized to CO_2 or will once again bond with the humic components (Bollag, 1992). This mechanism is responsible for the transformation of naturally occurring phenols (Bollag, 1992). This naturally occurring mechanism may be enhanced through the catalytic abilities of oxidoreductive enzymes such as peroxidases (Dec and Bollag, 1994b).

One of the concerns previously discussed was the formation of the precipitate product due to the polymerization of the free radicals and the inability to remove the precipitate from the soil matrix. The oxidative coupling between free radicals and soil is proposed to eliminate the polymerization of free radicals and the resulting precipitate (Dec and Bollag, 1994b). This may eliminate significant problems associated with precipitate removal. This may also indicate that without polymerization products that enzyme inactivation due to the entrapment of the enzyme or the blocking of the active site by the polymer may no longer be a concern.

2.3 Research Conditions and Results

Through careful selection of variables, the use of SBP for treatment of chlorinated phenols may be optimized. The following section examines the results of previous research for treatment of phenol contaminated wastewater. Although the conditions may not be optimal for soil or soil slurries, they do give some starting strategies for research.

2.3.1 Substrate

Although various substrates may be used from veratryl alcohol to aromatic amines, phenols are perhaps the most important, as they are one of the most commonly and broadly used toxic organic compounds. The entry of phenols into the environment is usually associated with the release of synthetic materials, environmental decomposition or metabolism of halogenated aromatic compounds, or as a by-product of production. Phenols are among the largest polluters in effluent streams from coal conversion procedures (Boyd, 1982). Exposure to phenols may lead to the following health

problems; cardiac dysrhythmia, dermal necrosis, and increased liver enzymes (Watts, 1998). In 1980, the Chemical and Engineering News placed annual production of phenols at 1.25 billion kg (Boyd, 1982).

Phenols are the building blocks for many organic compounds. Phenol is a benzene ring with an attached hydroxyl group, as shown in figure 2.5. Phenol provides the structure for various synthetic organics such as chemicals used for agriculture, dyes, textiles, resins, plastic, iron and steel production (Boyd, 1982; Bewtra et al., 1995).

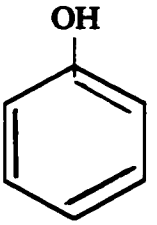


Figure 2.5. Phenol, a benzene ring with attached hydroxyl group – OH

Boyd (1982) hypothesized that the main mechanism for sorption of phenols to soil was hydrogen bonding between the phenolic hydroxyl and the H-bonding sites on the soils organic matter or clay particles, see figure 2.6. This theory was strengthened when Boyd (1982) examined different phenolic compounds and found that the addition of a substituent to the phenol has a direct affect on the sorption properties. Different compounds affect the pH of the phenol, and the steric hindrance of H-bond formation. Additionally, the bonding of various isomers was examined and found that with an increased electron-donating ability the sorption increased as well. This suggests that

hydrogen bonds form between the soil and the phenol as a result of phenols role as a proton acceptor. (Boyd, 1982)

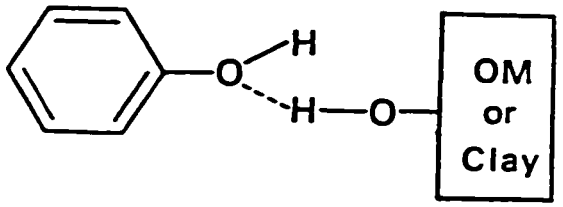


Figure 2.6. H-bonding between phenols and organic material or clay. (from Boyd, 1982)

Chlorinated phenols include the addition of chlorine molecules to the carbon on the phenol, shown in figure 2.7.

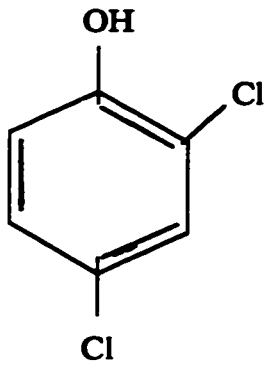


Figure 2.7. 2,4-dichlorophenol

Boyd (1982) found that the sorption of phenols to soil increased as the degree of chlorination increased. The Freundlich K value is an indication of contaminant sorption

to solids. As the Fruendlich K value increases the tendency to sorbed to the solid and organic soils increases. Boyd (1982) reported a Freundlich K value of 1.5 for monochlrorinated phenols to soil. Dichlorinated phenols had a Freundlich K value of 3.4, and trichlorinated phenols increased the Freundlich K value to 9.8.

2.3.2 Enzyme Dose

SBP is available as a pure salt-free dry powder, or can be mulched from soybean seed coat. The activity of soybean peroxidase is measured in units. Each unit of enzyme activity has the ability to degrade 1 micromole of phenol per minute for a specific set of conditions (pH of 7.4 and 25°C) (Nicell et al., 1992).

For a given set of conditions, the enzyme dose added to a reactor determines the percent removal from solution, however at some point there is little increase in phenol removal with a further increase in enzyme dose. On the other hand the number of turnovers or reactions catalyzed per enzyme unit will decrease once the optimum dose is reached. Enzyme levels should not exceed the optimum dose as inactivation increases with higher enzyme doses, most likely a result of the higher concentration of free radicals (Nicell et al., 1992). Research on soybean peroxidase dose at various pH levels has yielded results shown in figure 2.8.

The minimum dose of peroxidase to achieve 95% removal is given in units per mL, and is dependent on the concentration of phenol (C_{ph}) (Wu et al., 1993).

$$\text{Dose}_{min} = 0.0435 + 0.0048\, C_{ph} + 0.0031\, C^2_{ph} \qquad [2.5]$$

The optimum dose of enzymes in soil situations may exceed that for an aqueous reactor, as a result of enzyme sorbing to the soil, inactivation, or organics sorbing to the enzyme's active site.

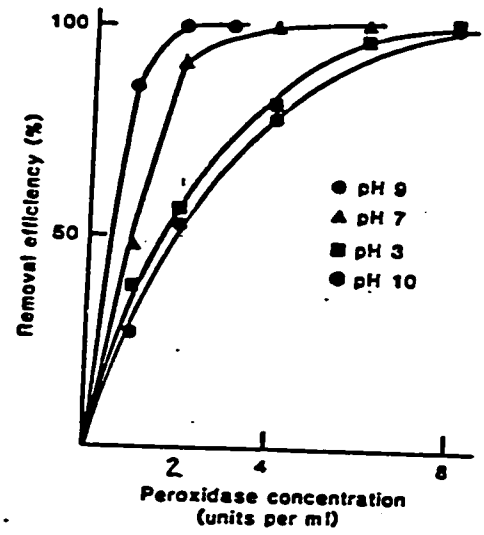


Figure 2.8. Removal efficiency as a function of peroxidase dose. (from Klibanov et al., 1983)

2.3.3 Hydrogen Peroxide Dose

Hyrdrogen peroxide is required to catalyse the reaction or oxidation of aromatic compounds. An insufficient dose results in H_2O_2 being the limiting reagent for the reaction, while excessive dosing will encourage inactivation of the enzyme (figure 2.9).

Nicell and Wright (1999), developed equation 4.2, based on regressions, for calculating the hydrogen peroxide concentration for various peroxidases which would result in maximum activity.

$$[H_2O_{2max}] = \left(\frac{\beta}{\gamma}\right)^{\frac{1}{2}} \qquad [2.6]$$

Where β and γ are variables. β and γ for soybean peroxidase 2.05×10^{-5} mol/L and 2.08×10^{2} $mol^{-1}L$ respectively, which yields a maximum hydrogen peroxide concentration of 3.31×10^{-4} mol/L for SBP. At concentrations less than H_2O_{2max} there is a decrease in activity as a result of hydrogen peroxide limiting the reaction. At concentrations greater than H_2O_{2max} there is also a decrease in activity as a result of excess hydrogen peroxide creating reactions which result in Compound III, an inactive form of the enzyme. (Wright and Nicell, 1999).

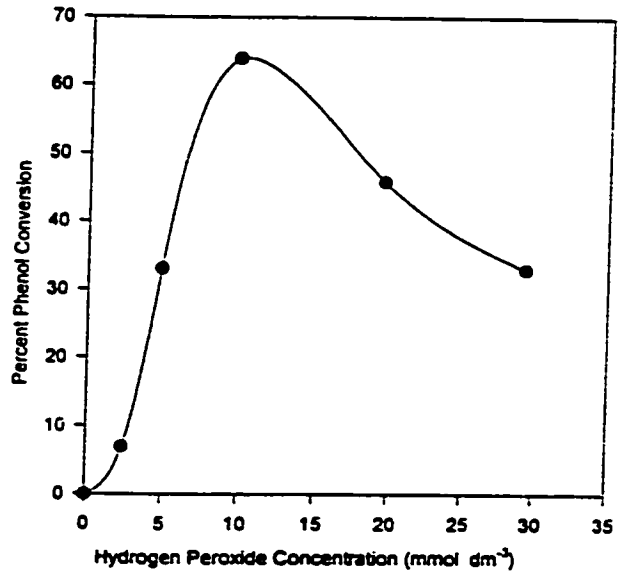


Figure 2.9. Phenol removal as a function of hydrogen peroxide. 1000 ppm Phenol, 40g soybean seed-hulls (from Flock et al., 1999)

Others have suggested a 1:1 molar ratio for hydrogen peroxide and phenol concentration (Wu et al., 1993). In addition the following relationship between H_2O_2 and the peroxidase concentration ratio has been suggested by Wu et al. (1994).

$$10 \leq [H_2O_2] / [\text{Peroxidase}] \leq 25 \qquad [2.7]$$

Fenton's Reagent processes indicate, an excess of H_2O_2 may be required to activate the enzymes in reactors containing soil, if any appreciable quantity of iron is present. Additionally excess H_2O_2 may be required to overcome any degradation of H_2O_2 due to interaction with soil organics.

2.3.4 Polyethylene Glycol Dose

The addition of PEG (a form of additive) prevents the inactivation of the peroxidase as a result of product polymerization, as opposed to inactivation by thermal condition, pH or hydrogen peroxide. The PEG protects the enzyme by forming bonds with the free radical polymers, as a result of the higher partitioning affinity the PEG has with the polymer rather than the additive.

The ability of PEG to prevent the suppression of enzymatic activity, due to polymerization, is largely a function of the dose and molecular weight of PEG. The addition of PEG increases the phenol removal efficiency (figure 2.10), and allows for a decreased enzyme dose. The removal efficiency of phenols increases as the PEG concentration is increased to an optimum concentration. As a result of PEG addition the enzyme dose may be reduced by up to 1/200 (Nakamoto and Machida, 1992). However

these reductions are for extremely high phenol concentrations, in the 10 to 30 g/L phenol range, which are higher concentration than that which is usually found in the environment (.1 to 1.0 g/L phenol) (Bewtra et al., 1995). The use of higher phenol concentrations may result in higher removal efficiency (Nicell et al., 1992). Others have placed the required enzyme dose at 1/10 to 1/132 depending on the additive concentration, phenol concentration, and compound being removed (Bewtra et al., 1995; Wu et al., 1993).

The minimum PEG dose (g/L), with average molecular weight of 3350 g/mol, for 95% phenol removal, is given by the following equation which is a function of the concentration of phenol (Wu et al., 1993).

$$PEG_{min} = 0.0065 + 0.0241\ C_{ph} \qquad [2.8]$$

In addition to the concentration of PEG, the molecular weight of the additive has an effect on the inactivation of the peroxidase, see table 2.2. The remaining enzymatic activity was higher when the molecular weight of PEG was 1000 g/mol or higher. Molecular weights at or below 400 g/mol were extremely ineffective at maintaining enzyme activity. At molecular weights between 1000 and 7500 g/mol there is little additional increase in residual enzymatic activity with an increase in molecular weight (Nakamoto and Machida, 1992).

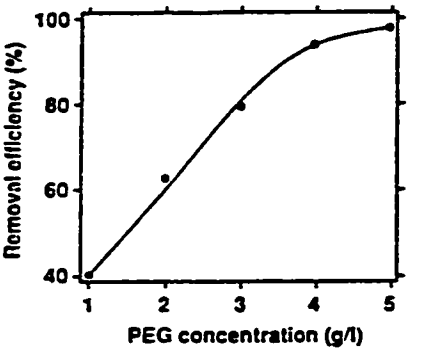


Figure 2.10. Phenol removal efficiency as a function of PEG dose for a pH of 6.0, with 10 mg/L HRP and 10 g/L phenol, conducted at room temperature, and 5 mmol of hydrogen peroxide was added at 0.175 mmol/min (from Nakamoto and Machida, 1992)

Table 2.2. The remaining activity of HRP in supernatants as a function of the molecular weight of PEG (from Nakamoto and Machida, 1992). Reactions were carried out at room temperature in GTA buffer, pH 6.0, containing 10 g/L phenol and 2 g/L peroxidase. Reaction mixture volume was 50 mL. A total of 1 mmol of hydrogen peroxide was added at a rate of 0.175 mmol/min to the mixture

PEG average molecular weight	% Residual peroxidase activity
200	1.5
300	1.3
400	1.3
600	62
1000	92
7500	97
20000	97

A recent peroxidase study has examined PEG and the precipitate. It has been suggested that the PEG and polymer products become covalently bonded. This results in the inability to separate the PEG from the precipitate, which eliminates any possibility of recycling the PEG as a cost saving measure (Wu et al., 1998).

2.3.5 Type of Reactor and Component Addition

Improved peroxidase efficiency for phenol removal has been shown when peroxidase and hydrogen peroxide are added gradually throughout the retention time, rather than all being added at once (Nicell et al., 1992; Klibanov et al., 1983; Alemany, 2000). This could be the result of the low concentration of the enzyme at any time, and the resulting low concentration of free radicals. This decreases the probability that a free radical will sorb with the active site. Additionally, due to the high concentration of contaminant as compared to the concentration of free radicals, the active site would more likely be occupied by contaminant rather than the free radical. (Nicell et al., 1992)

Trials with batch reactors indicated that there was no change in results regardless as to whether the reactor was stirred, shaken or left to stand (Klibanov et al., 1983), indicating that the enzymes may be mobile within the aqueous phase. This is one less obstacle facing in-situ remediation.

Experimentation with different types of reactors reveals that a continuously stirred tank reactor (CSTR) outperforms batch reactors when HRP is the peroxidase (Nicell et al., 1992, Nicell et al., 1993). Similar results should be seen for SBP. Nicell et al., 1992, explain that CSTR maintains the activity of the enzyme since the concentration of contaminant, hydrogen peroxide and enzyme are decreased once they enter the reactor,

reducing the potential for inactivation and increasing the number of turnovers. Similar results are seen in the research of Alemany (2000) when using SBP as the peroxidase enzyme. These experiments examined different methods of SBP and H_2O_2 addition. The mode of SBP addition are given in table 2.3, with the results in table 2.4. The most effective methods were those which broke up the SBP and hydrogen peroxide dose and applied a smaller dose over a time interval. The increased efficiency was attributed to the limiting of both the SBP and H_2O_2 at a given time, resulting in the reduction of H_2O_2 inactivation as a result of reduced presence of H_2O_2 as compared to a batch test (Alemany, 2000).

Table 2.3. Modes of soybean peroxidase and hydrogen peroxide addition (from Alemany (2000)).

TimeHrs		#1	#2a	#2b	#3a	#3b	#4
0	No Enzyme	All SBP, All H_2O_2	1/5 SBP, All H_2O_2	1/5 SBP, All H_2O_2	1/5 SBP, 1/5 H_2O_2	1/5 SBP, 1/5 H_2O_2	1/5 SBP, All H_2O_2
0.15			1/5 SBP		1/5 SBP, 1/5 H_2O_2		
0.30			1/5 SBP	1/5 SBP	1/5 SBP, 1/5 H_2O_2	1/5 SBP, 1/5 H_2O_2	1/5 SBP, All H_2O_2
0.45			1/5 SBP		1/5 SBP, 1/5 H_2O_2		
1.00			1/5 SBP	1/5 SBP	1/5 SBP, 1/5 H_2O_2	1/5 SBP, 1/5 H_2O_2	1/5 SBP, All H_2O_2
1.15							
1.30				1/5 SBP		1/5 SBP, 1/5 H_2O_2	1/5 SBP, All H_2O_2
1.45							
2.00				1/5 SBP		1/5 SBP, 1/5 H_2O_2	1/5 SBP, All H_2O_2
2.15							
2.30							
2.45							
3.00	Sample	Sample	Sample	Sample	Sample	Sample	Sample

Table 2.4. Results for different modes of enzyme and hydrogen peroxide addition (from Alemany (2000)).

	2,4-DCP Removal		
Test #	100 mg/l	200 mg/l	300 mg/l
1	62	52	58
2a	78	72	60
2b	76	64	62
3a	83	75	70
3b	84	76	72
4	55	47	30

2.4 Conclusions

Despite various indications that the use of enzymes to remediate soil is problematic, encouraging results have been shown with the application of humic acid to the reactor. Some of the potential problems that face the use of enzymes to remediate soil systems follow. Firstly, remediation efforts may be hindered by the sorption or sorbing of contaminants, hydrogen peroxide and/or enzymes. Secondly, excess hydrogen peroxide may be required to overcome sorption, or degradation due to interactions with organics, which may result in the accellerated inactivation of SBP. Thirdly, potential toxicity of the end product polymer, which is a function of the reaction between the toxic parent compound and the free radicals produced by the peroxidase. Finally, the high cost of enzyme treatment is problematic, although it may be drastically reduced if optimum design and application conditions are employed (Alemany, 2000).

What does seem obvious is that for peroxidase enzymes each soil type and contaminant have independent affinities for each situation. Although research may prove some conditions feasible and others infeasible, it will be difficult to draw conclusions

regarding the process as a whole. Therefore, it is important that before committing to extensive soil remediation with enzymes, each situation must undergo laboratory tests to determine the viability and optimum conditions for each individual case.

Chapter 3

Experimental Investigation

3.1 Materials and Methods

3.1.1 Materials

2,4-dichlorophenol (2,4-DCP), soybean peroxidase and PEG (with average molecular masses of 1000, 3350, and 8000 g/gmol), and hydrogen peroxide (30% by mass) were purchased from Sigma-Alderich Canada (Oakville, Ontario). Sphagnum peat moss was purchased from a local Home Depot outlet, manufactured under the brand name *The Gardner*. The peat moss was sieved using a size 16 sieve (1.18 mm openings), this allowed for removal of larger size particles, providing a more uniform sample. The moisture content of the peat moss was 8.5% and it contains 97% organics. Silica sand with an average diameter of 0.25 mm was purchased from a local hardware store.

3.1.2 Experimental Procedure

The first phase of the experiment, investigated the sorption of a buffered 2,4-DCP solution to a mixture of peat moss and silica sand. An initial 24 hour tumbling period allowed the sorption of 2,4-DCP to the soil and the 2,4-DCP to reach an equilibrium state between the liquid and solid phase. The equilibrium state appears to occur between the 6^{th} and 12^{th} hour, as shown in figure 3.1, but a 24 hour tumbling period was used for convenience. The following steps were performed to determine the sorption of 2,4-DCP to soil. Step 1, 20 mL of a known concentration of 2,4-DCP was added to a 60 mL serum bottle reactor containing a known mass of soil mixture (consisting of a 10% peat moss,

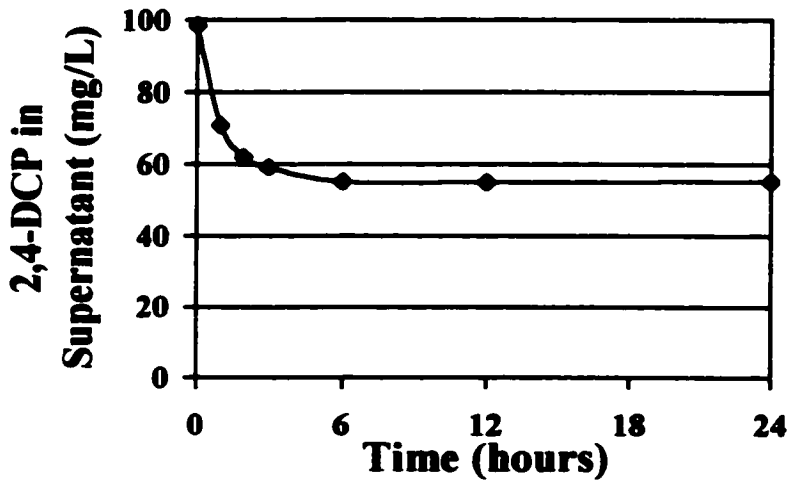


Figure 3.1 Sorption of 2,4-DCP with time. Initial concentration 99.6 mg/L at pH 6.

and 90% silica sand unless otherwise indicated) (figure 3.2). The temperature of the soil and liquid mixture was 22°C, unless otherwise specified, and kept at the same temperature throughout the experiments. Step 2, the addition (if any) of PEG to the reactor. PEG (with varying molecular weight) was added in a 1 mL dose resulting in a reactor concentration of 0.1 g/L to 5 g/L (this concentration range is commonly used in the literature investigated). Step 3, hydrogen peroxide was added to the reactor using a micro-syringe. The concentration of H_2O_2 was a 1:1 molar ratio to 2,4-DCP unless otherwise specified. Step 4, the reactors were sealed with teflon-coated crimp tops, and placed in a tumbler (figure 3.3) for 24 hours. The tumbler rotated around its longitudinal axis at a rate of 10 rpm, with a distance of 27 cm from the axle to the furthest point of the bottle. Step 5, after 24 hours a 1 mL sample was drawn and filtered to determine the 2,4-DCP concentrations as described in section 3.1.3. For several time-dependent tests, samples were drawn to analyze the 2,4-DCP concentrations after 1, 2, 3, 5, 6, 12, 15, 25,

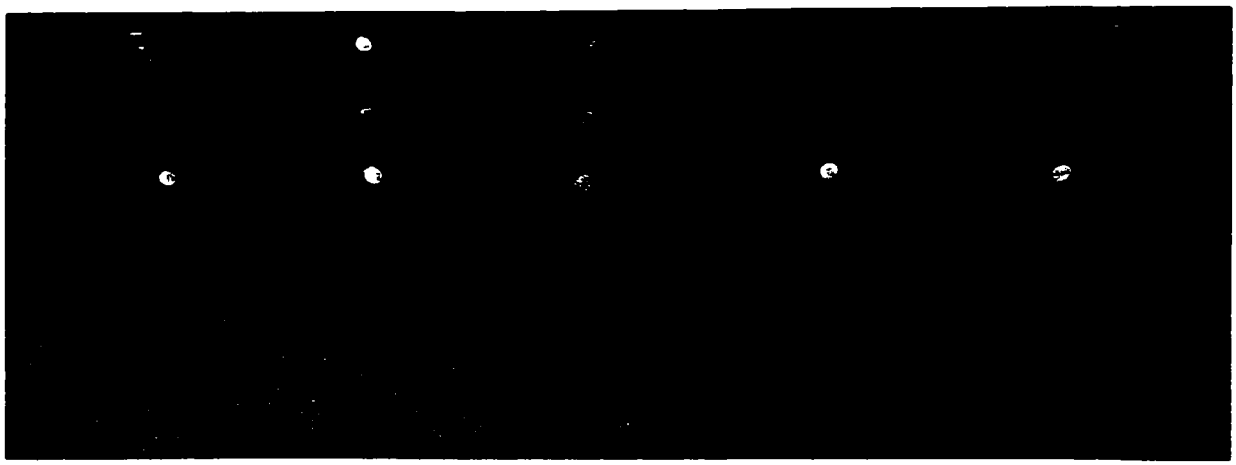


Figure 3.2. Reactor containing 0, 0.25, 0.5, 1.0 g soil. 20 mL of 2,4-DCP is added to reactors resulting in soil concentrations of 0, 12.5, 25.0, 37.5, 50 g/L soil.

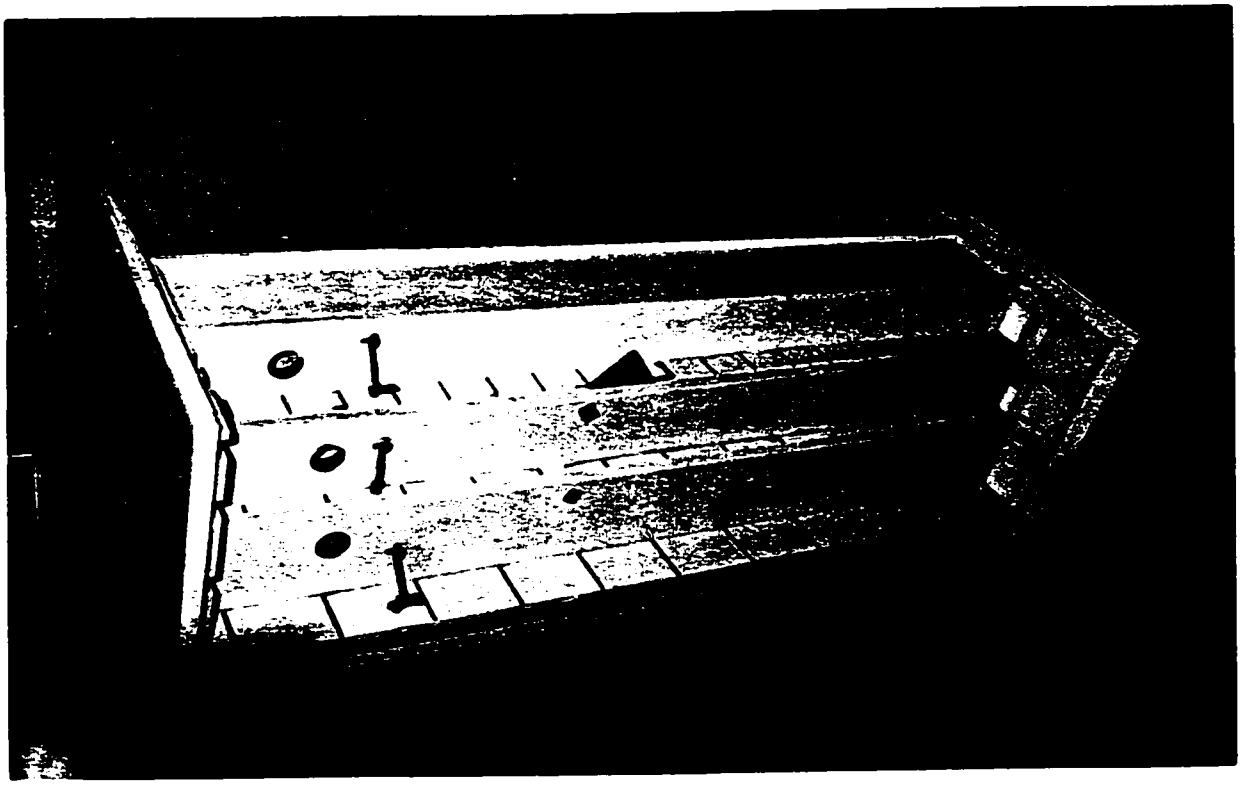


Figure 3.3. Tumbler used to ensure proper mixing of the reactors. The tumbler rotates around the horizontal axis.

and 30 minutes (to compare with current literature on the affect of soil addition to the reactor on the enzyme activity within the first 30 minutes). Enzyme kinetics are not discussed due to their extremely quick rate of reaction.

The procedure for the preparation of contaminant, enzyme, and PEG stock solutions as well as buffer preparation are given in Appendix A. Experimental conditions are given in table 3.1, and are those being referred in the following research unless stated otherwise.

In phase two of the peroxidase experiment, steps 1, 4 and 5 are performed, as described above, to allow sorption of 2,4-DCP to occur. Then 1 mL of SBP is added to the reactor, followed by the addition of 1 mL of PEG (if required) and rapid mixing. A sample was drawn and 2,4-DCP concentration analyzed to determine the concentration after binding and prior to SBP addition. Hydrogen peroxide was then added to initiate the reaction. The reactor bottle was sealed and returned to the tumbler for another 24 hours. At the end of this 24-hour period a 1 mL sample was filtered and used to measure the remaining 2,4-DCP concentration. Most of the experiments were performed in duplicate.

