Degradation of Phenolic Compounds Using Laccase or Peroxidase Enzymatic Treatment

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 9, 2010

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Abstract

To remove ammonia from wastewater most refineries use biological treatment employing suspended biomass because there is no secondary pollutant. The main purpose of biological treatment is to accelerate the oxidation process of the organic matter (nitrification), which happens naturally within the receiving waters. This accelerated treatment is usually accomplished by using the activated sludge process. Although this method of treatment may be the preferred way to remove ammonia due to its simplicity and economic benefits, this process does have some disadvantages. One of the major problems associated with treating refinery wastewater is the potential for inhibition of the nitrifying bacteria due to several phenolic compounds found in the wastewater.

The process of removing these phenolic compounds by using peroxidase and laccase has been researched by several researchers. This research has shown that these enzymes can react with aqueous phenolic compounds to create non-soluble compounds that can simply be removed from the aqueous phase using any conventional removal method.

It has been hypothesized that the addition of hydrogen peroxide and either laccase or peroxidase enzymes can reduce the concentration of selected phenolic compounds in refinery wastewater. This enzymatic approach had been researched before; however, the majority of the previous research was done using a synthetic

wastewater matrix, either de-ionized water or a buffer solution. Therefore, the main objective of this thesis was to determine the efficiency of the laccase and peroxidase enzymes at reducing the amount of phenols in an actual refinery wastewater matrix.

To accomplish the objective of this thesis, this study was conducted in three phases. The first phase involved the enzymatic treatment of phenolic compounds in a simple matrix (de-ionized water) to determine both the efficiency of the enzymes and also the effect of hydrogen peroxide on the enzymes. The second phase involved the enzymatic treatment of various refinery wastewaters (CITGO, Frontier, and Imperial Oil). Lastly, the third phase investigated the effect of enzymatic treatment on nitrification.

Phase one considered the efficiency of laccase and peroxidase treatment in both a simple matrix with only one individual compound present and a simple matrix with a defined mixture of phenolic compounds present. The testing of the individual compounds showed that the peroxidase/peroxide treatment had a slightly higher rate of removing the compounds from the simple matrix than the laccase/peroxide treatment (refer to Table 4.1). However, the overall percent remaining for all compounds were similar for both treatment options. The phenol and 2,4-dmp were reduced to about 25 to 30 percent remaining after one hour, whereas o-cresol and p-cresol were reduced to a remaining percent of about 45 to 50 percent (see Figures 4.5 and 4.6)

In the simple matrix with defined concentrations of phenolic compounds it was determined that the peroxidase/peroxide treatment had a faster rate of removal and was more efficient. Phenol and 2,4-dmp had higher reduction rates than the o-

cresol and p-cresol. Also noticed in this part of phase one is that the rates for removing the phenolic compounds in the defined mixture were lower than the rate for removing the compounds individually.

In Phase Two the results for each individual wastewater differed slightly from each other. One important observation is that the rates for reducing the target phenols were generally lower than the rates determined in Phase One. Another difference is with most of the wastewater samples, the majority of the reduction did not occur until the last 15 to 30 minutes of the reaction; whereas, the reaction occurred in the first 15 to 30 minutes in Phase One with the clean system. Table 4.12 shows the average first order reduction rate constants for all compounds in the defined mixture.

To confirm the finding in Phases One and Two, Phase Three testing was conducted using a commercially available culture of Nitrosomonas and Nitrobacter known as Nitrotox. Dilutions of treated wastewater were made over the range expected in the wastewater treatment process. The rate of nitrification in these dilutions was measured as the reduction in ammonia over time. Compared to the control (no enzymatic treatment), the ammonia reduction in the peroxidase/peroxide and laccase/peroxide treated samples was significantly higher.

The results of this research increase the overall understanding of this enzymatic approach to removing phenol from refinery wastewater. The reduction of phenolic compounds by laccase and peroxidase in synthetic and refinery wastewater are explored throughout this investigation.

Acknowledgments

First and foremost, I offer my sincerest gratitude to my advisor, Dr. Clifford R. Lange, who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my Masters degree to his encouragement and effort and without him this thesis, too, would not have been completed or written. One simply could not wish for a better or friendlier advisor.

Additionally, I would like to express my appreciation to CITGO, Frontier, and Imperial Oil for providing refinery wastewater samples for the purposes of research and analysis.

Furthermore, I offer my regards and blessings to my amazing wife Emily for all her support and motivation throughout the course of completing this challenging educational endeavor.

Lastly, I would like to thank my parents, other family and friends for supporting me throughout all my studies at and prior to Auburn University.

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Chapter 1

Introduction

There are approximately 140 operating oil refineries in the United States and many more world-wide. An oil refinery must fractionate crude oil into useable petroleum products such as diesel and gasoline. To separate these fractions, the crude oil must go through one or both of two main processes used at a refinery, separation and conversion (Hengstebeck 1959). Unfortunately, these processes form several aqueous waste products. Sour water is any wastewater that contains hydrogen sulfide, and is the main wastewater from oil refineries (Beychok 1967). In addition to hydrogen sulfide, most sour water also contains high concentrations of phenol, substituted phenolic compounds, ammonia, and cyanide (Meyers 1997). Wastewater containing high concentrations of nitrite, ammonia, and ammines is a major problem at refineries all over the world. Nitrate is considered a carcinogen and can also have harmful effects on human health, such as methylmyoglobia also known as the blue-baby syndrome (Wang et al. 2007). Complete removal of ammonia nitrogen from the wastewater is required due to its extreme toxicity to most fish species and other aquatic life, the high oxygen demand associated with ammonia, and the stimulation of aquatic plants and algae (Jorgensen and Weatherley 2003).

