PHENOL REMOVAL FROM SATURATED POROUS MEDIA USING HORSERADISH PEROXIDASE MEDIATED OXIDATIVE POLYMERIZATION PROCESS

by

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B.S., Changwon National University, Changwon, Korea, 1996
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Civil Engineering
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Abstract

Aquifers are frequently contaminated by phenolic compounds from spills, leaking underground storage tanks, or landfills. These compounds can be toxic to a variety of organisms including humans. Their disposal is restricted in many countries with strict limits for acceptable concentrations in drinking water. Phenols that are chlorinated have significantly greater toxicity and are resistant to aerobic biodegradation. Enzyme-mediated *in situ* stabilization has been advocated as an approach for the treatment of phenolic compounds in soils and groundwater. This research investigated the applicability of a luminol-based chemiluminescence assay to monitor transport of horseradish peroxidase (HRP) enzyme in saturated porous media. The chemiluminescence assay was optimized by varying solution conditions such as the concentration of luminol, p-iodophenol, hydrogen peroxide, ionic strength and pH. All assay components were found to affect the maximum chemiluminescene intensity. The study also evaluated the ability of HRP to mediate the removal of phenol from solution by catalyzing its oxidative polymerization in simulated aquifer conditions. HRP behaved as a conservative tracer in the column packed with Ottawa sand. The concentration of phenol in the column effluent was found to decrease by nearly 90% in the presence of HRP and H_2O_2 in the continuous flow system. HRP mediated oxidative polymerization of phenols resulted in the production of soluble and insoluble oligomeric products. Modification of porous media caused by the deposition of phenol polymerization products was studied and the impact of media modification on subsequent transport of phenolic contaminants was evaluated using 2,4-dichlorophenol (2,4-DCP) as a probe solute. The pore volume of the porous media was reduced due to the deposition of insoluble phenolic oligomers. The transport behavior of 2,4-DCP showed that the contaminant was retarded in the modified porous media.

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Table of Contents

List of Figures

List of Tables

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CHAPTER 1. GENERAL INTRODUCTION

Chemical contamination of soil and groundwater is widerspread and frequent and has become a great concern in the U.S. and around the world. Aquifers are frequently contaminated by mixtures of organic compounds from spills, leaking underground storage tanks, or landfills.

Several hazardous organic pollutants have been introduced into the soil and groundwater environment in past decades. Among these, phenols are of specific concern because of their toxicity, mobility, and widespread occurrence at contaminated sites. Chlorinated phenols are highly toxic and resistant to aerobic microbial degradation.

Groundwater pump and treat technologies frequently involve high cost and low efficiency. Intrinsic in situ bioremediation may be achieved at low cost, but proceeds very slowly. Therefore, alternative enhanced in situ remediation approaches are needed to treat contaminated sites and meet the nations's most pressing site cleanup and waste management needs.

Enzyme catalyzed polymerization processes have been proposed as alternative approaches for the treatment of phenolic pollutants in industrial waste water. Enzymes such as horseradish peroxidase (HRP) are capable of catlayzing the oxidation of phenolic compounds in the presence of hydrogen peroxide to produce insoluble oligomers that precipitate out of solution. This approach can be used for a wide wariety of phenolic compound. The reactions are stable at normal groundwater pH and over large concentration ranges of the contaminant. Enzymatic polymerization reactions occur at rates that are significantly higher than biological processes.

Enzyme-mediated *in situ* stabilization also has been advocated as a new approach for the treatment of phenolic compounds in soils and groundwater. However, since most past studies have focused on the application of this technology to surface water treatment, little information is available concerning for the application of this technology to the decontamination of groundwater and subsoil pollution.

Chapter 3 of this dissertation focuses on the development of a reliable assay to monitor HRP in water. The chapter presents the results of a luminol-based chemiluminescence assay for

detecting HRP in clear aqueous solutions. It also illustrates the results of HRP transport behavior under simulated aquifer conditions. Chapter 4 describes phenol removal using HRP-mediated polymerization in saturated porous media. It discusses parameter estimation for transport behavior of phenol and tracer. This chapter also discusses the modification of porous media resulting form the deposition of phenol polymerization products. Finally, Chapter 5 contains the results of parameter estimation of transport behavior of tracer and 2,4-dichlorophenol in the modified porous media and presents estimates of parameters that can be used to predict solute transport.

CHAPTER 2. RESEARCH GOAL AND HYPOTHESES

The overall goal of this research was to achieve an improved understanding of the factors impacting the ability of horseradish peroxidase (HRP)-mediated oxidative polymerization processes to remove phenol from the aqueous phase in saturated porous media under continuous flow conditions. The following research hypotheses were evaluated to achieve the research goal:

Hypothesis 1

The activity of HRP in clear aqueous solutions can be estimated using a luminol-based chemiluminescence assay.

Hypothesis 2

HRP behaves like a conservative tracer in a continuous flow system containing porous media comprised of porous media.

Hypothesis 3

Phenol entering a packed column under simulated aquifer conditions can be removed from the aqueous phase by injecting HRP and H_2O_2 into the flow.

Hypothesis 4

HRP-mediated phenol removal in continuous flow, packed column reactors is accompanied by the generation of soluble and insoluble oligomeric products.

Hypothesis 5

Phenol removal and the production of oligomeric products under the action of HRP and H_2O_2 are affected by the enzyme dose, solution pH and solution ionic strength.

Hypothesis 6

Deposition of insoluble phenol polymerization products in the packed column results in physical modification of the porous media.

Hypothesis 7

Hydrophobic solutes experience retardation in the modified porous media as a result of their interaction with the newly developed organic phase comprised of insoluble oligomeric products.

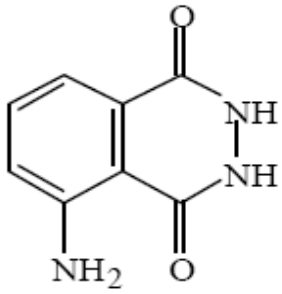
CHAPTER 3. DETERMINATION OF HORSERADISH PEROXIDASE USING AN ENHANCED CHEMILUMINESCENCE ASSAY AND HRP TRANSPORT IN SATURATED POROUS MEDIA

3.1 INTRODUCTION

Horseradish peroxidase (HRP) is a plant glycohemoprotein (Dunford, 1991). Its enzymatic activity arises from the cyclic reduction and oxidation of the iron atom in its hematin group (Chance, 1951). HRP has been widely studied and its catalytic activity has been quantified using colorimetric, fluorometric and chemiluminescence methods. Chemiluminescence methods utilizing a HRP-luminol-H_2O_2 system have been previously used as simple and sensitive assays for the quantification of HRP (Cercek et al., 1994, 1995; Yakunin and Hallenbeck, 1998).

Although the phenomenon of chemiluminescence has been studied for a long time, its analytical application for liquid-phase reactions has been reported only since the 1980s. The discovery of iodophenol in the mid-1980s, as a compound capable of significantly improving the chemiluminescence associated with luminol (5-amino-2,3 dihydrophthalazine-1,4-dione; Figure 3.1), resulted in the development of the enhanced chemiluminescence (ECL) assay (Kricka and Thorpe, 1983; Thorpe et al., 1985b; Thorpe and Kricka, 1986). ECL is produced when a compound such as p-iodophenol, known as an enhancer, is added to a HRP-luminol-H_2O_2 system. A variety of substituted phenols, including firefly luciferin and 6-hydroxybenzothiazole derivatives (Whitehead et al., 1983; Thorpe et al., 1985c), 4-iodophenol (Thorpe and Kricka, 1986) and 4- (4-hydroxyphenyl)thiazole (Ii et al., 1993), can serve as effective enhancers (Easton et al., 1996). The ECL assay has been reported to detect aqueous phase HRP at concentrations as low as 200 μM (Kricka et al., 1996) and has greatly extended the use of the HRP-luminol-H_2O_2 system in applications such as the monitoring of free radicals and reactive metabolites in cell-free, enzyme, cell, or organ systems, and for screening antioxidant activity (Krol et al., 1990; Yasaei et al., 1996; Yildiz and Demiryurek, 1998; Yildiz et al., 1998).

Figure 3.1. Chemical structure of luminol.



Although various parameters affecting the luminol-based ECL assay have been previously studied (Kricka and Thorpe, 1983), the HRP-luminol-H_2O_2 system has not been optimized for the ionic strength of the assay solution (reagent). In this study, we investigated the impacts of the following factors on the effectiveness of the luminol-H_2O_2 system quantification of HRP activity in clear aqueous samples: reagent pH, the concentrations of H_2O_2, HRP, and p-iodophenol in the assay solution, the H_2O_2/iodophenol ratio, and the reagent ionic strength

3.2 BACKGROUND

3.2.1 Chemiluminescence

Chemiluminescence is defined as the emission of electromagnetic radiation (ultraviolet, visible or near infrared) by an electronically excited intermediate of a chemical reaction. Chemiluminescence reactions generally result in reaction products that are in an electronically excited state and produce light when they return to the ground state (Figure 3.2). When this phenomenon occurs in living organisms, it is called bioluminescence. However, bioluminescence is not a common occurrence since most luminescence reactions also release energy as heat.

Radziszewski was the first to observe a chemiluminescence spectrum in a synthetic setting when, in 1877, he reported that lophine (2,4,5-triphenylimidazole) emitted green light upon reaction with oxygen in the presence of a strong base (Mestre et al., 2001 quoting Radiziszewski, 1877). A decade later, Wiedemann (1888) first used the term “luminescence” and

classified it into six different categories (Table 3.1) to distinguish light emission based on the method of excitation; chemiluminescence, crystalloluminescence, electroluminescence, photoluminescence, thermo-luminescence, and triboluminescence (Mestre et al., 2001 quoting Wiedemann, 1888).

Since the discovery of synthetically produced chemiluminescence, many other types of chemiluminescence reactions have been discovered. At the turn of the century, Trauzt (1905) reviewed all known examples of chemiluminescence and bioluminescence and described the luminescent properties of reactions of several organic compounds with various oxidants (Mestre et al., 2001 quoting Trauzt, 1905).

Figure 3.2. Schematic Diagram of Chemiluminescence Mechanism.

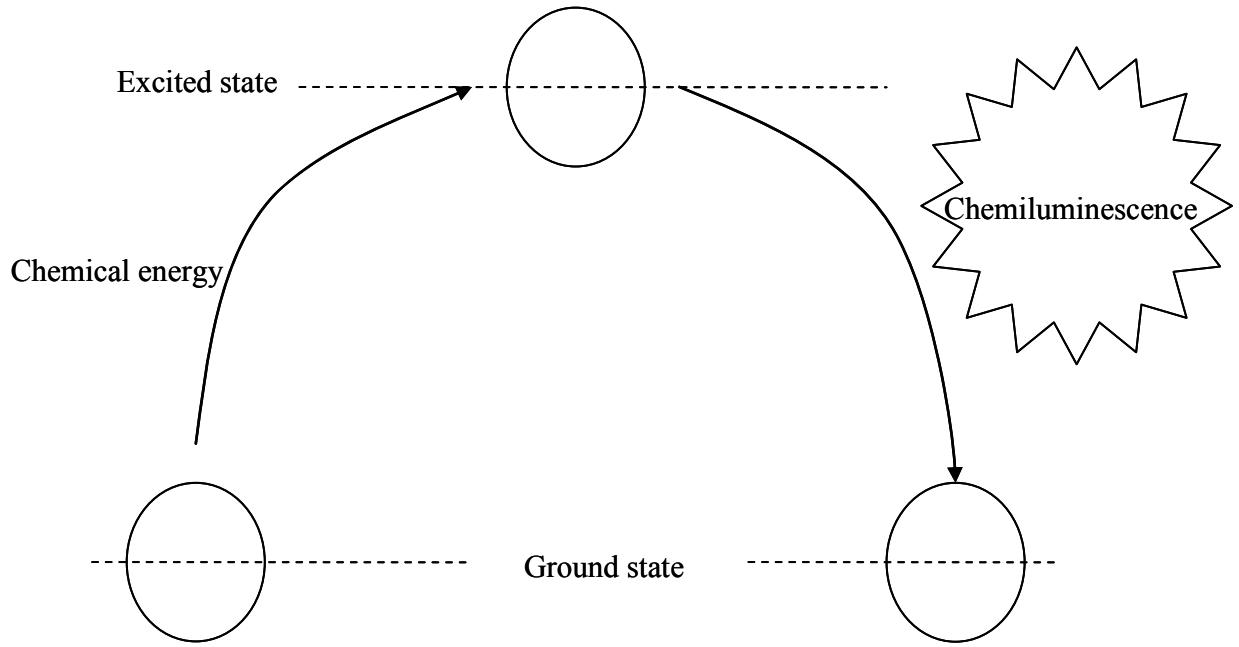


Table 3.1. Types of luminescence.

Name	Characteristics
Luminescence	The emission of ultraviolet, visible or near-infrared radiation from a molecule or an atom resulting from the transition of an electronically excited state to a lower energy state (usually ground state)
Photoluminescence	Luminescence caused by the absorption of light
Fluorescence	Short-lived photoluminescence from a singlet electronically excited state
Cristaloluminesecence	Luminescence produced as a result of crystallization
Bioluminescence	UV, visible or near infrared radiation emitted from living organisms
Chemiluminescence	Luminescence caused by a chemical reaction
Thermoluminescence	Luminescence produced by mild heating
Pyroluminescence	Luminescence caused by metal atoms in flames
Radioluminescence	Radiation arising from irradiation by gamma or X-ray
Electroluminescence	Luminescence produced in gases by an electric discharge
Triboluminescence	Radiation as a result of friction
Sonoluminescence	Radiation from ultrasonication of dissolved substance

(Robards and worsfold, 1992; Mestre et al., 2001)

Although many inorganic and organic chemical reactions produce chemiluminescence in the liquid phase, only a few systems have been used for analytical purposes. These include luminol, lucigenin (N,N-dimethyl-9,9-biacridinium nitrate), lophine (2,4,5-triphenylimidazole), bis (2,4,5-tricholorophenyl oxalate nitrate), and luciferin (1- (4,5-dimethoxy-2-nitrophenyl)ethyl ester). The intense luminescence associated with alkaline oxidation of luminol was first reported in 1928 (Mueller and Arnhold, 1995 quoting Albrecht, 1928). Since then, the chemiluminescence resulting from the reaction of luminol and an oxidant (H_2O_2 in particular), in a strongly basic medium, has been extensively studied (White et al., 1964; White and Bursey, 1964; White and Rosewell, 1985) and used for the quantification of several inorganic and organic solutes (Robards and Worsfold, 1992).

3.2.2 The Principle of Chemiluminescence

Chemiluminescence reactions usually involve cleavage of the oxygen-oxygen bond in organic peroxide compounds. Peroxides are commonly involved in light emitting reactions because the relatively weak peroxide bond is easily cleaved and the resulting molecular reorganization produces a large amount of energy.

Three necessary conditions have been described for chemiluminescence reactions (Mestre et al., 2001; Neil et al., 1998; Kricka and Thorpe, 1983). First, the reaction should release sufficient energy to produce an excited state molecule; second, the reaction pathway must favor the formation of an excited product; and finally, the excited state should be capable of transferring the energy to another molecule or be luminescent itself. The resulting chemiluminescence can be characterized using one or more of the following parameters: color, intensity, rate of production of luminescence, and decay rate of intensity. Among these, luminescence intensity is most frequently used for analytical purposes.

In order to achieve the highest levels of sensitivity, a chemiluminescent reaction must be as efficient as possible in generating photons of light. This depends on the rate of the reaction. Each molecule of a chemiluminescent compound can produce no more than one photon of light. A perfectly efficient reaction would have a chemiluminescence quantum yield (Φ_{CL}) of one, i.e. one photon per molecule. The intensity of chemiluminescence (I_{CL}) represents the number of photons emitted per second and is defined according to the following equation (Guilbault, 1973; Mestre, 2001):

$$I_{CL} = \Phi_{CL}\left(\frac{dP}{dt}\right) = \Phi_{EX}\Phi_{EM}\left(\frac{dP}{dt}\right) \qquad \text{Eq. (3.1)}$$

where, dP/dt represents reaction kinetics, i.e., the number of molecules reacting per unit time; Φ_{EX} is the excitation quantum yield, i.e., the number of excited states formed per reacting molecule; and Φ_{EM} is the emission quantum yield, i.e., the number of excited photons emitted per excited state.

The excitation quantum yield (Φ_{EX}) is the probability of generating an excited electronic state in a reaction and has a value between 0 and 1, with 0 being a completely dark reaction. When Φ_{EX} =1, all product molecules are generated in the excited state (Robards and Worsfold,

1992). The most useful chemiluminescence reactions have $\Phi_{EX}=10^{-3}$. The quantum yield of a reaction (Φ_{EM}) is the product of the efficiencies of the excitation and emission steps with values ranging from 10^{-15} to nearly 1 (Campbell, 1988; Paul, 1978).

Values of I_{CL} are affected by reaction kinetics and the duration of chemiluminescence. I_{CL} is also impacted by the properties of organic solvents used in the reaction assay. For example, chemiluminescence intensity was found to be affected when the volume of dimethyl sulfoxide (DMSO) in the assay solution was varied (Tadashi et al., 1995). Other reaction conditions such solution pH and temperature can also impact the progress of chemiluminescence reactions. Table 3.2 summarizes chemiluminescence wavelength and quantum yield (Φ_{EM}) values reported in the literature for a variety of chemical reactions.

Table 3.2. Properties of selected chemiluminescence reactions.

Reaction	**Color (λ_{max})**	**Quantum Yield**	**Reference**
Luminol oxidation in DMSO	Blue-violet (480-502 nm)	0.05	Campbell (1998)
Luminol oxidation in aqueous alkali	Blue (425 nm)	0.01	Campbell (1998) Kricka and Thorpe (1983)
Lucigenin oxidation in alkaline H_2O_2	Blue-green (440 nm)	0.016	Kricka and Thorpe (1983)
Lophine oxidation in alcoholic NaOH	Yellow (525 nm)	0.88	Kricka and Thorpe (1983), Paul (1978)
Pyrogallol in alkaline H_2O_2	Reddish pink		Campbell (1998)
Peroxyoxalate (TCPO) oxidation using 9,10-diphenylanthracene as fluorophore	Blue	0.07-0.5	Campbell (1998) Kricka and Thorpe (1983)

3.2.3 Luminol-Based Chemiluminescence

Luminol is among the most popular and widely used chemiluminescence reagents for chemical analyses conducted on-site and in the laboratory (Navas-Diaz et al., 1997). It is capable of producing chemiluminescence at a wavelength of 425 nm under many conditions and does not require mixing in an organic solvent like the peroxyoxalate system (Givens and Schowen, 1989; Orosz et al., 1996). Luminol finds extensive use in immunoassays, metabolic pathway monitoring, detection of a number of inorganic and organic compounds, determination of enzymatic reactions, and detection of blood at crime scenes (Briheim et al., 1984; Radi et al., 1993; Lundqvist and Dahlgren, 1996; Kricka and Ji, 1995).

Luminol can also be used in assays for the detection of horseradish peroxidase using hydrogen peroxide as a substrate (Rost et al., 1998). Phenolic compounds play the role of hydrogen donors in the HRP catalytic cycle and are transformed into phenoxy radicals during the cycle. Some phenolic compounds such as luminol, polyphenols (Segawa, et al. 1995; Nilsson et al., 1964; Halmann et al, 1979), xanthene dyes (O'Brien et al., 1978; De Toledo et al., 1980; Segawa et al., 1990; Segawa et al., 1992) and tyrosine (Ushijima et al., 1985), have been shown to emit chemiluminescence in the presence of HRP and hydrogen peroxide.

Luminol-based chemiluminescence has been extensively studied and used in analytical methods ever since Albrecht (1928) first reported the oxidation of luminol catalyzed by enzymes in basic solutions (Nieman, 1989). Luminol chemiluminescence is produced in aqueous solutions in the presence of supplemental oxidants such as hydrogen peroxide, permanganate, ferricyanide, iodine, hypochlorite, persulfate and metal derivatives such as hemin (Cunningham et al., 1998; Seitz, 1981). Among these oxidants, H_2O_2 is most effective under basic conditions.

Chemiluminescence reactions can utilize a wide variety of catalysts to produce longer and brighter light intensity. Peroxidase enzymes, such as HRP, are among the most effective catalysts that allow chemiluminescence reactions to precede at near-neutral pH values (8–8.5). Transition metal ions, such as Co(II), Cr(II), Cu(II), Fe(II), Fe(III), Hg (II), Mn(II), and their complexed forms, such as ferrocene and ferricyanide, can also be used as catalysts in chemiluminescence reactions (Nussbaum et al., 1987; Guilbault, 1990; Skoog et al., 1998). Among these, cobalt is the most effective metal catalyst. Some metals, however, can inhibit chemiluminescence at specific concentrations (Christophe and Blum, 2006).

HRP is a widely utilized enzyme in fundamental research and practical applications (Kitagawa et al., 1987; Broker et al., 1978). It has been used to determine aqueous concentrations of H_2O_2 and other compounds (Robards and worsfold, 1992). Its application has also been proposed for the removal of toxic chemicals from drinking water, industrial wastewater, groundwater and soils (Hirayama et al., 1997; Arakawa et al., 1999; Wang et al., 1991; Nicell et al., 1992, 1993a, 1993b; and Bollag et al., 1988). For example, Olsson (1982) reported that H_2O_2 concentrations of 3×10^{-6} to 1×10^{-2} mM in a pH 10 carbonate buffer could be accurately determined using chemiluminescence and an automated flow injection system. Chemiluminescence reactions that utilize myleoperoxidase, HRP, or lactoperoxidase in the oxidation of luminol by H_2O_2 have been well documented (Robards and Worsfold, 1992; Nakamura and Nakamura, 1998; Haqqani et al., 1999).

The chemiluminescence intensity of luminol-based reactions is generally enhanced at higher solution pHs. I_{CL} is directly proportional to the concentration of the reactants including the oxidant, the catalyst, and luminol itself (Garcia-Sanchez et al., 1995; Christophe and Blum, 2006). However, it is affected by the catalyst- transition metal (Seitz and Neary, 1974) or HRP (Seitz 1978; Schroeder et al., 1978).

Although the HRP-luminol-H_2O_2 assay is capable of producing well-defined calibration curves for substrates and peroxidase in the presence of organic material, there are several issues to be considered during the application of chemiluminescence techniques. Chemiluminescence emission is not constant since the light emission versus time profile – the chemiluminescence intensity profile (CIP) – can vary widely with reaction time in different chemiluminescence systems. The possibilities of enzyme inactivation, extremely high pHs, and the impact of organic solvents are all disadvantages that have to be overcome during the application of the luminol-based chemiluminescence assay to determine aqueous concentrations of target compounds (Yeh and Lin, 2002).

Although the luminol-based chemiluminescence method suffers from some limitations, there are several advantages that favor the consideration of this reaction chemistry for assay development (Kuroda et al., 2001). The advantages include high sensitivity, rapid measurements, a wide linear range, no chemical pre-treatment of the sample, inexpensive equipment required, and simple operation. These advantages explain the recent success of its utilization in

deoxyribonucleic acid (DNA) analysis (Garcia and Fox, 2003) and in flow injection analysis of phenylephrine hydrochloride and carbonyl (Mestre et al., 1999; Huertas-Pérez et al., 2005).

3.2.4 Enhanced Chemiluminescence

Enhanced chemiluminescence describes the phenomenon of increased light production during co-oxidation of luminol and a substrate called an enhancer mediated by HRP in the presence of H_2O_2 (Alfassi et al., 1986; Neta et al., 1978). A variety of enhancer substrates have been found to increase chemiluminescence intensity during reaction (Zhang and Dunford, 1993; Whitehead et al., 1983; Thorpe et al., 1985b; Thorpe and Kricka, 1986; Kricka and Ji, 1995; Kricka et al., 1996). The effectiveness of an enhancer appears to be based principally on its oxidation potential under the reaction conditions and its relation to the oxidation potential of the detectable substrate.

Enhanced chemiluminescence reactions have been used in immunoassays (Thorpe et al., 1984a; Thorpe et al., 1985a, 1985b) and in other analytical methods (Garcia and Sanchez, 1995). Numerous phenol derivatives such as aromatic amines (Kricka et al., 1996; Milbrath, 1987), certain heterocyclics, in particular benzothiazoles (Thorpe et al., 1985b, 1985c; Whitehead et al, 1983), and arylboronic acids (Kricka and Ji, 1995) are capable of enhancing the chemiluminescence produced in HRP-luminol-H_2O_2 systems (Navas-Diaz et al., 1997).

In spite of several reports in the literature (Lundin and Hallander, 1987; Merenyi et al., 1990; Nakamura and Nakamura, 1998; Hiner et al., 2002; Rodriguez-Lopez et al., 2001) and multiple studies examining the influence of factors such as pH, buffer, and organic ligands in the presence and absence of H_2O_2, the exact mechanisms of the peroxidase-catalyzed reaction and its luminol-chemiluminescence stoichiometry are still poorly understood and remain largely hypothetical.

The peroxidase-catalyzed oxidation of luminol (LH^-) involves the reaction of HRP with H_2O_2 via oxygen transfer (Chance, 1952; George, 1952). HRP is first converted into intermediary complexes (HRP-I and HRP-II) in the presence of luminol and H_2O_2. These intermediates abstract an electron from the substrate (LH−) to form the luminol radical ($L^{\cdot -}$) (Easton et al., 1996):

$$HRP + H_2O_2 \rightarrow HRP\text{-}I + H_2O \quad \text{Eq. (3.2)}$$

$$HRP\text{-}I + LH^- \rightarrow HRP\text{-}II + L^{-}\cdot + H_2O \quad \text{Eq. (3.3)}$$

$$HRP\text{-}II + LH^- \rightarrow HRP + L^{-}\cdot \quad \text{Eq. (3.4)}$$

The intermediate enzyme complexes can oxidize an enhancer substrate (EH) in a one-electron transfer process with the enhancer serving as an electron relay. The oxidized enhancer, in turn, oxidizes the substrate, which reacts with H_2O_2 or molecular oxygen to form luminol radicals (Thorpe and Kricka, 1987; Lundin and Hallander, 1987; Cercek et al., 1994) through the steps illustrated in equations 3.5 to 3.7. The enhancer is returned to its natural state in the process, and is ready to undergo further oxidation by the intermediate enzyme complexes. Enhancer radicals can also react with luminol, yielding luminol radicals that produce light.

$$HRP\text{-}I + EH \rightarrow HRP\text{-}II + E\cdot + H_2O \quad \text{Eq. (3.5)}$$

$$HRP\text{-}II + EH \rightarrow HRP + E\cdot \quad \text{Eq. (3.6)}$$

$$LH^- + E\cdot \rightarrow L^{-}\cdot + EH \quad \text{Eq. (3.7)}$$

These reactions produce luminol radicals (L−·) that enter a complex chemical pathway to finally generate luminol hydroperoxide (LO_2H-), a precursor to the light emitting excited 3-aminophthalate ion. Nitrogen gas is released during the reaction, leaving the luminol in the form of an excited 3-aminophthalate ion, $(AP^{2-})^*$, which emits light at 425 nm (Gilbault, 1967; Gilbault, 1973). The spectra of the unenhanced and enhanced chemiluminescence are similar (Kricka et al., 1991; Thorpe and Kricka, 1986; Whitehead et al., 1983; Navas-Diaz et al., 1995; García-Sanchez et al., 1995; Hori et al. 1994). This is because the chemiluminescence emission is from aminophthalate, which emits light at around 425 nm, and the enhancer simply accelerates the reaction. $(AP^{2-})^*$ is produced from luminol endoperoxide (LO_2^{2-}) which emits luminescence and is converted to ground state as illustrated in Equations 3.8 to 3.11 (Lundin and Hallander, 1987; Thorpe and Kricka, 1987; Cercek et al., 1994).

$$2L^{-}\cdot \rightarrow L + LH^- \quad \text{Eq. (3.8)}$$

$$L + H_2O_2 \rightarrow LO_2^{2-} \quad \text{Eq. (3.9)}$$

$$LO_2^{2-} \rightarrow (AP^{2-})^* + N_2 \quad \text{Eq. (3.10)}$$

$$(AP^{2-})^* \rightarrow AP^{2-} + h\nu\ (425\ nm) \qquad \text{Eq. (3.11)}$$

where L = diazaquinone; LO_2^{2-} = luminol endoperoxide; $(AP^{2-})^*$ = 3-aminophthalate excited; and AP^{2-} = 3-aminophthalate dianion.

Although the HRP-luminol-H_2O_2 assay is capable of producing well-defined calibration curves for substrates and peroxidase, chemiluminescence emission is not constant since the light emission versus time profile can vary widely with reaction time in different chemiluminescence systems due to the possibilities of enzyme inactivation at extremely high pHs. The effect of solution ionic strength is not also well studied.

3.2.5 One-Dimensional Advection and Dispersion Equation

Prediction of contaminant transport must be based on fundamental understanding of the geochemical mechanisms influencing contaminant migration in groundwater environments. Solutes dissolved in the porous media are influenced by a number of different processes. Contaminants can associate with the surfaces of the mineral grains of the aquifer, sorb to organic carbon in the aquifer, undergo chemical precipitation, or participate in oxidation-reduction reactions. As a result of these processes, some solutes move much more slowly through the aquifer than the groundwater that is transporting them; this effect is called retardation. Biodegradation and precipitation will decrease the concentration of solute in the plume but may not necessarily slow the rate of plume migration.

The macroscopic continuity equation in a volume element generates the advection dispersion transport equation. The advection dispersion equation (ADE) was developed in an attempt to quantify solute transport processes for one-dimensional, steady-state flow in a homogeneous soil column (Lapidus and Amundson, 1952). The one-dimensional advection-dispersion equation was later modified to include sorption and decay (Miller and Weber, 1984). Solute transport in isothermal, one-dimensional Darcian water flow in a variably saturated, rigid isotropic porous medium can be represented by the following equation:

$$R\frac{\partial C}{\partial t} = D_L\frac{\partial^2 C}{\partial x^2} - v_x\frac{\partial C}{\partial x} - \frac{B_d}{\theta}\frac{\partial C^*}{\partial t} + \left(\frac{\partial C}{\partial t}\right)_{rxn} \quad \text{Eq. (1.1)}$$

(dispersion) (advection) (sorption) (reaction)

where

C = concentration of solute in liquid phase

t = time

D_L = dispersion coefficient

v_x = average linear groundwater velocity

B_d = bulk density of aquifer

θ = volumetric moisture content or porosity for saturated media

C^* = amount of solute sorbed per unit weight of solid

rxn = subscript indicating a biological or chemical reaction of the solute (other than sorption)

In the absence of microbial or chemical decay or transformation, the ADE model for one-dimensional transport of reactive solutes Eq. (1.1) can be organized as following:

$$R\frac{\partial C}{\partial t} = D_L\frac{\partial^2 C}{\partial x^2} - v_x\frac{\partial C}{\partial x} - \frac{B_d}{\theta}\frac{\partial C^*}{\partial t}$$

In this study, we evaluated the impact of pH, solution ionic strength, ratio of substrate/[H_2O_2] on the chemiluminescence using HRP-luminol-H_2O_2 assay and HRP transport in saturated porous media using one-dimensional advection-dispersion equation.

Specific research hypotheses were:

Hypothesis 1

The activity of HRP in clear aqueous solutions can be estimated using a luminol-based chemiluminescence assay.

Hypothesis 2

HRP behaves like a conservative tracer in a continuous flow system containing porous media comprised of porous media.

3.3 EXPERIMENTAL APPROACH

This section presents the materials, reagents and experimental method for the chemiluminescence test using luminol-HRP-enhancer-H_2O_2 system.

3.3.1 Reagents, Materials and Equipment

Horseradish peroxidase (HRP, Type II, RZ: 2.2, 181 activity units/mg), luminol (5-amino-2, 3-dihydrophthalazine 1,2-dione), hydrogen peroxide (30% w/w), and *p*-iodophenol were purchased from Sigma Chemical (St. Louis, MO) and stored at 4 ^{o}C. Analytical grade potassium phosphate (mono and dibasic), sodium bicarbonate, dimethyl sulfoxide (DMSO) and Tris-HCl buffer solution (1 M, pH 8.5) were purchased from Fisher Scientific (Pittsburg, PA). All other reagents used were analytical grade. Standard solutions were prepared in distilled-deionized (DD) water, or in DMSO. DD water (conductivity < 0.06 μS/cm) was obtained from a Barnstead Nanopure water purification system (18.2 MΩ cm^{-1}, Dubuque, IA).

A stock solution of HRP (181 activity units/mg) with 30 activity units (AU) units per milliliter was prepared two hours before use. One AU was defined by Sigma Chemicals as the amount of HRP that formed 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20 ^{o}C. The stock solution of H_2O_2 (150 mM) was prepared by volumetric dilution of 30% (w/w) of H_2O_2 one hour before adding to the assay solution.

Luminol stock solutions (1, 0.5, and 0.1 M) were prepared daily by weighing 2.66 g of the pure compound and dissolving in 10 mL of DMSO. The luminol solution was stored at 4 ^{o}C and used within 3 days. A stock solution of *p*-iodophenol (0.3 M) was prepared by dissolving 0.66 g of the chemical in 10 mL of DMSO.

The chemiluminescence assay utilized luminol, *p*-iodophenol, HRP, and H_2O_2 solutions. All solutions used in the chemiluminescence measurements were prepared in phosphate and bicarbonate buffer solutions of pre-determined ionic strengths. The compositions of these buffers are summarized in Table 3.3. Tris-HCl buffer (1 M, pH 8.5) was used after diluting the solution

to adjust for desired ionic strength. All solutions in the kinetic studies were prepared in 50 mM Tris-HCl buffer (adjusted to an appropriate pH) in the absence or presence of 50 mM NaCl. Once the optimum pH of the assay solution was decided, Tris-HCl buffer solution (pH 8.5) was used in all experiments to achieve more consistent results.

To determine the optimum concentrations of the assay components and the effect of pH and ionic strength, the experiments in this study were first conducted for baseline reaction conditions consisting of 0.5 mM luminol, 0.1 mM *p*-iodophenol, 2AU/mL of HRP and 5 mM H_2O_2. Luminescence measurements were performed using a

EPP Miniature Fiber Optic Spectrometer (StellarNet, Inc. FL) with the light source switched off.

Table 3.3. Buffer compositions for various solution pHs and ionic strengths.

		Ionic Strength (mM)							
pH	**Molar Ratio**	**10**	**50**	**100**	**500**	**10**	**50**	**100**	**500**
	A/B	A = K_2HPO_4 (mM)				B = KH_2PO_4 (mM)			
6.5	0.20	6.3	31.3	62.6	312.8	1.2	6.2	12.5	62.4
7	0.63	3.5	17.3	34.6	172.8	2.2	10.9	21.8	109.1
7.5	2.00	1.4	7.2	14.3	71.6	2.9	14.3	28.6	142.8
8	6.31	0.5	2.5	5.0	25.1	3.2	15.8	31.7	158.3
8.5	19.95	0.2	0.8	1.6	8.2	3.3	16.4	32.8	163.9
		A = Na_2CO_3 (mM)				B = $NaHCO_3$ (mM)			
9	0.05	8.7	43.5	86.9	434.6	0.4	2.2	4.4	21.8
9.5	0.16	6.8	33.9	67.81	338.9	1.1	5.4	10.7	53.7
10	0.50	4.0	20.0	39.9	199.7	2.0	10.0	20.0	100.1
10.5	1.58	1.7	8.7	17.4	86.9	2.8	13.8	27.5	137.7

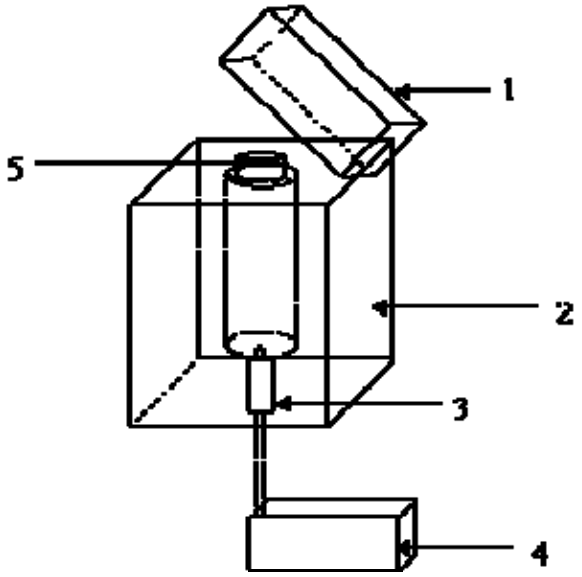
3.3.2 Experimental Approach of Chemiluminescence

A five-milliliter test tube was used to determine chemiluminescence at baseline conditions. One hundred microliters of each assay component (HRP, *p*-iodophenol, and luminol)

except H_2O_2 were transferred into the test tube and 50 mM Tris-HCl buffer (pH 8.5) was added to obtain a final volume of 2.9 mL. After 30 minutes, the test tube was placed in the dark chamber connected to a light detector. A 100 μL aliquot of H_2O_2 was transferred to the test tube with a Hamilton syringe to initiate the chemiluminescence reaction. The same procedure was performed using DD water as control instead of the assay solution.

The chamber-cap was immediately covered after injecting H_2O_2. All light sources in the laboratory were turned off to minimize interference. A schematic of a typical chemiluminescence detection system is illustrated in Figure 3.3. Light intensity from the chemiluminescence reaction (I_{CL}) was detected by the EPP Miniature Fiber Optic Spectrometer and analyzed using the built-in Spectrawiz Software. I_{CL} at 425 nm was determined at five-second intervals by averaging 10 measurements obtained every 300 microsecond. I_{CL} was recorded as a function of reaction time for the first 5 minutes of the assay yielding a chemiluminescence intensity profile (CIP) for the reaction conditions studied. H_2O_2 was injected 2s after chemiluminescence data collection was commenced.

Figure 3.3. Schematic diagram of chemiluminescence detection system.
The chamber cap (1), dark chamber (2), light detector probe (3), light detector (4), and test tube (5).



The maximum chemiluminescence intensity (I_{CLmax}) was measured and the total area under the CIP (A_{CIP}) was determined using the built-in software. I_{CLmax} values were utilized to develop calibration curves. The effect of HRP dose on chemiluminescence was evaluated at pHs of 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, and 10.5. The effect of reagent ionic strength was studied for ionic strengths ranging from 10 mM to 1 M. The effect of luminol concentration was evaluated using concentrations of 0.5, 0.63, 0.83, 1.25, 2.5, and 5 mM. All experiments were repeated three times at 20±2 ^{o}C. After the optimum concentrations for various assay components were determined, calibration curves for HRP and H_2O_2 were developed using the chemiluminescence assay.

3.3.3 Column Experiment

A column experiment was conducted to investigate the transport behavior of HRP in saturated porous media.

3.3.2.1 Reagent, Material and Equipment

Ottawa sand standard (20-30 Mesh) was purchased from Fisher Scientific. A column reactor consisting of a glass tube of length 11 cm and internal diameter of 4.1 cm was fabricated in the laboratory. Teflon-lined tubing, caps and connections were used to minimize sorption of enzyme to the reactor components. All other reagents, materials and equipments were used as described in section 3.3.1.1.

3.3.1.2 Experimental Approach

Ottawa sand was used for the porous media packing in the column. The experimental set up for studying HRP transport in the column system is illustrated in Figure 3.4. Potassium chloride (KCl) was used as the non-reactive tracer and measured indirectly using a conductivity meter. All experiments were conducted at room temperature (20 ± 2 ^{o}C).

HRP transport experiments were conducted in the upward direction. HRP solution (pH 7 and ionic strength 20 mM) was introduced through the inlet located at bottom of the column. A peristaltic pump (Buchler Instrument, Multistaltic pump) was used to introduce the enzyme and maintain a constant pore water velocity of 1.5 m/day..

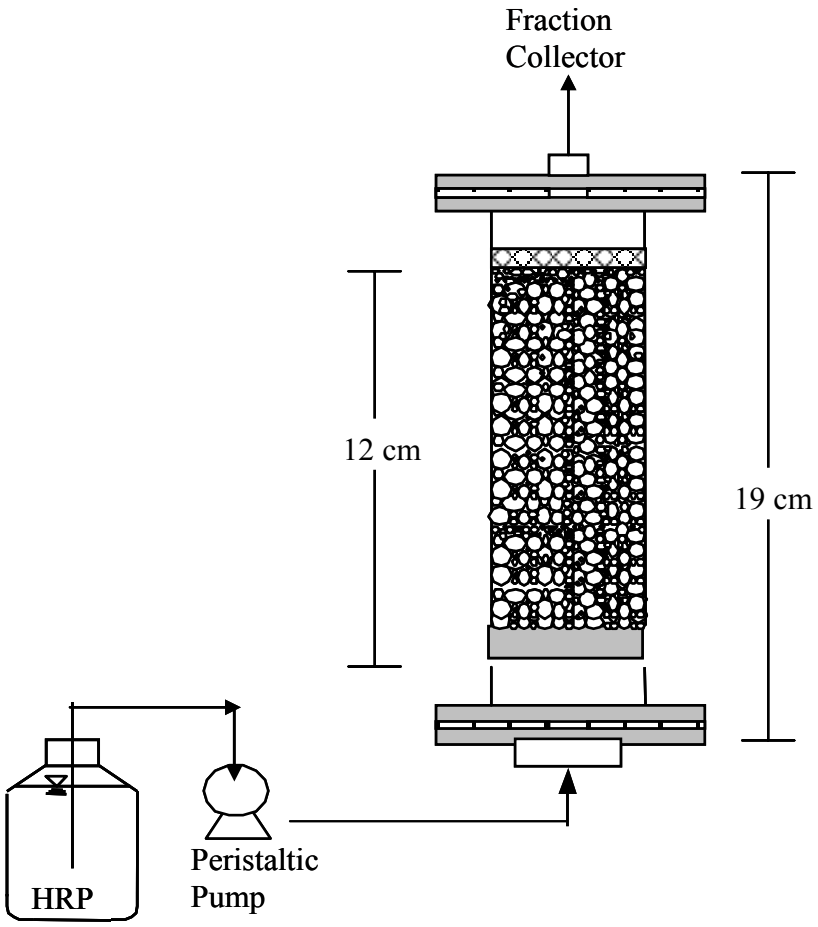
Samples (5 mL) were taken from the outlet of the column every 10 minutes using an automated fraction collector (ISCO, Foxy Jr.). Triplicate 100 μL of aliquots of column effluent

from each collected fraction were transferred into 5mL test tubes to determine HRP activity using the chemiluminescence assay. One hundred microliters each of luminol (0.5 mM) and *p*-iodophenol were transferred into the test tube and the solution made up to 2.9 mL using pH 8.5 Tris-HCl buffer solution. The test tube was placed in the dark chamber and 100 µL of 5 mM H_2O_2 was added to initiate the chemiluminescence assay as described in section 3.3.1.2.

HRP transport in the porous media was simulated with the one-dimensional advection dispersion equation (1-D ADE) by using CXTFIT software. A tracer test was conducted before conducting the HRP transport experiment to compare the difference in transport behavior.

Potassium chloride (250 mg/L) was used as a tracer and introduced into the column packed with Ottawa sand. The conductivity in the column effluent stream was measured every five minutes. The diffusion coefficient was estimated using CXTFIT software.

Figure 3.4. Schematic Diagram of Column Experiment for HRP Transport.



3.4 RESULTS AND DISCUSSION

This section presents results obtained during the development and optimization of the HRP-luminol-H_2O_2 chemiluminescence assay in the presence of the enhancer *p*-iodophenol. The effects of assay solution pH, ionic strength, and concentrations of H_2O_2, *p*-iodophenol, and HRP on I_{CL} and A_{CIP} are discussed. This is followed by results of a study where the enhanced chemiluminescence assay was used to quantify HRP activity and evaluate transport of the enzyme in saturated porous media.

3.4.1 Preliminary Studies

Preliminary experiments were conducted to assure that there was no reaction in the absence of H_2O_2 in the chemiluminescence assay. Preliminary tracer tests were also conducted to select a conservative tracer and ensure reliability of results measured by an in-line conductivity meter. Potassium chloride was selected as the tracer due to its highest reliability.

3.4.2 Development of the Enhanced Chemiluminescence Assay

Enhanced chemiluminescence describes the phenomenon of increased light emission during luminol oxidation catalyzed by HRP in the presence of an enhancer, such as *p*-iodophenol. In this work, the effect of pH, H_2O_2 concentration, enhancer concentration, and ionic strength were studied and calibration curves were developed to determine HRP concentrations in unknown samples by using a HRP-luminol-iodophenol system.

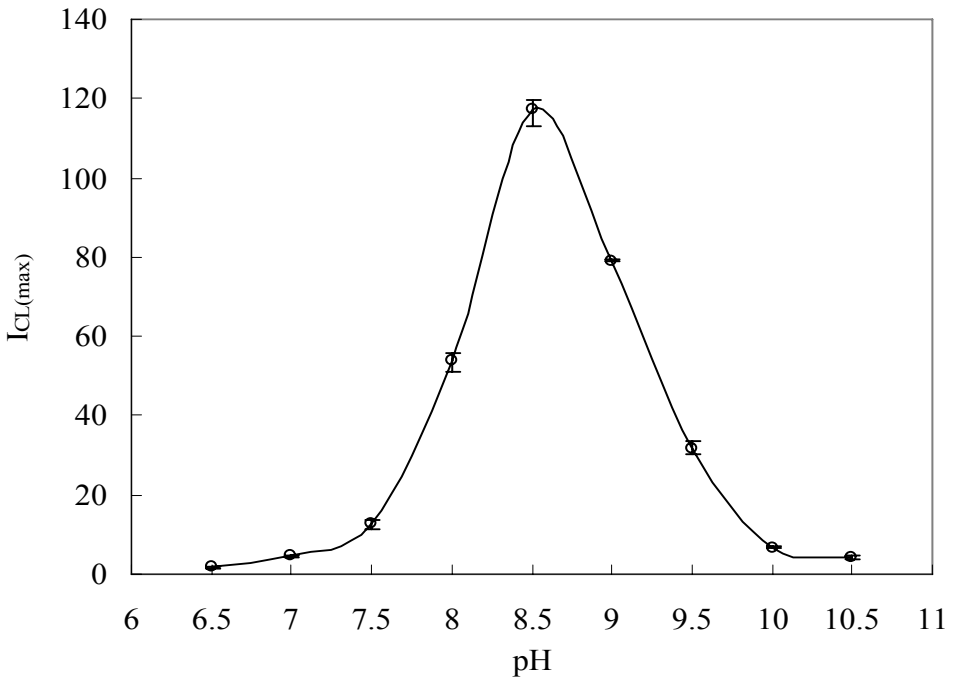
3.4.2.1. Reagent Solution pH

The impact of pH on I_{CL} and A_{CIP} was evaluated because of the reported dependence of luminol chemiluminescence on reagent pH (Thorpe et al., 1984b; Thorpe and Kricka et al., 1986; Ilyina et al., 2003). Concentrations of H_2O_2, HRP, luminol and enhancer were maintained constant and the pH of the buffer solution was altered between 5.5 and 10.5 using the buffering solutions described in Table 3.3.

Figure 3.5 illustrates the pH dependence of peak chemiluminescence intensity (I_{CLmax}) for a reagent ionic strength of 100 mM and H_2O_2, luminol, and *p*-iodophenol concentrations of 5 mM, 0.5 mM, 0.1 mM, respectively, and HRP activity of 1 AU/mL. I_{CLmax} was affected by reagent pH and reached a maximum value at pH 8.5. In their comparative study of chemiluminescence using HRP-catalyzed peroxidation of acridan (GZ-11) and luminol. Osman

et al. (2000) reported that the rate of light production decreased with the lowering of pH from 7.1 to 2.6 resulting in reduction in I_{CLmax}. Intermediate products formed during peroxidase-catalyzed reaction are pH-sensitive and more stable at slightly alkaline pH values. The assay solution pH also exerts a remarkable effect on the enzyme activity, yielding optimal response at pH 8.5. Navas-Diaz and coworkers (1995) reported results from their evaluation of chemiluminescence production during HRP mediated oxidation of luminol with fluorescence diacetate as an enhancer. Thorpe et al. (1985b) also reported that enhancement of chemiluminescence light intensity by *p*-iodophenol was markedly pH dependent with maximum intensity at approximately pH 8.6. Based on the peak chemiluminescence at pH 8.5, this pH was selected as the optimum reagent pH for further studies.

Figure 3.5. Effect of assay solution pH on observed maximum chemiluminescence intensity. [H_2O_2] = 5 mM, [luminol] = 0.5 mM, [p-iodophenol] = 0.1 mM, HRP = 1 AU/mL and [ionic strength] = 100 mM.



The impact of reagent ionic strength on the chemiluminescence intensity was evaluated by using 10 mM, 50 mM, 100 mM and 500 mM Tris-HCl solutions, all buffered at pH 8.5. Figure 3.6 illustrates the impact of the reagent ionic strength on I_{CLmax}. While I_{CLmax} values for the 10 mM and 50 mM buffers were similar, the 100 mM and 500 mM Tris-HCl buffers produced increasingly higher I_{CLmax} values.

When A_{CIP} values were plotted as a function of buffer pH (Figure 3.7), the maximum CIP area was observed for the 500 mM assay solution at pH 9. Based on the results shown in Figures 3.5, 3.6 and 3.7, the 100 mM ionic strength, pH 8.5 Tris-HCl buffer was selected for further assay development for the reliable and stable results.

Figure 3.6. Effect of assay solution pH and ionic strength on I_{CLmax}. [H_2O_2] = 5 mM, [luminol] = 0.5 mM, [*p*-iodophenol] = 0.1 mM and HRP = 1 AU/mL.

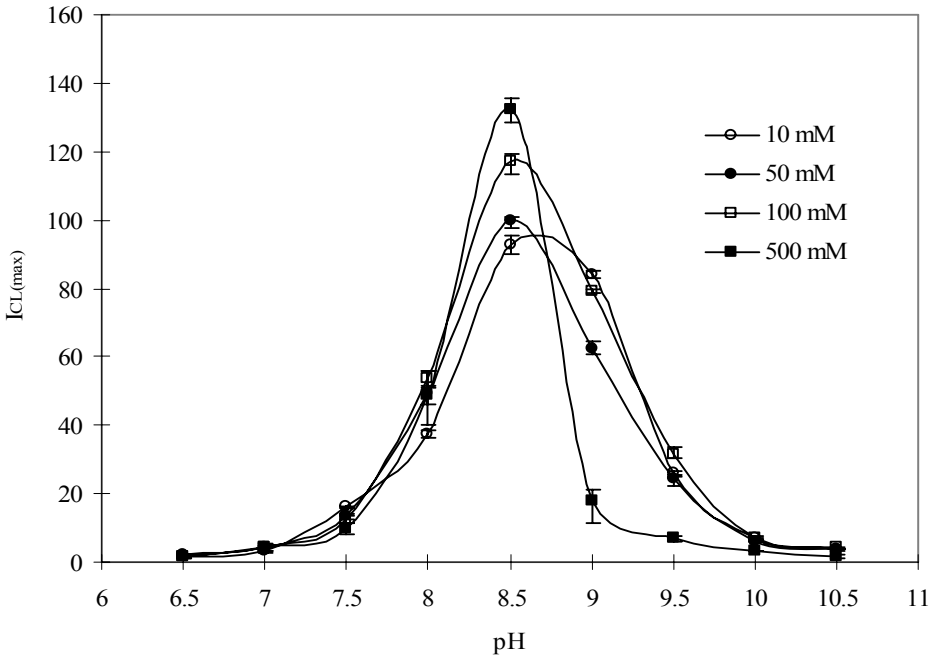
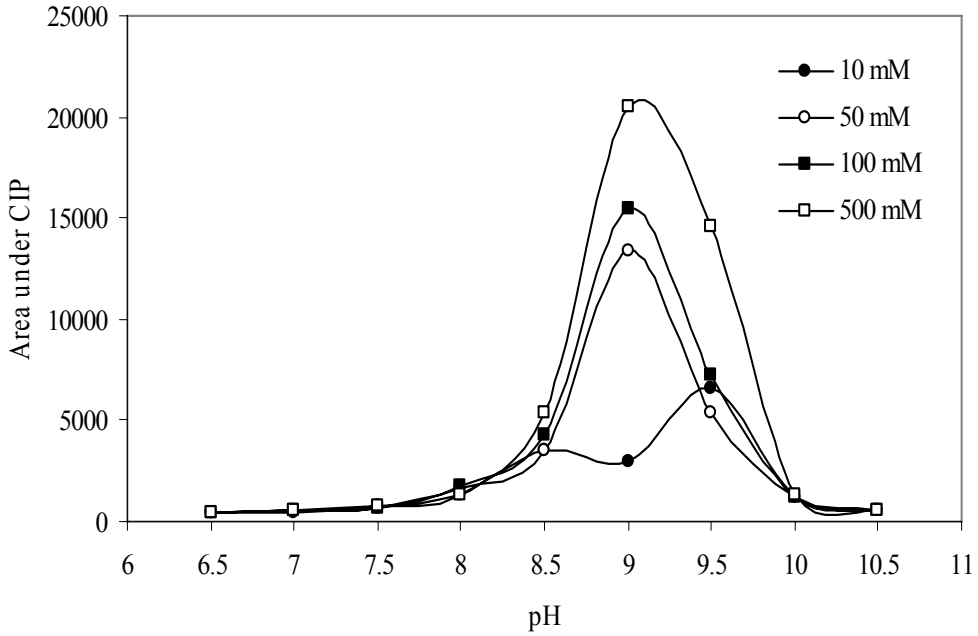


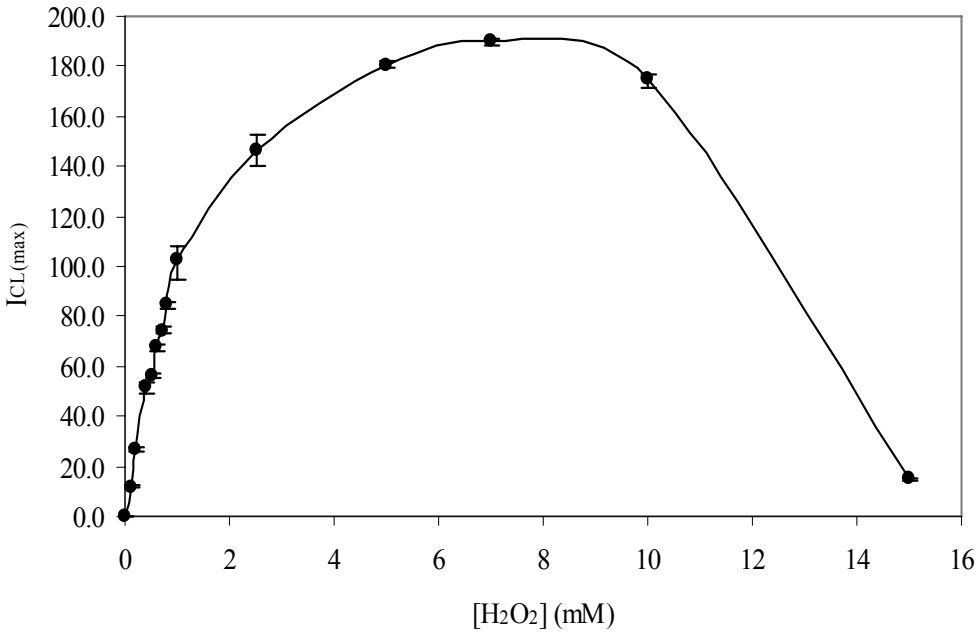
Figure 3.7. Effect of assay solution pH and ionic strength on A_{CIP} values. [H_2O_2] = 5 mM, [luminol] = 0.5 mM, [*p*-iodophenol] = 0.1 mM and HRP = 1 AU/mL.



3.4.2.2 H_2O_2 and Enhancer Concentration

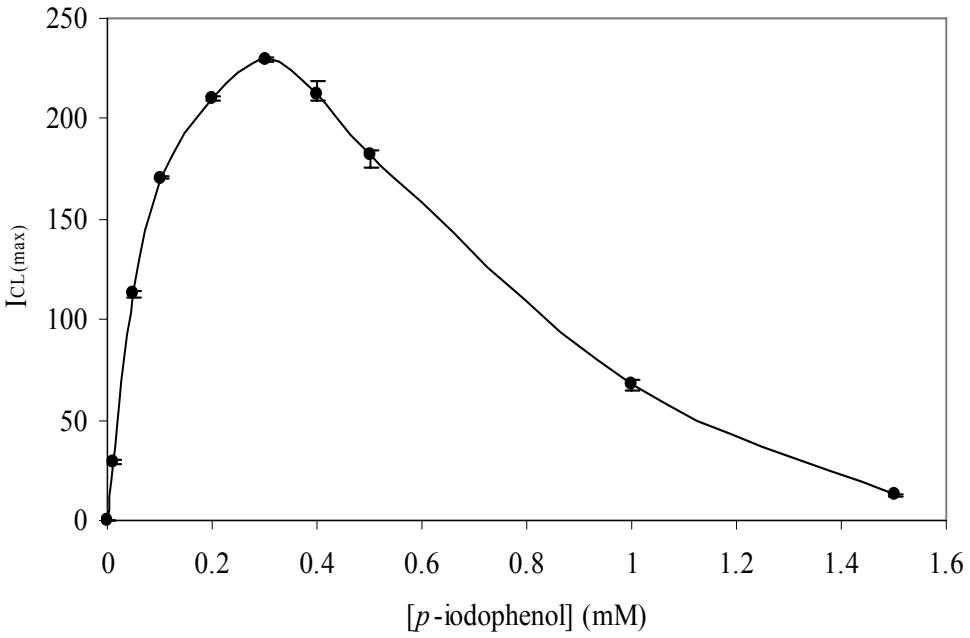
The effect of H_2O_2 concentration on the chemiluminescence intensity of HRP is shown in Figure 3.8. It can be seen that I_{CL} increased rapidly from zero to 3 mM H_2O_2 and, thereafter, more gradually upto 7 mM. Cormier and Prichard (1968) also reported an increase in the quantum yield of the chemiluminescence reaction when H_2O_2 concentration was increased from 0 to 6.5 mM under conditions similar to our study. Beyond a H_2O_2 concentration of 7 mM, chemiluminescence intensity was observed to decrease. Osman et al. (2000) reported that I_{CLmax} continued to increase with increasing H_2O_2, while Nicell et al. (1993a) found that an excess of H_2O_2 inactivated the enzyme. Wu et al. (1997) reported that the efficiency of HRP improved as the ratio of H_2O_2/substrate increased up to an optimum value, beyond which increase of H_2O_2 resulted in reduced efficiency of HRP. In our study, the optimum H_2O_2 concentration was observed to be between 5 and 8 mM. Therefore, 5 mM H_2O_2 was selected as the working concentration for subsequent assay development.

Figure 3.8. Effect of H_2O_2 concentration on I_{CLmax}. [Luminol] = 0.5 mM, [*p*-iodophenol] = 0.1 mM, HRP = 1 AU/mL, [ionic strength] = 50 mM and pH 8.5.



The enhancer concentration producing the maximum chemiluminescence depends on the nature of enhancer and the concentrations of luminol and H_2O_2 in the reagent mixture. The enhancer concentration revealed a similar trend as that manifested by H_2O_2 (Figure 3.9). Optimum chemiluminescence was observed at a *p*-iodophenol concentration of 0.3 mM. Thorpe et al. (1985b) had previously reported that the degree of chemiluminescence enhancement depended on the concentration of the enhancing agent used. Optimum enhancement in their study was observed at 0.34 mM *p*-iodophenol, a concentration similar to that obtained in our experiments. Yeh and Lin (2002) noted that I_{CLmax} values increased to various extents when a variety of enhancers were evaluated at different assay solution pHs. In our experiments, a value of approximately 0.75 I_{CLmax} was observed at a *p*-iodophenol concentration of 0.1 mM. The stability and repeatability of chemiluminescence observations were better at this enhancer concentration than at 0.3 mM. Hence, 0.1 mM *p*-iodophenol concentration was selected as the enhancer concentration for further assay development.

Figure 3.9. Effect of enhancer concentration on I_{CLmax}. [H_2O_2] = 5 mM, [luminol] = 0.5 mM, HRP = 1 AU/mL, [ionic strength] = 50 mM and pH 8.5.

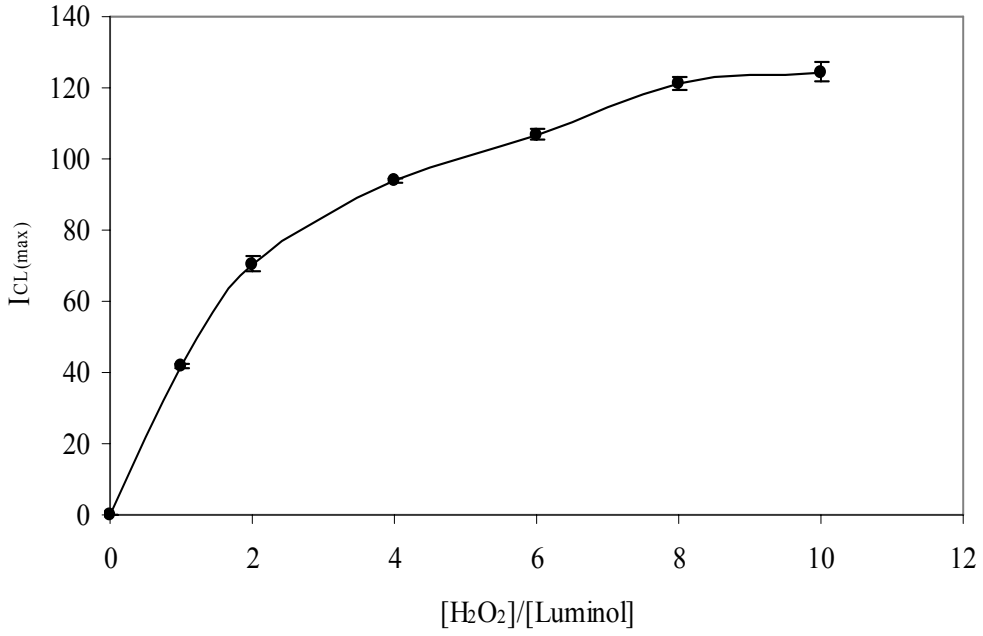


3.4.2.3 H_2O_2/Luminol Ratio

The effect of H_2O_2/luminol ratio on the observed maximum chemiluminescence intensity is shown in Figure 3.10. In this study, the H_2O_2 concentration was fixed at 5 mM and the luminol concentration was varied from 0.5 to 5 mM. I_{CLmax} values continually increased as a function of H_2O_2/luminol ratio illustrating that luminol was the limiting agent in the reaction. The chemiluminescence intensity followed a saturation-type behavior with respect to the H_2O_2/luminol ratio and appeared to level off at a value of approximately 130 beyond H_2O_2/luminol molar ratios of about 10.

In their study on micro-determination of proteins based on complexation with Cu(II), Li et al. (2004) reported that the chemiluminescence intensity continually increased as the luminol and H_2O_2 concentrations increased. However, they observed that the increases in I_{CL} were small for luminol concentrations greater than 0.5 mM, and H_2O_2/luminol ratios greater than 20. Based on the results of our experiment the concentrations of H_2O_2 and luminol were fixed at 5 mM and 0.5 mM luminol, respectively, corresponding to a H_2O_2/luminol ratio of 10 for subsequent work in this study.