3.1.3 2,4-DCP Compound Concentrations

2,4-DCP concentrations were measured using a Hewlett Packard (model 1090) High Performance Liquid Chromatograph (HPLC). The HPLC utilized a CSC Hypersil 120A/ODS, 5 μm particle size, 10 cm x 0.21 cm internal diameter column that was maintained at a constant temperature of 40°C. The HPLC detector consisted of an ultraviolet diode array detector set at a wavelength of 280 nm. The mobile phase flowrate was 0.3 mL/min of an isocratic mixture consisting of 40% HPLC grade

methanol at pH 4.7, and 60% 0.05M sodium acetate at pH 4.7. Standards were run before every set of tests, as well as after every 20 samples to ensure the accuracy of the calibration curve. A sample 2,4-DCP calibration curve is given in Appendix B.

The detection limit of the analysis was 0.4 mg/L of 2,4-DCP. For an initial concentration of 100 mg/L 2,4-DCP a non-detectable concentration of 0.4 mg/L would result in 99.6% removal. When the detection limit was not reached the remaining concentration was assumed to be 0.4 mg/L or less. The sample error associated with the procedure and HPLC analysis is approximately 1%.

Table 3.1. Experimental Conditions for Phase I and II

Experimental Variable	Experimental Condition used unless stated otherwise	Range of Variables for experimental tests
2,4-dichlorophenol	0.6 mM	0.3-1.2 mM
Soybean Peroxidase	0.01 units/mL	0.0001 to 2 units/mL
Soil Composition	10% peat moss, 90% silica sand by weight	upto 20% peat moss
Polyethylene glycol	none	0.1 g/L – 5 g/L, with molecular weights of 1000, 3350, and 8000 g/mol
Time	24 hours in tumbler without enzyme, and 24 hours in tumbler after enyzme addition.	24 hours in tumbler without enzyme, and 1, 2, 3, 5, 6, 12, 15, 25, and 30 minutes after enzyme addition.
Temperature	22°C	4°C
H_2O_2	0.6 mM	0.3 to 5 mM
pH	6	pH 3 – pH 9
Reactor Set-up	Batch	Batch
Light/Dark	Dark	Dark
Tumbler	10 rpm (27 cm from axle to bottle top	10 rpm (27 cm from axle to bottle top
Volume	20 mL	20 mL

3.2 Results and Discussion

Although the experiments performed with the conditions described above do not exactly describe in situ soil conditions, these experiments can be used as a starting point in determining soil, 2,4-DCP, SBP, and H_2O_2 interactions. The experiments to be discussed are the first step towards demonstrating the potential for enzymatic remediation of wastewater-soil systems using SBP. Furthermore potential may be shown for in situ soil remediation using SBP. The experiments have been designed to limit the variables and unknowns, so as to allow conclusions to be drawn regarding the interactions of 2,4-DCP, enzyme, and additives in a soil matrix.

The selection of silica sand, and peat moss to simulate soil was based on the different characteristics of both soils. Peat moss has a high organic content and tendency to sorb to materials, while the silica sand should not interfere with enzymatic activity due to sorbing and should provide improved mixing of reactor contents while in the tumbler. It is possible that many alternative soils may provide different results than found in this study as their composition differs (i.e., more or less organic composition, or substantial iron content possibly initiating Fenton's reaction), and as a result their interactions with some of the variables tested would be expected to differ. The soil used in this study has a negligible iron content and should not result in Fenton's reaction, though experiments to confirm this will be performed.

3.2.1 Phase I – Sorption of 2,4DCP with Soil

The goal of phase I is to determine the partitioning behavior of 2,4-DCP between soil and water. For the given 2,4-DCP concentration, sorption data should be valid for

soil concentrations between 12.5 g/L and 50.0 g/L for the soil mixture used in this study. Extrapolation of results are not made for different soil types or soil concentrations less than 12.5 g/L. For soil concentrations greater than 50.0 g/L the relationship may not be valid, as one moves into the non-linear range of partitioning, and the mass contaminant sorbed per mass soil decreased. Additionally for soil concentrations up to 50.0 g/L in a buffered 2,4-DCP solution; the peat moss does not alter the liquid pH level, however at higher quantities of soil this becomes a significant concern.

Initial experiments were conducted to determine whether glass reactors and teflon seals could be sorbing the contaminant. Figure 3.4 shows the 2,4-DCP concentration added to reactor bottles versus the 2,4-DCP supernatant concentration measured in a buffered solution at pH 6.0. The small changes in concentration of 2,4-DCP added to the reactor and the 2,4-DCP after 24 hours of tumbling are considered negligible as they are within the sample error of the analysis. Figure 3.5 shows the silica sand concentration versus the percent change in 2,4-DCP concentration. This test confirmed that any 2,4-DCP losses were within the expected sampling error and silica sand sorption was considered negligible.

Peat moss and silica sand experiments examined the partitioning of 2,4-DCP with soil, as pH, 2,4-DCP concentration, soil concentration, and percent organics were varied. For these experiments, the mass of soil did not exceed 50.0 g/L of solution. These restrictions ensured that the sorbing of contaminant to soil remained in the linear range of the partitioning curves.

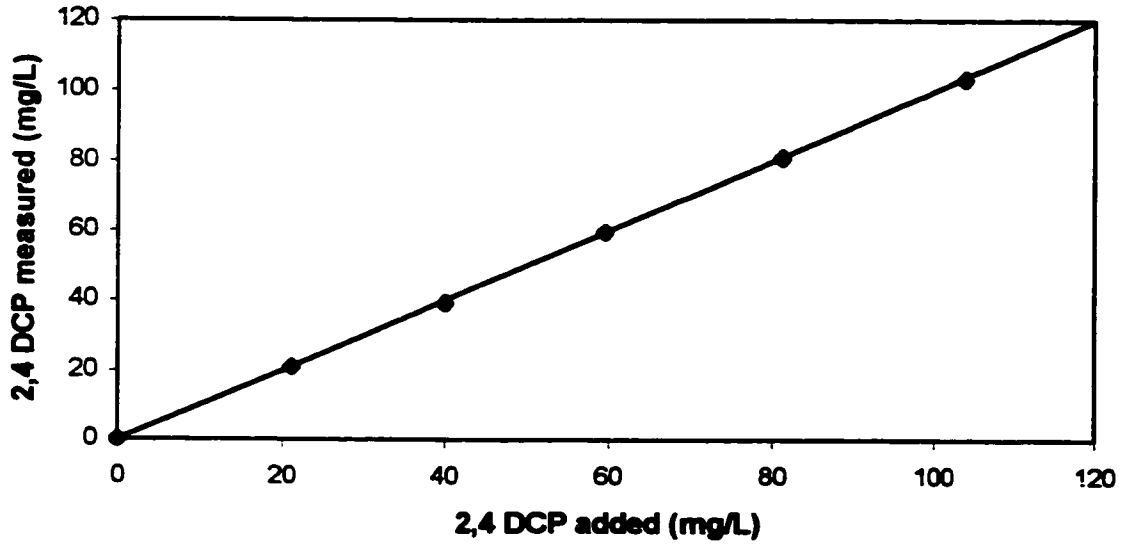


Figure 3.4. Sorption of 2,4-DCP to reactor bottles, cap, or seal. The 2,4-DCP was buffered to pH 6.0. The data is represented by a point on the graph, and the linear sequence would represent ideal relationship between the contaminant added and measured.

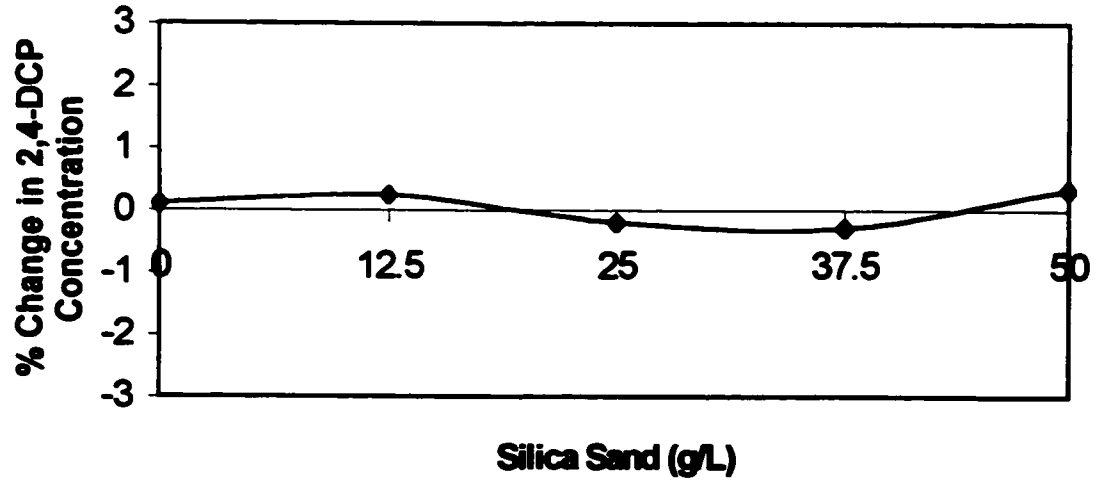


Figure 3.5. Sorption of 2,4 DCP to silica sand. Initial 2,4-DCP concentration 100 mg/L, at pH 6.0.

3.2.1.1 Effect of pH on 2,4-DCP Sorption to Soil

The isotherms for 2,4-DCP and soil are shown in figure 3.6a, b and c for pH 3, 6 and 8, respectively. The isotherms were developed using the following equation,

$$Q = kCe \qquad [3.1]$$

Where Q is the mass of sorbate (in mg 2,4-DCP/g soil) sorbing on the sorbent (soil), Ce is the equilibrium liquid-phase concentration (mg/L), and k is a proportionality constant. By plotting Ce versus Q, the value of k can be determined by the slope of the trendline. From figure 3.6, the k value for pH 3, 6 and 8 are 0.0098, 0.0141 and 0.0035 respectively. This indicates that sorption is highest at pH 6, followed by pH 3 and the lowest sorption at pH 8. The highest sorption seems to be found at a neutral pH and decreases with a decrease or increase in pH. Similar sorption trends as shown as function of pH were reported by Cooney (1999). The linear isotherm model appears to be appropriate for the sorption of SBP to the soil used in these experiments.

In chapter 5, these isotherms will be used to determine the mass of 2,4-DCP remaining sorbed to the soil after SBP application, under the assumption of reversible sorption of 2,4-DCP. The k values and equation 3.1 can be used to determine the mass of sorbate per mass of sorbent (Q value) in the reactor for a given effluent concentration. The mass of 2,4-DCP sorbed to the soil can then be determined and summed with the mass of 2,4-DCP in the solute to find the actual mass of 2,4-DCP remaining in the reactor.

The k values and the standard error and confidence intervals for the k values derived from the sorption isotherms for pH 3, 6 and 8 are given in table 3.2. The results of experiments with pH 3 and 6 indicate that they have greatly overlapping 95% confidence intervals. The confidence intervals for pH 3 and pH 6 overlap indicating that

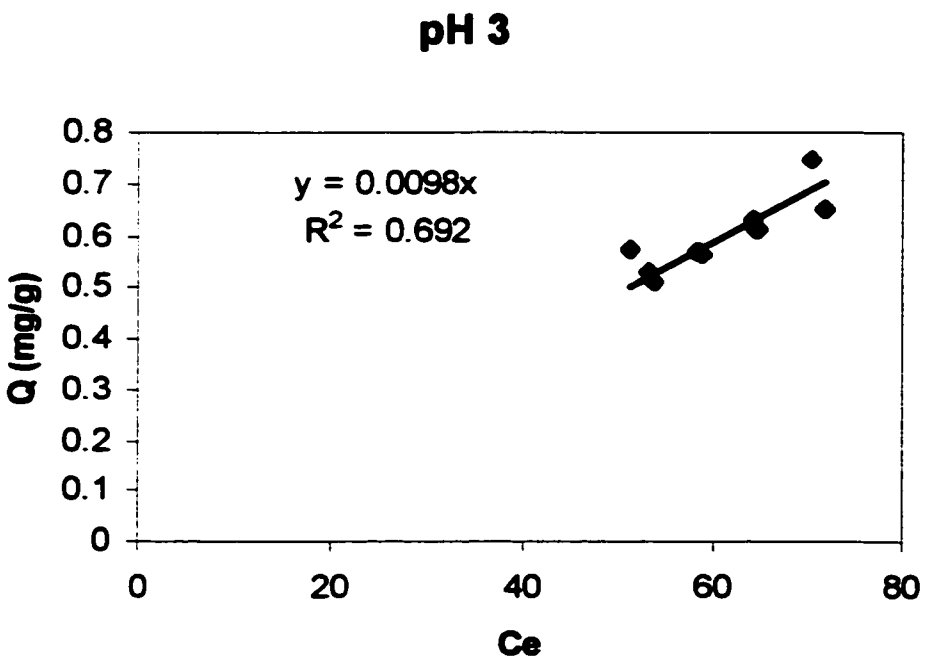


Figure 3.3a. Sorption isotherm for 2.4-DCP at pH 3.

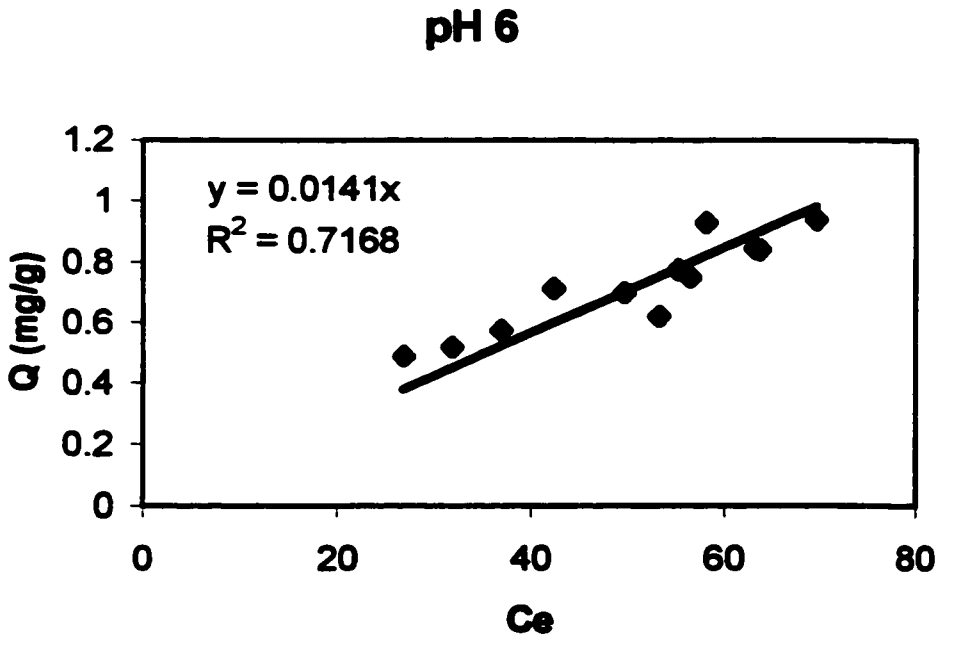


Figure 3.3b. Sorption isotherm for 2.4-DCP at pH 6.

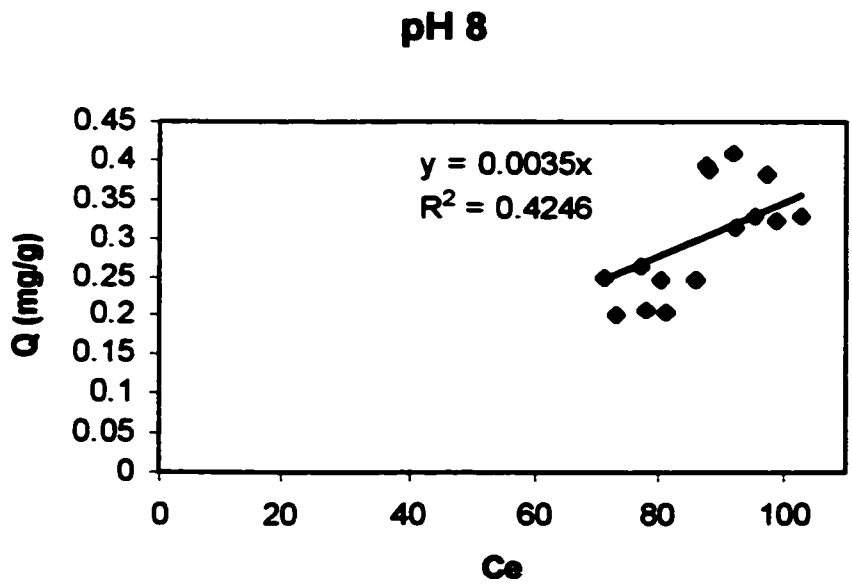


Figure 3.3c. Sorption isotherm for 2.4-DCP at pH 8.

k value for pH 3 falls between the lower and higher 95% confidence interval for pH 6, indicating that the k value for pH 3 can not be deemed different from that of pH 6.

Table 3.2. K values, standard error and confidence intervals for the k values derived from the isotherms developed for pH 3, 6 and 8.

pH	K value	Standard Error	Lower 95% Confidence Interval	Upper 95% Confidence Interval
3	0.0098	0.0017	0.0043	0.012
6	0.0141	0.0027	0.0073	0.019
8	0.0035	0.0015	0.0019	0.0084

A more consistent soil sample may have resulted in lower standard errors and a narrower confidence interval. The peat moss was sieved (1 mm opening sieve size) to provide a more uniform sample, but a smaller sieve opening would have provided even less variability within the sample. As a result of the fibrous nature of peat moss particles, a 1 mm sieve was unable to remove the larger fibers from the peat moss due to the long cylindrical shape of the fibers with small diameters. These larger spindly fibers are not as organically rich as the smaller particles. The peat moss fibers may not have the same binding properties as the smaller particles. As a result the variability in peat moss sample may have had an effect on the overall binding of the 2,4-DCP in solution.

Chapter 4

Removal of 2,4-Dichlorophenol in Soil Slurries with Soybean Peroxidase

Having characterized the physical interactions of the soil with 2,4-DCP, the second phase of the experiment is to evaluate the ion of 2,4-DCP in soil systems with SBP addition. In this chapter, a " black box " approach is taken for the removal of 2,4-DCP. The actual enzymatic removal due to SBP addition is not isolated from that of the contribution of soil to the removal of 2,4-DCP. Chapter 5 will make assumptions about the sorption of 2,4-DCP in order to predict the effect of soil on the 2,4-DCP removal efficiency of SBP.

The 2,4-DCP was first allowed to bind to the soil for 24 hours before the SBP was added to the system and activated. The soluble percent removal of 2,4-DCP is based on the change in 2,4-DCP supernatant concentration between time 24 hrs (after sorption) and time 48 hrs after SBP addition. The soluble percent removal of 2,4-DCP ($SR_{2,4\text{-DCP}}$) is calculated from the intermediate 2,4-DCP concentration (after sorption of 2,4-DCP immediately before enzyme activation) and the final 2,4-DCP concentration (24 hours after the enzyme activation) and is given in equation 4.1

$$SR_{2,4\text{-DCP}} = \frac{C_{int} - C_e}{C_{int}} \times 100\% \qquad [4.1]$$

Where C_{int} is the intermediate concentration (after sorption of 2,4-DCP and before SBP addition) in mg/L. $SR_{2,4\text{-DCP}}$ provides an accurate representation of the supernatant

removal, but does not consider the removal of 2,4-DCP bound to the soil. Alternatively total 2,4-DCP removal considers both the immobilization of 2,4-DCP due to sorption, as well as enzymatic removal of 2,4-DCP. Therefore the total percent removal of 2,4-DCP ($TR_{2,4\text{-}DCP}$) is calculated as the difference between the initial concentration before exposure to soil and the final effluent (equation 4.2).

$$TR_{2,4-DCP} = \frac{C_i - C_e}{C_i} \times 100\% \qquad [4.2]$$

Where C_i is the initial concentration (before sorption of 2,4-DCP to soil). $TR_{2,4\text{-}DCP}$ would provide the most optimistic removal by assuming that 2,4-DCP that is sorbed is irreversibly immobilized and no longer a concern (i.e. can be separated from supernatant and disposed of). For reactors without soil, the $SR_{2,4\text{-}DCP}$ and $TR_{2,4\text{-}DCP}$ are equal, since $C_{sorbed}=0$.

4.1 Influence of pH and SBP Dose

The first set of experiments determined whether silica sand interfered with the enzymatic removal of 2,4-DCP. Figure 4.1 shows the $TR_{2,4\text{-}DCP}$ as a function of silica sand concentration. No sorbing of 2,4-DCP occurred and hence the $SR_{2,4\text{-}DCP}$ and $TR_{2,4\text{-}DCP}$ are equal. The $SR_{2,4\text{-}DCP}$ and $TR_{2,4\text{-}DCP}$ was 66% for both the control (without soil) and reactors with silica sand only, regardless of the silica sand concentration for 0.01 units/mL SBP, 100 mg/L 2,4-DCP, and 0.6 mM H_2O_2. The presence of silica sand alone did not significantly alter the removal of contaminant from trials without silica sand. Therefore any changes in the removal of 2,4-DCP between samples with and without the

soil mixture are attributed to the peat moss portion of the soil. These results also indicated that any change in enzymatic removal of 2,4-DCP is due to characteristics of the peat moss rather than the silica sand.

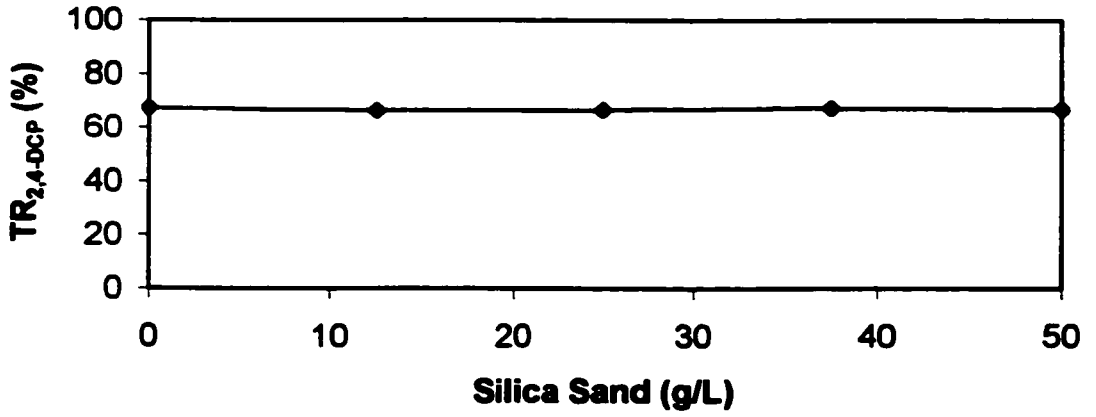


Figure 4.1. The $TR_{2,4\text{-DCP}}$ as a function of silica sand concentration. Reactor conditions were pH 6, 100 mg/L 2,4-DCP, 0.6 mM H_2O_2, 0.01 units/mL SBP.

Figure 4.2a shows $SR_{2,4\text{-DCP}}$ versus soil concentration for pH 3, 6 and 8. The removal of 2,4-DCP in the supernatant with soil addition was greatest at pH 6, followed by 8, and then 3. The differences in 2,4-DCP removal for pH levels became more pronounced as the amount of soil was increased indicating the soil may be affecting the ability of the enzyme to remediate the 2,4-DCP. For concentrations of 100 mg/L 2,4-DCP, the addition of 1.0 unit/mL SBP resulted in 99.6% or greater $SR_{2,4\text{-DCP}}$ (no soil) for pH 6 and 8, while at pH 3, 97% $SR_{2,4\text{-DCP}}$ occurred. With up to 25.0 g/L soil addition, reactors at pH 6 were able to maintain 99.6% $SR_{2,4\text{-DCP}}$, but removal decreased with 37.5 g/L soil or greater. The $SR_{2,4\text{-DCP}}$ for pH 8 fell below 99.6% with the addition of 12.5 g/L soil to the reactor vessel. For all pH levels tested with 1.0 units/mL SBP, $SR_{2,4\text{-DCP}}$ was

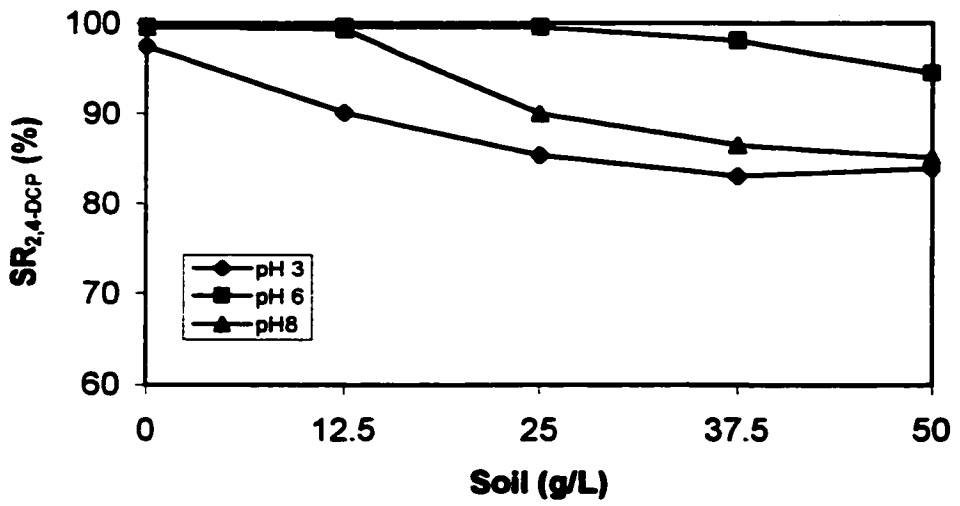


Figure 4.2a. $SR_{2,4\text{-DCP}}$ for various pH levels and soil concentrations. 1.0 unit/mL SBP, 0.6 mM H_2O_2, and soil was 10% peat, 90% silica sand. Initial 2,4-DCP concentrations after binding, and before SBP activation are pH 3 (80, 72, 64, 59, 51) mg/L, pH 6 (84, 68, 63, 55, 53) mg/L, and pH 8 (99, 92, 87, 80, 78) mg/L for 0, 12.5, 25.0, 37.5, and 50 g/L soil respectively.

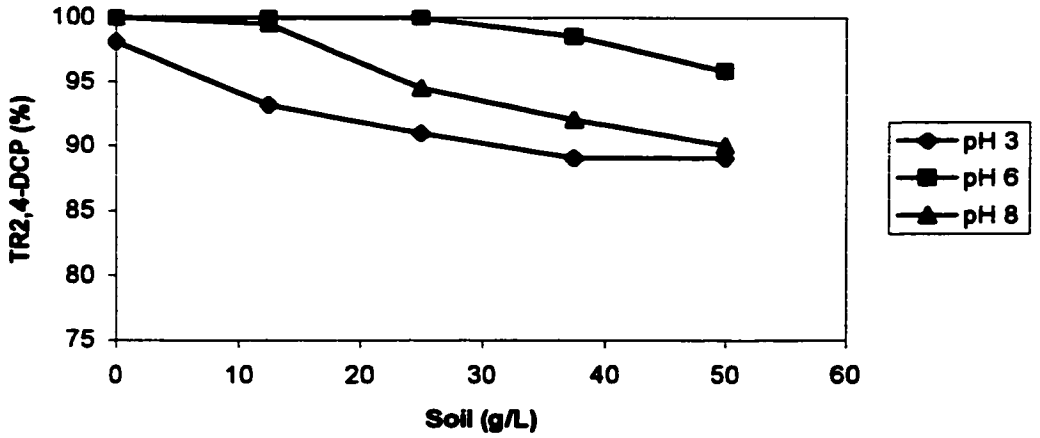


Figure 4.2b. $TR_{2,4\text{-DCP}}$ as a function of soil concentration for pH 3, 6, and 8 Conditions are the same as in figure 4.2a.

inversely related to the mass of soil. The $SR_{2,4\text{-DCP}}$ with 50.0 g/L of soil is greatest for pH 6, followed by pH 8, and pH 3 with 91%, 79%, and 74% respectively.