Nitrification is the most common way to remove ammonia from wastewater. Nitrification is the microbial oxidation of ammonia into nitrites (NO_2^-), and then into nitrate (NO_3^-). With this method, organic nitrogen and ammonia nitrogen is converted into nitrite and nitrate nitrogen in an aerobic environment, subsequently may be released into the atmosphere as nitrogen gas after anaerobic denitrification (Sintobrator 2002). To remove ammonia from wastewater most refineries use biological treatment employing suspended biomass because there is no secondary pollutant. The main purpose of biological treatment is to accelerate the oxidation process of the organic matter, which happens naturally within the receiving waters. This accelerated treatment is usually accomplished by using the activated sludge process (Ben-Youssef et al. 2009). Activated sludge was first recognized as a possible solution for wastewater treatment in 1914. Edward Arden and William T. Lockett noticed that bacterial binding material forms naturally during prolonged aeration of urban wastewaters. This allowed the "activated solids" to be recycled and process efficiency to be greatly increased. Thus, the activated sludge process was born. Some of the organic matter is changed into biomass (sludge) and the rest is mineralized to carbon dioxide and water. The bacterial binding material can then be separated out from the treated water using simple settling techniques. In addition to removing organics, the activated sludge process has been shown to oxidize ammonia into nitrate. This process is referred to as nitrification and occurs concurrently with organic carbon oxidation (Ben-Youssef et al. 2009).

Although this method of treatment may be the preferred way to remove ammonia due to its simplicity and economic benefits, this process does have some

disadvantages. One disadvantage is the susceptibility of the nitrifying bacteria to several environmental factors such as pH, temperature, alkalinity, and oxygen concentration (Liu et al. 2005). One of the major problems associated with treating refinery wastewater is the potential for inhibition of the nitrifying bacteria due to several phenolic compounds found in the wastewater. Kim et al. (2008) concluded that phenol and p-cresol when above 200 and 100 mg/L respectively could noticeably inhibit nitrification. Thus, the removal of phenols from refinery wastewater is a practical problem of great significance (Singh and Singh 2002).

There are several methods that can be used to remove phenol from wastewater, including; activated carbon, microbial degradation, incineration methods, chemical oxidation, solvent extraction, and irradiation (Miland et al. 1996; Tong et al. 1997). Even though these techniques can be used to remove phenol from the wastewater, high cost, long times, and inability to avoid the formation of and possible release of hazardous by products top the list of disadvantages (Lante et al. 2000). However, bioremediation strategies look to be an environmental-friendly alternative to thermal or chemical treatment of phenolic waste (Moeder et al. 2004).

An enzymatic approach to removing phenols from the wastewater using laccase and peroxidase enzymes was proposed as early as 1980 (Klibanov and Alberti 1981). The process of removing phenols by using peroxidase and laccase has been researched by several researchers. This research has shown that these enzymes can react with aqueous phenolic compounds to create non-soluble compounds that can simply be removed from the aqueous phase using any conventional removal method (Cooper and Nicell 1996).

It has been hypothesized that the addition of hydrogen peroxide and either laccase or peroxidase enzymes can reduce the concentration of selected phenolic compounds in refinery wastewater. This enzymatic approach had been researched before; however, the majority of the previous research was done using a synthetic wastewater matrix, either de-ionized water or a buffer solution. Therefore, the main objective of this thesis was to determine the efficiency of the laccase and peroxidase enzymes at reducing the amount of phenols in an actual refinery wastewater matrix.

To accomplish the objective of this thesis, several representative petroleum refinery wastewaters were obtained. The petroleum refineries that served as the source of test samples included two refineries from the United States (CITGO and Frontier), and three Canadian refineries (Imperial Oil). These five refineries were chosen because they represent the different types of crude being processed here in North America. The US refineries mostly process Texas "sweet crude" although they sometimes process Canadian or Venezuelan tar sand synthetic crude. The three Canadian refineries process a mixture of "sweet crude" and Canadian tar sand synthetic crude. "Sweet crude" is a type of crude that is not very complex and often easy to process, where as tar sand synthetic crude comes from adding several water soluble compounds such as ethylene glycol or propylene glycol to the tar sand mixture to produce the synthetic crude. Samples were taken at various points throughout wastewater treatment including: desalter, sour water, coker, DAF effluent, biox influent, and biox effluent. These locations were selected because they encompass the sources of refinery wastewater.

The hypothesis of this thesis is that the addition of hydrogen peroxide, and either peroxidase or laccase enzymes, can lower the concentration of phenolic compounds in actual refinery wastewater matrices. This hypothesis is based on the experimental data and anecdotal information given by previous research. However, one the significant limitations of these laboratory studies to refinery wastewater is the effect which the complex array of organic and inorganic contaminants in actual refinery wastewater have upon the hydrogen peroxide/ enzyme function. In this study the five refineries were chosen so that the wastewaters would vary in both phenolic compounds and the aqueous matrix, and represent a cross-section of refinery wastewater.

A three phase approach was utilized to determine the efficiency of peroxidase and laccase. In phase one of this study, the enzymatic approach to reducing phenols was tested in de-ionized water. Hydrogen peroxide and an enzyme were added to a synthetic wastewater, and then tested every fifteen minutes on the gas chromatograph. Also tested in this phase of the study was the effect of hydrogen peroxide on the enzymes. To determine if hydrogen peroxide affected the enzyme's ability to lower phenol concentrations, different concentrations of hydrogen peroxide were added to synthetic wastewater along with an enzyme, and then the sample was tested on the gas chromatograph over time.

In the second phase of this research, actual refinery wastewater was used to determine peroxidase and laccase enzyme's ability to reduce the concentration of certain phenolic compounds found in the wastewater. An enzyme and hydrogen

peroxide were added to the refinery wastewater samples, and then tested on the gas chromatograph over time.

The third phase of the study considered the effect of the enzymatic treatment on nitrification. Treated refinery wastewater was compared against untreated wastewater to determine the amount of ammonia oxidized. Since phenol inhibits the nitrifying bacteria, the untreated wastewater should have a lower amount of ammonia being oxidized.

The results of this research increase the overall understanding of this enzymatic approach to removing phenol from refinery wastewater. The reduction of phenolic compounds by laccase and peroxidase in synthetic and refinery wastewater are explored throughout this investigation and the results are presented herein. Therefore, by uniting the knowledge obtained from past research, summarized in the following chapter (Chapter 2), and that acquired by conducting this thesis (Chapters 3 and 4), perceptive familiarity with this enzymatic treatment approach should ensue, and hopefully will spark interest in connected research topics yet to be explored.