Figure 3.10. Effect of $[H_2O_2]$/[luminol] ratio on I_{CLmax} values. $[H_2O_2]$ = 5 mM, [*p*-iodophenol] = 0.1 mM, HRP = 1 AU/mL, [ionic strength] = 50 mM and pH 8.5.

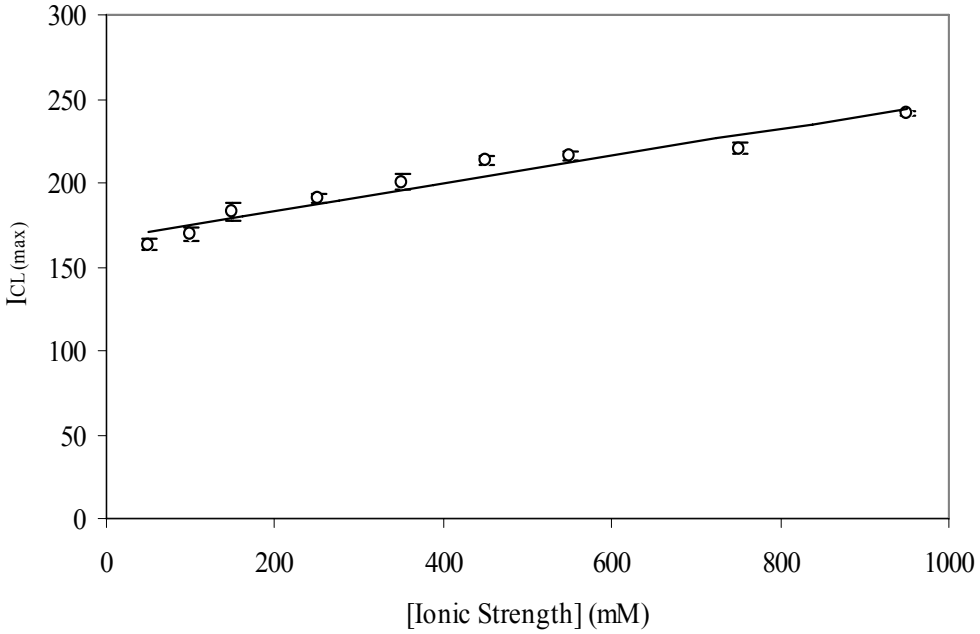


3.4.2.4 Ionic Strength

The effect of ionic strength on I_{CLmax} was evaluated for a pH 8.5 buffer with ionic strength ranging from 10 to 150 mM. NaCl was used to increase the ionic strength of the pH 8.5 Tris-HCl buffer solution. Figures 3.6 and 3.8 had illustrated that the solution pH and ionic strength impacted the maximum light intensity, although the impact of pH appeared to be greater than that of ionic strength.

Figure 3.11 shows the impact of reagent ionic strength on I_{CLmax} at pH 8.5. The maximum light intensity exhibited a linear relationship with ionic strength over the range evaluated. These results differ from those reported by Li et al. (2004) who observed that chemiluminescence light intensity gradually decreased with increasing buffer concentration based on calpain (Cu(II)-based enzyme) from 2 to 10 mM of ionic strength using a C(H_3BO_3) buffer solution. They explained that a high ionic strength could inhibit the formation of Cu(II)-protein complex.

Figure 3.11. Effect of reagent ionic strength (0 to 1000 mM) on observed chemiluminescence intensity. [H_2O_2] = 5 mM, [luminol] = 0.5 mM, [*p*-iodophenol] = 0.1 mM, HRP = 1 AU/mL and pH 8.5. R^2 value for linear fit = 0.937. Error bars indicate one standard deviation of triplicate samples.



Borgesa and Reis (2005) also reported that ionic strength may have an adverse effect on enzyme activity. These researchers presented experimental results showing a decrease in light emission by increasing ionic strength of a KBr buffer solution. In a study of the impact of solvent and ionic strength on electro-chemiluminescence efficiency, Maness et al. (1988) found a decrease in chemilunescence as the ionic strength of the solution was increased.

3.4.2.4 Enzyme Concentration

Figure 3.12 illustrates the effect of HRP concentration on the chemiluminescence intensity profile (CIP). I_{CLmax} was observed to increase as function of enzyme concentration. Motsenbocker and Knodo (1994) noted that the short delay in achieving the maximum chemiluminescence light intensity is associated with a delay in light emission after mixing of reactants (H_2O_2, luminol, enhancer and HRP). Since the lag time phenomenon affects HRP-induced chemiluminescence at low HRP concentrations, it makes the dependence of light output

on HRP concentration a nonlinear function with respect to time (Motsenbocker and Knodo, 1994).

No lag time was observed in our study, since a higher enzyme concentration was utilized. As shown in Figure 3.12, maximum chemiluminescence intensity occurred within 5s of H_2O_2 addition to the assay mixture. The I_{CLmax} values increased steadily with HRP dose. Since the area under the CIP did not yield a consistent relationship with respect to HRP activity at low enzyme concentrations, I_{CLmax} instead of A_{CIP}, was selected as the appropriate response factor to measure HRP activity in unknown solutions.

Figure 3.12. Effect of HRP dose on the chemiluminescence intensity profile (CIP) at pH 8.5. [H_2O_2] = 5 mM, [luminol] = 0.5 mM, and [*p*-iodophenol] = 0.1mM, [ionic strength] = 100 mM.

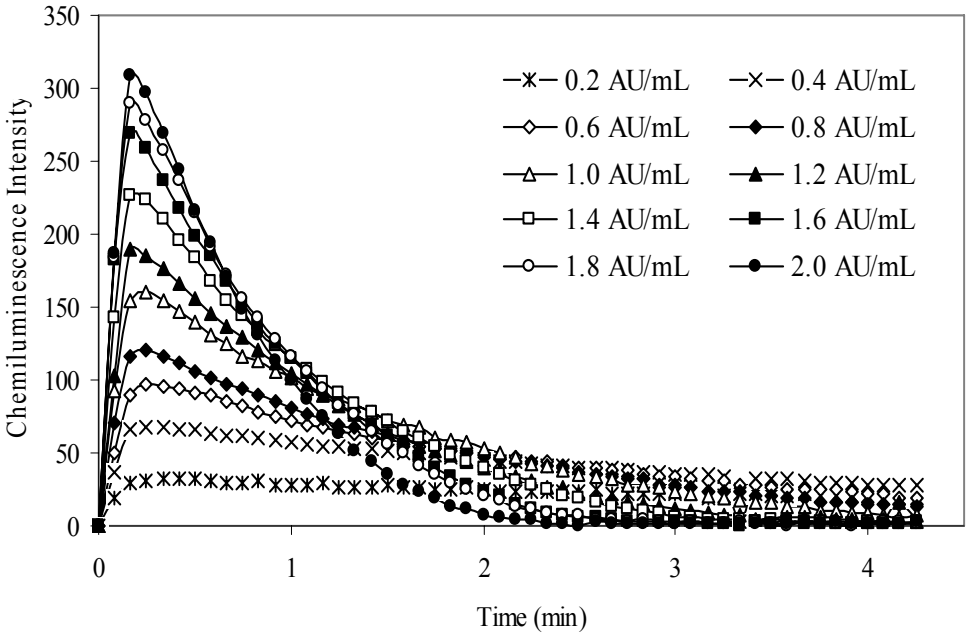
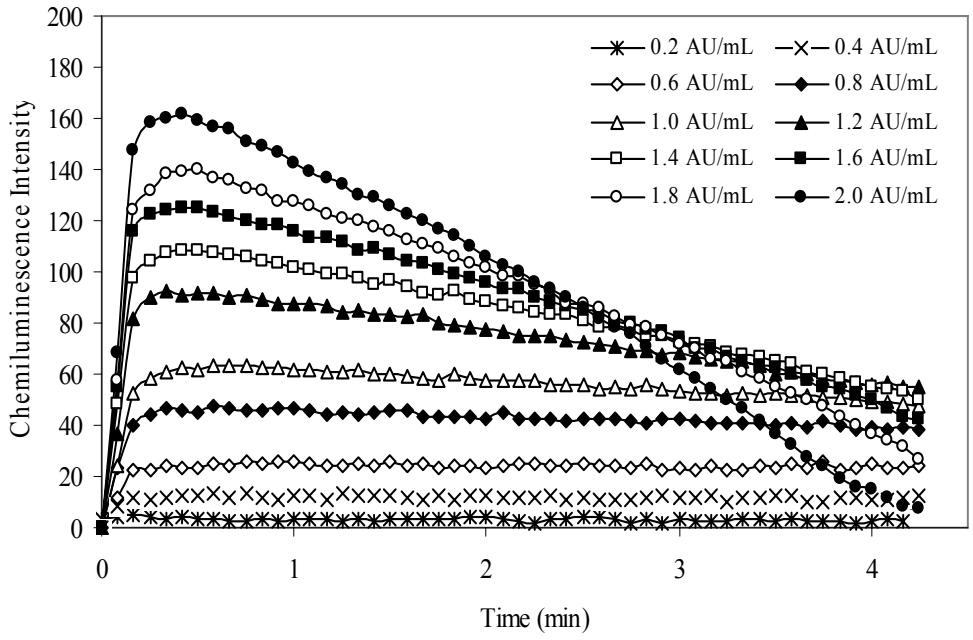


Figure 3.13 shows the CIP data under conditions identical to those in Figure 3.12 except at pH 9. It can be seen that the maximum intensity was about half of what was observed at pH 8.5 and the reaction rate profile was not sharp at the maxima. However, chemiluminescence continued to occur over a longer time period and produced larger CIP areas at pH 9.

Figure 3.13. Effect of HRP dose on chemiluminescence intensity profile (CIP) at pH 9.0. [H_2O_2] = 5 mM, [luminol] = 0.5 mM, and [*p*-iodophenol] = 0.1 mM, [ionic strength] = 100 mM.



3.4.2.4 Enzyme Calibration Curve

Since chemiluminescence can vary with the batch of commercial-grade HRP utilized in the reaction and the type of detector used in the assay, calibration curves are necessary for each HRP lot and detector type. A HRP calibration curve was developed for maximum chemiluminescence intensity at various enzyme activity and previously described assay conditions (Figure 3.14). The maximum chemiluminescence intensity was observed to increase proportionally with enzyme dose from 0 to 1 AU/mL. In this case, the relationship between I_{CLmax} and HRP concentration yielded a linear relationship expressed by the following equation:

$$I_{CLmax} = 168.44 \text{ (HRP dose)} \qquad \text{Eq. (3.12)}$$

The relationship between I_{CLmax} and HRP dose appeared to remain near-linear even across a larger HRP dose range of 0 to 2.0 AU/mL (Figure 3.15).

The impact of incubation time on the I_{CLmax}-HRP dose calibration curve was compared for zero and 5-days of enzyme pre-incubation prior to conducting the chemiluminescence assay (Figure 3.16). This study was performed since HRP introduced into natural or engineered environmental systems is likely to be 'pre-incubated' for varying periods of time. The calibration curve shown in Figure 3.16 represents the 5-day pre-incubation data and is similar to the curve shown in Figure 3.15 indicating no significant impact of pre-incubation. The data illustrated in Figure 3.16 can be described by the following linear equation:

$$I_{CLmax} = 159.55 \text{ (HRP dose)} \qquad \text{Eq. (3.13)}$$

Figure 3.14. Calibration curve developed for I_{CLmax} values versus HRP dose (0 to 1.0 AU/mL). [H_2O_2] = 5 mM, [luminol] = 0.5 mM, and [p-iodophenol] = 0.1 mM, [ionic strength] = 100 mM and pH 8.5. R^2 value for linear fit = 0.995.

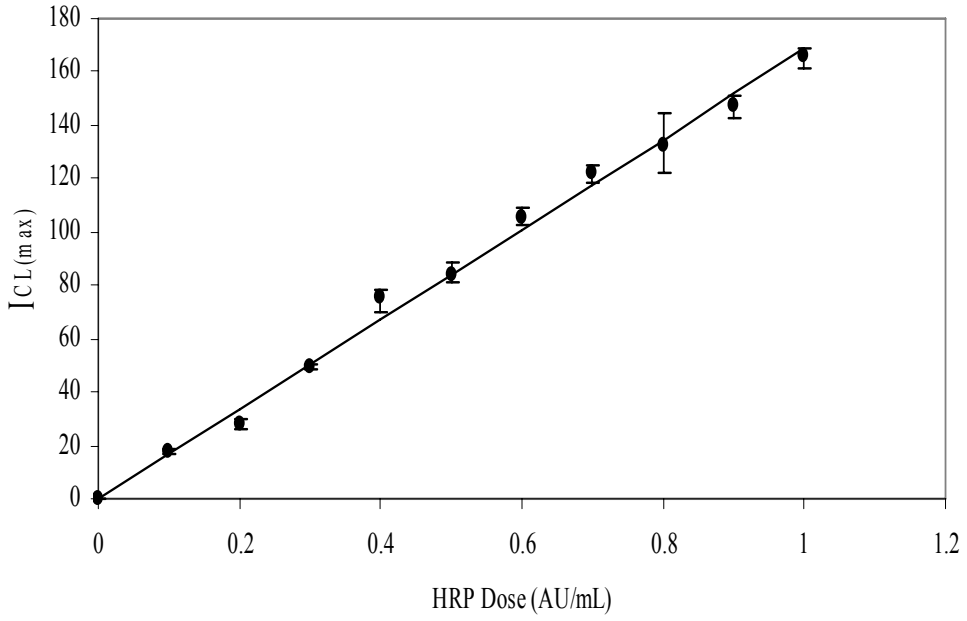


Figure 3.15. Calibration curve for I_{CLmax} values versus HRP dose (0 to 2.0 AU/mL). [H_2O_2] = 5 mM, [luminol] = 0.5 mM, and [*p*-iodophenol] = 0.1 mM, [ionic strength] = 100 mM and pH 8.5. R^2 value for linear fit = 0.997.

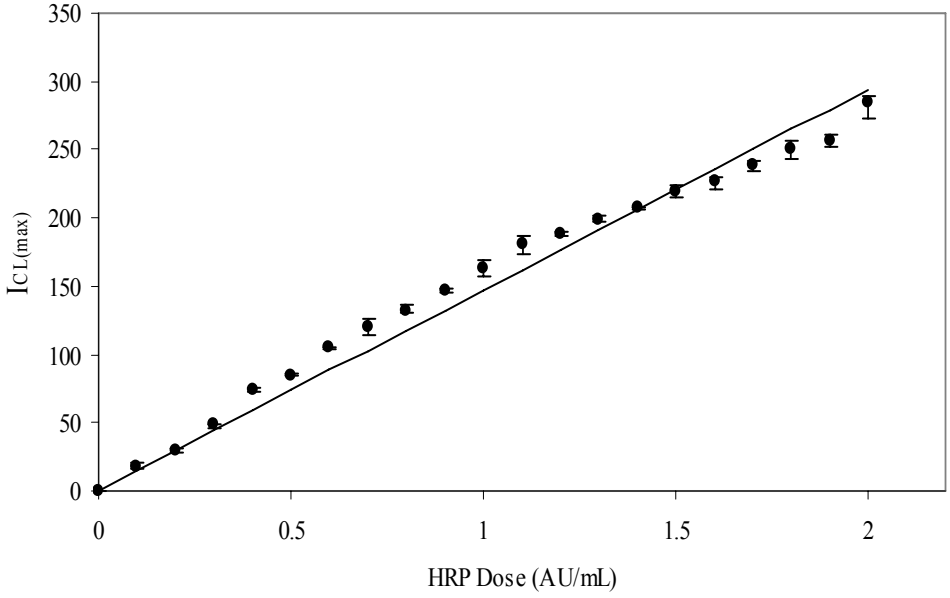
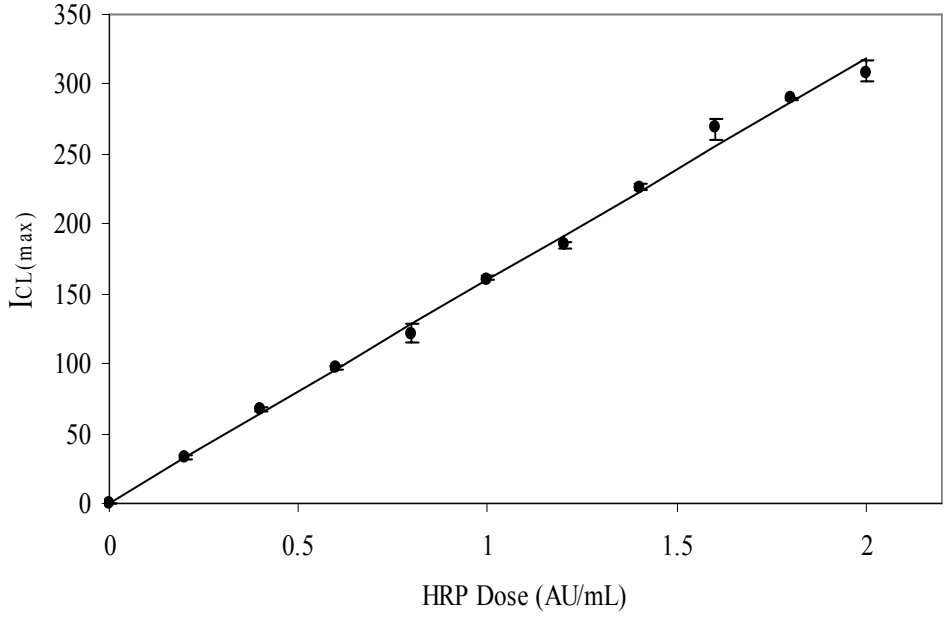


Figure 3.16. Impact of incubation time on the calibration curve for I_{CLmax} versus HRP dose (0 to 2.0 AU/mL). [H_2O_2] = 5 mM, [luminol] = 0.5 mM, and [*p*-iodophenol] = 0.1 mM, [ionic strength] = 100 mM and pH 8.5. R^2 value for linear fit = 0.996.

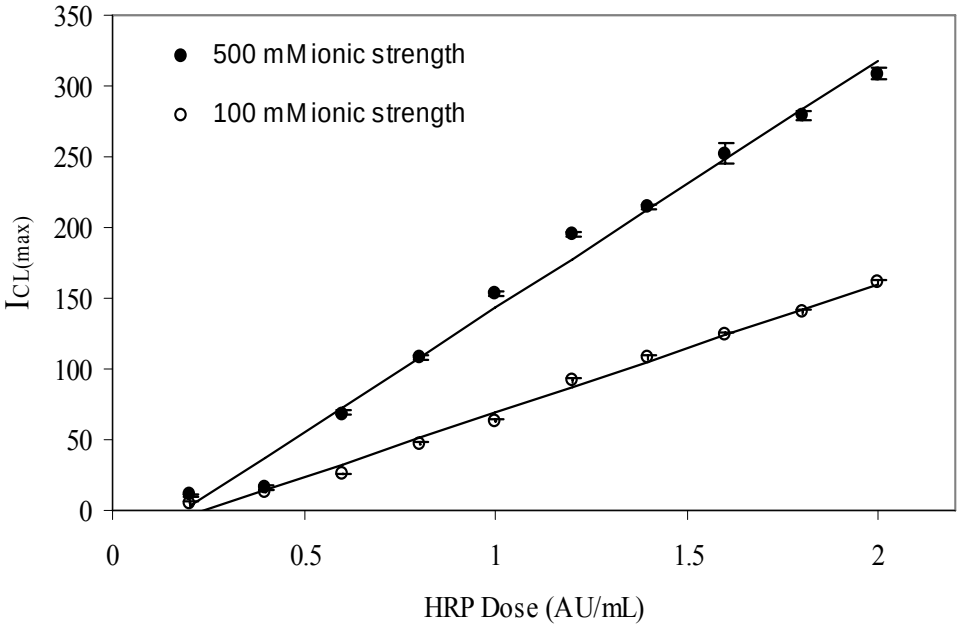


The ionic strength of the assay solution, however, had a significant impact on the calibration curve (Figure 3.17). The maximum intensity was found to increase substantially at the 500 mM versus 100 mM ionic strength while remaining linear. The use of higher assay solution ionic strengths, therefore, has the potential to significantly improve the HRP detection accuracy using the luminol-based chemiluminescence assay. The data illustrated in Figure 3.17 can be described by the following linear equation:

I_{CLmax} = 158.07 (HRP dose) -22.1 (500 mM of ionic strength) Eq. (3.14)

I_{CLmax} = 85.97 (HRP dose)-14.8 (100 mM of ionic strength) Eq. (3.15)

Figure 3.17. Calibration curve for I_{CLmax} versus HRP dose (0 to 2.0 AU/mL) at reagent ionic strengths of 100 mM and 500 mM. [H_2O_2] = 5 mM, [luminol] = 0.5 mM, and [*p*-iodophenol] = 0.1 mM and pH 8.5.



3.4.2.6 H_2O_2 Calibration Curve

A calibration curve was also developed to quantify H_2O_2 concentrations in unknown samples using the luminol-HRP-enhancer chemiluminescence assay. Concentrations of all components of the assay were maintained constant, including HRP (2 AU/mL), while varying the concentration of H_2O_2. The reaction was triggered by the addition of H_2O_2 five seconds after initiating recording. The calibration curve for I_{CLmax} versus H_2O_2 concentration is illustrated in Figure 3.18. Results show the reliability of this assay to detect H_2O_2 in aqueous solutions. The calibration curve revealed a linear relationship for H_2O_2 concentration raging from 0 to 1 mM with a relationship described by the equation:

$$I_{CLmax} = 106.8 \text{ } (H_2O_2 \text{ concentration in mM}) \qquad \text{Eq. (3.16)}$$

However, extending the range of H_2O_2 concentration beyond 1 mM produced a flattening of the curve (Figure 3.19) indicating a reaction limiting condition produced by some other reactant in the assay.

We also attempted to utilize phenol as an enhancer instead of *p*-iodophenol in the chemiluminescence assay solution. Some researchers (Candy and Jones, 1991; Ilyina et al., 2000; Huang et al, 2001) have reported that a wide range of chemicals, including phenol, could not be quantified using HRP because of the inhibitory effects on HRP activity from the derivatives of these compounds. However, Ilyina and coworkers (2003) reported quantification of phenol in water and organic solvent mixtures by utilizing the chemiluminescence assay. Our experiment was not successful in producing reliable quantification of phenol concentrations due to weak and oscillating light emission (Figure 3.20).

Figure 3.18. Calibration curve for I_{CLmax} versus H_2O_2 (0 to 1 mM). HRP = 1 AU/mL, [luminol] = 0.5 mM, [p-iodophenol] = 0.1 mM, [lonic strength] = 100 mM and pH 8.5. R^2 value for linear fit = 0.978.

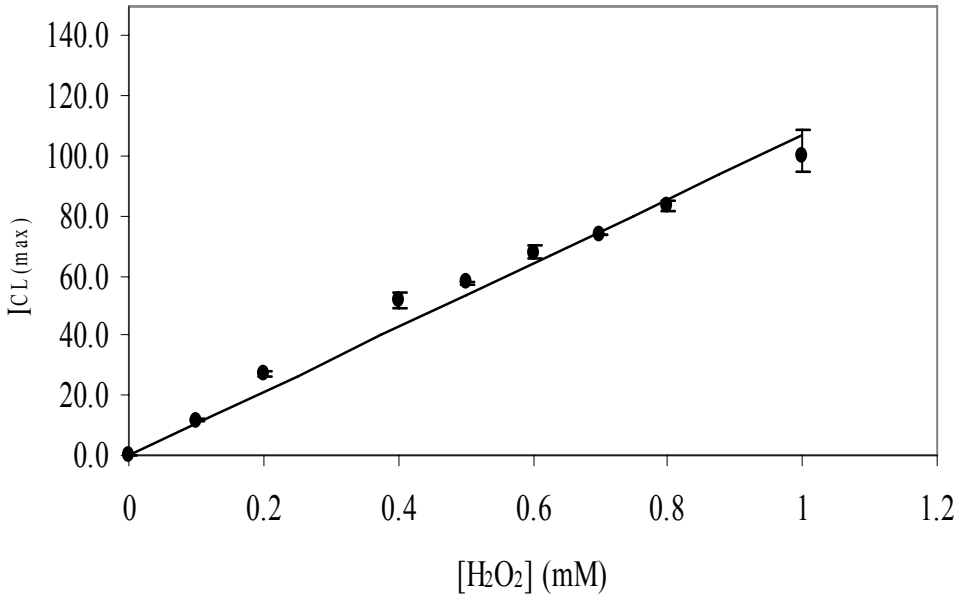


Figure 3.19. Calibration curve for I_{CLmax} versus H_2O_2 (0 to 2.5 mM). HRP = 1 AU/mL, [luminol] = 0.5 mM, [*p*-iodophenol] = 0.1 mM, [ionic strength] = 100 mM and pH 8.5.

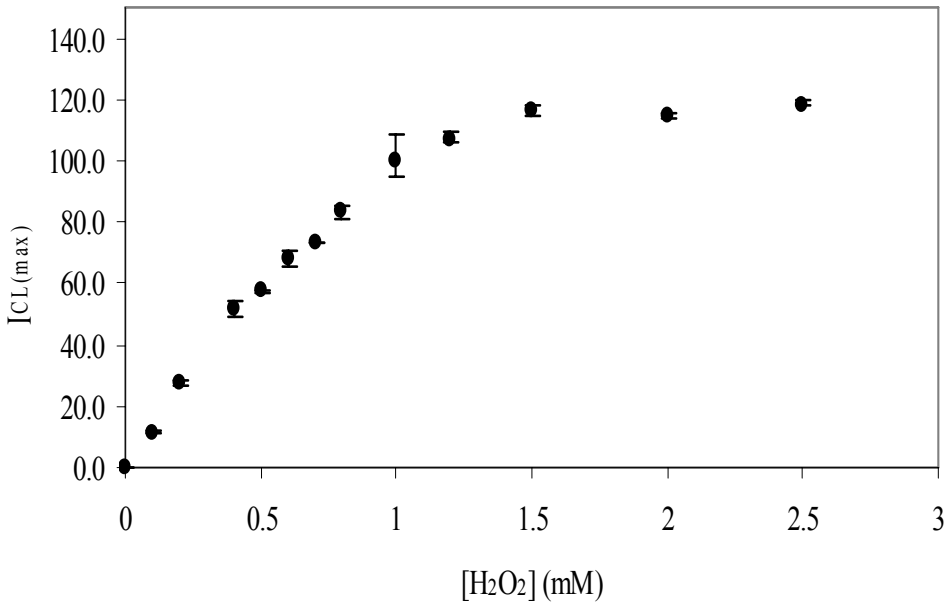
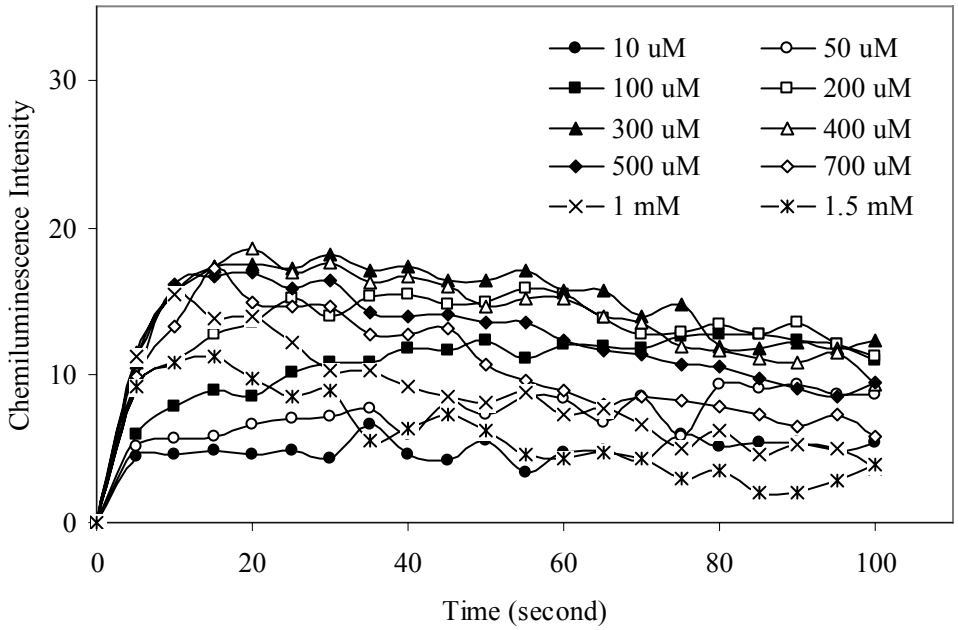


Figure 3.20. Chemiluminescence intensity profile (CIP) with phenol as enhancer. HRP = 1 AU/mL, [H_2O_2] = 5 mM, [luminol] = 0.5 mM, [ionic strength] = 100 mM and pH 8.5.



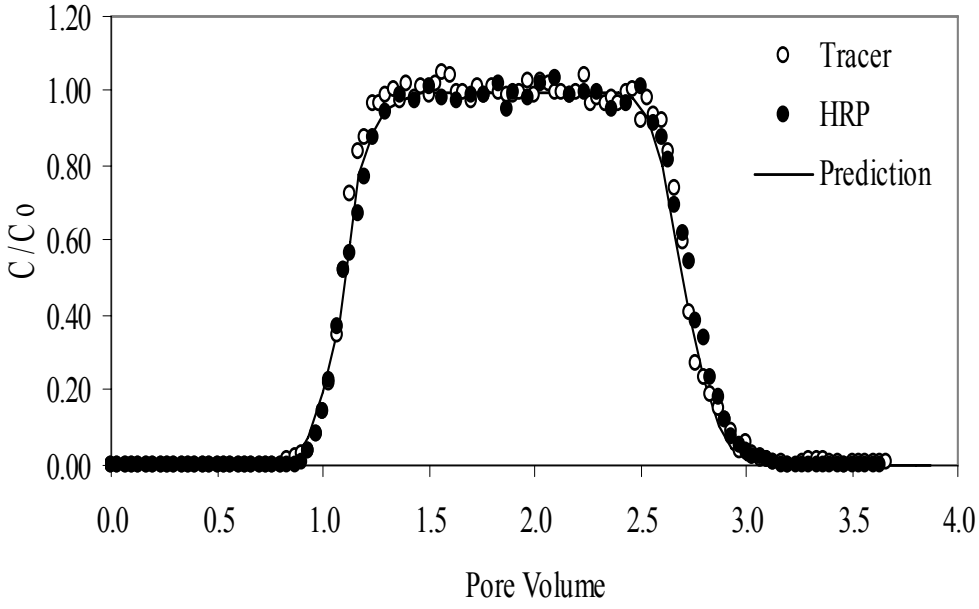
3.4.3 HRP Transport

Based on the results from the luminol-H_2O_2 chemiluminescence experiment, an assay was developed to monitor HRP enzyme concentration. It consisted of H_2O_2 (5 mM), enhancer (0.1 mM), luminol (0.5 mM) and buffer solution (50 mM) with pH 8.5. The assay was used to measure HRP in the column effluent. As described in experimental method (section 3.2.2), enzyme was injected into the glass column packed with Ottawa sand. The porosity of the column after packing with the porous media was about 0.38. Samples from the outlet were tested for HRP concentration with H_2O_2-luminol-(*p*-iodophenol) chemiluminescence assay method.

Figure 3.21 shows that no retardation was observed of enzyme transport in the column packed with Ottawa sand. The behavior of tracer and enzyme matched well with the prediction obtained using CXTFIT Software with retardation factor set to zero. In this case, only advection and dispersion terms were used in one-dimensional advection-dispersion equation for the prediction of enzyme transport. The results indicated that the HRP behaved the same as a

conservative tracer in the porous media. The dispersion coefficients of tracer and enzyme were estimated to be 0.007474 (R^2 = 0.996) and 0.01232 cm^2/min (R^2=0.997), respectively.

Figure 3.21. Breakthrough curves of enzyme and tracer. [H_2O_2] = 5 mM [Luminol] = 0.5 mM, [*p*-iodophenol] = 0.1 mM and in tris-HCl buffer ([Ionic strength] = 50 mM) at pH 8.5 with 15 AU/mL of Enzyme injection and washing in the column



3.5 SUMMARY AND CONCLUSIONS

This study investigated the impact of various assay conditions on chemiluminescence intensity in H_2O_2-lumiol-enhancer-HRP system. Results for these experiments showed that the activity of HRP in clear aqueous solution was quantified using the luminol-based chemiluminescence (Hypothesis 1) and HRP behaved as a conservative tracer in a continuous flow system (Hypothesis 2). All assay components affected the maximum chemiluminescence intensity. High or low concentration of H_2O_2 have attributed to less chemiluminescence intensity. Enhancer concentration had a specific range for the maximum chemiluminescence intensity. The chemiluminescence intensity was increased as increasing the ratio of H_2O_2/luminol. The

maximum intensity was decreased when the pH increased or decreased from pH 8.5. Ionic strength has significantly attributed to increase the maximum chemiluminescence intensity.

The calibration curves for the HRP dose versus the maximum intensity dose had a linear relationship. The linear relationship was maintained at ionic strength assay solution. The activity of HRP in clear aqueous solutions for the HRP transport was also estimated using a luminol-based chemiluminescence assay. There is no retardation on enzyme transport in the column packed with Ottawa sand. HRP behaves like a conservative tracer in a continuous flow system containing porous media comprised of Ottawa sand. Enzyme transport can be predicted using one-dimensional advection dispersion equation.

Real-World Implications

HRP in relatively clear groundwater can be monitored with a portable spectrometer using the CL assay developed in this study. The effects of pH and ionic strength should be considered to use the CL assay in groundwater. HRP transport is not retarded in porous media made of nonreactive mineral grains. The enzyme moves with the groundwater and with or ahead of soluble phenolic contaminants.

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CHAPTER 4. PHENOL REMOVAL USING ENZYME-MEDIATED COUPLING REACTION IN SATURATED POROUS MEDIA AND MODIFICATION OF SATURATED POUROUS MEDIA USING POLYMERIZATION PRODUCTS

4.1 INTRODUCTION

Phenol and its derivatives are the basic structural units for a wide variety of synthetic organics including many pesticides (Keith and Telliard, 1979). These chemicals have been introduced into the soil and water environment, through the application of pesticides and via manufacturing processes and waste disposal. Several phenolic compounds are toxic and can accumulate in the food chain (Kuivasniem et al., 1985). Because of their toxicity, phenolic compounds are restricted in many countries and require removal from wastewater before release into the environment (Karam and Nicell, 1997). In the United States, phenol is listed as a priority pollutant by the U.S. EPA. (Clean Water Act, 1985)

Phenols can be oxidized by peroxidase and other enzymes to produce oligomeric products (Klibanov et al., 1980). Horseradish peroxidase (HRP) has been proposed as an effective enzyme for the oxidative polymerization of phenols due to its stability, broad substrate specificity and its ability to operate at wide ranges of temperature and pH (Klibanov et al., 1980; Karam and Nicell, 1997; Ghioureliotis and Nicell, 1997).

HRP-mediated oxidative polymerization of phenol results in the formation of a variety of products. These reaction products consist of large molecular weight insoluble oligomers and low molecular weight soluble products (Klibanov and Morris, 1981; Schwartz and Hutchinson, 1981; Davidenko et al., 2004). Phenol polymerization products generally have high molecular weight and tend to precipitate out of solution. These properties of the oligomers allow peroxidases to

decontaminate water containing phenolic compounds to levels that are otherwise difficult to attain via conventional microbial degradation processes.

4.2 BACKGROUND

4.2.1 Phenol

Phenolic compounds are among major pollutants of environmental concern and are heavily regulated in the U.S. and many other countries due to their multiple toxic health effects (Clean Water Act, 1985). Phenols are also listed as hazardous constituents under the Resource Conservation and Recovery Act (ATSDR, 1989). Phenols and their derivatives are found in the wastewaters originating from a variety of industries including petroleum refining, pharmaceutical, petroleum and coal refining, resins and plastics, wood preservation, metal coating, dyes, leather, rubber textiles, and pulp and paper manufacturing, and phenol-containing products (Ates and Kilic, 2005; Chuphal et al., 2005; Aktas et al., 2000; Caza et al., 1999; Parkhurst et al., 1981). These chemicals can also be released into the environment via accidental spills, uncontrolled discharges or accumulation of intermediates during degradation of pesticide mixtures and gasoline constituents (Prpich and Daugulis, 2006; Gisi et al., 1997; Heitkamp and Cerniglia, 1988). The environmental release and transport of xenobiotic phenols needs effective control because of their tendency to persist and bioaccumulate in the ecosystem (Karam and Nicell, 1997).

4.2.1.1 Chemical and Physical Properties of Phenol

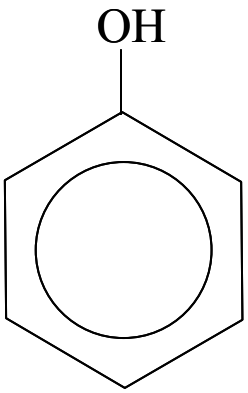
Phenol, a monosubstituted aromatic hydrocarbon (Figure 4.1), is a colorless solid in its pure state. It evaporates more slowly than water and is moderately soluble in water at room temperature (HSG 88, 1994). Phenol vapors are heavier than air and form explosive mixtures when exposed to heat. Phenol in air or soil is usually removed within a relatively short duration (less than 1 day and 5 days, respectively). However, when phenol is released in large quantities or over long periods, it is more persistent even in air or soil (GFM, 1995).

Phenol can be produced either synthetically or naturally. Animal wastes and decomposition of organic wastes are two natural sources of phenol (EPA, 1980). Forest fires can also result in the increase of phenol concentration in the ecosystem (Castelo et al., 2005; Hubble

et al., 1981). However, most environmental concerns arise from manufactured or xenobiotic phenols.

Phenol is obtained from the distillation of coal tar (Thurman, 1982). More than 90% of phenol produced in industry is used in the production of phenolic resins (phenol formaldehyde) for plywood adhesion, construction and the automobile industry. Other uses of phenol include production of an assortment of products including salicylic acid, dyes, metal cleaners, photographic chemicals, wood preservatives, and paint. Due to its toxicity to the bacteria and fungi, phenol is also used as an antiseptic and disinfectant (Budavari et al., 1989).

Figure 4.1. Molecular Structure of Phenol.



Phenol exists in a partially dissociated state at environmental pH values in water and moist soils. The transport and reactivity of phenol can be affected by pH since the pKa of phenol is 10 (Howard, 1989). Physical and chemical properties of phenol are listed in Table 4.1.

Phenol can be easily oxidized to generate phenolic radicals. These radicals are produced by removing the hydrogen atom from phenol's hydroxyl group using oxidizing agents. Phenolic radicals can react to form various products, such as dihydroxybenzenes, trihydroxybenzenes and quinones depending on the reaction conditions (Hwang et al., 1986). Light can accelerate the oxidation of phenol in air and water with photochemically produced hydroxyl and peroxy radicals (Hwang et al., 1986). Knoevenagel and Himmelreich (1976) reported a first-order dacay rate of 0.11/day for oxidation of phenol to CO_2 in the presence of oxygen and sunlight in water at 50 °C.

Table 4.1 Physical and Chemical Properties of Phenol.

Characteristic	Information	Reference
Chemical name	Phenol	Lide (1993)
Empirical formula	C_6H_6O	Lide (1993)
Density	1.07 g/cm^3 at 20°C	HSG 88 (1994)
Molecular weight	94.11 g	WHO (1994)
Boiling point:	181.75 C	WHO (1994)
Melting point:	40.8 C	WHO (1994)
Vapor pressure	0.2 hPa at 20°C	HSG 88 (1994)
Solubility	In water: 82 g/L, readily soluble in alcohol, ether, chloroform, fats and ethereal oils	HSG 88 (1994)
log K_{ow}	1.46	HSG 88 (1994)
pK_a	9.95	HSG 88 (1994)
Synonyms	Carbolic Acid; hydroxybenzene	WHO (1994)

Niessen et al. (1998) reported that the reaction of phenol with nitrate in dilute aqueous solutions can form dihydroxybenzenes, nitrophenols, nitrosophenol and nitroquinone. These researchers postulated that the phenol reaction with nitrate ions occurred by a radical mechanism involving hydroxyl and phenoxyl radicals. Other researchers have reported the reaction of phenol with various chemicals to form chlorophenols in chlorinated drinking water (Jarvis et al., 1985), *p*-benzoquinone in the presence of chlorine dioxide (Wajon et al., 1982), and cyanide in the presence of nitrous acid (Adachi et al., 1987).

4.2.1.2 Toxicity of Phenol

Phenol is a common pollutant from industrial wastewater and waste streams generated from agricultural activities. Considering the widespread use of phenols, it is not surprising that these compounds are ubiquitous in the environment and are listed as priority pollutants.

Phenol is a toxic and hazardous substance even at low concentrations (Clean Water Act, 1985; Li and Humphrey, 1989). The toxicity of phenol generally increases with the number of chlorine or nitrogen atoms substituted on the benzene ring. The most toxic compounds in the

chlorophenol and nitrophenol groups include pentachlorophenol and trinitrophenol (Jarvinen and Ankley, 1999).

Phenol is toxic to microorganisms as well as higher freshwater organisms. A toxicity threshold of 64 mg phenol/liter was found for bacteria (HSG 88, 1994). Similar threshold values for protozoa and fungi have also been reported (HSG 88, 1994). The membranes of Escherichia coli cells grown in the presence of phenol were significantly impaired by the presence of phenol (Keweloh et al., 1989; 1990). The toxicity of phenol to various organisms is illustrated by the data summarized in Table 4.2.

Table 4.2. Toxicity of Phenol.

Receptor	Toxic Dose
Human	1 g can be fatal
Rat	LD_{50} 414-530 mg/kg, oral
Rabbit	LD_{50} 400-600 mg/kg, oral
Cat	LD_{50} 100 mg/kg, oral
Dog	LD_{50} 500 mg/kg, oral
Pimephales promelas	LC_{50} 24-68 mg/l
Leuciscus idus melanotus	LC_{50} 25 mg/l (48h)
Daphnia	LC_{50} 12 mg/l (48h)
Scenedesmus quadricauda	EC_0 7.5-40 mg/l

(NIOSH 1991; WHO, 1994; HSG 88, 1994; GFM, 1995)

The mean lethal concentration (LC) for inhaling phenol vapor is reported to be about 316 mg/m^3 for rats and 177 mg/m^3 for mice (NIOSH, 1991). The oral lethal dose for 50% fatality (LD_{50}) was 317 mg/kg and 270 mg/kg for rats and mice, respectively (WHO, 1994).

Phenol is not expected to bioaccumulate significantly since the bioconcentration factors of phenol in various types of water organisms are low. However, phenol metabolites can be extremely toxic. For example, the incomplete combustion of 2,4,5-trichlorophenol can result in the formation of the significantly more toxic TCDD (dioxin) (GFM, 1995).

4.2.1.3 Health Effects of Phenol

Phenol is detectable by humans at 40 ppb in air and 1-8 ppm in water (ATSDR, 1998). The odor threshold has been reported to range from 0.021 to 20 mg/m^3 in air and 7.9 mg/liter in water. A taste threshold value of 0.3 mg/L of water has been suggested (HSG 88, 1994). Possible pathways for exposure to phenol are drinking contaminated water, eating contaminated food, and contacting products that have phenol in them. Phenol can cause headaches, dizziness, high blood pressure, heart problems, shallow breathing, wheezing, coughing, vomiting, and stomach ulceration when it is absorbed into the body (WHO, 1994).

Serious health effects such as diarrhea, nausea, mouth sores, and dark urine have been reported for people who consumed water contaminated with phenol (Jarvis et al., 1985; Baker et al., 1978). Short-term exposure to phenol in the air can also cause respiratory irritation, weight loss, headaches, dark urine and burning eyes, paralysis and severe injury to the heart, kidneys, liver, and lungs, followed by death in some cases (Hathaway et al., 1991; Clayton and Clayton, 1982; Parmeggiani, 1983). Humans who had skin exposure to high amounts of phenol experienced skin burns, liver damage, dark urine, and irregular heart beat.

Phenol does not appear to cause cancer in mice or rats that drank water containing phenol for up to two years, although the effects of exposure to phenol in human reproduction and fetus development are unknown (IARC, 1999; EPA, 2002). Phenol can be used for medical purposes and is an effective antiseptic for skin and mouth wash when used in small amounts.

4.2.1.4 Degradation, Decomposition and Treatment of Phenol

There are many approaches to treat phenol containing wastewaters. These methods include adsorption on activated carbon, steam distillation, extraction, chemical oxidation and irradiation. Recently, advanced oxidation processes (AOPs) have also been studied to treat phenol containing water and wastewater. These AOPs include ozonation (O_3), ultraviolet (UV), ultrasound (US), Fenton's reagent (Kang et al., 2002), and enzymatic oxidation (Tabrizi and Mehrvar, 2005).

The oxidation chemistry of Fenton's reagent is somewhat similar to the enzymatic treatment of phenol. In oxidation using Fenton's reagent, ferrous iron (Fe^{2+}) is oxidized to ferric iron (Fe^{3+}) in the presence of H_2O_2. Hydroxyl radicals produced during the reaction are very reactive and oxidize the phenol molecules in solution. Hydroxyl radicals cleave the benzene ring or create free radicals (Watts et al., 2005). Although a variety of AOPs are applicable for phenol

degradation, several are limited by high cost, incomplete contaminant destruction, formation of undesirable waste products, and low efficiency (Davidenko et al., 2004).

Phenol in water and soil can be degraded by abiotic reactions as well as microbial activity (Rubin and Alexander, 1983; Scott et al., 1982). Biodegradation has been identified as the most important factor responsible for phenol attenuation in contaminated soils and groundwater. Natural phenols are generally biodegradable resulting in no accumulation in plants or animals. A small percentage of the total microbial population in soil has the ability to utilize phenol (Hickman and Novak, 1989). However, repeated phenol exposure can result in acclimation of the microbial community in the soil and aquifer environments (Jung and Sofer, 1995; Tibbles and Baecker, 1989; Wiggins and Alexander, 1988).

Phenol can be broken down to CO_2 under aerobic conditions (Karlsson et al., 2000; Hoyle et al, 1995) or methane under strict anaerobic conditions (Satsangee and Ghosh, 1990), although anaerobic transformation is significantly slower than aerobic degradation (Baker and Mayfield, 1980). The biodegradation of phenol produces many intermediates such as benzoate, catechol, cis-cis-muconate, β-ketoadipate, succinate and acetate (Knoll and Winter, 1987). Factors affecting degradation of phenol include temperature (Onysko et al., 2000; Hwang et al., 1986), concentration of phenol (Loh and Tan 2000; Rozich and Colvin, 1985; Hwang et al., 1989) and the presence of other compounds (Mamma et al., 2004; Gonzalez et al., 2001; Cho et al., 2000, 1998).

4.2.1.5 Phenol Contamination

Phenol can be released inadvertently into the environment during its use in manufacturing, through accidental spills, inappropriate disposal, and leaching from hazardous waste sites and landfills (Xing et al., 1994). About 85,700 pounds and 1.2 million pounds of phenol were estimated to have been released into surface water and soil, respectively, from 689 domestic manufacturing and processing facilities in 2004 (TRI04, 2006).

Phenol has been found in leachate from landfills (Clark and Piskin, 1977), and hazardous waste sites (Plumb, 1987) and in the groundwater near these sites (Delfino and Dube, 1976). High levels of 1000 ppb phenol were reported in two aquifers (Howard, 1989) and up to 1130 ppm in nine wells in Wisconsin (Delfino and Dube, 1976). The U.S. National Priorities List (NPL) reported the presence of phenol at more than 595 out of the 1,678 contaminated sites

(ATSDR, 2006). Groundwater at these hazardous waste sites was contaminated with 2.48 to 85,000 ppb of phenol (ATSDR, 1989).

Although much higher levels of phenol have been reported, the contaminant is generally detected in the environment below 100 parts per billion (ppb) (Krogmann and Woyczechowski, 2000; Richard and Chadsey, 1990). If phenol concentration is less than one ppb in surface and groundwater, it is usually considered relatively unpolluted. Since phenol does not sorb to soil or aquifer materials, it is easily leaches into ground water and is highly mobile in the aquifer (Xing et al. 1994, Howard, 1989; Ehrlich et al., 1982).

The Environmental Protection Agency (EPA) regulates phenol in waters at 0.3 mg/L to protect human health from the possible exposure to phenol via drinking water and eating plants and animals exposed to the contaminated water (ATSDR, 2006).

4.2.2 Enzymes that Polymerize Phenols

Enzymes such as peroxidases are produced by plants and microorganisms. These enzymes can catalyze the polymerization of aromatic compounds such as phenols via oxidative coupling reactions, which occur in the presence of peroxides such as hydrogen peroxide (H_2O_2). Theses enzymes can be classified into two subclasses: polyphenol oxidases and peroxidase (Karam and Nicell, 1997).

Peroxidases such as horseradish peroxidase (HRP) and lignin peroxidase (LiP) have been used to evaluate the treatment of phenolic compounds in aqueous solutions in the laboratory. HRP is among the most widely used enzymes for the treatment of contaminated water and soils. When activated by H_2O_2, HRP can catalyze the oxidation of a wide variety of aromatic compounds such as phenol, biphenols, anilines, and benzidines (Klibanov et al., 1980; Karam and Nicell, 1997). The most important HRP isoenzymes reported in experimental works are isoenzyme A (acidic), isoenzyme C (neutral or lightly basic) and the strong basic HRP (Xu, 2002).

Lignin peroxidase, an extracellular enzyme of the white-rot fungus *Phanerochaete chrysosporium* (Aitken et al., 1994; Aitken et al., 1989; Venkatadri and Irvine, 1993) has a reaction mechanism similar to HRP (Aiken et al., 1989). A number of polycyclic and phenolic compounds can be oxidized by LiP (Aitken et al., 1994; Aiken et al., 1989). Aitken and

coworkers (1989) have reported the stability of LiP for the application to wastewater treatment. They observed that activity of LiP was increased with increasing pH.

Other peroxidases such as chloroperoxidase from the fungus *Caldariomyces fumago* (Aitken et al., 1994), manganese peroxidase from *Phanerochaete chrysosporium* (Aiken, 1989), and microbial peroxidase from *Coprinus macrorhizus* (Al-Kassim et al, 1994) have been reported to oxidize several phenolic compounds and aromatic dyes.

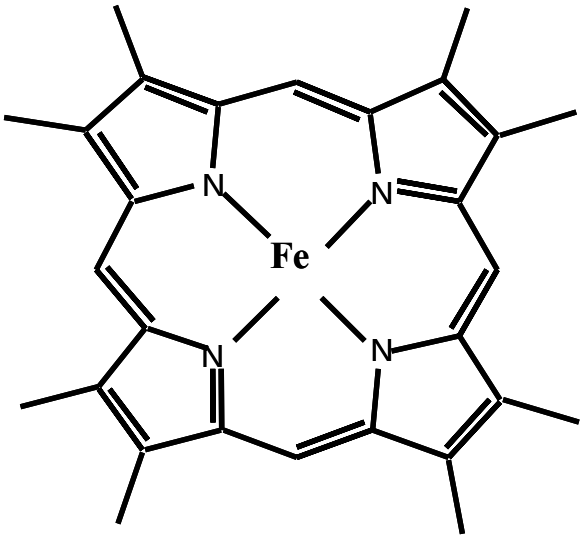
Polyphenol oxidases also catalyze the oxidation of phenolic compounds. Polyphenol oxidases require molecular oxygen instead of peroxide to activate them. Polyphenol oxidases can be further classified into laccases and tryosinases (Karam and Nicell, 1997).

Laccases from several fungi can oxidize phenolic compounds and reduce their toxicity by polymer formation. Bollag et al. (2003) investigated the transformation of a mixture of chlorinated phenols using a fungal laccase of *Tramates villosa*. Tyrosinases oxidize phenolic compounds into respective quinones through the hydroxylation of monophenols to *o*-diphenols and dehydrogenation of the *o*-diphenols to *o*-quinones. The quinones undergo non-enzymatic polymerization to produce polymerization products that have low solubility and high molecular weight (Arica, 2000).

4.2.2.1 Horseradish Peroxidase

Klibanov et al. (1980) were among the first to propose a HRP-mediated method to remove aromatics from aqueous solutions. HRP has since been widely studied as an agent suitable for enzymatic treatment of water and soils contaminated with phenolic compounds (Klibanov et al., 1980; ; Dunford, 1991; Bhandari and Xu, 2001; Bollag et al., 2003). When activated by H_2O_2, HRP can catalyze the oxidation of a wide variety of aromatic compounds such as phenol, biphenols, anilines, and benzidines (Klibanov et al., 1980; Karam and Nicell, 1997;). Soluble and insoluble polymers are produced as reaction by-products through a subsequent radical-mediated non-enzymatic process (Ghioureliotis and Nicell, 2000; Wagner and Nicell, 2003; Palomo and Bhandari, 2006).

Figure 4.2. Molecular structure of active site in HRP.



HRP is extracted from horseradish (*Amoracia rusticana*) roots and classified among the ferroprotoporphyrin group of peroxidases. HRP consists of 208 amino acid residues, a prosthetic group, two calcium ions per molecule and a ferric heme (iron protophophyrine type IX) (Shannon et al., 1988). The ferric heme constitutes the active site responsible for oxidation of substrate molecules (Figure 4.2). H_2O_2 binds to this iron center to yield the ferry1 iron (Fe^{4+}) porphyrin cation radical intermediate through the cleavage of the oxygen-oxygen bond of H_2O_2 to 'activate' the enzyme (Rodiguez-Lopez et al., 2001). The pH optimum of HRP is in the range of 6.0 to 6.5. It is suitable for wastewater treatment because its activity remains stable in the pH range of 5.0 to 9.0 and up to a temperature of 40°C (Nicell et al., 1993b; Schomberg et al., 1993; Dunford, 1991). HRP is reversibly inhibited by cyanide and sulfide at a concentration of 10^{-5} M (Zollner, 1993; Theorell, 1951).

4.2.2.2 HRP-Mediated Oxidation of Phenol

HRP has been shown to catalyze the oxidation of a wide variety of aromatic compounds including phenols, anilines and PAHs from aqueous solution over a wide range of solute concentrations (Klibanov and Morris, 1981; Everse et al., 1991; Dunford, 1991). HRP mediates the oxidation of phenolic substrates via a three-step catalytic cycle. The enzymatic mechanisms for phenol oxidation by HRP are relatively well understood and have been mathematically

modeled. The three-step catalytic cycle of phenol oxidation catalyzed by HRP can be described by the following chemical equations (Dunford, 1991):

$$E + H_2O_2 \rightarrow E\text{-}I + H_2O \quad \text{(Eq. 4.1)}$$

$$E\text{-}I + AH_2 \rightarrow E\text{-}II + AH\bullet \quad \text{(Eq. 4.2)}$$

$$E\text{-}II + AH_2 \rightarrow E + AH\bullet \quad \text{(Eq. 4.3)}$$

or:

$$2AH_2 + H_2O_2 \rightarrow 2\,AH\bullet + 2H_2O \quad \text{(Eq. 4.4)}$$

where E is the peroxidase enzyme, E-I and E-II are active enzyme intermediates, AH_2 is a reducing substrate such as phenol or aromatic amine, and $AH\bullet$ is a free radical product.

The ferric iron (Fe^{3+}) of the heme reactive center is oxidized by H_2O_2 to yield a phenoxy radical ($AH\bullet$) and an active enzyme intermediate (E-I) in the first step (Eq. 4,1). The enzyme intermediate (E-I) abstracts an electron from the phenolic substrate (AH_2) in the second step and transforms into another active enzyme intermediate (E-II), which is reduced by one electron. E-II reacts with a second phenol molecule to produce another phenolic radical ($AH\bullet$) (Eq. 4,2). These free radicals produced from the first and second stages diffuse from the active center of the enzyme into solution and undergo self-coupling or cross-coupling to form dimers, trimers and, eventually, larger oligomers with low water solubility (Dunford, 1991; Rodriguez-Lopez et al, 2001, Davidenko et al., 2004). Finally, the enzyme converts back to the original state in the third step (Eq. 4,3). Yamazaki et al. (1960) reported that one molecule of peroxidase can remove approximately 10^3 molecules of phenol. Two free radicals are generated for every molecule of peroxide consumed based on the catalytic cycle.

4.2.2.3 Enzyme Kinetics

The kinetics of enzymatic reactions can be expressed by the Michaelis-Menten model:

$$E + S \leftrightarrow ES \quad \text{(Eq. 4.5)}$$

$$ES \leftrightarrow E + P \quad \text{(Eq. 4.6)}$$

where *E* is the enzyme, *S* is a substrate, *ES* is the enzyme-substrate complex, and *P* is the reaction product. The enzyme reacts with substrate to form a reversible enzyme-substrate complex in the first step. The complex produced then breaks down to produce free enzyme and the product *P* in a second step (Lehninger et al., 1993).

The Michaelis-Menten equation is the simplest and idealized algebraic form of a rectangular hyperbola that displays saturation. For single substrate enzymatic reactions, the Michaelis-Menten equation for the reaction rate is written as:

$$Vo = \frac{(V_{\max}[S])}{K_M + [S]} \qquad \text{(Eq. 4.7)}$$

where *Vo* is the initial velocity (d[*P*]/dt or –d[*S*]/dt), V_{max} is the maximum reaction rate, K_M is the Michaelis-Menten constant, and *S* is the initial substrate concentration. K_M is the substrate concentration at which initial velocity is half of its maximum value.

Wu et al. (1993) reported that HRP-mediated oxidation of phenol was complete within 14 hours. However, other investigators have reported that over 90% of substrate was removed within few minutes and the reaction was completed within 3 hours (Nicell et al., 1993a; Aitken et al., 1994; Bollag et al., 2003; Cooper and Nicell, 1996; Kinsley, 1998; Caza et al., 1999; Duarte-Vazquez et al., 2002; Huang et al., 2005)

4.2.2.4 Kinetic Models

Several researchers have proposed models for HRP-mediated removal of phenolic contaminants (Buchanan and Nicell, 1999; Buchanan et al., 1998a, 1998b; Buchanan and Nicell, 1997; Nicell and Wright, 1997; and Nicell et al., 1993c). Nicell (1994) developed a kinetic model to predict the HRP-catalyzed removal of 4-chlorophenol in batch reactors. This model was capable of predicting the residual concentration of 4-chlorophenol but was unable to predict the concentration of other substrates such as phenol due to two major problems: over-prediction of E-III (temporarily inactivated enzyme) formation and the assumption of a fixed number of substrate molecules removed per enzyme molecule inactivated.

Yu et al. (1994) investigated phenol conversion and the production of dimeric intermediates using HRP-mediated oxidation. They proposed an inactivation model for peroxidase in the presence of polyethylene glycol (PEG). Phenol conversion followed a first-

order reaction with respect to phenol concentration at an equimolar ratio of $[H_2O_2]$/[phenol]. The kinetic behavior of HRP at pH 8 and room temperature was also studied by Vasudevan and Li (1996) who found that the enzyme-catalyzed reaction of phenol with H_2O_2 exhibited normal Michaelis-Menten saturation kinetics. These researchers reported second-order rate constants of 4.14×10^5 $M^{-1}s^{-1}$ and 5.54×10^4 $M^{-1}s^{-1}$ for the reaction of HRP with H_2O_2 and E-II with phenol, respectively. In another paper, Modi (1995) reported that the nature of heme had no effect on the reaction of the intermediate E-II with aromatic substrates.

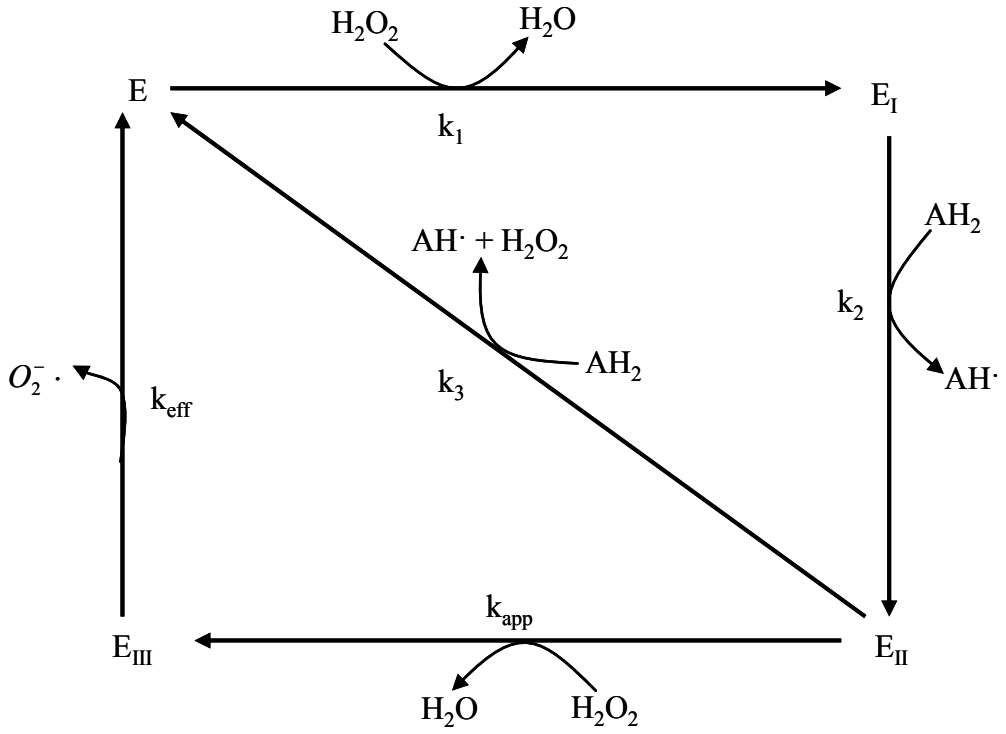
Nicell and Wright (1997) tested a steady-state kinetic model describing the dependence of soybean peroxidase (SBP) and HRP activity on H_2O_2 concentration. This model successfully described the inhibitory effect of H_2O_2 on catalytic activity over a wide range of H_2O_2 concentration. These researchers reported that HRP exhibited faster reaction kinetics than SBP and formed less E-III during the oxidation of phenol.

Buchanan and Nicell (1997) modified a pseudo steady-state kinetic model to incorporate the mechanisms of enzyme inactivation. Their model consisted of three differential equations and a mass balance equation. A fourth-order Runge-Kutta numerical method was used to simultaneously solve the set of nonlinear ordinary differential equations. The model was calibrated and validated for 0.5 to 6 mM of initial phenol concentration from 12 batch reactor runs and effectively described enzyme inactivation from interaction with free radical and formation of reaction products. In a separate study, Buchanan and Nicell (1998b) extended the application of their model to account for the presence of an additive such as PEG. The rate constant for inactivation by polymer interactions was reduced 20-fold in the presence of PEG.

Buchanan et al. (1988a) developed models for plug-flow reactors (PFR) and continuous-flow stirred tank reactors (CFSTR) utilized for the removal of aqueous phenol. They tested the validity of the models at pH 7 and 25 °C, both with and without PEG. Results showed that the rate of enzyme inactivation was lower in CFSTR than PFR for the same removal of phenol. However, they concluded that optimum reactor configuration depended on the initial substrate concentration, target concentration of effluent, and volume of the reactor.

The most recent version of the HRP catalytic cycle modeled by Buchanan and Nicell (1998b) is described in Figure 4.3 and by Equations 4.8 to 4.16.

Figure 4.3. HRP catalytic cycle (Buchanan et al., 1998).