The ability of SBP to remediate contaminated water is a function of the pH in the reactor (Alemany, 2000; Wright and Nicell, 1999; McEldoon et al., 1995). The removal of 2,4-DCP in water only by SBP is best at pH 8, followed by pH 6, then 3 (Alemany, 2000). However when PEG is added to wastewater the best 2,4-DCP removal was found for pH 6, followed by pH 8, and then pH 3 (Alemany, 2000; Caza et al., 1999). The influence of pH on the SBP enzymatic removal of 2,4-DCP when soil is added to the reactors is similar to that found with the addition of PEG in water only systems. However unlike that found for PEG addition, the addition of soil seems to interfere with the contaminant removal for 1.0 units/mL SBP or higher.

Figure 4.2b shows the $TR_{2,4\text{-DCP}}$ versus soil concentration for pH 3, 6 and 8. The $TR_{2,4\text{-DCP}}$ is higher than the $SR_{2,4\text{-DCP}}$ when soil is present. The $TR_{2,4\text{-DCP}}$ is highest for pH 6, followed by pH 8, and 3. At pH 3 the $TR_{2,4\text{-DCP}}$ is now just slightly less than pH 8 with the addition of 50.0 g/L soil. The improvement in $TR_{2,4\text{-DCP}}$ is due to the higher capacity of 2,4-DCP to sorb to the soil at pH 3 than at pH 8. While the $TR_{2,4\text{-DCP}}$ is higher than the $SR_{2,4\text{-DCP}}$ as a result of the immobilization through sorption, the $SR_{2,4\text{-DCP}}$ in figure 4.2a shows a possible decrease in activity with soil present and 1.0 units/mL SBP.

Figure 4.3a shows $SR_{2,4\text{-DCP}}$ versus soil concentration for various SBP doses between 0.25 and 2.0 units/mL. As the SBP dose is increased from 0.25 to 2.0 units/mL there appeared to be a decrease in $SR_{2,4\text{-DCP}}$ for reactors with 50 g/L soil. This may be the result of soil interfering with SBP. In fact the $SR_{2,4\text{-DCP}}$ is inversely related to the SBP

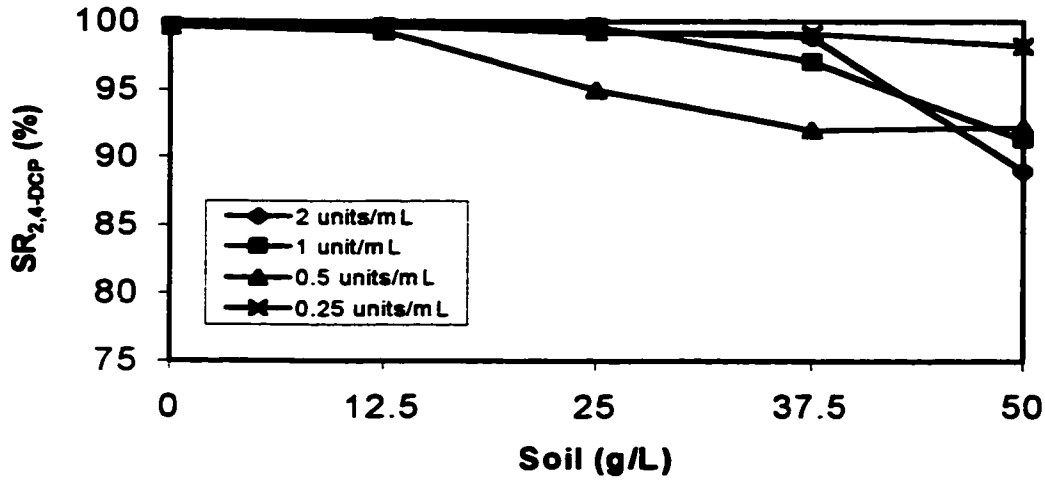


Figure 4.3a. $SR_{2,4\text{-DCP}}$ for varying soil concentrations for doses of SBP ranging from 0.25 to 2 units/mL. Reactor conditions are 0.6 mM H_2O_2, pH 6, with various doses of SBP. The initial 2,4-DCP concentrations after binding and enzyme addition (but before enzyme activation) for 2 units/mL are (81, 77, 69, 60, 53) mg/L, for 1 unit/mL (84, 68, 63, 55, 53)mg/L, for 0.5 units/mL (84, 76, 41, 53, 52) mg/L, and for 0.25 units/mL (80, 70, 65, 54, 53) mg/L for soil concentrations of 0, 12.5, 25.0, 37.5, and 50.0 g/L respectively.

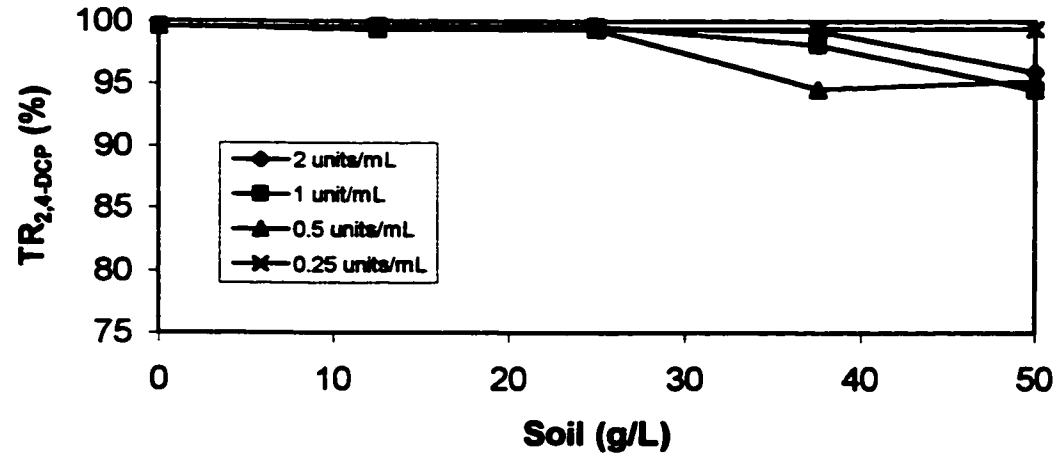


Figure 4.3b. $TR_{2,4\text{-DCP}}$ for varying soil concentrations. Enzyme doses range from 0.25 to 2 units/mL. Conditions are given in figure 4.3a

dose with 50 g/L of soil. When high doses of enzyme (0.50-2.0 unit/mL) are used for the removal of 2,4-DCP in wastewater, the presence of soil interferes with the removal of 2,4-DCP. As the soil concentration is increased from 12.5 to 50.0 g/L there is a decrease in $SR_{2,4\text{-DCP}}$. Previous research with wastewater contaminated with 2,4-DCP indicates that an increase in SBP dose results in an increase in removal (Wu et al., 1993; Bewtra et al., 1995; Alemany, 2000). It is inexplicable why a lower SBP dose would outperform higher SBP doses when soil is present in the reactor.

Figure 4.3b shows the $TR_{2,4\text{-DCP}}$ versus soil for various SBP doses between 0.25 and 2.0 units/mL. Similar patterns are found for $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ for samples with and without soil, however $TR_{2,4\text{-DCP}}$ is higher than $SR_{2,4\text{-DCP}}$ as a result of sorption. The highest total percent removal is found for 0.25 units/mL of SBP in reactors containing soil. Similar to the $SR_{2,4\text{-DCP}}$ it is difficult to explain why a lower dose of SBP would result in higher SBP efficiencies in the removal of 2,4-DCP.

Figures 4.4a,b shows the $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ as a function of SBP dose for each soil concentration. Figures 4.4a and b share the same data with figures 4.3a and b, but the soil concentration on the x-axis in figures 4.3a,b has been replaced with SBP dose in figures 4.4a, and b. The $SR_{2,4\text{-DCP}}$ is lowest at 0.50 units/mL SBP and the $SR_{2,4\text{-DCP}}$ improves with a decrease or increase in SBP dose (with the exception of 50 g/L of soil which shows decreased removal efficiency as the SBP dose is increased). The reduction in $SR_{2,4\text{-DCP}}$ may be up to 11% with the addition of 50.0 g/L of soil to the reactor. From this figure it appears that the addition of soil in the reactor decreases $SR_{2,4\text{-DCP}}$ for a given enzyme concentration. It also suggests that for reactors with soil, a dose of SBP greater

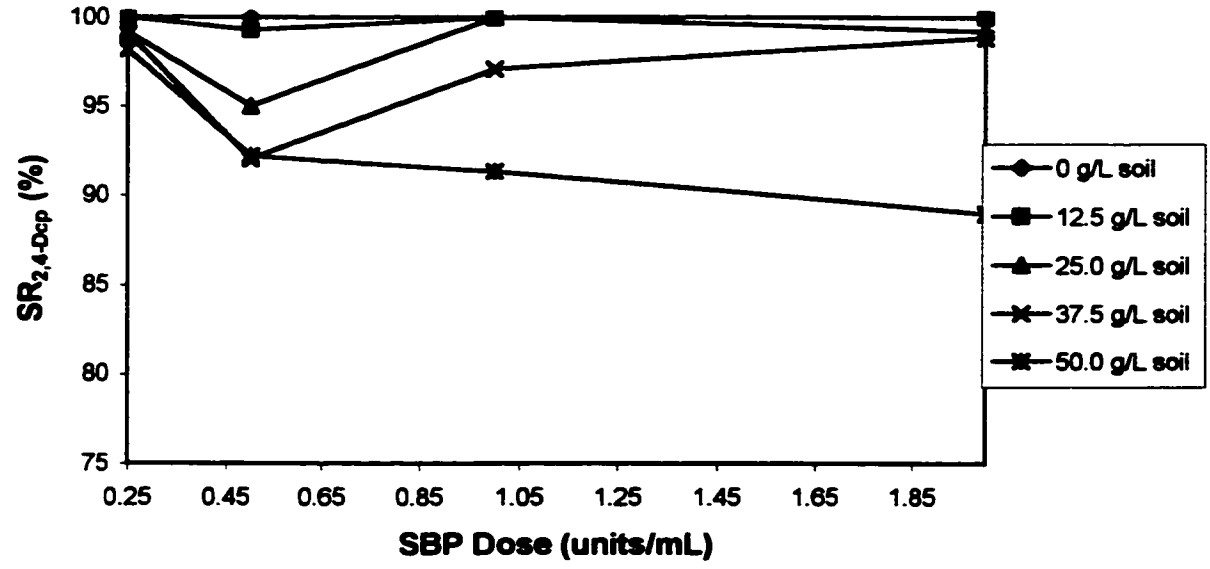


Figure 4.4a. $SR_{2,4\text{-DCP}}$ versus SBP dose for varying soil concentrations. Conditions are those for figure 4.3a.

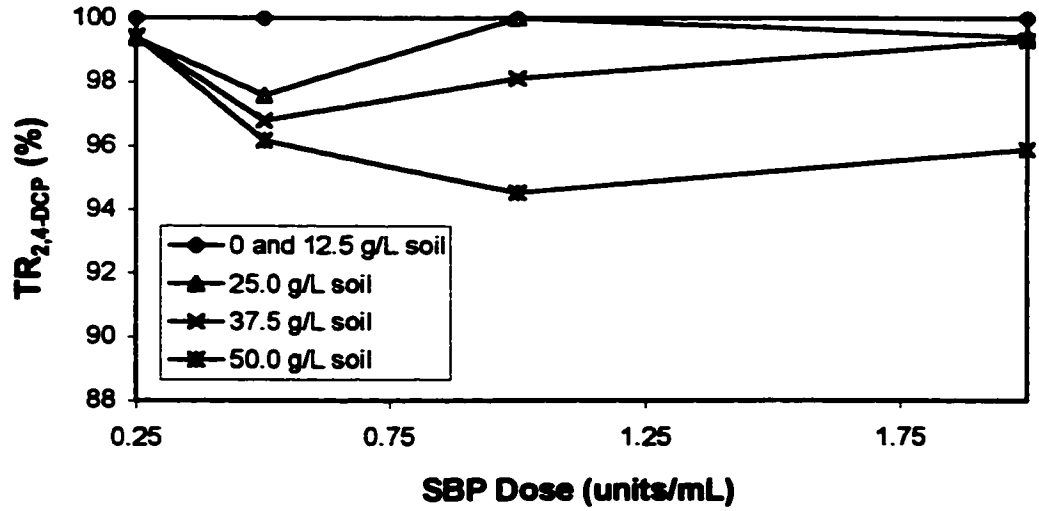


Figure 4.4b. $TR_{2,4\text{-DCP}}$ versus SBP dose for varying soil concentrations. Reactor conditions are the same as figure 4.3a.

than 0.5 units/mL is an inefficient dose since similar or higher $SR_{2,4\text{-DCP}}$ can be achieved with lower doses.

Figure 4.4b shows the $TR_{2,4\text{-DCP}}$ versus enzyme dose for various soil concentrations. The $TR_{2,4\text{-DCP}}$ for a given enzyme dose are higher than the $SR_{2,4\text{-DCP}}$ due to the effect of soil sorption. The figure also indicates that the addition of soil and increasing concentrations of soil hinders the $TR_{2,4\text{-DCP}}$. These figures indicate that soil systems are at a disadvantage to water alone when using SBP to remediate 2,4-DCP at SBP doses between 0.50 and 2.0 units/mL.

Aitken (1993) postulates that a potential problem facing the addition of enzymes to soil for remediation purposes is that the enzymes may bind to the soil and become inactivated. This may be the mechanism responsible for lowering the $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ at SBP doses between 0.50 units/mL and 2.0 units/mL.

Figure 4.5a shows the $SR_{2,4\text{-DCP}}$ as a function of soil concentration for lower doses of SBP (0.0050-0.10units/mL). Figure 4.5b shows the $TR_{2,4\text{-DCP}}$ as a function of soil concentrations for lower doses of SBP. Figures 4.5a, and b have the same reactor conditions as figures 4.4a and b, however figure 4.4 shows higher SBP doses. As a result of water only studies which indicate a decrease in removal efficiency with a decrease in SBP dose (Wu et al., 1993; Bewtra et al., 1995; Alemany, 2000), it was thought the same reduction in $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ would be found with reactors containing soil when the SBP dose was decreased. Contrarily, it appears that the addition of soil to the reactors increases the removal of 2,4-DCP at lower SBP doses. When the SBP dose was reduced to 0.050 units/mL, 61% $SR_{2,4\text{-DCP}}$ occurred in the wastewater without soil. For the same SBP dose the addition of 12.5 to 50 g/L of soil to the reactor resulted in 98% or greater

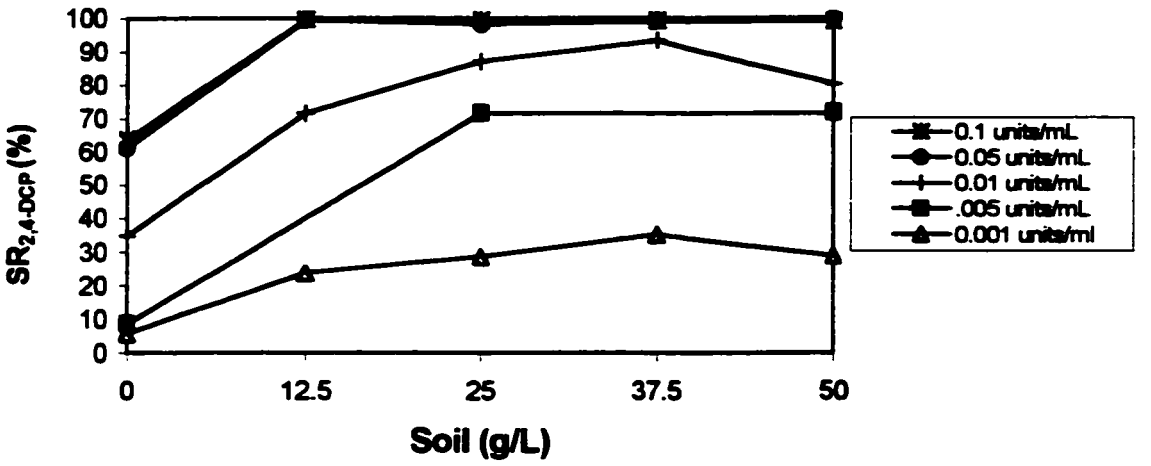


Figure 4.5a. $SR_{2,4\text{-DCP}}$ versus soil for doses of SBP ranging from 0.001 to 0.1 units/mL. Reactor conditions are 0.6 mM H_2O_2, with various doses of SBP at pH 6. Initial 2,4-DCP concentrations after binding and before enzyme activation for 0.005 units/mL are (83, 65, 53) mg/L, for 0.015 units/mL (87, 64, 50) mg/L for 0, 25.0, and 50.0 g/L soil respectively. Initial 2,4-DCP concentrations for 0.01 units/mL are (82, 72, 62, 56, 48) mg/L, for 0.05 units/mL are (82, 69, 62, 54, 50) mg/L, and for 0.1 units/mL are (81, 73, 62, 58, 49) mg/L for 0, 12.5, 25.0, 37.5, and 50.0 g/L respectively.

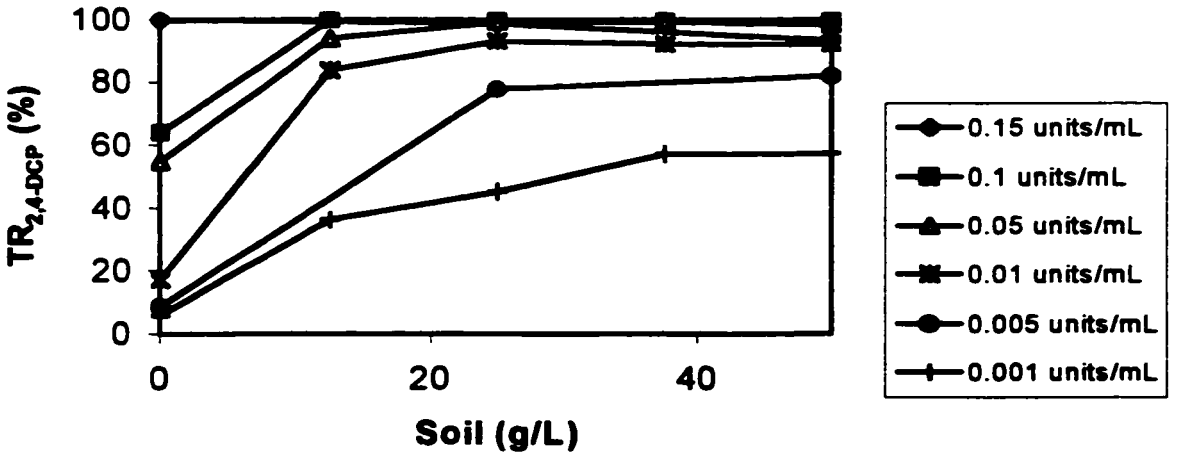


Figure 4.5b. $TR_{2,4\text{-DCP}}$ versus soil for doses of SBP between 0.001 and 0.1 units/mL. Reactor conditions are given in figure 4.5a.

$SR_{2,4\text{-DCP}}$. For 50 g/L soil the $SR_{2,4\text{-DCP}}$ was 99.6% or greater. In fact, for an SBP dose as low as 0.010 units/mL, there is 35% $SR_{2,4\text{-DCP}}$ in contaminated water without soil, while the addition of 12.5 g/L of soil doubles the $SR_{2,4\text{-DCP}}$ to 70%. Even at SBP concentrations as low as 0.0050 units/mL which achieve only 8.6% $SR_{2,4\text{-DCP}}$ for water alone, with the addition of soil (25.0 and 50.0 g/L) 72% $SR_{2,4\text{-DCP}}$ is achieved. The addition of soil resulted in an 8.4 fold increase in soluble percent removal.

At low SBP concentrations, the soluble 2,4-DCP removal on a mass basis (per unit volume) indicates the advantages of soil addition with SBP treatment. When 0.010 units/mL of SBP is added to the reactors with 0 g/L soil, 28.5 mg of the 81.7 mg of 2,4-DCP are removed, but when 50.0 g/L of soil is added to the reactors, 34.0 mg of the 48.5 mg of 2,4-DCP available is removed. Addition of 0.0050 units/mL of SBP resulted in the removal of 6.6 mg of the 82.6 mg of 2,4-DCP available without soil present while in reactors with 50.0 mg/L of soil, the removal was 38.3 mg of the 53.2 mg 2,4-DCP available in the supernatant. The addition of soil resulted in up to 5.8 fold increase in mass of 2,4-DCP removed with a 0.005 units/mL dose of SBP. The increase in mass of 2,4-DCP removed with the addition of soil indicates that the soil is positively affecting the SBP performance.

Aitken (1993) conjectured that the application of enzymes to soil could result in binding and inactivation of the enzyme. If this was occurring for SBP doses between 0.50 and 2.0 units/mL, then why is soil addition increasing the removal of 2,4-DCP at SBP doses below 0.25 units/mL? Since SBP is more efficient with reactors containing soil at lower SBP doses, it seems unlikely that the SBP is becoming inactive as a result of

binding. There must be another unknown mechanism which is responsible for decreasing the removal of 2,4-DCP at higher SBP doses.

A reduction in enzyme efficiency was found by Park et al. (1999) with the addition of enzymes and select humic acids when treating phenolic compounds. It was also reported that the addition of other humic acids had no effect on the enzyme efficiency or enhanced the removal of chlorinated phenols. Park et al. (1999) postulate that the enhanced removal due to humic acid addition is dependent on both the phenolic compound and the humic constituent having similar mechanisms of oxidative coupling. But they acknowledge that there is likely an additional controlling factor which would account for some combinations of humic acid and phenolic combinations not adhering to their theory of similar oxidative coupling mechanisms. Unfortunately, all their tests were performed with 1.5 unit/mL of enzyme, and the effect of enzyme concentration did not appear to be investigated in their study.

Figure 4.6a. shows $SR_{2,4\text{-DCP}}$ as a function of SBP doses for three concentrations of soil. With 0.050 units/mL of SBP, the $SR_{2,4\text{-DCP}}$ was 61% without soil, and 98% and 99.6% or better with 25 g/L and 50 g/L of soil addition, respectively. For 0.015 units/mL SBP, the $SR_{2,4\text{-DCP}}$ for wastewater without soil is 19%, with the addition of 25 and 50 g/L of soil the $SR_{2,4\text{-DCP}}$ is 98 and 89%, respectively. Therefore the $SR_{2,4\text{-DCP}}$ can be increased by up to 500% with soil addition and 0.015 units/mL of SBP.

Figure 4.6b. shows the $TR_{2,4\text{-DCP}}$ as a function of the SBP dose. For reactors with soil there is higher $TR_{2,4\text{-DCP}}$ than $SR_{2,4\text{-DCP}}$. This increase in removal is more pronounced for $TR_{2,4\text{-DCP}}$ due to sorption of soil. For 0.0050 units/mL SBP, there is 8.6% $TR_{2,4\text{-DCP}}$ without soil. The $TR_{2,4\text{-DCP}}$ is 77%, and 82% with the addition of 25 g/L and 50 g/L of

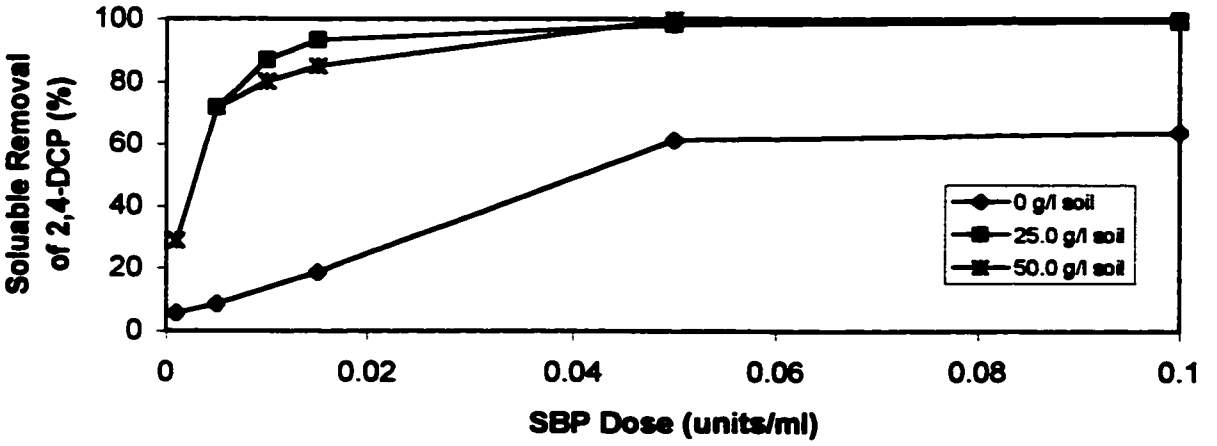


Figure 4.6a. $SR_{2,4\text{-DCP}}$ versus SBP dose for 0, 25, and 50 g/L of soil. Conditions are stated in Figure 4.5a.

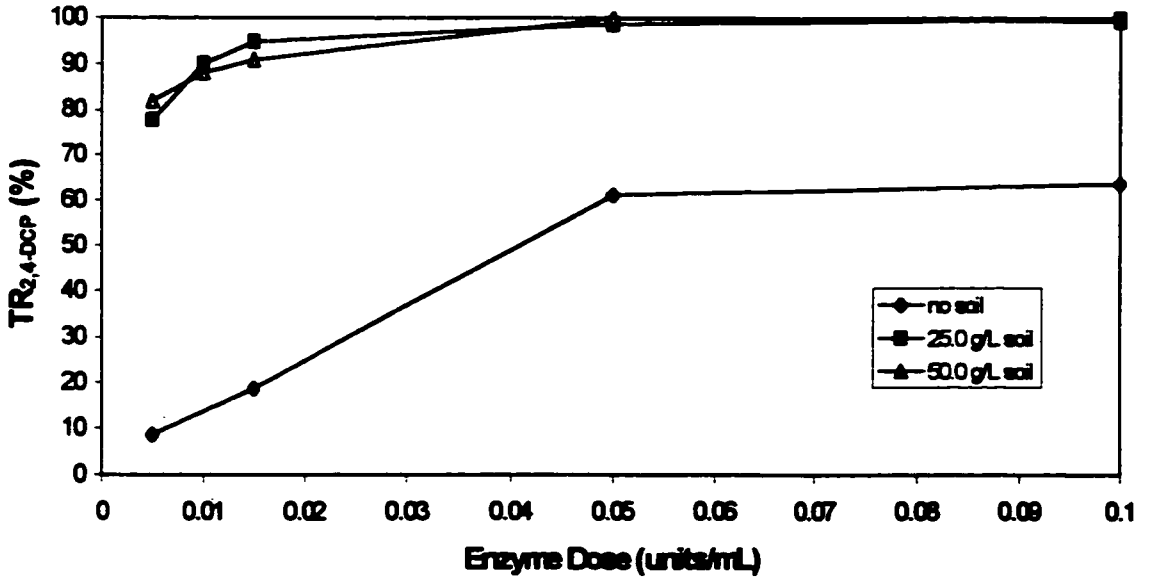


Figure 4.6b. $TR_{2,4\text{-DCP}}$ versus enzyme dose for 0, 25, and 50 g/L of soil. Conditions are stated in Figure 4.5a.

soil. The $TR_{2,4\text{-DCP}}$ for reactors containing 25 or 50 g/L is approximately 900% higher than reactors without soil addition. The $TR_{2,4\text{-DCP}}$ is substantially higher with soil possibly due to the an increase in SBP efficiency (as indicated in figure 4.6a) as well as the immobilization of 2,4-DCP due to sorption.

At lower SBP concentrations the addition of soil appears to have a positive effect on the SBP efficiency as shown by the increase in both $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ when soil is present.