Chapter 2

Literature Review

General Info

Crude oil can be described not as a chemical compound, but a mixture of many different chemical compounds. Some as simple as methane [CH_4]; others are very complex hydrocarbons. Most significantly though is when it is heated to a boiling point and held there, unlike water it will not all evaporate (Leffler 1979). According to the U.S. Energy Information Association (2009), crude oil can be used in many ways, the world uses petroleum products to help move people and merchandise by using gasoline and diesel in engines, and to help make plastics, and to even make things like crayons, CD's, ink, and bubble gum (Gary and Handwerk 1984).

In order to make a wide range of consumer products, different parts of the crude oil are separated into useable petroleum feed stocks at an oil refinery. There are around 165 operating oil refineries in North America. Today, most refineries process over half of every 42-gallon barrel of crude oil into gasoline (U.S. 2008). Since the properties of crude oils are so diverse and the number of products so abundant the name "petroleum refinery" gives little indication of what operations or equipment is used at a refinery. Most petroleum refineries have two main processes; separation

including distillation and conversion including cracking and reforming (Hengstebeck 1959).

In separation or distillation, the difference in heavy and light petroleum components or fractions allows the various chemical components to separate out. Modern separation involves sending the petroleum through a hot furnace (Waddams 1980). Then the resulting vapors and liquids are discharged into distillation towers. Once in these towers the vapors and liquids separate according to their boiling points. Figure 2.1 shows the different components of petroleum separating out of a distillation tower. The lightest components such as gasoline and liquid petroleum gas (LPG) vaporize and rise to the very top of the distillation tower where they then condense back to liquids. Kerosene, diesel oil distillates, naphtha and other medium weight liquids stay in the middle of the tower. Heavier liquids with the highest boiling points such as gas oils settle out at the bottom (Gary and Handwerk 1984).

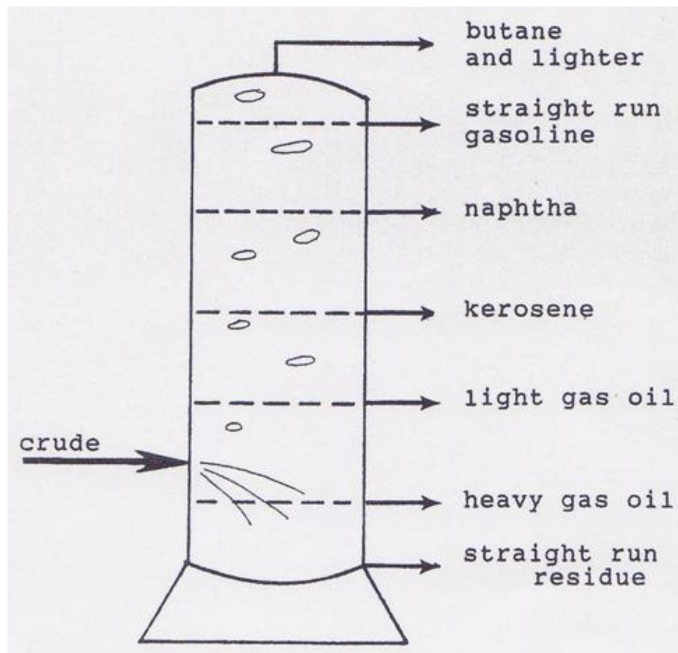

Figure 2.1: Distillation Tower (Huiyi)

The most commonly used conversion method is cracking, because it uses heat and pressure to "crack" complex and heavy hydrocarbon molecules into lighter ones. Cracking has been a part of the refinery process for a long time. Catalytic cracking, since its first successful application in 1936, has become the largest of all catalytic operations (Hengstebeck 1959). This cracking and rearranging takes the output from an earlier process, the heavy, low-valued feedstock, and changes it to something such as gasoline, a higher valued output. This is where output components from the distillation towers are transformed into intermediate components that ultimately are changed into the end products (Gary and Handwerk 1984).

Cracking is not the only form of conversion used in refining. Some processes rearrange the molecules to add value instead of splitting them. Take alkylation for example, which hit the industrial scene in 1955 (Hengstebeck 1959). In the alkylation process, which is basically cracking in reverse, several gaseous byproducts are combined to form different gasoline components (Meyers 1997).

Catalytic reforming is another form of conversion. Introduced in the 1950's, reforming was pioneered to help improve the octane number of gasoline. This process uses pressure, catalysts, and heat to convert poly aromatic hydrocarbons or naphtha (a low-value component of distillation) into high-octane liquid products called reformates. These reformates are components of gasoline (Leffler 1979).

Wastewater Characteristics

Sour condensate or sour water is the main waste water source by volume from petroleum plants. Sour water is defined as any water that has hydrogen sulfide in it

(CASTion 2008). The sour water from a refinery usually comes from using steam as a stripping medium in distillation and specifically the distillation reflux drum. Some refineries also inject wash water or steam to absorb corrosive compounds or control pH (Beychok 1967). Thus any steam or wash water that comes in contact with hydrogen sulfide is considered sour water (Meyers 1997). Furthermore, there is most always free NH_3 in the water thus causing the water to contain mostly NH_4SH rather than free H_2S or free NH_3. There are several points in the refining process that produce sour water; crude oil distillation, hydrotreating, catalytic reforming, catalytic cracking, thermal cracking, and hydrocracking (Beychok 1967). In addition to hydrogen sulfide and ammonia, sour water can also have phenol, substituted phenolic compounds, and cyanide (Meyers 1997). Traditionally, the sour water is treated to eliminate the hydrogen sulfide before being released. However, due to current regulations reducing nitrogen in estuaries, the removal of ammonia is now essential (CASTion 2008). The ammonia in refinery sour water comes from either the nitrogen in the crude oil or form when ammonia and/or amines are added into the crude fractionator in order to cut down on sulfide catalyzed corrosion (Meyers 1997).