The kinetics of the HRP-mediated oxidative process for the removal of phenol (AH_2) are derived from the system of reaction pathways illustrated in Figure 4.3. Native enzyme (E), intermediate compound I (E-I), and intermediate compound II (E-II) are the active forms of HRP in the catalytic cycle. A temporarily inactivated form of HRP (compound III or E-III) can be produced from the reaction between E-II and hydrogen peroxide while permanent inactivation results from interaction between the HRP and the free radical and polymerization products

$$\frac{dAH_2}{dt} = -(E_o - E_{III} - E_{inact})\left[\frac{1}{k_1[H_2O_2]} + \frac{1}{[AH_2]} + \left(\frac{k_2 + k_3}{k_2 k_3}\right)\right]^{-1} \qquad \text{(Eq. 4.8)}$$

$$[H_2O_2] = [H_2O_2]_o - ([AH_2]_o - [AH_2]) \qquad \text{(Eq. 4.9)}$$

$$\frac{dE_{III}}{dt} = \frac{k_{app}}{k_3}[H_2O_2](E_o - E_{III} - E_{inact})\left[\frac{[AH_2]}{k_1[H_2O_2]} + \left(\frac{k_2 + k_3}{k_2 k_3}\right)\right]^{-1} - k_{eff} E_{III} \quad \text{(Eq. 4.10)}$$

$$E_I = \frac{k_1[H_2O_2]}{k_2[AH_2]} E \quad \text{(Eq. 4.11)}$$

$$E_{II} = \frac{k_1[H_2O_2]}{k_3[AH_2]} E \quad \text{(Eq. 4.12)}$$

$$E = \frac{(E_o - E_{III} - E_{inact})}{1 + \left(\frac{k_1[H_2O_2]}{[AH_2]}\right)\left(\frac{k_2 + k_3}{k_2 k_3}\right)} \quad \text{(Eq. 4.13)}$$

$$\frac{dE_{inact}}{dt} = k_r (E_o - E_{inact})\sqrt{\frac{dAH_2}{dt}} - k_e \frac{dAH_2}{dt} \quad \text{(Eq. 4.14)}$$

where E_o, E_I, E_{II}, E_{III}, and E_{inact} represent the concentrations of the initial active enzyme, compound-I, compound-II, compound-III and inactive enzyme, respectively; k_1, k_2, k_3, k_{eff}, and k_{app} are the rate constants associated with the equations; k_r is a lumped rate constant associated with enzyme inactivation by free radicals; and k_e is a dimensionless proportionality constant associated with enzyme inactivation by polymerization products.

$$Adx\frac{\partial C}{\partial t} = QC_x - Q\left(C_x + \frac{\partial C}{\partial x}dx\right) + Adxr_c \quad \text{(Eq. 4.15)}$$

$$\frac{\partial C}{\partial x} = \frac{1}{u} r_c \quad \text{(Eq. 4.16)}$$

where Q is the volumetric flow rate, r_c is the volumetric flow rate, u is the linear velocity of flow in the axial direction (Q/A), and x is the distance from the reactor inlet ($0 \leq x \leq L$). L is the length of the plug flow reactor (Buchanan et al., 1998a).

Wu et al. (1999) simulated HRP-mediated oxidation of phenol with PEG using a kinetic model that contained a second-order Michaelis-Menten equation with respect to the concentrations of phenol and H_2O_2, and two inactivation equations for the influence of reaction end-product and H_2O_2. They obtained evidence that enzyme inactivation by polymer was a second-order reaction. These researchers also utilized a completely mixed batch reactor and plug-flow reactor for peroxidase-catalyzed removal of phenol. They observed similar outputs from semi-batch and plug-flow operations but recommended a plug-flow reactor for the removal of phenol in the presence of PEG.

Huang and Selig. (2002) developed a kinetic model to evaluate non-extractable product formation during HRP-catalyzed oxidative coupling of phenols. The model showed excellent prediction of non-extractable products. Gilabert et al. (2004) reported the catalytic constant and the second-order association constant using kinetic analyses. These researchers also calculated the first-order rate constant for the transformation of each phenol.

4.2.2.5 Factors Affecting HRP-Mediated Polymerization

There are many factors that affect the removal of phenolic solutes in HRP-catalyzed reactions. These include enzyme dose, substrate concentration, temperature, solution pH and solution ionic strength.

pH. Solution pH is an important factor for enzyme-mediated reactions (Wu et al., 1997). Many researchers have reported the effects of pH on the catalytic efficiencies of enzymatic reaction systems (Aitken et al., 1989; Dec and Bollag, 1990; Nakamoto and Machida, 1992; Nicell et al., 1993a; Wu et al., 1993; Bewtra et al., 1995; Wright and Nicell, 1999; Duarte-Vázquez et al., 2003; Xu, 2002). Dec and Bollag (1990) reported that solution pH affected properties of both the enzyme and the substrate during HRP catalyzed oxidation for 4-chlorophenol and 2,4-dichlorophenol (2,4-DCP). Klibanov et al. (1983) reported an optimum pH of 9.0 for treating phenols from coal-conversion wastewater using HRP. Optimum pH for the removal of 2,4-DCP was observed to be 6.5 using HRP (Bewtra et al., 1995) and 8.2 using soybean peroxidase (Kennedy et al., 2002).

Turnip peroxidase was used to remove aqueous phenolic compounds such as phenol, 2-chlorophenol, 3-chlorophenol, *o*-cresol, *m*-cresol, 2,4-DCP and bisphenol-A (Duarte-Vazquez et al., 2003). More than 85% of phenol derivatives were removed between pH 4 and 8. The

optimum pH for the removal of all phenolic compounds occurred between 5 and 7. These researchers observed that the lower removal efficiency at pH above 10 was due to the formation of the conjugated base of phenol since pK_a of phenol is 10 (Budavari, 1989). Nicell et al. (1992) reported that optimum pH was 6-9 when they treated eight different phenolic compounds using HRP.

HRP catalyzed the oxidation and detoxification of 0.05 mM of pentachlorophenol in distilled-deionized water within an optimal pH range of 4 to 5 (Zhang and Nicell, 2002). The main products generated were dimers. Masuda et al. (2002) reported the effect of temperature and pH on phenol removal using purified *Coprinus cinereus* peroxidase. The optimum pH value for phenol removal was 9.0 at 0^oC.

Huang et al. (2005) investigated the effect of solution pH, and the type and background concentration of solution ion on the precipitation of polymerization products. They observed that precipitation of polymerization products increased and then leveled off as salt concentration and pH increased. These researchers postulated that a fraction of the phenolic sites on the polymerization products was more acidic than phenol due to stronger resonance effects. Protonation of theses sites reduced the ionic character of the products and increased the tendency to precipitate when the solution pH was decreased.

Hydrogen Peroxide. H_2O_2 is an essential co-factor in HRP-mediated oxidation of phenols. The activity of peroxidases is determined largely by the ratio of the molar concentrations of H_2O_2 to substrate and the reaction conditions. Klibanov et al. (1980) reported that removal efficiency of *o*-chlorophenol (1 mM) was not enhanced by increasing H_2O_2 concentration higher than 1 mM. Nicell et al. (1992) determined the stoichiometric consumption of peroxide during HRP-mediated oxidation of aromatic substrates in wastewater. A molar ratio of 1:1 ($[H_2O_2]$:[substrate]) was reported as the optimum condition. Bassi et al. (2004) reported an optimum molar ratio of 1.0 for H_2O_2 and phenolic substrates in oxidation reactions mediated by soybean seed hulls.

Miland et al. (1996) demonstrated phenol removal using modified peroxidases and reported an optimum molar ratio of H_2O_2 and phenolic substrate of ~ 1.0. Caza et al. (1999) investigated the removal efficiency of phenolic compounds from synthetic wastewater using soybean peroxidase (SBP) with optimum molar ratios of $[H_2O_2]$/[substrates] of 0.6 for 3-

chlorophenol and 1.2 for both phenol and bisphenol A. They also reported SBP inactivation at higher [H_2O_2]/[substrates]. Duarte-Vazquez et al. (2001) showed that H_2O_2 concentrations greater than 1.2 mM caused inhibition of the enzyme by irreversible oxidation of the enzyme ferriheme group. Nicell et al. (1995) and Flock et al. (1999) also found that excess of H_2O_2 inactivated the enzyme. Kennedy et al (2002) attributed this inactivation to the formation of species that produced the catalytically inactivated form of peroxidases in the presence of excessive H_2O_2.

Duarte-Vazquez et al. (2003) reported that the maximum phenolic removal achieved using turnip peroxidase was at a [H_2O_2]/[phenolic compound] molar ratio of 1.6.

We et al. (1994) examined the effect of the mode of H_2O_2 addition on phenol removal in in the presence of PEG. The ratio of H_2O_2 concentration to HRP activity was studied versus phenol oxidation for substrate concentrations ranging from 1 to 10 mM. Optimum ratio of [H_2O_2]/[HRP] was observed between 10 and 25 μmol/activity unit.

Davidenko et al. (2004) reported that HRP was able to catalyze the effective transformation of phenol, o-chlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol. These researchers observed that the maximum removal of phenolic compounds occurred over a pH range of 6.0 to 7.0, a molar ratio 1:1 (H_2O_2/phenol), and an incubation period of 1 to 3 hours.

Additives. Enzymatic treatment process occasionally suffers from inactivation during the reaction and requires addition of more enzymes (Klibanov et al., 1983; Nakamoto and Machida, 1992; Baynton et al., 1994; Caza et al., 1999; Ibrahim et al., 2001). Inactivation of enzyme during the polymerization reaction results in high operating costs. To achieve a high efficiency of phenol removal, therefore, large amounts of enzyme are required to counteract the effects of enzyme inactivation (Karam and Nicell, 1997; Cooper and Nicell, 1996).

Klibanov et al. (1983) speculated that the main mechanism of inactivation was caused by binding of polymerization products to the active site of the enzyme resulting in blocking the access of solute to the active site of the enzyme. Nakamoto and Machida (1992) postulated that this interaction changed the geometric configuration of enzyme. Wagner and Nicell (2001a) also reported HRP inactivation due to reaction products and excess H_2O_2 concentration. Many researchers agree that a combination of these mechanisms result in eliminating the catalytic ability of HRP during the reaction.

Several researchers have reported that HRP can be stabilized in solution by using highly hydrophilic additives such as borate, gelatin and PEG, since these additives appear to have a greater affinity for the hydroxyl groups on the polymeric products than HRP (Wu et al., 1994; Zhang and Nicell, 2000; Kinsley and Nicell, 2000).

Nakamoto and Machida (1992) achieved ~200 fold reductions in enzyme requirement for the treatment of aqueous phenol in the range of 10 to 30g/L by using additives. They found that gelatin may be the most suitable additive for actual applications since gelatin is less pH dependent than PEG.

Wu et al. (1993) evaluated HRP-mediated removal of phenol at lower concentrations (50 – 1500 mg/L) and reported that the amount of enzyme required was reduced by 15 to 75 times by using gelatin and PEG. Cooper and Nicell (1996) also demonstrated that PEG reduced the enzyme required for achieving 97-99% of total phenol removal in foundry wastewater. Kennedy et al. (2003) also reported 10 and 50-fold increases in removal efficiency of 2, 4-dichlorophenol by addition of PEG-3350 or PEG-8000, respectively.

Sun et al. (1992) reported that another protective additive, chitosan, can covalently bind the quinones, which were identified as HRP catalyzed reaction products in oxidation of phenols.

Solution Ionic Strength Although other researchers have evaluated the impact of pH, substrate concentration, enzyme dose and H_2O_2 concentration on enzyme mediated polymerization reaction, the effect of ionic strength remains unclear since only one study has addressed this topic (Huang et al., 2005).

A variety of buffer solutions of different ionic strengths have been used in HRP-mediated polymerization experiments to control the pH. For example, Kazunga et al. (1999) used 67 mM phosphate buffer (pH 6.0) while Zhang and Nicell (2000) used 0.1 mM monobasic-dibasic sodium phosphate buffer (pH 6.0). Wu et al. (1993) demonstrated a protective effect of borate buffer to increase phenol removal.

Huang et al. (2005) investigated the effect of background ion on the precipitation of enzyme-catalyzed oxidative coupling products of phenol. They observed that the amount of precipitated product was significantly increased when each salt was added, indicating that certain dissolved products become more precipitable as background ion concentration increased. Twice

as much precipitate product was generated at 10 mM salt concentration (sodium chloride and potassium chloride) than at zero salt concentration. However, no further enhancement was observed with increasing salt concentration.

Wagner and Nicell (2002) investigated the effect of dissolved wastewater constituents on the phenol polymerization reaction and found inhibition of phenol transformation in the presence of sulfide, cyanide, Mn(II), NaCl, $CaCl_2$, $MgCl_2$, NH_4Cl and $(NH_4)_2SO_4$. Increasing the H_2O_2 dose reduced inhibition by sulfide but not by cyanide. They also investigated the effect of salts concentrations such as sodium chloride, calcium chloride, magnesium chloride, ammonium chloride and ammonium sulfate. They observed that phenol removal was initially increased with increasing salt concentration up to 0.05 M. beyond which there was no additional impact on phenol removal.

4.2.2.6 Phenol Polymerization Products

HRP-mediated oxidative polymerization of phenol results in the formation of a variety of products. These reaction products consist of large molecular weight insoluble oligomers and low molecular weight soluble products (Klibanov and Morris, 1981; Schwartz and Hutchinson, 1981; Davidenko et al., 2004).

Klibanov and Morris (1981) observed that products formed during HRP-mediated removal of aromatic amines could be removed by precipitation or filtration. Schwartz and Hutchinson (1981) observed the formation of high molecular weight polymers, but approximately three percent of the substrate was converted to soluble biphenols. Such compounds may be the main products at low substrate concentrations and may not be removed well using conventional filtration and flocculation processes.

In certain cases, a dark precipitate was observed at high substrate concentrations and no precipitation was observed at low concentrations. Davidenko et al. (2004) reported that the oxidation of all phenols using partially purified peroxidases resulted in the formation of insoluble red-brown polymer. The polymers were not soluble in diethyl ether, ethanol, acetone, chloroform and benzene. However, their research illustrated that treatment conditions could significantly affect the quality and quantity of the reaction products. For example, when PEG was used as a protective additive, there was an increase in the quantity of products that remained in solution following the treatment of phenol (Ghioureliotis and Nicell, 1986).

Minard et al. (1981) reported production of the dimeric quinines 2-(2,4-dichloropheoxy)-1,4-benzoquinone and 2-(2,4-dichlorophenoxy)-6-chloro-1,4-benzoquinone using mass spectrometry and ^{1}H-NMR analyses when they incubated 2,4-dichlorophenol (DCP) with a phenol oxidase from the fungus *Rhizoctonia praticola*.

Sun et al. (1992) reported that another protective additive, chitosan, was able to covalently bind the quinones produced during HRP catalyzed oxidation of phenols. They noted that oxidation of phenolic compounds in water at low concentrations of hydrogen peroxide (< 0.3 mM) resulted in the formation of soluble products which become larger polymers in the presence of higher peroxide concentrations. These larger polymers readily precipitated in the aqueous solution.

Yu et al. (1994) identified five dimeric and one trimeric products from the phenol polymerization reaction in aqueous solution. The structure of 4-(4-phenoxyphenoxy) phenol was determined by NMR spectrum. These authors reported more than 95% phenol removal from an initial phenol concentration of 188 ppm, and about 7% of the precipitate mass consisted of three dimers - *p, p*-biphenol, *o,o*-biphenol and *p*-phenoxyphenol.

4.2.2.7 Toxicity of Phenol Polymerization Products

The main objective of using peroxidase or catalysts for the removal of phenols is to remove them by precipitation or transformation to other nontoxic products without the necessity of completely mineralizing them. Although most phenol polymerization products precipitate out of solution, small amounts of low molecular weight byproducts remain in the aqueous phase. In certain cases, the soluble byproducts can be more toxic than the parent compounds.

Although many investigators have researched and characterized polymerization end products (Wagner and Nicell, 2002; Ghioureliotis and Nicell, 2000; Xu et al, 2005), the impact of these products on residual aqueous phase toxicity remains unclear because of the difficulty of identifying the products and assessing their toxicity. Heck et al. (1992) evaluated the toxicity of phenol polymerization products and reported that the toxicities of *o*-cresol, *p*-cresol, 2-chlorophenol, and 4-chlorophenol solutions decreased after polymerization while the toxicity (EC_{50} value) of *p*-cresol increased. However, most of researchers have stated that oligomer produced from enzymatic reaction were less toxic than parent compounds.

Aitken et al. (1994) studied the mutagenicity of reaction products resulting from the oxidation of phenols by enzyme. Toxicity analysis of polymerization products resulting from

chloroperoxidase, HRP, lignin peroxidase, manganese peroxidase and mushroom polyphenol oxidase showed that solutions containing 4-chlorophenol, *p*-cresol and pentachlorophenol (PCP) were detoxified by at least one of the enzymes tested, whereas 2-nitrophenol, 4-nitrophenol, 2-chlorophenol, *o*-cresol and phenol solutions either became more toxic or showed no effect depending on reaction conditions. In the case of PCP, the toxicity of reaction products generated at pH 4 was significantly less than those produced at natural pH.

Zhang and Nicell (2000) found that the residual toxicity of treated PCP solutions were higher than that accounted for residual PCP and attributed it to unidentified soluble products. Other researchers have demonstrated detoxification of PCP-contaminated media using peroxidase-mediated transformation to less chlorinated products (Hammel and Tardone, 1988; Ricotta et al., 1996).

Ghioureliotis and Nicell (2000) reported the toxicity of soluble products of soybean and horseradish peroxidase-catalyzed polymerization of substituted phenols and found that, in most cases, the residual toxicity was significantly lower than the initial toxicity of the solution. The decrease in toxicity was attributed to the polymeric products formed from the enzyme-catalyzed oxidation of parent phenols. There was no significant difference of residual toxicities using either SBP or HRP in most cases.

Wagner and Nicell (2002) investigated the stability of toxic soluble reaction products under ambient conditions (25 ^{o}C, pH 7) and explored ways to prevent and/or eliminate residual toxic compounds following enzymatic treatment. These researchers observed that soluble reaction products were mainly phenolic dimers after HRP-mediated polymerization reaction and toxic compounds were formed during the treatment of aqueous solutions of phenol, 2-chlorophenol, 4-chlorophenol, 2,4-dichloropehol and 2-methyphenol. However, the toxicities of HRP treated solutions decreased within 21 h after the completion of the enzymatic reaction, except in the case of two solutions that phenol was removed. Solutions that were treated in the presence of chitosan exhibited lower toxicities than solutions treated in its absence. Treatment in the presence of PEG resulted in significantly higher toxicities. These researchers concluded that the toxicity of solutions could be reduced by supplying additional H_2O_2 after completion of the enzymatic phenol removal reaction.

Wagner and Nicell (2003) continued their research to investigate the impact of the presence of solids on peroxidase-catalyzed treatment of aqueous phenol. Solids such as kaolin,

bentonite, cellulose and peat moss increased phenol transformation at pH 5.0 and 7.0. Phenolic solutions treated in the presence of bentonite, kaolin and peat moss were significantly less toxic than controls, indicating that these materials were able to partially neutralize precursors of toxic reaction products.

Many researchers have reported that HRP-mediate oxidation of phenol can alter the toxicity of the phenolic compounds under various reaction parameters such as the type of enzyme, initial substrate concentration, peroxide dose, enzyme dose and pH (Bollag et al., 1988; Aitken et al., 1994; Wagner and Nicell, 2002; Ghioureliotis and Nicell, 2000). For example, after phenol conversion, the toxicities remain very high in the case of 2-methyphenol, 2-chlorophenol, 4-chlorophenol and phenol, while the toxicities of other enzyme-treated phenolic solutions were partly or completely reduced (Wagner and Nicell, 2002). Research has shown that treatment conditions can affect the quality and quantity of reaction products. In one case, soluble polymers were not produced (Davidenko et al., 2004).

4.2.3 Peroxidase Mediated Removal of Phenols from Aqueous Solutions

The use of enzyme-mediated polymerization to remove phenols in aqueous solution has several advantages over conventional treatments. This approach can be effectively applied over wide ranges of pH, temperature, and substrate concentrations (Klibanov et al., 1983). Since enzymes are highly selective, this process can reduce the cost of operations by preventing undesirable and unnecessary reactions (Aitken et al., 1994). In addition, this process requires low retention times and operates over a broad range of substrate concentrations (Nicell et al., 1992; Bewtra et al., 1995).

Many researchers have demonstrated the impact of the HRP-based process for the treatment of synthetic wastewater consisting of selected aromatic compound (Klibanov et al., 1980; Dec and Bollag, 1990; Nicell et al., 1992).

Nakamoto and Machida (1992) speculated the effect of scale-up on the enzyme-mediated polymerization process from their experiments. Dec and Bollag (1994, 1995) determined the optimum operating conditions for the removal of phenols using this process. These researchers postulated the dehalogenation and detoxification of chlorinated phenol during the polymerization reaction from their observations. The enzymatic process was effective in the low drinking water

range of 2,4 DCP (0.0025 to 240 mg/) and much higher concentrations of phenol treatment without using additives (Maloney et al., 1984; Wu et al., 1993).

Fang and Barcelona (2002) demonstrated the coupled oxidation of aromatic hydrocarbons such as o-xylene-d10 and naphthalene-d8 with HRP and H_2O_2. The oxidation of aromatic hydrocarbons was tested as a function of HRP at a fixed concentration of H_2O_2 and constant HRP activity (4000 units/mL). Up to 54% and 51% of mass removal were observed for o-xylene-d10 and naphthalene-d8, respectively.

Bassi et al. (2004) investigated the feasibility of soybean seed hulls for enzymatic removal of phenol and chlorophenols. Eighty percent of phenol (10.6 mM), 96% of 2-chlorophenol (3.9 mM), 95% of 2,4-dichlorophenol (3.1 mM), and 94% of mixed phenol and chlorophenols were successfully removed using soybean seed hulls.

Huang and Weber (2005) investigated the feasibility and mechanisms for transformation and removal of bisphenol A (BPA) from aqueous phase via HRP-oxidative coupling reactions. They postulated that two BPA radicals are coupled primarily by the interaction of an oxygen atom on one radical and propyl-substituted aromatic carbon atom on another, followed by elimination of an isopropylphenol carbonation. They also concluded that catalyzed oxidative coupling reactions may be important natural transformation pathways for estrogenic phenolic compounds.

4.2.4 Wastewater Treatment Using Enzyme-Mediated Oxidative Coupling Reaction

Enzymatic treatment has been proposed as a potential alternative to overcome several limitations associated with conventional chemical and biological methods for the decontamination of wastewater, soil and groundwater (Klibanov et al., 1980, 1981; Nicell et al., 1993a; Aitken et al., 1989; Nakamoto and Machida, 1992; Nicell, 1994b; Caza et al., 1999). The enzymatic method can be used for a wide variety of phenolic compounds and it retains stabililty during sudden changes of pH and contaminant concentrations (Nicell et al., 1993a). It also allows high reaction rates and produces low volume by-products compared to traditional biological processes. Enzymatic treament methods can also be used to specifically target selected contaminants due to their specificity to individual species (Duran and Esposito, 2000; Caza et al., 1999). However, peroxidases are attractive agents of decontamination due to their low substrate

specificity. HRP can remove organic compounds by co-precipitation (Klibanov et al., 1980; Karam and Nicell, 1997). For example, Klibanov et al. (1983) observed that polychlorinated biphenyls could be coprecipitated with phenols from coal-conversion wastewater.

The possibility and feasibility of using peroxidase to treat wastewater have been extensively studied (Klibanov et al., 1983; Maloney et al., 1986; Nannipieri and Bollag, 1991; Bollag, 1998; Dec and Bollag, 1994; Zhang and Dunford, 1993; Duran and Esposito, 2000; Wagner and Nicell, 2001a, 2001b). Most applications of enzyme-mediated oxidation process have focused on the removal of phenolic contaminants (Aitken et al., 1994). Klibanov et al. (1980) first demonstrated that up to 99% of 30 different phenols and amines could be removed from wastewater using HRP. Ferrer et al. (1991) compared color removal from Kraft mill effluents by the use of HRP and lignin peroxidase. Both enzymes were found to have considerable potential.

Foundry wastewater containing 3.5 mM total phenol was treated using HRP and H_2O_2, resulting in 97 to 99% removal of total phenol and formation of water-insoluble polymer (Cooper and Nicell, 1996). The economic feasibility of HRP-mediated oxidation was also investigated by evaluating the use of an additive and examining a low purity enzyme. Results showed that HRP requirement was reduced up to 95% of original enzyme requirement in the presence of additive with crude and purified HRP. Wagner and Nicell (2001a) reported that treatment of a foul condensate from Kraft pulping with HRP and H_2O_2 reduced the concentration of total phenol below 1 ppm and toxicity by 46%. They observed that lignin derivatives in the wastewater protected the enzyme from inactivation by polymerization products.

Ibrahim et al. (2001) reported a feasibility study for enzyme-catalyzed removal of phenol from refinery wastewater. Phenol (2 mM) in petroleum refining wastewater was removed up to 95-99% using *Arthoromyces* ramosus peroxidase in batch and plug-flow reactors. PEG addition reduced enzyme requirement by more than 40%.

Wagner and Nicell (2002) investigated the impact of dissolved wastewater constituents on the treatment of synthetic phenol solution using HRP and H_2O_2. They found inhibition of phenol transformation in the presence of sulfide, cyanide, Mn(II), NaCl, $CaCl_2$, $MgCl_2$, NH_4Cl and $(NH_4)_2SO_4$. Increasing the H_2O_2 dose reduced inhibition by sulfide but not by cyanide.

4.2.5 Application of HRP-Mediated Oxidative Coupling to Soil Remediation

Soil organic matter (SOM) is the primary sorption domain in surface soils. The characteristics of SOM can, therefore, exert significant impact on the fate of contaminants in soils and sediments (Weber et al, 1992; Young and Weber, 1995; Xing and Pignatello, 1996; Huang et al., 1997). Many researchers have reported irreversible binding of phenolic compounds to SOM through the enzyme-catalyzed oxidative coupling reaction (Bhandari et al*.,* 1996; Burgos et al*.,* 1999; Xu and Bhandari 2003; Palomo and Bhandari, 2006).

The biogeochemical fate of natural organic matter can be explained by the pathways of degradation and humification. Degradation is a process through which large macromolecules decompose to smaller molecules while humification is a process whereby smaller molecules aggregate to form macromolecular substances.

Enzyme mediated oxidative coupling reactions play an important role in soil humification processes. Soils contain a large amount of extracellular enzymes such as peroxidases, laccases, and polyphenol oxidases that catalyze the oxidation of contaminants. Some microorganisms in soils also produce phenoloxidases (Bartha and Bordeleau, 1969; Dec and Bollag, 1994b). These extracellular enzymes easily bind with the soil matrix and remain active even after the decomposition of the plant.

Enzyme-catalyzed oxidative coupling reactions can result in the formation of covalent linkages between phenols and humic substances. Bhandari et al. (1996) investigated the binding of 4-chlorophenol (CP) to two soils and found that oxygen enhanced binding of 4-CP to soil and the binding was further increased with addition of H_2O_2.

Huang and Selig (2002) examine the incorporation of phenols on geosorbents using peroxidase-catalyzed oxidative coupling reaction. They found that each of these two different types of natural geosorbents increased the formation of non-extractable coupling products over that which occurred in solids-free systems. HRP inactivation by free radical attack was also significantly reduced in the presence of each geosorbents.

Weber and Haung (2003) investigate the behavior of phenanthrene in humification processes using HRP-catalyzed oxidative coupling reaction. They found that phenanthrene removal occurred not only by sorption but also by chemical bonding to precipitated reaction products.

Selig et al. (2003) evaluated the sorption and oxidative coupling reaction of phenol, *o*-cresol, and p-chlorophenol with natural geosorbents. They found that soils containing highly amorphous organic matter more adsorbed the contaminants than soils containing condensed organic matter. The sorption/desorption properties of the solutes were significantly altered in the presence of birnessite as a result of both cross-coupling reactions with reactive soil organic matter components and self-coupling reactions with each other to form polymeric species.

Palomo and Bhandari (2005) investigated sorption/desorption properties of 2,4-DCP to agricultural and woodland soils using an enzyme mediated polymerization reaction. They observed the sorption of DCP to the oligomeric precipitate and additional removal of DCP to soil. Their results are consistent with those of Weber and Haung (2003).

In this study, we evaluated the impact of pH, solution ionic strength and HRP concentration on polymerization process in saturated porous media.

Specific research hypotheses were:

Hypothesis 1

Phenol entering a packed column under simulated aquifer conditions can be removed from the aqueous phase by injecting HRP and H_2O_2 into the flow.

Hypothesis 2

HRP-mediated phenol removal in continuous flow, packed column reactors is accompanied by the generation of soluble and insoluble oligomeric products.

Hypothesis 3

Phenol removal and the production of oligomeric products under the action of HRP and H_2O_2 are affected by the enzyme dose, solution pH and solution ionic strength.

Hypothesis 4

Deposition of insoluble phenol polymerization products in the packed column results in physical modification of the porous media.

4.3 EXPERIMENTAL APPROACH

This section describes the reagents, materials, equipment and methods employed to conduct the research.

4.3.1 Reagents, Materials and Equipment

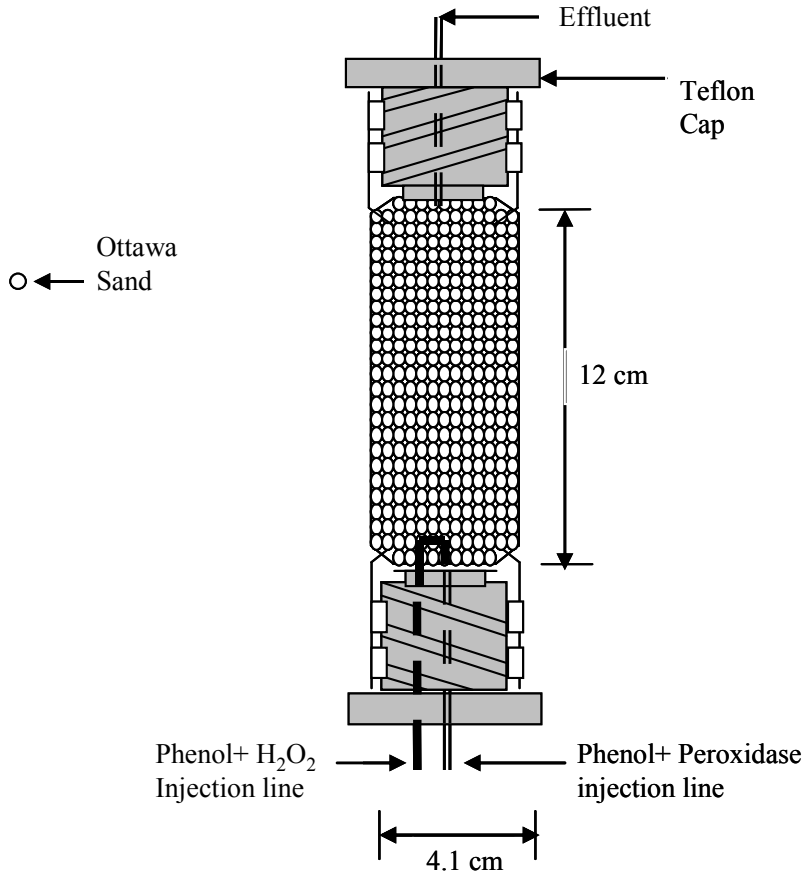
Horseradish peroxidase (Type II, RZ 2.2, 181 activity units/mg), hydrogen peroxide (30%, w/w, 8.2 M) and phenol (> 99%) were purchased from Sigma Chemicals (St. Louis, MO). Analytical grade potassium chloride, sodium chloride, potassium phosphate (mono and dibasic), acetic acid, methanol and scintillation cocktail (Fisher ScintiSafe, 50%) were obtained from Fisher Scientific (Pittsburg, PA). Uniformly ring-labeled ^{14}C-phenol (specific activity 40.1 mCi/mmol) was purchased from Sigma Chemicals. Working solutions of phenol were amended with ^{14}C-phenol for quantification of polymerization products generated after reaction with HRP and H_2O_2.

A glass column (41 mm internal diameter, 110 mm length) was fabricated in Kansas State University's glass blowing laboratory (Figures 4.4 and 4.5). Teflon end fittings and tubes (1.14 mm) were purchased from Fisher Scientific. Ottawa sand (20-30 mesh) was used as the granular media and was purchased from Fisher Scientific. A peristaltic pump (Bulcher, Model 426-2000) was used to introduce the solution into the column. An automated fraction collector (ISCO, Model Foxy Jr.) was used to collect samples from the column effluent. All other chemicals and materials used in this experiment were previously described in Section 3.3.1.

A high pressure liquid chromatograph (HPLC) system consisting of a gradient pump (Varian, Model ProStar 220 Solvent Delivery Module), a reverse-phase column (Varian RES ELUT 5 µ C18 90A, 150 mm x 4.6 mm), ProStar 410 AutoSampler and a photodiode array (PDA) detector (Varian, Model ProStar 335) were used to quantify total phenol concentration in solution. The PDA detector was set at a wavelength of 280 nm with an oven temperature of 40 ^{o}C. The flow rate of the mobile phase was set at 0.1 mL/min. The injection volume of the sample was set with 10 µL after 200 µL of methanol washing. The mobile phase consisted of 60% HPLC grade methanol and 40% HPLC grade water containing 2% acetic acid. The mobile phase was degassed prior to use. The column was stabilized for 1 hr with mobile phase prior to analysis. The HPLC was calibrated before and after sample analysis. The calibration curves

remained relatively constant (R^2= 0.98 to1.0) throughout the analysis. Run time of HPLC was set at 6 min since the phenol peak appeared at 4.95 min.

Figure 4.4. Schematic diagram of upflow column.



A liquid scintillation counter (Beckman, Model 6500) was used for quantification of the total ^{14}C-activity in aqueous solution as disintegrations per minute (dpm). An appropriate amount (250 to 300 μL) of the sample collected in the fraction collector was transferred into 7 mL of scintillation vials containing 5 mL of scintillation cocktail. The LSC was operated in Autocounting mode and enumerated the beta-activity twice for each sample. The LSC was calibrated with a ^{14}C-standard prior to initiating an Auto DPM program. A flowthrough conductivity meter (Accument, Model AR20) was used to measure conductivity of solutions during tracer tests. The calibration of conductivity meter was conducted 1 hr prior to analysis.

Table 4.3. Properties of porous material.

Granular Media	Properties
ρ (g/cm^3)	2.65
ρ_b (g/cm^3)	1.64
ε	0.38

ρ : solid density, ρ_b: bulk density, ε : porosity,

Table 4.4. Column parameters.

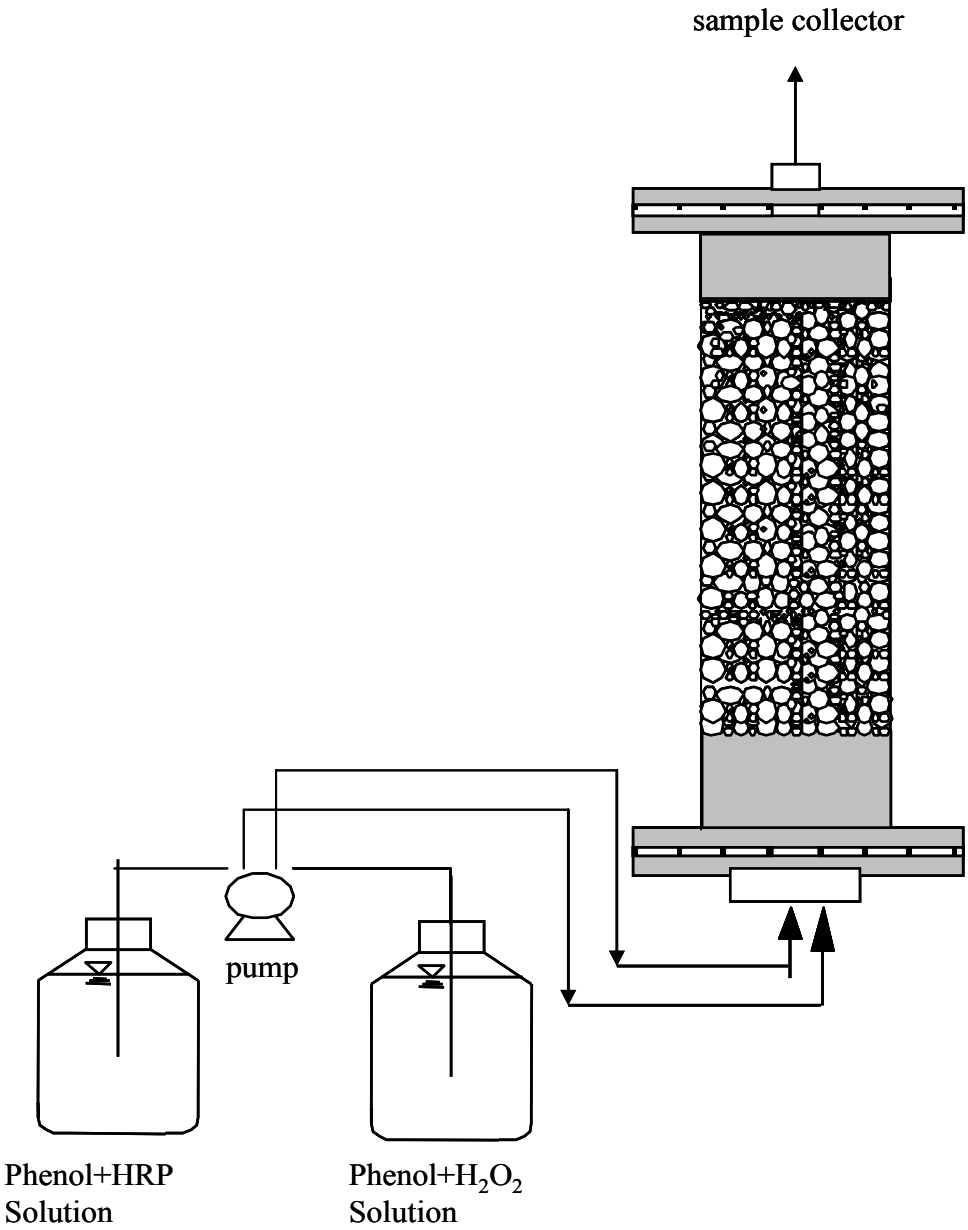
Parameter	Value
L (cm)	11
A (cm^2)	13.20
v_w (m/d)	1.5
V_p (cm^3)	55.15

L: column length; A: cross-sectional area of column;
v_w: pore water velocity; V_p: pore volume

4.3.2 Experimental Method

The experimental set up for studying phenol polymerization in porous media is illustrated in Figure 4.4. Ottawa sand was packed as uniformly as possible in a glass column (Table 4.3). The packed column was saturated and flushed with 20 pore volumes of distilled-deionized water. A tracer test using KCl as a nonreactive tracer was conducted to determine the hydrodynamic properties of the packed column.

Figure 4.5. Schematic diagram of upflow column system used to conduct the experiment.



The tracer test consisted of pumping 1.5 pore volumes of KCl solution through the column before flushing it with two pore volumes of distilled-deionized water. Effluent samples were analyzed directly using an in-line conductivity meter. The results were plotted as relative concentration (C/C_0; effluent concentration divided by influent concentration) versus the number of pore volume (discharge volume divided by water retention volume of column). All experiments were performed at room temperature ($20 \pm 2\ ^{o}C$).

The glass column was packed with 215 grams of Ottawa sand and operated with flow in the upward direction. The column consisted of two closely spaced inlet lines. One line delivered a solution consisting of enzyme and phenol in a buffer solution. The other line delivered a solution containing H_2O_2 and phenol in an identical buffer solution. The two solutions contained enzyme and H_2O_2 at twice their target 'in-column' concentrations, which were achieved when the two flows merged immediately after entering the column. Two separate lines were used for solution delivery to initiate polymerization within the saturated porous media. The buffer pH was controlled by utilizing appropriate ratios of KH_2PO_4 and K_2HPO_4 in solution, while the buffer ionic strength was adjusted using NaCl.

The column study consisted of the following sequence: (i) injection of a conservative tracer (KCl, 250 mg/L); (ii) washing of column with deionized water;

(iii) injection of phenol to saturate column with phenol solution; (iv) injection of HRP and H_2O_2 with phenol to facilitate in-situ polymerization of phenol in the porous media; (v) washing of column with deionized water; (vi) injection of tracer; and (vii) washing of column with deionized water. The *in-situ* polymerization of phenol was evaluated for the combinations of experimental conditions summarized in Table 4.5.

Table 4.5. Experimental conditions evaluated in column experiments.

Experimental parameter	Value
Influent phenol concentration	500 μM
Influent H_2O_2 concentration	500 μM
Influent HRP concentrations	0.5, 1.0 and 2.0 AU/mL
Solution ionic strengths	5, 20, 100 mM
Solution pHs	5, 7, 9

A fraction collector loaded with 10 mL glass test tubes was used to collect samples from the column outlet every five minutes during column operation. In the case of tracer test, effluent samples were analyzed directly using a flow-through conductivity meter. A 250 mL aliquot of each effluent sample was transferred from the test tube into 7-mL scintillation vials containing 5 mL of scintillation cocktail. These samples were analyzed for total ^{14}C-activity using liquid scintillation counting. A one-milliliter subsample from each test tube was transferred into a 2-µL HPLC vial for subsequent quantification of total phenol using the HPLC system.

4.3.3 Modeling

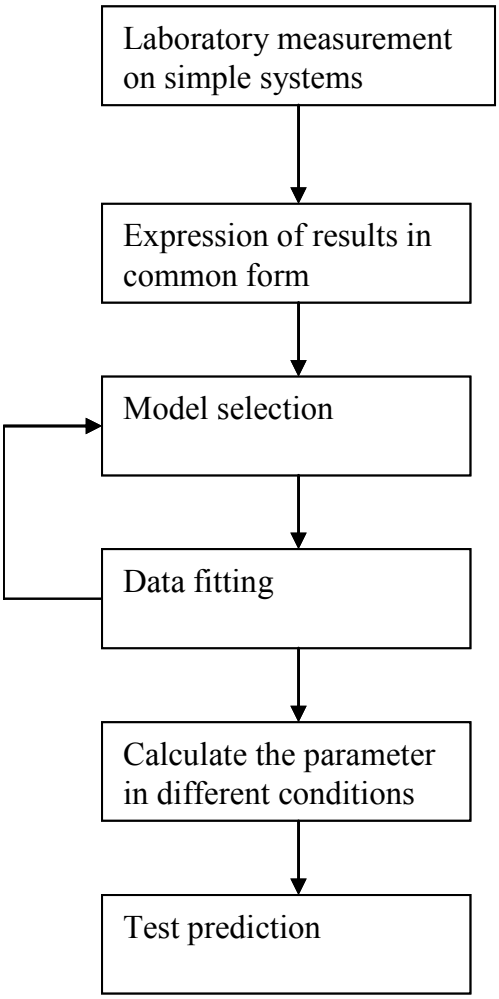
For the modeling process, a free STANMOD (STudio of Analytical MODels version 2.0) software package was downloaded from http://pc-progress.cz/Pg_Stanmod.htm and used to evaluate solute transport in porous media using analytical solutions of the advection-dispersion equation. This software has been verified against a number of test cases. Version 2.0 of STANMOD includes the following models (Simunek et al., 1999):

CXTFIT 2.1 [Toride et al., 1995], CFITM [van Genuchten, 1980],
CFITIM [van Genuchten, 1981], CHAIN [van Genuchten, 1985],
3DADE [Leij and Bradford, 1994] and N3DADE [Leij and Toride, 1997].

This software package also include a modified and updated version of CXTFIT code published by van Genuchten (1979, 1981), Parker and van Genuchten (1984) and Toride et al. (1995) for estimating solute transport parameters using a nonlinear least-squares parameter optimization method (Simunek et al., 1999). The inverse estimation method can estimate the parameters by minimizing an objective function that consists of the sum of the squared differences between observed and fitted concentrations using a nonlinear least-squares inversion method (Marquardt, 1963).

The CXTFIT 2.1 program in the STANMOD software package was used to estimate parameters in several analytical models for solute transport during steady one-dimensional flow by fitting analytical solutions for the models to observed laboratory (Simunek et al., 1999). The modeling step-by-step cycle is summarized in Figure 4.6.

Figure 4.6 Modeling Procedures.



The experimental results were fitted to the mathematical solutions of theoretical transport model using inverse parameter estimation method, based on the one-dimensional advection-dispersion equation (Simunek et al., 1999). The parameters estimated were further used to predict the transport behavior of other solutes or estimate the other parameters such as concentration as a function of time and/or position using direct or inverse method (Simunek et al., 1999).

4.4 RESULTS AND DISCUSSION

This section presents results of experiments evaluating peroxidase-mediated phenol removal and accumulation of polymerization products in saturated porous media. The results of nonreactive tracer tests before and after polymerization are also presented and discussed. The impact of solution pH, ionic strength and HRP dose on phenol polymerization in the column system are discussed.

4.4.1 Preliminary Studies

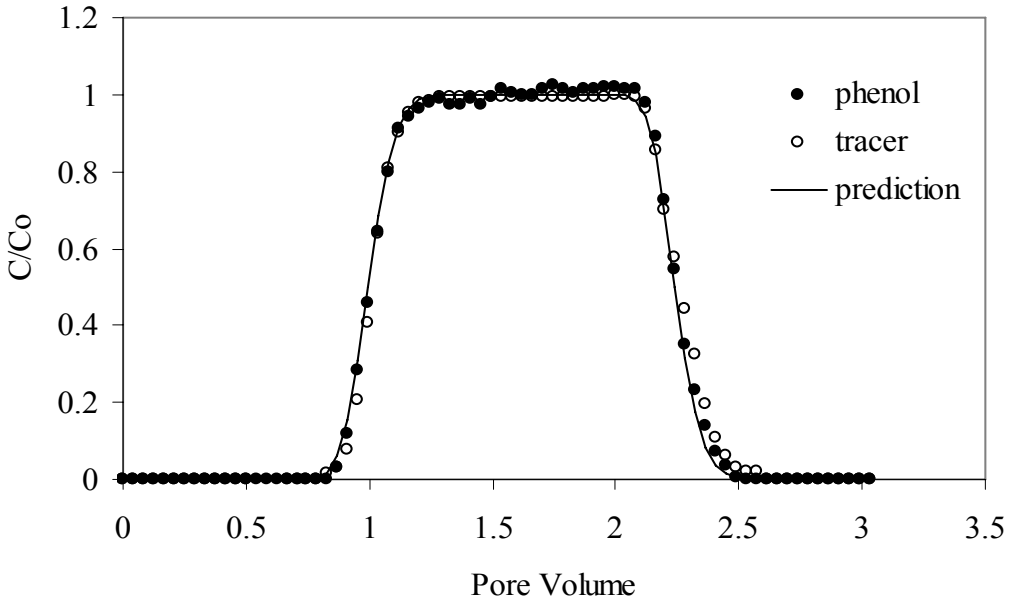
Preliminary experiments were conducted to ensure that phenol polymerization did not occur in the absence of H_2O_2. Polymerization of phenol was also evaluated under various condition based on information obtained from published literature. Results of these preliminary investigations (not included in this document) showed that polymerization did not occur in the absence of any component of the polymerization solution such as H_2O_2, HRP and phenol. Preliminary tracer tests were also conducted to select a conservative tracer and assure the reliability of results measured by a conductivity meter using sodium chloride, calcium chloride and potassium chloride. Potassium chloride was selected as the tracer due to its reliable detection using an inline conductivity meter.

4.4.2 Tracer Test

The breakthrough curve obtained for the nonreactive tracer (KCl) is shown in Figure 4.7. The tracer appeared in the effluent after approximately 0.8 pore volumes had passed through the column reactor. As illustrated in Figure 4.7, the behavior of tracer and phenol in saturated porous media were nearly identical. The relative concentration (C/C_0) reached a value of 1 at approximately 1.2 pore volumes. When the mobile phase in the column was replaced with distilled-deionized water, the relative concentration of the tracer in the effluent dropped to zero within one pore volume.

The symmetrical breakthrough curve of the tracer indicated the uniformity of the porous material in the column. The sand column appeared to have been packed uniformly since the tracer demonstrated an ideal transport behavior in the column.

Figure 4.7. Transport behavior of tracer and phenol through the packed column reactor. Influent phenol concentration = 500 µM, solution pH = 7, solution ionic strength = 20 mM.



The model prediction (solid line) for phenol transport in Ottawa sand is also illustrated in Figure 4.7. The transport behavior of phenol was predicted using dispersion coefficient of tracer without retardation parameter. The dispersion coefficient of tracer was estimated to be 0.004389 cm^2/min (R^2=0.998) using CXTFIT software. The phenol curve appears to coincide with that of the nonreactive tracer. Phenol does not experience any retardation during flow through the saturated porous media. The breakthrough curves also verified that the tracer was conserved since more than 98% of both tracer and phenol injected were recovered in the effluent. The column packing in all other experiments had similar hydrodynamic properties since all breakthrough curves had very similar shapes and retention times.

4.4.3 Effect of Enzyme Dose

In general, phenol removal using HRP-mediated oxidative coupling reaction is observed to increase with increasing enzyme dose (Nicell et al., 1992). However, since an inefficient use of the enzyme in enzyme-mediated treatment can result in high operational costs, the process should be optimized before deployment. Based on preliminary experiment results, up to 98% of phenol was removed using 2 AU/mL of HRP in batch reaction tests. Therefore, the enzyme doses selected for the column experiments were 0.5, 1.0, and 2.0 AU/mL.

The results of phenol polymerization and transport in continuous flow, saturated porous media for various enzyme doses are illustrated in Figures 4.8 to 4.10. The effluent phenol concentration sharply increased after 0.8 pore volume. Since phenol was continuously supplied to the column, the relative phenol concentration ($C_e^{phenol} / C_0^{phenol} = 1$) was expected to be maintained under saturated conditions when no HRP was added (as indicated by the dashed line, ---). HRP and H_2O_2 were introduced into the column inlet once the column was saturated with phenol. Phenol polymerization reaction in the porous column was expected to occur as soon as HRP and H_2O_2 were added.

Injection of 0.5 or 1 AU/mL of HRP (Figures 4.8 and 4.9, respectively) into the column, resulted in a 70% reduction in the effluent phenol concentration. The effect of 2 AU/mL HRP dose is illustrated in Figure 4.10. Approximately 0.8 pore volumes after HRP addition was initiated, the phenol breakthrough curve (o) sharply dropped to a relative phenol concentration less than 0.1, indicating 90% removal of the phenol in the flowing solution. Soluble polymer was also observed in the flow exiting the column at approximately 0.8 pore volumes after HRP addition. The relative concentration of soluble polymer (secondary y-axis in figure) increased and steadied at a value of 0.2 indicating that 20% of the influent phenol was converted to soluble polymers that were not retained in the column. Phenol concentration decreased and steadied at ~10% of the initial phenol concentration after about 4 pore volumes of HRP addition.

The relative concentration of soluble polymer in the effluent was found to increase with HRP dose. The fraction of influent phenol exiting the column as soluble polymer was about 0.05, 0.1, and at HRP doses of 0.5, 1 and 2 AU/mL, respectively. This trend was attributed to greater phenol polymerization at higher HRP dose.

Figure 4.8. Phenol transport behavior through the packed column reactor coincides with soluble polymer resulting from polymerization reaction (2AU/mL). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 0.5 AU/mL, and H_2O_2 concentration = 500 µM.

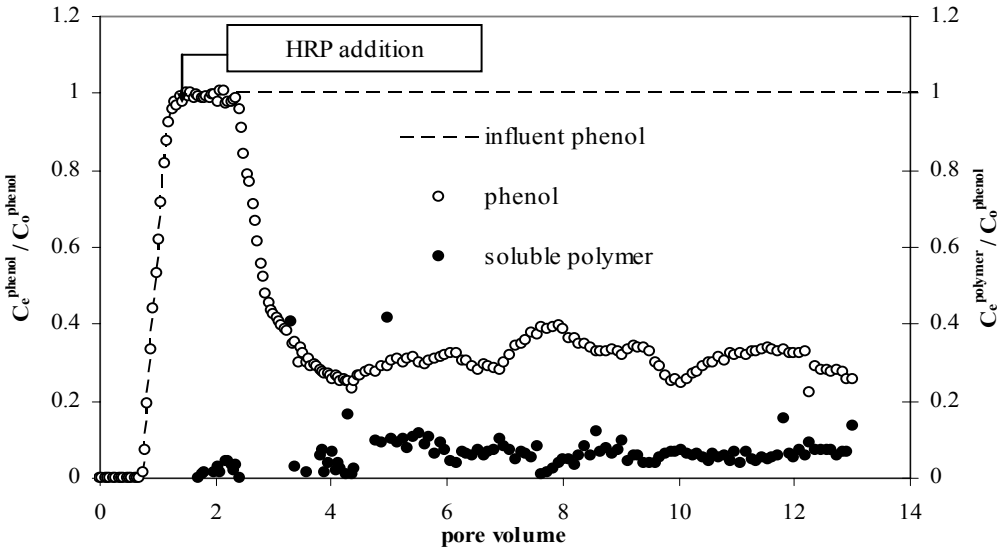


Figure 4.9. Phenol transport behavior through the packed column reactor coincides with soluble polymer resulting from polymerization reaction (1 AU/mL). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 1.0 AU/mL, and H_2O_2 concentration = 500 µM.

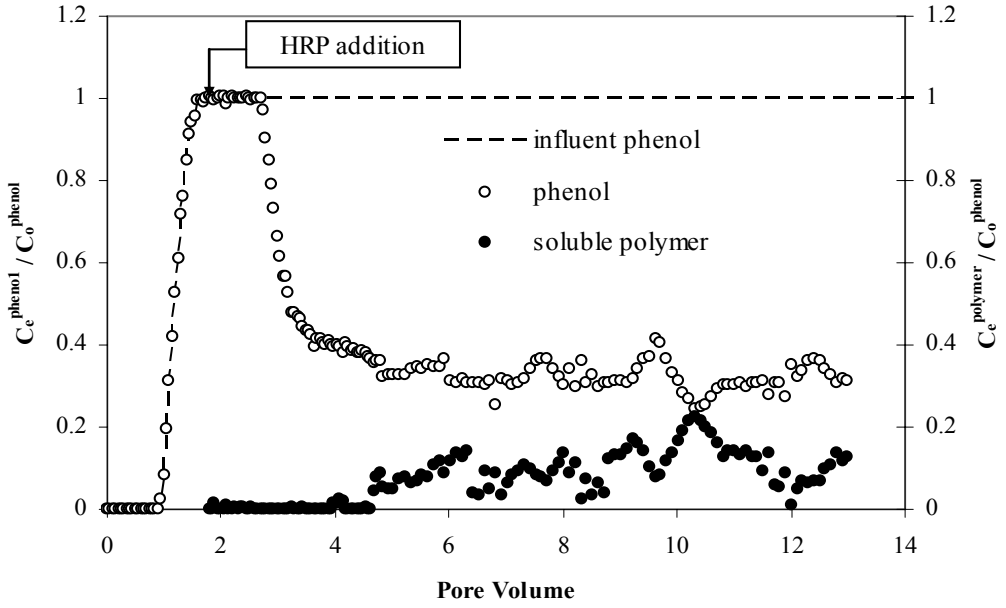
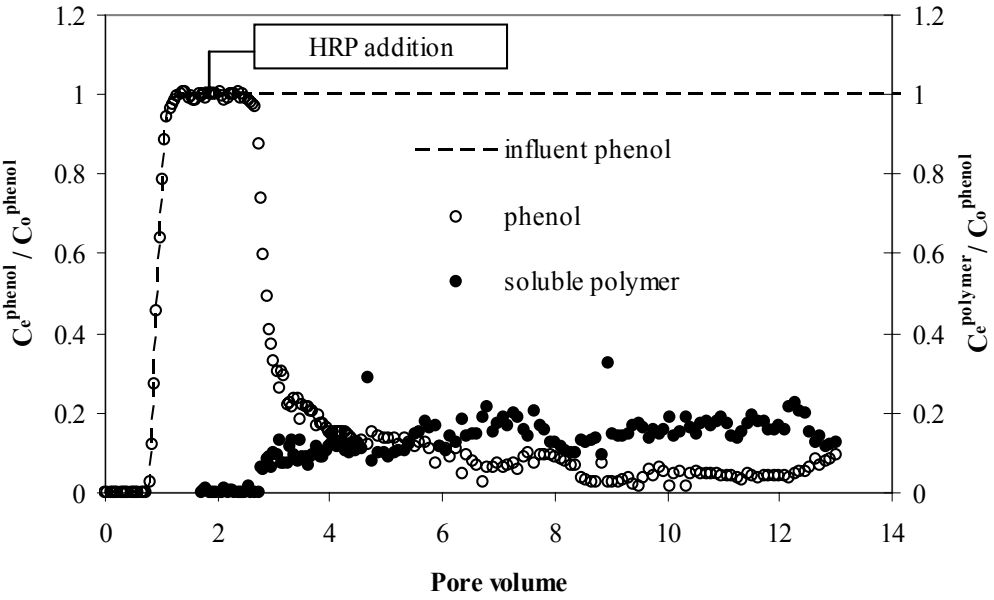


Figure 4.10. Phenol transport behavior through the packed column reactor coincides with soluble polymer resulting from polymerization reaction (2 AU/mL). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM



Figures 4.11 to 4.13 and Table 4.6 illustrate the mass balance of phenol for the column system experiments. These figures and Table 4.6 show the mass of influent and effluent phenol as well as the soluble and insoluble polymerization products generated as a consequence of HRP and H_2O_2 injection. The insoluble polymer consisted of the polymer accumulated in the sand column and was calculated by subtracting the mass of soluble polymer and effluent phenol from the total phenol injected. In the case of 2 AU/mL HRP dose, 65.5 % of the injected phenol was found to accumulate in the column as insoluble polymer while 59.3 % and 51.1 % of the influent phenol was retained in the porous media at HRP doses of 1 and 0.5 AU/mL. More insoluble polymers were produced at higher enzyme dose due to more complete polymerization of the influent phenol.

Figure 4. 11. Mass balance of phenol, soluble polymer and insoluble polymer (0.5 AU/mL). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 0.5 AU/mL, and H_2O_2 concentration = 500 µM.

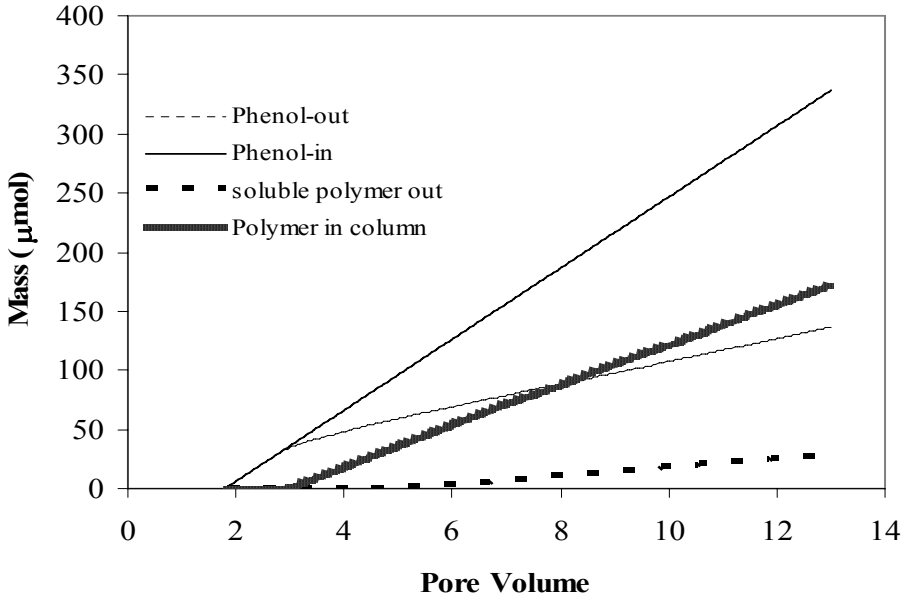


Figure 4.12. Mass balance of phenol, soluble polymer and insoluble polymer (1.0 AU/mL). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 1.0 AU/mL, and H_2O_2 concentration = 500 µM.

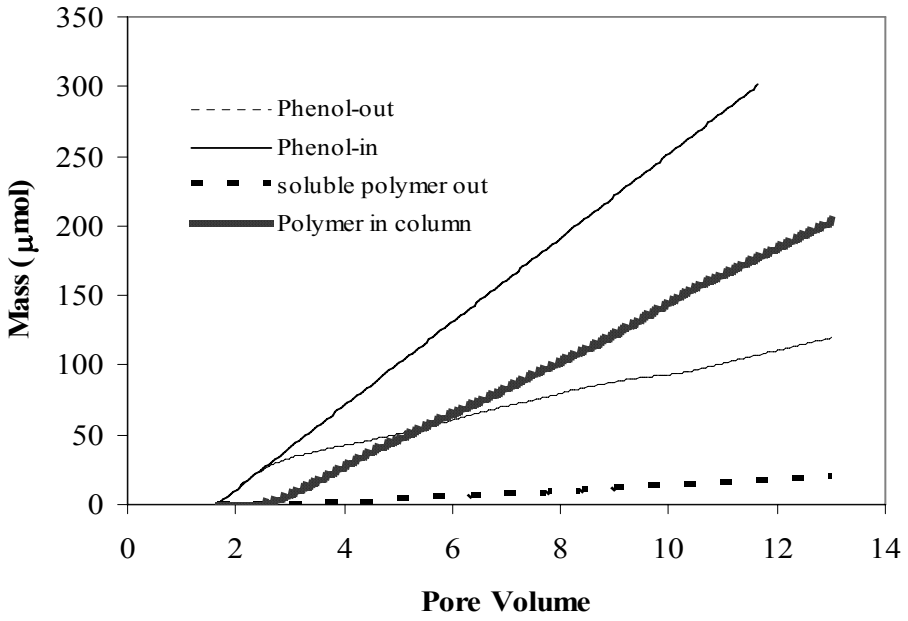
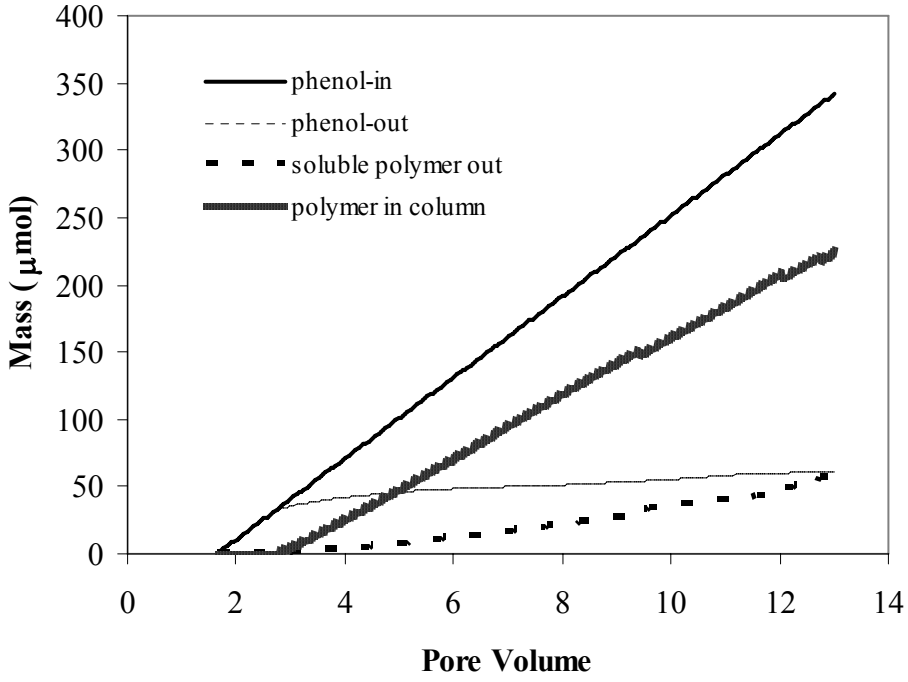


Figure 4.13. Mass balance of phenol, soluble polymer and insoluble polymer (2.0 AU/mL). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.