Figure 4.7a shows the $SR_{2,4\text{-DCP}}$ as a function of pH with 0.010 units/mL SBP. For operating conditions between pH 4, and 8 (the pH is adjusted using a buffered solution) the $SR_{2,4\text{-DCP}}$ is substantially higher for reactors with soil. Between pH 4 and 8, there is up to a 500% increase in $SR_{2,4\text{-DCP}}$ as a result of soil addition. At pH 3, and 9 there is a lower $SR_{2,4\text{-DCP}}$ with the addition of soil. Previous investigations by Wright and Nicell (1999) indicate that the use of SBP was most effective between pH 6 and 9. This is similar to results from Alemany (2000) and Caza et al. (1999) who found that the enzymatic removal with SBP is highest at pH 8. Yet the results of the current study indicate that the removal is highest at pH 6, and quite efficient between pH 5 and 8. Reactors with soil seem to achieve maximum $SR_{2,4\text{-DCP}}$ efficiency in a somewhat lower pH range, similar to water alone results with PEG addition (Alemany, 2000).

Figure 4.7b shows the $TR_{2,4\text{-DCP}}$ as a function of pH. The $TR_{2,4\text{-DCP}}$ trends are similar to those found for $SR_{2,4\text{-DCP}}$ in figure 4.7a. A notable difference is at pH 3. While the addition of soil to the reactor at pH 3 hinders the $SR_{2,4\text{-DCP}}$, the $TR_{2,4\text{-DCP}}$ is higher when soil is added to the reactor. This is mainly a result of the low $SR_{2,4\text{-DCP}}$ with SBP addition but the high sorption at pH 3.

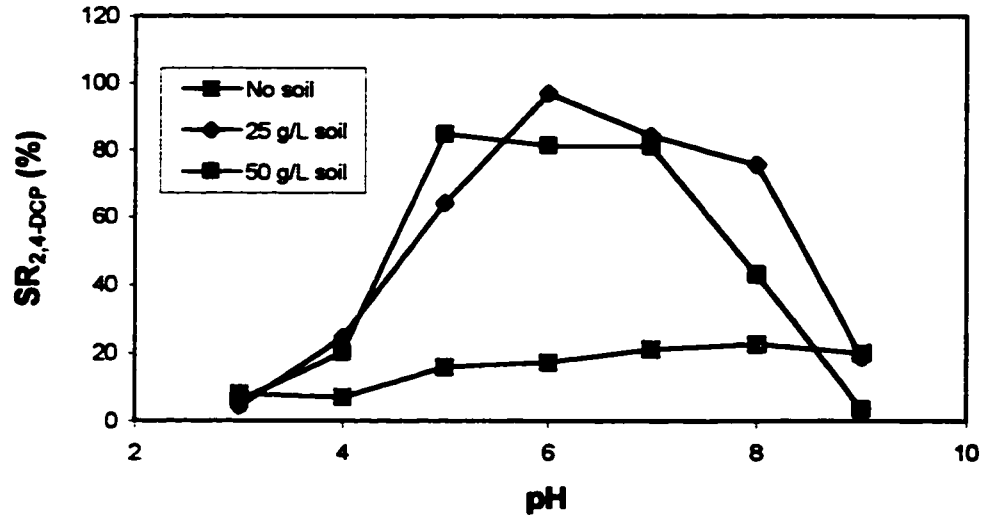


Figure 4.7a. $SR_{2,4\text{-DCP}}$ for pH 3 to 9 with no soil, 25 g/L, and 50 g/L of soil, with 0.01 units/mL of SBP activated with 0.6 mM H_2O_2. Initial concentrations after binding, and activation for pH 3 are (89, 65, 51) mg/L, for pH 4 are (87, 71, 52) mg/L, for pH 5 are (85, 65, 75) mg/L, for pH 6 are (86, 65, 51) mg/L, for pH 7 are (91, 70, 56) mg/L, for pH 8 are (93, 80, 63) mg/L, for pH 9 are (93, 86, 85) mg/L for 0, 25, and 50 g/L.

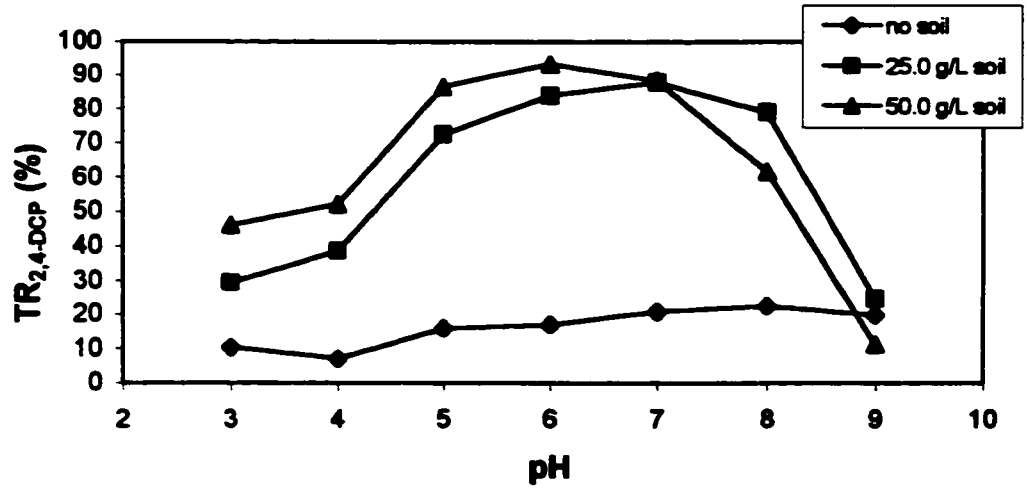


Figure 4.7b. $TR_{2,4\text{-DCP}}$ versus pH for various soil concentrations. Reactor conditions are the same for figure 4.7a.

It is unclear why the higher removal of 2,4-DCP is achieved without soil at high SBP concentrations, but at lower SBP concentrations there is a dramatic increase in the removal of 2,4-DCP with soil in reactors operating between pH 4 and 8 compared to reactors without soil. The ability to maintain high $SR_{2,4\text{-DCP}}$ with low SBP doses over a broad pH range when soil is present, is an advantage that increases the feasibility of SBP use.

It is possible that the soil added to the reactor is acting as an additive, as $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ curves for soil (figure 4.8b) are similar to those reported for PEG addition (Alemany, 2000) (figure 4.8a). Figure 4.8a shows the percent of 2,4-DCP remaining as a function of SBP concentration and is taken from experiments performed by Alemany (2000) with the addition of PEG 8000. Figure 4.8b shows the 2,4-DCP remaining in solution as a function of SBP dose. Figures 4.8a and b shows similar trends whether soil or PEG is added. The addition of soil, like that of PEG, results in a decrease in the necessary enzyme dose to achieve a certain degree of removal. Results obtained by Alemany (2000) indicate that the SBP dose required to achieve 85% removal can be reduced from 0.10 units/mL SBP to less than 0.0050 units/mL with the addition of PEG. Similarly to results with soil, the addition of soil to the reactor the SBP dose required to remove 85% could be reduced from 0.13 units/mL to 0.010 units/mL. While the addition of PEG in water alone is quite effective between pH 6 to 7 (Alemany, 2000), the improved removal shown with soil is apparent at pH 4 through 8 as reported in figure 4.7a. Essentially PEG is a high molecular weight, organic compound. The characteristics of PEG are similar to peat moss as peat moss is also a high molecular weight organic compound. PEG is thought to prevent

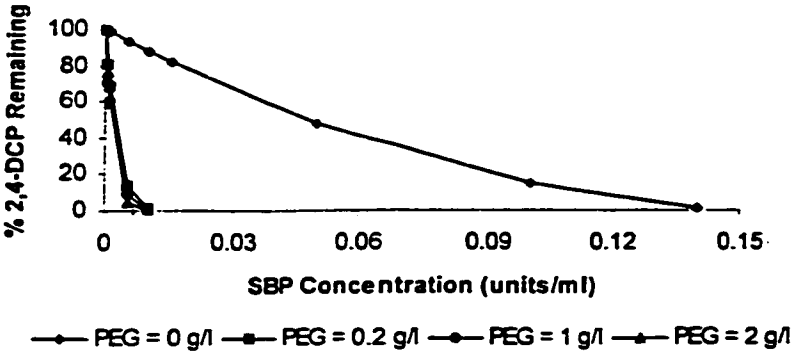


Figure 4.8a Percentage of 100 mg/L 2,4-DCP remaining versus SBP concentration for various concentrations of PEG8000, at pH 6.2. (from Allemany, 2000)

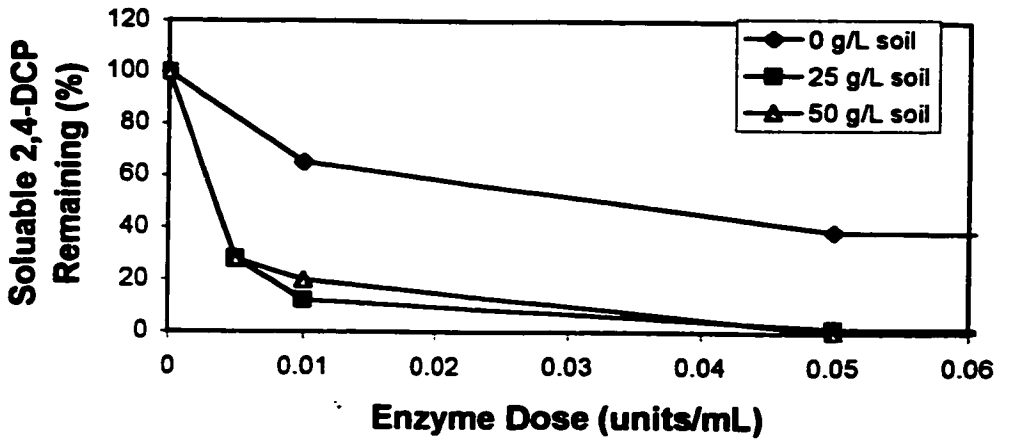


Figure 4.8b. The percent soluble 2,4-DCP remaining for various concentrations of soil, at pH 6.0. Initial concentrations after binding, and before enzyme activation are 82, 62, and 49 mg/L 2,4-DCP for reactions with 0, 25.0, and 50.0 g/L of soil respectively. 0.6 mM H_2O_2 added.

mechanism-based inactivation (Nakamoto and Machida, 1992), i.e., the entrapment of enzymes in the insoluble polymer product or the binding of a phenoxy radical to the active site of the enzyme. It appears that the addition of soil produces a similar effect on remediation by SBP. Research by Park et al. (1999) found that the addition of some humic components improved the removal of chlorinated phenols with peroxidase enzymes. Interestingly the humic components which improved the removal were those with higher molecular weight, while low molecular weight humic additions reduced the removal efficiency of the peroxidases. If soil is acting as an additive then the SBP or end product may be chelating to the PEG or soil (perhaps as a function of the highly adsorptive nature of the organic components found in PEG or soil), rather than binding between the enzyme and the product of the catalytic cycle.

While the improved $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ in reactors with soil and low SBP doses (<0.25 units/mL) may be a result of soil acting as an additive, it is unclear why similar results are not seen at higher SBP doses (>0.25units/mL).

Figures 4.9a-e plot the enzyme dose versus $SR_{2,4\text{-DCP}}$ for a given soil concentration. The required enzyme concentration to achieve a given $SR_{2,4\text{-DCP}}$ can be determined for each soil concentration using these graphs. The effective SBP concentration to remove a given percent of contaminants referred to as the ECX (where X represents a percent removal between 0 and 99.6%) can then be plotted against the soil concentration, to determine the effect of soil addition on the enzyme.

By graphing the enzyme dose versus soil concentration for a given percent removal ability, the capacity of soil to reduce the SBP dose is apparent. The reduction in SBP dose is important since the cost of the enzyme itself is the majority of the remedial

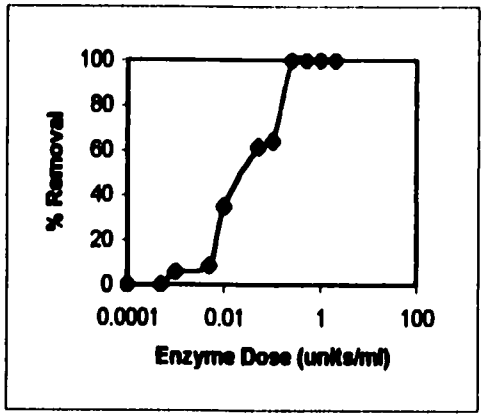


Figure 4.9a. $SR_{2,4\text{-DCP}}$ for 0 g/L soil for various SBP doses. Reactor at pH 6, 86 mg/L 2,4-DCP,with 0.6mMol hydrogen peroxide

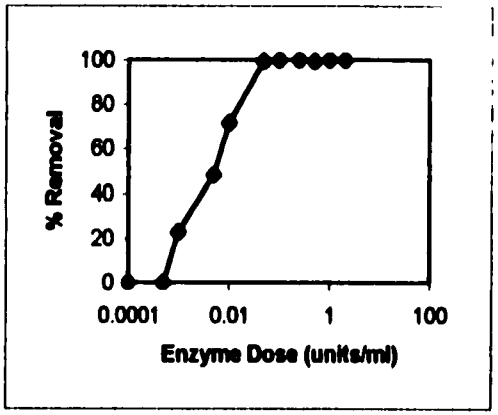


Figure 4.9b. $SR_{2,4\text{-DCP}}$ for 12.5 g/L soil for various SBP doses. Reactor at pH 6, 75 mg/L 2,4-DCP,with 0.6mMol hydrogen peroxide

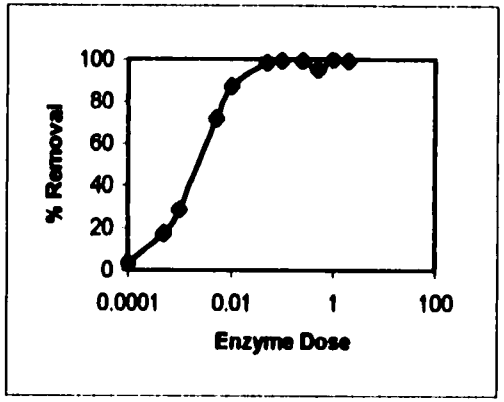


Figure 4.9c. $SR_{2,4\text{-DCP}}$ for 25 g/L soil for various SBP doses. Reactor at pH 6, 65 mg/L 2,4-DCP,with 0.6mMol hydrogen peroxide

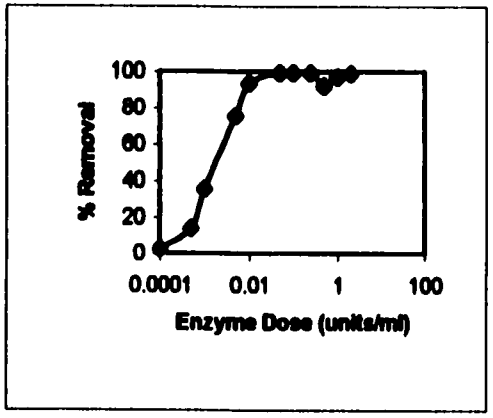


Figure 4.9d. $SR_{2,4\text{-DCP}}$ for 37.5 g/L soil for various SBP doses. Reactor at pH 6, 57 mg/L 2,4-DCP,with 0.6mMol hydrogen peroxide

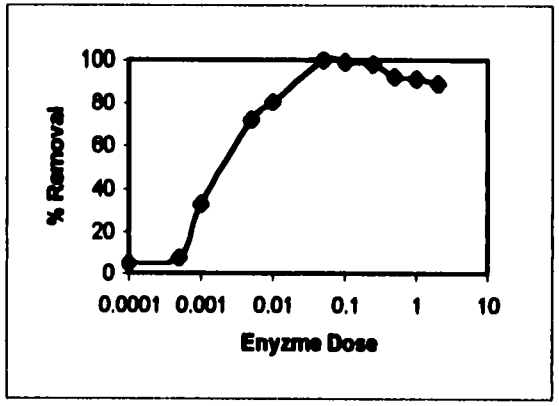


Figure 4.9e. $SR_{2,4\text{-DCP}}$ for 50 g/L soil for various SBP doses. Reactor at pH 6, 50 mg/L 2,4-DCP,with 0.6mMol hydrogen peroxide

cost. The implications of this graph indicate that the SBP and hence cost can be reduced by manipulating the soil concentration in the reactor.

Figure 4.10a shows the EC80, the enzyme concentration required to achieve 80% $SR_{2,4\text{-DCP}}$ as a function of soil concentration. The EC80 indicates that as a result of the addition of soil to the reactors, the SBP dose can be reduced to 4.2% of the SBP dose required without soil to achieve the same $SR_{2,4\text{-DCP}}$. The EC50 is plotted in figure 4.10b. The EC50 indicates that the addition of soil to the reactors allows the dose of SBP to be reduced to 10% of the dose required to achieve the same $SR_{2,4\text{-DCP}}$ of 50% without soil. The reduction of the required SBP dose is substantial for 50% and 80% $SR_{2,4\text{-DCP}}$ resulting in a lower total SBP requirement and lower associated SBP costs. Thus to achieve a given removal, the addition of soil results in a lower required SBP dose.

4.2 Hydrogen Peroxide

Previous research indicated that when excess hydrogen peroxide is available it may react with Compound II to form Compound III, an inactive enzyme form rather than completing the cycle and returning to the original form of the enzyme (Arnao et al., 1990). Figure 4.11 shows the $SR_{2,4\text{-DCP}}$ as a function of soil concentration for various concentrations of H_2O_2. By manipulating the H_2O_2 dose in reactors it was found that wastewater without soil has an optimum H_2O_2:substrate molar ratio of 2:1. For a dose of 0.050 units/mL SBP, there is 94% $SR_{2,4\text{-DCP}}$ with a 2:1 ratio of H_2O_2:substrate ratio. For 1:1 and 4:1 H_2O_2:substrate ratio the $SR_{2,4\text{-DCP}}$ decreases to 61 and 54%, respectively. The optimum H_2O_2:substrate ratio has been reported to be between 0.7 to 1.6 units/mL range for SBP (Flock et al., 1999; Caza et al., 1999) and horseradish peroxidase (Wu et al., 1993; Wu et al., 1999; Nicell et al., 1992). Figure 4.12 shows the $SR_{2,4\text{-DCP}}$ as a function

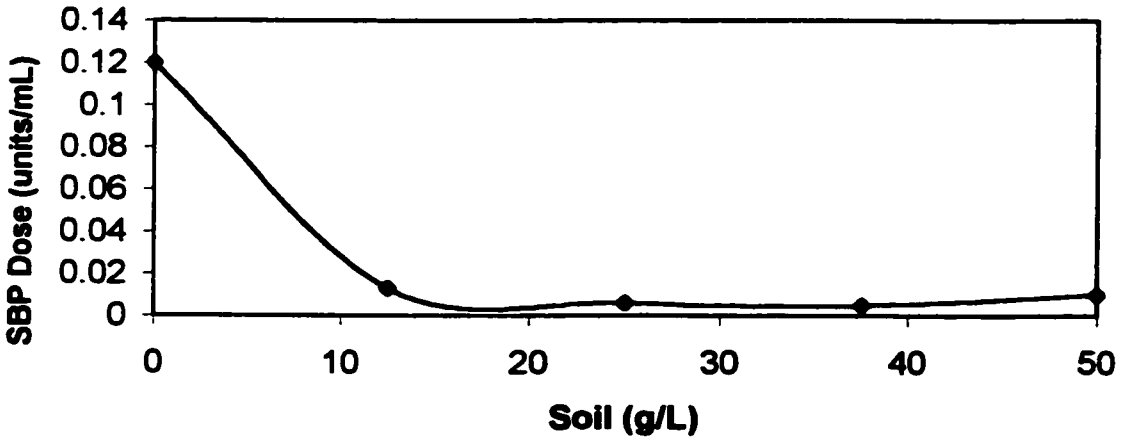


Figure 4.10a. The EC80 as a function of soil concentration. Conditions are the same as figure 4.9

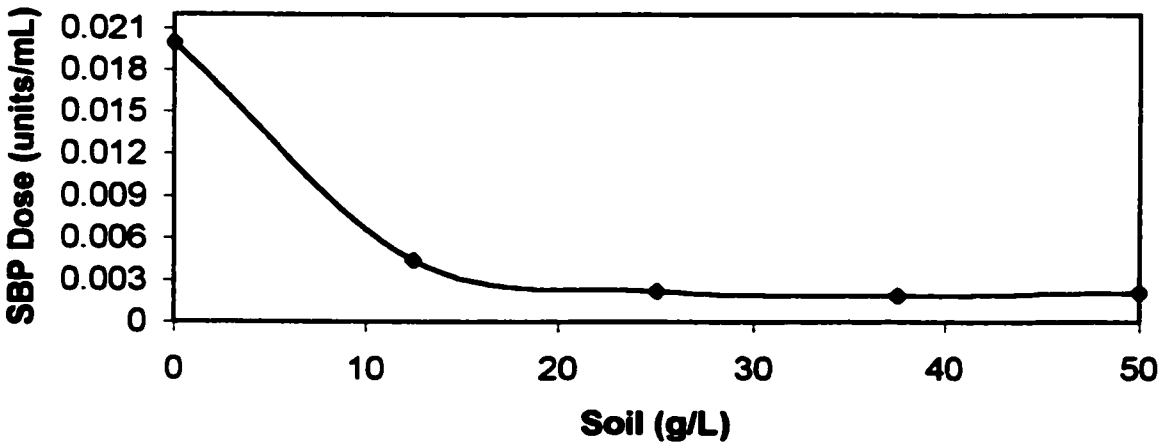


Figure 4.10b. The EC50 as a function of soil concentration. Conditions are the same as figure 4.9.

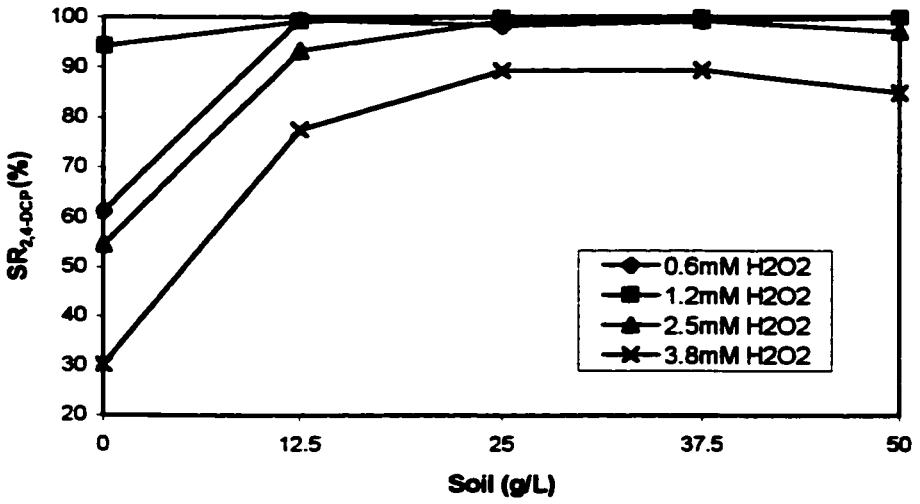


Figure 4.11. $SR_{2,4\text{-DCP}}$ as a function soil concentration for various hydrogen peroxide dose. Reactor conditions were pH 6, with 0.05 units/mL SBP added. Initial 2,4 DCP concentrations after binding, and before enzyme activation for experiments run with 0.6 m M H_2O_2 are (82, 69, 62, 54, 49) mg/L, for those with 1.2 mM H_2O_2 are (82, 73, 62, 55, 48) mg/L, for those with 2.5 mM H_2O_2 are (81.0, 72.7, 66.9, 57.1, 38.6) mg/L , for those with 3.75 mM H_2O_2 added are (77.5, 71.9, 61.5, 55.1, 47.9) mg/L for 0, 12.5, 25.0, 37.5, and 50.0 g/L soil respectively.

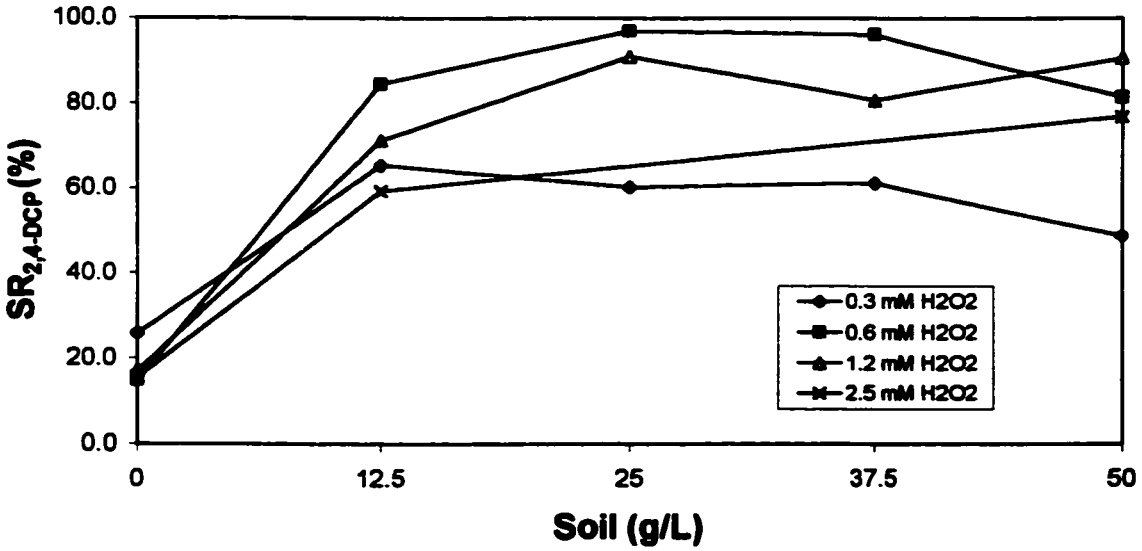


Figure 4.12. $SR_{2,4\text{-DCP}}$ as a funciton of soil concentration for various hydrogen peroxide dose. Reactor were run at pH 6, with 0.01 units/mL SBP. Initial concentrations of 2,4-DCP after binding and before enzyme activation for the addition of 0.306 mM H_2O_2 are (78.7, 71.2, 64.3, 56.03, 49.9) mg/L, for the addition of 0.613 mM H_2O_2 are (86.0, 72.6, 65.1, 57.8, 51.7) mg/L, for the addition of 1.25 mM H_2O_2 are (83.2, 72.6, 62.2, 58.2,

of soil concentration for various concentrations of H_2O_2. This plot is similar to figure 4.11 but a lower SBP dose of 0.010 units/mL was used to keep $SR_{2,4\text{-DCP}}$ below 99.6%. Figure 4.12 indicates that the optimum H_2O_2:substrate ratio is 1:1 when soil is present in the reactor. It is possible that the reactors with soil require less H_2O_2 as a result of soil acting as an additive and making the SBP more efficient at lower SBP doses. The optimum H_2O_2:substrate ratio for 2-chlorophenol without PEG was 1.6 (Flock et al., 1999), for 2-chlorophenol with PEG addition the optimum ratio was 0.8 (Caza et al., 1999). It seems possible that the addition of an additive, such as PEG, may decrease the optimum H_2O_2:substrate ratio. Similarly, if soil is functioning as an additive as previously discussed, then the lower H_2O_2 requirement seems reasonable. $TR_{2,4\text{-DCP}}$ was not considered as the goal was to optimize the efficiency of the $SR_{2,4\text{-DCP}}$ with SBP.

One of the concerns with the use of SBP in soil systems was that excess H_2O_2 would be required to overcome the potential degradation of H_2O_2 by organic components in the soil, leading to increased inactivation of SBP. It appears from the results presented in figures 4.11 and 4.12 that excess H_2O_2 may not be necessary for the soil concentrations investigated. This may be related to the rate at which the reaction proceeds. It appears the SBP may not be inactived through H_2O_2 inactivation and therefore higher concentrations of H_2O_2 are available for the activation of the enzyme. H_2O_2 would not be limiting the reaction.