Ammonia Removal

The pollution of ground water with nitrate is a severe problem in many places all over the world. Nitrate is considered a carcinogen and can also have harmful effects on human health, such as methemoglobinemia (blue-baby syndrome) (Wang et al. 2007). Complete removal of ammonia is required due to these harmful effects as well as extreme aquatic toxicity, and the high oxygen demand. (Jorgensen and Weatherley 2003). There are various methods of removing nitrogen, each with

advantages and disadvantages. Although, nitrification, the biological treatment method, is the most commonly used at oil refineries, other methods are also employed, including: sour water stripping and ion exchange. These processes available for ammonia removal, and their advantages and disadvantages, will be discussed in the following sections.

Nitrification

The most common way to remove ammonia from refinery wastewater is through the microbial oxidation of ammonia into nitrite, and then into nitrate, which is referred to as nitrification. In nitrification, organic nitrogen and ammonia nitrogen is converted into nitrite and nitrate nitrogen in an aerobic environment, and is disposed of into the atmosphere as nitrogen gas following anaerobic denitrification (Sintobrator 2002). Russian microbiologist Sergei Winogradsky discovered that by isolating certain soil bacteria he could clearly show the process of nitrification. Thus, he concluded that this process was the result was due to bacterial action. In the first step of nitrification, ammonia-oxidizing bacteria oxidize ammonia to nitrite according this equation: $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$ (Watson et al. 1981). Of all the bacteria that can perform this step, *Nitrosomonas* is the most commonly known genus associated with this process, and is also usually used in activated sludge (Fang et al. 1993). In the second step of nitrification, nitrite oxidizing bacteria oxidize nitrite to nitrate according to this equation: $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$ (Watson et al. 1981). *Nitrobacter* is the main genus isolated from nitrifying activated sludge (Fang et al. 1993).

Biological treatment, using suspended biomass, is currently the most common treatment solution used to treat wastewaters. The main purpose is to accelerate the oxidation process of the organic matter, which happens naturally within the receiving waters. This accelerated treatment is usually accomplished by using the activated sludge technique (Ben-Youssef et al. 2009). Activated sludge was first recognized as a possible solution in 1914. Edward Arden and William T. Lockett noticed that bacterial binding material forms naturally during prolonged aeration of urban wastewaters. Thus the activated sludge process was born. Some of the organic matter is changed into biomass and the rest is mineralized to carbon dioxide and water. The bacterial binding material can then be separated out from the treated water using simple settling techniques. Originally, both aeration and sedimentation phases were done in the same tank or container. This process is currently referred to as sequencing batch reactor (SBR) technology. Presently, the sedimentation phase is preformed in a second tank to allow for a continuous operating process. Today the activated sludge process consist of two different tanks: an aeration tank with suitable biomass for settling and a settling tank where the purified water is separated from the activated sludge through settling.

Although this method of treatment may be preferred in some places due to its simplicity and economic benefits, this process does have its disadvantages. Nitrifying bacteria such as ones listed above are susceptible to several environmental factors including pH, substrate concentration, temperature, oxygen concentration, light, heavy metals, and heavy organic compounds or solvents (Liu et al. 2005). Kim et al. concluded that phenol and p-cresol when above 200 and 100 mg/L respectively could

noticeably inhibit nitrification (2008). Temperature is also a major problem since refinery wastewater is often 100° F or higher.

Sour Water Stripping

Sour water stripping or ammonia stripping is one way to remove ammonia from industrial wastewater. Through this process ammonia is separated from the aqueous solution due to their differences in boiling points and vapor pressures (Elston and Karmarkar 2007). However, the sour water must first be prepped by first being pumped into a flash drum to remove hydrocarbon vapors and liquids. The sour water is then pumped to a storage tank to allow for further hydrocarbon removal and longer mixing time to minimize feed composition fluctuations. The hydrocarbons need to be fully removed before the next step otherwise the sour water stripper would become a volatile hydrocarbon stripper. From the storage tank, the sour water is pumped on to sour water stripper. The sour water is heated in a heat exchanger by hot stripped water from the stripper bottoms and then fed into the stripping tower. As the sour water fall down the down the tower, ammonia and also hydrogen sulfide are stripped by steam entering the tower from below. Thus, the hydrogen sulfide, ammonia, and steam rise to the cooling section of the tower, which is cooled by sour water from the middle part of the tower being pumped around it. After being cooled, stripped water is then sent off site for further water processing (Armstrong et al. 1996).

Among the several at-source treatment options, steam stripping may offer a general and simple method. In addition to its ability to remove several organic compounds, steam stripping offers several advantages over other treatment methods. A stripper can be built with well developed, but simple equipment (Hwang et al.

1992). However, when designing a stripper it is important to balance the investment costs with the operating costs. There are also several operating variables that can affect the stripping of the ammonia and hydrogen sulfide in the water. These include the steam rate, the tower pressure, and the pH of the sour water (Lee et al. 2002). Increasing the stripping rate is one way o raise the stripping rate of ammonia and hydrogen sulfide, although the recommended steam rate is about three pounds of steam per gallon of sour water (Armstrong et al. 1996). pH is a little complicated. Raising the pH will aid in releasing ammonia, but hydrogen sulfide tends to stay in solution. Conversely a low pH will tend to hold ammonia in solution, and improve the stripping of hydrogen sulfide. Therefore, it is essential to determine what specific species are present and at what concentrations levels in the sour water (Melin et al. 1975).

Ion Exchange

Today, biological treatment is the traditional method for ammonia and organic removal, however, ion exchange has been around since the early 1850s, when agriculturist Harry S. Thompson and chemist John Holmes Way independently reported the phenomenon. They discovered that upon treating soil with either ammonium sulfate or ammonium carbonate, the majority of the ammonia was absorbed and calcium was released into solution. Ion exchange can be defined as the as a reversible exchange of ions involving a solid and a liquid in which the structure of the solid is not significant altered. Also the ion exchanger can be regenerated or overloaded with desirable ions by washing with an excess of these ions (Dow Chemical Company. 1958).