The deposition of phenolic oligomers in the column was expected to result in the modification of hydraulic properties of the porous media. Figures 4.14 through 4.16 illustrate the results of nonreactive tracer tests conducted in the saturated porous media before and after phenol polymerization. In all cases, the tracer in the modified media appeared at less than 0.8 pore volumes and disappeared earlier than in the original saturated porous media. It is considered that insoluble polymer was deposited in the porous media during the polymerization resulting in a reduced pore volume of the sand column. The change in transport behavior of tracer in the modified porous media was more pronounced at the highest HRP dose. The dispersion coefficients and retardation factors were also estimated and summarized in Table 4.7.

Table 4.6. Mass balance with enzyme dose.

HRP Dose (AU/ mL)	Total phenol in (μM)	Total phenol out (μM)	Total soluble polymer out (μM as phenol)	Total insoluble polymer in column (μM as phenol)
0.5	337.5	136.7	28.3	172.5
1.0	342.4	119.9	19.32	203.2
2.0	342.4	61.1	57.23	224.1

Figure 4.14. Transport behavior of KCl (250 mg/L, nonreactive tracer) before and after polymerization in the saturated porous media (0.5 AU/mL). Polymerization condition: influent phenol concentration = 500 μM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 0.5 AU/mL, and H_2O_2 concentration = 500 μM.
TBP = Tracer before polymerization, MTBP = Modeling before polymerization, TAP = Tracer after polymerization, MTAP = Modeling tracer after polymerization.

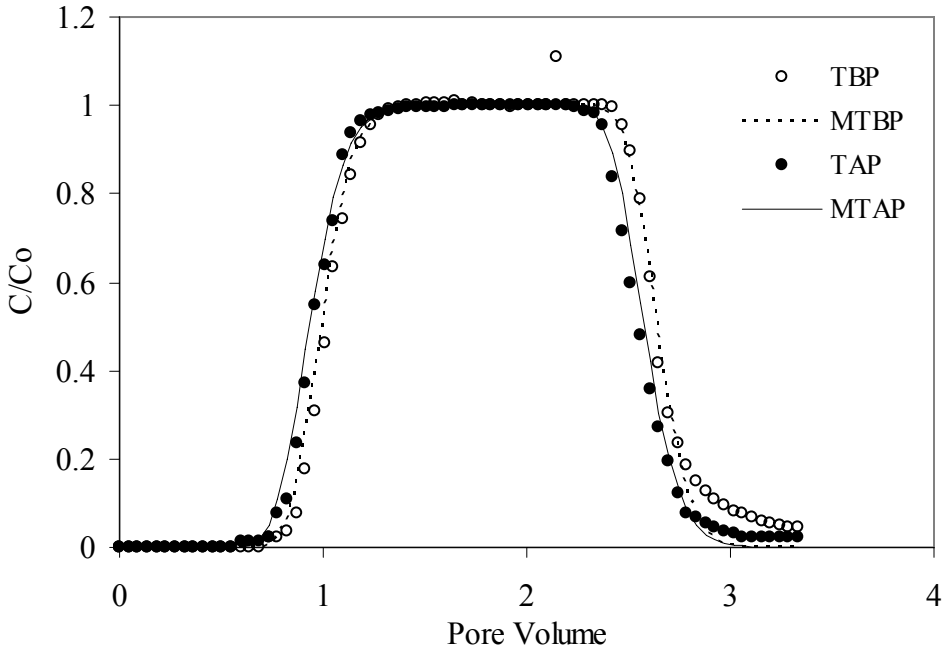
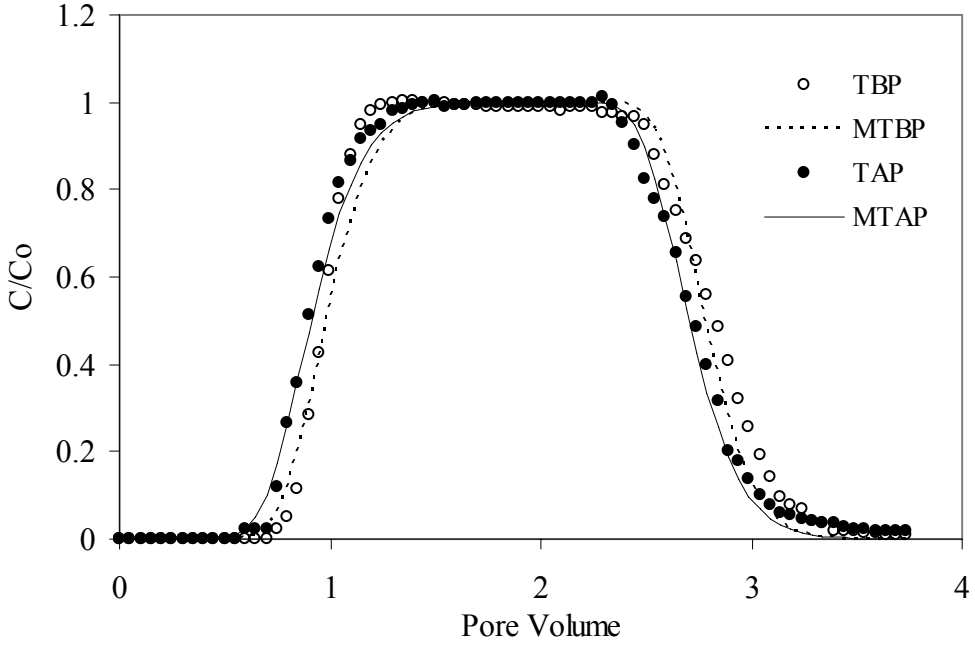
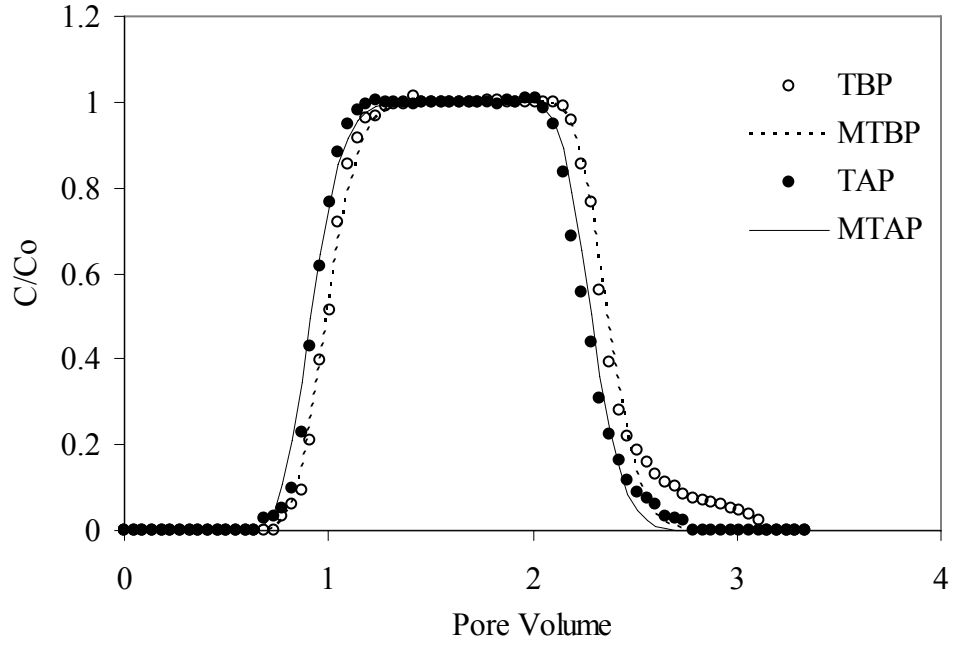


Figure 4.15. Transport behavior of KCl (250 mg/L, nonreactive tracer) before and after polymerization in the saturated porous media (1.0 AU/mL). Polymerization condition: influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 1.0 AU/mL, and H_2O_2 concentration = 500 µM.
TBP = Tracer before polymerization, MTBP = Modeling before polymerization, TAP = Tracer after polymerization, MTAP = Modeling tracer after polymerization.



The retardation factors (R) and dispersion coefficients (D) were estimated with inverse parameter estimation method using CXTFIT program of STANMOD software package version 2.0. Typically, sorption/desorption plays a dominant roll in determining retardation factor. As a result of sorption process (R>1), some solutes move slower than groundwater. If R<1, breakthrough curve appears faster than that of R=1(no retardation). Results obtained in these experiments showed that retardation factors of tracer after polymerization decreased. Since the tracer used in this experiment showed no retardation (R=1), these results are a strong evidence that the pore volume was decreased by polymer produced and deposited from polymerization reaction.

Figure 4.16. Transport behavior of KCl (250 mg/L, nonreactive tracer) before and after polymerization in the saturated porous media (2.0 AU/mL). Polymerization condition: influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.
TBP = Tracer before polymerization, MTBP = Modeling before polymerization, TAP = Tracer after polymerization, MTAP = Modeling tracer after polymerization.



The change in retardation factors estimated by modeling processes were increased with increasing enzyme dose, indicating that highest enzyme dose produced more oligomeric precipitates that modified the physical structure of the porous media. Dispersion coefficients also increased when the enzyme dose was increased although the variation of dispersion coefficient was not significant because the sensitivity of dispersion coefficient to the 1-D advection-dispersion equation much less than retardation factor. These results are consistent with that of mass balance. The values of dispersion and retardation estimated are summarized in Table 4.7.

Table 4.7. Parameters estimated with variation of enzyme dose.

Enzyme dose	Condition	v (cm/min)	D (cm^2/min)	R	R^2
0.5 (AU/mL)	MTBP	0.1096	0.0101	1	0.993
	MTAP	0.1096	0.0146	0.9419	0.996
1 (AU/mL)	MTBP	0.1120	0.0230	1	0.984
	MTAP	0.1120	0.0323	0.9313	0.995
2 (AU/mL)	MTBP	0.1096	0.0100	1	0.994
	MTAP	0.1096	0.0114	0.9224	0.995

MTBP: Modeling Tracer before polymerization

MTAP: Modeling Tracer after polymerization

v: pore water velocity, D: dispersion coefficient, R: retardation, R^2=least square

Based on these parameters estimated, pore volume decreased (pore volume occupied by polymer) was also estimated by simulating model with parameters. Since tracer showed no retardation, retardation factor was set at R=1. Simulation for the breakthrough curve of tracer after polymerization was conducted by varying pore water velocity and dispersion coefficient with no retardation. Variation of pore water velocity may be a clue to calculate the variation of porosity. Pore volume decrease was calculated by subtracting the estimated pore volume from original pore volume. Re-estimated parameters and pore volume decrease are summarized in Table 4.8.

These results are consistent with observation in mass balance and tracer tests. About 7.8 % of pore volume was decreased when 2.0 AU/mL of HRP was used. Reduction in pore volume as increased with enzyme dose.

Table 4.8. Estimation of pore volume occupied by polymer deposition with enzyme dose.

Enzyme dose (AU/mL)	estimated pore water velocity (cm/min)	estimated dispersion coefficient (cm^2/min)	R^2	flow rate (mL/min)	estimated porosity	pore volume decrease (%)
0.5	0.1164	0.01549	0.996	0.55	0.358	5.8
1	0.1284	0.03466	0.995	0.60	0.354	6.9
2	0.1189	0.01236	0.995	0.55	0.350	7.8

4.4.2 Effect of Solution pH

Several researchers have reported the importance of solution pH for enzyme-mediated polymerization (Aitken et al., 1989; Wu et al., 1983; Nicell et al., 1993b). Solution pH may affect the catalytic efficiency of the enzymatic reaction system. The pH can also affect the speciation of the weakly acidic phenol molecules.

The phenol removal and polymer production data at pH 7.0 was illustrated in Figure 4.9. Figures 4.17 and 4.18 show the transport and removal of phenol at pHs 5.0 and 9.0. The concentration of soluble polymer in the effluent was highest at pH 7, followed by pH 5 and pH 9. It is likely that the solution pH affected the configuration of oligomers produced.

Figure 4.19 illustrates that the insoluble polymer deposited in the column was highest at pH 7 and lowest at pH 9. Huang et al. (2005) reported that precipitation of coupling products increased significantly as solution pH decreased in batch polymerization reaction tests, especially in the range from pH 5 to pH 3. They reported total phenol conversion did not vary with pH and postulated that a fraction of the phenolic sites on the products may be dissociable around neutral pH (Huang and Weber, 2005) and protonation of proton-disassociated sites reduced the ionic character of the products and increased their tendency to precipitate when solution pH dropped. In these experiments, phenol removal was highest at pH 7.0, which is the optimum pH for the HRP-mediated polymerization reaction. Removal efficiency of phenol was higher at pH 5.0 compared to pH 9.0. These results agree with the observations of Huang et al. (2005).

In the case of pH 7.0, 65.5 % of the injected phenol was found to accumulate in the column as insoluble polymer while 51.7 % and 48.2 % of the influent phenol was retained in the porous media at pH 5.0 and pH 9.0 (Table 4.9).

Figure 4.17. Phenol transport behavior through the packed column reactor coincides with soluble polymer resulting from polymerization reaction (pH 5). Influent phenol concentration = 500 µM, solution pH = 5.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

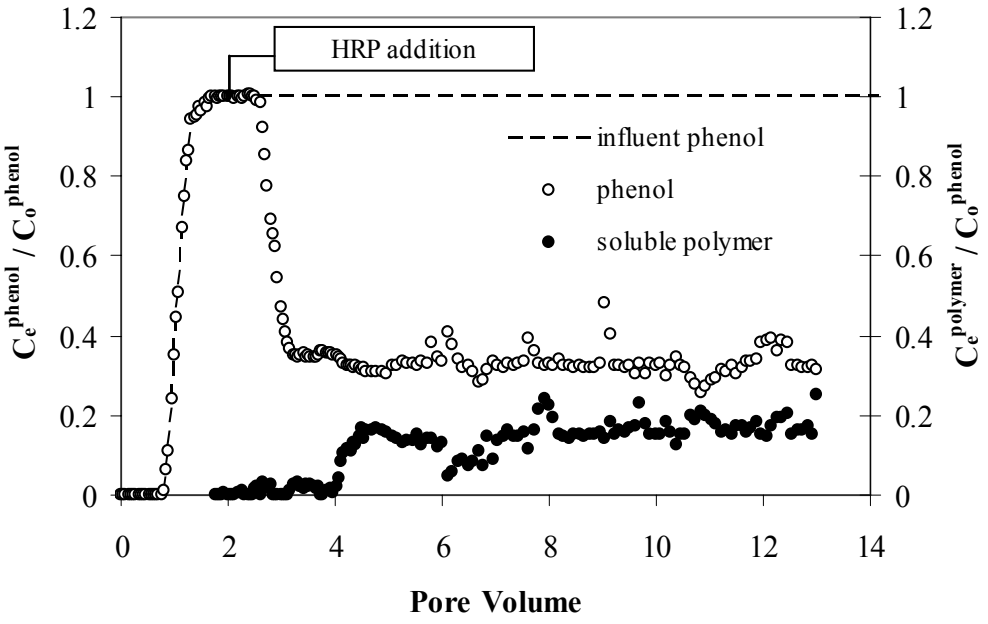


Figure 4.18. Phenol transport behavior through the packed column reactor coincides with soluble polymer resulting from polymerization reaction (pH 9). Influent phenol concentration = 500 µM, solution pH = 9.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

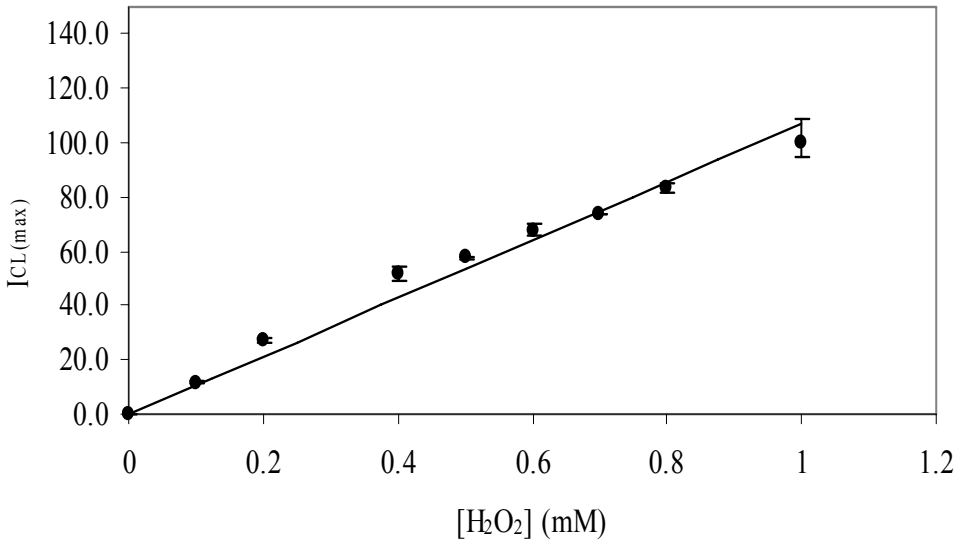


Figure 4.19. Mass balance of insoluble polymer (pH 5, 7 and 9). Influent phenol concentration = 500 µM, solution pH = 5, 7 and 9, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

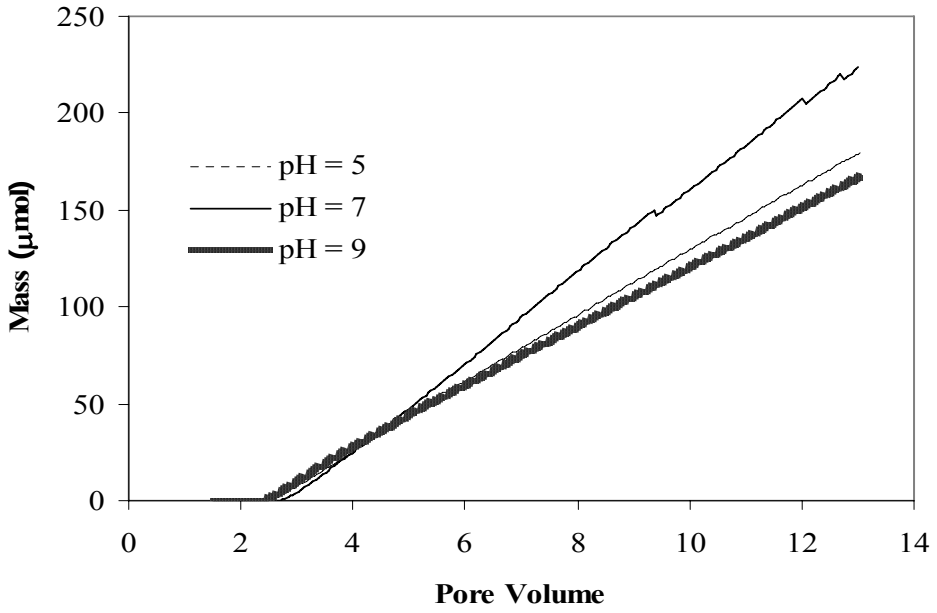


Table 4.9. Mass balance with pH.

Solution pH	Total phenol in (μM)	Total phenol out (μM)	Total soluble polymer out (μM as phenol)	Total insoluble polymer in column (μM as phenol)
5	347.5	133.3	34.48	179.7
7	342.4	61.1	57.23	224.1
9	347.5	133.2	45.9	167.4

Figure 4.20. Transport behavior of KCl (250 mg/L, nonreactive tracer) before and after polymerization in the saturated porous media (pH 5). Polymerization condition: influent phenol concentration = 500 μM, solution pH = 5.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 μM.
TBP = Tracer before polymerization, MTBP = Modeling before polymerization, TAP = Tracer after polymerization, MTAP = Modeling tracer after polymerization.

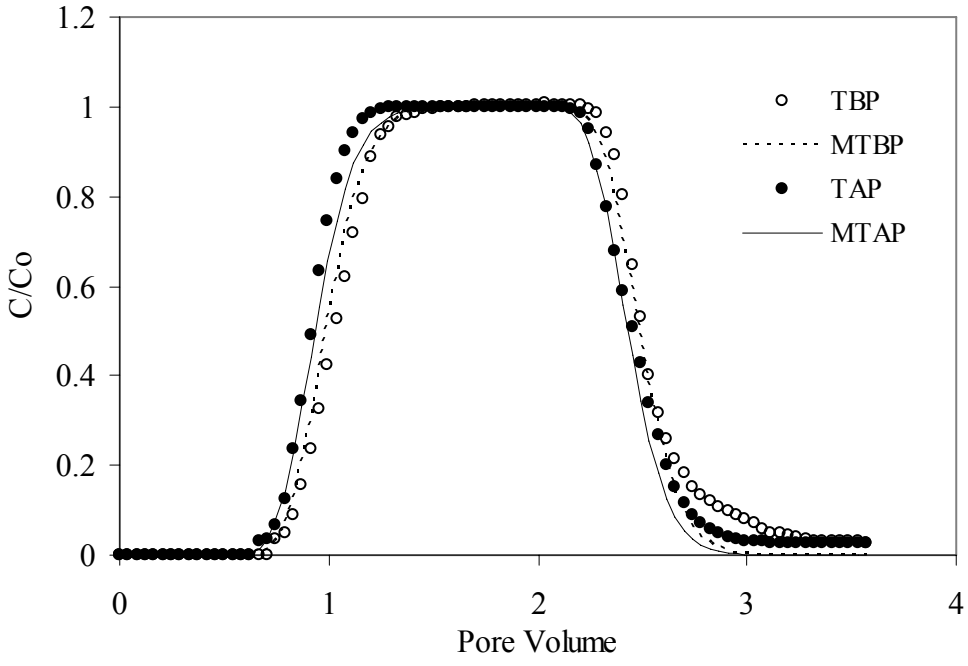
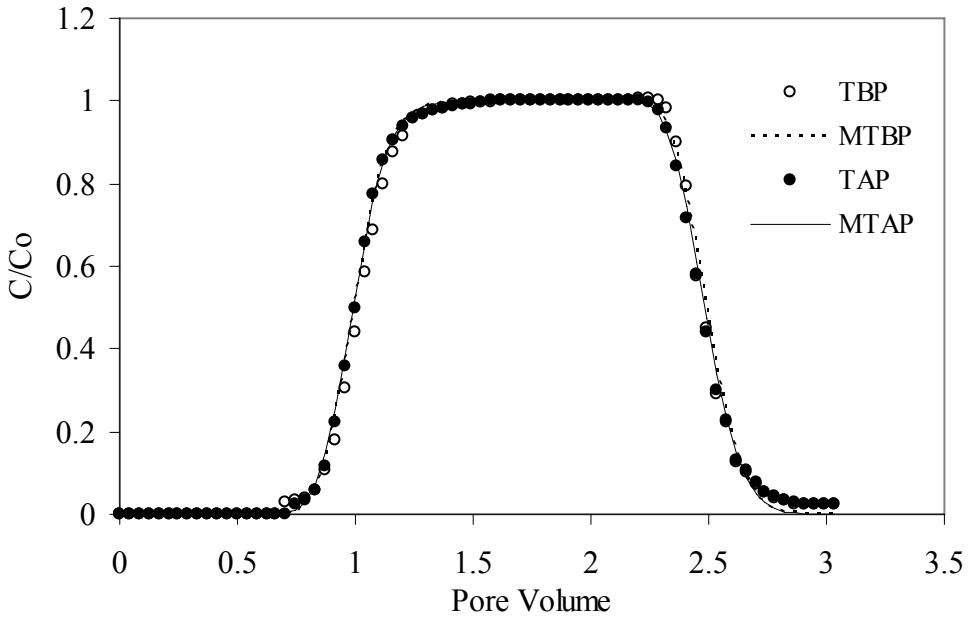


Figure 4.21. Transport behavior of KCl (250 mg/L, nonreactive tracer) before and after polymerization in the saturated porous media (pH 9). Polymerization condition: influent phenol concentration = 500 µM, solution pH = 9.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.
TBP = Tracer before polymerization, MTBP = Modeling tracer before polymerization, TAP = Tracer after polymerization, MTAP = Modeling tracer after polymerization.



The breakthrough curves of tracer for the modified saturated porous media show that the tracer appeared earlier at pH 5 than at pH 9 (Figures 4.20 and 4.21). It was considered that insoluble polymer was deposited in the column and reduced the pore volume. Retardation factors were decreased after polymerization in all cases (Table 4.10). The highest decrease of retardation factor was at pH 7 and lowest at pH 9. However, In the case of pH 9, almost no retardation was observed. There was little difference in the tracer test before and after polymerization since less insoluble polymer was deposited in the column. Estimates of pore volume decrease with pH are summarized in Table 4.11. In the case of pH 9, it can be considered that there was a experimental error or very less amount of polymer deposition in the porous media.

Table 4.10. Parameters estimated with variation of pH.

pH	Condition	v (cm^2/min)	D (cm/min)	R	R^2
5	MTBP	0.997	0.0081	1	0.997
	MTAP	0.997	0.0087	0.995	0.999
7	MTBP	0.110	0.0100	1	0.994
	MTAP	0.110	0.0114	0.922	0.995
9	MTBP	0.997	0.0140	1	0.989
	MTAP	0.997	0.0152	0.947	0.994

MTBP: Modeling Tracer before polymerization

MTAP: Modeling Tracer after polymerization

v: pore water velocity, D: dispersion coefficient, R: retardation, R^2=least square

Table 4.11. Estimation of pore volume occupied by polymer deposition with pH.

pH	estimated pore water velocity (cm/min)	estimated dispersion coefficient (cm^2/min)	R^2	flow Rate (cm^3/min)	estimated Porosity	pore volume decrease (%)
5	0.1052	0.0161	0.994	0.50	0.360	5.26
7	0.1189	0.0124	0.995	0.50	0.350	7.79
9	0.1189	0.0087	0.999	0.55	0.378	0.43

4.4.3 Effect of Solution Ionic Strength

Figures 4.22 and 4.23 illustrate the impact of solution ionic strength on phenol polymerization and polymer production in the continuous flow packed column. Three different ionic strengths (5, 20 and 100 mM) were selected to observe the behavior of phenol, soluble polymer and in soluble polymer. The highest phenol removal was observed at an ionic strength of 20 mM.In the case of 5 mM ionic strength, phenol removal was reduced to ~ 70% (Figure 4.22) and effluent phenol concentration continued to decrease with time (volume). The soluble concentration exiting the column was lowest at the 100 mM ionic strength (Table 4.12), most likely due to a combination of configurational changes in the polymeric macromolecules and the 'salting out' effect at the high ionic strength solution (Schwarzenbach et al., 1993). Huang et al. (2005) observed similar results when they increased solution ionic strength in batch polymerization of phenol using HRP. They found that phenol conversion itself was not responsive to salt addition. It is evident that certain dissolved coupling products become more 'precipitable' as background ion concentrations increase.

Figure 4.22. Phenol transport behavior through the packed column reactor coincides with soluble polymer resulting from polymerization reaction (IS 5 mM). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 5 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

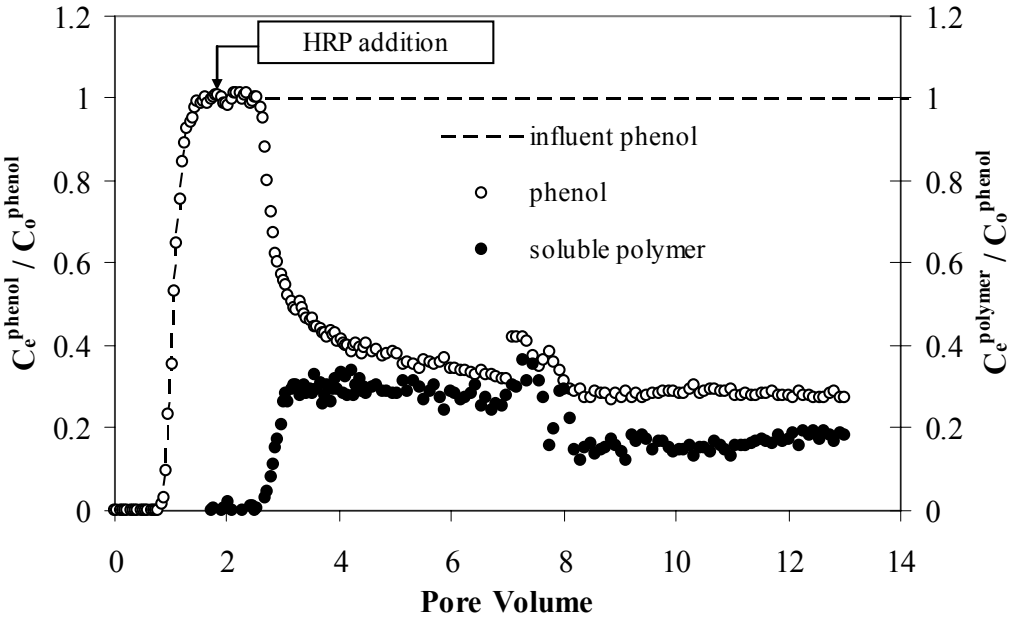


Figure 4.23. Phenol transport behavior through the packed column reactor coincides with soluble polymer resulting from polymerization reaction (IS 100 mM). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 100 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

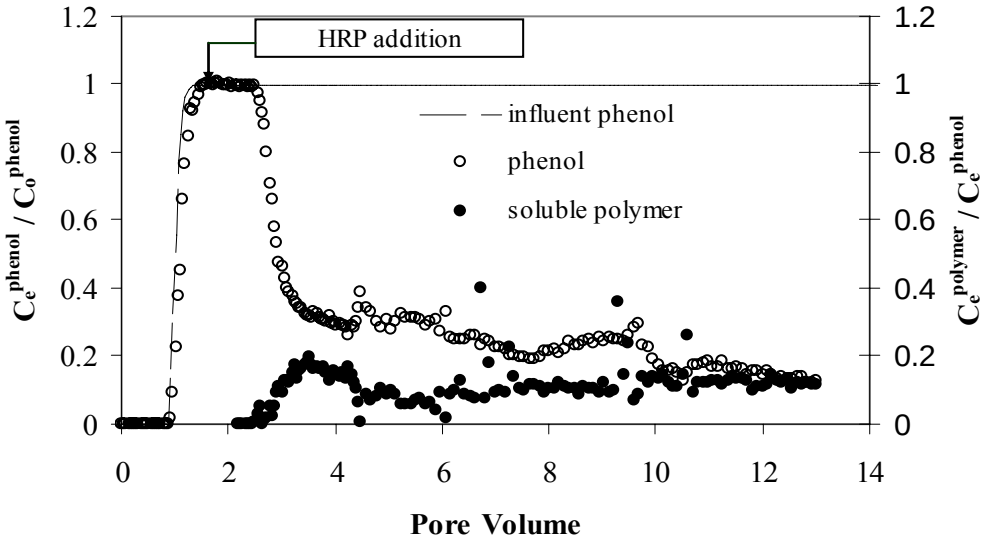


Figure 4.24. Mass balance of insoluble polymer (IS 5, 20, and 100 mM). Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 5, 20 and 100 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

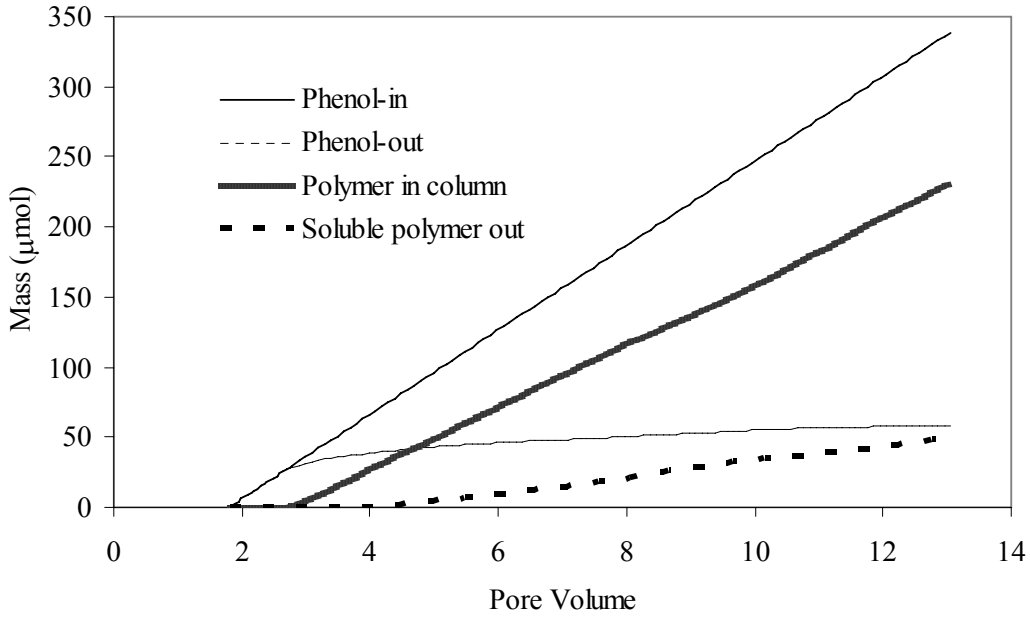


Table 4.12. Mass balance with ionic strength.

Ionic strength (mM)	Total phenol in (µM)	Total phenol out (µM)	Total soluble polymer out (µM as phenol)	Total insoluble polymer in column (µM as phenol)
5	347.5	129.2	73.5	144.8
20	342.4	61.1	57.2	224.1
100	347.5	99.2	37.3	211.1

There was minimal difference in tracer behavior before and after polymerization at 5 mM ionic strength solution (Figure 4.25). However, the nonreactive tracer appeared earlier after polymerization in 100 mM ionic strength as shown in Figure 4.26. Deposition of insoluble polymer was significantly higher at 20 and 100 mM ionic strength solution than at 5 mM ionic strength. The retardation factors for both 20 mM and 100 mM are similar, which is consistent with the mass balance results. These results indicate that high ionic strength positively affects polymer deposition (Table 4.13). About 8 % of pore volume was decreased using 100 mM of ionic strength. The decrease of pore volume was increased with ionic strength as shown in Table 4.14. However, higher than 20 mM of ionic strength does not significantly affected the deposition of polymer in porous media.

Figure 4.25. Transport behavior of KCl (250 mg/L, nonreactive tracer) before and after polymerization in the saturated porous media (IS 5 mM). Polymerization condition: influent phenol concentration = 500 µM, solution pH = 7, solution ionic strength = 5 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

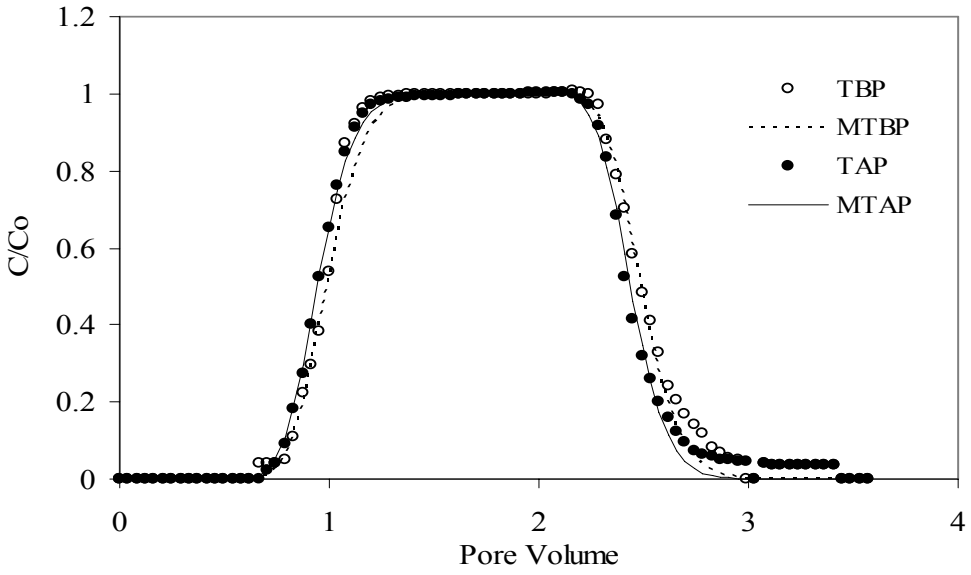


Figure 4.26. Transport behavior of KCl (250 mg/L, nonreactive tracer) before and after polymerization in the saturated porous media (IS 100 mM). Polymerization condition: influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 100 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

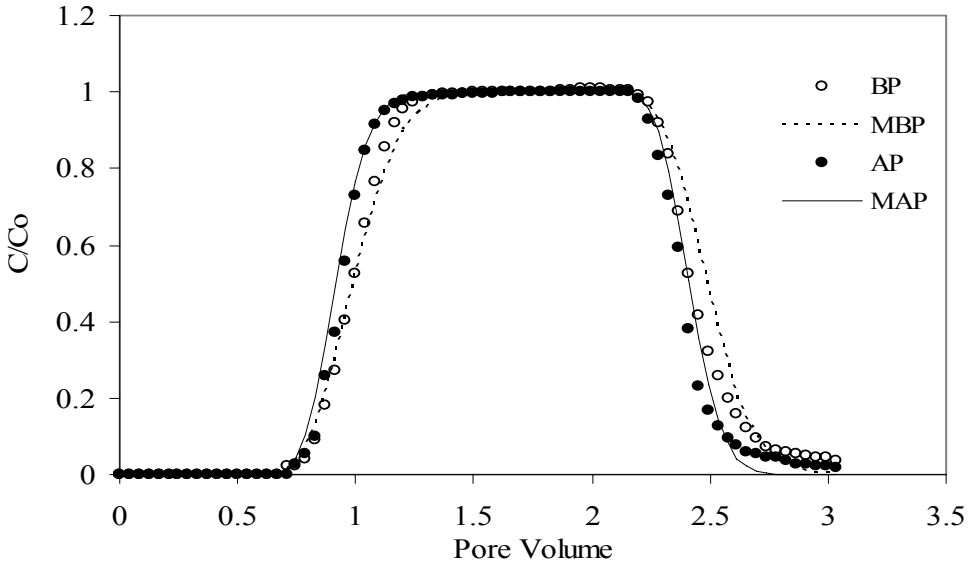


Table 4.13. Parameters estimated with variation of ionic strength.

Ionic strength (mM)	Condition	v (cm^2/min)	D (cm^2/min)	R	R^2
5	MTBP	0.997	0.0127	1	0.993
	MTAP	0.997	0.0126	0.952	0.997
20	MTBP	0.110	0.0100	1	0.994
	MTAP	0.110	0.0114	0.922	0.995
100	MTBP	0.997	0.0142	1	0.990
	MTAP	0.997	0.0083	0.921	0.993

MTBP: Modeling Tracer before polymerization

MTAP: Modeling Tracer after polymerization

v: pore water velocity, D: dispersion coefficient, R: retardation, R^2=least square

Table 4.14. Estimation of pore volume occupied by polymer deposition with ionic strength.

Ionic strength (mM)	estimated pore water velocity (cm/min)	estimated dispersion coefficient (cm^2/min)	R^2	flow rate (cm^3/min)	estimated porosity	pore volume decrease (%)
5	0.105	0.0132	0.993	0.50	0.362	4.81
20	0.119	0.0124	0.995	0.55	0.350	7.89
100	0.108	0.0090	0.995	0.50	0.350	7.80

4.5 CONCLUSIONS AND IMPLICATIONS

This study investigated the impact of polymerization reaction condition in continuous flow-saturated porous media. Results for these experiments showed that phenol entering a packed column under simulated aquifer conditions was removed from the aqueous phase by injecting HRP and H_2O_2 into the flow (Hypothesis 1). Removal of phenol increased with HRP dose. More than 90% of the influent phenol was removed after about 1.5 pore volume from injection of 2.0 AU/mL of HRP dose. HRP-mediated phenol removal in continuous flow-saturated porous media was accompanied by the generation of soluble and insoluble oligomeric products (Hypothesis 2). Soluble reaction products and insoluble products increased with HRP dose. While about 20% of the influent phenol exited the column as soluble polymerization products, nearly 62.6% of the total influent phenol was retained in the porous media as precipitated products.

Phenol removal and the production of oligomeric products under the action of HRP and H_2O_2 was affected by the enzyme dose, solution pH and solution ionic strength (Hypothesis 3). The concentration of soluble reaction products exiting the column was lowest at the 100 mM ionic strength. Deposition of insoluble polymer was significantly higher at 20 and 100 mM ionic strength solution than at 5 mM ionic strength. Optimum polymer deposition occurred at pH 7. The amount of soluble polymer produced was also highest at pH 7, followed by pH 5 and pH 9.

Deposition of insoluble phenol polymerization products in the packed column resulted in physical modification of the porous media (Hypothesis 4). Modification of porous media could be explained by modeling process. The variation of retardation factors was increased with increasing enzyme dose and ionic strength while the variation of dispersion coefficient was negligible. Maximum 8 % of pore volume (100 mM of ionic strength) was deposited by insoluble polymer from the polymerization reaction. The highest decrease of retardation factor was at pH 7 and lowest at pH 9.

Real-World Implications

The HRP-mediated oxidative polymerization process has a potential for application as a remediation technique for rapidly moving phenol plumes in saturated aquifers. HRP-mediated in

situ contaminant stabilization works best under most groundwater conditions (pH and ionic strength) and for the most soluble (and mobile) phenolic contaminants. HRP-mediate in situ stabilization of phenol should be carefully managed as it can result in desirable or undesirable clogging of the porous media.

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CHAPTER 5. IMPACTS OF MODIFIED SATURATED POROUS MEDIA ON TRANSPORT BEHAVIOR OF 2,4-DCP USING HRP-MEDIATED COUPLING REACTION OF PHENOL

5.1 INTRODUCTION

Chemical contamination of soil and groundwater is widespread and frequent and has become a great concern in the U.S. and around the world (Madsen et al., 1991; Tursman and Cork, 1992). Aquifers are frequently contaminated by mixtures of organic compounds from spills, leaking underground storage tanks, or landfills. For example, more than half of the Department of Energy sites are contaminated with mixtures of two or more compound classes (Riley, 1992). These compounds include chlorinated solvents, aviation fuel hydrocabons (mostly aromatics), and pesticides.

Groundwater pump and treat technologies frequently involve high cost and low efficiency. Intrinsic in situ bioremediation may be achieved at low cost, but proceeds very slowly. Therefore, alternative enhanced in situ remediation approaches are needed to treat contaminated sites and meet the nations's most pressing site cleanup and waste management needs.

Enzyme-mediated *in situ* stabilization has been advocated as a new approach for the treatment of phenolic compounds in soils and groundwater. Peroxidase enzymes catalyze the oxidation of hydroxylated aromatic compounds in the presence of hydrogen peroxide producing insoluble oligomers that precipitate out of solution. This approach can be used for a wide wariety of phenolic compounds. The reactions are stable at normal groundwater pH and over large concentration ranges of the contaminant. Enzymatic polymerization reactions occur at rates that are significantly higher than biological processes (Karam and Nicell, 1997).

Although many researchers have discussed the advantages and applications of this method to groundwater or subsurface cleanup, few experiments have been performed to evaluate the applicability of this method in saturated porous media. Chapter 4 of this dissertation discussed results from column experiments that clearly showed the removal of phenol and deposition of phenol polymerization products in a saturated column packed with Ottawa sand. Deposition of polymerization products resulted in modification of the porous media.

The depostion of phenol polymerization product resulted in the creation of a previously nonexistent organic phase in the porous media. The presence of this organic domain has the potential to greatly impact the transport of upstream contaminants. Thus, the motivation for this work was to study the impact of the synthetically produced organic phase on the transport of hydrophobic contaminants in the modified porous media under saturated flow conditions.

5.2 BACKGROUND

5.2.1 2,4-Dichlorophenol

Chlorinated phenols have been widely used in industrial and agricultural products and can be found in both domestic and industrial wastewater (Schellenberg et al., 1984).

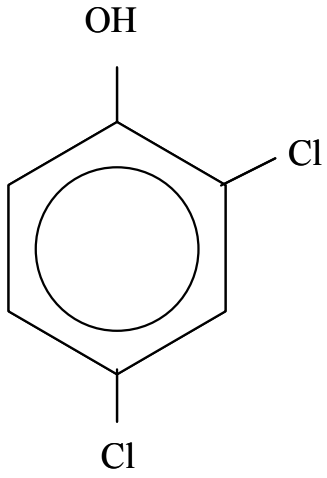
Chlorophenols are synthetic chemicals that are formed by reacting chlorine gas with phenol. Chlorophenols are normally released into the environment from leaching of lumber and agricultural run off. Among these, 2,4-dichlorophenol (2,4-DCP) is of significant environmental concern because of its use in moth proofing and as an antiseptic. Because of their inherent toxicity and relative persistence, many countries restrict the use and disposal of these compounds.

DCP is also used as an ingredient in the production of herbicides such as 2,4-dichlorophenoxyacetic acid. This chemical is also used in a feedstock mixture to produce wood preservative (Kent, 1983). Because of the inherent toxicity and relative persistence of chlorinated organics, the pesticide industry has largely substituted the use of chlorine in pesticides with phosphorous. This raw material substitution has produced a significant decrease in the persistence, and a large drop in the environmental release of chlorinated phenols.

5.2.1.1 Chemical and Physical Properties of 2,4-DCP

Chlorophenols are organic chemicals formed from phenol by substitution in the phenol ring with one or more chlorine atoms (Esposito et al. 1980). The structure of 2,4-dichlorophenol consists of a benzene ring, to which are attached a hydroxyl (OH) group and two chlorine atoms in positions 2 and 4 as shown in Figure 5.1 (Kiefer et al. 1998).

Figure 5.1. Molecular structure of 2,4-DCP.



Chlorinated phenols are weak acids with pKa values that generally decrease with increasing number of chlorine atoms on the phenol ring (Kishino and Kobayashi, 1994). The pKa of 2,4-DCP is 7.9 allowing it to exist in both protonated and ionic forms in the natural water and soil environments (pH 6.0-8.0). The pH can significantly affect the properties of DCP including its fate and transport in natural and engineered environments (EPA 1987; Kiefer et al. 1998). At pH 7.0, approximately 83% of DCP exists in its unionized form (Palomo, 2003).

The half-life of DCP can vary with environmental conditions. The half-life values of 2, 4-DCP in various environments are summarized in Table 5.1. Other key properties are listed in Table 5.2.

Table 5.1. Half-Life Values for DCP in Various Environments.

Environment	Half-Life (days)
Air	0.89 to 8.8
Distilled water	Summer: 0.03 Winter: 0.13
Sea water	Summer: 5 Winter: 23
Groundwater	133 to 1032
Sediment	Summer: 47 Winter: 116

(Mackay et al., 1997)

Table 5.2. Selected Physical and Chemical Properties of 2,4-DCP.

Property	Value
Molecular Weight (g/mol)	163
Aqueous Solubility (mg/L)	4,500
Melting Point (^{o}C)	45
Boiling Point (^{o}C)	210
Density (g/cm^{3})	1.38
pKa	7.68
log K_{ow}	3.06
log K_{oc}	2.49

(Mackay et al., 1997; ATSDR, 1997)

2,4-DCP is usually considered a polar compound due to the H-bonding ability of the hydroxyl group although it has the significant hydrophobic surface areas. The hydroxyl group also permits orientational interactions with the sorbent.

5.2.1.2 Toxicity of 2,4-DCP

The toxicity of chlorinated phenols is dependent on the number and position of the chlorine substituents in the benzene ring (Kishino and Kobayashi 1994). Toxicity increases with greater chlorine substitution. Pentachlorophenol (PCP) which is occupied by chlorine in all five available substituent positions is known to be the most toxic among the chlorinated phenols (Kiefer et al. 1998; Janik and Wolf 1992). The LD_{50} (lethal dose for 50% kill) values of selected chlorinated phenols are summarized in the Table 5.3.

Table 5.3. LD_{50} Values of Chlorinated Phenols.

Compound	Species	LD_{50} (mg/kg)
2,4-DCP	Rat, oral	580
2,3,4,6-TCP	Rat, oral	140
PCP	Rat, oral	27-78

(Verschueren, 1977)

Mackay et al. (1977) reported that 2,4-DCP has a bioaccumulation factor of 1.0 for trout, 1.53 for goldfish, and 2.41 for algae in food chain uptake. Algae appear to be some of the most sensitive organisms to chlorophenol exposure (Ruckdeschel et al., 1987).

Crespin et al (2001) reported that 2,4-DCP metabolites are more toxic than the parent compound when absorbed through the skin. DCP is extremely toxic to some plants. Huang and Gloyna (1968) observed the total destruction of chlorophyll after applying 100,000 μg/L of DCP to *Chlorella pyrenoidosa*.

For humans and mammals, 2,4-DCP can be readily adsorbed through breathing and cause a risk of cancer (Hill et al., 1989). EPA has reported that exposure to high concentrations of DCP shows carcinogenic traits and can damage the immune system and liver (ATSDR, 1999).

5.2.1.3 Environmental Contamination by 2,4-DCP

Chlorophenols have been found in the water, air and soil of many ecosystems. DCP may be produced from the chlorination of water and the breakdown of other chemicals found in wastewater, drinking water, and soil (Kerkvliet et al., 1985). Peuravouri et al (2002) reported the

presence of DCP in soil, air, water and wastewater indicating contamination from DCP is ubiquitous at mostly ng/L level.

DCP has also been found in the effluents from industries associated with the production of iron and steel, electrical components, photographic equipment, pharmaceuticals, and paper and pulp (EPA, 1987). It was estimated that 741,000 pounds of 2,4-DCP were released in 1977 from U.S. production facilities, mainly as industrial discharges (ATSDR, 1999). In 2000, the total on-site releases of DCP were 6,600 pounds (TRI, 2000).

Release of DCP in soil occurs primarily due to pesticide use and deposition from the atmosphere. It is estimated that 12,000 pounds of 2,4-DCP were discharged directly to land in 1998 from production facilities (ASTDR, 1999). DCP was found at 14 out of 471 waste disposal sites and detected in leachates collected at municipal landfills (ATSDR, 1997).

The primary source of air emissions of chlorophenols is by volatilization during production-related activities and the manufacturing of other end-use products. In 1991, 1432 pounds of 2,4-DCP was released as air emissions (TRI, 1991). 2,4-DCP has also been detected in the combustion of hazardous waste, coal, wood, and municipal solid wastes.

5.2.1.4 Environmental Regulations

DCP was listed in priority list of hazardous substance by section 104 of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) due to the high solubility, mobility and potential for human exposure (ATSDR, 2001). Discharge permits (National Pollutant Discharge Elimination System) are required for DCP and any release must be reported by all industries subject to TRI reporting. The 24-hour discharge limitation of DCP in wastewater is 112 mg/L and ambient water quality criteria of 2,4-DCP is 3.08 mg/l (ATSDR 1990). The maximum concentration of

PCP in the groundwater was 2,000 µg/L, compared to the drinking water maximum contaminant level (MCL) of 5 µg/L. These requirements are regulated according to the 1987 Clean Water Act. On an International level, DCP was classified as group 2B carcinogens by the international Agency for Research and Cancer (IARC) indicating the limited evidence for human carcinogenicity (IARC, 1987).

5.2.1.5 Treatment of 2,4-DCP

Biological treatment is normally the cheapest and simplest technology to remove organic chemicals. However, biological oxidation of chlorophenols is often difficult because of their toxicity and electron deficient characteristics. It is known that several commercial products from plants and microorganisms can potentially transform DCP to less or non-toxic products such as peroxidase.

Ozone pretreatment is one of the possible methods to enhance chlorophenol biodegradation. Agustina et al., (2005) reported that ozone pretreatment of DCP showed promising results and much higher levels of COD removal. Freedman et al. (1989) reported enzymatic detoxification using horseradish peroxidase enzyme to remove chlorophenol from drinking water and wastewater. In the presence of hydrogen peroxide, HRP caused enzymatic cross-linking of the substrate forming insoluble polymers that were removed from solution by precipitation or filtration.

5.2.2 Solute Fate and Transport in Porous Media

The predictions of contaminant transport and strategies to remediate existing problems or improve containment must be based on an understanding of the geochemical factors influencing contaminant migration in groundwater environments. Transport of contaminants in aquifer systems is affected by a number of processes. These include advection, dispersion, diffusion, adsorption and degradation. These processes can work together or separately in groundwater flow.

5.2.2.1 Advection

Advection is the process that transports dissolved solutes with groundwater flow. It is the most dominant mass transport process (Domenico, 1987) and critical to understanding or predicting solute transport in groundwater flow system. For one-dimensional flow normal to a unit cross-sectional area of the porous media, the rate and direction of groundwater flow can be characterized by the average linear velocity (v_x) which is the flux of water across the unit cross-sectional area of pore space occurs. The average linear velocity of a fluid flowing in a porous media is determined using Darcy's law (Domenico, 1987):

$$v_x = \frac{K}{n_e}\frac{dh}{dl} \qquad \text{Eq. (5.1)}$$

where:

v_x = average linear velocity [L/T]

K = hydraulic conductivity tensor of the porous medium [L/T]

n_e = effective porosity

dh/dl = hydraulic gradient [L/L]

Effective porosity and hydraulic conductivity are both properties of the porous media. The effective porosity is the pore space through which water can flow. It does not include noninterconnected and dead-end pores. The hydraulic gradient can be determined by solving the equations of flow using appropriate initial and boundary conditions (Domenico, 1987).

5.2.2.2 Dispersion

Water in porous media generally moves faster or slower than the average linear velocity. This behavior is caused by three phenomena:

1) water moves faster in the center of the pore than along the edges because of pore friction at pore walls;
2) some water molecules travel along longer flow paths in the porous media than other particles; and
3) sizes of pores are different, and water moves faster through smaller pores.

Dispersion produces the spreading of solute beyond the region that would be affected by advection. Mechanical dispersion and diffusion are the two processes responsible for hydrodynamic dispersion in porous media (Domenico, 1987).

Mechanical dispersion is the mixing of solute-containing water and background water due to movement through complex pore structures. A dilution of the solute at the advancing edge of flow is generally observed. If a solute is diluted in the porous medium it follows the path of

normal flow and is called lateral dispersion. Transverse dispersion can also occur with longitudinal dispersion. Lateral dispersion is generally much greater than transverse dispersion.

Mechanical dispersion is defined as the product of dynamic dispersivity and average linear velocity (Clark, 1996):

$$D_i = \alpha_i v_x \qquad \text{Eq.(5.2)}$$

where:

D_i = dispersion coefficient in the i^{th} direction [L^2/T]

α_i = the dynamic dispersivity in the i^{th} direction [L]

v_x = the average linear velocity in the x-direction [L/T]

Dispersivity is a property of the porous medium that is proportional to the scale of the system under consideration (Gelhar et al., 1992). The dispersivity of solute in a porous medium in a laboratory column would be considerably smaller than the dispersivity in an aquifer through which contaminated groundwater is flowing over distances of hundreds of meters. Presumably, this scale-effect is due to the increased spreading caused by variations in velocities due to larger scale heterogeneities (Domenico, 1987).

Diffusion is a process that can occur in the absence of advection. Molecular diffusion is the movement of molecules from regions of high concentration to those of low concentration. Diffusion is not important in systems with high velocity because the effects of advection are much larger than those of diffusion. Molecular diffusion is generally found to have affects at very low velocities and is usually ignored in modeling contaminant transport (Clark, 1996).

Diffusion processes should be considered with mechanical dispersion in the porous media system. The hydrodynamic dispersion coefficient is defined as a sum of mechanical dispersion and effective diffusion coefficient (Bear, 1969):

$$D_i = \alpha_i v_i + D^* \qquad \text{Eq. (5.3)}$$

where,

D_i = hydrodynamic dispersion coefficient in the i^{th} direction of flow

D^* = effective diffusion coefficient

5.2.3 Sorption

Sorption is a key phenomenon controlling the fate and transport of organic solutes in aquifers. Although most aquifer materials generally have a relatively low organic matter content, as little as 0.1% organic matter can produce significant impacts on the behavior of organic pollutants in groundwater systems (Seol and Lee, 2000). If the sorption process is rapid compared to flow velocity, this phenomenon can be described by several equilibrium sorption models including the partitioning model, the Langmuir model and the Freundlich Model. Other models are used to describe non-equilibrium sorption or irreversible binding of solutes to the porous media.

5.2.3.1 The Partitioning Model

The partitioning or linear sorption model assumes a proportional relationship between the concentration of the solute in the aqueous phase and its concentration on the solid phase at equilibrium. The linear adsorption isotherm in natural waters can be described by the equation (Domenico, 1987):

$$q_e = K_d C \qquad \text{Eq. (5.4)}$$

where:

q_e = sorbed concentration [M solute / M solids]

K_d = distribution coefficient [L^3/M]

C = dissolved solute concentration in solution [M/L^3]

When a linear equilibrium condition is established without chemical reaction, Eq. (1.1) reduces to an equilibrium advection dispersion equation,

$$R\frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial x^2} - v_x \frac{\partial C}{\partial x} \qquad \text{Eq. (5.5)}$$

where R is the dimensionless retardation factor and is given by

$$R = 1 + \frac{\rho k_d}{\theta} \qquad \text{Eq. (5.6)}$$

5.2.3.2 The Langmuir Model

The Langmuir adsorption model describes the thermodynamics and kinetics of solute phase distribution between a sorbent surface and solution (Langmuir, 1918). The sorbent surface is considered to contain a fixed number of energetically identical active sites where solute molecules may be chemically bound. The model has the following form:

$$q_e = \frac{QbC_e}{1 + bC_e} = \frac{q_m C_e}{1 + bC_e} \qquad \text{Eq. (5.7)}$$

where q_e is the solid-phase solute concentration at equilibrium; Q is the monolayer adsorption capacity or the maximum number of moles adsorbed per mass of sorbent when the surface sites are saturated with solute; *b* is an empirical constant related to the affinity of the surface for the solute; and q_m refers to the maximum sorption capacity.

Key assumption of the Langmuir model are that (i) adsorption energy in a fixed number of sorption sites is homogeneous, (ii) there is no interaction between adjacent adsorbed molecules on the sorbent surface, and (iii) the adsorbent surface can hold only one layer of solute molecules (Ruthven, 1984).

The energy of sorption may vary because real surfaces are heterogeneous. It is, therefore, considered that the Langmuir model is generally inappropriate to describe equilibrium sorption of HOCs on soils and other natural sorbents (Weber et al., 1998). In practice, the actual number of sites per unit surface area is unknown, and the primary interest is the number of moles of sorbate per mass of sorbent at equilibrium, q_e.

If the sorption characteristics can be described by Langmuir sorption iostherm, Equation (1.1) can be organized as follows:

$$\frac{\partial C}{\partial t}\left[1 + \frac{B_d}{\theta}\left(\frac{\alpha\beta C}{(1+\alpha C)^2}\right)\right] = D_L \frac{\partial^2 C}{\partial x^2} - v_x \frac{\partial C}{\partial x} \qquad \text{Eq. (5.8)}$$

5.2.3.3 The Freundlich Model

The Freundlich sorption isotherm (Freundlich, 1926) attempts to supplement the Langmuir model's assumption that the energy of sorption is the same for all surface sites and independent of the degree of coverage. This model assumes that the frequency of sites associated with a free energy of adsorption decreases exponentially with increasing free energy.

The Freundlich sorption equation is widely used for describing nonlinear sorption of organic solutes on soils and sediments. It is often regarded as an empirical model with the following mathematical representation:

$$q_e = K_F C_e^n \qquad \text{Eq. (5.9)}$$

where C_e is the equilibrium aqueous concentration of solute, q_e is the solid-phase solute concentration in equilibrium with C_e. The pre-exponential term K_F is the Freundlich sorption capacity of the solid, and the exponent *n* is the isotherm nonlinearity parameter which is indicative of the sorbent's surface heterogeneity distribution.

Equation (5.9) is often linearized as follows:

$$\log q_e = \log K_F + n \log C_e \qquad \text{Eq. (5.10)}$$

When $n > 1$, a convex isotherm (Type I) is produced. The sorption constant, K_F, increases with increasing solution concentration, perhaps reflecting an increase in the hydrophobic character of the surface after a monolayer has been established.

A concave isotherm (Type III) is produced when $n < 1$. K_F decreases with solution concentration as the low energy sites are occupied and modified by previously sorbed molecules. In most cases, the sorption of hydrophobic solutes to geosorbents can be represented by Type III Freundlich isotherms.

When solute sorption characteristics in the porous media can be described by the Freundlich sorption isotherm, the ADE equation (Eq. 1.1) can be written as:

$$\frac{\partial C}{\partial t}\left(1+\frac{B_d KnC^{n-1}}{\theta}\right)=D_L\frac{\partial^2 C}{\partial x^2}\quad -v_x\frac{\partial C}{\partial x} \qquad \text{Eq. (5.11)}$$

5.2.3 Groundwater Treatment

Groundwater systems contaminated with phenols pose a high risk to ecosystem health because of the multiple toxic effects associated with these chemicals even at very low concentration. Groundwater pump and treat technologies frequently involve high cost and low efficiency. Intrinsic in situ physicochemical and biological techniques may be available and achieved at low cost, but proceeds very slowly.

Therefore, alternative enhanced in situ remediation approaches are needed to treat contaminated sites for the decontamination of phenolic contaminants in groundwater system. Enzyme-mediated *in situ* stabilization has been also advocated as a new approach for the treatment of phenolic compounds in soils and groundwater. Its reaction rates are significantly higher than biological process. Furthermore, these reactions are stable at normal groundwater pH and over high concentration range of the contaminant.

However, there is no availability on information to apply this method to groundwater syst em from recent literature review. To obtain the basic knowledge of HRP-mediated oxidative cou pling polymerization in the presence of porous media, removal of target chemical and impacts of porous media modified by polymer produced should be studied.

In this study, we investigated the phenol removal using the optimized condition of HRP-mediated oxidative polymerization process in saturated porous media and the impacts of modified saturated porous media on the hydrophobic solute transport.

Specific research hypotheses were:

Hypothesis 1

Deposition of insoluble phenol polymerization products in the packed column results in physical modification of the porous media.

Hypothesis 2

Hydrophobic solutes experience retardation in the modified porous media as a result of their interaction with the newly developed organic phase comprised of insoluble oligomeric products.

5.3 EXPERIMENTAL APPROACH

5.3.1 Reagents, Materials and Equipment

The target chemical, 2,4-dichlorophenol (2,4-DCP, > 99%) was purchased from Sigma Chemicals (St. Louis, MO). An Orion Sage Syringe Pump (Orion M365) was used to study the transport of 2,4-DCP in the saturated porous media. All other chemicals, materials and equipment used in this study were previously described in Chapter 4, section 4.3.1.

Phenol was selected to obtain the fundamental understanding of polymerization reaction in saturated porous media and to investigate the impact of enzyme-mediated polymerization reaction in saturated porous media. The presence of this organic domain has the potential to greatly impact the transport of downstream contaminants. 2,4-DCP was used to study the impact of the synthetically produced organic phase on the transport of hydrophobic contaminants in the modified porous media under saturated flow conditions

5.3.2 Experimental Method

Most experimental procedures utilized in this work have been described in section 4.3.2 of Chapter 4. The impact of phenol polymer deposition on transport of 2,4-DCP in the saturated porous media was evaluated by injecting the solute into the column before and after *in-situ* phenol polymerization.