4.3 Removal of 2,4-DCP as a Function of Time

The time required for the enzyme reaction to reach completion is an important aspect of the economic feasibility of enzyme remediation. Figure 4.13a is taken from

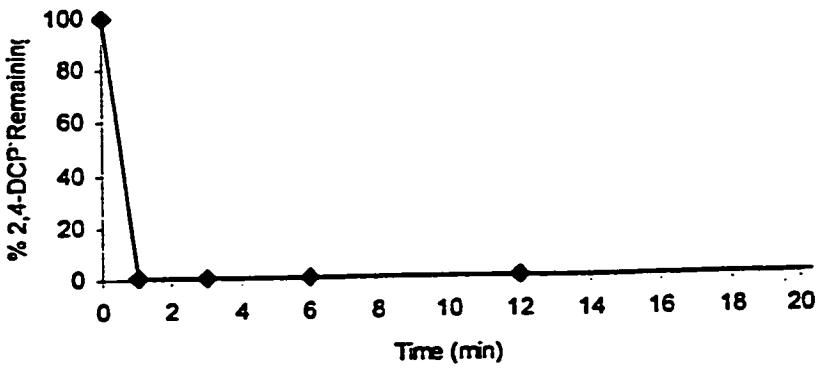


Figure 4.13a. The percent remaining of 100 mg/L 2,4-DCP with time after the activation of 1 unit/mL SBP at 22°C with 0.613 mM H_2O_2 at pH 6.2. (from Allemany, 2000)

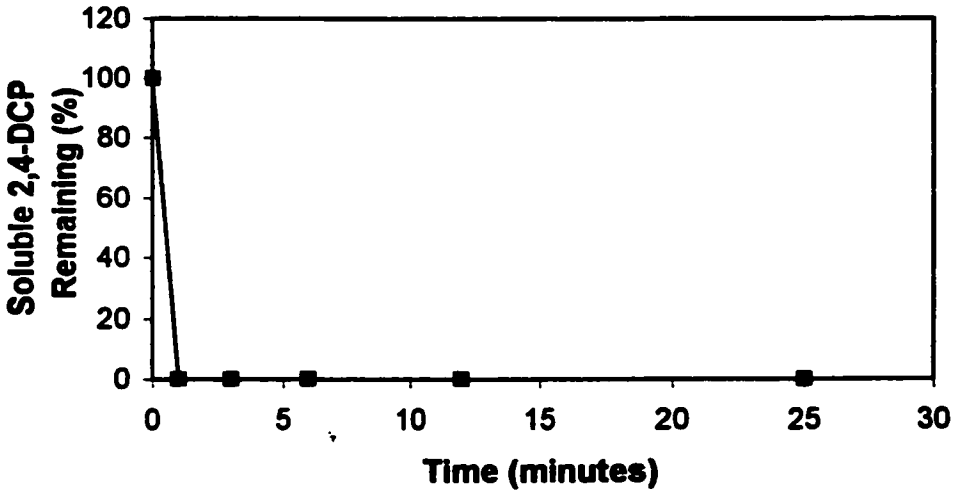


Figure 4.13b. The percent soluble 2,4-DCP remaining as a function of time at 22°C with soil. Activation of 1 unit/mL SBP with 0.613 mM H_2O_2, in reactor with 50 g/L of soil at pH 6. Initial concentration 98 mg/L, after binding and before enzyme activation the initial concentrations were 49 mg/L.

Alemany (2000), it shows the percent of 2,4-DCP remaining as a function of time. Alemany (2000) found that one minute after SBP activation 99.6% or greater $SR_{2,4\text{-DCP}}$ had occurred (initial conditions: 100 mg/L 2,4-DCP, 1 unit/mL SBP, 0.613 M H_2O_2) at 22°C. The number of turnovers (TO) describe the number of times the catalytic cycle is completed. If the time and substrate removal are known the turnover rate can be calculated (calculations described in section 4.7). The rate of turnover at 22°C was 31.2 TO/s for wastewater assuming the reaction took exactly 1 minute to complete (Alemany, 2000). Figure 4.13b shows the percent $SR_{2,4\text{-DCP}}$ as a function of time for reactors with 50 g/L of soil. With soil in the reactor (supernatant concentration of 51 mg/L) 99.6% or greater $SR_{2,4\text{-DCP}}$ occurred within the first minute. This resulted in a turnover rate of 15.8 TO/s. This turnover rate reflects only the change in substrate in the supernatant concentration and could be up to twice as high if the 2,4-DCP bound to the soil is also being consumed. $TR_{2,4\text{-DCP}}$ was not plotted as the plot was very similar to $SR_{2,4\text{-DCP}}$.

4.4 Removal of 2,4-DCP as a Function of Temperature and Time

Alemany (2000) used time dependent tests to show that the rate of SBP removal is slower at lower temperatures. To achieve reactions at 4°C the reactor bottles were maintained in a 4°C refrigerator. Figure 4.14a from Alemany (2000) shows the percent of 2,4-DCP remaining as a function of time at 4°C. Although data showed the same removal or number of turnovers at 4°C, the process took 180 minutes to reach completion. After 20 minutes, it was found that 95% $SR_{2,4\text{-DCP}}$ occurred, resulting in a average turnover rate of 1.48 TO/s with wastewater at 4°C (Alemany, 2000). Figure 4.14b shows the percent soluble remaining of 2,4-DCP as a function of time for reactors with 50 g/L of soil. When these tests were performed with 50 g/L of soil (resulting in a

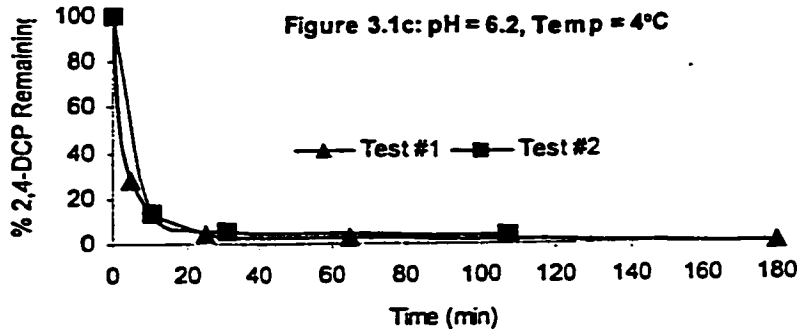


Figure 4.14a. The percent remaining of 100 mg/L 2,4-DCP with time after the activation of 1 unit/mL SBP with 0.613 mM H_2O_2 at 4°C, and pH 6. (from Allemany, 2000)

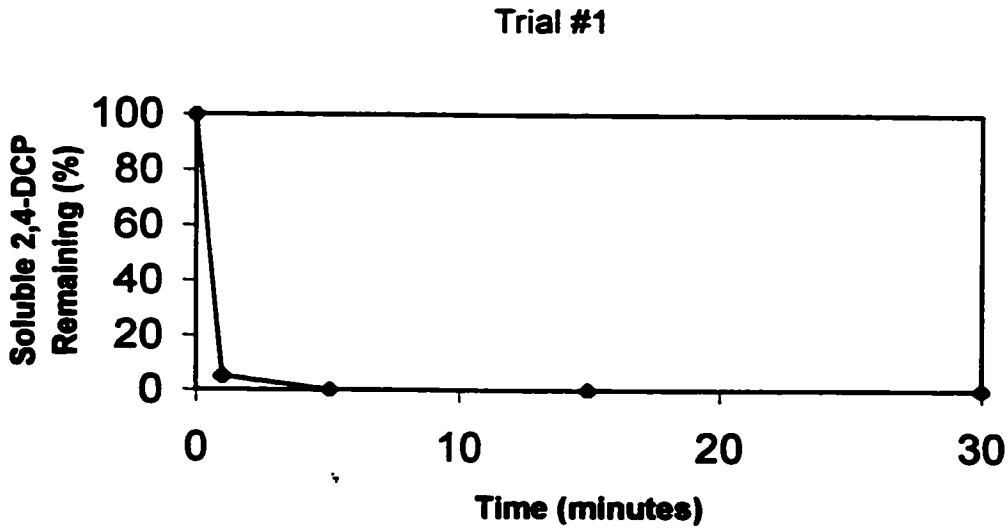


Figure 4.14b. The percent soluble 2,4-DCP remaining as a function of time at 4°C with soil in the reactor. Activation of 1 unit/mL SBP with 0.613 mM H_2O_2, in reactor with 50 g/L of soil at pH 6. Initial concentrations were 98 mg/L, after binding and before enzyme activation the initial concentrations were 49 mg/L.

supernatant concentration of 51 mg/L 2,4-DCP after binding), 97% $SR_{2,4\text{-}DCP}$ occurred within the first minute. This resulted in an average rate of 15.5 TO/s. Again this turnover rate is conservative as it considers only the change in substrate present in the supernatant. The turnover rate could be up to twice as high if the 2,4-DCP bound to the soil is being remediated. Therefore even at low temperatures, the addition of soil seems to increase the rate at which enzymatic removal occurs. The resulting effect of the soil acting as an additive and protecting the enzyme allows the enzyme to perform unhindered. While it appears from Alemany's (2000) research that the rate of the SBP reaction is slower at 4°C, the enzyme may also be becoming inactivated. Whereas with the addition of soil the reaction may also be proceeding slowly, but is not experiencing the inactivation that may have occurred without the soil.

Tests performed with soil at 4°C resulted in a ten fold increase in turnover rate compared to those which Alemany (2000) found without soil. The implications of these results is that soil slurries with 2,4-DCP would require a lower hydraulic retention time (HRT) to remediate, and thus would require smaller reactor volumes. This investigation reveals some potential for groundwater remediation, where groundwater is typically at much lower temperatures.

4.5 Polyethylene Glycol

Previous tests dealing with the use of SBP to remediate contaminated water have shown significant increases in removal efficiency of chlorinated phenols with the addition of polyethylene glycol (Alemany, 2000; Caza et al., 1999). Additionally, the removal increased even more dramatically as the concentration and molecular weight of

PEG increased (Alemany, 2000). Therefore, it was necessary to determine if this same trend would be found in the remediation of water in the presence of soil.

Figure 4.15 shows the $SR_{2,4\text{-DCP}}$ as a function of soil concentration for various doses of PEG at pH 6. At pH 6, the addition of PEG appeared to slightly hinder the $SR_{2,4\text{-DCP}}$. The effects of concentration and molecular weight of the PEG is not discernable. With water alone the efficiency of SBP should be improved with the addition of PEG (Alemany, 2000). SBP reactors with soil do not produce similar results with PEG addition. However, at higher soil concentrations PEG appears to have a positive effect.

Figure 4.16 shows the $SR_{2,4\text{-DCP}}$ as a function of soil concentration for various doses of PEG at pH 8. At pH 8, the $SR_{2,4\text{-DCP}}$ appeared to be slightly improved for an increase in concentration as almost every test with PEG slightly outperformed those without PEG. The highest $SR_{2,4\text{-DCP}}$ was found when PEG was added at a concentration of 5.0 g/L and with a molecular weight of 8000 g/gmol. The next highest $SR_{2,4\text{-DCP}}$ was found for 1.0 g/L of PEG8000, and then 0.1 g/L PEG1000. Despite the slight improvement in the $SR_{2,4\text{-DCP}}$ when PEG was added, this effect was insignificant compared to the documented material regarding HRP or SBP and PEG for wastewater treatment. The literature suggests that the HRP doses be reduced to 0.030 (Bewtra et al., 1995), or 0.025 to 0.013 (Wu et al., 1993). While the majority of research to date focuses on the effect of PEG on HRP, significant improvements in SBP efficiency have also been documented by Alemany (2000), and Caza et al. (1999). Alemany (2000) reported an increase in 2,4-DCP removal from 14 to 99% with PEG addition to SBP reactors. Similar reductions in SBP dose as a result of PEG addition do not seem feasible for systems that contain the soil examined in this study. The inability of PEG to increase the

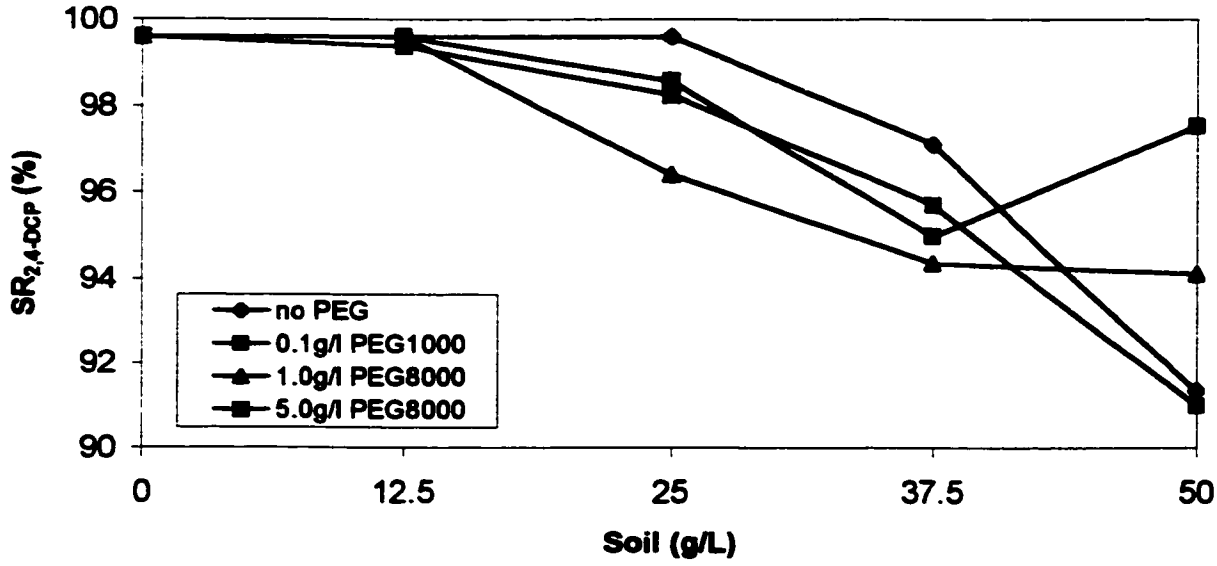


Figure 4.15. $SR_{2,4\text{-DCP}}$ at pH 6 for various additions of PEG. The soil was composed of 10% peat, and 90% silica sand by weight. 1 unit/mL of SBP, and 0.613mM of H_2O_2 were added. The concentrations after binding and before enzyme inactivation for experiments with no PEG were (84.3, 67.8, 63.1, 55.2) mg/L, for 0.1 g/L PEG1000 were (80.7, 67.8, 73.7, 59.4) mg/L, for 1.0 g/L PEG8000 were (82.0, 71.8, 62.1, 56.9) mg/L, for 5.0 g/L PEG800 were (85.9, 74.7, 63.1, 56.4) mg/L for 0, 12.5, 25.0, and 37.5 g/L of soil respectively.

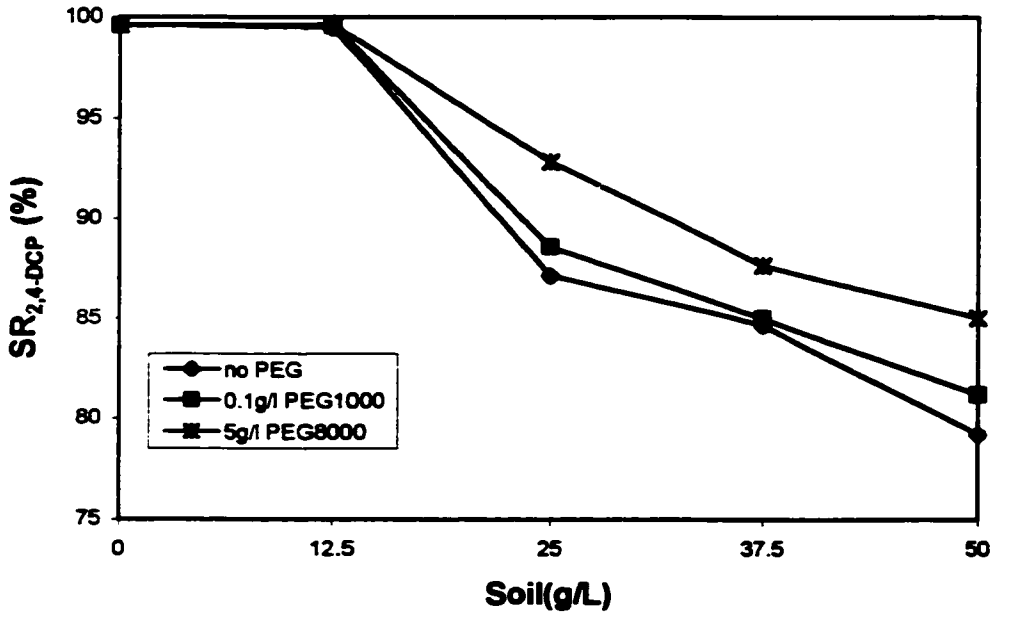


Figure 4.16. $SR_{2,4\text{-DCP}}$ as a function of soil at pH 8 for various additions of PEG. The soil was composed of 10% peat, and 90% silica sand by weight. 1 unit/mL of SBP, and and 0.613mM of H_2O_2 were added. The concentration of the contaminant after binding and before enzyme activation for experiments without PEG were (86.4, 80.4, 78.2, 73.9, 78.7) mg/L, for 0.1 g/L PEG1000 were (84.4, 81.6, 78.8, 76.0, 71.4) mg/L, for 5.0 g/L PEG8000 were (99.5, 92.2,87.1, 80.7, 78.1) mg/L for soil concentrations 0, 12.5, 25.0, 37.5, and 50 g/L respectively.

efficiency of the $SR_{2,4\text{-DCP}}$ of SBP may be due to the fact that the soil is already working as an additive. Results with PEG indicate that the incremental removal of contaminant decreases with an incremental increase in PEG at higher PEG concentrations. This may be the result of the addition of soil providing a saturated additive environment, and any addition of PEG does not provide any significant benefit to the SBP.

The total percent removal plots were not presented as they did not significantly differ from the soluble percent removal plots.

4.6 Performance of SBP with Higher Soil Concentrations

The removal of 2,4-DCP in the presence of up to 600 g/L of soil at low enzyme concentration was examined. Figure 4.17a shows the $SR_{2,4\text{-DCP}}$ as a function of soil concentration. Due to the acidic nature of the soil, when 200 g/L of soil was added to the system the pH dropped from 6 to 5.9, with 400 g/L of soil the pH dropped to 5.4, and with the addition of 600 g/L the pH dropped to 4.9. This indicates a potential problem which may occur when large quantities of soil are added to the reactor or for *in situ* soil remediation. As soil concentrations increase, the pH decreases as a result of the organic and humic components in soil. The drop in pH may result in non-optimum conditions for enzymatic removal. The optimum removal between 0 and 600 g/L of soil was found to occur around 25 g/L, and begins to decrease with further soil addition. As the amount of soil increases beyond 400 g/L there is little improvement over wastewater only systems. At 600 g/L of soil, the enzyme performance is below that without soil. With the addition of 600 g/L of soil there was a drop in pH to 4.9 and only 8% $SR_{2,4\text{-DCP}}$. Alternate tests performed at pH 5.0 under the same conditions showed 64%, and 85%

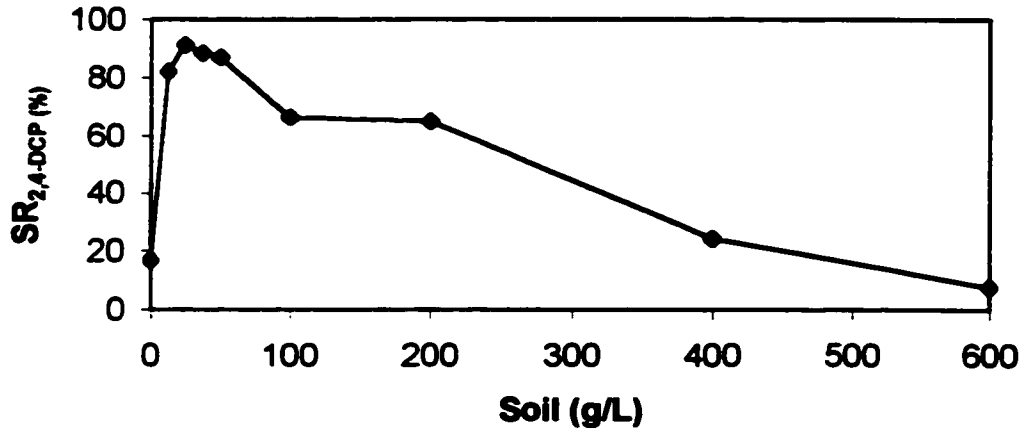


Figure 4.17a. $SR_{2,4\text{-DCP}}$ as a function of soil concentration. Reactors were at pH 6, 0.01 u/mL SBP, 0.6 mM 2,4-DCP and 1:1 substrate to H_2O_2 molar ratio. Concentrations after binding and before enzyme activation were (86, 74, 66, 58, 53, 38, 22, 11, 7) mg/L 2,4-DCP for reactors with (0, 12.5, 25.0, 37.5, 50.0, 100, 200, 400, 600) g/L soil

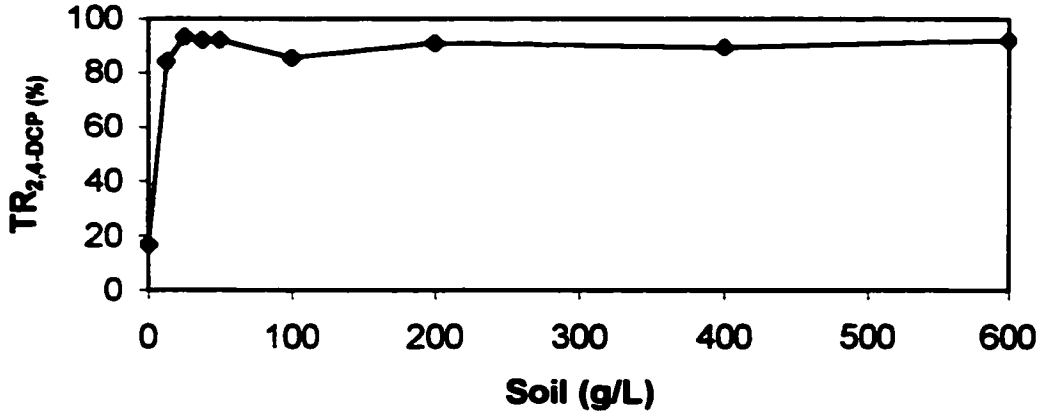


Figure 4.17b. $TR_{2,4\text{-DCP}}$ for various concentrations of soil. Reactor conditions are given in figure 4.17a.

$SR_{2,4\text{-DCP}}$ for 25 g/L and 50 g/L of soil respectively. This may indicate that the enzyme interference is a result of higher concentrations of soil not merely a decrease in $SR_{2,4\text{-DCP}}$ due to the lower pH. For 600 g/l of soil the $SR_{2,4\text{-DCP}}$ is approximately 10% of the $SR_{2,4\text{-DCP}}$ found for 50 g/L of soil at approximately the same pH. While the addition of soil up to about 400 g/L may improve the removal of 2,4-DCP over wastewater only, the addition of larger quantities of soil seems to interfere with the $SR_{2,4\text{-DCP}}$.

Figure 4.17b shows the $TR_{2,4\text{-DCP}}$ as a function of soil concentration. Despite the decrease in $SR_{2,4\text{-DCP}}$ as a result of adding larger quantities of soil, the $TR_{2,4\text{-DCP}}$ remains fairly stable at approximately 90% with soil concentrations of 25 g/L or greater and SBP concentration of 0.010 units/mL. At lower soil concentrations the SBP is responsible for the majority of mass removal while at higher soil concentrations the majority of the mass removal is occurring as a result of binding with the soil with minimal enzymatic removal.

It appears that the use of soil as a method of immobilization may be feasible in addition to enzymatic removal. Unfortunately, the SBP loses much of its efficiency with the addition of larger concentrations of soil. This indicates that remediation of in situ soil may not be feasible since the concentrations of soil in situ would be much higher than examined here, and results have indicated that larger concentrations of soil resulted in lower enzyme activity. The decrease in SBP activity may be attributable to the degradation of H_2O_2 with larger quantities of soil and organics. If this was occurring, the soil would still be behaving as an additive but the H_2O_2 would be limiting the reaction. Therefore an increase in SBP activity may be possible with an increase in H_2O_2 dose. With the addition of excess H_2O_2 there may be an increased risk of SBP inactivation.

Future research should examine the effect of adding much higher doses of H_2O_2 to determine if SBP activity can be increased with larger soil concentrations.

4.7 Implications

As the soil is added, the increase in $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ occurs as the soil concentration is increased at low SBP doses for pH 4 to 8, while at pH 3 and 9 the 2,4-DCP removal efficiency declines. Between pH 4 and 8, the increase in removal may be due to the increase in enzyme activity with the addition of soil may if the soil acting as an additive. At pH 3, and 9 the soil would also act as an additive, but previous research with SBP indicates that the addition of PEG has very little effect on wastewater at pH levels other than 6 to 7 (Alemany, 2000). Therefore it is likely that if soil is acting as an additive it would have little or no effect at pH 3, and 9.

Additionally, if soil was acting as an additive it would partially explain why there was very little effect of PEG addition to the reactor when soil was present. Perhaps the ineffectiveness of PEG in soil systems is due to the fact that the soil has already created an environment saturated with additives. While this would account for the increase in $SR_{2,4\text{-DCP}}$ at low enzyme doses it does not explain why at high enzyme doses the $SR_{2,4\text{-DCP}}$ decreases with the addition of soil. If the addition of soil is acting as an additive at lower SBP doses then it should also act as an additive at higher SBP doses. The reason this statement does not hold true is inexplicable.

The investigation of contaminant removal in wastewater with soil indicates that there is potential for 2,4-DCP remediation of soil slurry systems. Despite promising results that SBP can be used effectively to remove contaminants from the wastewater

when exposed to soil, there was no investigation into the 2,4-DCP sorbed to the soil and whether it was remediated. In spite of these concerns, the 2,4-DCP was removed effectively with relatively low doses of SBP, indicating there is potential for applications of SBP to soil systems. Improved removal of 2,4-DCP with SBP occurs both at low doses of SBP and decreases the time required for the reaction to occur at 4°C. At 4°C the addition of soil seems to reduce the time necessary to reach 95% removal by up to a factor of 20. This would result in lower HRT for reactors, consequently requiring a smaller volume, possibly culminating in lower operating and capital costs.

An initial concern regarding the addition of SBP to soil for the treatment of 2,4-DCP was that the process may require excess H_2O_2 to overcome the degradation of H_2O_2 by soil organics. In fact, it was found that lower doses of H_2O_2 are required for soil systems (<50 g/L soil) as opposed to wastewater-alone. This may result in higher SBP efficiency as a result of less inactivation. With soil concentrations greater than 50 g/L, the SBP activity decreases. In systems that contain higher soil concentrations, higher doses of H_2O_2 may be required to overcome its degradation. If higher doses of H_2O_2 are applied there may be an increase in SBP efficiency if H_2O_2 is no longer limiting. Alternatively higher doses of H_2O_2 may result in decreased the removal of 2,4-DCP as a result of SBP inactivation.

While in this study it has not been determined whether the 2,4-DCP remains sorbed to the soil or has also been remediated, the 2,4-DCP has been effectively removed from the supernatant. Further concerns would be the desorption of 2,4-DCP and the toxicity of the end-product polymer, and its removal from the reactor and/or soil disposal.

While the possibility of in situ soil remediation exists, the following would be of concern: a decrease in pH due to the acidic nature of soil and organic matter resulting in lower SBP efficiency, and the likely degradation of H_2O_2, as well as increased H_2O_2 doses. Despite this, it is encouraging that the addition of soil results in higher removal per time at 4°C than without, as soil is typically at much lower temperatures than 22°C.