In municipal and industrial wastewater treatment systems designed to remove ammonium ions, organic pollutants are likely to be present. Some heterotrophic bacteria that can consume these organic species hinder the growth and activity of nitrifying bacteria used in biological treatment. In this case, ion exchange can offer an alternative solution to the removal of ammonia. The most common used ion exchanger is zeolites, due to their porous structure and high cation-exchange capacity (Wang and Peng 2010). There are many different natural zeolites, ones such as clinoptilolite, which is the most abundant and has a high affinity for ammonium ions, sepiolite, and select other natural zeolites can effectively remove ammonia from wastewater (Jorgensen and Weatherley 2003). In one study, high concentrations of ammonia were reduced by around 60% through the use of sepiolite as the ion exchanger (Balci and Dincel 2002). In another, clinoptilolite was used as the ion exchanger and a removal of 80% was reached (Park et al. 2002).

Ion exchange has drawn interest from researchers as a method of removing ammonia due since it is hardly influenced by temperature change, its ease of operation, and it has a relative low cost (Wang et al. 2007). Although ion exchange has these advantages, one of the most significant issues in the application of it in industrial wastewater is the lack of research on the effects of other contaminants in the wastewater on the techniques used (Jorgensen and Weatherley 2003). Another disadvantage is that free product (NAPL oil) can interfere with ion exchange. Also the zeolites that are used either need to be discarded or regenerated, and the major drawback to this is the high cost (Li et al. 2010).

Phenol Removal

Phenols can occur in a number of industrial wastewaters, wastewater such as that from petroleum refining, wood and dye industries, high temperature coal conversion, and resin and plastic industries (Tong et al. 1997). It is known to be a carcinogen and toxic at high levels (Miland et al. 1996). Thus, the removal of phenols from industrial wastewater is a practical problem of great significance (Singh and Singh 2002).

When it comes to removing phenol from wastewater, there are several methods that can be used including; using activated carbon, microbial degradation, incineration methods, chemical oxidation, solvent extraction, and irradiation are available (Miland et al. 1996; Tong et al. 1997). Although these techniques can remove phenol from an aqueous matrix, the formation of and possible release of hazardous by-products from the polychlorophenols during incineration or even composting is often tough to avoid. Other conventional techniques such as solvent extraction, adsorption on to activated carbon, and chemical oxidation also have some serious disadvantages. High cost, incompleteness of purification, and again the formation of hazardous by-products top the list of disadvantages (Lante et al. 2000). However, biological treatment strategies look to be an environmental-friendly alternative to thermal or chemical treatment of phenolic waste (Moeder et al. 2004).

There are several enzymatic approaches to removing the phenols from the industrial wastewater using laccase and peroxidase enzymes, plus ways using other enzymes such as phenolase. This approach was first proposed in the early 1980s, and was able to remove over 30 different phenols from water with an efficiency of 99% in

some wastewaters (Klibanov and Alberti 1981). A catalytic process of removing phenol by using peroxidase and laccase has been research by several researchers (Alemzadeh and Nejati 2009). This research demonstrated that these enzymes can react with aqueous phenolic compounds to create non-soluble compounds that can simply be removed from the aqueous phase using any conventional removal method (Cooper and Nicell 1996). In this process, phenols are oxidized to form the equivalent free radicals in the presence of hydrogen peroxide as an oxidant. The free radicals are then polymerized into less soluble compounds than the original matter and are then removed via settling and/or sorption methods (Singh and Singh 2002). This process has many advantages over the current methods listed above, and has been shown to be applicable for the treatment of many industrial wastewaters (Nakamoto and Machida 1992; Nicell et al. 1993). However, enzyme cost has to be significantly reduced in order to make this treatment option economically competitive (Cooper and Nicell 1996). The following is several case studies done on this enzymatic approach.

By using immobilized turnip seed peroxidase in the presence of hydrogen peroxide, Singh and Singh (2002) were able to treat and remove 95 % of phenol from a synthetic wastewater. In their experiments 200 µL of hydrogen peroxide and 90-100 units of peroxidase enzyme were added to 10 mL of synthetic industrial wastewater. In another study by Almezdeh and Nejati (2009) focused on immobilized horseradish peroxidase's ability to removal phenol in synthetic wastewater. Cooper and Nicell (1996) studied the effects of horseradish peroxidase on foundry wastewater. They used two different enzyme stocks of different purities,

and results showed that 97 and 99% of phenolic compounds were removed. Klibanov and Morris (1981)successfully utilized horseradish peroxidase in polymerizing phenol in the effluent of coal-conversion wastewater. They also showed that this enzymatic treatment was effective over a wide range of pH, temperatures, and phenol concentrations (Alberti and Klibanov 1981; Klibanov 1982). In a study by Moeder et al. (2004) laccase and horseradish peroxidase were used to study the degradation of several phenolic compounds. They found that most of the phenols in the buffer solution were efficiently removed within 48 hrs. In another study, immobilized commercial laccase was investigated. This laccase was tested in a model phenol solution containing several phenolic substrates. The enzyme laccase could remove phenols from this solution although it also can convert them in to intermediates such as catechol or guaiacol.

Chapter 3

Materials and Methods

The goal of the research presented in this thesis was to determine if the addition of laccase and peroxidase enzymes along with hydrogen peroxide can reduce the concentration of phenolic compounds commonly found in refinery wastewater. To accomplish this goal, several representative petroleum refinery wastewaters were obtained. The petroleum refineries that served as the source of test samples included two refineries from the United States (CITGO and Frontier), and three Canadian refineries (Imperial Oil). These five refineries were chosen because they represent the different types of crude being processed here in North America. The US refineries mostly process Texas "sweet crude" although they sometimes process Canadian or Venezuelan tar sand synthetic crude. The three Canadian refineries process a mixture of "sweet crude" and Canadian tar sand synthetic crude. "Sweet crude" is a type of crude that is not very complex and often easy to process, where as tar sand synthetic crude comes from adding several water soluble compounds such as ethylene glycol or propylene glycol to the tar sand mixture to produce the synthetic crude. Samples were taken at various points throughout wastewater treatment including: desalter, sour water, coker, DAF effluent, biox influent, and biox effluent. These locations were selected because they encompass the sources of refinery wastewater.