The column study consisted of the following sequence of experiments:

(1) injection of a conservative tracer (KCl, 250 mg/L);
(2) washing of column with deionized water;
(3) injection of buffer solution (pH 7.0, phosphate buffer, ionic strength 20 mM)
(4) injection of 2,4-DCP (200 μM) to evaluate transport behavior in porous media
(5) washing of column with buffer solution

(6) injection of phenol to saturate the column with phenol solution

(7) injection of HRP and H_2O_2 with phenol to facilitate *in-situ* polymerization of phenol in the porous media

(8) washing of column with deionized water

(9) injection of tracer (KCl, 250 mg/L) to evaluate modification of porous media resulting from polymer deposition

(10) washing of column with deionized water

(11) injection of buffer solution

(12) injection of 2,4-DCP (200 μM) to evaluate transport behavior in modified porous media

(13) washing of column with buffer solution.

Properties of the porous media and the column condition used were identical to those summarized in Tables 4.3 and 4.4 of Chapter 4. Selected parameters are summarized in Table 5.4. Material and procedures of modeling are also discussed in Chapter 4.3.3.

Table 5.4 Experimental conditions used in column experiment.

Experimental parameter	Value
Influent phenol concentration	500 μM
Influent H_2O_2 concentration	500 μM
Influent HRP concentration	2.0 AU/mL
Solution ionic strength	20 mM
Solution pH	7
Influent 2,4-DCP concentration	200 μM

5.4 RESULTS AND DISCUSSION

This section presents results of laboratory-scale experiments conducted to evaluate the transport of 2,4-DCP in unmodified and modified saturated porous media. Modification of the porous media was a consequence of the deposition of polymeric material during peroxidase mediated treatment of phenol contaminated water.

5.4.1 DCP Transport in Unmodified Saturated Porous Media

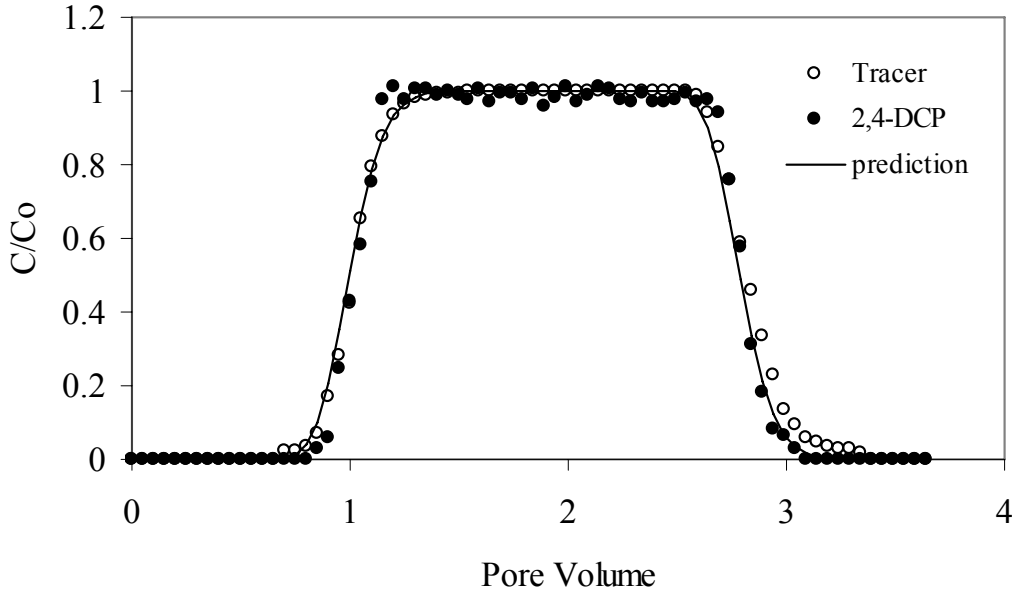
DCP transport was evaluated in a column packed with Ottawa sand. DCP was added to a pH 7.0 and 20 mM ionic strength buffer to yield an influent solute concentration of 200 μM. At this pH approximately 83% of DCP was expected to be protonated while about 17% was ionized. The breakthrough curve for DCP is shown in Figure 5.2. The figure also shows the breakthrough curve obtained for the nonreactive tracer (KCl). The two curves appear to overlap indicating that DCP was not retarded in the porous media. The tracer and DCP appeared in the effluent after ~ 0.8 pore volumes had passed through the column reactor. The C/C_0 values approached 1.0 at approximately 1.2 pore volumes.

When the mobile phase in the column was replaced with distilled-deionized water, the relative concentration of the tracer in the effluent dropped to zero within one pore volume. The symmetrical breakthrough curve of the tracer is indicative of the uniformity of the porous material in the column. The sand column appeared to have been packed uniformly since the tracer demonstrated an ideal transport behavior in the column.

The breakthrough curves also verified that the tracer was conserved since more than 98% of the injected tracer and 2,4-DCP were recovered in the effluent. The column packing in all other experiments had similar hydrodynamic properties since all breakthrough curves had very similar shapes and retention times.

The transport behavior of 2,4-DCP and tracer was estimated using CXTFIT software. The dispersion coefficients for the tracer and 2,4-DCP were estimated to be 0.01118 cm^2/min (R^2=0.993) and 0.005767 (R^2=0.996) without retardation parameter, respectively. Since the variation of dispersion coefficient is negligible, 2,4-DCP curve appears to coincide with that of the nonreactive tracer. 2,4-DCP does not experience any retardation during flow through the saturated porous media.

Figure 5.2. Transport behavior of tracer and 2,4-DCP through the packed column reactor. Influent 2,4-DCP concentration, = 200 μM, solution pH = 7, solution ionic strength = 20 mM.



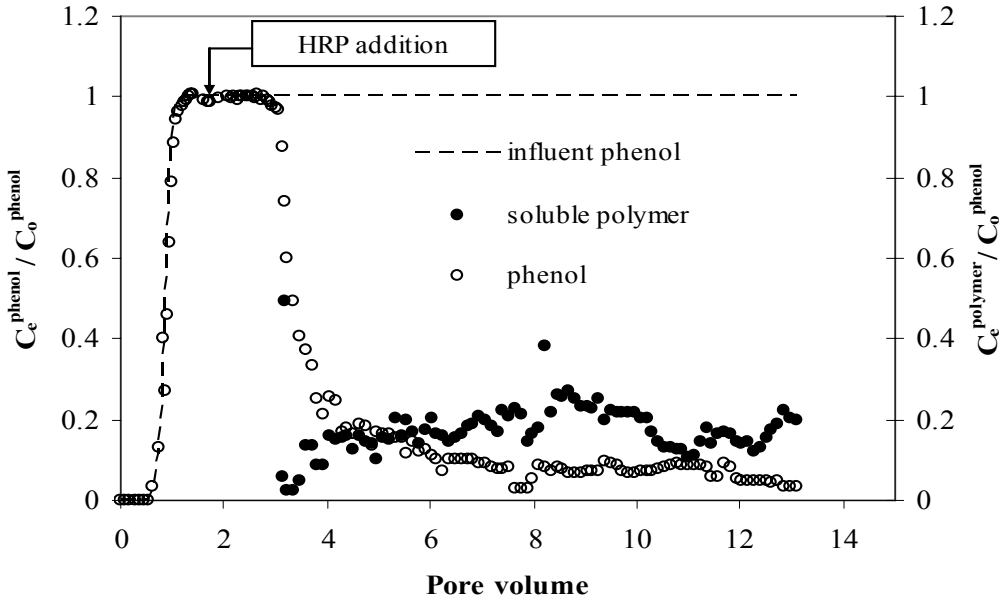
5.4.2. Phenol Polymer Production and Deposition in Porous Media

Phenol polymers were produced and allowed to deposit in the porous media using experimental protocols similar to those described in Chapter 4. Results of phenol removal and deposition in the column are illustrated in Figure 5.3. Since the experimental conditions were the same with those in Figure 4.10, the results were similar indicating reliable repeatability of the experiment. The effluent phenol concentration sharply increased after 0.8 pore volume. Since phenol was continuously supplied to the column, a relative phenol concentration $C_e^{phenol} / C_0^{phenol}$ = 1 was expected to be maintained under saturated conditions when no HRP was added (as indicated by the dashed line, ---). HRP and H_2O_2 were introduced into the column inlet once the column was saturated with phenol. Phenol polymerization reaction in the porous column was expected to occur as soon as HRP and H_2O_2 were added.

Injection of 2.0 AU/mL of HRP with H_2O_2 (500 μM) into the column resulted in a 90% reduction in the effluent phenol concentration within about 4 pore volumes after enzyme injection. Approximately 0.8 pore-volumes after HRP addition was initiated, the phenol

breakthrough curve (o) sharply dropped. A relative phenol concentration less than 0.1, indicating 90% removal of the phenol in the flowing solution, was obtained 4 pore volume of HRP addition. Phenol continued to be removed from the influent with simultaneous deposition of insoluble polymerization products in the porous media. Soluble polymers were observed in the flow exiting the column at approximately 0.8 pore volumes after HRP addition. The relative concentration of soluble polymer (secondary *y*-axis in figure) increased and steadied at a value of around 0.2 indicating that 20% of the influent phenol was converted to soluble polymers that were not retained in the column. Phenol concentration decreased and steadied at ~10% of the initial phenol concentration after about 4 pore-volume of HRP addition.

Figure 5.3. Phenol removal and in-situ polymer production mediated by HRP in saturated porous media. Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.



A mass balance on phenol yielded the curves illustrated in Figure 5.4. Approximately 340 micromoles of phenol (1 µmole phenol = 94 µg) had been injected into the column within 13 pore volumes. Less than 60 micromoles were recovered in the effluent indicating > 68 % phenol transformation. About 60 micromole of the influent phenol (or 17%) was converted into soluble

polymers that exited the column (Table 5.5). The insoluble polymer consisted of the polymer accumulated in the sand column and was calculated by subtracting the mass of soluble polymer and effluent phenol from the total phenol injected. About 65.8% of the injected phenol accumulated in the column as insoluble polymers.

Figure 5.4. Mass balance of phenol, soluble polymer and insoluble polymer. Influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.

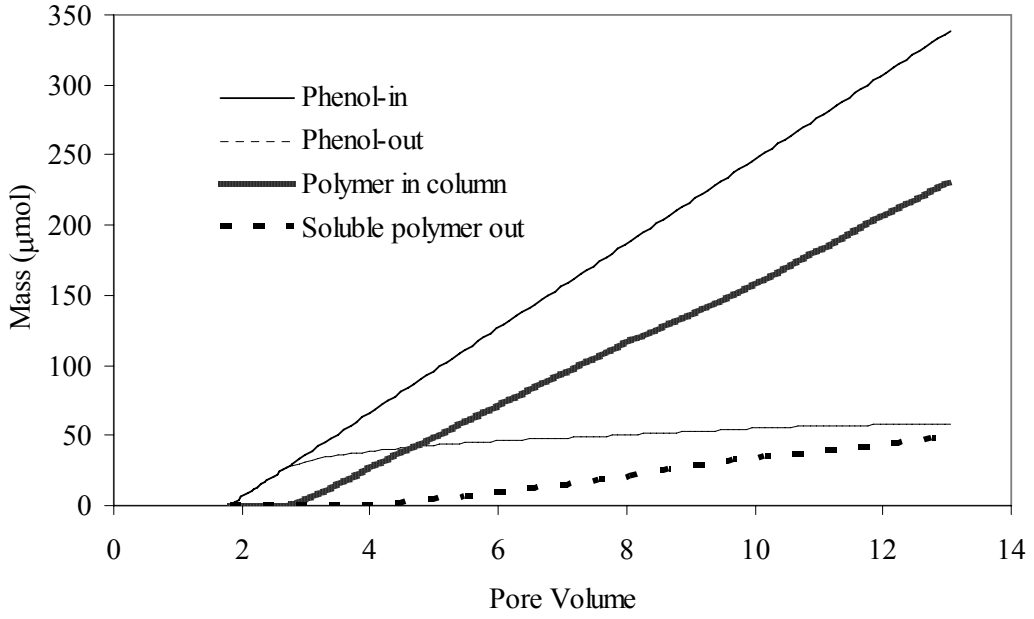


Table 5.5. Mass balance of phenol, soluble polymer and insoluble polymer.

Experimental Condition	Total phenol in (µmol)	Total phenol out (µmol)	Total soluble polymer out (µmol as phenol)	Total insoluble polymer in column (µmol as phenol)
2 AU/mL pH 7 IS 20 (mM)	339	58.3	48.77	231.7 (21.8 mg of phenol)

The deposition of phenolic oligomers in the column was expected to result in the modification of hydraulic properties of the porous media. Figure 5.5 illustrates the results of the nonreactive tracer tests conducted in the saturated porous media after phenol polymerization. The figure also includes the tracer curve obtained for the virgin column. The tracer in the modified media appeared earlier in the modified porous media indicating pore volume reduction due to polymer deposition. The tracer also disappeared faster when the column containing modified porous media was washed with clean buffer.

Estimations of model parameter such as dispersion coefficient and retardation factor are summarized in Table 5.6. The retardation parameter was decreased to 0.9206 while the variation of dispersion coefficient is negligible indicating pore volume degreased. The estimation of pore volume decreased was also summarized in Table 5.7.

Since tracer showed no retardation, by setting the retardation prarameter, R=1, dispersion coefficient and pore water velocity was estimated using inverse parameter estimation method of CXTFIT program. These results were consistent with those of experiment in figure 4.16. A similar pore volume was decreased by deposition of polymer from HRP-mediated polymerization reaction.

Table 5.6. Parameters estimated for tracer before and after polymerization.

Condition	**v** (cm/min)	**D** (cm^2/min)	**R**	**R^2**
MTBP	0.119598	0.01118	1	0.993
MTAP	0.119598	0.00975	0.9206	0.995

MTBP: Modeling tracer before polymerization;

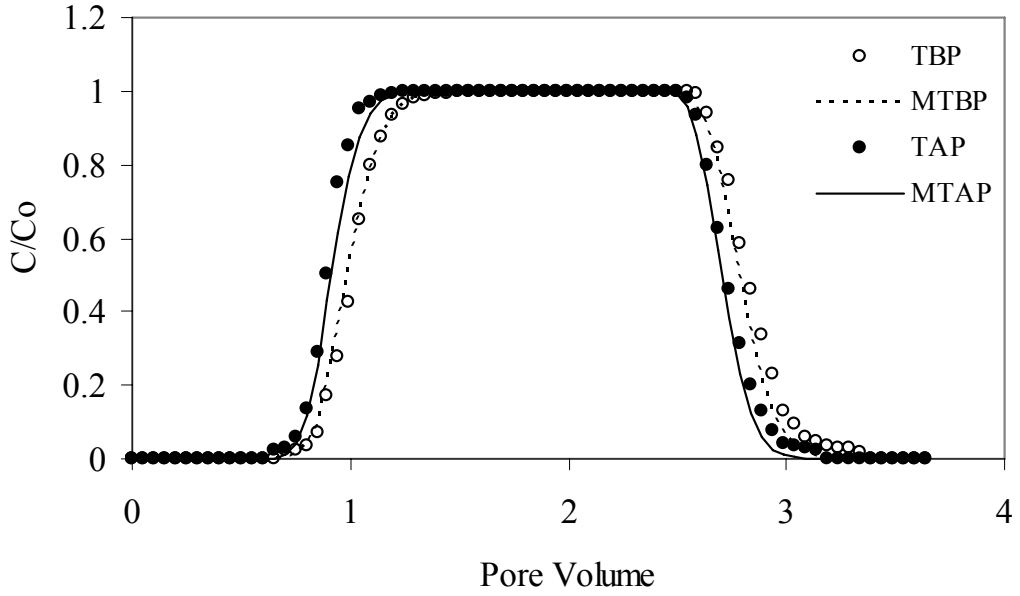
MTAP: Modeling tracer after polymerization;

v: pore water velocity,D: dispersion coefficient, R: retardation, R^2=least square

Table 5.7. Estimation of pore volume occupied by polymer deposition with enzyme dose.

Parameters		Values
estimated pore water velocity	(cm/min)	0.1299
estimated dispersion coefficient	(cm^2/min)	0.0106
R^2		0.995
flow rate (cm^3/min)		0.60
specific discharge (cm/min)		0.0417
estimated porosity		0.3499
pore volume decreased (mL)		4.7746

Figure 5.5. Tracer transport before and after deposition of phenol polymerization products in the saturated porous media.
TBP = Tracer before polymerization, MTBP = Modeling tracer before polymerization, TAP = Tracer after polymerization, MTAP = Modeling tracer after polymerization.



5.4.3 DCP Transport in Modified Porous Media

The modification of porous media resulting from polymer deposition was expected to alter DCP transport in the column. Figure 5.6 illustrates results collected from DCP transport experiments conducted on the virgin column and the same column modified as a result of phenol polymer deposition. While no solute retardation was observed for DCP transport in the virgin column, significant deviation from ideal advection-dispersion behavior was noted for DCP transport through the modified porous media. Furthermore, transport behavior of 2,4-DCP after polymer deposition should be compared with not that of tracer or 2,4-DCP before polymer deposition but that of tracer after polymer deposition since parameters such as porosity, dispersion coefficient and pore water velocity were varied.

Figure 5.6. Transport behavior of 2,4-DCP (200 µM) before and after polymerization in the saturated porous media. Polymerization condition: influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.
DCPAP = DCP after polymerization, MDCPAP = Modeling DCP after polymerization and DCPBP = DCP before polymerization.

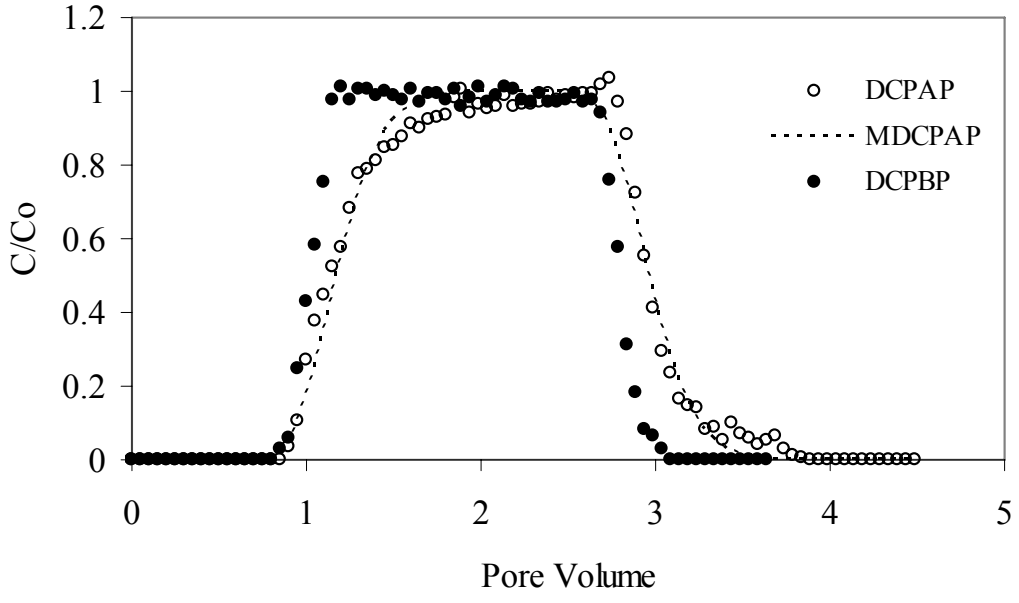
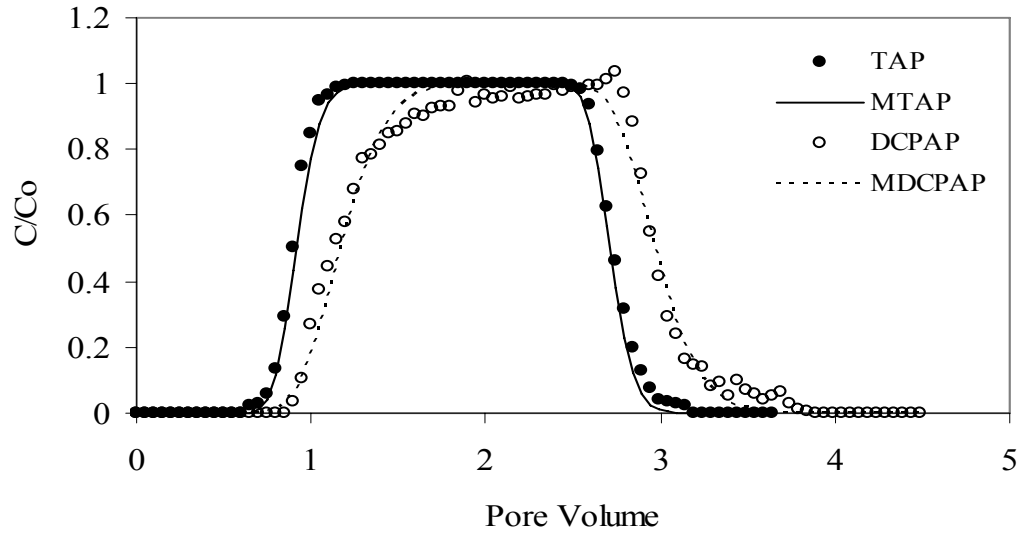


Figure 5.7 illustrates the comparison of transport behavior of tracer and 2,4-DCP after polymer deposition. Tracer after polymerization (TAP) was appeared earlier than tracer before polymerization (TBP) with the value of 0.92 retardation (Table 5.6 and figure 5.5). DCP transport did not coincide with tracer transport in the modified media. The tracer had appeared earlier in the effluent of the modified porous media due to pore volume reduction in the column. DCP appearance in the effluent was greatly delayed in the modified media indicating that other factors impacted solute transport.

Paloma and Bhandari (2005) had observed that DCP removal in batch reactors containing soil particles was larger than that in soil-free solution. They attributed the additional solute removal to sorption of the polymerization products to soil particles. It is likely that the observed retardation in DCP transport through the modified column was caused by its sorption to the polymeric precipitates deposited in the column. Effluent DCP concentration remained less than the influent concentration even after 1.7 pore volumes of flow. Xu and Bhandari (2000) were also able to describe the sorption and desorption rate of 2,4-DCP on two different types of soils using equilibrium sorption model (Freudlich sorption model).

Figure 5.7. Comparison of Breakthrough Curves of 2,4-DCP (200 µM) and Tracer. Polymerization condition: influent phenol concentration = 500 µM, solution pH = 7.0, solution ionic strength = 20 mM, HRP dose = 2.0 AU/mL, and H_2O_2 concentration = 500 µM.



Since the all parameters of the transport model were varied after polymer deposition, the modeling parameters of TAP was re-estimated by setting the retardation factor =1 as summarized in Table 5.7. By standardizing the TAP and using the pore water velocity estimated, dispersion coefficient and retardation factor of 2,4-DCP was estimated based on one-dimensional advection-dispersion equation. The retardation and dispersion coefficient of 2,4-DCP obtained from the simulation are summarized in Table 5.8. Assuming linear sorption, distribution coefficient was calculated with 0.00587 L/kg base on equation (5.7) in the matrix structure of soil and polymer.

Table 5.8. Parameters estimated for 2,4-DCP.

Parameters	Values
v (cm/min)	0.130
D (cm^2/min)	0.023
R	1.289
R^2	0.988

5.5 SUMMARY AND CONCLUSIONS

The study investigated the phenol removal using HRP-mediated oxidative polymerization process in saturated porous media. HRP and cofactor H_2O_2 were injected to the column representing groundwater system to evaluate the effectiveness of enzyme based oxidative polymerization. Impact of porous media modified by deposition of polymer produced was also investigated. Transport behavior of tracer and 2,4-DCP before and after polymerization was also investigated. Their transport behavior was fitted with one-dimensional advection-dispersion model. Experimental results revealed that more than 90% of the influent phenol concentration was removed within 2 pore volume after injection of 2.0 AU/mL of HRP dose and 500 μM H_2O_2 at pH 7.0 in continuous flow porous media system. These optimized conditions show the

potential of HRP-mediated oxidative coupling polymerization process to remediate the groundwater system.

While about 15 % of the influent phenol exited the column as soluble polymerization products, nearly 70 % of the influent phenol was retained in the porous media as precipitated products. HRP-mediated polymerization process produces soluble and insoluble polymers. These polymer produced can be deposited in groundwater porous media system and modify the structure of microscopic and macroscopic path of groundwater. Results of tracer tests indicated that the saturated porous media was modified during HRP-mediated removal of phenol as a result of polymer deposition (Hypothesis 1). The modified porous media shows the characteristics of nearly constant dispersion coefficients but significant variation in retardation. Generally, retardation factor are influenced by sorption/desorption process, for example, the value of retardation factors are usually decreased. However, the results of our preliminary experiment revealed that tracer showed no retardation with polymerization products.

Results of these experiments also showed hydrophobic solutes (2,4-DCP) experienced retardation in the modified porous media as a result of their interaction with the newly developed organic phase comprised of insoluble oligomeric products (Hypothesis 2). The results of tracer test after polymerization and estimation of model parameter shows the value of retardation was decreased ($R<1$) indicating groundwater moves faster while dispersion coefficient was not varied much. The effluent concentration of phenol continued to decrease as polymerization products accumulated in the porous media. This can be considered that there is adsorption of phenol on the polymer deposited in porous media. These results and recent literature review confirmed that polymerization products deposited in saturated porous media has the absorption ability. HRP-mediated coupling polymerization process showed not only removal of target chemical but also the secondary potential of remediation in contaminated groundwater.

HRP-mediate in situ stabilization of phenol should be carefully managed as it can result in desirable or undesirable clogging of the porous media. Deposition of phenolic oligomers in the porous media can be optimized to generate in situ hydraulic barriers that allow some degree of plume control. Alternately, oligomer deposition can also be engineered to create in situ permeable reactive barriers at the leading edge of a plume capable of removing or retarding hydrophobic pollutants via sorption.

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CAHPTER 6. CONCLUSIONS AND IMPLICATIONS

This study investigated the feasibility of using enzyme-mediated *in situ* stabilization for managing phenol contamination in soils and groundwater. The research optimized a luminol-based chemiluminescence assay to monitor horseradish peroxidase (HRP) transport in satruated porous media. The impact of HRP-mediated oxidative coupling on phenol removal under simulated aquifer conditions was assessed. The modification of porous media resulting from the deposition of insoluble phenol polymerization products was studied and its impact on column hydraulics and the transport behavior of a hydrophobic contaminant such as 2,4-DCP was evaluated.

All assay components in the H_2O_2-luminol-enhancer-HRP system affected the maximum chemiluminescence intensity. An optimum chemiluminescence intensity was observed at pH 8.5. The ionic strength of the assay solution had a significant impact on the maximum chemiluminescence intensity. HRP concentration was directly related to the observed chemiluminescence intensity. No retardation was observed for HRP transport in the column packed with Ottawa sand.

Removal of phenol increased with HRP dose up to 2.0 AU/mL and more than 90% removal of influent phenol was observed under optimum conditions. Soluble and insoluble reaction products were generated by injection of H_2O_2 and HRP into the phenol saturated column. Approximately 8% reduction in pore volume was noted due to deposition of insoluble phenol polymerization products at pH 7, 2 AU/mL enzyme dose and 100 mM ionic strength. The concentration of soluble reaction products exiting the column was low at higher ionic strengths while deposition of insoluble polymer was significantly higher.

Greater deposition of insoluble phenol polymerization products was observed with increasing enzyme dose and ionic strength while the variation of dispersion coefficient was relatively negligible. Transport of 2,4-DCP was significantly affected in the modified porous media. Retardation of 2,4-DCP was significant due to the sorption of 2,4-DCP on insoluble polymer deposited in porous media.

The following hypotheses were proven to be true as a result of this research:

Hypothesis 1

The activity of HRP in clear aqueous solutions can be estimated using a luminol-based chemiluminescence assay.

Hypothesis 2

HRP behaves like a conservative tracer in a continuous flow system containing porous media comprised of porous media.

Hypothesis 3

Phenol entering a packed column under simulated aquifer conditions can be removed from the aqueous phase by injecting HRP and H_2O_2 into the flow.

Hypothesis 4

HRP-mediated phenol removal in continuous flow, packed column reactors is accompanied by the generation of soluble and insoluble oligomeric products.

Hypothesis 5

Phenol removal and the production of oligomeric products under the action of HRP and H_2O_2 are affected by the enzyme dose, solution pH and solution ionic strength.

Hypothesis 6

Deposition of insoluble phenol polymerization products in the packed column results in physical modification of the porous media.

Hypothesis 7

Hydrophobic solutes experience retardation in the modified porous media as a result of their interaction with the newly developed organic phase comprised of insoluble oligomeric products.

Real-World Implications

• HRP in relatively clear groundwater can be monitored with a portable spectrometer using the CL assay developed in this study.

• The effects of pH and ionic strength should be considered to use the CL assay in groundwater.

• HRP transport is not retarded in porous media made of nonreactive mineral grains. The enzyme moves with the groundwater and with or ahead of soluble phenolic contaminants.

• The HRP-mediated oxidative polymerization process has a potential for application as a remediation technique for rapidly moving phenol plumes in saturated aquifers.

• HRP-mediated in situ contaminant stabilization works best under most groundwater conditions (pH and ionic strength) and for the most soluble (and mobile) phenolic contaminants.

• HRP-mediate in situ stabilization of phenol should be carefully managed as it can result in desirable or undesirable clogging of the porous media.

• Deposition of phenolic oligomers in the porous media can be optimized to generate in situ hydraulic barriers that allow some degree of plume control.

• Alternately, oligomer deposition can also be engineered to create in situ permeable reactive barriers at the leading edge of a plume capable of removing or retarding hydrophobic pollutants via sorption.

• HRP-mediated oxidative process can be applicable to treat the phenol contaminated groundwater and information obtained in this research can be used to apply this process to groundwater system.

Appendix A - Data for Chapter 3

Table A.1. Data for Figures 3.5, 3.6 and 3. 7.

Time (sec)	Ionic strength 10 mM								
	6.5	7	7.5	8	8.5	9	9.5	10	10.5
0	0	0	0	0	0	0	0	0	0
5	1.4	1.4	14.5	34.1	63.9	77	13.4	3.7	3.7
10	1.7	1.7	16.2	37.2	95.7	114	24	6.9	1.7
15	1.3	1.3	10.7	27.5	72.1	96.5	25.5	5.1	1.3
20	0.4	0.4	9.3	18.2	54.8	76.7	25.8	5.4	0.4
25	0.7	0.7	6.3	15.4	41.7	60.3	26.1	5.2	0.7
30	1.1	1.1	4.8	11.9	32.2	46.9	25.4	5.8	1.1
35	2.5	2.5	4.5	9.9	27.4	36.3	24.3	4.9	2.5
40	1.5	1.5	4.5	7.7	23.9	28.4	26	4.3	1.5
45	1.9	1.9	3.1	5.2	19	21.8	25.1	3.7	1.9
50	1.7	1.7	2.3	4.1	15.8	18.2	25.7	4.7	1.7
55	2	2	2	4	13.5	14	27.1	4.8	2
60	2.1	2.1	2.1	4.3	11.4	12.7	25.2	5.9	2.1
65	2.8	2.8	2.8	3.5	9.2	8.9	24.7	4.3	2.8
70	2.2	2.2	2.2	2.6	8.2	7.9	26.5	5	2.2
75	2.2	2.2	2.2	1.8	8	5.8	26.6	4.4	2.2
80	2.8	2.8	2.8	2.8	7.3	4.7	26.9	4.9	2.8
85	3.4	3.4	3.4	3.4	6.3	6.5	26.8	4.3	3.4
90	1.7	1.7	1.7	1.7	5.3	4.3	26	4.9	1.7
95	1.4	1.4	1.4	1.4	5.5	4.2	25.6	5.6	1.4
100	2.3	2.3	2.3	2.3	4.8	3.2	24.4	5.3	2.3
105	1.4	1.4	1.4	1.4	4.4	3.5	26.8	5.1	1.4
110	1.6	1.6	1.6	1.6	3.2	2.6	26.8	5.1	1.6
115	1.2	1.2	1.2	1.2	3.9	2.9	25.4	5.4	1.2
120	2.6	2.6	2.6	2.6	3.8	2.6	27.5	5.6	2.6
125	3.3	3.3	3.3	3.3	3	3.3	25.8	4.4	3.3
130	1.5	1.5	1.5	1.5	2.8	1.5	26.3	4.8	1.5
135	0.6	0.6	0.6	0.6	2.9	0.6	26.4	4.8	0.6
140	1.7	1.7	1.7	1.7	4	1.7	27	4.7	1.7
145	1.6	1.6	1.6	1.6	2.1	1.6	24.1	4.9	1.6
150	2.3	2.3	2.3	2.3	2.3	2.3	25.8	4.8	2.3
155	1.8	1.8	1.8	1.8	1.8	1.8	26.9	4.6	1.8
160	2.1	2.1	2.1	2.1	2.1	2.1	26	4.5	2.1
165	2.3	2.3	2.3	2.3	2.3	2.3	26.1	4.2	2.3
170	2.6	2.6	2.6	2.6	2.6	2.6	26.1	4.8	2.6
175	1.9	1.9	1.9	1.9	1.9	1.9	26.1	5.1	1.9

180	1.7	1.7	1.7	1.7	1.7	1.7	25.5	6	1.7
185	2.3	2.3	2.3	2.3	2.3	2.3	26.4	4.7	2.3
190	2.1	2.1	2.1	2.1	2.1	2.1	24.6	4.6	2.1
195	3.1	3.1	3.1	3.1	3.1	3.1	24.9	5.3	3.1
200	2.8	2.8	2.8	2.8	2.8	2.8	26.3	3.9	2.8
205	1.1	1.1	1.1	1.1	1.1	1.1	27.2	6	1.1
210	1.6	1.6	1.6	1.6	1.6	1.6	25.1	5.6	1.6
215	2.6	2.6	2.6	2.6	2.6	2.6	25.9	4.9	2.6
220	2.7	2.7	2.7	2.7	2.7	2.7	26.4	4.3	2.7
225	0.7	0.7	0.7	0.7	0.7	0.7	26.8	4.8	0.7
230	1.8	1.8	1.8	1.8	1.8	1.8	26.9	4.4	1.8
235	2.6	2.6	2.6	2.6	2.6	2.6	26.7	3.9	2.6
240	1.3	1.3	1.3	1.3	1.3	1.3	25.3	5.3	1.3
245	2.1	2.1	2.1	2.1	2.1	2.1	26.8	5.2	2.1
250	2.1	2.1	2.1	2.1	2.1	2.1	27.3	4.7	2.1
255	1.3	1.3	1.3	1.3	1.3	1.3	26.5	5.7	1.3

Intensity vs pH at 50 mM

Time (sec)	Ionic strength 50 mM								
	6.5	7	7.5	8	8.5	9	9.5	10	10.5
0	0	0	0	0	0	0	0	0	0
5	2.2	4.6	13.3	41.9	80	34.6	13.6	4.9	3.6
10	1.7	1.7	13	49.5	94.9	56.8	23	6.2	2.5
15	1.3	1.3	9.5	36.2	77.8	59.6	22.4	5.9	2.5
20	0.4	0.4	5.7	27.7	62.2	61.6	24.2	4.8	2.4
25	0.7	0.7	4.2	20.2	51.2	62.6	20.9	4.7	2.5
30	1.1	1.1	2.8	16.2	42.3	61.4	22.5	4.1	3.2
35	2.5	2.5	3.1	12.8	35.5	62.4	22.4	5.2	1.7
40	1.5	1.5	2.6	11.2	27.8	61.9	22.2	6.1	1.7
45	1.9	1.9	2.7	9.9	25.7	60.5	22.3	5.3	1.7
50	1.7	1.7	1.7	8	21.1	60.2	22.3	4.8	2.1
55	2	2	2	7.1	18.5	59.5	21.8	5.9	1.1
60	2.1	2.1	2.1	5.5	15.4	59.8	22.4	5.2	2
65	2.8	2.8	2.8	4.2	15	59.8	21.8	4	2.1
70	2.2	2.2	2.2	3.4	13.7	59.3	21.3	4.7	2.8
75	2.2	2.2	2.2	3.4	10	59.4	21.8	5.8	2.2
80	2.8	2.8	2.8	3.1	8.8	58.4	21.7	4.6	2.2
85	3.4	3.4	3.4	3.3	8.4	58.8	21.9	4.1	2.8

90	1.7	1.7	1.7	1.4	7.1	57.2	21.1	5.9	3.4
95	1.4	1.4	1.4	3	6.3	56.6	21.5	4.9	1.7
100	2.3	2.3	2.3	2	5.9	56.3	20.9	5.5	1.4
105	1.4	1.4	1.4	2.3	5.1	55.4	21.7	5.1	2.3
110	1.6	1.6	1.6	2	4.2	55.3	20.8	5.1	1.4
115	1.2	1.2	1.2	1.2	4.4	54.9	20.9	5.8	1.6
120	2.6	2.6	2.6	2.6	4.7	53.8	20.9	4.9	1.2
125	3.3	3.3	3.3	3.3	4.8	55.6	20.7	5.4	2.6
130	1.5	1.5	1.5	1.5	3.8	54.4	21.2	5.3	3.3
135	0.6	0.6	0.6	0.6	3.7	53.7	21.5	5.7	1.5
140	1.7	1.7	1.7	1.7	2.8	52.5	21.1	3.8	0.6
145	1.6	1.6	1.6	1.6	2.2	52.7	20.8	4.7	1.7
150	2.3	2.3	2.3	2.3	2.6	51.2	21.7	4.5	1.6
155	1.8	1.8	1.8	1.8	2.5	51.5	20.4	5.4	2.3
160	2.1	2.1	2.1	2.1	2.1	50.6	20.1	5.1	1.8
165	2.3	2.3	2.3	2.3	2.3	50.4	20.2	4	2.1
170	2.6	2.6	2.6	2.6	2.6	49.6	21.1	4.6	2.3
175	1.9	1.9	1.9	1.9	1.9	49.2	21.1	4	2.6
180	1.7	1.7	1.7	1.7	1.7	49	22	4	1.9
185	2.3	2.3	2.3	2.3	2.3	48.6	20.9	4.9	1.7
190	2.1	2.1	2.1	2.1	2.1	46.8	21	4.3	2.3
195	3.1	3.1	3.1	3.1	3.1	46.5	20.4	5.8	2.1
200	2.8	2.8	2.8	2.8	2.8	46.1	20.6	4.2	3.1
205	1.1	1.1	1.1	1.1	1.1	46.2	20.8	4.6	2.8
210	1.6	1.6	1.6	1.6	1.6	45.1	19.2	3.9	1.1
215	2.6	2.6	2.6	2.6	2.6	45.7	21.9	4.8	1.6
220	2.7	2.7	2.7	2.7	2.7	44.3	19.8	4.4	2.6
225	0.7	0.7	0.7	0.7	0.7	43.6	20.3	5.3	2.7
230	1.8	1.8	1.8	1.8	1.8	42.6	20.3	5	0.7
235	2.6	2.6	2.6	2.6	2.6	41.8	20.4	5.1	1.8
240	1.3	1.3	1.3	1.3	1.3	41.2	19.9	5.2	2.6
245	2.1	2.1	2.1	2.1	2.1	41.7	19.8	3.8	1.3
250	2.1	2.1	2.1	2.1	2.1	40.7	19.7	4.9	2.1
255	1.3	1.3	1.3	1.3	1.3	39.3	19	4.5	2.1

Intensity vs pH at 100 mM

Time (sec)	Ionic strength 100 mM pH								
	6.5	7	7.5	8	8.5	9	9.5	10	10.5
0	0	0	0	0	0	0	0	0	0
5	1.4	1.9	11.1	44.7	72.1	47.1	16.7	6.1	3
10	1.7	4.5	12.6	53.9	117.1	75.4	29.1	6.8	4.2
15	1.3	4.3	8.8	39.3	95.4	77.2	30.1	5.7	2.2
20	0.4	3.1	6.1	27.6	75.8	79.2	30.6	5.3	3
25	0.7	2.7	5	21.1	61.6	78	31.3	4.8	2.4
30	1.1	1.2	3.8	16.7	51.5	78.9	31.6	4.3	2.7
35	2.5	1	4.1	13.7	42.1	77.8	31.4	4	2.3
40	1.5	1.5	3.4	11.2	35.5	76	31.3	4.1	3.1
45	1.9	1.9	3.1	8.9	32.1	76.5	31.4	5.2	2.4
50	1.7	1.7	1.8	8.2	26.4	74.4	29.9	4.1	3.6
55	2	2	1.9	5.5	24.2	72.9	30.9	5.9	2.6
60	2.1	2.1	0.8	4.9	26.5	72.4	30.7	4.3	2.9
65	2.8	2.8	1.7	3.6	18.6	73.3	30.5	4.8	3.1
70	2.2	2.2	2.2	3.4	16.1	73.1	30.1	4.1	2.7
75	2.2	2.2	2.2	5.4	14.7	71.8	30.3	4.8	2.2
80	2.8	2.8	2.8	3.1	13.3	70	30.8	4.6	2.8
85	3.4	3.4	3.4	2.2	12.9	68.6	29.6	4.6	3.4
90	1.7	1.7	1.7	2.9	11.8	67.3	29.7	4.6	1.7
95	1.4	1.4	1.4	3.1	9.1	68.1	30.4	4.3	1.4
100	2.3	2.3	2.3	2.1	8.1	65.8	29.9	4.8	2.3
105	1.4	1.4	1.4	1.4	6.7	65.4	28.8	3.9	1.4
110	1.6	1.6	1.6	1.6	7.1	64.2	30.2	3.7	1.6
115	1.2	1.2	1.2	1.2	5.8	62.2	29.7	3.9	1.2
120	2.6	2.6	2.6	2.6	6	62.6	28.5	3.8	2.6
125	3.3	3.3	3.3	3.3	4.3	61.9	29.2	5	3.3
130	1.5	1.5	1.5	1.5	4	60.1	28.8	3.5	1.5
135	0.6	0.6	0.6	0.6	3.6	58.7	29.4	5.4	0.6
140	1.7	1.7	1.7	1.7	2.7	58.6	28.6	4.1	1.7
145	1.6	1.6	1.6	1.6	2.9	58	30.1	4.4	1.6
150	2.3	2.3	2.3	2.3	2.4	57.3	28.2	4.2	2.3
155	1.8	1.8	1.8	1.8	3.6	57.5	27.3	3.5	1.8
160	2.1	2.1	2.1	2.1	4	55.5	28	4.5	2.1
165	2.3	2.3	2.3	2.3	2.8	56	27.9	4.6	2.3
170	2.6	2.6	2.6	2.6	2.2	53.5	27.7	3.9	2.6
175	1.9	1.9	1.9	1.9	2.6	53.5	28.1	5.1	1.9

180	1.7	1.7	1.7	1.7	1.7	54.2	27.1	5.8	1.7
185	2.3	2.3	2.3	2.3	2.3	53.5	26.5	5	2.3
190	2.1	2.1	2.1	2.1	2.1	52.5	27.1	5	2.1
195	3.1	3.1	3.1	3.1	3.1	51.1	26.9	4.9	3.1
200	2.8	2.8	2.8	2.8	2.8	49.5	26.7	5.4	2.8
205	1.1	1.1	1.1	1.1	1.1	48.8	27.1	4.9	1.1
210	1.6	1.6	1.6	1.6	1.6	48.5	26.6	4.6	1.6
215	2.6	2.6	2.6	2.6	2.6	47.8	27.3	3.8	2.6
220	2.7	2.7	2.7	2.7	2.7	47.7	27.2	4.2	2.7
225	0.7	0.7	0.7	0.7	0.7	46.2	26.8	5	0.7
230	1.8	1.8	1.8	1.8	1.8	44.2	27.3	5	1.8
235	2.6	2.6	2.6	2.6	2.6	44.5	26.9	3.8	2.6
240	1.3	1.3	1.3	1.3	1.3	41.8	27.6	2.9	1.3
245	2.1	2.1	2.1	2.1	2.1	42.4	27.2	4.6	2.1
250	2.1	2.1	2.1	2.1	2.1	44.2	26.1	4.3	2.1
255	1.3	1.3	1.3	1.3	1.3	42	26.5	4.4	1.3

Intensity vs pH at 500 mM

Time (sec)	Ionic strength 500 mM pH								
	6.5	7	7.5	8	8.5	9	9.5	10	10.5
0	0	0	0	0	0	0	0	0	0
5	1.4	4.6	9.7	44.8	97.8	72.6	33.7	5.7	3.4
10	1.7	2.3	7.8	48.6	132.3	123.7	58.4	5.7	2.1
15	1.3	1.6	6.5	34.2	109.5	134.5	64.3	6.9	2.1
20	0.4	2.7	6.5	24.7	91.6	136	65.7	6.9	2.7
25	0.7	2.3	4.7	19.8	76.3	134.6	67.7	6.7	0.9
30	1.1	3	2.9	15.9	65	132.3	67.2	4.5	1.1
35	2.5	2	3.3	13	55.4	130.5	67.8	6	2.5
40	1.5	1.5	1.9	10.6	48.8	125.7	67.4	5.1	1.5
45	1.9	1.9	1.5	8.6	41.3	125.1	65.8	5.7	1.9
50	1.7	1.7	30.4	7.3	36	121.1	65.7	4.5	1.7
55	2	2	2.2	6.6	33.7	118.2	64.9	3.9	2
60	2.1	2.1	2.1	5.1	29.1	116.9	64.9	4.1	2.1
65	2.8	2.8	2.8	5.3	26.2	113.4	64.6	5.1	2.8
70	2.2	2.2	2.2	3.3	22.1	112.2	62.7	5.1	2.2
75	2.2	2.2	2.2	2.8	21	108.1	63	5.1	2.2
80	2.8	2.8	2.8	3.1	18.2	106.1	62.8	5	2.8
85	3.4	3.4	3.4	2	17	102.1	61.2	5.5	3.4

90	1.7	1.7	1.7	3.5	14.2	99.1	60.5	3.9	1.7
95	1.4	1.4	1.4	2.2	13.7	96.2	60.6	4.9	1.4
100	2.3	2.3	2.3	1.9	11.5	94.2	59.6	4.5	2.3
105	1.4	1.4	1.4	2.4	7.3	92.5	60.4	3.7	1.4
110	1.6	1.6	1.6	2.7	8.4	91	60.9	5	1.6
115	1.2	1.2	1.2	1.2	8.3	87.4	59.9	5.6	1.2
120	2.6	2.6	2.6	2.6	8.1	85.9	57.9	4.6	2.6
125	3.3	3.3	3.3	3.3	7.2	82.1	57.6	4.9	3.3
130	1.5	1.5	1.5	1.5	7.3	80.7	57.8	6.6	1.5
135	0.6	0.6	0.6	0.6	6.9	79.5	57.7	4.4	0.6
140	1.7	1.7	1.7	1.7	4.4	76.5	56.6	5.3	1.7
145	1.6	1.6	1.6	1.6	3.9	72.9	56.7	5.2	1.6
150	2.3	2.3	2.3	2.3	4.7	72	56.1	4	2.3
155	1.8	1.8	1.8	1.8	3.4	68.8	56.8	5.5	1.8
160	2.1	2.1	2.1	2.1	4.5	68.5	55	4.7	2.1
165	2.3	2.3	2.3	2.3	4.8	65.3	55.1	5.3	2.3
170	2.6	2.6	2.6	2.6	3.7	63	53.3	6.2	2.6
175	1.9	1.9	1.9	1.9	2.8	62.2	54.1	5.3	1.9
180	1.7	1.7	1.7	1.7	2.9	59.7	54.3	5.4	1.7
185	2.3	2.3	2.3	2.3	2.2	55.4	52.9	4.5	2.3
190	2.1	2.1	2.1	2.1	2.1	54.8	52.7	5	2.1
195	3.1	3.1	3.1	3.1	3.1	52.3	52.5	4.7	3.1
200	2.8	2.8	2.8	2.8	2.8	49.2	52.9	5.2	2.8
205	1.1	1.1	1.1	1.1	1.1	48.7	52	5.6	1.1
210	1.6	1.6	1.6	1.6	1.6	46	51.1	5.7	1.6
215	2.6	2.6	2.6	2.6	2.6	41.3	50.4	6.5	2.6
220	2.7	2.7	2.7	2.7	2.7	39	50.2	6.8	2.7
225	0.7	0.7	0.7	0.7	0.7	37.3	49	4.7	0.7
230	1.8	1.8	1.8	1.8	1.8	35.1	48.4	3.7	1.8
235	2.6	2.6	2.6	2.6	2.6	33.2	48	5.6	2.6
240	1.3	1.3	1.3	1.3	1.3	30.5	48.4	6.4	1.3
245	2.1	2.1	2.1	2.1	2.1	28.8	47.6	6.3	2.1
250	2.1	2.1	2.1	2.1	2.1	25.7	46.8	5.6	2.1
255	1.3	1.3	1.3	1.3	1.3	23.5	47.2	6	1.3

Table A.2. Data for Figure 3.8.

	H_2O_2 (mM)					
Time (sec)	0.5	1	2	3	4	5
0	0	0	0	0	0	0
5	13.4	12.6	24.6	41.7	51	44.4
10	42.7	70.6	93.2	107.1	120.5	124
15	31.1	56.4	83.5	95.7	110.5	116.6
20	25.1	48.3	69.4	86.6	98.1	105
25	20.5	40.3	58.9	74.2	87.7	95.8
30	16.3	33.7	53.2	68.8	80.1	88.6
35	13.3	30.4	46.8	61.7	72.3	82.3
40	10.8	25	43.2	57.6	67.1	75.5
45	10	23	37.9	52.3	60.8	70.8
50	9	21.1	35.6	48.6	58	63.9
55	6.3	16.9	30.4	44.2	51.7	59.9
60	5.8	16.5	28.6	41.6	49	57.5
65	4.7	15.6	25.3	36	45.5	54.2
70	5.1	12.6	23.1	32.6	42.2	49.8
75	3.1	11.3	21.7	30.1	39.9	46.9
80	2.9	10.8	20.5	29	37.5	44.3
85	3.2	8.7	18.4	27.6	34.5	42.9
90	3.6	9.1	17	23.6	32.3	39.2
95	2.5	7	15.6	23.1	29.9	38.1
100	4	6.3	15.5	20.9	27.9	33.9
105	3.5	7	13.3	20.3	28.6	33.2
110	2.6	5.9	12	18.3	26.1	31
115	2.3	7	12.2	17.3	23.9	30.3
120	3.7	6	11.3	16	22.2	26.2
125	3.1	5.7	9.4	14	21.1	26
130	3.7	5.6	9.9	14.7	19.4	25.3
135	2.6	4.9	9.5	13.7	19.5	24
140	3.9	4.8	7.5	12.7	17.6	22
145	3.5	3.9	7.2	11.7	16.6	22.2
150	1.4	2.6	7.9	11.5	17	20.6
155	2.3	4.4	7.7	11	15.9	19.2
160	3.2	3.4	6	9.7	14.9	18.6
165	2.4	3.3	6.2	9.6	12.5	18.3
170	3.3	3.7	4.8	9.3	11.8	15.7
175	2.3	3	6.2	7.7	12.6	15.9
180	3.5	4.4	5.2	8	11.3	15.1

185	3	2.5	6.8	7.6	10.3	13.7
190	3.9	2.1	5.3	7.3	10.9	12.5
195	2.7	2.8	4.4	6.8	10.2	12.1
200	2.9	3.1	4.2	6.5	9.2	11.2
205	3.2	4.1	3.7	7.5	9	12
210	2	2.9	4.3	6	8.8	10.6
215	3.6	2.5	4.6	6.3	7	10.3
220	3.6	1.8	4.1	6.4	6.7	10.5
225	2.5	3.3	3.2	4.8	7.4	8.9
230	4	3.4	3.8	5.9	7.6	8.9
235	3.8	3	4.1	5.4	6.5	9.2
240	2.6	3	5.4	3.8	5.3	9.2
245	2.4	3.6	2.8	2.7	6.6	8.4
250	4.2	3.4	3.8	5	4.8	6.2
255	3.8	2.8	4.2	4.6	7.1	7.4

Table A.3. Data for Figure 3.8.

	H_2O_2 (mM)					
Time (sec)	0.5	1	2	3	4	5
0	0	0	0	0	0	0
5	13.4	12.6	24.6	41.7	51	44.4
10	42.7	70.6	93.2	107.1	120.5	124
15	31.1	56.4	83.5	95.7	110.5	116.6
20	25.1	48.3	69.4	86.6	98.1	105
25	20.5	40.3	58.9	74.2	87.7	95.8
30	16.3	33.7	53.2	68.8	80.1	88.6
35	13.3	30.4	46.8	61.7	72.3	82.3
40	10.8	25	43.2	57.6	67.1	75.5
45	10	23	37.9	52.3	60.8	70.8
50	9	21.1	35.6	48.6	58	63.9
55	6.3	16.9	30.4	44.2	51.7	59.9
60	5.8	16.5	28.6	41.6	49	57.5
65	4.7	15.6	25.3	36	45.5	54.2
70	5.1	12.6	23.1	32.6	42.2	49.8
75	3.1	11.3	21.7	30.1	39.9	46.9
80	2.9	10.8	20.5	29	37.5	44.3
85	3.2	8.7	18.4	27.6	34.5	42.9
90	3.6	9.1	17	23.6	32.3	39.2

95	2.5	7	15.6	23.1	29.9	38.1
100	4	6.3	15.5	20.9	27.9	33.9
105	3.5	7	13.3	20.3	28.6	33.2
110	2.6	5.9	12	18.3	26.1	31
115	2.3	7	12.2	17.3	23.9	30.3
120	3.7	6	11.3	16	22.2	26.2
125	3.1	5.7	9.4	14	21.1	26
130	3.7	5.6	9.9	14.7	19.4	25.3
135	2.6	4.9	9.5	13.7	19.5	24
140	3.9	4.8	7.5	12.7	17.6	22
145	3.5	3.9	7.2	11.7	16.6	22.2
150	1.4	2.6	7.9	11.5	17	20.6
155	2.3	4.4	7.7	11	15.9	19.2
160	3.2	3.4	6	9.7	14.9	18.6
165	2.4	3.3	6.2	9.6	12.5	18.3
170	3.3	3.7	4.8	9.3	11.8	15.7
175	2.3	3	6.2	7.7	12.6	15.9
180	3.5	4.4	5.2	8	11.3	15.1
185	3	2.5	6.8	7.6	10.3	13.7
190	3.9	2.1	5.3	7.3	10.9	12.5
195	2.7	2.8	4.4	6.8	10.2	12.1
200	2.9	3.1	4.2	6.5	9.2	11.2
205	3.2	4.1	3.7	7.5	9	12
210	2	2.9	4.3	6	8.8	10.6
215	3.6	2.5	4.6	6.3	7	10.3
220	3.6	1.8	4.1	6.4	6.7	10.5
225	2.5	3.3	3.2	4.8	7.4	8.9
230	4	3.4	3.8	5.9	7.6	8.9
235	3.8	3	4.1	5.4	6.5	9.2
240	2.6	3	5.4	3.8	5.3	9.2
245	2.4	3.6	2.8	2.7	6.6	8.4
250	4.2	3.4	3.8	5	4.8	6.2
255	3.8	2.8	4.2	4.6	7.1	7.4

Table A.4. Data for Figure 3.9.

Time (sec)	para-iodophenol (uM)								
	10	50	100	200	300	400	500	1000	1500
0		0	0	0	0	0	0	0	
5		53.3	74.2	132	96	86.4	92.6	42.7	5.3
10	29.9	114	170.3	211	229	209	185	69.2	11.5
15	21.7	108	154.4	176	199.7	185	164	63.1	5
20	15.3	100	135.1	145	166.1	153	137	60.2	2.3
25	12.1	92.6	122	124	140.8	121	110	54.3	2.8
30	9	86.6	109.5	106	115.3	95.7	83.5	53.1	2.6
35	6.2	80.7	95.7	92.5	98.3	72.8	62.7	50.3	2.1
40	3.6	76.7	89.3	79	83	56.3	45.1	45.2	1.9
45	4.5	71.7	81.7	67.8	68.4	42.5	32.1	42.3	2
50	3.9	66.7	73	55.2	56	32.2	18.7	41.8	2.1
55	3.1	63.1	66.7	48.2	45.8	22.4	11.5	38.9	1.6
60	2.7	58.5	61.7	42.5	37.9	15.5	5	34.6	2.3
65	2.1	56.2	56.7	34.1	29.8	9.6	2.3	22.4	2.5
70	3.1	53.5	51.2	30	21.7	6.5	2.8	20	1.9
75	2.4	51.2	47.6	25	15.3	3.1	2.6	15.2	2
80	1.3	49.6	44.2	21.5	12.1	2.5	2.1	7.8	2
85	2.4	44.6	41.4	17.6	9	3.2	1.9	3.4	2.3
90	1.2	43.8	37.9	14.3	6.2	1.6	2	2	2.2
95	2	40.2	32.8	10.6	3.6	1.5	2.1	1.8	2.9
100	1.9	38.7	32.1	8.6	4.5	1.8	1.9	1.5	1.9
105	2	38.3	28.9	6.7	3.9	2.6	1.2	0.8	2.2
110	1.6	35.9	25.8	5.3	3.1	2	1.7	1.5	1.6
115	1.5	34.5	24.3	5.2	2.5	0.3	0.7	2	1.3
120	1.8	34.8	23.7	4	1.9	1.3	1.1	3.1	0.9
125	1.6	30.7	21.7	3.3	2	0.3	1.2	0.7	1.7
130	1.2	29.9	19.8	3.1	2	1.3	1.3	0.9	1
135	0.6	29.1	18.6	2.7	2.3	1.8	1.5	1	1.4
140	1.9	28.2	16.8	2.1	2.2	1.7	0.8	0.5	0.9
145	1	26.8	15.4	3.1	2.9	0.8	0.6	3.1	2
150	2.4	24.5	13.8	2.4	1.9	1.3	1.7	2.4	1.4
155	0.3	24.8	11.2	1.3	2.2	2.7	2.3	1.8	0.3
160	1.4	23.3	12.8	2.4	1.6	2.5	0.6	1.7	1.6
165	0.9	21.4	9.8	1.2	1.3	2.9	0.9	1.3	1.7
170	2	22.3	9.1	2	1.4	2.1	1.7	0.5	1.4

175	1.4	20.9	7.7	1.9	2.2	0.5	1	1.4	0.3
180	1.5	19.9	9.9	2	0.7	1.7	1.4	3.4	0
185	1.8	20	9.8	1.6	1.2	0.6	0.9	3.4	2
190	1.6	19	6.5	1.5	1.8	1.2	2	1.2	1.5
195	1.2	18.2	6.9	1.8	2.1	0	1.4	0.9	1.4
200	0.6	16.8	6.2	1.6	0.1	0	0.3	1.3	0.3
205	1.9	16.6	6.5	1.2	0.9	2.3	1.6	1.5	0
210	1.5	15.3	4.8	0.6	1.4	1.4	1.7	1.4	2
215	1.8	15.4	5.2	1.9	1.9	1.5	1.4	1.6	1.5
220	1.6	16	5	1	1.3	1.8	0.3	0.8	1.4
225	1.2	14.9	5.1	2.4	0.9	1.6	0	1.7	1.7
230	0.6	12.7	4.2	0.3	1.1	1.2	2	0.6	0.9
235	1.9	15.3	5.2	1.4	1.4	0.6	1.5	1.2	1.2
240	1.7	12.4	4.8	1.7	1.5	1.9	1.8	1.2	1.2
245	0.7	12.9	4.2	0.7	0.4	2	0.9	1.5	0.7
250	1.3	11.6	4.6	1.2	2.1	1	0.9	0.8	1.6
255	1.2	10.8	3.7	1.2	2.4	2	2.2	1.2	1.8

Table A.5. Data for Figures 3.13 and 3.16.

Time (sec)	HRP dose (AU/mL)									
	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
0	0	0	0	0	0	0	0	0	0	0
5	3.3	8.1	12	23	24.5	36.7	48.1	54.8	57.5	68.5
10	4.4	11.4	22.2	40.4	52.2	81.4	97.1	115.8	123.8	147.5
15	5.3	10.9	22.7	43.8	58.1	89.7	104.3	122.1	131.9	158.3
20	4	11.8	24.4	46.5	61.1	92.2	107.7	124.1	138.6	160.1
25	3	12.7	23	46.1	62.1	91.2	108.5	124.9	138.8	161.4
30	4	12.3	23.5	45.1	61.6	91.8	108.7	125	140	159.4
35	3.6	13.1	24.7	47.3	63.5	91.9	107.5	123.3	136.5	156.7
40	3.7	11.8	24.3	46.3	63.7	89.7	106.4	121.4	135.9	155.5
45	2.1	13.4	25.8	46.1	63.2	91.1	105.5	119.8	132.3	150.9
50	2.8	12	25.3	45.7	62.7	88.8	104.1	118.3	131.3	149.3
55	3.1	10.9	25.5	46.7	62.5	87.8	103	118.3	127.3	146.3
60	2.5	12.7	25.8	46.5	61.5	87.6	101.7	115.8	127.5	142.2
65	3.6	12.8	25.2	45.6	61.6	87.1	101	113.6	125.5	139.5
70	3.7	11.2	24.4	44.1	60.6	87	99.3	113.6	122.6	136.3
75	3.7	13.5	24.4	45.1	61	84.2	99.1	111.9	120.9	134.5
80	2.9	12.3	24.6	44.3	61.5	85.3	97.6	108.7	119.9	130.2

85	3.7	12.1	24.3	44.6	59.8	83.2	95	108.9	117.7	129.4
90	2.8	12.5	25.6	45.9	60.2	83.2	96.6	106.6	115.9	126
95	3.4	12	23.9	46.2	58.9	82.9	94.5	104.3	112.9	122.5
100	3.1	11.1	24.9	43.5	58.7	83	92	103.7	110.6	119.6
105	3.2	12.3	23.7	43	57.3	79.7	91	100.8	109.2	116.7
110	3.2	11.1	23.9	43.5	59.7	79.5	92.2	99.4	105.9	114.5
115	3.4	12.3	24	43.4	58.5	78.1	88.8	97.8	103.4	110.4
120	3.8	12.1	23.5	42.9	57.6	77.6	88.3	96	101.7	106.1
125	3.8	11.5	24.1	44.6	57.7	77	86.8	93.3	98.6	102.5
130	3.7	11.7	25	42.4	57.3	75.3	86	93.6	98.4	100.3
135	2.9	12	25.1	42.5	57.3	75	84.2	90.2	95.3	96.2
140	1.8	12.4	24.3	42.2	56.2	75	83	88.6	92.7	93.3
145	3.2	10.6	24	42	56	73.2	83.4	86.8	87.8	89.7
150	3.2	11.9	24.6	42.6	55.5	72.9	81.1	85.3	87.8	86
155	4.3	11.1	24.5	42.7	54.1	71.7	78.6	83.8	85.5	81.5
160	3.9	10.8	23.9	42.6	55.1	71.1	79.3	81.2	82.9	78.2
165	3	10.5	23.2	41.8	54.5	69.1	77.4	80.3	79	75.9
170	1.4	11.3	24.7	40.9	55.8	69.3	74.6	77.7	78.4	70.9
175	3	12.5	22.5	42.5	53.8	67.9	74.8	76.5	75.4	65.5
180	1.9	11.9	23.4	42.8	53.6	68.7	72.7	74.3	71.6	61.7
185	3.5	11.4	22.4	41.6	52.7	66.3	72	70.4	70.4	58.5
190	2.6	12.6	23.8	41.1	52.6	66	70.7	69.6	66.2	54.5
195	2.3	10.4	22.8	41.2	51.3	64.9	68.7	66.6	64.7	49.6
200	2.7	12	22.8	40.5	52.9	64.6	67.2	65.3	60.8	46.8
205	3.6	11.6	24.5	41.1	51.8	63	66.3	63.3	58.4	41.3
210	3.6	12.1	23.7	40.4	52.5	60.4	64.7	62.3	55.3	36.4
215	2.8	12.7	24.6	40.7	51.5	60.6	64.2	59.7	52.5	32.7
220	3.2	10.1	23	39.3	51.5	60.4	61	57.5	49.9	27.5
225	2.8	9.8	25.6	41.3	50.6	58.2	60	55.1	47.2	24.1
230	2.1	11.3	22.6	40.1	51.2	57.3	58.5	53.9	43.1	19.3
235	2.7	11.4	23.2	38.5	49.8	56.4	56.7	51.9	39.7	15.9
240	1.9	11	24.7	39	48.8	55.8	55.2	50	36.8	14.9
245	2.6	11	23.2	38.1	49.4	56.5	54	47	34.3	11.9
250	3.3	11.3	23.2	39.1	48.2	54.7	53.2	43.4	31.7	8
255	2.4	12.1	23.8	38.5	47.6	54.7	49.8	42.1	27	7.8

Table A.6. Data for Figure 3.12.