Chapter 5

Isolating the SBP Activity

While the Chapter 4 has taken a " black box " approach to the removal of 2,4-DCP, chapter 5 will examine the contribution of the SBP to remediate the 2,4-DCP under the assumptions of both reversible and irreversible sorption conditions. Figures 4.2 to 4.5 will be revisited in the following figures using the sorption isotherms developed in chapter 3 to determine the final mass of 2,4-DCP sorbed to the soil and hence the final mass of 2,4-DCP in the reactor. Therefore this chapter will use a mass balance to determine the removal of 2,4-DCP that includes the 2,4-DCP that sorbs to the soil. This will allow conclusions to be drawn about the activity of the SBP and the removal is based on the entire change in 2,4-DCP. The final mass of 2,4-DCP sorbed to the soil under completely reversible sorption conditions is based on the sorption isotherms from chapter 3 and the following relationship:

$$Q = kCe \qquad [5.1]$$

Under reversible sorption conditions, the final mass of 2,4-DCP in the reactor can be found using the k value (from chapter 3), the effluent concentration (Ce) and equation 5.1 to determine the mass of 2,4-DCP sorbed to the soil. For irreversible sorption the initial mass sorbed to the soil in the initial 24 hours prior to SBP addition is assumed to remain in the reactor at the final 2,4-DCP analysis after SBP addition. This can be calculated by subtracting the 2.4-DCP concentration after the 24 hour binding period (before SBP addition) from the initial concentration of 2,4-DCP

Figure 5.1a plots the soil concentration versus percent removal of 2,4-DCP for the same set of experiments as in figure 4.2a. However figure 4.2a examines only the soluble

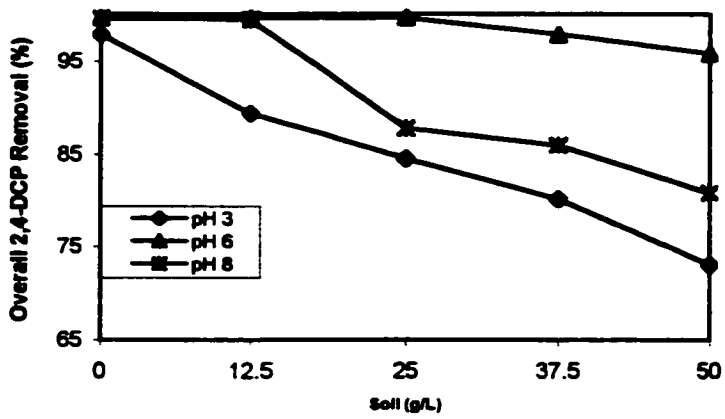


Figure 5.1a Removal of 2,4-DCP for various pH and soil concentrations assuming reversible sorption of 2,4-DCP. Reactor conditions are the same as figure 4.2a

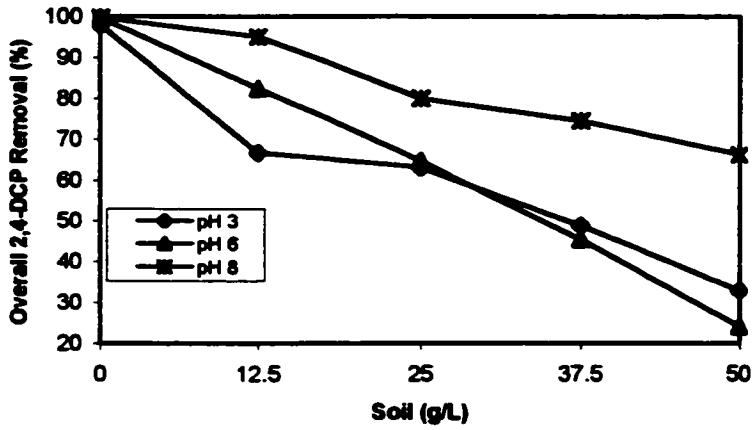


Figure 5.1b Removal of 2,4-DCP for various pH and soil concentrations assuming irreversible sorption of 2,4-DCP. Reactor conditions are the same as figure 4.2a.

removal of 2,4-DCP (i.e. change in supernatant concentration), figure 5.1a assumes reversible sorption of 2,4-DCP and uses a mass balance to determine removal. Figure 5.1 shows lower removal of 2,4-DCP than in figure 4.2a as the soil concentration is increased. Similar to figure 4.2a, with the addition of soil the highest removal is found for reactors at pH 6, followed by pH 8 and 3. Figure 5.1b plots the soil concentration versus percent removal of 2,4-DCP under irreversible sorption conditions. For this case, since the sorption is highest for pH 6, there is more 2,4-DCP bound to the soil than at pH 3 or 8 and higher mass of 2,4-DCP remaining in the reactor. Therefore as the mass of soil is increased the removal of 2,4-DCP decreases more at pH 6 than at pH 3 or 8. At pH 6 and 50 g/L of soil the enzymatic removal of 2,4-DCP decreases from 99.6% to 24%.

Figure 5.2a plots the concentration of soil versus the enzymatic percent removal of 2,4-DCP under reversible sorption conditions. This figure is similar to figure 4.3a, but exhibits slightly lower enzymatic removal of 2,4-DCP due to the bound residual 2,4-DCP assumed to remain in the reactor under reversible sorption conditions. Similar to figure 4.3 the highest enzymatic removal of 2,4-DCP is found for an SBP dose of 0.25 u/mL. Higher doses of SBP reduce the activity of the SBP. Figure 5.2b plots the same data but assumes irreversible sorption. The graph indicates that the enzymatic removal is similar regardless of the SBP dose. There is a linear decrease in the enzymatic removal as the soil concentration increases. This is due to the bound residual limiting the enzymatic removal under irreversible sorption conditions.

Figure 5.3a plots the SBP concentration versus the removal of 2,4-DCP. This figure is similar to figure 3.3a but assumes reversible sorption of 2,4-DCP. Figure 5.3a

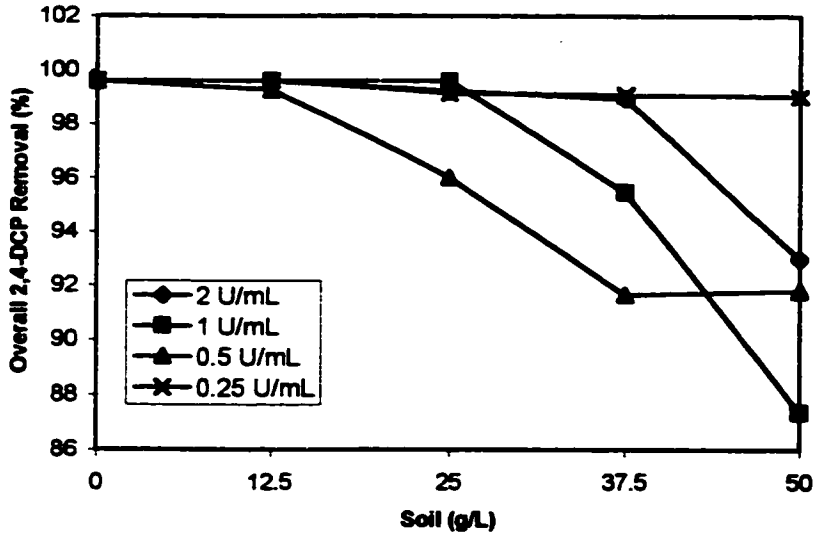


Figure 5.2b Removal of 2,4-DCP for varying soil concentrations and doses of SBP ranging from 0.25 to 2 units/mL assuming reversible sorption of 2,4-DCP. Conditions are given in figure 4.3a.

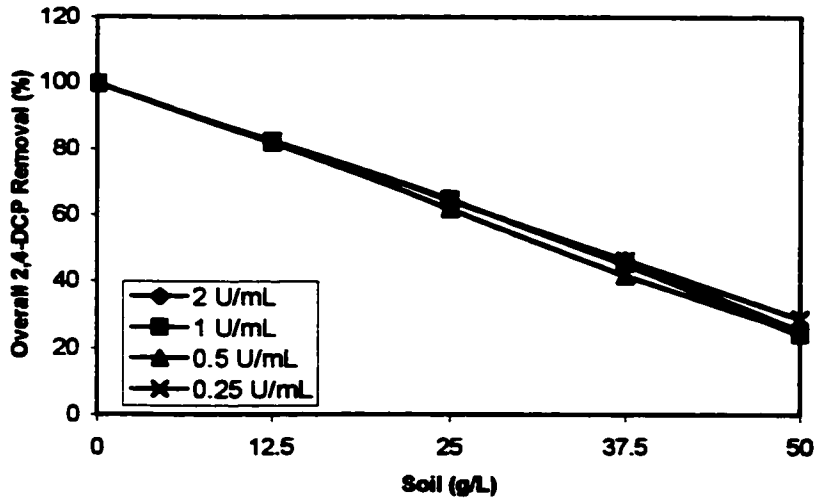


Figure 5.2b Removal of 2,4-DCP for varying soil concentrations and doses of SBP ranging from 0.25 to 2 units/mL assuming irreversible sorption of 2,4-DCP. Conditions are given in figure 4.3a.

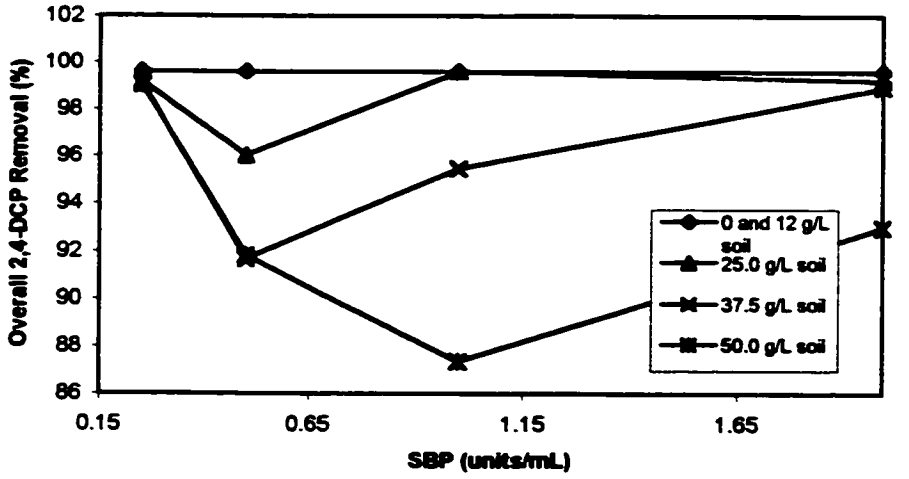


Figure 5.3a The removal of 2,4-DCP versus SBP dose for varying soil concentrations assuming reversible sorption. Conditions are the same as figure 4.4a.

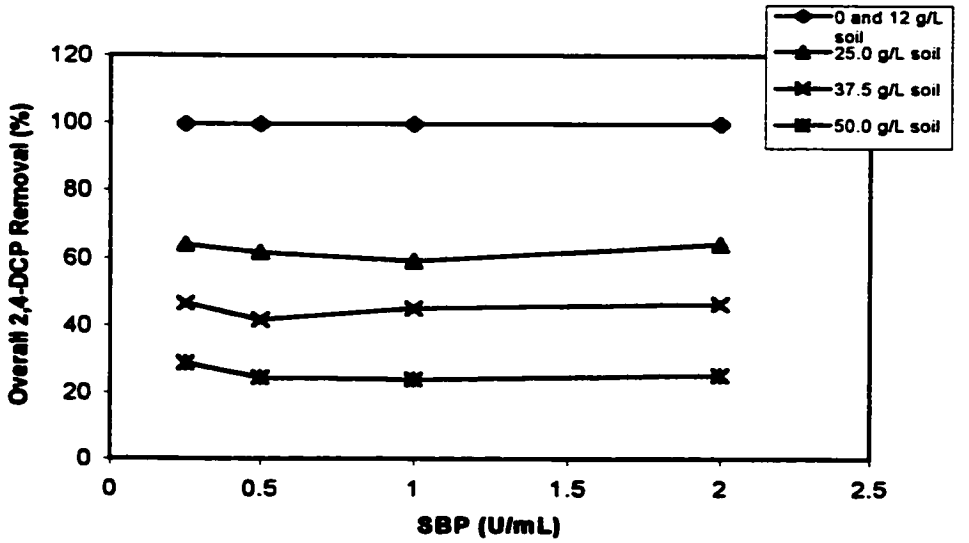


Figure 5.3b The removal of 2,4-DCP versus SBP dose for varying soil concentrations assuming irreversible sorption. Conditions are the same as figure 4.4a.

indicates that the removal of 2,4-DCP is highest without soil and with 12.5 g/L of soil. The removal of 2,4-DCP decreases as the concentration of soil increases. While 99.6% or greater removal is achieved with 0 and 12.5 g/L of soil, the addition of 50 g/L of soil results in 87% removal of 2,4-DCP with 1 u/mL SBP. But at lower SBP concentrations the percent removal of 2,4-DCP increases, similar to figure 4.4a. With 0.25 units/mL of SBP, 99% or greater removal of 2,4-DCP is achieved regardless of soil concentration.

Assuming irreversible sorption of 2,4-DCP, figure 5.3b plots the SBP concentration versus the removal of 2,4-DCP. Under irreversible sorption conditions the removal of 2,4-DCP varies very little with change in SBP concentration, but the percent removal varies with SBP dose. While 99.6% or greater removal is achieved with 0 and 12.5 g/L of soil; the percent removal decreases to approximately 60% removal of 2,4-DCP regardless of SBP concentration. The addition of 37.5 g/L of soil reduces the removal approximately 45%, and the addition of 50 g/L of soil to 25% removal of 2,4-DCP.

Figures 5.3a and b show that under either reversible or irreversible sorption conditions that the addition of soil interferes with the enzymatic removal of 2,4-DCP at SBP doses above 0.25 units/mL.

Figure 5.4a plots the concentration of soil versus the percent removal of 2,4-DCP at low SBP concentrations (0.001 to 0.1 units/mL). This figure is similar to figure 4.5a but assumes that the sorption of 2,4-DCP is reversible. Similar trends are seen between the two figures with higher removal of 2,4-DCP as the concentration of soil is increased. In figure 4.5a, the soluble percent removal of 2,4-DCP with 0.005 units/mL increased 8.4 fold with the addition of 25 g/L of soil versus 0g/L of soil. Assuming reversible sorption,

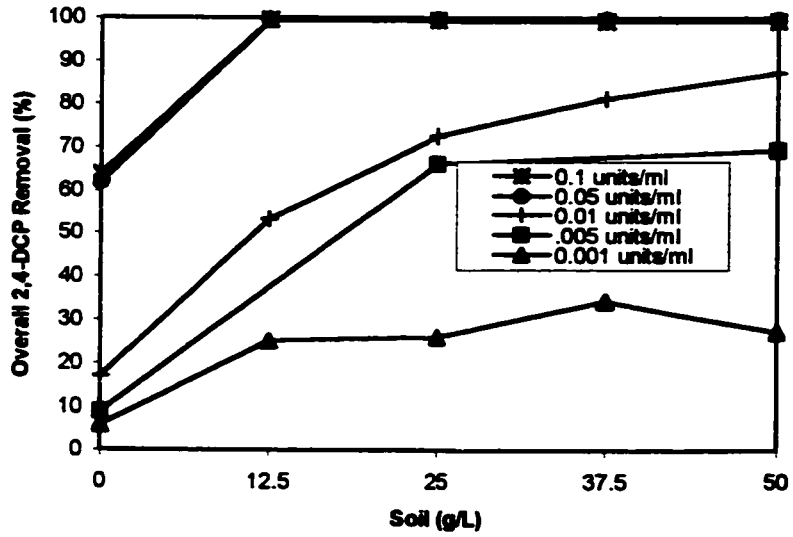


Figure 5.4a Removal of 2,4-DCP versus soil for doses of SBP ranging from 0.001 to 0.1 units/mL, assuming reversible sorption of 2,4-DCP to soil. Conditions are the same as in figure 4.5a.

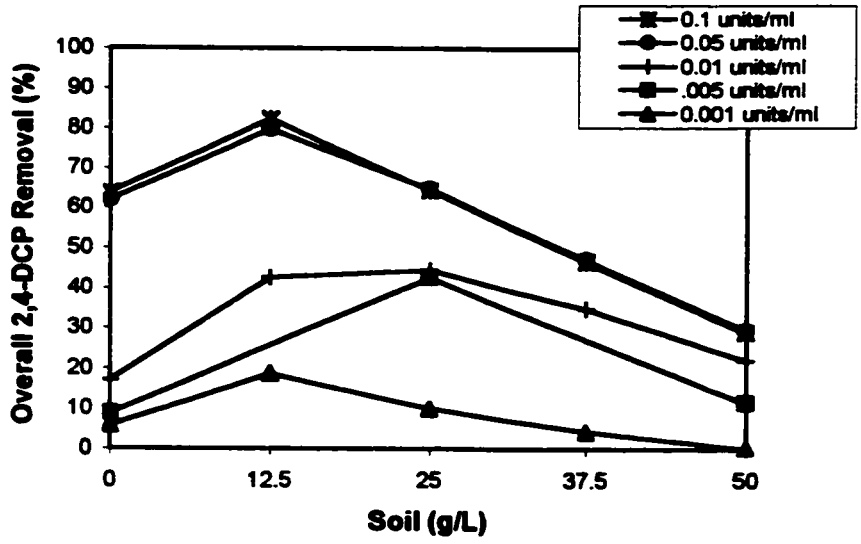


Figure 5.4a Removal of 2,4-DCP versus soil for doses of SBP ranging from 0.001 to 0.1 units/mL, assuming irreversible sorption of 2,4-DCP to soil. Conditions are the same as in figure 4.5a.

the removal of 2,4-DCP increased from 8.6% without soil to 66.2% with 25 g/L of soil. This represents a 7.7 fold increase in percent removal of 2,4-DCP with the addition of 25 g/L of soil. Figure 5.4a indicates that the addition of soil to the reactors increases the activity of SBP, as shown through the removal of 2,4-DCP.

Figure 5.4b plots the concentration of soil versus the percent removal of 2,4-DCP at low SBP concentrations assuming irreversible sorption. Figure 5.4b indicates that the highest percent removal of 2,4-DCP generally occurs with the addition of 12.5 g/L of soil and decreases with higher soil concentrations. With the addition of 0.05 units/mL of SBP 62% removal of 2,4-DCP is achieved without soil while 80% removal of 2,4-DCP can be accomplished with the addition of 12.5 g/L of soil. With 25 g/L of soil the removal of 2,4-DCP is 64%. At higher soil concentrations the percent removal of 2,4-DCP is similar to reactors without soil. Figure 5.4b shows that even under irreversible sorption conditions, SBP with the addition of 12.5 g/L of soil to the reactor outperforms reactors without soil.

Figures 5.4a and b indicate that with low SBP doses (< 0.25 units/mL), the addition of soil in small concentrations (0.001 to 0.10 units/mL) increases the activity of the SBP under either the assumption of reversible or irreversible sorption conditions.

5.1 Turnovers

The number of "turnovers" characterizes the activity of an enzyme throughout its life. More precisely it is the number of substate molecules precipitated out of solution for each molecule of enzyme consumed (Nicell et al., 1993). The number of turnovers indicates the number of times the enzyme was able to complete the catalytic process

indicates the number of times the enzyme was able to complete the catalytic process before becoming inactivated. The number of turnovers can be calculated using a proportionality constant (function of substrate and SBP molecular weight), and the amount of contaminant removed, as given by Alemany (2000), in equation 4.3.

$$\text{Number of Turnovers} = 18.75 \times \frac{C_i - C_e(\text{mg/L})}{EC(\text{units/mL})} \qquad [4.3]$$

Where EC is the enzyme concentration and C_i - C_e is the change in 2,4-DCP (this takes into account the 2,4-DCP sorbed to the soil under reversible or irreversible assumptions). Therefore for a given substrate removal and enzyme concentration the number of turnovers can be calculated (table 5.1).

Table 5.1. Turnovers for various soil concentrations at pH 6 with a SBP dose of 0.01 units/mL under reversible and irreversible assumptions. Reactor conditions are given in figure 4.5a.

Soil Concentration (g/L)	# of Turnovers under reversible conditions	# of Turnovers under irreversible conditions
0	26537	26537
12.5	71879	66255
25	112885	69191
37.5	126326	54221
50	135702	34301

Table 4.1, indicates that the SBP activity increases with the addition of soil to the reactor under the assumption of both reversible and irreversible conditions. Under reversible conditions the number of turnovers continues to increase as the soil concentration is increased. Under irreversible conditions the addition of soil to the reactor results in a higher number of turnovers, and the maximum number of turnovers is achieved with the addition of 12.5 g/L of soil.

It is unknown whether the actual sorption is reversible or irreversible or between the two extremes but the SBP has been shown to be more effective with the addition of soil regardless of the actual sorption behavior and fate of the sorbed 2,4-DCP.

Chapter 6

Conclusions

The main conclusion for the soil mixture and conditions used in this study is that the addition of soil can improve the removal of 2,4-DCP and the SBP efficiency under certain conditions. The presence of soil in reactors provides better $SR_{2,4\text{-DCP}}$ and $TR_{2,4\text{-DCP}}$ than reactors without soil at SBP concentrations below 0.25 units/mL. The improved efficiency of SBP seems to be optimized with 12.5 g/L of soil in the reactor at SBP concentrations below 0.25 units/mL under the assumptions of both reversible and irreversible sorption. While the addition of soil to the reactor may increase the removal of 2,4-DCP and SBP efficiency, too much soil may result in decreased removal of 2,4-DCP.

The addition of an optimal concentration of soil, has several advantages over treatment of wastewater-only systems. First, the increased SBP efficiency and 2,4-DCP removal when soil is added is most likely effected by the behavior of the peat moss acting as an additive, therefore the addition of PEG is not necessary. Secondly, the soil also improves removal in a broader pH range (4 to 8) than does PEG (6 to 7). As a result of cost reductions due to the absence of PEG, and lower enzyme doses the process has become more economical.

It was also found that the soil does not delay the rate of the reaction at 22°C, and the reaction appears to proceed more quickly with the addition of soil at 4°C than without soil. This overcomes one of the main concerns with remediation of soil systems, that reactions may proceed too slowly as a result of colder temperatures, as has been shown

by Alemany (2000) with wastewater. Remediation of high concentrations of soil (i.e., in situ) does not seem feasible under the conditions examined in this study. Although it may be the result of H_2O_2 limiting the reaction and activity of the SBP. Soil remediation may be feasible by optimizing the H_2O_2 dose as a function of organic components as well as 2,4-DCP concentration.

While the addition of soil to the reactors achieved greater removal of 2,4-DCP than the reactors without soil, the results presented in chapter 5 indicate that the addition of soil to the reactors also results in an increase in SBP activity. The increase in SBP activity was shown under both reversible and irreversible sorption assumptions. Since irreversible sorption would be the worst case scenario it is possible to conclude, regardless of the sorption behavior, that the addition of soil to the reactors increased the SBP activity and performance. The degree to which the activity of SBP increases with soil addition is a function of the actual sorption behavior of 2,4-DCP between the reversible and irreversible sorption extremes.

6.1 Recommendations for Future Research

To assess the increase in SBP activity, future research should focus on the desorption of 2,4-DCP and whether the sorption is reversible or irreversible.

Additional research should focus on the fate of sorbed residuals, and whether they remain sorbed to the soil or are remediated. If sorbed contaminants are being remediated there is potential to remediate soil in situ. Alternatively if the contaminant remains sorbed to the soil there would be concerns as to the disposal of contaminated soil.

One of the major obstacles facing the use of soybean peroxidase for remediation of chlorinated phenols is the unknown nature of the end-product polymers. Further research is required to determine the exact toxicity of the precipitate that forms as a result of the enzyme cycle.

Initial investigations indicate that there is exciting potential for the use of SBP in soil systems. Future research should continue to examine condition optimization in soil slurries, as well as investigating the in situ application of enzymes.

References

Aitken, M.D. (1993) Waste Treatment Applications of Enzymes: Opportunities and Obstacles, *The Chemical Engineering Journal*. **52**, B49.

Aitken, M.D. (1994) Characterization of Reaction Products from the Enzyme Catalyzed Oxidation of Phenolic Pollutants, *Water Resources*. **28**, *9*, 1879.

Alemany, K. (2000) Treatment of 2,4-dichlorophenol using Soybean Peroxidase. Master's Dissertation Submitted to The University of Ottawa.

Arnao, M.B., Acosta, M., del Rio, J.A., Varon, R. and Garcia-Canovas, F. (1990) A Kinetic Study on the Suicide Inactivation of Peroxidase by Hydrogen Peroxide, *Biochimica et Biophysica Acta*. **1041**, 43.

Banci, L. (1997) Structural Properties of Peroxidases, *Journal of Biotechnology*. **53**, 253.

Bewtra, J.K., Biswas, N., Henderson, W.D., and Nicell, J.A. (1995) Recent Advances in Treatment of Selected Hazardous Wastes, *Water Quality Resources J. Canada*. **30**, *1*, 115-125.

Bollag, J. (1992) Decontaminating Soil with Enzymes: An in Situ Method Using Phenolic and Anilinic Compounds, *Environ. Sci. Technol.* **25**, *10*, 1876.

Boyd, S.A. (1982) Adsorption of Substituted Phenols by Soil, *Soil Science*. **134**, *5*, 337.

Buchanan, I.D. and Nicell, J.A. (1996) Model Development for Horseradish Peroxidase Catalyzed Removal of Aqueous Phenol, *Biotechnology and Bioengineering*. **54**. 251.

Caza, N., Bewtra, J.K., Biswas, N., and Taylor, K.E. (1999) Removal of Phenolic Compounds from Synthetic Wastewater using Soybean Peroxidase, *Water Resources*. **33**, 13,3012.

Cooney, D.O. (1999) Adsorption Design for Wastewater Treatment. Lewis Publishers. New York.

Dec, J. and Bollag, J.M. (1994a) Use of Plant Material for the Decontamination of Water Polluted with Phenols, *Biotechnology and Bioengineering*. **44**, 1132.

Dec, J. and Bollag, J.M. (1994b) Dehalogenation of Chlorinated Phenols during Oxidative Coupling, *Environ. Sci. Technol.* **28**, *3*, 484.

Dunford, H.B. and Stillman, J.S. (1976) On the Function and Mechanisms of Action of Peroxidases, *Coordination Chemistry Review*. **19**, 187.

Flock, C., Bassi, A. and Gijzen, M. (1999) Removal of Aqueous Phenol and 2-Chlorophenol With Purified Soybean Peroxidase and Raw Soybean Hulls, Journal Of Chemical Technology and Biotechnology. **74**, 303.

Goetz, R.J. (1996) New Test Kit Adds 'Green' Value To Soybeans. www.purdue.edu/UNS/html4ever/9605.Vierling.html

Gomori, G. (1955). Preparations of Buffers for use in Enzyme Studies. Methods in Enzymology, Volume 1. Edited by Sidney P. Colowick and Nathan O. Kaplan. Academic Press Inc. New York. pp. 138-146.

Heck, P.E., Massey, I.J. and Aitken, M.D. (1992) Toxicity of Reaction Products from Enzymatic Oxidation of Phenolic Pollutants, *Water Science Technology*. **26**, *9*, 2369.

Icpet.www.icpet.nrc.ca/projects/soil_e.html

Isaacson, P.J. and Frink, C.R. (1984) Nonreversible Sorption of Phenolic Compounds by Sediment Fractions: The Role of Sediment Organic Matter, *Environmental Science Technology*. **18**, 43.

Kakarla, P. and Watts, R.J. (1997) Depth of Fenton-Like Oxidation in Remediation of Surface Soil, *Journal of Environmental Engineering*. **123**, *1*, 11.

Klibanov, A.M., Alberti, B.N., Morris, E.D. and Felshin, L.M. (1981) Enzymatic Removal of Toxic Phenols and Anilines from Waste Waters, *Journal of Applied Biochemistry*. **2**, 414.

Klibanov, A.M., Tu, T.M., and Scott, K.P. (1983) Peroxidase-Catalyzed Removal of Phenols from Coal-Conversion Waste Waters, *Science*. **221**. 259.