The hypothesis of the research is that the addition of hydrogen peroxide and either laccase or peroxidase enzymes can reduce the concentration of phenolic compounds in these wastewaters. This hypothesis is based on the experimental data and anecdotal information given by previous research. However, one the significant limitations of these laboratory studies to refinery wastewater is the effect which contaminants in actual refinery wastewater have upon the technique used. In this study five refineries were chosen so that the wastewaters would vary in both phenolic compounds and the aqueous matrix, and any adverse effects could be seen. Therefore, to test the hypothesis, the wastewater samples were exposed to various concentrations of the enzymes and hydrogen peroxide. The resulting changes in phenol and phenolic compound concentrations were measured using gas chromatography. The experimental study approach is outlined in this section.

Experimental Study Approach

The research was conducted in three phases. The first phase involved the enzymatic treatment of phenolic compounds in de-ionized water. Enzyme and hydrogen peroxide were added to this synthetic wastewater, and then the sample was tested on the gas chromatograph over time. Also studied in this phase was the effect of hydrogen peroxide on the enzymes.

The second phase involved the enzymatic treatment of refinery wastewater. Enzyme and hydrogen peroxide were added to the refinery wastewater samples. The sample was then tested on the gas chromatograph over time.

The third phase of the study investigated the effect of enzymatic treatment on nitrification. This was determined by analyzing the amount of ammonia being oxidized in each of the samples.

Phase 1: Synthetic Wastewater

In this phase of the research, the laccase and peroxidase were tested fro the ability to remove common phenolic compounds individually and in mixtures of simulated wastewater. Phenol, 3-methyl phenol (m-cresol), 4-methyl phenol (p-cresol), and 2, 4-dimethyl phenol were chosen for the study based on their high concentrations and common occurrence in refinery wastewater. For each of the individual test, 100 ppm of the selected compound was added to de-ionized water. This mixture was tested via the gas chromatograph (GC) to determine the starting concentrations. Once complete, an enzyme and hydrogen peroxide were added and tested in 15 minute intervals over the span of one hour. The make-up of the synthetic wastewater was as follows: 100 ppm phenol, 100 ppm m-cresol, 100 ppm p- cresol, and 50 ppm 2-4 dimehyl phenol in 100mL de-ionized water. This mixture was tested via the gas chromatograph to determine intial concentrations of the compounds added. Then 0.1 ml of enzyme (approximately 2,600 units of peroxidase or 200 units of laccase) and 1 mL of hydrogen peroxide (300 mg/L) were added to the sample. The sample was then test over time every 15 minutes for 60 minutes via the gas chromatograph.

Also tested in this phase of the study was the effect of hydrogen peroxide on the enzymes. A synthetic wastewater was created using 100ppm of phenol, 100ppm of o-cresol, 100ppm of p-cresol, and 50ppm of 2,4-dimethyl phenol. Once the

synthetic wastewater was made, 2600 units of peroxidase or 200 units of laccase and varied concentrations of hydrogen peroxide were added to the sample. This testing was done to determine if the concentration of hydrogen peroxide affected the reduction of these phenolic compounds by the enzyme. The concentrations used in this part of testing were 600 mg/L, 300 mg/L, 150 mg/L, and 75 mg/L of hydrogen peroxide. Samples were also tested with peroxide strips to determine the hydrogen peroxide concentrations in the sample over time intervals of 0, 15, 30, 45, and 60 minutes. The reason for this test was to ensure that hydrogen peroxide was not limiting the enzymes' ability to oxidize the phenols.

Phase 2: Refinery Wastewater

In phase 2, the ability of laccase and peroxidase enzymes to remove the four target phenolic compounds from refinery wastewater obtained from five working refineries was tested. The experiments differed from phase one testing by the fact that the phenolic compounds were in an actual refinery wastewater matrix. The wastewaters tested were obtained from various sites in the refineries, including: i) coker, ii) desalter, iii) Dissolved Air Floatation (DAF) effluent, iv) Biological treatment (Biox) influent, and v) Biox effluent. Since the refineries differ in production and wastewater treatment operations, not all of these samples were obtained from each refinery. The individual samples tested for each of the five refineries are summarized in Table 3.1.

Table 3.1: Refinery Wastewater Sources

Refinery	Sour Water	Coker	Desalter	DAF	Biox influent	Biox effluent
Lake Charles (CITGO)	X	X	X	X		
Cheyenne, WY (Frontier)	X		X			
Dartmouth (Imperial Oil)			X	X	X	
Nanticoke (Imperial Oil)			X		X	X
Sarnia (Imperial Oil)		X	X		X	X

The concentrations of phenolic compounds were measured in the wastewater using gas chromatography. In addition, the concentrations of other organic compounds (phenolic and non-phenolic) in the wastewater were measured. Once initial concentrations were determined, 2600 units of peroxidase or 200 units of laccase (0.1 mL of enzyme) were added along with 300 mg/L (1 mL) of hydrogen peroxide to 100 mL of the selected refinery wastewater. This mixture was then tested at time intervals of 0, 15, 30, 45, and 60 minutes on the gas chromatograph.

Phase 3: Nitrifying

In phase three of this study, the samples used in phase two were also tested to determine if nitrification was being affected by this enzymatic treatment. This can be shown by the amount of ammonia being oxidized in the sample. It is hypothesized that the amount of ammonia being oxidized will be lower in the control wastewater, or the non-treated sample, verses the samples treated with peroxidase or laccase. Samples included in this phase of testing were: CITGO stripped sour water, Frontier

stripped sour water, Dartmouth biox influent, Nanticoke biox influent, and Sarnia biox influent.