Time (sec)	HRP Dose (AU/mL)									
	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
5	19.7	37.3	49.5	71.1	93	103	143.1	181.8	184.4	186.7
10	29.5	66.5	89.6	116	153.8	190.4	226.5	269.5	289.7	308.1
15	30.8	67.8	97	120.4	160.6	185.3	223.1	259.1	278.4	297.4
20	31.7	67.3	95.6	116.3	154.8	175.9	210.6	237	256.8	269.3
25	32.3	65.9	93.4	112.3	147.3	166.3	195.9	217.5	236.2	243.7
30	32.4	66.3	90.5	106.3	139.8	155.3	183.1	199.2	214.3	216.2
35	31.4	63.7	89.2	101.4	130.8	145.5	168.3	184.8	191.8	194
40	30	62.4	85.4	96.6	124.4	136.7	154.5	167.9	171.4	170.3
45	30.1	61.1	82	93.4	116.8	128.7	144.7	152.6	156	149.1
50	30.9	60.6	78.6	89.7	113.6	120.2	133.5	138.5	142.1	131
55	27.4	58.4	75.5	86	106.2	111.4	123.7	125.2	127.9	113.6
60	28.5	57.4	72.4	81	101.1	105	115	114.1	116.9	100
65	28.3	55.5	69.4	76.6	94.4	97.7	107.1	104.5	106	87.2
70	29.4	54.9	67.8	73.6	89.1	90.1	98.3	93.1	93.4	74.4
75	27	54.1	65.2	69.7	87.3	82.1	91.5	83.7	82.5	62.7
80	26.9	53.5	63.7	68	82.7	75.9	84.5	76.8	76.6	51.3
85	27	52.4	60.4	64.7	78.4	70.4	78.3	68.5	65.4	43.4
90	27.7	51.2	58.4	61.6	72.7	63.6	71.9	62	55.9	34.8
95	26.6	49.4	56.3	58.7	69.2	59.9	64.7	54.1	49.5	27.7
100	27.1	49.3	54.8	57.1	67.1	54.8	60.7	46.5	41.4	23.3
105	24.8	48.5	53	54.2	60.9	51.6	54.6	39.2	34.8	18.8
110	24.6	47	52.6	51.9	58.6	47.9	49.1	34	29.7	13.5
115	26	45.1	49.7	50.8	57.3	43.6	43.5	28.5	25.2	11.5
120	25.3	46	48.3	47.7	52.7	39.3	40.1	23.7	20.8	7.8
125	23.8	44.5	47.3	46.3	50.3	37.8	35.9	19.9	17	6.2
130	24	44.5	46.9	44.7	47.7	33.1	31	14.5	13.9	4.4
135	23.9	42.1	43.9	42.3	42.3	30.6	28.5	11.8	12.4	4
140	23.5	41.4	43.6	40.7	41.9	27.2	24.7	9.1	8.7	2.2
145	24	40.6	41.3	39.6	38.1	25.9	20.8	7.8	6.9	2
150	23.8	40.4	39.7	37	34.8	21.5	19	5.2	7.1	0.6
155	24.6	39.2	39	34.9	34.2	20.4	15.6	6.3	4.6	2.9
160	21.9	39.8	37.5	33.8	32.1	19.7	13.6	3.9	3.7	1.6
165	22.1	38.8	37.7	32.9	28.4	16	11.2	2.6	3.4	1.1
170	22.7	36.7	35.2	31.8	28.3	14.6	9.8	3.3	3.9	0.9
175	22.2	37	33.9	27.8	26.3	13.2	8.1	3.6	3.3	1.3
180	21.7	35.3	33.2	28.3	23.4	11.9	6.5	3.2	1	0.9
185	22.5	35.4	31.8	26	22.4	9.8	6.2	3	2.6	1.6
190	19.7	34.8	30.4	23.8	19.9	8.5	5.1	1.8	1.5	0.9
195	20.8	34.1	30.3	23.4	18.5	7.9	4.1	0.9	2	1.3
200	21.8	3.5	27.4	23.6	17.8	7.7	4.2	0.3	3.2	0.9
205	21.6	31.9	27.6	21.1	16.5	4.8	2.4	2.6	2.8	1.8

210	21.1	32.4	26.5	20.7	15.9	5	3.9	1.8	2.1	1.6
215	20.7	32.1	25.4	18.8	12.7	6.4	3.2	2.5	0.4	0.8
220	22.1	30.6	24.6	18.7	13.4	5.9	2.3	1.8	2.3	2.5
225	21	29.5	24.9	16.7	11	5.4	2.5	1.2	1.6	1.9
230	19.9	29.8	24.4	16.4	10.4	3.7	2.2	2.1	1.2	2.7
235	20.3	29.6	23.7	16	8.8	4.1	2	1.8	0.4	2
240	19.9	28.5	21.4	14.7	8.4	3.3	2.6	2.2	1.1	1.4
245	19.4	28.2	21.8	14.2	7.6	3.9	0.9	1.9	2.3	0.8
250	19.3	27.8	19.4	14.7	6.4	16	1.4	1	2.3	2
255	18.8	27.5	19.3	12.7	7.5	2.9	2.5	2	1.7	2.3

Table A.7. Data for Figure 3.15.

Enzyme (unit/mL)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Time (second)	Intensity									
0	0	0	0	0	0	0	0	0	0	0
5	6.98	13.3	39.6	74.5	85.2	104.7	120.7	132.6	146.9	163.5
10	13.3	26.6	48.5	72.7	82.7	101.6	109.8	119.2	127.6	134.3
15	15.9	28.9	47.5	69.4	79.6	92.9	96.7	104	110.1	111.9
20	16.9	29.3	46.6	65.1	72.1	82.9	89.3	92.6	97.1	97
25	17.2	28.7	44.1	60.7	68.9	75.1	79.7	82.3	83.8	85.8
30	15.9	28.5	42.8	57.5	63.6	70.7	70.1	74.6	74.6	75.4
35	17.9	27.4	41.6	54.9	59.7	64.1	63.7	65.4	66.9	67.4
40	15.9	25.9	39	50.7	55	58.8	58.5	58.2	61	61.3
45	16.8	25.9	35.8	47.5	52.7	54.8	54.7	55.2	54.9	55.2
50	16.6	26.4	35.8	43	47.8	51.8	51.1	49.4	51.4	49
55	16.7	24.8	36.5	41.5	45.1	48.7	47.8	47	47.1	45.1
60	15.6	25	35.1	40.5	43.2	45.9	44.5	43.8	43.3	39.1
65	16.5	25.3	33	39.4	39.9	42.7	40.9	40.6	41.9	37.8
70	15.3	23.8	31.9	38.4	39.2	40.4	39.6	37.6	38.3	35.7
75	15.1	23	31.5	35.5	38	37.5	37.5	35.4	36.5	32.4
80	15.3	23.3	30.2	32.5	35.7	35.5	35.2	32.9	33.8	29.3
85	13.9	21.4	29.4	33.1	34.5	34.6	33.9	30.8	31.8	27.7
90	14.8	21.7	27.3	31.6	32.8	32.8	31.7	29.3	29.5	26.7
95	15.1	19.7	27.3	31	31.4	30.3	29.6	26.8	28.7	23.8
100	14.1	20.5	26.4	28.2	30.3	29	28.6	25.6	26.6	22.5
105	13.3	19.3	24.1	27.9	28.7	28.7	27	24.8	24	22.4

110	13.5	19.5	25	27.7	27.3	27	25.6	23.6	24.2	19.1
115	14.3	19	23.4	26.1	25.5	26.3	24	22.4	23.1	17.9
120	12.6	18.8	23.9	24	25.5	25.4	23.3	21	21.6	17.7
125	14.4	17.9	22.9	21.7	25	24.6	21.3	19.1	20.2	17.3
130	14	17.9	22.5	22.5	23.8	23.8	22.7	19	19.5	14.6
135	15.1	18.9	21.7	24.3	22.2	21.9	20.2	18.1	19.1	14.8
140	12.3	17.1	21	21.3	22.8	21.3	19.1	17.8	16.5	13.7
145	12.1	17.5	19.1	20.5	21	21.2	20.2	16.3	15.6	13

Enzyme (unit/mL)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Time (second)	Intensity									
0	0	0	0	0	0	0	0	0	0	0
5	180.7	188.6	199	207.1	220	226.5	257.2	250	257.2	284.3
10	154.4	172.7	167.7	153.9	17.4	170.5	183.4	176.4	188.7	218.5
15	128.9	137	143.7	124.1	12.5	133	137.1	133.7	143.5	163.2
20	105.7	107.3	119	103.3	98.4	107.8	104	106.4	112.7	114.4
25	89.5	88.9	98	88.5	70.2	90.2	82.2	76.9	86.7	82.4
30	75.7	78.2	83	76.6	54.8	74.8	65.7	58.8	70.2	63.8
35	66.7	66.9	72.7	65.8	45.1	64.5	54.6	47.7	57.3	52.3
40	60.3	57.3	63.4	55.5	38.4	56.6	46.4	38.7	46.9	46.2
45	60.3	51.4	57.6	48.8	32.7	49.3	39.5	33.4	38.9	39.9
50	52.7	45.7	52	43.2	28	43.2	35.3	27.7	32.9	33.2
55	47.9	39.5	45.7	40.4	24.3	39.7	29.4	23.6	27.7	27.1
60	42.4	38.1	40.4	34.7	19.9	36.6	26.5	18.5	24.1	23.5
65	39.3	33.3	38	32.5	16.4	31.5	21.6	16.6	20.9	20.4
70	35.9	29.4	34.8	29.5	14.5	28.8	18.6	13.7	17	17.2
75	31.7	26	31.3	26.8	11.2	26	15.8	11.7	14.8	15.1
80	31.4	25.1	28.7	23.5	10.5	23.1	14.4	10.3	14.1	11.1
85	28.3	22.4	27.1	22.3	8.9	21.9	10.8	9	11.5	10.3
90	26.9	20.6	26	21.3	8.3	19.6	10.7	7.3	11.4	9.1
95	24.4	19.6	21.9	18.6	7.3	19.3	8	7.3	8.7	7.7
100	23	15.6	21.7	16.3	6.7	17.2	7.2	5.5	7.2	7.5
105	21.6	14.2	20.4	15.9	5	13.8	6.3	3.8	8	4.9
110	20.2	12.8	17.6	13.5	4.6	14.1	6	4.3	5	5.1
115	17.5	12.5	16	12.7	4.4	12.1	5.7	4.5	5	4.2
120	17.8	9.3	15.9	11.9	3.1	11	5.6	3.7	4.6	4.7
125	16.1	10.6	16	10.3	3.9	11.4	5.4	3.8	3.9	3.7
130	14	8.7	14.6	9.2	3.5	9.6	4.4	3.4	3.5	3.7
135	13.7	9.2	12.5	8.8	4.4	8.2	3.1	2.4	3.5	2.2
140	14.1	9.3	11.5	7.8	4.1	7.8	3.1	3.5	4.5	3.1
145	10.2	7.1	11.4	6.5		8.4			2.8	

Table A.8. Data for Figure 3.17.

Enzyme (AU/mL)	Max. intensity	
0	0	0
0.2	10.6	5.3
0.4	16.7	13.4
0.6	68.3	25.8
0.8	108.3	47.3
1	152.9	63.5
1.2	195.6	92.2
1.4	214.7	108.7
1.6	252.2	125
1.8	278.6	140
2	307.8	161.4

Table A.9. Data for Figure 20.

Time (sec)	phenol concentration (uM)									
	10	50	100	200	300	400	500	700	1000	1500
0	0	0	0	0	0	0	0	0	0	0
5	4.5	5.2	6	9.2	10.8	11.5	9.8	9.9	11.2	9.2
10	4.6	5.7	7.9	10.9	15.6	15.5	16.2	13.3	15.4	10.8
15	4.9	5.9	8.9	12.7	17.3	17.3	16.7	17.2	13.8	11.2
20	4.6	6.7	8.6	13.6	17.5	18.6	16.9	14.9	14	9.7
25	4.9	7.1	10.2	15.2	17.2	16.9	15.9	14.6	12.2	8.6
30	4.4	7.2	10.8	14	18.2	17.7	16.4	14.6	10.3	9
35	6.6	7.7	10.9	15.3	17.1	16.3	14.3	12.7	10.3	5.6
40	4.6	6	11.8	15.4	17.4	16.7	14	12.8	9.2	6.4
45	4.2	8.1	11.7	14.8	16.4	16	14.1	13.1	8.6	7.3
50	5.6	7.3	12.3	14.9	16.4	14.7	13.6	10.7	8.2	6.2
55	3.4	8.6	11.1	15.9	17.1	15.2	13.5	9.6	8.8	4.6
60	4.8	8.4	12.1	15.4	15.8	15.2	12.4	9	7.3	4.4
65	4.9	6.8	11.9	13.9	15.7	14	11.6	8	7.7	4.7
70	4.2	8.5	11.8	12.7	14	13.6	11.4	8.6	6.6	4.3
75	6	5.9	12.6	12.9	14.8	11.9	10.7	8.3	5	3
80	5.1	9.4	12.8	13.4	11.9	11.6	10.6	7.9	6.2	3.5
85	5.4	9.1	12.7	12.8	11.8	11.1	9.8	7.3	4.6	2
90	5.4	9.3	12.4	13.6	12.2	10.8	9.1	6.5	5.3	2.1
95	4.9	8.7	12.1	12.1	11.8	11.5	8.6	7.3	5	2.9
100	5.4	8.7	11	11.3	12.3	9.3	9.5	5.9	3.6	3.9

Table A.10. Data for Figure 3.21.

No.	pore volume	Distance	Time	Tracer C/Co	MT Fitted	HRP C/Co	MHRP Fitted
1	0	12	0	0	0	0	0
2	0.041528	12	5	0	0	0	0
3	0.083056	12	10	0	0	0	0
4	0.124585	12	15	0	0	0	0
5	0.166113	12	20	0	0	0	0
6	0.207641	12	25	0	0	0	0
7	0.249169	12	30	0	0	0	0
8	0.290698	12	35	0	0	0	0
9	0.332226	12	40	0	0	0	0
10	0.373754	12	45	0	0	0	0
11	0.415282	12	50	0	0	0	0
12	0.456811	12	55	0	0	0	0
13	0.498339	12	60	0	0	0	0
14	0.539867	12	65	0	0	0	0
15	0.581395	12	70	0	0	0	0.0001
16	0.622924	12	75	0	0	0	0.0005
17	0.664452	12	80	0.0013	0.0001	0	0.0025
18	0.70598	12	85	0.0042	0.001	0.0021	0.0088
19	0.747508	12	90	0.0087	0.0052	0.0103	0.0245
20	0.789037	12	95	0.0164	0.0191	0.0355	0.0562
21	0.830565	12	100	0.0385	0.0537	0.081	0.1098
22	0.872093	12	105	0.0793	0.1207	0.1469	0.188
23	0.913621	12	110	0.1729	0.2254	0.2241	0.2878
24	0.95515	12	115	0.35	0.3611	0.3662	0.4016
25	0.996678	12	120	0.5178	0.5102	0.5231	0.5192
26	1.038206	12	125	0.7254	0.6523	0.5683	0.6305
27	1.079734	12	130	0.8361	0.7712	0.6748	0.7281
28	1.121262	12	135	0.879	0.8602	0.769	0.8082
29	1.162791	12	140	0.9683	0.9204	0.875	0.8698
30	1.204319	12	145	0.9655	0.9576	0.9434	0.9149
31	1.245847	12	150	0.9879	0.9788	0.9866	0.9463
32	1.287375	12	155	1.0037	0.9899	0.9721	0.9671
33	1.328904	12	160	0.9739	0.9955	1.0103	0.9805
34	1.370432	12	165	1.0219	0.9981	0.9828	0.9887
35	1.41196	12	170	0.9795	0.9992	0.9745	0.9937
36	1.453488	12	175	1.0111	0.9997	0.9917	0.9965
37	1.495017	12	180	0.9874	0.9999	0.9916	0.9981

38	1.536545	12	185	1.0209	1	1.0197	0.999
39	1.578073	12	190	1.046	1	0.95	0.9995
40	1.619601	12	195	1.0414	1	0.9791	0.9997
41	1.66113	12	200	1.0001	1	1.02	0.9999
42	1.702658	12	205	0.9953	1	1.034	0.9999
43	1.744186	12	210	0.9767	1	0.9897	1
44	1.785714	12	215	1.0139	1	0.9938	1
45	1.827243	12	220	1.0361	1	1.0702	1
46	1.868771	12	225	1.0093	1	0.9993	1
47	1.910299	12	230	1.0023	1	1.0097	1
48	1.951827	12	235	0.986	1	0.9483	1
49	1.993355	12	240	1.0001	1	1.032	1
50	2.034884	12	245	0.9907	1	0.9624	1
51	2.076412	12	250	1.0006	1	1.0023	0.9999
52	2.11794	12	255	1.0279	1	1.0109	0.9995
53	2.159468	12	260	0.986	0.9999	0.978	0.9975
54	2.200997	12	265	1.0687	0.999	0.995	0.9912
55	2.242525	12	270	1.0186	0.9948	0.9901	0.9755
56	2.284053	12	275	0.987	0.9809	0.9808	0.9438
57	2.325581	12	280	0.956	0.9463	0.8748	0.8902
58	2.36711	12	285	0.8403	0.8793	0.8128	0.812
59	2.408638	12	290	0.7426	0.7746	0.6924	0.7122
60	2.450166	12	295	0.5946	0.6389	0.6203	0.5984
61	2.491694	12	300	0.407	0.4898	0.5448	0.4808
62	2.533223	12	305	0.2737	0.3477	0.3817	0.3695
63	2.574751	12	310	0.2315	0.2288	0.3428	0.2719
64	2.616279	12	315	0.1907	0.1398	0.2314	0.1918
65	2.657807	12	320	0.1483	0.0796	0.1779	0.1302
66	2.699336	12	325	0.1203	0.0424	0.1197	0.0851
67	2.740864	12	330	0.0924	0.0212	0.0776	0.0537
68	2.782392	12	335	0.0412	0.0101	0.0531	0.0329
69	2.82392	12	340	0.0598	0.0045	0.0372	0.0195
70	2.865449	12	345	0.0319	0.0019	0.0248	0.0113
71	2.906977	12	350	0.018	0.0008	0.0197	0.0063
72	2.948505	12	355	0.0133	0.0003	0.0124	0.0035
73	2.990033	12	360	0.0086	0.0001	0.0072	0.0019
74	3.031561	12	365	0.004	0	0	0.001
75	3.07309	12	370	0.004	0	0	0.0005
76	3.114618	12	375	0.004	0	0	0.0003
77	3.156146	12	380	0.0086	0	0	0.0001
78	3.197674	12	385	0.0133	0	0	0.0001

79	3.239203	12	390	0.018	0	0	0
80	3.280731	12	395	0.0133	0	0	0
81	3.322259	12	400	0.0086	0	0	0
82	3.363787	12	405	0	0	0	0
83	3.405316	12	410	0	0	0	0
84	3.446844	12	415	0	0	0	0
85	3.488372	12	420	0	0	0	0
86	3.5299	12	425	0	0	0	0
87	3.571429	12	430	0	0	0	0
88	3.612957	12	435	0	0	0	0
89		12	440	0	0	0	0
90		12	445	0	0	0	0
91		12	450	0	0	0	0

Appendix B - Data for Chapter 4

Table B.1. Data for Figures 4.8 and 4.11.

Sample No.	Time (min)	Pore vol. (mL)	phenol in	phenol out	Soluble polymer out	polymer in column
0	180	1.79	0	0	0	0
1	185	1.84	1.5	1.5	0	0
2	190	1.89	3	2.99	0	0
3	195	1.94	4.5	4.49	0	0
4	200	1.99	6	6	0	0
5	205	2.04	7.5	7.51	0	0
6	210	2.09	9	8.98	0	0
7	215	2.14	10.5	10.48	0	0
8	220	2.19	12	11.99	0	0
9	225	2.24	13.5	13.49	0	0
10	230	2.29	15	14.99	0	0
11	235	2.34	16.5	16.49	0	0
12	240	2.39	18	17.98	0	0
13	245	2.44	19.5	19.49	0	0
14	250	2.49	21	20.99	0	0
15	255	2.54	22.5	22.48	0	0
16	260	2.59	24	23.99	0	0
17	265	2.64	25.5	25.48	0	0
18	270	2.69	27	26.98	0	0
19	275	2.74	28.5	28.44	0	0
20	280	2.79	30	29.8	0	0.2
21	285	2.84	31.5	31.07	0	0.43
22	290	2.89	33	32.26	0	0.74
23	295	2.94	34.5	33.35	0	1.15
24	300	2.99	36	34.34	0	1.66
25	305	3.04	37.5	35.26	0	2.24
26	310	3.09	39	36.12	0	2.88
27	315	3.14	40.5	36.96	0	3.54
28	320	3.19	42	37.76	0	4.24
29	325	3.24	43.5	38.47	0.01	5.02
30	330	3.29	45	39.19	0.01	5.81
31	335	3.34	46.5	39.89	0.01	6.6
32	340	3.39	48	40.59	0.01	7.41
33	345	3.44	49.5	41.25	0.01	8.24
34	350	3.49	51	41.9	0.01	9.09
35	355	3.54	52.5	42.55	0.01	9.94
36	360	3.59	54	43.18	0.01	10.81
37	365	3.64	55.5	43.78	0.01	11.71
38	370	3.69	57	44.4	0.01	12.59
39	375	3.74	58.5	45.02	0.01	13.47
40	380	3.79	60	45.63	0.01	14.36
41	385	3.84	61.5	46.23	0.01	15.26
42	390	3.89	63	46.85	0.01	16.14

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
43	395	3.94	64.5	47.45	0.01	17.04
44	400	3.99	66	48.04	0.03	17.93
45	405	4.04	67.5	48.64	0.05	18.81
46	410	4.09	69	49.22	0.09	19.69
47	415	4.14	70.5	49.8	0.12	20.59
48	420	4.19	72	50.41	0.12	21.47
49	425	4.24	73.5	51	0.12	22.38
50	430	4.29	75	51.58	0.12	23.3
51	435	4.34	76.5	52.16	0.12	24.22
52	440	4.39	78	52.73	0.12	25.15
53	445	4.44	79.5	53.3	0.12	26.08
54	450	4.49	81	53.88	0.12	27
55	455	4.53	82.5	54.45	0.13	27.93
56	460	4.58	84	55.01	0.13	28.87
57	465	4.63	85.5	55.56	0.13	29.82
58	470	4.68	87	56.09	0.19	30.72
59	475	4.73	88.5	56.63	0.31	31.56
60	480	4.78	90	57.17	0.44	32.39
61	485	4.83	91.5	57.65	0.52	33.33
62	490	4.88	93	58.14	0.59	34.27
63	495	4.93	94.5	58.63	0.67	35.2
64	500	4.98	96	59.12	0.78	36.1
65	505	5.03	97.5	59.61	0.89	37
66	510	5.08	99	60.12	0.99	37.89
67	515	5.13	100.5	60.64	1.09	38.77
68	520	5.18	102	61.15	1.22	39.63
69	525	5.23	103.5	61.68	1.34	40.48
70	530	5.28	105	62.2	1.5	41.3
71	535	5.33	106.5	62.71	1.67	42.11
72	540	5.38	108	63.26	1.8	42.94
73	545	5.43	109.5	63.73	1.98	43.79
74	550	5.48	111	64.19	2.19	44.62
75	555	5.53	112.5	64.66	2.38	45.46
76	560	5.58	114	65.12	2.59	46.29
77	565	5.63	115.5	65.58	2.65	47.26
78	570	5.68	117	66.05	2.7	48.25
79	575	5.73	118.5	66.5	2.84	49.16
80	580	5.78	120	66.97	2.91	50.12
81	585	5.83	121.5	67.35	3.05	51.11
82	590	5.88	123	67.82	3.1	52.08
83	595	5.93	124.5	68.29	3.19	53.02
84	600	5.98	126	68.74	3.32	53.94
85	605	6.03	127.5	69.2	3.45	54.85
86	610	6.08	129	69.67	3.62	55.71

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
87	615	6.13	130.5	70.19	3.76	56.55
88	620	6.18	132	70.73	3.89	57.38
89	625	6.23	133.5	71.28	4	58.22
90	630	6.28	135	71.83	4.1	59.07
91	635	6.33	136.5	72.34	4.24	59.92
92	640	6.38	138	72.82	4.41	60.76
93	645	6.43	139.5	73.28	4.62	61.6
94	650	6.48	141	73.79	4.76	62.45
95	655	6.53	142.5	74.24	4.92	63.34
96	660	6.58	144	74.78	4.96	64.26
97	665	6.63	145.5	75.25	5.06	65.19
98	670	6.68	147	75.74	5.12	66.15
99	675	6.73	148.5	76.18	5.21	67.1
100	680	6.78	150	76.64	5.27	68.09
101	685	6.83	151.5	77.11	5.45	68.94
102	690	6.88	153	77.57	5.65	69.78
103	695	6.93	154.5	78.04	5.84	70.62
104	700	6.98	156	78.5	6.06	71.44
105	705	7.03	157.5	78.97	6.32	72.21
106	710	7.08	159	79.49	6.56	72.96
107	715	7.13	160.5	80.03	6.77	73.7
108	720	7.18	162	80.59	6.92	74.49
109	725	7.23	163.5	81.21	7.04	75.25
110	730	7.28	165	81.81	7.17	76.02
111	735	7.33	166.5	82.36	7.34	76.8
112	740	7.38	168	82.86	7.55	77.59
113	745	7.43	169.5	83.33	7.79	78.38
114	750	7.48	171	83.75	8.08	79.17
115	755	7.52	172.5	84.16	8.4	79.94
116	760	7.57	174	84.52	8.74	80.74
117	765	7.62	175.5	84.89	9.06	81.54
118	770	7.67	177	85.27	9.37	82.36
119	775	7.72	178.5	85.68	9.65	83.17
120	780	7.77	180	86.12	9.89	83.99
121	785	7.82	181.5	86.58	10.08	84.85
122	790	7.87	183	87.03	10.29	85.68
123	795	7.92	184.5	87.49	10.5	86.51
124	800	7.97	186	87.95	10.7	87.35
125	805	8.02	187.5	88.39	10.91	88.2
126	810	8.07	189	88.85	11.1	89.05
127	815	8.12	190.5	89.31	11.29	89.9
128	820	8.17	192	89.78	11.43	90.79
129	825	8.22	193.5	90.2	11.63	91.67
130	830	8.27	195	90.66	11.72	92.62

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
131	835	8.32	196.5	91.12	11.8	93.58
132	840	8.37	198	91.53	11.93	94.54
133	845	8.42	199.5	92.05	11.95	95.5
134	850	8.47	201	92.54	12.02	96.44
135	855	8.52	202.5	93.04	12.12	97.34
136	860	8.57	204	93.58	12.21	98.2
137	865	8.62	205.5	94.13	12.32	99.05
138	870	8.67	207	94.67	12.42	99.9
139	875	8.72	208.5	95.19	12.57	100.75
140	880	8.77	210	95.68	12.73	101.59
141	885	8.82	211.5	96.14	12.93	102.43
142	890	8.87	213	96.61	13.1	103.28
143	895	8.92	214.5	97.08	13.29	104.13
144	900	8.97	216	97.54	13.49	104.97
145	905	9.02	217.5	97.99	13.69	105.82
146	910	9.07	219	98.44	13.91	106.65
147	915	9.12	220.5	98.93	14.08	107.49
148	920	9.17	222	99.39	14.29	108.33
149	925	9.22	223.5	99.88	14.44	109.18
150	930	9.27	225	100.34	14.63	110.02
151	935	9.32	226.5	100.8	14.84	110.86
152	940	9.37	228	101.26	15.06	111.68
153	945	9.42	229.5	101.71	15.34	112.45
154	950	9.47	231	102.09	15.71	113.2
155	955	9.52	232.5	102.56	16	113.94
156	960	9.57	234	103.08	16.19	114.73
157	965	9.62	235.5	103.63	16.39	115.49
158	970	9.67	237	104.12	16.62	116.26
159	975	9.72	238.5	104.65	16.82	117.04
160	980	9.77	240	105.19	16.98	117.83
161	985	9.82	241.5	105.79	17.09	118.62
162	990	9.87	243	106.34	17.25	119.41
163	995	9.92	244.5	106.86	17.45	120.18
164	1000	9.97	246	107.35	17.67	120.98
165	1005	10.02	247.5	107.83	17.89	121.78
166	1010	10.07	249	108.34	18.06	122.6
167	1015	10.12	250.5	108.89	18.2	123.41
168	1020	10.17	252	109.35	18.42	124.23
169	1025	10.22	253.5	109.8	18.61	125.09
170	1030	10.27	255	110.19	18.89	125.92
171	1035	10.32	256.5	110.65	19.1	126.75
172	1040	10.37	258	111.12	19.3	127.58
173	1045	10.42	259.5	111.51	19.58	128.42
174	1050	10.47	261	111.96	19.78	129.26

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
175	1055	10.51	262.5	112.42	20.08	130
176	1060	10.56	264	112.86	20.29	130.84
177	1065	10.61	265.5	113.32	20.56	131.62
178	1070	10.66	267	113.78	20.76	132.45
179	1075	10.71	268.5	114.25	20.95	133.31
180	1080	10.76	270	114.71	21.13	134.16
181	1085	10.81	271.5	115.17	21.32	135.01
182	1090	10.86	273	115.72	21.38	135.9
183	1095	10.91	274.5	116.24	21.43	136.83
184	1100	10.96	276	116.73	21.57	137.7
185	1105	11.01	277.5	117.2	21.64	138.66
186	1110	11.06	279	117.72	21.77	139.51
187	1115	11.11	280.5	118.26	21.83	140.41
188	1120	11.16	282	118.72	21.92	141.36
189	1125	11.21	283.5	119.18	22.04	142.28
190	1130	11.26	285	119.56	22.18	143.26
191	1135	11.31	286.5	120.02	22.34	144.13
192	1140	11.36	288	120.5	22.49	145.01
193	1145	11.41	289.5	120.94	22.61	145.94
194	1150	11.46	291	121.4	22.73	146.87
195	1155	11.51	292.5	121.86	22.92	147.71
196	1160	11.56	294	122.33	23.21	148.47
197	1165	11.61	295.5	122.79	23.41	149.3
198	1170	11.66	297	123.25	23.71	150.04
199	1175	11.71	298.5	123.8	23.92	150.78
200	1180	11.76	300	124.32	24.19	151.49
201	1185	11.81	301.5	124.81	24.39	152.3
202	1190	11.86	303	125.36	24.57	153.07
203	1195	11.91	304.5	125.88	24.76	153.86
204	1200	11.96	306	126.37	24.95	154.69
205	1205	12.01	307.5	126.84	25.01	155.65
206	1210	12.06	309	127.35	25.06	156.59
207	1215	12.11	310.5	127.88	25.2	157.42
208	1220	12.16	312	128.37	25.27	158.37
209	1225	12.21	313.5	128.91	25.47	159.12
210	1230	12.26	315	129.44	25.65	159.92
211	1235	12.31	316.5	129.92	25.83	160.74
212	1240	12.36	318	130.38	26.03	161.59
213	1245	12.41	319.5	130.83	26.23	162.44
214	1250	12.46	321	131.28	26.45	163.27
215	1255	12.51	322.5	131.74	26.62	164.14
216	1260	12.56	324	132.2	26.83	164.97
217	1265	12.61	325.5	132.66	26.98	165.85
218	1270	12.66	327	133.13	27.18	166.69

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
219	1275	12.71	328.5	133.59	27.38	167.53
220	1280	12.76	330	134.13	27.6	168.26
221	1285	12.81	331.5	134.66	27.65	169.19
222	1290	12.86	333	135.14	27.79	170.06
223	1295	12.91	334.5	135.62	27.86	171.02
224	1300	12.96	336	136.13	28.06	171.8
225	1305	13.01	337.5	136.66	28.24	172.6

Table B.2. Data for Figures 4.9 and 4.12.

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
0	180	1.64	0	0	0	0
1	185	1.69	1.38	1.37	0	0
2	190	1.74	2.75	2.73	0	0
3	195	1.78	4.13	4.08	0	0
4	200	1.83	5.5	5.44	0	0
5	205	1.87	6.88	6.8	0	0
6	210	1.92	8.25	8.18	0	0
7	215	1.96	9.63	9.55	0	0
8	220	2.01	11	10.9	0	0
9	225	2.06	12.38	12.28	0	0
10	230	2.1	13.75	13.66	0	0.09
11	235	2.15	15.13	15	0	0.13
12	240	2.19	16.5	16.34	0	0.16
13	245	2.24	17.88	17.68	0	0.19
14	250	2.28	19.25	19.03	0	0.22
15	255	2.33	20.63	20.39	0	0.24
16	260	2.38	22	21.7	0	0.3
17	265	2.42	23.38	22.96	0	0.42
18	270	2.47	24.75	24.11	0	0.64
19	275	2.51	26.13	25.2	0	0.93
20	280	2.56	27.5	26.25	0	1.25
21	285	2.6	28.88	27.24	0	1.64
22	290	2.65	30.25	28.15	0	2.1
23	295	2.7	31.63	29	0	2.63
24	300	2.74	33	29.76	0	3.24
25	305	2.79	34.38	30.48	0	3.89
26	310	2.83	35.75	31.14	0	4.61
27	315	2.88	37.13	31.77	0	5.36
28	320	2.92	38.5	32.37	0	6.13
29	325	2.97	39.88	32.95	0	6.92
30	330	3.01	41.25	33.53	0	7.72
31	335	3.06	42.63	34.09	0	8.54
32	340	3.11	44	34.63	0	9.37
33	345	3.15	45.38	35.17	0	10.21
34	350	3.2	46.75	35.69	0	11.06
35	355	3.24	48.13	35.69	0.56	11.87
36	360	3.29	49.5	36.17	0.56	12.77
37	365	3.33	50.88	36.65	0.6	13.62
38	370	3.38	52.25	37.07	0.6	14.58
39	375	3.43	53.63	37.53	0.6	15.49
40	380	3.47	55	37.98	0.6	16.42
41	385	3.52	56.38	38.4	0.62	17.36
42	390	3.56	57.75	38.82	0.62	18.31

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
43	395	3.61	59.13	39.22	0.62	19.28
44	400	3.65	60.5	39.63	0.62	20.25
45	405	3.7	61.88	40.03	0.62	21.22
46	410	3.75	63.25	40.41	0.7	22.13
47	415	3.79	64.63	40.8	0.8	23.03
48	420	3.84	66	41.17	0.82	24.01
49	425	3.88	67.38	41.54	0.88	24.96
50	430	3.93	68.75	41.91	0.88	25.97
51	435	3.97	70.13	42.26	0.97	26.89
52	440	4.02	71.5	42.63	1	27.88
53	445	4.07	72.88	42.98	1.05	28.84
54	450	4.11	74.25	43.33	1.08	29.84
55	455	4.16	75.63	43.69	1.08	30.86
56	460	4.2	77	44.03	1.09	31.88
57	465	4.25	78.38	44.38	1.32	32.68
58	470	4.29	79.75	44.7	1.33	33.72
59	475	4.34	81.13	45.04	1.37	34.72
60	480	4.39	82.5	45.41	1.37	35.72
61	485	4.43	83.88	45.78	1.37	36.73
62	490	4.48	85.25	46.16	1.37	37.73
63	495	4.52	86.63	46.54	1.37	38.72
64	500	4.57	88	46.92	1.5	39.58
65	505	4.61	89.38	47.32	1.62	40.43
66	510	4.66	90.75	47.72	2.2	40.84
67	515	4.71	92.13	48.14	2.33	41.65
68	520	4.75	93.5	48.56	2.46	42.48
69	525	4.8	94.88	48.97	2.6	43.31
70	530	4.84	96.25	49.4	2.71	44.15
71	535	4.89	97.63	49.83	2.85	44.94
72	540	4.93	99	50.24	3.01	45.75
73	545	4.98	100.38	50.65	3.13	46.59
74	550	5.02	101.75	51.07	3.28	47.4
75	555	5.07	103.13	51.5	3.37	48.26
76	560	5.12	104.5	51.93	3.5	49.07
77	565	5.16	105.88	52.37	3.6	49.91
78	570	5.21	107.25	52.82	3.66	50.77
79	575	5.25	108.63	53.26	3.71	51.65
80	580	5.3	110	53.69	3.8	52.51
81	585	5.34	111.38	54.1	3.89	53.38
82	590	5.39	112.75	54.5	3.97	54.28
83	595	5.44	114.13	54.89	4.07	55.17
84	600	5.48	115.5	55.29	4.15	56.06
85	605	5.53	116.88	55.69	4.24	56.95
86	610	5.57	118.25	56.08	4.34	57.83

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
87	615	5.62	119.63	56.47	4.48	58.68
88	620	5.66	121	56.88	4.59	59.53
89	625	5.71	122.38	57.32	4.69	60.37
90	630	5.76	123.75	57.79	4.76	61.2
91	635	5.8	125.13	58.27	4.85	62
92	640	5.85	126.5	58.76	4.94	62.8
93	645	5.89	127.88	59.28	5.01	63.58
94	650	5.94	129.25	59.79	5.13	64.33
95	655	5.98	130.63	60.33	5.14	65.15
96	660	6.03	132	60.86	5.16	65.97
97	665	6.08	133.38	61.4	5.2	66.78
98	670	6.12	134.75	61.95	5.25	67.55
99	675	6.17	136.13	62.48	5.32	68.33
100	680	6.21	137.5	62.98	5.39	69.13
101	685	6.26	138.88	63.48	5.43	69.97
102	690	6.3	140.25	63.96	5.51	70.78
103	695	6.35	141.63	64.44	5.63	71.56
104	700	6.4	143	64.9	5.7	72.4
105	705	6.44	144.38	65.35	5.87	73.16
106	710	6.49	145.75	65.8	5.96	73.99
107	715	6.53	147.13	66.26	6.06	74.8
108	720	6.58	148.5	66.72	6.15	75.63
109	725	6.62	149.88	67.17	6.26	76.45
110	730	6.67	151.25	67.61	6.39	77.25
111	735	6.72	152.63	68.07	6.45	78.11
112	740	6.76	154	68.54	6.53	78.93
113	745	6.81	155.38	69	6.61	79.76
114	750	6.85	156.75	69.47	6.66	80.62
115	755	6.9	158.13	69.92	6.72	81.49
116	760	6.94	159.5	70.33	6.77	82.4
117	765	6.99	160.88	70.73	6.84	83.3
118	770	7.03	162.25	71.1	6.93	84.22
119	775	7.08	163.63	71.45	7.02	85.16
120	780	7.13	165	71.8	7.11	86.08
121	785	7.17	166.38	72.14	7.21	87.02
122	790	7.22	167.75	72.49	7.3	87.96
123	795	7.26	169.13	72.86	7.38	88.88
124	800	7.31	170.5	73.24	7.46	89.8
125	805	7.35	171.88	73.64	7.54	90.7
126	810	7.4	173.25	74.05	7.6	91.6
127	815	7.45	174.63	74.46	7.68	92.48
128	820	7.49	176	74.89	7.76	93.35
129	825	7.54	177.38	75.31	7.84	94.23
130	830	7.58	178.75	75.76	7.89	95.1

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
131	835	7.63	180.13	76.19	7.99	95.94
132	840	7.67	181.5	76.64	8.04	96.82
133	845	7.72	182.88	77.08	8.13	97.67
134	850	7.77	184.25	77.53	8.2	98.52
135	855	7.81	185.63	77.98	8.26	99.38
136	860	7.86	187	78.45	8.33	100.22
137	865	7.9	188.38	78.91	8.4	101.06
138	870	7.95	189.75	79.37	8.47	101.9
139	875	7.99	191.13	79.83	8.56	102.74
140	880	8.04	192.5	80.29	8.77	103.45
141	885	8.09	193.88	80.73	8.86	104.28
142	890	8.13	195.25	81.18	8.93	105.14
143	895	8.18	196.63	81.63	9.03	105.97
144	900	8.22	198	82.08	9.11	106.81
145	905	8.27	199.38	82.38	9.24	107.75
146	910	8.31	200.75	82.78	9.34	108.63
147	915	8.36	202.13	83.17	9.44	109.52
148	920	8.41	203.5	83.56	9.53	110.41
149	925	8.45	204.88	83.94	9.63	111.3
150	930	8.5	206.25	84.33	9.71	112.21
151	935	8.54	207.63	84.7	9.81	113.11
152	940	8.59	209	85.06	9.9	114.04
153	945	8.63	210.38	85.41	10.09	114.88
154	950	8.68	211.75	85.76	10.18	115.81
155	955	8.73	213.13	86.12	10.26	116.75
156	960	8.77	214.5	86.45	10.35	117.7
157	965	8.82	215.88	86.77	10.43	118.67
158	970	8.86	217.25	87.07	10.53	119.65
159	975	8.91	218.63	87.37	10.61	120.64
160	980	8.95	220	87.69	10.68	121.62
161	985	9	221.38	88.02	10.76	122.6
162	990	9.04	222.75	88.34	10.84	123.57
163	995	9.09	224.13	88.66	10.91	124.56
164	1000	9.14	225.5	88.98	11	125.52
165	1005	9.18	226.88	89.3	11.08	126.49
166	1010	9.23	228.25	89.6	11.19	127.46
167	1015	9.27	229.63	89.9	11.27	128.45
168	1020	9.32	231	90.19	11.37	129.44
169	1025	9.36	232.38	90.46	11.73	130.19
170	1030	9.41	233.75	90.71	11.8	131.25
171	1035	9.46	235.13	90.92	11.89	132.31
172	1040	9.5	236.5	91.11	11.99	133.4
173	1045	9.55	237.88	91.31	12.08	134.48
174	1050	9.59	239.25	91.52	12.17	135.57

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
175	1055	9.64	240.63	91.73	12.25	136.64
176	1060	9.68	242	91.95	12.34	137.72
177	1065	9.73	243.38	92.12	12.45	138.8
178	1070	9.78	244.75	92.3	12.57	139.89
179	1075	9.82	246.13	92.5	12.67	140.96
180	1080	9.87	247.5	92.7	12.78	142.01
181	1085	9.91	248.88	92.93	12.88	143.06
182	1090	9.96	250.25	93.15	12.98	144.13
183	1095	10	251.63	93.37	13.09	145.17
184	1100	10.05	253	93.62	13.16	146.22
185	1105	10.1	254.38	93.86	13.25	147.26
186	1110	10.14	255.75	94.1	13.35	148.3
187	1115	10.19	257.13	94.34	13.44	149.34
188	1120	10.23	258.5	94.59	13.53	150.38
189	1125	10.28	259.88	94.85	13.61	151.42
190	1130	10.32	261.25	95.09	13.7	152.47
191	1135	10.37	262.63	95.34	13.79	153.49
192	1140	10.42	264	95.67	13.9	154.43
193	1145	10.46	265.38	96.03	13.97	155.37
194	1150	10.51	266.75	96.46	14.07	156.22
195	1155	10.55	268.13	96.91	14.16	157.05
196	1160	10.6	269.5	97.37	14.26	157.87
197	1165	10.64	270.88	97.83	14.35	158.7
198	1170	10.69	272.25	98.29	14.44	159.52
199	1175	10.74	273.63	98.77	14.52	160.33
200	1180	10.78	275	99.29	14.6	161.12
201	1185	10.83	276.38	99.82	14.69	161.87
202	1190	10.87	277.75	100.3	14.78	162.66
203	1195	10.92	279.13	100.78	14.88	163.47
204	1200	10.96	280.5	101.2	14.95	164.34
205	1205	11.01	281.88	101.62	15.05	165.21
206	1210	11.05	283.25	102.05	15.14	166.06
207	1215	11.1	284.63	102.47	15.24	166.92
208	1220	11.15	286	102.89	15.32	167.79
209	1225	11.19	287.38	103.29	15.39	168.69
210	1230	11.24	288.75	103.72	15.49	169.54
211	1235	11.28	290.13	104.14	15.58	170.4
212	1240	11.33	291.5	104.54	15.68	171.28
213	1245	11.37	292.88	104.96	15.75	172.16
214	1250	11.42	294.25	105.37	15.85	173.04
215	1255	11.47	295.63	105.77	15.95	173.91
216	1260	11.51	297	106.14	16.04	174.82
217	1265	11.56	298.38	106.5	16.13	175.74
218	1270	11.6	299.75	106.87	16.23	176.65

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
219	1275	11.65	301.13	107.24	16.3	177.58
220	1280	11.69	302.5	107.65	16.4	178.46
221	1285	11.74	303.88	108.12	16.49	179.26
222	1290	11.79	305.25	108.69	16.57	179.99
223	1295	11.83	306.63	109.09	16.65	180.88
224	1300	11.88	308	109.52	16.72	181.76
225	1305	11.92	309.38	109.94	16.81	182.63
226	1310	11.97	310.75	110.34	16.88	183.53
227	1315	12.01	312.13	110.76	16.96	184.4
228	1320	12.06	313.5	111.17	17.05	185.28
229	1325	12.11	314.88	111.57	17.15	186.15
230	1330	12.15	316.25	111.94	17.25	187.05
231	1335	12.2	317.63	112.3	17.35	187.98
232	1340	12.24	319	112.67	17.45	188.89
233	1345	12.29	320.38	113.15	17.53	189.69
234	1350	12.33	321.75	113.63	17.62	190.5
235	1355	12.38	323.13	114.05	17.72	191.36
236	1360	12.43	324.5	114.47	17.78	192.25
237	1365	12.47	325.88	114.9	17.87	193.11
238	1370	12.52	327.25	115.32	17.96	193.97
239	1375	12.56	328.63	115.74	18.06	194.83
240	1380	12.61	330	116.14	18.16	195.7
241	1385	12.65	331.38	116.57	18.26	196.55
242	1390	12.7	332.75	116.99	18.35	197.41
243	1395	12.75	334.13	117.4	18.44	198.29
244	1400	12.79	335.5	117.8	18.51	199.19
245	1405	12.84	336.88	118.17	18.6	200.11
246	1410	12.88	338.25	118.53	18.69	201.03
247	1415	12.93	339.63	118.89	18.79	201.95
248	1420	12.97	341	119.38	18.88	202.74
249	1425	13.02	342.38	119.86	18.97	203.55

Table B.3. Data for Figures 4.10 and 4.13.

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
0	180	1.64	0	0	0	0
1	185	1.69	1.38	1.37	0	0
2	190	1.74	2.75	2.75	0	0
3	195	1.78	4.13	4.11	0	0.02
4	200	1.83	5.5	5.48	0	0.02
5	205	1.87	6.88	6.86	0	0.01
6	210	1.92	8.25	8.24	0	0.01
7	215	1.96	9.63	9.62	0	0.01
8	220	2.01	11	11	0	0
9	225	2.06	12.38	12.37	0	0.01
10	230	2.1	13.75	13.72	0	0.03
11	235	2.15	15.13	15.09	0	0.04
12	240	2.19	16.5	16.46	0	0.04
13	245	2.24	17.88	17.84	0	0.04
14	250	2.28	19.25	19.21	0	0.04
15	255	2.33	20.63	20.59	0	0.03
16	260	2.38	22	21.96	0	0.04
17	265	2.42	23.38	23.33	0	0.04
18	270	2.47	24.75	24.7	0	0.05
19	275	2.51	26.13	26.06	0	0.07
20	280	2.56	27.5	27.4	0	0.1
21	285	2.6	28.88	28.74	0	0.13
22	290	2.65	30.25	30.07	0	0.18
23	295	2.7	31.63	31.28	0	0.34
24	300	2.74	33	32.29	0.09	0.62
25	305	2.79	34.38	33.12	0.17	1.09
26	310	2.83	35.75	33.8	0.28	1.67
27	315	2.88	37.13	34.35	0.4	2.37
28	320	2.92	38.5	34.87	0.49	3.14
29	325	2.97	39.88	35.32	0.63	3.93
30	330	3.01	41.25	35.74	0.76	4.76
31	335	3.06	42.63	36.1	0.93	5.59
32	340	3.11	44	36.52	1.04	6.44
33	345	3.15	45.38	36.93	1.14	7.31
34	350	3.2	46.75	37.23	1.24	8.28
35	355	3.24	48.13	37.54	1.4	9.18
36	360	3.29	49.5	37.84	1.58	10.08
37	365	3.33	50.88	38.17	1.71	10.99
38	370	3.38	52.25	38.49	1.82	11.94
39	375	3.43	53.63	38.74	2	12.88
40	380	3.47	55	39.05	2.12	13.83
41	385	3.52	56.38	39.34	2.24	14.79
42	390	3.56	57.75	39.64	2.34	15.78

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
43	395	3.61	59.13	39.92	2.46	16.75
44	400	3.65	60.5	40.2	2.58	17.72
45	405	3.7	61.88	40.43	2.74	18.7
46	410	3.75	63.25	40.7	2.88	19.68
47	415	3.79	64.63	40.93	3	20.69
48	420	3.84	66	41.17	3.12	21.71
49	425	3.88	67.38	41.4	3.27	22.71
50	430	3.93	68.75	41.61	3.42	23.72
51	435	3.97	70.13	41.77	3.62	24.73
52	440	4.02	71.5	41.98	3.8	25.72
53	445	4.07	72.88	42.19	3.95	26.73
54	450	4.11	74.25	42.4	4.13	27.72
55	455	4.16	75.63	42.61	4.29	28.73
56	460	4.2	77	42.82	4.43	29.75
57	465	4.25	78.38	43.02	4.6	30.76
58	470	4.29	79.75	43.21	4.74	31.8
59	475	4.34	81.13	43.4	4.88	32.85
60	480	4.39	82.5	43.57	5.03	33.9
61	485	4.43	83.88	43.75	5.19	34.94
62	490	4.48	85.25	43.92	5.35	35.98
63	495	4.52	86.63	44.09	5.74	36.79
64	500	4.57	88	44.3	5.85	37.84
65	505	4.61	89.38	44.5	5.99	38.89
66	510	4.66	90.75	44.68	6.13	39.94
67	515	4.71	92.13	44.87	6.25	41.01
68	520	4.75	93.5	45.05	6.38	42.06
69	525	4.8	94.88	45.22	6.53	43.13
70	530	4.84	96.25	45.41	6.67	44.17
71	535	4.89	97.63	45.58	6.84	45.2
72	540	4.93	99	45.74	7.04	46.21
73	545	4.98	100.38	45.92	7.25	47.2
74	550	5.02	101.75	46.09	7.49	48.16
75	555	5.07	103.13	46.24	7.72	49.16
76	560	5.12	104.5	46.34	7.95	50.21
77	565	5.16	105.88	46.5	8.11	51.27
78	570	5.21	107.25	46.65	8.25	52.35
79	575	5.25	108.63	46.77	8.45	53.41
80	580	5.3	110	46.93	8.62	54.45
81	585	5.34	111.38	46.99	8.87	55.51
82	590	5.39	112.75	47.12	9.07	56.56
83	595	5.44	114.13	47.23	9.27	57.62
84	600	5.48	115.5	47.33	9.48	58.7
85	605	5.53	116.88	47.36	9.74	59.77
86	610	5.57	118.25	47.45	10.03	60.77

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
87	615	5.62	119.63	47.54	10.23	61.85
88	620	5.66	121	47.64	10.47	62.89
89	625	5.71	122.38	47.72	10.73	63.92
90	630	5.76	123.75	47.82	10.96	64.97
91	635	5.8	125.13	47.92	11.24	65.97
92	640	5.85	126.5	47.99	11.5	67.01
93	645	5.89	127.88	48.12	11.71	68.04
94	650	5.94	129.25	48.25	11.91	69.09
95	655	5.98	130.63	48.35	12.19	70.09
96	660	6.03	132	48.48	12.41	71.11
97	665	6.08	133.38	48.61	12.63	72.14
98	670	6.12	134.75	48.74	12.81	73.21
99	675	6.17	136.13	48.86	12.98	74.29
100	680	6.21	137.5	48.98	13.13	75.39
101	685	6.26	138.88	49.08	13.28	76.51
102	690	6.3	140.25	49.17	13.42	77.66
103	695	6.35	141.63	49.26	13.56	78.8
104	700	6.4	143	49.31	13.74	79.95
105	705	6.44	144.38	49.35	13.91	81.11
106	710	6.49	145.75	49.39	14.1	82.26
107	715	6.53	147.13	49.42	14.29	83.42
108	720	6.58	148.5	49.52	14.41	84.56
109	725	6.62	149.88	49.56	14.86	85.46
110	730	6.67	151.25	49.59	15.06	86.6
111	735	6.72	152.63	49.63	15.26	87.74
112	740	6.76	154	49.67	15.45	88.88
113	745	6.81	155.38	49.72	15.65	90
114	750	6.85	156.75	49.75	15.88	91.12
115	755	6.9	158.13	49.77	16.12	92.24
116	760	6.94	159.5	49.83	16.34	93.34
117	765	6.99	160.88	49.91	16.52	94.45
118	770	7.03	162.25	49.96	16.74	95.55
119	775	7.08	163.63	50.05	16.93	96.64
120	780	7.13	165	50.12	17.15	97.73
121	785	7.17	166.38	50.14	17.41	98.82
122	790	7.22	167.75	50.21	17.6	99.93
123	795	7.26	169.13	50.28	17.81	101.03
124	800	7.31	170.5	50.3	18.08	102.12
125	805	7.35	171.88	50.37	18.3	103.21
126	810	7.4	173.25	50.44	18.5	104.31
127	815	7.45	174.63	50.51	18.74	105.38
128	820	7.49	176	50.57	18.98	106.45
129	825	7.54	177.38	50.63	19.22	107.53
130	830	7.58	178.75	50.7	19.46	108.59

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
131	835	7.63	180.13	50.76	19.72	109.65
132	840	7.67	181.5	50.81	19.96	110.73
133	845	7.72	182.88	50.87	20.15	111.85
134	850	7.77	184.25	50.92	20.33	113
135	855	7.81	185.63	50.97	20.54	114.11
136	860	7.86	187	51.03	20.78	115.18
137	865	7.9	188.38	51.09	21.05	116.23
138	870	7.95	189.75	51.15	21.3	117.31
139	875	7.99	191.13	51.2	21.54	118.38
140	880	8.04	192.5	51.26	21.76	119.48
141	885	8.09	193.88	51.32	21.97	120.58
142	890	8.13	195.25	51.37	22.2	121.67
143	895	8.18	196.63	51.43	22.42	122.77
144	900	8.22	198	51.49	22.71	123.8
145	905	8.27	199.38	51.55	23.02	124.8
146	910	8.31	200.75	51.63	23.31	125.82
147	915	8.36	202.13	51.7	23.58	126.84
148	920	8.41	203.5	51.79	23.79	127.92
149	925	8.45	204.88	51.91	23.97	129
150	930	8.5	206.25	52	24.16	130.09
151	935	8.54	207.63	52.11	24.32	131.19
152	940	8.59	209	52.23	24.48	132.29
153	945	8.63	210.38	52.36	24.65	133.36
154	950	8.68	211.75	52.48	24.98	134.29
155	955	8.73	213.13	52.6	25.24	135.28
156	960	8.77	214.5	52.72	25.54	136.24
157	965	8.82	215.88	52.85	25.8	137.23
158	970	8.86	217.25	52.99	26.04	138.22
159	975	8.91	218.63	53.1	26.25	139.28
160	980	8.95	220	53.2	26.43	140.37
161	985	9	221.38	53.3	26.62	141.45
162	990	9.04	222.75	53.4	26.78	142.56
163	995	9.09	224.13	53.51	26.95	143.66
164	1000	9.14	225.5	53.61	27.16	144.73
165	1005	9.18	226.88	53.7	27.39	145.79
166	1010	9.23	228.25	53.78	27.67	146.8
167	1015	9.27	229.63	53.89	27.9	147.84
168	1020	9.32	231	53.99	28.14	148.87
169	1025	9.36	232.38	54.08	28.45	149.84
170	1030	9.41	233.75	54.16	32.41	147.17
171	1035	9.46	235.13	54.22	32.73	148.17
172	1040	9.5	236.5	54.27	33	149.23
173	1045	9.55	237.88	54.3	33.3	150.28
174	1050	9.59	239.25	54.36	33.56	151.33

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
175	1055	9.64	240.63	54.38	33.87	152.38
176	1060	9.68	242	54.42	34.18	153.4
177	1065	9.73	243.38	54.5	34.43	154.45
178	1070	9.78	244.75	54.6	34.68	155.46
179	1075	9.82	246.13	54.71	34.91	156.51
180	1080	9.87	247.5	54.81	35.15	157.53
181	1085	9.91	248.88	54.93	35.4	158.55
182	1090	9.96	250.25	55.06	35.64	159.55
183	1095	10	251.63	55.22	35.92	160.48
184	1100	10.05	253	55.38	36.12	161.5
185	1105	10.1	254.38	55.53	36.31	162.53
186	1110	10.14	255.75	55.68	36.49	163.57
187	1115	10.19	257.13	55.82	36.75	164.55
188	1120	10.23	258.5	55.96	36.99	165.55
189	1125	10.28	259.88	56.08	37.2	166.6
190	1130	10.32	261.25	56.21	37.39	167.66
191	1135	10.37	262.63	56.33	37.57	168.73
192	1140	10.42	264	56.44	37.73	169.82
193	1145	10.46	265.38	56.57	37.91	170.9
194	1150	10.51	266.75	56.71	38.11	171.93
195	1155	10.55	268.13	56.84	38.34	172.95
196	1160	10.6	269.5	56.99	38.55	173.96
197	1165	10.64	270.88	57.12	38.72	175.03
198	1170	10.69	272.25	57.27	38.92	176.06
199	1175	10.74	273.63	57.41	39.07	177.14
200	1180	10.78	275	57.55	39.24	178.21
201	1185	10.83	276.38	57.7	39.41	179.27
202	1190	10.87	277.75	57.83	39.65	180.27
203	1195	10.92	279.13	57.96	39.85	181.31
204	1200	10.96	280.5	58.09	40.04	182.37
205	1205	11.01	281.88	58.23	40.23	183.42
206	1210	11.05	283.25	58.36	40.39	184.5
207	1215	11.1	284.63	58.5	40.56	185.57
208	1220	11.15	286	58.63	40.76	186.61
209	1225	11.19	287.38	58.76	41	187.62
210	1230	11.24	288.75	58.89	41.19	188.67
211	1235	11.28	290.13	59	41.34	189.78
212	1240	11.33	291.5	59.1	41.51	190.89
213	1245	11.37	292.88	59.2	41.68	191.99
214	1250	11.42	294.25	59.31	41.92	193.02
215	1255	11.47	295.63	59.41	42.12	194.09
216	1260	11.51	297	59.48	42.31	195.21
217	1265	11.56	298.38	59.55	42.52	196.31
218	1270	11.6	299.75	59.62	42.71	197.42

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
219	1275	11.65	301.13	59.67	42.89	198.56
220	1280	11.69	302.5	59.73	43.06	199.71
221	1285	11.74	303.88	59.79	43.23	200.86
222	1290	11.79	305.25	59.84	43.43	201.98
223	1295	11.83	306.63	59.89	43.66	203.07
224	1300	11.88	308	59.94	43.86	204.2
225	1305	11.92	309.38	59.99	44.01	205.37
226	1310	11.97	310.75	60.04	44.18	206.53
227	1315	12.01	312.13	60.09	44.49	207.54
228	1320	12.06	313.5	60.14	48.45	204.91
229	1325	12.11	314.88	60.19	48.77	205.91
230	1330	12.15	316.25	60.21	49.04	207
231	1335	12.2	317.63	60.26	49.33	208.03
232	1340	12.24	319	60.31	49.6	209.1
233	1345	12.29	320.38	60.36	49.9	210.11
234	1350	12.33	321.75	60.41	50.22	211.12
235	1355	12.38	323.13	60.46	50.46	212.2
236	1360	12.43	324.5	60.49	50.72	213.29
237	1365	12.47	325.88	60.5	50.92	214.45
238	1370	12.52	327.25	60.55	51.16	215.54
239	1375	12.56	328.63	60.6	51.35	216.67
240	1380	12.61	330	60.65	51.5	217.84
241	1385	12.65	331.38	60.7	51.67	219
242	1390	12.7	332.75	60.75	51.98	220.02
243	1395	12.75	334.13	60.79	55.95	217.39
244	1400	12.79	335.5	60.84	56.26	218.4
245	1405	12.84	336.88	60.89	56.47	219.52
246	1410	12.88	338.25	60.93	56.66	220.66
247	1415	12.93	339.63	60.97	56.86	221.79
248	1420	12.97	341	61.02	57.05	222.93
249	1425	13.02	342.38	61.05	57.24	224.09

Table B.4. Data for Figures 4.17 and 4.19.