Marten, D.A. and Williams, T.F. (1995) Enhanced Degradation of Polycyclic Aromatic Hydrocarbons in Soil Treated with an Advanced Oxidative Process - Fenton's Reagent, *Journal of Soil Contamination*. **4**, *2*,1.

McEldoon, J.P., Pokora, A.R. and Dordick, J.S. (1995) Lignin Peroxidase-Type Activity Of Soybean Peroxidase, *Enzyme and Microbial Technology*. **17**, 359.

McEldoon, J.P., Pokora, A.R. and Dordick, J.S. (1996) Unusual Thermal Stability of Soybean Peroxidase, *Biotechnol. Prog*. **12**, *4*, 555.

Nakamoto, S. and Machida, N. (1992) Phenol Removal from Aqueous Solutions by Peroxidase-Catalyzed Reaction Using Additives. *Wat. Res.* **26**, *1*, 49.

Nicell, J.A., Bewtra, J.K., Biswas, N., and Taylor, E. (1993) Reactor Development for Peroxidase Catalyzed Polymerization and Precipitation of Phenols from Wastewater, *Water Resources*. **27**, *11*, 1629.

Nicell, J.A., Bewtra, J.K., Taylor, K.E., Biswas, N., and St. Pierre, C. (1992) Enzyme Catalyzed Polymerization and Precipitation of Aromatic Compounds from Wastewater, *Wat. Sci. Tech.* **25**, *3*, 157.

Nicell, J.A., and Wright, H. (1997) A Model of Peroxidase Activity with Inhibition by Hydrogen Peroxide, *Enzyme and Microbial Technology*. **21**, *19*, 302.

Park, J., Dec, J., Kim, J. and Bollag, J. (1999) Effect of Humic Constituents on the Transformation of Chlorinated Phenols and Anilines in the Presence of Oxido-Reductive Enzymes or Birnessite, *Environ. Sci. Tech.* **33**, 2028.

Ravikumar, J.X., and Gurol, M.D. (1994) Chemical Oxidation of Chlorinated Organics By Hydrogen Peroxide in the Presence of Sand, *Environ. Sci. Technol.* **28**, *3*, 394.

Trombly, J. (1995) Engineering Enzymes for Better Bioremediation: Efforts to Identify And Manipulate These Active Biochemical Agents May Lead to More Effective Bioremediation Applications, *Environmental Science & Technology*. **29**, *12*, 560A.

Watts, R.J., Udell, M.D. and Monsen, R.M. (1993) Use of Iron Minerals in Optimizing The Peroxide Treatment of Contaminated Soils, *Water Environment Research*. **65**, *7*, 839.

Watts, R.J. (1998) *Hazardous Wastes: Sources Pathway Receptors*, New York, John Wiley & Sons Inc.

Wright, H. and Nicell, J.A. (1999) Characteriazation of Soybean Peroxidase for the Treatment of Aqueous Phenols, *Bioresource Technology*. **70**, 69.

Wu, J., Bewtra, J.K., Biswas, N., and Taylor, K.E. (1994) Effect of H_2O_2 Addition Mode On Enzymatic Removal of Phenol from Wastewater in the Presence of Polyethylene Glycol, *The Canadian Journal of Chemical Engineering*. **72**, 881.

Wu, J., Taylor, K.E., Bewtra, J.K. and Biswas, N. (1993) Optimization of the Reaction Conditions for Enzymatic Removal of Phenol from Wastewater in the Presence of Polyethylene Glycol, *Water Resources*. **27**, *12*, 1701.

Wu, Y., Taylor, K.E., Biswas, N., and Bewtra, J.K. (1998) A Model for the Protective Effect of Additives on the Activity of Horseradish Peroxidase in the Removal of Phenol, *Enzyme and Microbial Technology*. **22**. 315.

Appendix A – Buffer Preparation

Preparation of 2,4 DCP

A stock solution was prepared using the following method. Weigh approximately 0.2 g 2,4 DCP (VS) into a tared scintillation vial tared (VT). Preweigh 150 mL beaker (BS) with a magnetic stirrer. Rinse 2,4-DCP into the beaker with 8 mL of NaOH, rinsing twice again, each time with 1 mL NaOH. Add approximately 60 mL of Milli-Q water, stirring and adding heat as required to dissolve. Weigh the beaker and all its contents (BT). To calculate the stock concentration, the following equation is used.

[CP] (mg/L) = [VS(g) – VT(g)]/[BS(G) – BT(G)] * 1000(mg/g) *1000(mL/l)

Knowing this concentration, secondary solutions could be diluted and buffered.

Preparation of Buffer Solutions

Two types of buffers were used for these experiments; tris(hydroxymethyl) aminomethane (Tris) buffer, and a citrate-phosphate buffer. They were prepared according to Gomori's methods (Gomori, 1955) and are described below.

Tris(hydroxymethyl) aminomethane (Tris) Buffer

Solution "A" is 24.2g of Tris in 1000 mL of Milli-Q water to give a molar concentration of 0.2. Solution "B" is 0.2M HCl, prepared by diluting 1.66 mL HCl in 100 mL. To achieve a desired pH, the buffer was prepared by adding "x" mL of solution "B" to 50 mL of solution "A" then diluting to reach a final volume of 200 mL (including 2,4 DCP). The values for "x" are given below as a function of the pH.

Table A.1. Preparation of Tris(hydroxymethyl) aminomethane (Tris) Buffer

PH	"x" (mL) Solution "B"
9.0	5.0
8.8	8.1
8.6	12.2
8.4	16.5
8.2	21.9
8.0	26.8
7.8	32.5
7.6	38.4
7.4	41.4
7.2	44.2

Citrate-Phosphate Buffer

This buffer was prepared by combining various combinations of 0.1M solution of citric acid (solution "A"), and 0.2 M solution of diabasic sodium phosphate (solution "B"). The citric acid was prepared by diluting 19.21 g citric to 1000 mL using Milli-Q water. The diabasic sodium phosphate was prepared by diluting 28.4 g of sodium phosphate dibasic in 1000 mL of Milli-Q water. To reach the desired pH the following

table gives "x" mL of solution "A", and "y" mL of solution "B" then diluting to 200 mL including 2,4 DCP.

Table A.2. Preparation of Citrate-Phosphate Buffer

pH	"x" (mL) of solution "A"	"y" (mL) of solution "B"
2.6	44.6	5.4
2.8	42.2	7.8
3.0	39.8	10.2
3.2	37.7	12.3
3.4	35.9	14.1
3.6	33.9	16.1
3.8	32.3	17.7
4.0	30.7	19.3
4.2	39.4	20.6
4.4	27.8	22.2
4.6	26.7	23.3
4.8	25.2	24.8
5.0	24.3	25.7
5.2	23.3	26.7
5.4	22.2	27.8
5.6	21.0	29.0
5.8	19.7	30.3
6.0	17.9	32.1
6.2	16.9	33.1
6.4	15.4	34.6
6.6	13.6	36.4
6.8	9.1	40.9
7.0	6.5	43.5

Preparation of Enzyme Solution

5,000 units of soybean peroxidase were dissolved in 52.8 mL of Milli-Q water. This gave a concentration of 88 units/mL. From this stock, secondary dilutions were made to achieve the desired concentrations. Both primary and secondary solutions were kept at 4°C to avoid any reduction in enzyme activity.

Preparation of Polyethylene Glycol

Stock solution of PEG was prepared by dissolving 11.0 g of PEG in a 100 mL volumetric flask. This gave a concentration of 110 g/L, which could then be diluted if necessary. The addition of 1 mL of 110 g/L concentration into the reactor with a total of 22 mL (including PEG) resulted in a reactor concentration of 5 g/L.

Appendix B – HPLC Calibration Curves

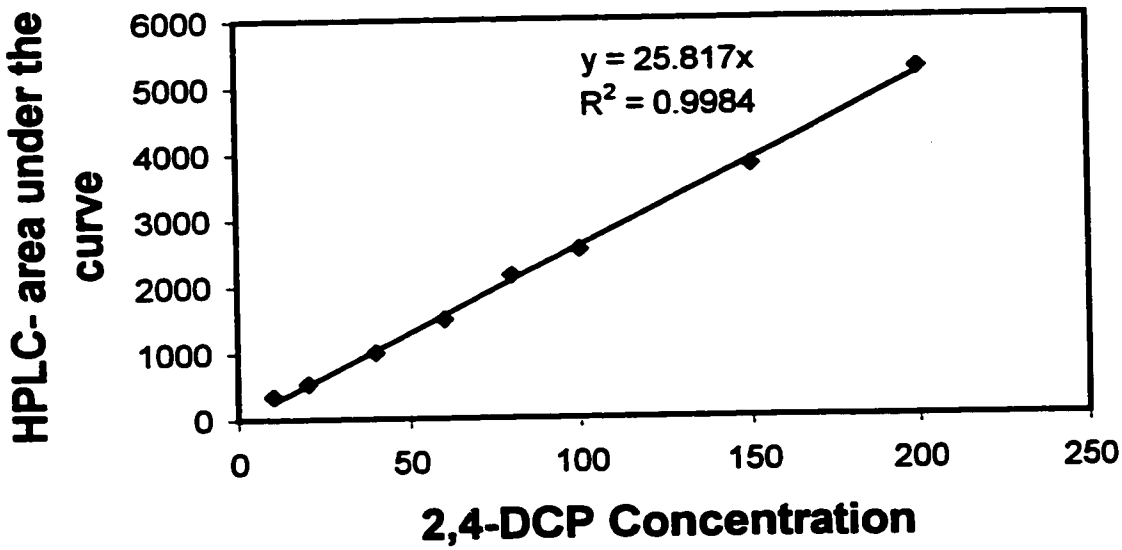
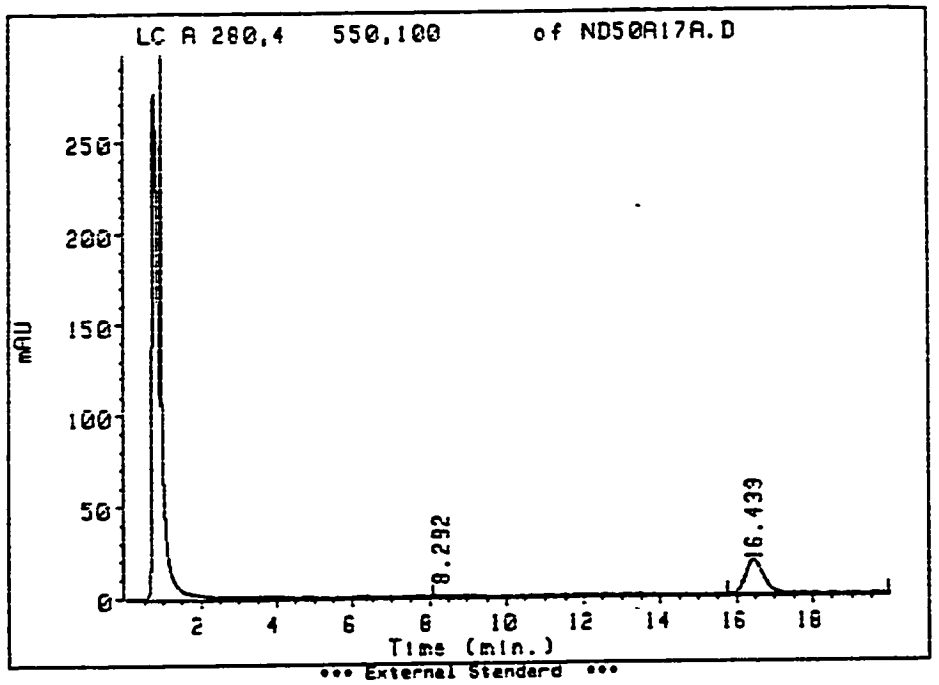


Figure B1. Example of calibration curves developed with testing of standards. For a slope of 25.817, the inverse (0.3873) represents the factor used to multiply the area to find the concentration

Appendix C - HPLC Chromatographs



Operator: NICOLE 18 Feb 90 2:35 pm
Method File Name : KEVIN.M
Sample Info : 4078
Misc Info:
Integration File Name : DATA:ND50A17A.I
consisting of channels : 1, A 280,4 550,100 of ND50A17A.D
Sequence Index: 1 Bottle Number : 17 Repetition Number: 1

Calibration file: DATA:KEV3.Q Last Update: 17 Feb 90 6:50 pm
Reference Peak Window: 15.00 % of Retention Time
Non-Reference Peak Window: 15.00 % of Retention Time
Sample Amount: 0.000 Uncalibrated Peak RF: 0.000 Multiplier: 1.000

••• 2,4-DCP •••

Peak Num	Type	Int Type	Ret Time	Signal Description	Compound Name	Area	Amount
1		IHBAS	16.439	A 280,4 550,100	2,4-DCP	621.48	22.715 ng/ul

••• No Additional Qualifiers •••

Counts • Response Factor = Corrected Amt
621 • 0.03658 = 22.72

Figure C1. Sample HPLC chromatograph for 2,4-DCP from experiment #407. The final concentration after addition of 0.05 unit/mL SBP, and 1.25 mM H_2O_2 to 0.613 mM 2,4-DCP, reactor conditions are pH 3, and 200 g/L soil at 22°C.

APPENDIX D

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
1	100	3	0	10					85.2		85.2	0	0
2	100	3	12.5	10					85.2		80.36	4.84	5.680751
3	100	3	25	10					85.2		77.04	8.16	9.577465
4	100	3	37.5	10					85.2		77.01	8.19	9.612676
5	100	3	50	10					85.2		73.37	11.83	13.88498
6	100	8	0	10					103.67		103.67	0	0
7	100	8	12.5	10					103.67		101.42	2.25	2.170348
8	100	8	25	10					103.67		95.43	8.24	7.948297
9	100	8	37.5	10					103.67		91.95	11.72	11.3051
10	100	8	50	10					103.67		82.66	21.01	20.26623
11	50	6	0	10					51.25		51.25	0	0
12	50	6	12.5	10					51.25		42.34	8.91	17.38537
13	50	6	25	10					51.25		36.88	14.37	28.03902
14	50	6	37.5	10					51.25		31.83	19.42	37.89268
15	50	6	50	10					51.25		26.82	24.43	47.66829
16	100	6	0	10	0.1	1000			106.88		106.88	0	0
17	100	6	12.5	10	0.1	1000			106.88		87.17	19.71	18.44124
18	100	6	25	10	0.1	1000			106.88		75.46	31.42	29.39746
19	100	6	37.5	10	0.1	1000			106.88		67.75	39.13	36.61115
20	100	6	50	10	0.1	1000			106.88		61.53	45.35	42.43076
21	100	6	0	10	5	8000			99.24		99.24	0	0
22	100	6	12.5	10	5	8000			99.24		89.03	10.21	10.28819
23	100	6	25	10	5	8000			99.24		77.01	22.23	22.40024
24	100	6	37.5	10	5	8000			99.24		70.22	29.02	29.24224
25	100	6	50	10	5	8000			99.24		61.35	37.89	38.18017
26	100	6	0	10	0.1	1000	1.25		96.52		96.52	0	0
27	100	6	12.5	10	0.1	1000	1.25		96.52		86.48	10.04	10.40199
28	100	6	25	10	0.1	1000	1.25		96.52		74.83	21.69	22.47203
29	100	6	37.5	10	0.1	1000	1.25		96.52		65.96	30.56	31.66183
30	100	6	50	10	0.1	1000	1.25		96.52		59.8	36.72	38.04393
31	100	6	0	10					102.1		102.1	0	0
32	100	6	12.5	10					102.1		98.19	3.91	3.829579
33	100	6	37.5	10					102.1		67.84	34.26	33.55534
34	200	6	0	10					197.84		197.84	0	0
35	200	6	12.5	10					197.84		190.12	7.72	3.902143
36	200	6	37.5	10					197.84		156.38	41.46	20.95633
37	100	3	0	10	0.1	1000			96.27		96.27	0	0
38	100	3	25	10	0.1	1000			96.27		76.19	20.08	20.858
39	100	3	50	10	0.1	1000			96.27		62.57	33.7	35.00571
40	100	3	0	10	5	8000			95.81		95.81	0	0
41	100	3	25	10	5	8000			95.81		78.87	16.94	17.68083
42	100	3	50	10	5	8000			95.81		60.59	35.22	36.76025
43	100	3	0	10	1	8000			96.66		96.66	0	0
44	100	3	25	10	1	8000			96.66		77.33	19.33	19.99793
45	100	3	50	10	1	8000			96.66		63.17	33.49	34.64722
46	100	3	0	10	5	8000			95.72		95.72	0	0
47	100	3	25	10	5	8000			95.72		75.38	20.34	21.24948
48	100	3	50	10	5	8000			95.72		60.56	35.16	36.73214
49	100	8	0	10	0.1	1000			100.6		100.6	0	0
50	100	8	25	10	0.1	1000			100.6		93.61	6.99	6.94831
51	100	8	50	10	0.1	1000			100.6		82.81	17.79	17.6839
52	100	8	0	10	5	8000			98.8		98.8	0	0
53	100	8	25	10	5	8000			98.8		95.85	2.95	2.98583
54	100	8	50	10	5	8000			98.8		73.79	25.01	25.31377
55	100	8	0	10	1	8000			98.08		98.08	0	0
56	100	8	25	10	1	8000			98.08		92.19	5.89	6.005302

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
57	100	8	50	10	1	8000			98.08		83.22	14.86	15.1509
58	100	8	0	10	5	8000	1.25		100.8		100.8	0	0
59	100	8	25	10	5	8000	1.25		100.8		91.31	9.49	9.414683
60	100	8	50	10	5	8000	1.25		100.8		80.86	19.94	19.78175
61	100	6	0	10	5	8000	1.25		93.8		93.8	0	0
63	100	6	25	10	5	8000	1.25		93.8		71.2	22.6	24.09382
64	100	6	50	10	5	8000	1.25		93.8		47.44	46.36	49.42431
65	100	6	0	10			1.25		99.28		99.28	0	0
67	100	6	25	10			1.25		99.28		75.55	23.73	23.9021
68	100	6	50	10			1.25		99.28		49.8	49.48	49.83884
69	100	6	0	10	0.1	1000	2.5		93.04		93.04	0	0
70	100	6	25	10	0.1	1000	2.5		93.04		87.6	5.44	5.846948
71	100	6	50	10	0.1	1000	2.5		93.04		58.29	34.75	37.34953
72	100	6	0	10	0.1	1000	0.625		93.15		93.15	0	0
73	100	6	25	10	0.1	1000	0.625		93.15		75.71	17.44	18.72249
74	100	6	50	10	0.1	1000	0.625		93.15		58.33	34.82	37.38057
75	100	3	0	10	5	8000	1.25		89.8		89.8	0	0
76	100	3	25	10	5	8000	1.25		89.8		73.72	16.08	17.90646
77	100	3	50	10	5	8000	1.25		89.8		57.86	31.94	35.56793
78	100	3	0	10			1.25		94.38		94.38	0	0
79	100	3	25	10			1.25		94.38		79.4	14.98	15.87201
80	100	3	50	10			1.25		94.38		62.22	32.16	34.07502
81	100	6	0	20					94.85		94.85	0	0
82	100	6	12.5	20					94.85		85.94	8.91	9.39378
83	100	6	25	20					94.85		60.31	34.54	36.41539
84	100	6	37.5	20					94.85		48.7	46.15	48.65577
85	100	6	50	20					94.85		39.62	55.23	58.22878
86	100	3	0	20					92.72		92.72	0	0
87	100	3	25	20					92.72		61.5	31.22	33.67127
88	100	3	50	20					92.72		54.15	38.57	41.59836
89	100	8	0	20					106.4		106.4	0	0
90	100	8	25	20					106.4		83.46	22.94	21.56015
91	100	8	50	20					106.4		59.45	46.95	44.12594
92	100	6	0	10					106.67		106.67	0	0
93	100	6	25	10					106.67		78.81	27.86	26.11793
94	100	6	50	10					106.67		61.22	45.45	42.60804
95	200	6	0	10					181.17		181.17	0	0
96	200	6	25	10					181.17		163.03	18.14	10.0127
97	200	6	50	10					181.17		122.6	58.57	32.32875
A	100	BOTTLE							103.96		103.67	0.29	0.278953
B	80	BOTTLE							81.25		81.11	0.14	0.172308
C	60	BOTTLE							59.62		59.5	0.12	0.201275
D	40	BOTTLE							40.07		39.22	0.85	2.121288
E	20	BOTTLE							21.13		20.88	0.25	1.183152
F	100	0							101.12		101.01	0.11	0.108782
G	100	12.5 ss							101.12		100.88	0.24	0.237342
H	100	25 ss							101.12		101.32	-0.2	-0.19778
I	100	37.5 ss							101.12		101.41	-0.29	-0.28679
J	100	50 ss							101.12		100.78	0.34	0.336234
K	100	ERR calc.											
L	100	ERR calc.											
M	100	ERR calc.											
N	100	ERR calc.											
O	100	ERR calc.											
P	10	ERR calc.											
Q	10	ERR calc.											
R	10	ERR calc.											
S	10	ERR calc.											
T	10	ERR calc.											

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
101	100	3	0	10			1.25	1	93.47	79.87	1.953	77.917	97.55478
102	100	3	12.5	10			1.25	1	93.47	71.76	7.94	63.82	88.93534
103	100	3	25	10			1.25	1	93.47	64.11	11.681	52.429	81.77975
104	100	3	37.5	10			1.25	1	93.47	58.74	13.53	45.21	76.96629
105	100	3	50	10			1.25	1	93.47	51.24	12.88	38.36	74.86339
106	100	6	0	10			1.25	1	94.32	84.3	0.4	83.9	99.5255
107	100	6	12.5	10			1.25	1	94.32	67.83	0.4	67.43	99.41029
108	100	6	25	10			1.25	1	94.32	63.14	0.4	62.74	99.36649
109	100	6	37.5	10			1.25	1	94.32	55.21	1.595	53.615	97.11103
110	100	6	50	10			1.25	1	94.32	53.23	4.6104	48.6166	91.33823
111	100	8	0	10			1.25	1	106.11	99.54	0.4	99.14	99.59815
112	100	8	12.5	10			1.25	1	106.11	92.25	0.463	91.787	99.4981
113	100	8	25	10			1.25	1	106.11	87.07	11.21	75.86	87.1253
114	100	8	37.5	10			1.25	1	106.11	80.7	12.37	68.33	84.67162
115	100	8	50	10			1.25	1	106.11	78.11	16.23	61.88	79.22161
116	100	3	0	10			1.25	2	93.47	81.55	0.4	81.15	99.5095
117	100	3	12.5	10			1.25	2	93.47	77.3	4.339	72.961	94.3868
118	100	3	25	10			1.25	2	93.47	68.69	4.869	63.821	92.91163
119	100	3	37.5	10			1.25	2	93.47	60.33	5.332	54.998	91.16194
120	100	3	50	10			1.25	2	93.47	52.97	8.558	44.412	83.84369
121	100	6	0	10			1.25	2	102.42	92.93	0.4	92.53	99.56957
122	100	6	12.5	10			1.25	2	102.42	75.79	0.4	75.39	99.47223
123	100	6	25	10			1.25	2	102.42	69.5	0.5424	68.9576	99.21957
124	100	6	37.5	10			1.25	2	102.42	58.11	0.649	57.461	98.88315
125	100	6	50	10			1.25	2	102.42	34.63	3.82	30.81	88.9691
126	100	8	0	10			1.25	2	106.11	94.03	3.071	90.959	96.73402
127	100	8	12.5	10			1.25	2	106.11	88.31	2.714	85.596	96.92674
128	100	8	25	10			1.25	2	106.11	87.03	7.073	79.957	91.87292
129	100	8	37.5	10			1.25	2	106.11	76.06	13.6	62.46	82.11938
130	100	8	50	10			1.25	2	106.11	79.65	11.84	67.81	85.13497
131	100	6	0	10			1.25	0.5	94.37	84.21	0.4	83.81	99.525
132	100	6	12.5	10			1.25	0.5	94.37	75.73	0.5331	75.1969	99.29605
133	100	6	25	10			1.25	0.5	94.37	41.12	0.4715	40.6485	98.85336
134	100	6	37.5	10			1.25	0.5	94.37	57.57	4.591	52.979	92.02536
135	100	6	50	10			1.25	0.5	94.37	52.05	4.044	48.006	92.23055
136	100	3	0	10	0.1	1000	1.25	1	94.37	81.43	0.4	81.03	99.50878
137	100	3	12.5	10	0.1	1000	1.25	1	94.37	69.88	6.922	62.958	90.09445
138	100	3	25	10	0.1	1000	1.25	1	94.37	63.27	11.94	51.33	81.1285
139	100	3	37.5	10	0.1	1000	1.25	1	94.37	59.27	10.33	48.94	82.57128
140	100	3	50	10	0.1	1000	1.25	1	94.37	48.94	13.36	35.58	72.70127
141	100	3	0	10	1	8000	1.25	1	94.37	84.48	2.455	82.025	97.09399
142	100	3	12.5	10	1	8000	1.25	1	94.37	70.82	6.882	63.938	90.28241
143	100	3	25	10	1	8000	1.25	1	94.37	66.21	8.664	57.546	86.91436
144	100	3	37.5	10	1	8000	1.25	1	94.37	51.76	12.43	39.33	75.98532
145	100	3	50	10	1	8000	1.25	1	94.37	49.17	9.012	40.158	81.67175
146	100	3	0	10	5	8000	1.25	1	94.37	84.13	3.065	81.065	96.35683
147	100	3	12.5	10	5	8000	1.25	1	94.37	73.25	5.377	67.873	92.65939
148	100	3	25	10	5	8000	1.25	1	94.37	65.23	8.794	56.436	86.51847
149	100	3	37.5	10	5	8000	1.25	1	94.37	49.17	11.21	37.96	77.20155
150	100	3	50	10	5	8000	1.25	1	94.37	59.62	14.41	45.21	75.83026
151	100	6	0	10	0.1	1000	1.25	1	94.37	80.68	0.4	80.28	99.50421
152	100	6	12.5	10	0.1	1000	1.25	1	94.37	67.81	0.4	67.41	99.41012
153	100	6	25	10	0.1	1000	1.25	1	94.37	73.66	1.05	72.61	98.57453
154	100	6	37.5	10	0.1	1000	1.25	1	94.37	59.4	2.986	56.414	94.97306
155	100	6	50	10	0.1	1000	1.25	1	94.37	50.81	1.252	49.558	97.53592
156	100	6	0	10	1	8000	1.25	1	94.37	82.05	0.4	81.65	99.51249
157	100	6	12.5	10	1	8000	1.25	1	94.37	71.77	0.4	71.37	99.44266