To test this hypothesis, 5 mL of *Nitrosomonas* was transferred to an empty beaker. Then a 15 mL solution of the wastewater to be tested was added to the beaker, followed by the addition of a 9.5 mL buffer solution. Finally, a 0.5 mL ammonium solution was added to a concentration of 50 mg $NH_4 – N\ L^{-1}$ in the beaker. The total test volume in the beaker was 30 mL and the pH at the start of testing was 8.3. The beakers were incubated on a rotary shaker in darkness for one hour. Aliquots of 1 mL were taken and the ammonium concentration was analyzed.

The buffer solution (pH ~ 9.0) was made of equal parts of 0.5 M phosphate buffer and 0.6 M carbonate buffer according to Sato *et al*. (1985). The ammonium solution added to the beakers contained 5 g $N\ L^{-1}$ as $(NH_4)_2SO_4$. The wastewater samples were treated with 2600 units of peroxidase or 200 units of laccase and 300 mg/L of hydrogen peroxide over a 30 minute reaction time. Dissolved oxygen was maintained above 4.0 ppm during the testing by vigorous shaking.

Analytical Methods

An Agilent 6890 Series gas chromatograph (GC) with an FID detector was used to determine the concentration of the phenolic compounds tested in this study. The column used in the gas chromatograph was a HP5 30m by 320μm by 0.25μm with 5 percent phenyl methyl siloxane. For full methodology refer to appendix A1.

EM Quant 10081-1 Peroxide Test strips were used to determine the amount of peroxide in the samples colorimetrically. The test strips could be used for concentrations from 1 – 100 mg/L of hydrogen peroxide.

Chemicals

The two enzymes used in this study were obtained from Novozymes, Inc. The first enzyme used in the study was peroxidase (Novozyme ® 51004). Once activated by hydrogen peroxide, the peroxidase enzyme catalyzes the oxidation of aqueous aromatic compounds to produce high molecular weight polymers of low solubility. The enzyme was produced by submerged fermentation of a genetically modified microorganism, which produces the peroxidase at a high rate. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism. The production organism was a fungi, *Aspergillus oryzae*. The declared activity is 10,000 POXU/g. The color of the enzyme solution is brown. Although the color can vary from batch to batch, it is not an indication of enzyme activity. The physical form of the enzyme is liquid, with an approximate density of 1.28 g/mL. For optimum results peroxidase should be stored with in the temperature range of 0-25˚ C (32-77˚ F). The enzyme has a shelf life in excess of one year.

One peroxidase unit (POXU) is defined as the amount of enzyme which catalyzes the conversion of one micromole of hydrogen peroxide per minute at 30 degrees Celsius in a 0.1 molar phosphate buffer at pH 7.0, using 0.88 millimoles hydrogen peroxide, and 1.67 millimoles of 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS). After 15 seconds of mixing, the reaction is followed for 60 seconds by the change in absorbance at 418 nanometers, which should be in the range of 0.15 to 0.30. For the calculation of activity, an absorption coefficient of oxidized ABTS of 36 $mM^{-1}cm^{-1}$ and a stoichiometry of one micromole of hydrogen peroxide converted per two micromoles ABTS oxidized are used (Baaziz, 1989).

The second enzyme used in this study was laccase (Novozymes ® 51003). Laccase was also produced by submerged fermentation of a genetically modified microorganism, *Aspergillus oryzae* (fungi). The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism. Laccase activity is expressed in Laccase Myceliophthora Units (LAMU). One LAMU is defined as the amount of enzyme that under standard conditions (pH 7.5; 30◦C) oxidizes 1 micromole syringaldazine per minute. The declared activity the enzyme used in this study is 1,000 LAMU/g. The color of the laccase enzyme solution is brown. Once more, the color can vary from batch to batch and is not an indication of enzyme activity. The physical form of the enzyme is liquid, with an approximate density of 1.05 g/mL. For optimum results laccase should be stored with in the temperature range of 0-25˚C (32-77˚F).

Phenol, m-cresol, p-cresol, and 2-4 dimethyl phenol were also used in this study. The particular phenolic compounds were chosen as these are the most common phenols found in refinery wastewater. The phenol was obtained from Acros organics. The cresols and the 2-4 dimethyl phenol were obtained from Aldrich Chemical Company.

Refinery Descriptions

Five refineries were chosen for this study. Two refineries are located in the United States, and three are located in Canada. They were chosen to represent the different types of wastewater that can be found at a refinery, as they each have their own unique mixture of crudes that are processed at each refinery. The two main types of crude that these refineries use are Texas/Gulf Coast “sweet crude” and

Canadian or Venezuelan synthetic crude which is derived from tar sand. The following sections are brief descriptions of the five refineries used in this study.

CITGO

CITGO is one of the leading manufacturers of high quality transportation fuels, lubricants, and petrochemicals. CITGO's Lake Charles Refinery in Louisiana is a modern, deep-conversion facility that has a crude oil capacity of 425,000 - 440,000 barrels per day. This refinery processes mostly Texas "sweet crude" although some imported Venezuelan synthetic crude is also processed. The complex is located on the banks of the Calcasieu Ship Channel, and encompasses 2,000 acres. Lake Charles Refinery is the fourth largest refinery in the nation, and is primarily suited to process heavy crudes into high-octane, unleaded gasoline. However, it also produces turbine fuel and heating oils. Additionally the CITGO refinery produces petrochemicals such as benzene and propylene that are used to manufacture numerous everyday products.

Frontier

The Frontier Refinery, located in Cheyenne, Wyoming, has a permitted capacity of 52,000 barrels of crude oil per day. The crude oil used at the Cheyenne Refinery is obtained from local producers (Texas and Midwest "sweet crude") as well as imported from Canada via the Express Pipeline (Canadian synthetic crude oil). The Cheyenne Refinery can process extensive amounts of heavy crude oil for use as a feedstock due to the coking unit on site. Although capable of utilizing heavy crude, this process mostly uses Texas "sweet crude" and some Canadian synthetic crude oil. The ability to process heavy crude oil lowers the cost of feed stock because lighter types of crude oil are usually more costly than heavy crude oil. The Cheyenne

refinery has historically used heavy crude oil, as it has accounted for 85 percent or more of the total crude oil charge. The Cheyenne Refinery's product mix usually consist of 41% gasoline, 30% diesel fuel, and asphalt and other refined petroleum products make up the remaining 29%.