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
0	180	1.5	0	0	0	0
1	185	1.54	1.25	1.25	0	0
2	190	1.58	2.5	2.48	0	0.02
3	195	1.62	3.75	3.71	0	0.04
4	200	1.66	5	4.96	0	0.04
5	205	1.7	6.25	6.22	0	0.03
6	210	1.74	7.5	7.48	0	0.02
7	215	1.79	8.75	8.75	0	0
8	220	1.83	10	9.99	0	0.01
9	225	1.87	11.25	11.24	0	0.01
10	230	1.91	12.5	12.5	0	0
11	235	1.95	13.75	13.76	0	-0.01
12	240	1.99	15	15.02	0	-0.02
13	245	2.03	16.25	16.29	0	-0.04
14	250	2.08	17.5	17.53	0	-0.03
15	255	2.12	18.75	18.78	0	-0.03
16	260	2.16	20	20.05	0	-0.05
17	265	2.2	21.25	21.28	0	-0.03
18	270	2.24	22.5	22.52	0	-0.02
19	275	2.28	23.75	23.8	0	-0.05
20	280	2.33	25	25.08	0	-0.08
21	285	2.37	26.25	26.31	0	-0.06
22	290	2.41	27.5	27.44	0.04	0.02
23	295	2.45	28.75	28.48	0.12	0.15
24	300	2.49	30	29.4	0.18	0.42
25	305	2.53	31.25	30.24	0.29	0.73
26	310	2.57	32.5	31	0.35	1.15
27	315	2.62	33.75	31.69	0.43	1.62
28	320	2.66	35	32.3	0.57	2.13
29	325	2.7	36.25	32.85	0.69	2.71
30	330	2.74	37.5	33.35	0.79	3.37
31	335	2.78	38.75	33.83	0.93	4
32	340	2.82	40	34.29	1.09	4.62
33	345	2.87	41.25	34.72	1.27	5.26
34	350	2.91	42.5	35.15	1.38	5.96
35	355	2.95	43.75	35.59	1.52	6.64
36	360	2.99	45	36.02	1.64	7.34
37	365	3.03	46.25	36.45	1.78	8.02
38	370	3.07	47.5	36.86	1.88	8.76
39	375	3.11	48.75	37.27	2.02	9.46
40	380	3.16	50	37.67	2.14	10.19
41	385	3.2	51.25	38.07	2.29	10.89
42	390	3.24	52.5	38.46	2.37	11.67

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
43	395	3.28	53.75	38.85	2.47	12.43
44	400	3.32	55	39.26	2.59	13.15
45	405	3.36	56.25	39.67	2.74	13.84
46	410	3.41	57.5	40.04	2.85	14.61
47	415	3.45	58.75	40.44	2.98	15.33
48	420	3.49	60	40.82	3.13	16.04
49	425	3.53	61.25	41.2	3.27	16.78
50	430	3.57	62.5	41.57	3.37	17.56
51	435	3.61	63.75	41.95	3.5	18.3
52	440	3.65	65	42.34	3.61	19.05
53	445	3.7	66.25	42.72	3.7	19.83
54	450	3.74	67.5	43.08	3.81	20.61
55	455	3.78	68.75	43.45	3.93	21.37
56	460	3.82	70	43.83	4.09	22.09
57	465	3.86	71.25	44.18	4.21	22.86
58	470	3.9	72.5	44.53	4.33	23.64
59	475	3.95	73.75	44.88	4.45	24.42
60	480	3.99	75	45.26	4.57	25.18
61	485	4.03	76.25	45.63	4.67	25.95
62	490	4.07	77.5	46	4.78	26.72
63	495	4.11	78.75	46.37	4.91	27.47
64	500	4.15	80	46.71	5.06	28.23
65	505	4.19	81.25	47.03	5.18	29.04
66	510	4.24	82.5	47.36	5.28	29.86
67	515	4.28	83.75	47.72	5.4	30.63
68	520	4.32	85	48.08	5.55	31.37
69	525	4.36	86.25	48.41	5.68	32.16
70	530	4.4	87.5	48.75	5.74	33.01
71	535	4.44	88.75	49.11	5.88	33.76
72	540	4.49	90	49.47	6.01	34.52
73	545	4.53	91.25	49.81	6.13	35.32
74	550	4.57	92.5	50.17	6.23	36.1
75	555	4.61	93.75	50.55	6.37	36.84
76	560	4.65	95	50.88	6.49	37.63
77	565	4.69	96.25	51.21	6.63	38.41
78	570	4.73	97.5	51.54	6.78	39.18
79	575	4.78	98.75	51.87	6.89	39.99
80	580	4.82	100	52.21	7.01	40.78
81	585	4.86	101.25	52.54	7.19	41.52
82	590	4.9	102.5	52.83	7.29	42.38
83	595	4.94	103.75	53.17	7.43	43.15
84	600	4.98	105	53.47	7.54	43.99
85	605	5.02	106.25	53.78	7.66	44.81
86	610	5.07	107.5	54.1	7.79	45.61

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
87	615	5.11	108.75	54.4	7.92	46.43
88	620	5.15	110	54.69	8.05	47.26
89	625	5.19	111.25	55.01	8.18	48.06
90	630	5.23	112.5	55.36	8.32	48.82
91	635	5.27	113.75	55.71	8.42	49.63
92	640	5.32	115	56.09	8.52	50.39
93	645	5.36	116.25	56.46	8.64	51.16
94	650	5.4	117.5	56.83	8.79	51.88
95	655	5.44	118.75	57.19	8.91	52.66
96	660	5.48	120	57.58	9.04	53.38
97	665	5.52	121.25	57.96	9.15	54.14
98	670	5.56	122.5	58.38	9.27	54.85
99	675	5.61	123.75	58.79	9.36	55.59
100	680	5.65	125	59.22	9.52	56.26
101	685	5.69	126.25	59.63	9.68	56.94
102	690	5.73	127.5	60.06	9.82	57.62
103	695	5.77	128.75	60.5	9.97	58.29
104	700	5.81	130	60.92	10.15	58.92
105	705	5.86	131.25	61.36	10.32	59.57
106	710	5.9	132.5	61.79	10.49	60.21
107	715	5.94	133.75	62.22	10.67	60.86
108	720	5.98	135	62.63	10.85	61.52
109	725	6.02	136.25	63.02	10.99	62.24
110	730	6.06	137.5	63.41	11.17	62.92
111	735	6.1	138.75	63.8	11.33	63.62
112	740	6.15	140	64.19	11.47	64.34
113	745	6.19	141.25	64.58	11.62	65.05
114	750	6.23	142.5	64.97	11.76	65.77
115	755	6.27	143.75	65.36	11.91	66.49
116	760	6.31	145	65.75	12.06	67.2
117	765	6.35	146.25	66.14	12.23	67.88
118	770	6.4	147.5	66.53	12.38	68.59
119	775	6.44	148.75	66.91	12.52	69.31
120	780	6.48	150	67.3	12.66	70.04
121	785	6.52	151.25	67.71	12.81	70.73
122	790	6.56	152.5	68.1	12.98	71.42
123	795	6.6	153.75	68.49	13.12	72.14
124	800	6.64	155	68.89	13.24	72.87
125	805	6.69	156.25	69.29	13.41	73.55
126	810	6.73	157.5	69.69	13.96	73.85
127	815	6.77	158.75	69.69	14.06	75
128	820	6.81	160	70.12	14.17	75.71
129	825	6.85	161.25	70.53	14.31	76.41
130	830	6.89	162.5	70.97	14.48	77.06

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
131	835	6.94	163.75	71.37	14.63	77.76
132	840	6.98	165	71.78	14.8	78.42
133	845	7.02	166.25	72.19	14.9	79.16
134	850	7.06	167.5	72.6	15.05	79.84
135	855	7.1	168.75	73.01	15.16	80.58
136	860	7.14	170	73.42	15.28	81.3
137	865	7.18	171.25	73.85	15.41	81.99
138	870	7.23	172.5	74.27	15.53	82.7
139	875	7.27	173.75	74.7	15.66	83.38
140	880	7.31	175	75.14	15.78	84.07
141	885	7.35	176.25	75.6	15.9	84.75
142	890	7.39	177.5	76.04	16.02	85.43
143	895	7.43	178.75	76.48	16.12	86.15
144	900	7.48	180	76.93	16.25	86.81
145	905	7.52	181.25	77.39	16.39	87.47
146	910	7.56	182.5	77.85	16.51	88.14
147	915	7.6	183.75	78.33	16.61	88.8
148	920	7.64	185	78.78	16.73	89.49
149	925	7.68	186.25	79.2	16.85	90.2
150	930	7.72	187.5	79.59	16.96	90.95
151	935	7.77	188.75	79.98	17.08	91.69
152	940	7.81	190	80.35	17.2	92.45
153	945	7.85	191.25	80.75	17.34	93.16
154	950	7.89	192.5	81.15	17.49	93.85
155	955	7.93	193.75	81.58	17.61	94.57
156	960	7.97	195	82.03	17.79	95.18
157	965	8.01	196.25	82.43	17.92	95.9
158	970	8.06	197.5	82.86	18.01	96.63
159	975	8.1	198.75	83.32	18.14	97.29
160	980	8.14	200	83.75	18.24	98.01
161	985	8.18	201.25	84.15	18.35	98.75
162	990	8.22	202.5	84.56	18.46	99.48
163	995	8.26	203.75	84.94	18.57	100.24
164	1000	8.31	205	85.34	18.68	100.98
165	1005	8.35	206.25	85.72	18.81	101.72
166	1010	8.39	207.5	86.12	18.92	102.47
167	1015	8.43	208.75	86.5	19.06	103.19
168	1020	8.47	210	86.91	19.18	103.91
169	1025	8.51	211.25	87.31	19.32	104.62
170	1030	8.55	212.5	87.74	19.46	105.3
171	1035	8.6	213.75	88.16	19.6	105.99
172	1040	8.64	215	88.57	19.75	106.68
173	1045	8.68	216.25	88.98	19.9	107.36
174	1050	8.72	217.5	89.39	19.99	108.12

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
175	1055	8.76	218.75	89.86	20.12	108.77
176	1060	8.8	220	90.28	20.22	109.49
177	1065	8.85	221.25	90.69	20.34	110.23
178	1070	8.89	222.5	91.09	20.47	110.93
179	1075	8.93	223.75	91.51	20.59	111.65
180	1080	8.97	225	91.93	20.71	112.36
181	1085	9.01	226.25	92.35	20.83	113.06
182	1090	9.05	227.5	92.79	20.93	113.78
183	1095	9.09	228.75	93.23	21.06	114.46
184	1100	9.14	230	93.68	21.2	115.12
185	1105	9.18	231.25	94.13	21.31	115.81
186	1110	9.22	232.5	94.56	21.42	116.51
187	1115	9.26	233.75	95.02	21.53	117.2
188	1120	9.3	235	95.48	21.66	117.86
189	1125	9.34	236.25	95.94	21.77	118.54
190	1130	9.39	237.5	96.42	21.89	119.19
191	1135	9.43	238.75	96.87	22.01	119.87
192	1140	9.47	240	97.29	22.15	120.56
193	1145	9.51	241.25	97.67	22.3	121.28
194	1150	9.55	242.5	98.06	22.42	122.02
195	1155	9.59	243.75	98.43	22.54	122.78
196	1160	9.63	245	98.84	22.65	123.51
197	1165	9.68	246.25	99.24	22.79	124.22
198	1170	9.72	247.5	99.66	22.92	124.92
199	1175	9.76	248.75	100.11	23.05	125.59
200	1180	9.8	250	100.52	23.2	126.29
201	1185	9.84	251.25	100.94	23.33	126.98
202	1190	9.88	252.5	101.41	23.48	127.61
203	1195	9.93	253.75	101.83	23.63	128.28
204	1200	9.97	255	102.26	23.72	129.02
205	1205	10.01	256.25	102.68	23.85	129.72
206	1210	10.05	257.5	103.09	23.96	130.45
207	1215	10.09	258.75	103.5	24.07	131.18
208	1220	10.13	260	103.91	24.21	131.88
209	1225	10.17	261.25	104.38	24.32	132.55
210	1230	10.22	262.5	104.8	24.44	133.25
211	1235	10.26	263.75	105.21	24.57	133.98
212	1240	10.3	265	105.61	24.66	134.72
213	1245	10.34	266.25	106.03	24.8	135.43
214	1250	10.38	267.5	106.45	24.93	136.12
215	1255	10.42	268.75	106.87	25.05	136.83
216	1260	10.47	270	107.31	25.21	137.48
217	1265	10.51	271.25	107.75	25.76	137.74
218	1270	10.55	272.5	108.2	25.86	138.44

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
219	1275	10.59	273.75	108.65	25.98	139.13
220	1280	10.63	275	109.08	26.11	139.8
221	1285	10.67	276.25	109.54	26.28	140.43
222	1290	10.71	277.5	110	26.43	141.07
223	1295	10.76	278.75	110.46	26.61	141.68
224	1300	10.8	280	110.85	26.71	142.44
225	1305	10.84	281.25	111.24	26.86	143.15
226	1310	10.88	282.5	111.63	26.97	143.9
227	1315	10.92	283.75	112.02	27.09	144.64
228	1320	10.96	285	112.41	27.22	145.37
229	1325	11	286.25	112.8	27.33	146.11
230	1330	11.05	287.5	113.19	27.47	146.84
231	1335	11.09	288.75	113.58	27.59	147.59
232	1340	11.13	290	113.96	27.71	148.33
233	1345	11.17	291.25	114.38	27.83	149.05
234	1350	11.21	292.5	114.76	27.93	149.81
235	1355	11.25	293.75	115.15	28.06	150.54
236	1360	11.3	295	115.55	28.19	151.26
237	1365	11.34	296.25	115.95	28.29	152
238	1370	11.38	297.5	116.35	28.41	152.74
239	1375	11.42	298.75	116.82	28.54	153.39
240	1380	11.46	300	117.24	28.66	154.1
241	1385	11.5	301.25	117.66	28.78	154.81
242	1390	11.54	302.5	118.09	28.9	155.51
243	1395	11.59	303.75	118.49	29	156.26
244	1400	11.63	305	118.9	29.13	156.97
245	1405	11.67	306.25	119.31	29.26	157.68
246	1410	11.71	307.5	119.72	29.38	158.39
247	1415	11.75	308.75	120.13	29.55	159.07
248	1420	11.79	310	120.55	30.1	159.36
249	1425	11.84	311.25	120.96	30.2	160.09
250	1430	11.88	312.5	121.37	30.31	160.82
251	1435	11.92	313.75	121.83	30.45	161.47
252	1440	11.96	315	122.26	30.62	162.12
253	1445	12	316.25	122.66	30.77	162.82
254	1450	12.04	317.5	123.07	30.94	163.49
255	1455	12.08	318.75	123.48	31.06	164.21
256	1460	12.13	320	123.9	31.18	164.91
257	1465	12.17	321.25	124.33	31.3	165.62
258	1470	12.21	322.5	124.76	31.4	166.34
259	1475	12.25	323.75	125.2	31.53	167.01
260	1480	12.29	325	125.66	31.66	167.68
261	1485	12.33	326.25	126.1	31.77	168.38
262	1490	12.38	327.5	126.54	31.88	169.08

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
263	1495	12.42	328.75	126.99	32.02	169.74
264	1500	12.46	330	127.45	32.13	170.41
265	1505	12.5	331.25	127.91	32.25	171.08
266	1510	12.54	332.5	128.31	32.38	171.82
267	1515	12.58	333.75	128.7	32.47	172.58
268	1520	12.62	335	129.09	32.61	173.31
269	1525	12.67	336.25	129.47	32.74	174.04
270	1530	12.71	337.5	129.86	32.86	174.78
271	1535	12.75	338.75	130.28	33.02	175.45
272	1540	12.79	340	130.69	33.57	175.74
273	1545	12.83	341.25	131.11	33.67	176.47
274	1550	12.87	342.5	131.54	33.79	177.17
275	1555	12.92	343.75	131.98	33.92	177.84
276	1560	12.96	345	132.44	34.09	178.47
277	1565	13	346.25	132.88	34.24	179.13
278	1570	13.04	347.5	133.32	34.42	179.76

Table B.5. Data for Figures 4.18 and 4.19.

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
0	180	1.5	0	0	0	0
1	185	1.54	1.25	1.25	0	0
2	190	1.58	2.5	2.5	0	0
3	195	1.62	3.75	3.75	0	0
4	200	1.66	5	5	0	0
5	205	1.7	6.25	6.26	0	-0.01
6	210	1.74	7.5	7.51	0	-0.01
7	215	1.79	8.75	8.76	0	-0.01
8	220	1.83	10	10.01	0	-0.01
9	225	1.87	11.25	11.26	0	-0.01
10	230	1.91	12.5	12.51	0.01	-0.02
11	235	1.95	13.75	13.76	0.01	-0.02
12	240	1.99	15	15.01	0.02	-0.03
13	245	2.03	16.25	16.26	0.02	-0.03
14	250	2.08	17.5	17.52	0.02	-0.04
15	255	2.12	18.75	18.77	0.02	-0.05
16	260	2.16	20	20.02	0.02	-0.05
17	265	2.2	21.25	21.27	0.05	-0.07
18	270	2.24	22.5	22.51	0.07	-0.09
19	275	2.28	23.75	23.74	0.07	-0.06
20	280	2.33	25	24.9	0.11	-0.01
21	285	2.37	26.25	25.97	0.13	0.15
22	290	2.41	27.5	26.94	0.16	0.4
23	295	2.45	28.75	27.81	0.19	0.75
24	300	2.49	30	28.62	0.19	1.18
25	305	2.53	31.25	29.4	0.19	1.66
26	310	2.57	32.5	30.08	0.19	2.22
27	315	2.62	33.75	30.67	0.19	2.89
28	320	2.66	35	31.22	0.19	3.59
29	325	2.7	36.25	31.73	0.19	4.33
30	330	2.74	37.5	32.21	0.19	5.1
31	335	2.78	38.75	32.67	0.2	5.87
32	340	2.82	40	33.11	0.24	6.65
33	345	2.87	41.25	33.55	0.27	7.43
34	350	2.91	42.5	33.99	0.3	8.21
35	355	2.95	43.75	34.43	0.33	8.99
36	360	2.99	45	34.87	0.35	9.77
37	365	3.03	46.25	35.31	0.39	10.56
38	370	3.07	47.5	35.75	0.41	11.34
39	375	3.11	48.75	36.18	0.45	12.12
40	380	3.16	50	36.61	0.47	12.91
41	385	3.2	51.25	37.04	0.49	13.71

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
42	390	3.24	52.5	37.48	0.52	14.5
43	395	3.28	53.75	37.93	0.52	15.3
44	400	3.32	55	38.39	0.52	16.09
45	405	3.36	56.25	38.83	0.53	16.89
46	410	3.41	57.5	39.28	0.54	17.68
47	415	3.45	58.75	39.72	0.56	18.47
48	420	3.49	60	40.16	0.57	19.26
49	425	3.53	61.25	40.6	0.6	20.05
50	430	3.57	62.5	41.03	0.65	20.82
51	435	3.61	63.75	41.45	0.75	21.54
52	440	3.65	65	41.87	0.89	22.24
53	445	3.7	66.25	42.27	1.03	22.95
54	450	3.74	67.5	42.68	1.18	23.64
55	455	3.78	68.75	43.09	1.32	24.34
56	460	3.82	70	43.48	1.48	25.03
57	465	3.86	71.25	43.89	1.64	25.72
58	470	3.9	72.5	44.29	1.82	26.39
59	475	3.95	73.75	44.68	2.03	27.03
60	480	3.99	75	45.08	2.21	27.71
61	485	4.03	76.25	45.47	2.41	28.37
62	490	4.07	77.5	45.85	2.61	29.03
63	495	4.11	78.75	46.24	2.82	29.69
64	500	4.15	80	46.62	3.02	30.35
65	505	4.19	81.25	47	3.22	31.03
66	510	4.24	82.5	47.41	3.4	31.69
67	515	4.28	83.75	47.82	3.58	32.35
68	520	4.32	85	48.23	3.75	33.02
69	525	4.36	86.25	48.65	3.92	33.69
70	530	4.4	87.5	49.06	4.09	34.36
71	535	4.44	88.75	49.46	4.27	35.01
72	540	4.49	90	49.89	4.43	35.68
73	545	4.53	91.25	50.3	4.61	36.34
74	550	4.57	92.5	50.78	4.78	36.94
75	555	4.61	93.75	51.21	4.94	37.61
76	560	4.65	95	51.62	5.1	38.27
77	565	4.69	96.25	52.13	5.16	38.96
78	570	4.73	97.5	52.6	5.24	39.66
79	575	4.78	98.75	53.03	5.34	40.38
80	580	4.82	100	53.43	5.45	41.12
81	585	4.86	101.25	53.83	5.54	41.87
82	590	4.9	102.5	54.22	5.64	42.64
83	595	4.94	103.75	54.57	5.78	43.39
84	600	4.98	105	54.93	5.88	44.19

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
85	605	5.02	106.25	55.32	6.06	44.86
86	610	5.07	107.5	55.74	6.17	45.58
87	615	5.11	108.75	56.15	6.34	46.26
88	620	5.15	110	56.55	6.53	46.92
89	625	5.19	111.25	56.96	6.73	47.56
90	630	5.23	112.5	57.37	6.91	48.21
91	635	5.27	113.75	57.79	7.1	48.86
92	640	5.32	115	58.21	7.3	49.5
93	645	5.36	116.25	58.7	7.44	50.11
94	650	5.4	117.5	59.16	7.64	50.71
95	655	5.44	118.75	59.57	7.91	51.27
96	660	5.48	120	59.98	8.21	51.81
97	665	5.52	121.25	60.39	8.49	52.37
98	670	5.56	122.5	60.8	8.73	52.97
99	675	5.61	123.75	61.22	8.92	53.61
100	680	5.65	125	61.63	9.1	54.27
101	685	5.69	126.25	62.03	9.28	54.94
102	690	5.73	127.5	62.43	9.46	55.61
103	695	5.77	128.75	62.83	9.66	56.26
104	700	5.81	130	63.23	9.84	56.93
105	705	5.86	131.25	63.63	10.03	57.59
106	710	5.9	132.5	64.03	10.22	58.25
107	715	5.94	133.75	64.44	10.42	58.9
108	720	5.98	135	65.04	10.59	59.36
109	725	6.02	136.25	65.55	10.82	59.88
110	730	6.06	137.5	65.95	11.02	60.53
111	735	6.1	138.75	66.36	11.22	61.17
112	740	6.15	140	66.76	11.41	61.83
113	745	6.19	141.25	67.16	11.62	62.46
114	750	6.23	142.5	67.54	11.84	63.12
115	755	6.27	143.75	67.95	12.13	63.67
116	760	6.31	145	68.33	12.35	64.32
117	765	6.35	146.25	68.74	12.54	64.97
118	770	6.4	147.5	69.15	12.73	65.62
119	775	6.44	148.75	69.56	12.92	66.27
120	780	6.48	150	69.93	13.15	66.92
121	785	6.52	151.25	70.34	13.34	67.57
122	790	6.56	152.5	70.77	13.5	68.23
123	795	6.6	153.75	71.18	13.69	68.88
124	800	6.64	155	71.57	13.88	69.55
125	805	6.69	156.25	71.94	14.13	70.18
126	810	6.73	157.5	72.29	14.36	70.84
127	815	6.77	158.75	72.62	14.62	71.51

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
128	820	6.81	160	72.96	14.87	72.17
129	825	6.85	161.25	73.32	15.1	72.83
130	830	6.89	162.5	73.69	15.33	73.49
131	835	6.94	163.75	74.08	15.52	74.15
132	840	6.98	165	74.46	15.73	74.81
133	845	7.02	166.25	74.87	15.92	75.46
134	850	7.06	167.5	75.25	16.13	76.12
135	855	7.1	168.75	75.65	16.35	76.75
136	860	7.14	170	76.07	16.55	77.39
137	865	7.18	171.25	76.49	16.75	78.01
138	870	7.23	172.5	76.92	16.98	78.6
139	875	7.27	173.75	77.39	17.17	79.18
140	880	7.31	175	77.88	17.36	79.76
141	885	7.35	176.25	78.37	17.58	80.3
142	890	7.39	177.5	78.82	17.82	80.86
143	895	7.43	178.75	79.31	18.06	81.38
144	900	7.48	180	79.79	18.32	81.89
145	905	7.52	181.25	80.2	18.51	82.54
146	910	7.56	182.5	80.6	18.71	83.19
147	915	7.6	183.75	81	18.91	83.84
148	920	7.64	185	81.4	19.13	84.47
149	925	7.68	186.25	81.8	19.32	85.13
150	930	7.72	187.5	82.2	19.64	85.67
151	935	7.77	188.75	82.61	19.86	86.29
152	940	7.81	190	82.99	20.1	86.9
153	945	7.85	191.25	83.41	20.28	87.56
154	950	7.89	192.5	83.82	20.47	88.21
155	955	7.93	193.75	84.22	20.67	88.86
156	960	7.97	195	84.6	20.9	89.51
157	965	8.01	196.25	85.01	21.08	90.17
158	970	8.06	197.5	85.39	21.28	90.82
159	975	8.1	198.75	85.82	21.45	91.49
160	980	8.14	200	86.21	21.67	92.12
161	985	8.18	201.25	86.6	21.87	92.78
162	990	8.22	202.5	87	22.06	93.43
163	995	8.26	203.75	87.41	22.27	94.08
164	1000	8.31	205	87.8	22.47	94.73
165	1005	8.35	206.25	88.21	22.68	95.37
166	1010	8.39	207.5	88.61	22.86	96.02
167	1015	8.43	208.75	89.02	23.11	96.62
168	1020	8.47	210	89.43	23.3	97.27
169	1025	8.51	211.25	89.84	23.49	97.92
170	1030	8.55	212.5	90.26	23.66	98.58

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
171	1035	8.6	213.75	90.67	23.86	99.23
172	1040	8.64	215	91.07	24.06	99.87
173	1045	8.68	216.25	91.4	24.33	100.52
174	1050	8.72	217.5	91.79	24.53	101.18
175	1055	8.76	218.75	92.2	24.71	101.84
176	1060	8.8	220	92.62	24.88	102.5
177	1065	8.85	221.25	92.99	25.12	103.14
178	1070	8.89	222.5	93.4	25.3	103.8
179	1075	8.93	223.75	93.81	25.48	104.46
180	1080	8.97	225	94.21	25.67	105.11
181	1085	9.01	226.25	94.66	25.87	105.72
182	1090	9.05	227.5	95.12	26.05	106.33
183	1095	9.09	228.75	95.56	26.28	106.91
184	1100	9.14	230	96.01	26.47	107.52
185	1105	9.18	231.25	96.45	26.67	108.13
186	1110	9.22	232.5	96.89	26.87	108.74
187	1115	9.26	233.75	97.33	27.08	109.34
188	1120	9.3	235	97.76	27.3	109.94
189	1125	9.34	236.25	98.18	27.59	110.48
190	1130	9.39	237.5	98.6	27.81	111.09
191	1135	9.43	238.75	99	28	111.75
192	1140	9.47	240	99.41	28.18	112.4
193	1145	9.51	241.25	99.82	28.37	113.06
194	1150	9.55	242.5	100.21	28.6	113.68
195	1155	9.59	243.75	100.63	28.8	114.33
196	1160	9.63	245	101.03	28.96	115.01
197	1165	9.68	246.25	101.44	29.14	115.67
198	1170	9.72	247.5	101.89	29.33	116.28
199	1175	9.76	248.75	102.34	29.58	116.83
200	1180	9.8	250	102.78	29.82	117.4
201	1185	9.84	251.25	103.19	30.08	117.98
202	1190	9.88	252.5	103.59	30.26	118.66
203	1195	9.93	253.75	104	30.42	119.33
204	1200	9.97	255	104.4	30.67	119.93
205	1205	10.01	256.25	104.81	30.84	120.6
206	1210	10.05	257.5	105.22	31.03	121.25
207	1215	10.09	258.75	105.64	31.22	121.89
208	1220	10.13	260	106.04	31.42	122.54
209	1225	10.17	261.25	106.45	31.6	123.21
210	1230	10.22	262.5	106.85	31.82	123.83
211	1235	10.26	263.75	107.25	32.02	124.48
212	1240	10.3	265	107.65	32.22	125.14
213	1245	10.34	266.25	108.05	32.42	125.79

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
214	1250	10.38	267.5	108.45	32.62	126.43
215	1255	10.42	268.75	108.86	32.84	127.05
216	1260	10.47	270	109.46	33.03	127.51
217	1265	10.51	271.25	109.96	33.22	128.07
218	1270	10.55	272.5	110.37	33.45	128.68
219	1275	10.59	273.75	110.78	33.64	129.33
220	1280	10.63	275	111.18	33.8	130.02
221	1285	10.67	276.25	111.63	33.99	130.63
222	1290	10.71	277.5	112.07	34.18	131.25
223	1295	10.76	278.75	112.48	34.43	131.85
224	1300	10.8	280	112.88	34.66	132.46
225	1305	10.84	281.25	113.29	34.92	133.04
226	1310	10.88	282.5	113.69	35.1	133.7
227	1315	10.92	283.75	114.1	35.27	134.39
228	1320	10.96	285	114.51	35.45	135.05
229	1325	11	286.25	114.93	35.67	135.65
230	1330	11.05	287.5	115.33	35.87	136.3
231	1335	11.09	288.75	115.74	36.07	136.95
232	1340	11.13	290	116.14	36.27	137.59
233	1345	11.17	291.25	116.52	36.48	138.25
234	1350	11.21	292.5	116.93	36.69	138.88
235	1355	11.25	293.75	117.31	36.88	139.56
236	1360	11.3	295	117.72	37.07	140.21
237	1365	11.34	296.25	118.13	37.3	140.82
238	1370	11.38	297.5	118.54	37.49	141.47
239	1375	11.42	298.75	118.91	37.65	142.18
240	1380	11.46	300	119.32	37.84	142.84
241	1385	11.5	301.25	119.75	38.04	143.46
242	1390	11.54	302.5	120.16	38.22	144.13
243	1395	11.59	303.75	120.55	38.45	144.75
244	1400	11.63	305	120.92	38.64	145.44
245	1405	11.67	306.25	121.27	38.84	146.14
246	1410	11.71	307.5	121.59	39.04	146.87
247	1415	11.75	308.75	121.93	39.25	147.57
248	1420	11.79	310	122.3	39.47	148.24
249	1425	11.84	311.25	122.72	39.76	148.77
250	1430	11.88	312.5	123.12	39.98	149.4
251	1435	11.92	313.75	123.53	40.2	150.02
252	1440	11.96	315	123.93	40.4	150.67
253	1445	12	316.25	124.31	40.61	151.33
254	1450	12.04	317.5	124.72	40.82	151.96
255	1455	12.08	318.75	125.1	41.11	152.53
256	1460	12.13	320	125.51	41.34	153.15

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
257	1465	12.17	321.25	125.92	41.53	153.81
258	1470	12.21	322.5	126.33	41.71	154.46
259	1475	12.25	323.75	126.7	41.9	155.14
260	1480	12.29	325	127.11	42.13	155.76
261	1485	12.33	326.25	127.54	42.33	156.38
262	1490	12.38	327.5	127.86	42.49	157.15
263	1495	12.42	328.75	128.2	42.68	157.86
264	1500	12.46	330	128.57	42.89	158.54
265	1505	12.5	331.25	128.99	43.11	159.15
266	1510	12.54	332.5	129.39	43.4	159.71
267	1515	12.58	333.75	129.8	43.62	160.32
268	1520	12.62	335	130.21	43.81	160.98
269	1525	12.67	336.25	130.63	44	161.62
270	1530	12.71	337.5	131.04	44.19	162.27
271	1535	12.75	338.75	131.44	44.42	162.89
272	1540	12.79	340	131.77	44.61	163.61
273	1545	12.83	341.25	132.16	44.77	164.32
274	1550	12.87	342.5	132.57	44.96	164.97
275	1555	12.92	343.75	132.99	45.16	165.6
276	1560	12.96	345	133.42	45.37	166.21
277	1565	13	346.25	133.82	45.59	166.84
278	1570	13.04	347.5	134.23	45.88	167.39

Table B.6. Data for Figures 4.22 and 4.24.

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
0	180	1.5	0	0	0	0
1	185	1.54	1.25	1.24	0	0.01
2	190	1.58	2.5	2.5	0	0
3	195	1.62	3.75	3.76	0	0
4	200	1.66	5	5.01	0	-0.01
5	205	1.7	6.25	6.27	0	-0.02
6	210	1.74	7.5	7.5	0	0
7	215	1.79	8.75	8.74	0	0.01
8	220	1.83	10	9.96	0	0.04
9	225	1.87	11.25	11.21	0	0.04
10	230	1.91	12.5	12.47	0	0.03
11	235	1.95	13.75	13.74	0	0.01
12	240	1.99	15	15	0	0
13	245	2.03	16.25	16.25	0	0
14	250	2.08	17.5	17.51	0	-0.02
15	255	2.12	18.75	18.78	0	-0.03
16	260	2.16	20	20.01	0.01	-0.03
17	265	2.2	21.25	21.26	0.03	-0.03
18	270	2.24	22.5	22.51	0.03	-0.04
19	275	2.28	23.75	23.76	0.04	-0.05
20	280	2.33	25	24.98	0.04	-0.02
21	285	2.37	26.25	26.17	0.04	0.04
22	290	2.41	27.5	27.27	0.07	0.16
23	295	2.45	28.75	28.27	0.13	0.35
24	300	2.49	30	29.17	0.23	0.6
25	305	2.53	31.25	30.02	0.37	0.86
26	310	2.57	32.5	30.8	0.56	1.15
27	315	2.62	33.75	31.55	0.78	1.43
28	320	2.66	35	32.26	1.03	1.7
29	325	2.7	36.25	32.96	1.36	1.93
30	330	2.74	37.5	33.64	1.72	2.15
31	335	2.78	38.75	34.29	2.04	2.42
32	340	2.82	40	34.92	2.41	2.67
33	345	2.87	41.25	35.53	2.79	2.93
34	350	2.91	42.5	36.14	3.17	3.19
35	355	2.95	43.75	36.77	3.52	3.46
36	360	2.99	45	37.38	3.89	3.73
37	365	3.03	46.25	37.98	4.27	4
38	370	3.07	47.5	38.56	4.63	4.31
39	375	3.11	48.75	39.14	5	4.61
40	380	3.16	50	39.72	5.35	4.93
41	385	3.2	51.25	40.28	5.76	5.21

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
42	390	3.24	52.5	40.84	6.13	5.53
43	395	3.28	53.75	41.39	6.52	5.84
44	400	3.32	55	41.93	6.84	6.22
45	405	3.36	56.25	42.47	7.19	6.59
46	410	3.41	57.5	43	7.57	6.93
47	415	3.45	58.75	43.54	7.9	7.31
48	420	3.49	60	44.07	8.27	7.66
49	425	3.53	61.25	44.61	8.67	7.97
50	430	3.57	62.5	45.13	9.04	8.33
51	435	3.61	63.75	45.64	9.46	8.65
52	440	3.65	65	46.15	9.81	9.03
53	445	3.7	66.25	46.65	10.22	9.38
54	450	3.74	67.5	47.15	10.57	9.78
55	455	3.78	68.75	47.63	10.99	10.13
56	460	3.82	70	48.13	11.34	10.53
57	465	3.86	71.25	48.64	11.72	10.89
58	470	3.9	72.5	49.14	12.12	11.24
59	475	3.95	73.75	49.61	12.48	11.66
60	480	3.99	75	50.11	12.85	12.04
61	485	4.03	76.25	50.61	13.2	12.43
62	490	4.07	77.5	51.09	13.58	12.83
63	495	4.11	78.75	51.58	13.96	13.21
64	500	4.15	80	52.04	14.32	13.64
65	505	4.19	81.25	52.52	14.68	14.05
66	510	4.24	82.5	53	15.03	14.47
67	515	4.28	83.75	53.48	15.39	14.88
68	520	4.32	85	53.92	15.78	15.3
69	525	4.36	86.25	54.37	16.14	15.74
70	530	4.4	87.5	54.81	16.54	16.15
71	535	4.44	88.75	55.25	16.91	16.6
72	540	4.49	90	55.7	17.24	17.05
73	545	4.53	91.25	56.15	17.6	17.49
74	550	4.57	92.5	56.6	17.98	17.93
75	555	4.61	93.75	57.05	18.32	18.38
76	560	4.65	95	57.51	18.63	18.87
77	565	4.69	96.25	57.93	18.98	19.33
78	570	4.73	97.5	58.37	19.34	19.79
79	575	4.78	98.75	58.79	19.68	20.28
80	580	4.82	100	59.22	20.02	20.76
81	585	4.86	101.25	59.64	20.37	21.24
82	590	4.9	102.5	60.05	20.75	21.7
83	595	4.94	103.75	60.47	21.07	22.2
84	600	4.98	105	60.88	21.42	22.7

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
85	605	5.02	106.25	61.29	21.72	23.24
86	610	5.07	107.5	61.7	22.05	23.76
87	615	5.11	108.75	62.1	22.36	24.29
88	620	5.15	110	62.5	22.71	24.79
89	625	5.19	111.25	63.02	23.09	25.13
90	630	5.23	112.5	63.54	23.47	25.48
91	635	5.27	113.75	64.07	23.92	25.76
92	640	5.32	115	64.58	24.32	26.1
93	645	5.36	116.25	65.05	24.76	26.44
94	650	5.4	117.5	65.49	25.15	26.86
95	655	5.44	118.75	65.94	25.5	27.31
96	660	5.48	120	66.42	25.69	27.88
97	665	5.52	121.25	66.87	25.94	28.44
98	670	5.56	122.5	67.29	26.3	28.9
99	675	5.61	123.75	67.69	26.67	29.39
100	680	5.65	125	68.06	26.95	30
101	685	5.69	126.25	68.42	27.13	30.7
102	690	5.73	127.5	68.79	27.28	31.44
103	695	5.77	128.75	69.13	27.47	32.16
104	700	5.81	130	69.47	27.67	32.86
105	705	5.86	131.25	69.83	27.84	33.58
106	710	5.9	132.5	70.18	28.02	34.3
107	715	5.94	133.75	70.54	28.21	35
108	720	5.98	135	70.88	28.43	35.7
109	725	6.02	136.25	71.23	28.62	36.4
110	730	6.06	137.5	71.57	28.8	37.13
111	735	6.1	138.75	71.94	28.96	37.86
112	740	6.15	140	72.28	29.18	38.54
113	745	6.19	141.25	72.64	29.39	39.22
114	750	6.23	142.5	72.98	29.62	39.9
115	755	6.27	143.75	73.32	29.83	40.59
116	760	6.31	145	73.68	30.02	41.3
117	765	6.35	146.25	74.03	30.22	41.99
118	770	6.4	147.5	74.39	30.43	42.68
119	775	6.44	148.75	74.75	30.62	43.38
120	780	6.48	150	75.11	30.8	44.09
121	785	6.52	151.25	75.47	30.98	44.8
122	790	6.56	152.5	75.83	31.16	45.51
123	795	6.6	153.75	76.19	31.36	46.2
124	800	6.64	155	76.57	31.52	46.9
125	805	6.69	156.25	76.93	31.71	47.61
126	810	6.73	157.5	77.29	31.9	48.31
127	815	6.77	158.75	77.65	32.08	49.01

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
128	820	6.81	160	78.02	32.29	49.69
129	825	6.85	161.25	78.38	32.48	50.39
130	830	6.89	162.5	78.74	32.67	51.09
131	835	6.94	163.75	79.11	32.83	51.81
132	840	6.98	165	79.46	33.02	52.52
133	845	7.02	166.25	79.81	33.22	53.22
134	850	7.06	167.5	80.16	33.42	53.92
135	855	7.1	168.75	80.51	33.62	54.62
136	860	7.14	170	80.86	33.83	55.31
137	865	7.18	171.25	81.21	34.04	56
138	870	7.23	172.5	81.57	34.25	56.68
139	875	7.27	173.75	81.93	34.45	57.37
140	880	7.31	175	82.27	34.68	58.05
141	885	7.35	176.25	82.62	34.89	58.74
142	890	7.39	177.5	82.97	35.1	59.43
143	895	7.43	178.75	83.32	35.33	60.1
144	900	7.48	180	83.67	35.53	60.79
145	905	7.52	181.25	84.02	35.77	61.46
146	910	7.56	182.5	84.37	36	62.13
147	915	7.6	183.75	84.71	36.24	62.79
148	920	7.64	185	85.06	36.46	63.48
149	925	7.68	186.25	85.4	36.7	64.15
150	930	7.72	187.5	85.75	36.93	64.82
151	935	7.77	188.75	86.11	37.14	65.5
152	940	7.81	190	86.45	37.38	66.17
153	945	7.85	191.25	86.79	37.6	66.85
154	950	7.89	192.5	87.15	37.81	67.54
155	955	7.93	193.75	87.48	38.06	68.21
156	960	7.97	195	87.83	38.31	68.87
157	965	8.01	196.25	88.16	38.54	69.55
158	970	8.06	197.5	88.48	38.79	70.23
159	975	8.1	198.75	88.81	39.04	70.9
160	980	8.14	200	89.13	39.3	71.56
161	985	8.18	201.25	89.46	39.54	72.25
162	990	8.22	202.5	89.79	39.77	72.93
163	995	8.26	203.75	90.12	40.01	73.62
164	1000	8.31	205	90.44	40.26	74.3
165	1005	8.35	206.25	90.76	40.53	74.97
166	1010	8.39	207.5	91.08	40.77	75.65
167	1015	8.43	208.75	91.39	41.02	76.34
168	1020	8.47	210	91.72	41.25	77.03
169	1025	8.51	211.25	92.02	41.53	77.7
170	1030	8.55	212.5	92.34	41.79	78.37

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
171	1035	8.6	213.75	92.64	42.05	79.06
172	1040	8.64	215	92.96	42.3	79.74
173	1045	8.68	216.25	93.28	42.55	80.42
174	1050	8.72	217.5	93.6	42.81	81.09
175	1055	8.76	218.75	93.91	43.08	81.77
176	1060	8.8	220	94.21	43.36	82.43
177	1065	8.85	221.25	94.53	43.61	83.12
178	1070	8.89	222.5	94.83	43.89	83.78
179	1075	8.93	223.75	95.14	44.17	84.44
180	1080	8.97	225	95.45	44.43	85.12
181	1085	9.01	226.25	95.76	44.72	85.78
182	1090	9.05	227.5	96.06	45.07	86.37
183	1095	9.09	228.75	96.42	45.44	86.89
184	1100	9.14	230	96.76	45.8	87.44
185	1105	9.18	231.25	97.12	46.21	87.92
186	1110	9.22	232.5	97.47	46.58	88.46
187	1115	9.26	233.75	97.82	46.96	88.97
188	1120	9.3	235	98.16	47.29	89.55
189	1125	9.34	236.25	98.51	47.64	90.1
190	1130	9.39	237.5	98.87	48.02	90.61
191	1135	9.43	238.75	99.22	48.34	91.19
192	1140	9.47	240	99.58	48.71	91.71
193	1145	9.51	241.25	99.94	49.12	92.2
194	1150	9.55	242.5	100.3	49.48	92.72
195	1155	9.59	243.75	100.66	49.9	93.19
196	1160	9.63	245	101.01	50.26	93.73
197	1165	9.68	246.25	101.38	50.66	94.21
198	1170	9.72	247.5	101.76	51.01	94.73
199	1175	9.76	248.75	102.11	51.43	95.2
200	1180	9.8	250	102.48	51.78	95.74
201	1185	9.84	251.25	102.84	52.17	96.24
202	1190	9.88	252.5	103.21	52.57	96.73
203	1195	9.93	253.75	103.57	52.92	97.26
204	1200	9.97	255	103.93	53.29	97.78
205	1205	10.01	256.25	104.3	53.65	98.3
206	1210	10.05	257.5	104.64	54.02	98.83
207	1215	10.09	258.75	104.99	54.41	99.35
208	1220	10.13	260	105.35	54.76	99.89
209	1225	10.17	261.25	105.7	55.12	100.43
210	1230	10.22	262.5	106.05	55.48	100.98
211	1235	10.26	263.75	106.4	55.83	101.52
212	1240	10.3	265	106.75	56.23	102.02
213	1245	10.34	266.25	107.11	56.59	102.55

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
214	1250	10.38	267.5	107.46	56.98	103.06
215	1255	10.42	268.75	107.78	57.35	103.62
216	1260	10.47	270	108.09	57.69	104.22
217	1265	10.51	271.25	108.39	58.05	104.81
218	1270	10.55	272.5	108.7	58.25	105.54
219	1275	10.59	273.75	109.01	58.46	106.28
220	1280	10.63	275	109.32	58.65	107.03
221	1285	10.67	276.25	109.67	58.83	107.75
222	1290	10.71	277.5	110.01	59.01	108.48
223	1295	10.76	278.75	110.38	59.19	109.18
224	1300	10.8	280	110.72	59.39	109.89
225	1305	10.84	281.25	111.08	59.55	110.62
226	1310	10.88	282.5	111.44	59.74	111.31
227	1315	10.92	283.75	111.81	59.93	112.01
228	1320	10.96	285	112.17	60.11	112.72
229	1325	11	286.25	112.53	60.32	113.4
230	1330	11.05	287.5	112.9	60.51	114.09
231	1335	11.09	288.75	113.25	60.69	114.81
232	1340	11.13	290	113.59	60.86	115.55
233	1345	11.17	291.25	113.95	61.05	116.25
234	1350	11.21	292.5	114.3	61.25	116.95
235	1355	11.25	293.75	114.65	61.45	117.65
236	1360	11.3	295	115	61.65	118.35
237	1365	11.34	296.25	115.31	61.86	119.09
238	1370	11.38	297.5	115.62	62.07	119.81
239	1375	11.42	298.75	115.92	62.28	120.55
240	1380	11.46	300	116.23	62.48	121.29
241	1385	11.5	301.25	116.58	62.71	121.96
242	1390	11.54	302.5	116.93	62.92	122.66
243	1395	11.59	303.75	117.29	63.13	123.33
244	1400	11.63	305	117.63	63.36	124.01
245	1405	11.67	306.25	117.99	63.56	124.7
246	1410	11.71	307.5	118.36	63.8	125.34
247	1415	11.75	308.75	118.72	64.16	125.87
248	1420	11.79	310	119.08	64.53	126.39
249	1425	11.84	311.25	119.44	64.91	126.9
250	1430	11.88	312.5	119.8	65.27	127.43
251	1435	11.92	313.75	120.17	65.63	127.95
252	1440	11.96	315	120.52	65.98	128.5
253	1445	12	316.25	120.87	66.34	129.05
254	1450	12.04	317.5	121.22	66.73	129.54
255	1455	12.08	318.75	121.57	67.09	130.09
256	1460	12.13	320	121.92	67.49	130.59

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
257	1465	12.17	321.25	122.27	67.86	131.12
258	1470	12.21	322.5	122.63	68.2	131.68
259	1475	12.25	323.75	122.99	68.55	132.21
260	1480	12.29	325	123.33	68.76	132.91
261	1485	12.33	326.25	123.65	68.97	133.63
262	1490	12.38	327.5	123.96	69.16	134.38
263	1495	12.42	328.75	124.27	69.33	135.15
264	1500	12.46	330	124.58	69.51	135.91
265	1505	12.5	331.25	124.88	69.7	136.67
266	1510	12.54	332.5	125.2	69.9	137.4
267	1515	12.58	333.75	125.51	70.25	137.99
268	1520	12.62	335	125.81	70.65	138.54
269	1525	12.67	336.25	126.12	71.01	139.12
270	1530	12.71	337.5	126.43	71.4	139.67
271	1535	12.75	338.75	126.74	71.77	140.24
272	1540	12.79	340	127.09	72.11	140.8
273	1545	12.83	341.25	127.43	72.47	141.35
274	1550	12.87	342.5	127.8	72.67	142.03
275	1555	12.92	343.75	128.14	72.88	142.73
276	1560	12.96	345	128.5	73.07	143.43
277	1565	13	346.25	128.84	73.25	144.17
278	1570	13.04	347.5	129.19	73.43	144.89

Table B.7. Data for Figures 4.23 and 4.24.

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
0	180	1.5	0	0	0	0
1	185	1.54	1.25	1.25	0	0
2	190	1.58	2.5	2.5	0	0
3	195	1.62	3.75	3.76	0	-0.01
4	200	1.66	5	5.01	0	-0.01
5	205	1.7	6.25	6.26	0	-0.01
6	210	1.74	7.5	7.51	0	-0.01
7	215	1.79	8.75	8.76	0	-0.01
8	220	1.83	10	10.01	0	-0.01
9	225	1.87	11.25	11.25	0	0
10	230	1.91	12.5	12.49	0	0.01
11	235	1.95	13.75	13.74	0	0.01
12	240	1.99	15	14.98	0	0.02
13	245	2.03	16.25	16.22	0	0.03
14	250	2.08	17.5	17.47	0	0.03
15	255	2.12	18.75	18.72	0	0.03
16	260	2.16	20	19.96	0	0.04
17	265	2.2	21.25	21.2	0	0.05
18	270	2.24	22.5	22.45	0.01	0.05
19	275	2.28	23.75	23.67	0.04	0.04
20	280	2.33	25	24.85	0.11	0.04
21	285	2.37	26.25	26	0.11	0.14
22	290	2.41	27.5	27.1	0.12	0.27
23	295	2.45	28.75	28.1	0.15	0.5
24	300	2.49	30	28.99	0.22	0.8
25	305	2.53	31.25	29.81	0.24	1.2
26	310	2.57	32.5	30.54	0.31	1.66
27	315	2.62	33.75	31.21	0.42	2.12
28	320	2.66	35	31.8	0.56	2.64
29	325	2.7	36.25	32.38	0.68	3.19
30	330	2.74	37.5	32.91	0.83	3.75
31	335	2.78	38.75	33.42	0.99	4.34
32	340	2.82	40	33.9	1.15	4.95
33	345	2.87	41.25	34.37	1.34	5.54
34	350	2.91	42.5	34.82	1.55	6.13
35	355	2.95	43.75	35.26	1.72	6.77
36	360	2.99	45	35.7	1.91	7.39
37	365	3.03	46.25	36.12	2.11	8.01
38	370	3.07	47.5	36.53	2.33	8.63
39	375	3.11	48.75	36.93	2.56	9.26
40	380	3.16	50	37.33	2.81	9.86
41	385	3.2	51.25	37.73	3.02	10.5

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
42	390	3.24	52.5	38.14	3.23	11.13
43	395	3.28	53.75	38.55	3.44	11.76
44	400	3.32	55	38.94	3.64	12.41
45	405	3.36	56.25	39.33	3.85	13.08
46	410	3.41	57.5	39.71	4.06	13.73
47	415	3.45	58.75	40.08	4.25	14.42
48	420	3.49	60	40.48	4.41	15.11
49	425	3.53	61.25	40.85	4.59	15.82
50	430	3.57	62.5	41.22	4.78	16.5
51	435	3.61	63.75	41.58	4.96	17.21
52	440	3.65	65	41.94	5.14	17.92
53	445	3.7	66.25	42.31	5.32	18.62
54	450	3.74	67.5	42.68	5.51	19.32
55	455	3.78	68.75	43.03	5.68	20.04
56	460	3.82	70	43.36	5.88	20.76
57	465	3.86	71.25	43.72	6.06	21.47
58	470	3.9	72.5	44.08	6.22	22.21
59	475	3.95	73.75	44.45	6.34	22.95
60	480	3.99	75	44.88	6.42	23.7
61	485	4.03	76.25	45.37	6.43	24.46
62	490	4.07	77.5	45.79	6.54	25.17
63	495	4.11	78.75	46.2	6.63	25.92
64	500	4.15	80	46.58	6.73	26.69
65	505	4.19	81.25	46.94	6.85	27.45
66	510	4.24	82.5	47.32	6.96	28.22
67	515	4.28	83.75	47.67	7.08	29
68	520	4.32	85	48.05	7.19	29.77
69	525	4.36	86.25	48.45	7.26	30.54
70	530	4.4	87.5	48.84	7.33	31.33
71	535	4.44	88.75	49.23	7.4	32.12
72	540	4.49	90	49.62	7.49	32.89
73	545	4.53	91.25	50	7.58	33.67
74	550	4.57	92.5	50.36	7.66	34.48
75	555	4.61	93.75	50.74	7.74	35.27
76	560	4.65	95	51.12	7.79	36.08
77	565	4.69	96.25	51.46	7.91	36.88
78	570	4.73	97.5	51.87	7.93	37.7
79	575	4.78	98.75	52.19	8.04	38.53
80	580	4.82	100	52.5	8.16	39.34
81	585	4.86	101.25	52.81	8.32	40.12
82	590	4.9	102.5	53.13	8.43	40.95
83	595	4.94	103.75	53.45	8.53	41.77
84	600	4.98	105	53.78	8.62	42.6

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
85	605	5.02	106.25	54.07	9.13	43.05
86	610	5.07	107.5	54.39	9.22	43.89
87	615	5.11	108.75	54.69	9.45	44.62
88	620	5.15	110	54.97	9.56	45.46
89	625	5.19	111.25	55.25	9.68	46.31
90	630	5.23	112.5	55.53	9.8	47.17
91	635	5.27	113.75	55.78	10.08	47.89
92	640	5.32	115	56.04	10.25	48.71
93	645	5.36	116.25	56.28	10.38	49.59
94	650	5.4	117.5	56.53	10.5	50.47
95	655	5.44	118.75	56.77	10.65	51.33
96	660	5.48	120	57.01	10.79	52.2
97	665	5.52	121.25	57.26	10.93	53.06
98	670	5.56	122.5	57.53	11.05	53.92
99	675	5.61	123.75	57.8	11.18	54.78
100	680	5.65	125	58.07	11.31	55.62
101	685	5.69	126.25	58.33	11.46	56.46
102	690	5.73	127.5	58.61	11.6	57.29
103	695	5.77	128.75	58.91	11.73	58.11
104	700	5.81	130	59.2	11.86	58.94
105	705	5.86	131.25	59.49	11.97	59.79
106	710	5.9	132.5	59.79	12.11	60.6
107	715	5.94	133.75	60.1	12.23	61.41
108	720	5.98	135	60.4	12.37	62.23
109	725	6.02	136.25	60.73	12.48	63.04
110	730	6.06	137.5	61.03	12.63	63.83
111	735	6.1	138.75	61.35	12.75	64.65
112	740	6.15	140	61.67	12.87	65.46
113	745	6.19	141.25	61.98	13.32	65.95
114	750	6.23	142.5	62.28	13.5	66.71
115	755	6.27	143.75	62.61	13.8	67.34
116	760	6.31	145	62.97	13.88	68.15
117	765	6.35	146.25	63.34	13.99	68.92
118	770	6.4	147.5	63.63	14.17	69.7
119	775	6.44	148.75	63.91	14.32	70.51
120	780	6.48	150	64.16	14.5	71.35
121	785	6.52	151.25	64.38	14.66	72.21
122	790	6.56	152.5	64.58	14.84	73.08
123	795	6.6	153.75	64.77	15	73.98
124	800	6.64	155	64.97	15.13	74.9
125	805	6.69	156.25	65.16	15.27	75.82
126	810	6.73	157.5	65.32	15.45	76.72
127	815	6.77	158.75	65.51	15.78	77.46

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
128	820	6.81	160	65.73	15.89	78.37
129	825	6.85	161.25	65.95	16.04	79.26
130	830	6.89	162.5	66.17	16.2	80.13
131	835	6.94	163.75	66.4	16.35	81
132	840	6.98	165	66.62	16.5	81.88
133	845	7.02	166.25	66.83	16.66	82.76
134	850	7.06	167.5	67.06	16.81	83.64
135	855	7.1	168.75	67.26	16.97	84.52
136	860	7.14	170	67.46	17.14	85.4
137	865	7.18	171.25	67.67	17.31	86.27
138	870	7.23	172.5	67.88	17.47	87.15
139	875	7.27	173.75	68.06	17.63	88.05
140	880	7.31	175	68.26	17.76	88.98
141	885	7.35	176.25	68.46	17.89	89.9
142	890	7.39	177.5	68.64	18.03	90.83
143	895	7.43	178.75	68.83	18.18	91.74
144	900	7.48	180	69.02	18.36	92.63
145	905	7.52	181.25	69.19	18.52	93.55
146	910	7.56	182.5	69.36	18.67	94.47
147	915	7.6	183.75	69.53	18.83	95.39
148	920	7.64	185	69.7	18.96	96.35
149	925	7.68	186.25	69.87	19.11	97.27
150	930	7.72	187.5	70.04	19.25	98.2
151	935	7.77	188.75	70.2	19.41	99.14
152	940	7.81	190	70.35	19.55	100.1
153	945	7.85	191.25	70.51	19.69	101.04
154	950	7.89	192.5	70.67	19.84	101.99
155	955	7.93	193.75	70.83	19.99	102.93
156	960	7.97	195	70.98	20.14	103.88
157	965	8.01	196.25	71.14	20.28	104.83
158	970	8.06	197.5	71.29	20.42	105.79
159	975	8.1	198.75	71.45	20.55	106.75
160	980	8.14	200	71.62	20.66	107.72
161	985	8.18	201.25	71.79	20.77	108.69
162	990	8.22	202.5	71.98	20.87	109.65
163	995	8.26	203.75	72.18	20.96	110.61
164	1000	8.31	205	72.38	21.08	111.54
165	1005	8.35	206.25	72.59	21.2	112.46
166	1010	8.39	207.5	72.81	21.3	113.39
167	1015	8.43	208.75	73.03	21.43	114.28
168	1020	8.47	210	73.26	21.55	115.19
169	1025	8.51	211.25	73.48	21.68	116.09
170	1030	8.55	212.5	73.7	21.82	116.98

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
171	1035	8.6	213.75	73.94	21.95	117.86
172	1040	8.64	215	74.2	22.07	118.73
173	1045	8.68	216.25	74.45	22.19	119.61
174	1050	8.72	217.5	74.71	22.33	120.46
175	1055	8.76	218.75	74.97	22.45	121.33
176	1060	8.8	220	75.23	22.58	122.19
177	1065	8.85	221.25	75.49	22.72	123.04
178	1070	8.89	222.5	75.75	22.86	123.89
179	1075	8.93	223.75	76	23	124.75
180	1080	8.97	225	76.26	23.09	125.66
181	1085	9.01	226.25	76.51	23.16	126.58
182	1090	9.05	227.5	76.75	23.24	127.5
183	1095	9.09	228.75	77	23.3	128.45
184	1100	9.14	230	77.24	23.41	129.35
185	1105	9.18	231.25	77.48	23.43	130.34
186	1110	9.22	232.5	77.73	23.54	131.23
187	1115	9.26	233.75	78	23.67	132.09
188	1120	9.3	235	78.27	23.83	132.91
189	1125	9.34	236.25	78.54	23.93	133.77
190	1130	9.39	237.5	78.8	24.03	134.66
191	1135	9.43	238.75	79.08	24.13	135.55
192	1140	9.47	240	79.38	24.63	135.99
193	1145	9.51	241.25	79.67	24.73	136.85
194	1150	9.55	242.5	79.96	24.95	137.59
195	1155	9.59	243.75	80.26	25.07	138.42
196	1160	9.63	245	80.58	25.19	139.23
197	1165	9.68	246.25	80.88	25.3	140.07
198	1170	9.72	247.5	81.07	25.58	140.85
199	1175	9.76	248.75	81.29	25.76	141.71
200	1180	9.8	250	81.5	25.89	142.61
201	1185	9.84	251.25	81.73	26.01	143.51
202	1190	9.88	252.5	81.96	26.16	144.39
203	1195	9.93	253.75	82.27	26.3	145.18
204	1200	9.97	255	82.58	26.44	145.98
205	1205	10.01	256.25	82.89	26.55	146.81
206	1210	10.05	257.5	83.22	26.68	147.6
207	1215	10.09	258.75	83.57	26.82	148.36
208	1220	10.13	260	83.95	26.96	149.09
209	1225	10.17	261.25	84.19	27.11	149.96
210	1230	10.22	262.5	84.41	27.24	150.86
211	1235	10.26	263.75	84.61	27.37	151.77
212	1240	10.3	265	84.8	27.48	152.72
213	1245	10.34	266.25	85	27.61	153.64

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
214	1250	10.38	267.5	85.19	27.74	154.57
215	1255	10.42	268.75	85.35	27.87	155.52
216	1260	10.47	270	85.54	27.99	156.47
217	1265	10.51	271.25	85.76	28.14	157.35
218	1270	10.55	272.5	85.98	28.26	158.26
219	1275	10.59	273.75	86.2	28.38	159.17
220	1280	10.63	275	86.43	28.83	159.74
221	1285	10.67	276.25	86.65	29.01	160.59
222	1290	10.71	277.5	86.86	29.3	161.34
223	1295	10.76	278.75	87.12	29.39	162.24
224	1300	10.8	280	87.39	29.5	163.11
225	1305	10.84	281.25	87.7	29.68	163.88
226	1310	10.88	282.5	87.99	29.83	164.68
227	1315	10.92	283.75	88.28	30	165.47
228	1320	10.96	285	88.58	30.17	166.25
229	1325	11	286.25	88.89	30.35	167.01
230	1330	11.05	287.5	89.09	30.5	167.91
231	1335	11.09	288.75	89.27	30.64	168.84
232	1340	11.13	290	89.47	30.78	169.76
233	1345	11.17	291.25	89.65	30.96	170.64
234	1350	11.21	292.5	89.82	31.28	171.4
235	1355	11.25	293.75	89.99	31.4	172.36
236	1360	11.3	295	90.16	31.53	173.32
237	1365	11.34	296.25	90.33	31.67	174.26
238	1370	11.38	297.5	90.5	31.78	175.22
239	1375	11.42	298.75	90.67	31.91	176.16
240	1380	11.46	300	90.83	32.05	177.12
241	1385	11.5	301.25	90.98	32.19	178.08
242	1390	11.54	302.5	91.14	32.33	179.03
243	1395	11.59	303.75	91.3	32.5	179.95
244	1400	11.63	305	91.46	32.67	180.87
245	1405	11.67	306.25	91.61	32.83	181.8
246	1410	11.71	307.5	91.77	33	182.73
247	1415	11.75	308.75	91.93	33.12	183.7
248	1420	11.79	310	92.08	33.26	184.66
249	1425	11.84	311.25	92.25	33.39	185.61
250	1430	11.88	312.5	92.42	33.54	186.54
251	1435	11.92	313.75	92.61	33.72	187.42
252	1440	11.96	315	92.81	33.88	188.31
253	1445	12	316.25	93.01	34.03	189.2
254	1450	12.04	317.5	93.22	34.19	190.09
255	1455	12.08	318.75	93.44	34.32	190.99
256	1460	12.13	320	93.67	34.47	191.87

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	soluble polymer out	polymer in column
257	1465	12.17	321.25	93.89	34.62	192.74
258	1470	12.21	322.5	94.11	34.77	193.62
259	1475	12.25	323.75	94.33	34.91	194.51
260	1480	12.29	325	94.57	35.06	195.37
261	1485	12.33	326.25	94.83	35.2	196.22
262	1490	12.38	327.5	95.08	35.35	197.07
263	1495	12.42	328.75	95.34	35.5	197.91
264	1500	12.46	330	95.6	35.64	198.76
265	1505	12.5	331.25	95.86	35.78	199.6
266	1510	12.54	332.5	96.12	35.91	200.46
267	1515	12.58	333.75	96.38	36.02	201.35
268	1520	12.62	335	96.63	36.13	202.24
269	1525	12.67	336.25	96.89	36.23	203.13
270	1530	12.71	337.5	97.14	36.33	204.03
271	1535	12.75	338.75	97.38	36.44	204.93
272	1540	12.79	340	97.63	36.56	205.81
273	1545	12.83	341.25	97.87	36.67	206.71
274	1550	12.87	342.5	98.11	36.8	207.59
275	1555	12.92	343.75	98.36	36.91	208.48
276	1560	12.96	345	98.63	37.04	209.33
277	1565	13	346.25	98.9	37.18	210.17
278	1570	13.04	347.5	99.17	37.31	211.02

Table B.8. Data for Figure 4.14.