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
158	100	6	25	10	1	8000	1.25	1	94.37	62.06	2.226	59.834	96.41315
159	100	6	37.5	10	1	8000	1.25	1	94.37	56.86	3.211	53.649	94.3528
160	100	6	50	10	1	8000	1.25	1	94.37	49.05	2.892	46.158	94.10398
161	100	6	0	10	5	8000	1.25	1	94.37	85.91	0.4	85.51	99.5344
162	100	6	12.5	10	5	8000	1.25	1	94.37	74.37	0.4649	73.9051	99.37488
163	100	6	25	10	5	8000	1.25	1	94.37	63.15	0.7519	62.3981	98.80934
164	100	6	37.5	10	5	8000	1.25	1	94.37	56.39	0.4	55.99	99.29065
165	100	6	50	10	5	8000	1.25	1	94.37	51.65	4.65	47	90.9971
166	100	8	0	10	0.1	1000	1.25	1	91.2	84.42	0.4	84.02	99.52618
167	100	8	12.5	10	0.1	1000	1.25	1	91.2	81.64	0.4	81.24	99.51004
168	100	8	25	10	0.1	1000	1.25	1	91.2	78.83	8.993	69.837	88.59191
169	100	8	37.5	10	0.1	1000	1.25	1	91.2	76.01	11.41	64.6	84.98882
170	100	8	50	10	0.1	1000	1.25	1	91.2	71.36	13.39	57.97	81.23599
171	100	8	0	10	1	8000	1.25	1	91.2	83.55	0.4	83.15	99.52124
172	100	8	12.5	10	1	8000	1.25	1	91.2	80.99	4.093	76.897	94.94629
173	100	8	25	10	1	8000	1.25	1	91.2	76.99	7.64	69.35	90.07663
174	100	8	37.5	10	1	8000	1.25	1	91.2		0.4	-0.4	#DIV/0!
175	100	8	50	10	1	8000	1.25	1	91.2	71.15	12.23	58.92	82.81096
176	100	8	0	10	5	8000	1.25	1	91.2	86.4	0.4	86	99.53704
177	100	8	12.5	10	5	8000	1.25	1	91.2	80.45	0.4	80.05	99.5028
178	100	8	25	10	5	8000	1.25	1	91.2	78.25	5.582	72.668	92.86645
179	100	8	37.5	10	5	8000	1.25	1	91.2	73.89	9.121	64.769	87.65598
180	100	8	50	10	5	8000	1.25	1	91.2	78.7	11.77	66.93	85.04447
181	100	6	0	10			0.625	1	94.37	84.53	15.25	69.28	81.95907
182	100	6	12.5	10			0.625	1	94.37	71.8	20.59	51.21	71.32312
183	100	6	25	10			0.625	1	94.37	50.52	13.38	37.14	73.51544
184	100	6	37.5	10			0.625	1	94.37	58.24	12.47	45.77	78.5886
185	100	6	50	10			0.625	1	94.37	50.12	11.31	38.81	77.43416
186	100	6	0	10			2.5	1	94.37	84.02	0.4	83.62	99.52392
187	100	6	12.5	10			2.5	1	94.37	72.73	0.4	72.33	99.45002
188	100	6	25	10			2.5	1	94.37	67.61	0.4	67.21	99.40837
189	100	6	37.5	10			2.5	1	94.37	544.74	0.4	544.34	99.92657
190	100	6	50	10			2.5	1	94.37	48.03	0.4	47.63	99.16719
191	100	6	0	10	1	8000	1.25	2	91.39	80.52	0.4	80.12	99.50323
192	100	6	12.5	10	1	8000	1.25	2	91.39	72.04	0.4	71.64	99.44475
193	100	6	25	10	1	8000	1.25	2	91.39	63.83	9.72	54.11	84.77205
194	100	6	37.5	10	1	8000	1.25	2	91.39	53.28	4.72	48.56	91.14114
195	100	6	50	10	1	8000	1.25	2	91.39	48.86 u		#VALUE!	#VALUE!
196	100	6	0	10			1.25	0.25	91.39	80.38	0.4	79.98	99.50236
197	100	6	12.5	10			1.25	0.25	91.39	70.32	0.4	69.92	99.43117
198	100	6	25	10			1.25	0.25	91.39	64.61	0.5104	64.0996	99.21003
199	100	6	37.5	10			1.25	0.25	91.39	53.86	0.4794	53.3806	99.10991
200	100	6	50	10			1.25	0.25	91.39	25.81	0.4639	25.3461	98.20263
201													
202													
203													
204													
205													
206													
207													
208													
209													
210													
211													
212													
213													
214													
215													
216													

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
217													
218													
219													
220													
221	100	8	0	10	1	1000	1.25	1	91.2	83.07	1.195	81.875	98.56145
222	100	8	12.5	10	1	1000	1.25	1	91.2	78.55	3.473	75.077	95.57861
223	100	8	25	10	1	1000	1.25	1	91.2	75.84	9.839	66.001	87.02664
224	100	8	37.5	10	1	1000	1.25	1	91.2	76.03	14.09	61.94	81.46784
225	100	8	50	10	1	1000	1.25	1	91.2	71.45	14.24	57.21	80.06998
226	100	6	0	10			1.25	0.1	91.39	81.32	29.4	51.92	63.84653
227	100	6	12.5	10			1.25	0.1	91.39	73.22	0.4	72.82	99.4537
228	100	6	25	10			1.25	0.1	91.39	62.01	0.318	61.692	99.48718
229	100	6	37.5	10			1.25	0.1	91.39	57.96	0.4112	57.5488	99.29055
230	100	6	50	10			1.25	0.1	91.39	48.72	0.4225	48.2975	99.1328
231	100	6	0	10			1.25	0.05	91.39	82.29	31.84	50.45	61.30757
232	100	6	12.5	10			1.25	0.05	91.39	68.77	0.387	68.383	99.43725
233	100	6	25	10			1.25	0.05	91.39	62.09	1.081	61.009	98.25898
234	100	6	37.5	10			1.25	0.05	91.39	53.83	0.4819	53.3481	99.10477
235	100	6	50	10			1.25	0.05	91.39	49.37	0.4	48.97	99.18979
236	100	6	0	10			2.5	0.05	91.39	81.83	4.721	77.109	94.23072
237	100	6	12.5	10			2.5	0.05	91.39	73.36	0.5881	72.7719	99.19834
238	100	6	25	10			2.5	0.05	91.39	62.16	0.4	61.76	99.3565
239	100	6	37.5	10			2.5	0.05	91.39	55.84	0.4	55.44	99.28367
240	100	6	50	10			2.5	0.05	91.39	48.3	0.4	47.9	99.17184
241	100	6	0	10			5	0.05	91.39	81	36.8	44.2	54.5679
242	100	6	12.5	10			5	0.05	91.39	72.66	4.893	67.767	93.2659
243	100	6	25	10			5	0.05	91.39	66.88	0.5908	66.2892	99.11663
244	100	6	37.5	10			5	0.05	91.39	57.09	0.4234	56.6666	99.25836
245	100	6	50	10			5	0.05	91.39	38.61	1.172	37.438	96.96452
246	100	6	0	10			7.5	0.05	91.39	77.51	54.13	23.38	30.16385
247	100	6	12.5	10			7.5	0.05	91.39	71.86	16.122	55.738	77.56471
248	100	6	25	10			7.5	0.05	91.39	61.5	6.6	54.9	89.26829
249	100	6	37.5	10			7.5	0.05	91.39	55.08	5.855	49.225	89.37001
250	100	6	50	10			7.5	0.05	91.39	47.89	7.187	40.703	84.99269
251	100	6	0	ss			1.25	0.25	91.39	80.44	0.4	80.04	99.50273
252	100	6	12.5	ss			1.25	0.25	91.39	79.86	0.4	79.46	99.49912
253	100	6	25	ss			1.25	0.25	91.39	80.12	0.4	79.72	99.50075
254	100	6	37.5	ss			1.25	0.25	91.39	80.76	0.4	80.36	99.50471
255	100	6	50	ss			1.25	0.25	91.39	79.97	0.4	79.57	99.49981
256	100	6	0	10			1.25	0.5	91.39	79.6	0.4	79.2	99.49749
257	100	6	12.5	10			1.25	0.5	91.39	71.66	0.4	71.26	99.44181
258	100	6	25	10			1.25	0.5	91.39	61.91	1.946	59.964	96.85673
259	100	6	37.5	10			1.25	0.5	91.39	57.43	2.564	54.866	95.53543
260	100	6	50	10			1.25	0.5	91.39	48.36	3.049	45.311	93.6952
261	100	8	0	10			1.25	0.5	91.2	81.33	0.5952	80.7348	99.26817
262	100	8	12.5	10			1.25	0.5	91.291.2	77.8	1.633	76.167	97.90103
263	100	8	25	10			1.25	0.5	91.2	77.94	7.388	70.552	90.52091
264	100	8	37.5	10			1.25	0.5	91.2	78.3	2.327	75.973	97.0281
265	100	8	50	10			1.25	0.5	91.2	76.82	2.244	74.576	97.07889
266	100	6	0	10			2.5	2	91.39	83.73	0.4	83.33	99.52227
267	100	6	12.5	10			2.5	2	91.39	73.43	0.4	73.03	99.45526
268	100	6	25	10			2.5	2	91.39	67.68	0.4	67.28	99.40898
269	100	6	37.5	10			2.5	2	91.39	57.11	0.4	56.71	99.2996
270	100	6	50	10			2.5	2	91.39	49.92	0.4	49.52	99.19872
271	100	6	0	10			2.5	0.05	91.39	64.94	34.09	30.85	47.50539
272	100	6	12.5	10			2.5	0.05	91.39	72.62	1.841	70.779	97.46489
273	100	6	25	10			2.5	0.05	91.39	64.6	0.4	64.2	99.3808
274	100	6	37.5	10			2.5	0.05	91.39	56.23	0.4	55.83	99.28864
275	100	6	50	10			2.5	0.05	91.39	48.75	0.9807	47.7693	97.98831

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
276	100	6	0	10			2.5	0.01	91.39	85.81	na		
277	100	6	12.5	10			2.5	0.01	91.39	69.3	0.4	68.9	99.4228
278	100	6	25	10			2.5	0.01	91.39	61.93	0.4	61.53	99.35411
279	100	6	37.5	10			2.5	0.01	91.39	54.31	0.4	53.91	99.26349
280	100	6	50	10			2.5	0.01	91.39	52.65	0.7035	51.9465	98.66382
281	100	6	0	10			2.5	0.01	91.39	82.86	68.7	14.16	17.08907
282	100	6	12.5	10			2.5	0.01	91.39	72.03	32.93	39.1	54.28294
283	100	6	25	10			2.5	0.01	91.39	64.02	16.75	47.27	73.8363
284	100	6	37.5	10			2.5	0.01	91.39	57.21	10.13	47.08	82.29331
285	100	6	50	10			2.5	0.01	91.39	48.12	6.152	41.968	87.2153
286	100	6	0	10			1.25	0.01	91.39	81.74	53.2	28.54	34.91559
287	100	6	12.5	10			1.25	0.01	91.39	72.12	20.483	51.637	71.59872
288	100	6	25	10			1.25	0.01	91.39	61.87	7.985	53.885	87.09391
289	100	6	37.5	10			1.25	0.01	91.39	56.08	3.612	52.468	93.5592
290	100	6	50	10			1.25	0.01	91.39	48.49	9.569	38.921	80.26603
291	100	8	0	10			2.5	1	91.2	81.25	0.4	80.85	99.50769
292	100	8	12.5	10			2.5	1	91.2	77.91	0.4	77.51	99.48659
293	100	8	25	10			2.5	1	91.2	77.14	0.4	76.74	99.48146
294	100	8	37.5	10			2.5	1	91.2	74.75	3.273	71.477	95.6214
295	100	8	50	10			2.5	1	91.2	73.12	0.825	72.295	98.87172
296	100	3	0	10			1.25	1	90.87	79.57	1.901	77.669	97.61089
297	100	3	12.5	10			1.25	1	90.87	73.11	4.766	68.3437	93.48058
298	100	3	25	10			1.25	1	90.87	64.27	5.237	59.0332	91.85181
299	100	3	37.5	10			1.25	1	90.87	58.19	5.516	52.6738	90.52043
300	100	3	50	10			1.25	1	90.87	51.92	5.105	46.8153	90.16816
301	100	6	0	10	5	8000	2.5	0.01	91.39	80.23	3.893	76.337	95.1477
302	100	6	12.5	10	5	8000	2.5	0.01	91.39	69.5	5.42	64.08	92.20144
303	100	6	25	10	5	8000	2.5	0.01	91.39	61.73	5.047	56.683	91.82407
304	100	6	37.5	10	5	8000	2.5	0.01	91.39	54.59	1.182	53.408	97.83477
305	100	6	50	10	5	8000	2.5	0.01	91.39	47.02	5.48	41.54	88.34538
306	100	8	0	10			2.5	0.05	91.2	83.18	1.933	81.247	97.67612
307	100	8	12.5	10			2.5	0.05	91.2	80.04	0.4	79.64	99.50025
308	100	8	25	10			2.5	0.05	91.2	77.96	6.517	71.443	91.64058
309	100	8	37.5	10			2.5	0.05	91.2	77.7	16.05	61.65	79.34363
310	100	8	50	10			2.5	0.05	91.2	73	17.24	55.76	76.38356
311	100	3	0	10			2.5	2	90.87	79.88	0.4	79.48	99.49925
312	100	3	12.5	10			2.5	2	90.87	72.76	2.729	70.0311	96.2495
313	100	3	25	10			2.5	2	90.87	64.58	6.37396	58.206	90.13014
314	100	3	37.5	10			2.5	2	90.87	58.56	8.800	49.7595	84.97183
315	100	3	50	10			2.5	2	90.87	51.22	9.750	41.4696	80.96361
316	100	3	0	10			2.5	1	90.87	79.98	2.038	77.9418	97.45156
317	100	3	12.5	10			2.5	1	90.87	73.46	3.880	69.5797	94.71776
318	100	3	25	10			2.5	1	90.87	66.87	14.14	52.7338	78.86025
319	100	3	37.5	10			2.5	1	90.87	59.03	16.27	42.759	72.43599
320	100	3	50	10			2.5	1	90.87	52.23	18.07	34.1558	65.39496
321	100	3	0	10			2.5	0.5	90.87	79.66	23.2299	56.4301	70.83873
322	100	3	12.5	10			2.5	0.5	90.87	72.45	29.21	43.2392	59.68145
323	100	3	25	10			2.5	0.5	90.87	65.14	31.27	33.8661	51.98969
324	100	3	37.5	10			2.5	0.5	90.87	57.98	28.57	29.4075	50.72
325	100	3	50	10			2.5	0.5	90.87	50.94	26.09	24.8476	48.77812
326	100	3	0	10			2.5	0.25	90.87	80.23	43.41	36.8169	45.88922
327	100	3	12.5	10			2.5	0.25	90.87	72.13	43.7926	28.3374	39.28653
328	100	3	25	10			2.5	0.25	90.87	64.32	41.5575	22.7625	35.38944
329	100	3	37.5	10			2.5	0.25	90.87	58.06	37.5951	20.4649	35.24786
330	100	3	50	10			2.5	0.25	90.87	50.88	35.4089	15.4711	30.4071
331	100	3	0	10			2.5	0.05	90.87	79.46	71.1405	8.31946	10.47
332	100	3	12.5	10			2.5	0.05	90.87	74.01	65.9757	8.03426	10.85564
333	100	3	25	10			2.5	0.05	90.87	64.78	58.0477	6.73229	10.39254
334	100	3	37.5	10			2.5	0.05	90.87	57.99	54.1917	3.79835	6.55

Soybean Peroxidase Data

Test nc.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
335	100	3	50	10			2.5	0.05	90.87	50.93	49.2748	1.65523	3.25
336	100	3	0	10			2.5	0.01	90.87	80.03	77.3402	2.68977	3.36095
337	100	3	12.5	10			2.5	0.01	90.87	73.05	72.3926	0.65745	0.9
338	100	3	25	10			2.5	0.01	90.87	64.89	64.3894	0.50056	0.771399
339	100	3	37.5	10			2.5	0.01	90.87	58.42	57.5028	0.91719	1.57
340	100	3	50	10			2.5	0.01	90.87	51.45	50.9561	0.49392	0.96
341													
342													
343													
344													
345													
346	100	8	0	10			2.5	0.5	117.32	108.8	1.865	106.935	98.28585
347	100	8	12.5	10			2.5	0.5	117.32	101.1	0.517	100.583	99.48863
348	100	8	25	10			2.5	0.5	117.32	98.92	1.8166	97.1034	98.16357
349	100	8	37.5	10			2.5	0.5	117.32	91.72	2.542	89.178	97.22852
350	100	8	50	10			2.5	0.5	117.32	87.65	3.231	84.419	96.31375
351	100	8	0	10			2.5	0.25	117.32	107	1.509	105.491	98.58972
352	100	8	12.5	10			2.5	0.25	117.32	100.8	3.155	97.645	96.87004
353	100	8	25	10			2.5	0.25	117.32	97.6	1.762	95.838	98.19467
354	100	8	37.5	10			2.5	0.25	117.32	90.32	2.274	88.046	97.48229
355	100	8	50	10			2.5	0.25	117.32	86	3.982	82.018	95.36977
356	100	8	0	10			2.5	0.01	117.32	106.23	47.74	58.49	55.05978
357	100	8	12.5	10			2.5	0.01	117.32	104.7	2.03	102.67	98.06113
358	100	8	25	10			2.5	0.01	117.32	97.51	5.775	91.735	94.07753
359	100	8	37.5	10			2.5	0.01	117.32	92.52	4.384	88.136	95.26157
360	100	8	50	10			2.5	0.01	117.32	87.34	5.674	81.666	93.50355
361	100	8	0	10			1.25	0.5	117.32	106.9	0.4	106.5	99.62582
362	100	8	12.5	10			1.25	0.5	117.32	102.8	0.481	102.319	99.5321
363	100	8	25	10			1.25	0.5	117.32	97.36	0.7301	96.6299	99.2501
364	100	8	37.5	10			1.25	0.5	117.32	90.24	2.142	88.098	97.62633
365	100	8	50	10			1.25	0.5	117.32	87.18	2.089	85.091	97.60381
366													
367													
368													
369													
370													
371													
372													
373													
374													
375													
376	100	8	0	10			2.5	0.05	100.85	91.99	0.4	91.59	99.56517
377	100	8	12.5	10			2.5	0.05	100.85	85.88	0.4	85.48	99.53423
378	100	8	25	10			2.5	0.05	100.85	78.02	0.4	77.62	99.48731
379	100	8	37.5	10			2.5	0.05	100.85	67.47	0.4	67.07	99.40714
380	100	8	50	10			2.5	0.05	100.85	61.87	0.4	61.47	99.35348
381													
382													
383													
384													
385													
386													
387													
388													
389													
390													
391	100	6	0	10	1	8000	1.25	0.01	93.83	84.59	0.4	84.19	99.52713
392	100	6	12.5	10	1	8000	1.25	0.01	93.83	75.34	0.4	74.94	99.46907
393	100	6	25	10	1	8000	1.25	0.01	93.83	63.58	2.149	61.431	96.62001

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
394	100	6	37.5	10	1	8000	1.25	0.01	93.83	56.46	7.33	49.13	87.01736
395	100	6	50	10	1	8000	1.25	0.01	93.83	49.62	9.617	40.003	80.6187
396	100	6	0	10	0.1	1000	1.25	0.01	93.83	85.17	69.4	15.77	18.51591
397	100	6	12.5	10	0.1	1000	1.25	0.01	93.83	72.62	22.04	50.58	69.65023
398	100	6	25	10	0.1	1000	1.25	0.01	93.83	57.08	6.009	51.071	89.47267
399	100	6	37.5	10	0.1	1000	1.25	0.01	93.83	58.2	8.758	49.442	84.95189
400	100	6	50	10	0.1	1000	1.25	0.01	93.83	49.17	12.08	37.09	75.43217
401	100	3	100	10			2.5	0	99.68	42.25	38.23	4.02	9.514793
402	100	3	100	10			2.5	1	99.68	42.07	15.63	26.44	62.84763
403	100	3	100	10			2.5	0.5	99.68	42.79	25.23	17.56	41.03763
404	100	3	100	10			2.5	0.25	99.68	42.23	na		
405	100	3	100	10			2.5	0.05	99.68	42.42	36.66	5.76	13.5785
406	100	3	100	10			2.5	0.01	99.68	44.31	36.4	7.91	17.8515
407	100	3	200	10			2.5	0.05	99.68	27.78	22.72	5.06	18.21454
408	100	6	0	10			1.25	0.015	91.98	86.82	67.35	19.47	22.42571
409	100	6	25	10			1.25	0.015	91.98	64.04	4.169	59.871	93.49001
410	100	6	50	10			1.25	0.015	91.98	50.05	7.417	42.633	85.18082
411	100	6	0	10			1.25	0.005	91.98	82.57	75.48	7.09	8.586654
412	100	6	25	10			1.25	0.005	91.98	64.96	18.32	46.64	71.79803
413	100	6	50	10			1.25	0.005	91.98	53.17	14.86	38.31	72.05191
414	100	6	0	10			1.25	0.15	91.98	84.63	0.4	84.23	99.52735
415	100	6	25	10			1.25	0.15	91.98	64.6	1.004	63.596	98.44582
416	100	6	50	10			1.25	0.15	91.98	51.58	5.524	46.056	89.29042
417													
418													
419													
420													
421													
422													
423													
424													
425													
426													
427													
428													
429													
430													
431													
432													
433													
434													
435													
436													
437													
438													
439													
440													
441													
442													
443													
444													
445													
446	100	6	0	10			0.625	0.01	91.34	78.68	58.38	20.3	25.8
447	100	6	12.5	10			0.625	0.01	91.34	71.16	24.63	46.53	65.4
448	100	6	25	10			0.625	0.01	91.34	64.3	25.55	38.75	60.3
449	100	6	37.5	10			0.625	0.01	91.34	56.03	21.72	34.31	61.2
450	100	6	50	10			0.625	0.01	91.34	49.93	25.57	24.36	48.8
451	100	6	0	10			1.25	0.01	91.34	86	71.22	14.78	17.2
452	100	6	12.5	10			1.25	0.01	91.34	72.64	11.22	61.42	84.6

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
453	100	6	25	10			1.25	0.01	91.34	65.13	1.932	63.198	97.0
454	100	6	37.5	10			1.25	0.01	91.34	57.79	2.253	55.537	96.1
455	100	6	50	10			1.25	0.01	91.34	51.75	9.622	42.128	81.4
456	100	6	0	10			2.5	0.01	91.34	83.18	69.04	14.14	17.0
457	100	6	12.5	10			2.5	0.01	91.34	72.62	20.9	51.72	71.2
458	100	6	25	10			2.5	0.01	91.34	62.24	5.62	56.62	91.0
459	100	6	37.5	10			2.5	0.01	91.34	58.16	11.22	46.94	80.7
460	100	6	50	10			2.5	0.01	91.34	51.32	4.853	46.467	90.5
461	100	6	0	10			5	0.01	91.34	82.44	69.56	12.88	15.6
462	100	6	12.5	10			5	0.01	91.34	69.86	28.44	41.42	59.3
463	100	6	25	10			5	0.01	91.34	na			
464	100	6	37.5	10			5	0.01	91.34	58.41	13.51	44.9	76.9
465	100	6	50	10			5	0.01	91.34	50.23	na		
466													
467													
468													
469													
470													
471													
472													
473													
474													
475													
476	100	4	0	10			1.25	0.01	102.4	87.12	80.93	6.19	7.1
477	100	4	25	10			1.25	0.01	102.4	70.77	53.48	17.29	24.4
478	100	4	50	10			1.25	0.01	102.4	52.09	41.63	10.46	20.1
479	100	5	0	10			1.25	0.01	94.63	85.51	71.91	13.6	15.9
480	100	5	25	10			1.25	0.01	94.63	65.55	23.52	42.03	64.1
481	100	5	50	10			1.25	0.01	94.63	75.94	11.51	64.43	84.8
482	100	7	0	10			1.25	0.01	101.1	90.63	71.51	19.12	21.1
483	100	7	25	10			1.25	0.01	101.1	88.64	11.07	77.57	87.5
484	100	7	50	10			1.25	0.01	101.1	55.94	10.61	45.33	81.0
485	100	3	0	10			1.25	0.01	94.44	88.64	81.49	7.15	8.1
486	100	3	25	10			1.25	0.01	94.44	65.49	62.48	3.01	4.6
487	100	3	50	10			1.25	0.01	94.44	51.01	47.81	3.2	6.3
488	100	8	0	10			1.25	0.01	104.4	93.37	72.29	21.08	22.6
489	100	8	25	10			1.25	0.01	104.4	80.46	19.59	60.87	75.7
490	100	8	50	10			1.25	0.01	104.4	62.84	35.82	27.02	43.0
491	100	9	0	10			1.25	0.01	103.7	92.55	74.14	18.41	19.9
492	100	9	25	10			1.25	0.01	103.7	85.89	69.71	16.18	18.8
493	100	9	50	10			1.25	0.01	103.7	85.27	82.16	3.11	3.6
494	100	6	100	10			1.25	0.01	98.59	38.6	13	25.6	66.3
495	100	6	200	10			1.25	0.01	98.59	22.38	4.44	17.94	80.2
496	100	6	400	10			1.25	0.01	98.59	10.88	5.64	5.24	48.2
497	100	6	800	10			1.25	0.01	98.59	6.99	5.92	1.07	15.3
498	100	6	100	10			1.25	0.01	98.59	34.86	11.74	23.12	66.3
499	100	6	200	10			1.25	0.01	98.59	21.91	11.01	10.9	49.7
500	100	6	400	10			1.25	0.01	98.59	12.03	12.27	-0.24	-2.0
501	100	6	800	10			1.25	0.01	98.59	7.31	7.33	-0.02	-0.3
502													
503													
504													
505													
506	100	6	0	10			1.25	0.01	94.89	85.09	7.049	78.041	91.7
507	100	6	12.5	10			1.25	0.01	94.89	74.73	13.73	61	81.6
508	100	6	25	10			1.25	0.01	94.89	65.67	5.76	59.91	91.2
509	100	6	37.5	10			1.25	0.01	94.89	58.09	6.55	51.54	88.7
510	100	6	50	10			1.25	0.01	94.89	52.59	6.56	46.03	87.5
511	100	6	0	10			1.25	0.01	94.89	84.87	70.65	14.22	16.8

Soybean Peroxidase Data

Test no.	(mg/L) 2,4 DCP	pH	(g/L) Soil	%org	(g/L) PEG	MW PEG	(uL) H2O2	(u/ml) SBP	(mg/L) Actual	(mg/L) 24hour	(mg/L) Final	Change	% loss
512	100	6	12.5	10			1.25	0.01	94.89	77.26	13.25	64.01	82.9
513	100	6	25	10			1.25	0.01	94.89	64.88	5.54	59.34	91.5
514	100	6	37.5	10			1.25	0.01	94.89	58.92	6.62	52.3	88.8
515	100	6	50	10			1.25	0.01	94.89	52.92	6.89	46.03	87.0
516	100	6	50	10			1.25	1	97.59	51.24	0.4	50.84	99.2
517	100	6	50	10			1.25	1	97.59	51.09	0.4	50.69	99.2
518	100	6	50	10			1.25	1	97.59	50.97	0.4	50.57	99.2
519	100	6	50	10			1.25	1	97.59	50.83	0.4	50.43	99.2
520	100	6	50	10			1.25	1	97.59	51.32	0.4	50.92	99.2
521	100	6	50	10			1.25	1	97.59	50.85	0.4	50.45	99.2
522	100	6	50	10			1.25	1	97.59	51.32	0.4	50.92	99.2
523	100	6	50	10			1.25	1	97.59	50.54	0.4	50.14	99.2
524	100	6	50	10			1.25	1	97.59	51.09	0.4	50.69	99.2
525	100	6	50	10			1.25	1	97.59	50.97	0.4	50.57	99.2
526	100	6	50	10			1.25	1	97.59	50.51	2.57	47.94	94.9
527	100	6	50	10			1.25	1	97.59	47.97	0.4	47.57	99.2
528	100	6	50	10			1.25	1	97.59	49.65	0.4	49.25	99.2
529	100	6	50	10			1.25	1	97.59	60.55	0.4	60.15	99.3
530	100	6	50	10			1.25	1	97.59	53	0.4	52.6	99.2
531	100	6	50	10			1.25	1	97.59	51.36	0.4	50.96	99.2
532	100	6	50	10			1.25	1	97.59	53.54	0.4	53.14	99.3
533	100	6	50	10			1.25	1	97.59	53	0.4	52.6	99.2
534	100	6	50	10			1.25	1	97.59	50.51	0.4	50.11	99.2
535	100	6	50	10			1.25	1	97.59	53.54	0.4	53.14	99.3
536	100	6	50	10			1.25	1	97.59	60.55	0.4	60.15	99.3
537	100	6	50	10			1.25	1	97.59	49.65	0.4	49.25	99.2
538	100	6	50	10			1.25	1	97.59	47.97	0.4	47.57	99.2
539	100	6	50	10			1.25	1	97.59	51.36	0.4	50.96	99.2

SS - indicates silica sand only