Sample No.	pore volume	Distance (cm)	Time (min)	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
1	0	12	0	0	0	0	0
2	0.045681	12	5	0	0	0	0
3	0.091362	12	10	0	0	0	0
4	0.137043	12	15	0	0	0	0
5	0.182724	12	20	0	0	0	0
6	0.228405	12	25	0	0	0	0
7	0.274086	12	30	0	0	0	0
8	0.319767	12	35	0	0	0	0
9	0.365449	12	40	0	0	0	0
10	0.41113	12	45	0	0	0	0
11	0.456811	12	50	0	0	0	0
12	0.502492	12	55	0	0	0	0
13	0.548173	12	60	0	0	0	0.0001
14	0.593854	12	65	0	0	0.0118	0.0011
15	0.639535	12	70	0	0.0002	0.0118	0.0054
16	0.685216	12	75	0	0.0013	0.0141	0.0189
17	0.730897	12	80	0.0204	0.0065	0.0245	0.0507
18	0.776578	12	85	0.0224	0.0234	0.075	0.1098
19	0.822259	12	90	0.0343	0.0639	0.1076	0.2001
20	0.86794	12	95	0.0757	0.1391	0.2365	0.3168
21	0.913621	12	100	0.1763	0.2517	0.372	0.4479
22	0.959302	12	105	0.308	0.3919	0.5483	0.5783
23	1.004983	12	110	0.4636	0.5405	0.64	0.6952
24	1.050664	12	115	0.6349	0.6776	0.74	0.7911
25	1.096346	12	120	0.7418	0.7897	0.8868	0.8638
26	1.142027	12	125	0.8427	0.872	0.9387	0.9152
27	1.187708	12	130	0.9133	0.927	0.9653	0.9494
28	1.233389	12	135	0.9569	0.9608	0.9782	0.9709
29	1.27907	12	140	0.9791	0.9801	0.9825	0.9839
30	1.324751	12	145	0.9911	0.9904	0.9875	0.9914
31	1.370432	12	150	0.9969	0.9956	0.9922	0.9955
32	1.416113	12	155	1.0007	0.9981	0.994	0.9977
33	1.461794	12	160	1.0022	0.9992	0.995	0.9989
34	1.507475	12	165	1.0038	0.9997	0.9958	0.9995
35	1.553156	12	170	1.0051	0.9999	0.9962	0.9997
36	1.598837	12	175	1.0051	0.9999	0.997	0.9999
37	1.644518	12	180	1.0084	1	0.9992	0.9999
38	1.690199	12	185	1.0029	1	1	1
39	1.73588	12	190	1.0047	1	1.0003	1
40	1.781561	12	195	1.0004	1	1	1
41	1.827243	12	200	1.0029	1	1.0005	1
42	1.872924	12	205	0.9998	1	1	1

Sample No.	pore volume	Distance (cm)	Time (min)	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
43	1.918605	12	210	0.9976	1	1	1
44	1.964286	12	215	0.9998	1	1	1
45	2.009967	12	220	1.0009	1	1	1
46	2.055648	12	225	1.0004	1	1	1
47	2.101329	12	230	1.0016	1	1.0008	1
48	2.14701	12	235	1.109	1	1	1
49	2.192691	12	240	1.0018	1	1.001	0.9999
50	2.238372	12	245	1.0013	1	0.995	0.9989
51	2.284053	12	250	1.002	0.9998	0.9875	0.9946
52	2.329734	12	255	1.0013	0.9987	0.9848	0.9811
53	2.375415	12	260	1.0029	0.9935	0.9555	0.9493
54	2.421096	12	265	0.9984	0.9766	0.8363	0.8902
55	2.466777	12	270	0.9542	0.9361	0.7175	0.7999
56	2.512458	12	275	0.898	0.8609	0.5988	0.6832
57	2.55814	12	280	0.7885	0.7483	0.481	0.5521
58	2.603821	12	285	0.6118	0.6081	0.3595	0.4217
59	2.649502	12	290	0.4162	0.4595	0.2727	0.3048
60	2.695183	12	295	0.3013	0.3224	0.1942	0.2089
61	2.740864	12	300	0.2358	0.2103	0.1225	0.1362
62	2.786545	12	305	0.1853	0.128	0.0753	0.0848
63	2.832226	12	310	0.1473	0.073	0.068	0.0506
64	2.877907	12	315	0.1257	0.0392	0.0541	0.0291
65	2.923588	12	320	0.1092	0.0199	0.0431	0.0161
66	2.969269	12	325	0.0944	0.0096	0.0377	0.0086
67	3.01495	12	330	0.0827	0.0044	0.03	0.0045
68	3.060631	12	335	0.0765	0.0019	0.0242	0.0023
69	3.106312	12	340	0.0671	0.0008	0.0231	0.0011
70	3.151993	12	345	0.0603	0.0003	0.0221	0.0005
71	3.197674	12	350	0.0564	0.0001	0.022	0.0003
72	3.243355	12	355	0.0476	0.0001	0.022	0.0001
73	3.289037	12	360	0.0465	0	0.022	0.0001
74	3.334718	12	365	0.0441	0	0.022	0
75		12	370	0.0465	0	0.022	0
76		12	375	0.0441	0	0.022	0

Table B.9. Data for Figure 4.7.

Sample No.	Pore volume	C/Co	phenol C/Co	Tracer C/Co	Fitted
1	0	0	0	0	0
2	0.041528	5	0	0	0
3	0.083056	10	0	0	0
4	0.124585	15	0	0	0
5	0.166113	20	0	0	0
6	0.207641	25	0	0	0
7	0.249169	30	0	0	0
8	0.290698	35	0	0	0
9	0.332226	40	0	0	0
10	0.373754	45	0	0	0
11	0.415282	50	0	0	0
12	0.456811	55	0	0	0
13	0.498339	60	0	0	0
14	0.539867	65	0		0
15	0.581395	70	0		0
16	0.622924	75	0		0
17	0.664452	80	0	0	0
18	0.70598	85	0	0	0
19	0.747508	90	0	0	0.0004
20	0.789037	95	0	0	0.0031
21	0.830565	100	0.001846	0.016789	0.0166
22	0.872093	105	0.031866	0.028559	0.0596
23	0.913621	110	0.120629	0.079708	0.1554
24	0.95515	115	0.280953	0.206952	0.3108
25	0.996678	120	0.460832	0.408977	0.5014
26	1.038206	125	0.641298	0.636326	0.6846
27	1.079734	130	0.795984	0.808351	0.8261
28	1.121262	135	0.911278	0.900418	0.9163
29	1.162791	140	0.943103	0.951983	0.9646
30	1.204319	145	0.962759	0.978288	0.9867
31	1.245847	150	0.980081	0.985804	0.9955
32	1.287375	155	0.986917	0.991649	0.9987
33	1.328904	160	0.971886	0.991649	0.9996
34	1.370432	165	0.972333	0.991649	0.9999
35	1.41196	170	0.991237	0.991649	1
36	1.453488	175	0.971643	0.991649	1
37	1.495017	180	0.993651	0.991649	1
38	1.536545	185	1.012272	0.991649	1
39	1.578073	190	1.00568	0.991649	1
40	1.619601	195	1	0.991649	1

Sample No.	Pore volume	C/Co	phenol C/Co	Tracer C/Co	Fitted
41	1.66113	200	1	0.991649	1
42	1.702658	205	1.015233	0.991649	1
43	1.744186	210	1.023063	0.991649	1
44	1.785714	215	1.014199	0.993111	1
45	1.827243	220	1.006085	0.993111	1
46	1.868771	225	1.014016	0.993111	1
47	1.910299	230	1.016552	0.993111	1
48	1.951827	235	1.021643	0.993111	1
49	1.993355	240	1.020041	0.997286	0.9996
50	2.034884	245	1.013854	0.997286	0.9969
51	2.076412	250	1.012475	0.994363	0.9834
52	2.11794	255	0.980041	0.960752	0.9404
53	2.159468	260	0.889189	0.853445	0.8446
54	2.200997	265	0.724361	0.700626	0.6892
55	2.242525	270	0.546126	0.574739	0.4986
56	2.284053	275	0.349594	0.441545	0.3154
57	2.325581	280	0.234016	0.32547	0.1739
58	2.36711	285	0.138519	0.196388	0.0837
59	2.408638	290	0.073286	0.108914	0.0354
60	2.450166	295	0.037241	0.05977	0.0133
61	2.491694	300	0.004665	0.032547	0.0045
62	2.533223	305	2.03E-05	0.021649	0.0013
63	2.574751	310	0	0.018163	0.0004
64	2.616279	315	0	0	0.0001
65	2.657807	320	0	0	0
66	2.699336	325	0	0	0
67	2.740864	330	0	0	0
68	2.782392	335	0	0	0
69	2.82392	340	0	0	0
70	2.865449	345	0	0	0
71	2.906977	350	0	0	0
72	2.948505	355	0	0	0
73	2.990033	360	0	0	0
74	3.031561	365	0	0	0

Table B.10. Data for Figure 4.15.

No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
1	0	12	0	0	0	0	0
2	0.049834	12	5	0	0	0	0
3	0.099668	12	10	0	0	0	0
4	0.149502	12	15	0	0	0	0
5	0.199336	12	20	0	0	0	0
6	0.249169	12	25	0	0	0	0
7	0.299003	12	30	0	0	0	0
8	0.348837	12	35	0	0	0	0
9	0.398671	12	40	0	0	0	0
10	0.448505	12	45	0	0	0	0.0003
11	0.498339	12	50	0	0	0	0.0018
12	0.548173	12	55	0	0.0004	0	0.0074
13	0.598007	12	60	0	0.0023	0.0208	0.0221
14	0.647841	12	65	0	0.009	0.0212	0.052
15	0.697674	12	70	0	0.026	0.0221	0.1022
16	0.747508	12	75	0.0224	0.0605	0.119	0.1738
17	0.797342	12	80	0.0524	0.118	0.2669	0.2637
18	0.847176	12	85	0.1123	0.1996	0.3555	0.3655
19	0.89701	12	90	0.2818	0.301	0.512	0.471
20	0.946844	12	95	0.4241	0.4138	0.6213	0.5729
21	0.996678	12	100	0.6137	0.5279	0.7333	0.6653
22	1.046512	12	105	0.7797	0.6345	0.8135	0.7449
23	1.096346	12	110	0.8814	0.7274	0.8668	0.8104
24	1.146179	12	115	0.9467	0.8037	0.9144	0.8624
25	1.196013	12	120	0.9798	0.8631	0.933	0.9022
26	1.245847	12	125	0.9921	0.9073	0.949	0.9318
27	1.295681	12	130	0.9995	0.939	0.9786	0.9533
28	1.345515	12	135	1.0029	0.9608	0.9843	0.9685
29	1.395349	12	140	1.0037	0.9754	0.9927	0.979
30	1.445183	12	145	1	0.9849	0.9998	0.9862
31	1.495017	12	150	1	0.9909	1.0018	0.9911
32	1.54485	12	155	1	0.9946	0.9909	0.9943
33	1.594684	12	160	0.9946	0.9968	0.9941	0.9964
34	1.644518	12	165	0.9946	0.9982	0.9952	0.9977
35	1.694352	12	170	0.9946	0.999	0.997	0.9986
36	1.744186	12	175	0.9892	0.9994	1	0.9991
37	1.79402	12	180	0.9892	0.9997	1	0.9995
38	1.843854	12	185	0.9892	0.9998	1	0.9997
39	1.893688	12	190	0.9892	0.9999	1	0.9998
40	1.943522	12	195	0.9892	0.9999	1	0.9999
41	1.993355	12	200	0.9892	1	1	0.9999

No	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
42	2.043189	12	205	0.9892	1	1	1
43	2.093023	12	210	0.9789	1	1	1
44	2.142857	12	215	0.9892	1	1	1
45	2.192691	12	220	0.9892	1	1	1
46	2.242525	12	225	0.9892	1	1	0.9997
47	2.292359	12	230	0.9748	1	1.0112	0.9982
48	2.342193	12	235	0.9756	0.9996	0.9954	0.9926
49	2.392027	12	240	0.9673	0.9977	0.9522	0.9779
50	2.44186	12	245	0.9649	0.991	0.9007	0.948
51	2.491694	12	250	0.9496	0.974	0.8262	0.8978
52	2.541528	12	255	0.8803	0.9395	0.7789	0.8262
53	2.591362	12	260	0.8091	0.882	0.7374	0.7363
54	2.641196	12	265	0.7534	0.8004	0.6534	0.6345
55	2.69103	12	270	0.6858	0.699	0.5564	0.529
56	2.740864	12	275	0.6366	0.5862	0.4869	0.4271
57	2.790698	12	280	0.559	0.4721	0.3979	0.3347
58	2.840532	12	285	0.4875	0.3655	0.3152	0.2551
59	2.890365	12	290	0.406	0.2726	0.2012	0.1896
60	2.940199	12	295	0.3226	0.1963	0.1795	0.1376
61	2.990033	12	300	0.2586	0.1369	0.1389	0.0978
62	3.039867	12	305	0.1946	0.0927	0.1002	0.0682
63	3.089701	12	310	0.1428	0.061	0.08	0.0467
64	3.139535	12	315	0.0979	0.0392	0.0612	0.0315
65	3.189369	12	320	0.0761	0.0246	0.053	0.021
66	3.239203	12	325	0.0665	0.0151	0.0455	0.0138
67	3.289037	12	330	0.0395	0.0091	0.0399	0.0089
68	3.33887	12	335	0.0378	0.0054	0.0364	0.0057
69	3.388704	12	340	0.019	0.0032	0.0346	0.0036
70	3.438538	12	345	0.0181	0.0018	0.0296	0.0023
71	3.488372	12	350	0.0168	0.001	0.0251	0.0014
72	3.538206	12	355	0.0143	0.0006	0.0228	0.0009
73	3.58804	12	360	0.0119	0.0003	0.0205	0.0005
74	3.637874	12	365	0.0095	0.0002	0.0205	0.0003
75	3.687708	12	370	0.0095	0.0001	0.0205	0.0002
76	3.737542	12	375	0.0095	0.0001	0.0205	0.0001

Table B.11. Data for Figure 4.16.

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
1	0	12	0	0	0	0	0
2	0.045681	12	5	0	0	0	0
3	0.091362	12	10	0	0	0	0
4	0.137043	12	15	0	0	0	0
5	0.182724	12	20	0	0	0	0
6	0.228405	12	25	0	0	0	0
7	0.274086	12	30	0	0	0	0
8	0.319767	12	35	0	0	0	0
9	0.365449	12	40	0	0	0	0
10	0.41113	12	45	0	0	0	0
11	0.456811	12	50	0	0	0	0
12	0.502492	12	55	0	0	0	0
13	0.548173	12	60	0	0	0	0
14	0.593854	12	65	0	0	0	0.0005
15	0.639535	12	70	0	0.0002	0	0.0031
16	0.685216	12	75	0	0.0012	0.0301	0.0137
17	0.730897	12	80	0	0.0063	0.0346	0.0437
18	0.776578	12	85	0.034	0.0229	0.0522	0.1064
19	0.822259	12	90	0.0606	0.063	0.0996	0.2091
20	0.86794	12	95	0.0953	0.138	0.2268	0.3455
21	0.913621	12	100	0.2105	0.2507	0.4286	0.4972
22	0.959302	12	105	0.3972	0.3913	0.6152	0.6422
23	1.004983	12	110	0.5132	0.5405	0.7653	0.7638
24	1.050664	12	115	0.7207	0.6781	0.8837	0.8548
25	1.096346	12	120	0.854	0.7905	0.9485	0.9165
26	1.142027	12	125	0.9149	0.8729	0.9825	0.9549
27	1.187708	12	130	0.9626	0.9277	0.9964	0.977
28	1.233389	12	135	0.9666	0.9614	1.002	0.9888
29	1.27907	12	140	0.9918	0.9805	1.0009	0.9948
30	1.324751	12	145	0.9956	0.9906	0.9977	0.9977
31	1.370432	12	150	0.9942	0.9957	0.9971	0.999
32	1.416113	12	155	1.014	0.9981	0.9955	0.9996
33	1.461794	12	160	1.0009	0.9992	0.9982	0.9998
34	1.507475	12	165	1.0012	0.9997	1	0.9999
35	1.553156	12	170	1.0012	0.9999	1	1
36	1.598837	12	175	1.0012	1	1	1
37	1.644518	12	180	1.0012	1	1	1
38	1.690199	12	185	1.0012	1	1	1
39	1.73588	12	190	1.0012	1	1	1
40	1.781561	12	195	1.003	1	1	1
41	1.827243	12	200	1.0035	1	0.9968	1
42	1.872924	12	205	0.9988	1	1.0045	1

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
43	1.918605	12	210	0.9998	1	1	1
44	1.964286	12	215	0.9988	1	1.0093	0.9995
45	2.009967	12	220	1	0.9998	1.0077	0.9969
46	2.055648	12	225	1.0012	0.9988	0.9873	0.9863
47	2.101329	12	230	1.0005	0.9937	0.9478	0.9563
48	2.14701	12	235	0.9897	0.9771	0.8349	0.8936
49	2.192691	12	240	0.9574	0.937	0.6841	0.7909
50	2.238372	12	245	0.8557	0.862	0.5562	0.6545
51	2.284053	12	250	0.7675	0.7493	0.4399	0.5028
52	2.329734	12	255	0.5616	0.6087	0.31	0.3578
53	2.375415	12	260	0.3906	0.4595	0.2237	0.2362
54	2.421096	12	265	0.2807	0.3219	0.1632	0.1452
55	2.466777	12	270	0.2208	0.2095	0.1169	0.0835
56	2.512458	12	275	0.1874	0.1271	0.09	0.0451
57	2.55814	12	280	0.1569	0.0723	0.0725	0.023
58	2.603821	12	285	0.131	0.0386	0.0589	0.0112
59	2.649502	12	290	0.1126	0.0195	0.0346	0.0052
60	2.695183	12	295	0.1006	0.0094	0.0291	0.0023
61	2.740864	12	300	0.0819	0.0043	0.0247	0.001
62	2.786545	12	305	0.0749	0.0019	0	0.0004
63	2.832226	12	310	0.0678	0.0008	0	0.0002
64	2.877907	12	315	0.0632	0.0003	0	0.0001
65	2.923588	12	320	0.0585	0.0001	0	0
66	2.969269	12	325	0.0531	0	0	0
67	3.01495	12	330	0.0444	0	0	0
68	3.060631	12	335	0.0374	0	0	0
69	3.106312	12	340	0.0234	0	0	0
70	3.151993	12	345	0	0	0	0
71	3.197674	12	350	0	0	0	0
72	3.243355	12	355	0	0	0	0
73	3.289037	12	360	0	0	0	0
74	3.334718	12	365	0	0	0	0

Table B.12. Data for Figure 4.17.

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
1	0	12	0	0	0	0	0
2	0.041528	12	5	0	0	0	0
3	0.083056	12	10	0	0	0	0
4	0.124585	12	15	0	0	0	0
5	0.166113	12	20	0	0	0	0
6	0.207641	12	25	0	0	0	0
7	0.249169	12	30	0	0	0	0
8	0.290698	12	35	0	0	0	0
9	0.332226	12	40	0	0	0	0
10	0.373754	12	45	0	0	0	0
11	0.415282	12	50	0	0	0	0
12	0.456811	12	55	0	0	0	0
13	0.498339	12	60	0	0	0	0
14	0.539867	12	65	0	0	0	0.0002
15	0.581395	12	70	0	0.0002	0	0.0013
16	0.622924	12	75	0	0.0011	0	0.005
17	0.664452	12	80	0	0.0044	0.0299	0.0153
18	0.70598	12	85	0	0.0133	0.0378	0.0379
19	0.747508	12	90	0.0345	0.0331	0.0686	0.0789
20	0.789037	12	95	0.0491	0.0694	0.1253	0.1421
21	0.830565	12	100	0.0876	0.1267	0.2344	0.2275
22	0.872093	12	105	0.1571	0.2057	0.3443	0.3301
23	0.913621	12	110	0.2383	0.3029	0.4929	0.4416
24	0.95515	12	115	0.3263	0.4111	0.6316	0.5528
25	0.996678	12	120	0.4245	0.5216	0.747	0.6556
26	1.038206	12	125	0.5258	0.6261	0.8377	0.7445
27	1.079734	12	130	0.6181	0.7186	0.901	0.8171
28	1.121262	12	135	0.7183	0.7957	0.942	0.8734
29	1.162791	12	140	0.7924	0.8566	0.9717	0.915
30	1.204319	12	145	0.8872	0.9025	0.9861	0.9446
31	1.245847	12	150	0.9349	0.9356	0.9947	0.9648
32	1.287375	12	155	0.9537	0.9587	0.9977	0.9782
33	1.328904	12	160	0.9756	0.9741	0.9991	0.9868
34	1.370432	12	165	0.9798	0.9842	1	0.9922
35	1.41196	12	170	0.9851	0.9905	1	0.9954
36	1.453488	12	175	0.9927	0.9945	1	0.9974
37	1.495017	12	180	0.9944	0.9968	1	0.9985
38	1.536545	12	185	1	0.9982	1	0.9992
39	1.578073	12	190	1	0.999	1	0.9996
40	1.619601	12	195	1	0.9995	1	0.9998
41	1.66113	12	200	1	0.9997	1	0.9999

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
42	1.702658	12	205	1.0042	0.9998	1	0.9999
43	1.744186	12	210	1.0042	0.9999	1	1
44	1.785714	12	215	1.0042	1	1	1
45	1.827243	12	220	1.0042	1	1	1
46	1.868771	12	225	1.0042	1	1	1
47	1.910299	12	230	1.0042	1	1.0005	1
48	1.951827	12	235	1.0042	1	1	1
49	1.993355	12	240	1.0042	1	1	1
50	2.034884	12	245	1.0065	1	0.9977	0.9998
51	2.076412	12	250	1.0056	0.9998	0.9977	0.9987
52	2.11794	12	255	1.0053	0.9989	0.9977	0.995
53	2.159468	12	260	1.0048	0.9956	0.9965	0.9847
54	2.200997	12	265	1.0053	0.9867	0.9868	0.9621
55	2.242525	12	270	0.9964	0.9669	0.9492	0.9211
56	2.284053	12	275	0.9837	0.9306	0.8709	0.8579
57	2.325581	12	280	0.9416	0.8733	0.7749	0.7725
58	2.36711	12	285	0.8931	0.7943	0.6777	0.6699
59	2.408638	12	290	0.8027	0.6971	0.5887	0.5584
60	2.450166	12	295	0.6453	0.5889	0.5087	0.4472
61	2.491694	12	300	0.5297	0.4784	0.4262	0.3444
62	2.533223	12	305	0.4018	0.3739	0.338	0.2555
63	2.574751	12	310	0.3171	0.2814	0.2694	0.1829
64	2.616279	12	315	0.2568	0.2043	0.2006	0.1266
65	2.657807	12	320	0.2127	0.1434	0.1513	0.085
66	2.699336	12	325	0.1814	0.0975	0.1142	0.0554
67	2.740864	12	330	0.1517	0.0644	0.0871	0.0352
68	2.782392	12	335	0.1348	0.0413	0.0707	0.0218
69	2.82392	12	340	0.121	0.0259	0.0561	0.0132
70	2.865449	12	345	0.1065	0.0158	0.0474	0.0078
71	2.906977	12	350	0.0976	0.0095	0.0417	0.0046
72	2.948505	12	355	0.0898	0.0055	0.0371	0.0026
73	2.990033	12	360	0.0819	0.0032	0.0325	0.0015
74	3.031561	12	365	0.0701	0.0018	0.0301	0.0008
75	3.07309	12	370	0.0561	0.001	0.0301	0.0004
76	3.114618	12	375	0.0505	0.0005	0.0278	0.0002
77	3.156146	12	380	0.0477	0.0003	0.0278	0.0001
78	3.197674	12	385	0.0449	0.0002	0.0278	0.0001
79	3.239203	12	390	0.0421	0.0001	0.0278	0
80	3.280731	12	395	0.0365	0	0.0278	0
81	3.322259	12	400	0.0309	0	0.0278	0
82	3.363787	12	405	0.0309	0	0.0278	0
83	3.405316	12	410	0.0309	0	0.0278	0
84	3.446844	12	415	0.0309	0	0.0278	0

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
85	3.488372	12	420	0.0309	0	0.0278	0
86	3.5299	12	425	0.0309	0	0.0278	0
87	3.571429	12	430	0.0278	0	0.0278	0

Table B.13. Data for Figure 4.18.

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
1	0	12	0	0	0	0	0
2	0.041528	12	5	0	0	0	0
3	0.083056	12	10	0	0	0	0
4	0.124585	12	15	0	0	0	0
5	0.166113	12	20	0	0	0	0
6	0.207641	12	25	0	0	0	0
7	0.249169	12	30	0	0	0	0
8	0.290698	12	35	0	0	0	0
9	0.332226	12	40	0	0	0	0
10	0.373754	12	45	0	0	0	0
11	0.415282	12	50	0	0	0	0
12	0.456811	12	55	0	0	0	0
13	0.498339	12	60	0	0	0	0
14	0.539867	12	65	0	0	0	0
15	0.581395	12	70	0	0	0	0
16	0.622924	12	75	0	0	0	0.0001
17	0.664452	12	80	0	0.0002	0	0.0004
18	0.70598	12	85	0.0314	0.0016	0	0.0025
19	0.747508	12	90	0.0325	0.0071	0.0254	0.0099
20	0.789037	12	95	0.0376	0.0236	0.0332	0.0302
21	0.830565	12	100	0.056	0.0616	0.0573	0.0737
22	0.872093	12	105	0.1083	0.1313	0.118	0.1488
23	0.913621	12	110	0.1809	0.2359	0.2214	0.2565
24	0.95515	12	115	0.3041	0.3681	0.3565	0.3882
25	0.996678	12	120	0.4411	0.5117	0.5	0.528
26	1.038206	12	125	0.5864	0.6481	0.6601	0.659
27	1.079734	12	130	0.6856	0.7635	0.7763	0.7692
28	1.121262	12	135	0.7971	0.8515	0.8563	0.8533
29	1.162791	12	140	0.8762	0.9125	0.9057	0.9121
30	1.204319	12	145	0.9153	0.9514	0.9385	0.9502
31	1.245847	12	150	0.9564	0.9745	0.9581	0.9732

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
32	1.287375	12	155	0.9693	0.9873	0.9692	0.9863
33	1.328904	12	160	0.9796	0.994	0.9773	0.9933
34	1.370432	12	165	0.9842	0.9973	0.9826	0.9968
35	1.41196	12	170	0.99	0.9988	0.9871	0.9986
36	1.453488	12	175	0.9915	0.9995	0.9904	0.9994
37	1.495017	12	180	0.9949	0.9998	0.9927	0.9997
38	1.536545	12	185	0.9961	0.9999	0.9957	0.9999
39	1.578073	12	190	0.9973	1	1	1
40	1.619601	12	195	0.9995	1	1	1
41	1.66113	12	200	0.9995	1	1	1
42	1.702658	12	205	0.9995	1	1	1
43	1.744186	12	210	0.9995	1	1	1
44	1.785714	12	215	0.9995	1	1	1
45	1.827243	12	220	1	1	1	1
46	1.868771	12	225	1	1	1	1
47	1.910299	12	230	1	1	1	1
48	1.951827	12	235	1	1	1	1
49	1.993355	12	240	1.0024	1	1.0008	1
50	2.034884	12	245	1.0024	1	1.001	1
51	2.076412	12	250	1.0024	1	1.001	1
52	2.11794	12	255	1.0024	1	1.0008	0.9999
53	2.159468	12	260	1.0024	0.9998	1.0008	0.9996
54	2.200997	12	265	1.0066	0.9984	1.001	0.9975
55	2.242525	12	270	1.0061	0.9929	0.9955	0.9901
56	2.284053	12	275	1.0017	0.9764	0.9783	0.9698
57	2.325581	12	280	0.983	0.9384	0.9327	0.9263
58	2.36711	12	285	0.8995	0.8687	0.8417	0.8512
59	2.408638	12	290	0.7934	0.7641	0.7151	0.7435
60	2.450166	12	295	0.5781	0.6319	0.5802	0.6118
61	2.491694	12	300	0.4491	0.4883	0.4407	0.472
62	2.533223	12	305	0.2883	0.3519	0.2988	0.341
63	2.574751	12	310	0.2206	0.2365	0.2286	0.2308
64	2.616279	12	315	0.1267	0.1485	0.1313	0.1467
65	2.657807	12	320	0.1021	0.0875	0.1058	0.0879
66	2.699336	12	325	0.0748	0.0486	0.0775	0.0498
67	2.740864	12	330	0.0526	0.0255	0.0545	0.0268
68	2.782392	12	335	0.0398	0.0127	0.0412	0.0137
69	2.82392	12	340	0.0318	0.006	0.0329	0.0067
70	2.865449	12	345	0.0262	0.0027	0.0271	0.0032
71	2.906977	12	350	0.0234	0.0012	0.0242	0.0014
72	2.948505	12	355	0.0236	0.0005	0.0245	0.0006
73	2.990033	12	360	0.0231	0.0002	0.024	0.0003
74	3.031561	12	365	0.0229	0.0001	0.0234	0.0001

Table B.14. Data for Figure 4.25.

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
1	0	12	0	0	0	0	0
2	0.041528	12	5	0	0	0	0
3	0.083056	12	10	0	0	0	0
4	0.124585	12	15	0	0	0	0
5	0.166113	12	20	0	0	0	0
6	0.207641	12	25	0	0	0	0
7	0.249169	12	30	0	0	0	0
8	0.290698	12	35	0	0	0	0
9	0.332226	12	40	0	0	0	0
10	0.373754	12	45	0	0	0	0
11	0.415282	12	50	0	0	0	0
12	0.456811	12	55	0	0	0	0
13	0.498339	12	60	0	0	0	0
14	0.539867	12	65	0	0	0	0
15	0.581395	12	70	0	0.0001	0	0.0004
16	0.622924	12	75	0	0.0006	0	0.002
17	0.664452	12	80	0.0399	0.0028	0	0.0076
18	0.70598	12	85	0.04	0.0096	0.0219	0.0227
19	0.747508	12	90	0.0417	0.0261	0.0425	0.0545
20	0.789037	12	95	0.0506	0.0588	0.0901	0.1099
21	0.830565	12	100	0.1111	0.1133	0.181	0.1917
22	0.872093	12	105	0.2223	0.1916	0.2719	0.2967
23	0.913621	12	110	0.2983	0.2909	0.4017	0.4159
24	0.95515	12	115	0.3839	0.4036	0.5238	0.5377
25	0.996678	12	120	0.5398	0.5197	0.6531	0.6513
26	1.038206	12	125	0.727	0.6296	0.7605	0.7489
27	1.079734	12	130	0.8724	0.7261	0.8499	0.827
28	1.121262	12	135	0.9232	0.8055	0.9132	0.8857
29	1.162791	12	140	0.9621	0.8671	0.949	0.9273
30	1.204319	12	145	0.9818	0.9124	0.9708	0.9554
31	1.245847	12	150	0.9905	0.9441	0.9821	0.9736
32	1.287375	12	155	0.9949	0.9655	0.9872	0.9848
33	1.328904	12	160	0.9947	0.9793	0.9906	0.9915
34	1.370432	12	165	0.9983	0.9879	0.9918	0.9954
35	1.41196	12	170	1	0.9931	0.9932	0.9975
36	1.453488	12	175	1	0.9961	0.9942	0.9987
37	1.495017	12	180	1	0.9979	0.9952	0.9993
38	1.536545	12	185	1	0.9989	0.9952	0.9997
39	1.578073	12	190	1	0.9994	0.9952	0.9998
40	1.619601	12	195	1	0.9997	0.9976	0.9999
41	1.66113	12	200	1	0.9998	0.9976	1

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
42	1.702658	12	205	1	0.9999	0.9976	1
43	1.744186	12	210	1	1	0.9976	1
44	1.785714	12	215	1	1	1	1
45	1.827243	12	220	1	1	1	1
46	1.868771	12	225	1	1	1	1
47	1.910299	12	230	1	1	1	1
48	1.951827	12	235	1	1	1.0029	1
49	1.993355	12	240	1	1	1.0029	1
50	2.034884	12	245	1	1	1.0029	1
51	2.076412	12	250	1.0046	0.9999	1.0017	0.9996
52	2.11794	12	255	1.0051	0.9994	1.0017	0.998
53	2.159468	12	260	1.0061	0.9972	1	0.9924
54	2.200997	12	265	1.0058	0.9904	0.9862	0.9773
55	2.242525	12	270	0.9993	0.9739	0.9705	0.9455
56	2.284053	12	275	0.9696	0.9412	0.9156	0.8901
57	2.325581	12	280	0.8816	0.8867	0.8347	0.8083
58	2.36711	12	285	0.7909	0.8084	0.6848	0.7033
59	2.408638	12	290	0.7027	0.7091	0.5233	0.5841
60	2.450166	12	295	0.5862	0.5964	0.4136	0.4623
61	2.491694	12	300	0.4826	0.4803	0.321	0.3487
62	2.533223	12	305	0.4094	0.3704	0.2589	0.2511
63	2.574751	12	310	0.3265	0.2739	0.1996	0.173
64	2.616279	12	315	0.2413	0.1945	0.1581	0.1143
65	2.657807	12	320	0.2075	0.1329	0.1239	0.0727
66	2.699336	12	325	0.1696	0.0876	0.0943	0.0446
67	2.740864	12	330	0.1398	0.0559	0.0725	0.0264
68	2.782392	12	335	0.1198	0.0345	0.0637	0.0152
69	2.82392	12	340	0.0837	0.0207	0.0573	0.0085
70	2.865449	12	345	0.0695	0.0121	0.052	0.0046
71	2.906977	12	350	0.0547	0.0069	0.0491	0.0025
72	2.948505	12	355	0.0488	0.0039	0.046	0.0013
73	2.990033	12	360	0	0.0021	0.0434	0.0007
74	3.031561	12	365	0	0.0011	0	0.0003
75	3.07309	12	370	0.043	0.0006	0.043	0.0002
76	3.114618	12	375	0.0382	0.0003	0.0382	0.0001
77	3.156146	12	380	0.0382	0.0002	0.0382	0
78	3.197674	12	385	0.0382	0.0001	0.0382	0
79	3.239203	12	390	0.0382	0	0.0382	0
80	3.280731	12	395	0.0382	0	0.0382	0
81	3.322259	12	400	0.0382	0	0.0382	0
82	3.363787	12	405	0.0382	0	0.0382	0
83	3.405316	12	410	0.0382	0	0.0382	0
84	3.446844	12	415	0	0	0	0

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
85	3.488372	12	420	0	0	0	0
86	3.5299	12	425	0	0	0	0
87	3.571429	12	430	0	0	0	0

Table B.15. Data for Figure 4.26.

Sample No	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
1	0	12	0	0	0	0	0
2	0.041528	12	5	0	0	0	0
3	0.083056	12	10	0	0	0	0
4	0.124585	12	15	0	0	0	0
5	0.166113	12	20	0	0	0	0
6	0.207641	12	25	0	0	0	0
7	0.249169	12	30	0	0	0	0
8	0.290698	12	35	0	0	0	0
9	0.332226	12	40	0	0	0	0
10	0.373754	12	45	0	0	0	0
11	0.415282	12	50	0	0	0	0
12	0.456811	12	55	0	0	0	0
13	0.498339	12	60	0	0	0	0
14	0.539867	12	65	0	0	0	0
15	0.581395	12	70	0	0.0002	0	0.0001
16	0.622924	12	75	0	0.0012	0	0.0005
17	0.664452	12	80	0	0.0046	0	0.0032
18	0.70598	12	85	0.0207	0.0139	0	0.0136
19	0.747508	12	90	0.022	0.0341	0.0263	0.0427
20	0.789037	12	95	0.0427	0.0709	0.0549	0.104
21	0.830565	12	100	0.0905	0.1285	0.0994	0.2053
22	0.872093	12	105	0.1819	0.2076	0.2578	0.3416
23	0.913621	12	110	0.2733	0.3045	0.3722	0.4947
24	0.95515	12	115	0.4037	0.4121	0.5562	0.6421
25	0.996678	12	120	0.5264	0.5219	0.729	0.766
26	1.038206	12	125	0.6563	0.6257	0.8475	0.8583
27	1.079734	12	130	0.7641	0.7176	0.9153	0.9202
28	1.121262	12	135	0.854	0.7944	0.9511	0.958
29	1.162791	12	140	0.9177	0.8551	0.9697	0.9792
30	1.204319	12	145	0.9536	0.9011	0.9791	0.9903
31	1.245847	12	150	0.9755	0.9344	0.9851	0.9957
32	1.287375	12	155	0.9869	0.9577	0.9883	0.9982
33	1.328904	12	160	0.992	0.9734	0.9908	0.9993

Sample No.	pore volume	Distance	Time	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
34	1.370432	12	165	0.9954	0.9836	0.992	0.9997
35	1.41196	12	170	0.9966	0.9901	0.9931	0.9999
36	1.453488	12	175	0.9981	0.9942	0.9946	1
37	1.495017	12	180	0.999	0.9966	0.9946	1
38	1.536545	12	185	1	0.9981	0.9946	1
39	1.578073	12	190	1	0.9989	0.9946	1
40	1.619601	12	195	1	0.9994	1	1
41	1.66113	12	200	1	0.9997	1	1
42	1.702658	12	205	1.0024	0.9998	1	1
43	1.744186	12	210	1.0024	0.9999	1	1
44	1.785714	12	215	1.0024	1	1	1
45	1.827243	12	220	1.0024	1	1	1
46	1.868771	12	225	1.0049	1	1	1
47	1.910299	12	230	1.0049	1	1	1
48	1.951827	12	235	1.0078	1	1	1
49	1.993355	12	240	1.0078	1	1	1
50	2.034884	12	245	1.0078	1	1	1
51	2.076412	12	250	1.0066	0.9998	1	0.9999
52	2.11794	12	255	1.0066	0.9988	1.002	0.9995
53	2.159468	12	260	1.0049	0.9954	0.9991	0.9968
54	2.200997	12	265	0.991	0.9861	0.9837	0.9864
55	2.242525	12	270	0.9752	0.9659	0.9273	0.9573
56	2.284053	12	275	0.9201	0.9291	0.8352	0.896
57	2.325581	12	280	0.8387	0.8715	0.7296	0.7947
58	2.36711	12	285	0.6881	0.7924	0.5911	0.6584
59	2.408638	12	290	0.5259	0.6955	0.3808	0.5053
60	2.450166	12	295	0.4156	0.5879	0.2327	0.3579
61	2.491694	12	300	0.3226	0.4781	0.1654	0.234
62	2.533223	12	305	0.2601	0.3743	0.1251	0.1417
63	2.574751	12	310	0.2006	0.2824	0.0949	0.0798
64	2.616279	12	315	0.1589	0.2056	0.0761	0.042
65	2.657807	12	320	0.1245	0.1449	0.0607	0.0208
66	2.699336	12	325	0.0947	0.0989	0.0523	0.0097
67	2.740864	12	330	0.0729	0.0656	0.0458	0.0043
68	2.782392	12	335	0.0641	0.0423	0.0438	0.0018
69	2.82392	12	340	0.0576	0.0266	0.0374	0.0007
70	2.865449	12	345	0.0523	0.0164	0.0291	0.0003
71	2.906977	12	350	0.0493	0.0099	0.0272	0.0001
72	2.948505	12	355	0.0462	0.0058	0.0246	0
73	2.990033	12	360	0.0436	0.0034	0.0212	0
74	3.031561	12	365	0.0376	0.0019	0.0203	0

Appendix C - Data for Chapter 5

Table C.1. Data for Figure 5.2.

				Tracer		2,4-DCP
Pore volume	No.	Distance	Time (min)	C/Co	Fitted	C/Co
0	1	12	0	0	0	0
0.049834	2	12	5	0	0	0
0.099668	3	12	10	0	0	0
0.149502	4	12	15	0	0	0
0.199336	5	12	20	0	0	0
0.249169	6	12	25	0	0	0
0.299003	7	12	30	0	0	0
0.348837	8	12	35	0	0	0
0.398671	9	12	40	0	0	0
0.448505	10	12	45	0	0	0
0.498339	11	12	50	0	0	0
0.548173	12	12	55	0	0	0
0.598007	13	12	60	0	0	0
0.647841	14	12	65	0	0.0003	0
0.697674	15	12	70	0.0219	0.0022	0
0.747508	16	12	75	0.0238	0.0112	0
0.797342	17	12	80	0.0335	0.0392	0
0.847176	18	12	85	0.0688	0.1019	0.028
0.89701	19	12	90	0.1696	0.2087	0.06165
0.946844	20	12	95	0.2806	0.3532	0.24655
0.996678	21	12	100	0.4235	0.5141	0.43215
1.046512	22	12	105	0.6505	0.6653	0.5843
1.096346	23	12	110	0.7959	0.7883	0.7529
1.146179	24	12	115	0.8745	0.8766	0.97555
1.196013	25	12	120	0.9332	0.9333	1.01315
1.245847	26	12	125	0.963	0.9664	0.9756
1.295681	27	12	130	0.9821	0.9841	1.00665
1.345515	28	12	135	0.9895	0.9929	1.0037
1.395349	29	12	140	0.9931	0.997	0.99085
1.445183	30	12	145	0.9959	0.9988	0.9999
1.495017	31	12	150	0.9969	0.9995	0.9889
1.54485	32	12	155	0.9982	0.9998	0.9776
1.594684	33	12	160	0.9987	0.9999	1.00855
1.644518	34	12	165	0.9997	1	0.9709
1.694352	35	12	170	0.9997	1	0.99405
1.744186	36	12	175	0.9997	1	0.9951
1.79402	37	12	180	0.9997	1	0.9769
1.843854	38	12	185	0.9997	1	1.00375
1.893688	39	12	190	1.0005	1	0.95965
1.943522	40	12	195	1.0005	1	0.9816
1.993355	41	12	200	1.0005	1	1.01455
2.043189	42	12	205	1.0005	1	0.96955

Pore volume	No	Distance	Time (min)	Tracer C/Co	Fitted	2,4-DCP C/Co
2.093023	43	12	210	1.0005	1	0.9895
2.142857	44	12	215	1.0005	1	1.014
2.192691	45	12	220	1.0005	1	1.00395
2.242525	46	12	225	1.0005	1	0.97895
2.292359	47	12	230	1.0005	1	0.9684
2.342193	48	12	235	1.0005	1	0.99255
2.392027	49	12	240	1.0005	1	0.96915
2.44186	50	12	245	1.0005	0.9997	0.9724
2.491694	51	12	250	1.0005	0.9978	0.9759
2.541528	52	12	255	1.0005	0.9888	0.99435
2.591362	53	12	260	0.9906	0.9608	0.9733
2.641196	54	12	265	0.9385	0.8981	0.97645
2.69103	55	12	270	0.8457	0.7913	0.9387
2.740864	56	12	275	0.7561	0.6468	0.76165
2.790698	57	12	280	0.5867	0.4859	0.57815
2.840532	58	12	285	0.4592	0.3347	0.31135
2.890365	59	12	290	0.3365	0.2117	0.1826
2.940199	60	12	295	0.2321	0.1234	0.08275
2.990033	61	12	300	0.1329	0.0667	0.0661
3.039867	62	12	305	0.0969	0.0336	0.0315
3.089701	63	12	310	0.0615	0.0159	0
3.139535	64	12	315	0.0447	0.0071	0
3.189369	65	12	320	0.0371	0.003	0
3.239203	66	12	325	0.0313	0.0012	0
3.289037	67	12	330	0.0306	0.0005	0
3.33887	68	12	335	0.0153	0.0002	0
3.388704	69	12	340	0	0.0001	0
3.438538	70	12	345	0	0	0
3.488372	71	12	350	0	0	0
3.538206	72	12	355	0	0	0
3.58804	73	12	360	0	0	0
3.637874	74	12	365	0	0	0

Table C.2. Data for Figures 5.3 and 5.4.

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
0	180	1.79402	0	0	0	0
1	185	1.843854	1.5	1.497196	0	0.002804
2	190	1.893688	3	2.997196	0	0.002804
3	195	1.943522	4.5	4.48315	0	0.01685
4	200	1.993355	6	5.982028	0	0.017972
5	205	2.043189	7.5	7.485112	0	0.014888
6	210	2.093023	9	8.988617	0	0.011383
7	215	2.142857	10.5	10.48949	0	0.010514
8	220	2.192691	12	11.9954	0	0.004598
9	225	2.242525	13.5	13.49389	0	0.006112
10	230	2.292359	15	15.00564	0	-0.00564
11	235	2.342193	16.5	16.49467	0	0.005327
12	240	2.392027	18	17.9945	0	0.005495
13	245	2.44186	19.5	19.48393	0	0.016065
14	250	2.491694	21	20.96383	0	0.036168
15	255	2.541528	22.5	22.43243	0	0.06757
16	260	2.591362	24	23.89489	0	0.105112
17	265	2.641196	25.5	25.34748	0	0.152523
18	270	2.69103	27	26.65929	0	0.34071
19	275	2.740864	28.5	27.76808	0	0.731916
20	280	2.790698	30	28.66483	0	1.335168
21	290	2.890365	33	30.14795	0	2.852047
22	300	2.990033	36	31.36904	0	4.630963
23	310	3.089701	39	32.48263	0	6.517374
24	320	3.189369	42	33.48014	0	8.51986
25	330	3.289037	45	34.24068	0	10.75932
26	340	3.388704	48	34.88128	0	13.11872
27	350	3.488372	51	35.6458	0	15.3542
28	360	3.58804	54	36.37977	0	17.62023
29	370	3.687708	57	36.89442	0.144286	19.96129
30	380	3.787375	60	37.4255	0.292598	22.2819
31	390	3.887043	63	37.9235	0.360383	24.71611
32	400	3.986711	66	38.48593	0.427779	27.08629
33	410	4.086379	69	39.04377	0.574765	29.38147
34	420	4.186047	72	39.44711	0.974473	31.57841
35	430	4.285714	75	39.95549	1.385643	33.65887
36	440	4.385382	78	40.44407	1.649959	35.90598
37	450	4.48505	81	40.94397	1.910817	38.14521
38	460	4.584718	84	41.40597	2.387717	40.20631
39	470	4.684385	87	41.86848	2.831901	42.29962
40	480	4.784053	90	42.2213	3.296579	44.48212

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
41	490	4.883721	93	42.65801	3.782505	46.55949
42	500	4.983389	96	43.02536	4.162708	48.81194
43	510	5.083056	99	43.39567	4.646666	50.95766
44	520	5.182724	102	43.73577	5.078471	53.18576
45	530	5.282392	105	44.04608	5.506445	55.44747
46	540	5.38206	108	44.25821	5.804271	57.93751
47	550	5.481728	111	44.56511	6.264787	60.1701
48	560	5.581395	114	44.86691	6.720293	62.4128
49	570	5.681063	117	45.17767	7.328917	64.49341
50	580	5.780731	120	45.48446	7.810217	66.70532
51	590	5.880399	123	45.7853	8.404318	68.81038
52	600	5.980066	126	46.06775	8.918518	71.01373
53	610	6.079734	129	46.34229	9.333782	73.32393
54	620	6.179402	132	46.59345	9.852482	75.55407
55	630	6.27907	135	46.82588	10.45957	77.71456
56	640	6.378738	138	47.05679	10.96036	79.98284
57	650	6.478405	141	47.29842	11.43289	82.26869
58	660	6.578073	144	47.38421	11.87539	84.74039
59	670	6.677741	147	47.47729	12.34079	87.18193
60	680	6.777409	150	47.56813	12.83516	89.59671
61	690	6.877076	153	47.73237	13.38028	91.88735
62	700	6.976744	156	47.99188	13.94772	94.06039
63	710	7.076412	159	48.24461	14.56758	96.18781
64	720	7.17608	162	48.46145	15.16497	98.37357
65	730	7.275748	165	48.7153	15.71036	100.5743
66	740	7.375415	168	48.94807	16.22479	102.8271
67	750	7.475083	171	49.15039	16.89285	104.9568
68	760	7.574751	174	49.35375	17.52058	107.1257
69	770	7.674419	177	49.55957	18.20937	109.2311
70	780	7.774086	180	49.77408	18.84923	111.3767
71	790	7.873754	183	49.99848	19.28443	113.7171
72	800	7.973422	186	50.21175	19.77693	116.0113
73	810	8.07309	189	50.50413	20.3124	118.1835
74	820	8.172757	192	50.78484	21.45419	119.761
75	830	8.272425	195	51.0464	22.11371	121.8399
76	840	8.372093	198	51.25956	22.89052	123.8499
77	850	8.471761	201	51.45588	23.66644	125.8777
78	860	8.571429	204	51.66488	24.48159	127.8535
79	870	8.671096	207	51.88195	25.2349	129.8832
80	880	8.770764	210	52.10044	25.93702	131.9625
81	890	8.870432	213	52.31309	26.6359	134.051
82	900	8.9701	216	52.54361	27.32426	136.1321
83	910	9.069767	219	52.79433	28.08562	138.12

Sample No.	Time (min)	Pore vol.	phenol in	phenol out	Soluble polymer out	polymer in column
84	920	9.169435	222	53.05581	28.68184	140.2623
85	930	9.269103	225	53.32591	29.35491	142.3192
86	940	9.368771	228	53.59056	30.00706	144.4024
87	950	9.468439	231	53.8518	30.66592	146.4823
88	960	9.568106	234	54.11986	31.31419	148.566
89	970	9.667774	237	54.38228	31.96832	150.6494
90	980	9.767442	240	54.63018	32.58378	152.786
91	990	9.86711	243	54.80841	33.19766	154.9939
92	1000	9.966777	246	54.98044	33.71161	157.308
93	1010	10.06645	249	55.25305	34.14966	159.5973
94	1020	10.16611	252	55.49637	34.54225	161.9614
95	1030	10.26578	255	55.64963	34.93374	164.4166
96	1040	10.36545	258	55.79377	35.31707	166.8892
97	1050	10.46512	261	55.94061	35.68962	169.3698
98	1060	10.56478	264	56.08575	36.01059	171.9037
99	1070	10.66445	267	56.23104	36.34196	174.427
100	1080	10.76412	270	56.38227	36.77723	176.8405
101	1090	10.86379	273	56.51919	37.31047	179.1703
102	1100	10.96346	276	56.65909	37.72523	181.6157
103	1110	11.06312	279	56.76508	38.22151	184.0134
104	1120	11.16279	282	56.86181	38.7333	186.4049
105	1130	11.26246	285	56.95946	39.22205	188.8185
106	1140	11.36213	288	57.0529	39.65983	191.2873
107	1150	11.46179	291	57.14237	40.0819	193.7757
108	1160	11.56146	294	57.22444	40.52226	196.2533
109	1170	11.66113	297	57.31335	40.88435	198.8023
110	1180	11.7608	300	57.4104	41.28019	201.3094
111	1190	11.86047	303	57.50993	41.74068	203.7494
112	1200	11.96013	306	57.61828	42.26078	206.1209
113	1210	12.0598	309	57.73459	42.83192	208.4335
114	1220	12.15947	312	57.84064	43.50548	210.6539
115	1230	12.25914	315	57.93441	44.1172	212.9484
116	1240	12.3588	318	58.01187	44.71618	215.272
117	1250	12.45847	321	58.08076	45.33734	217.5819
118	1260	12.55814	324	58.14382	45.99329	219.8629
119	1270	12.65781	327	58.20292	46.64923	222.1479
120	1280	12.75748	330	58.26455	47.28144	224.454
121	1290	12.85714	333	58.34359	47.81686	226.8396
122	1300	12.95681	336	58.43232	48.30655	229.2611
123	1310	13.05648	339	58.53701	48.7795	231.6835

Table C.3. Data for Figure 5.5.

Sample No.	pore volume (mL)	Distance (cm)	Time (min)	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
1	0	12	0	0	0	0	0
2	0.05	12	5	0	0	0	0
3	0.1	12	10	0	0	0	0
4	0.15	12	15	0	0	0	0
5	0.2	12	20	0	0	0	0
6	0.25	12	25	0	0	0	0
7	0.3	12	30	0	0	0	0
8	0.35	12	35	0	0	0	0
9	0.4	12	40	0	0	0	0
10	0.45	12	45	0	0	0	0
11	0.5	12	50	0	0	0	0
12	0.55	12	55	0	0	0	0
13	0.6	12	60	0	0	0	0
14	0.65	12	65	0	0	0.02	0
15	0.7	12	70	0.02	0	0.03	0.01
16	0.75	12	75	0.02	0.01	0.06	0.04
17	0.8	12	80	0.03	0.04	0.14	0.12
18	0.85	12	85	0.07	0.1	0.29	0.26
19	0.9	12	90	0.17	0.21	0.5	0.43
20	0.95	12	95	0.28	0.35	0.75	0.62
21	1	12	100	0.42	0.51	0.85	0.77
22	1.05	12	105	0.65	0.67	0.95	0.88
23	1.1	12	110	0.8	0.79	0.97	0.94
24	1.15	12	115	0.87	0.88	0.99	0.97
25	1.2	12	120	0.93	0.93	1	0.99
26	1.25	12	125	0.96	0.97	1	1
27	1.3	12	130	0.98	0.98	1	1
28	1.35	12	135	0.99	0.99	1	1
29	1.4	12	140	0.99	1	1	1
30	1.45	12	145	1	1	1	1
31	1.5	12	150	1	1	1	1
32	1.54	12	155	1	1	1	1
33	1.59	12	160	1	1	1	1
34	1.64	12	165	1	1	1	1
35	1.69	12	170	1	1	1	1
36	1.74	12	175	1	1	1	1
37	1.79	12	180	1	1	1	1
38	1.84	12	185	1	1	1	1
39	1.89	12	190	1	1	1	1
40	1.94	12	195	1	1	1	1
41	1.99	12	200	1	1	1	1

Sample No.	pore volume (mL)	Distance (cm)	Time (min)	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
42	2.04	12	205	1	1	1	1
43	2.09	12	210	1	1	1	1
44	2.14	12	215	1	1	1	1
45	2.19	12	220	1	1	1	1
46	2.24	12	225	1	1	1	1
47	2.29	12	230	1	1	1	1
48	2.34	12	235	1	1	1	1
49	2.39	12	240	1	1	1	1
50	2.44	12	245	1	1	1	1
51	2.49	12	250	1	1	1	0.99
52	2.54	12	255	1	0.99	0.98	0.96
53	2.59	12	260	0.99	0.96	0.93	0.88
54	2.64	12	265	0.94	0.9	0.8	0.74
55	2.69	12	270	0.85	0.79	0.62	0.57
56	2.74	12	275	0.76	0.65	0.46	0.38
57	2.79	12	280	0.59	0.49	0.31	0.23
58	2.84	12	285	0.46	0.33	0.2	0.12
59	2.89	12	290	0.34	0.21	0.13	0.06
60	2.94	12	295	0.23	0.12	0.08	0.03
61	2.99	12	300	0.13	0.07	0.04	0.01
62	3.04	12	305	0.1	0.03	0.03	0
63	3.09	12	310	0.06	0.02	0.03	0
64	3.14	12	315	0.04	0.01	0.02	0
65	3.19	12	320	0.04	0	0	0
66	3.24	12	325	0.03	0	0	0
67	3.29	12	330	0.03	0	0	0
68	3.34	12	335	0.02	0	0	0
69	3.39	12	340	0	0	0	0
70	3.44	12	345	0	0	0	0
71	3.49	12	350	0	0	0	0
72	3.54	12	355	0	0	0	0
73	3.59	12	360	0	0	0	0
74	3.64	12	365	0	0	0	0

Table C.4. Data for Figure 5.7.

Sample No.	pore volume (mL)	Distance (cm)	Time (min)	TA C/Co	MTA Fitted	2,4- DCP C/Co	MADCP Fitted
1	0	12	0	0	0	0	0
2	0.049834	12	5	0	0	0	0
3	0.099668	12	10	0	0	0	0
4	0.149502	12	15	0	0	0	0
5	0.199336	12	20	0	0	0	0
6	0.249169	12	25	0	0	0	0
7	0.299003	12	30	0	0	0	0
8	0.348837	12	35	0	0	0	0
9	0.398671	12	40	0	0	0	0
10	0.448505	12	45	0	0	0	0
11	0.498339	12	50	0	0	0	0
12	0.548173	12	55	0	0	0	0
13	0.598007	12	60	0	0.0001	0	0
14	0.647841	12	65	0.0231	0.0015	0	0.0002
15	0.697674	12	70	0.0313	0.0098	0	0.0011
16	0.747508	12	75	0.06	0.0414	0	0.0041
17	0.797342	12	80	0.135	0.1193	0	0.0121
18	0.847176	12	85	0.292	0.2557	0	0.029
19	0.89701	12	90	0.5041	0.4343	0.0333	0.0597
20	0.946844	12	95	0.7484	0.6176	0.1033	0.1078
21	0.996678	12	100	0.8498	0.7704	0.2711	0.1744
22	1.046512	12	105	0.9508	0.8769	0.3766	0.2575
23	1.096346	12	110	0.9676	0.9407	0.4455	0.3524
24	1.146179	12	115	0.9894	0.9741	0.5241	0.4527
25	1.196013	12	120	0.9957	0.9896	0.5781	0.5517
26	1.245847	12	125	0.9993	0.9962	0.6819	0.6437
27	1.295681	12	130	1	0.9987	0.7744	0.725
28	1.345515	12	135	1	0.9996	0.7863	0.7935
29	1.395349	12	140	1	0.9999	0.8143	0.8489
30	1.445183	12	145	1	1	0.8465	0.892
31	1.495017	12	150	1	1	0.8543	0.9246
32	1.54485	12	155	1	1	0.8787	0.9484
33	1.594684	12	160	1	1	0.9094	0.9654
34	1.644518	12	165	1	1	0.8999	0.9772
35	1.694352	12	170	1	1	0.9254	0.9852
36	1.744186	12	175	1	1	0.9301	0.9906
37	1.79402	12	180	1	1	0.9335	0.9941
38	1.843854	12	185	1	1	0.9796	0.9963
39	1.893688	12	190	1	1	1.0061	0.9977
40	1.943522	12	195	1	1	0.944	0.9986
41	1.993355	12	200	1	1	0.9675	0.9992

Sample No.	pore volume (mL)	Distance (cm)	Time (min)	BT C/Co	MBT Fitted	AT C/Co	MAT Fitted
42	2.043189	12	205	1	1	0.9515	0.9995
43	2.093023	12	210	1	1	0.9579	0.9997
44	2.142857	12	215	1	1	0.9885	0.9998
45	2.192691	12	220	1	1	0.9559	0.9999
46	2.242525	12	225	1	1	0.9618	0.9999
47	2.292359	12	230	1	1	0.963	1
48	2.342193	12	235	1	1	0.9685	1
49	2.392027	12	240	1	0.9999	0.9924	1
50	2.44186	12	245	1	0.9985	0.9777	0.9998
51	2.491694	12	250	0.997	0.9902	0.9891	0.9989
52	2.541528	12	255	0.9809	0.9586	0.9807	0.9959
53	2.591362	12	260	0.934	0.8807	0.995	0.9879
54	2.641196	12	265	0.7989	0.7443	0.996	0.971
55	2.69103	12	270	0.624	0.5657	1.0154	0.9403
56	2.740864	12	275	0.4622	0.3824	1.0364	0.8922
57	2.790698	12	280	0.3136	0.2296	0.9692	0.8256
58	2.840532	12	285	0.201	0.1231	0.8826	0.7425
59	2.890365	12	290	0.1309	0.0593	0.7243	0.6476
60	2.940199	12	295	0.0753	0.0259	0.5512	0.5473
61	2.990033	12	300	0.0429	0.0104	0.4145	0.4483
62	3.039867	12	305	0.033	0.0038	0.2947	0.3563
63	3.089701	12	310	0.0281	0.0013	0.2379	0.275
64	3.139535	12	315	0.0248	0.0004	0.1657	0.2065
65	3.189369	12	320	0	0.0001	0.1486	0.1511
66	3.239203	12	325	0	0	0.1398	0.108
67	3.289037	12	330	0	0	0.0809	0.0754
68	3.33887	12	335	0	0	0.091	0.0516
69	3.388704	12	340	0	0	0.0526	0.0346
70	3.438538	12	345	0	0	0.1005	0.0228
71	3.488372	12	350	0	0	0.0719	0.0148
72	3.538206	12	355	0	0	0.061	0.0094
73	3.58804	12	360	0	0	0.0403	0.0059
74	3.637874	12	365	0	0	0.053	0.0037

Appendix D - Pictures of chemiluminescence and polymerization

Figure D.1. Chemiluminescence reaction with various enzyme doses.

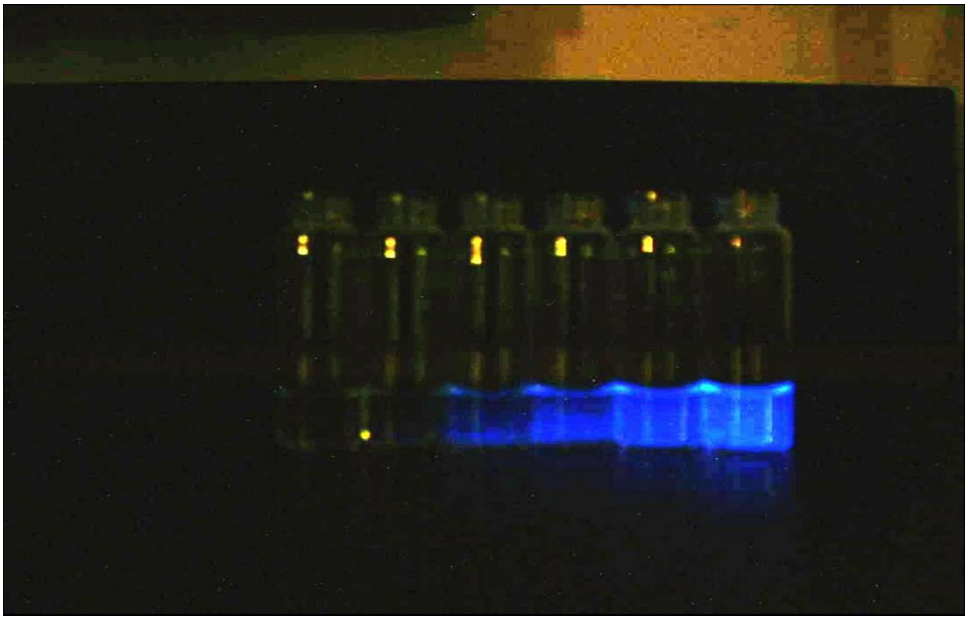

Figure D.2. Column experiment for HRP transport.

Figure D.3. Batch reaction of polymerization.

Figure D.4. Polymerization (a) and coagulation (b) using HRP-mediated oxidative reaction.

(a) (b)

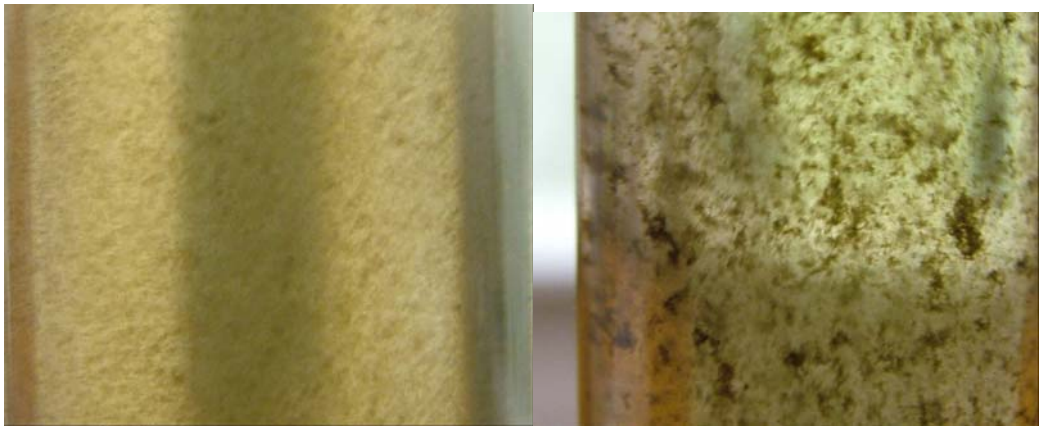

Figure D.5. Polymer precipitates (a) and air-dried polymers (b).

(a) (b)

Figure D.6. Polymer deposition (a) and coating (b) on porous media.

(a) (b)