Imperial Oil

Imperial Oil (Esso) began in 1880 and is one of Canada's top corporations. It has been a leading member of the petroleum industry for more than 125 years. Through these years Imperial Oil has been a contributor to the growth of the petroleum industry and to Canada's economic and social development. Imperial Oil is also an internationally renowned industrial cooperative, devoted to environmental protection. Imperial Oil produces several hundred different products. The crude processed to make these products is mainly synthetic crude that is derived from the tar sands there in Canada; however, these refineries do process some “sweet crude” from the United States or the Middle East as well. Samples from Imperial Oil included sample sets from three separate refineries: the Dartmouth Refinery located in Halifax, Nova Scotia; the Nanticoke Refinery located on the northern shore of Lake Erie just southwest of Hamilton, Ontario; and the Sarnia Refinery located southwestern Ontario, in the city of Sarnia.

The Dartmouth Refinery began production in 1918 so that the demands of World War I would be met. Since that time, it has grown continually, and now encompasses 650 acres. Dartmouth Refinery now has a processing capacity of 89,000 barrels of crude oil per day, producing 52 different products. These products include gasoline, jet fuel, diesel, and several heating oils and petrochemicals. The main crude

oil processed at Dartmouth is Canadian tar sand synthetic crude oil. Facilities at the Dartmouth Refinery include crude distillation and catalytic cracking.

The Nanticoke Refinery began shipping product in November of 1978 as part Texaco Canada. Imperial Oil purchased the Nanticoke Refinery in 1989 as part of its merger with Texaco. Since that time several investments have been made to try and improve the gasoline and diesel fuel being produce. Facilities have been upgraded and added to improve and reduce the sulfur levels in the fuels. The refinery has a processing capacity of 120,000 barrels of crude oil per day. Key products include unleaded gasoline, jet fuel, diesel, heavy fuel oil, asphalt and petrochemicals. Crude distillation, catalytic reforming, catalytic cracking, and sulfur recovery are the key facilities at Nanticoke.

The Imperial Oil's Sarnia Refinery was commissioned in 1897, and was the largest refinery in Canada with a 900 barrel per day capacity. Today Sarnia is still one of the most complex refineries in Canada, and has a capacity of 119,000 barrels per day of crude oil. Several improvements since the 1980s has increased its ability to process a wide range of crude oil into many quality products such as gasoline, jet fuel, diesel, and many other fuels, oils, and petrochemicals. One unique development is that since 2003 the Sarnia and Nanticoke refineries have been connected or integrated. Through this two refinery integrated system, Sarnia has been able to produce ultra low-sulfur gasoline and diesel, less than 30 ppm and 15 ppm respectively. Sarnia also manufactures over 250 lubricating oils, 60% is transported to customers via tank trucks and rail cars, and the other 40% is packaged into small containers on site. The chemical division at Sarnia and Nanticoke is one of Canada's

largest producers of petrochemicals, and today produces over one million tons of production per year.

Chapter 4

Results and Discussion

It is hypothesized that the addition of hydrogen peroxide coupled with either laccase or peroxidase enzymes can reduce the concentration of selected phenolic compounds in refinery wastewaters. This hypothesis is based on the experimental data and anecdotal information given by previous research. However, one the major limitations of these laboratory studies to refinery wastewater is that this research was conducted in either buffer solution or de-ionized water. A second limitation is that single compounds or a mixture of several compounds were employed rather than the complex mixtures actually present in refinery wastewater. Thus the effect which contaminants in actual refinery wastewater have upon this enzymatic approach is not reflected in these studies.

The research of this thesis was conducted in three phases. The first phase involved the enzymatic treatment of phenolic compounds in a simple matrix (de-ionized water). The second phase involved the enzymatic treatment of various refinery wastewaters, and the third phase of the study considered the effect of enzymatic treatment on nitrification. The results of these three phases of testing are present below.

Phase One: Synthetic Wastewater

It was theorized that the concentration of phenolic compounds in refinery wastewater could be reduced using hydrogen peroxide coupled with either laccase or peroxidase. In the first phase of testing this hypothesis, the enzymatic approach was tested in synthetic wastewater created by adding the phenolic compounds to de-ionized water. There were three different studies conducted in phase one using a simple matrix. The first study examined the efficiency of laccase and peroxidase to reduce the individual phenolic compounds in de-ionized water. The second study determined the enzyme's ability to lower the concentrations of a mixture of phenolic compounds in a synthetic wastewater. The final study conducted in phase one was to determine the effect of hydrogen peroxide concentration on the efficiency of the enzymatic treatment of phenolic compounds. The results of this phase are presented in the following sections for each enzyme, and then summarized for both.

Individual Testing of Each Compound in a simple Matrix

The efficacy of laccase and peroxidase enzymes for removing phenol, o-cresol, p-cresol, 2,4-dimethyl phenol was studied using individual compounds in a synthetic de-ionized water solution. The synthetic solution used in this phase of the study was created by adding each individual compound to de-ionized water. Laccase and peroxidase treatment were tested on the individual samples to examine the efficiency of removal. The samples were tested using gas chromatography with a flame ionization detector to determine the initial concentrations of each compound. Either 200 units of laccase or 2600 units of peroxidase were added along with 300 mg/L of hydrogen peroxide. Concentrations of each compound were then determined

by gas chromatography at 15 minute intervals. The results of this experiment are discussed in this section.

The effects of laccase and peroxidase on a pure synthetic solution containing phenol are shown in Figure 4.1. Laccase reduced the concentration of phenol from 130.7 mg/L to 33.6 mg/L over 60 minutes. The reduction in phenol by laccase was 74.3 percent after 60 minutes. The concentration of phenol decreased from 127.3 mg/L to 31.8 mg/L using the peroxidase enzyme. Over the 60 minutes of reaction time peroxidase lowered the concentration of phenol by 75 percent.

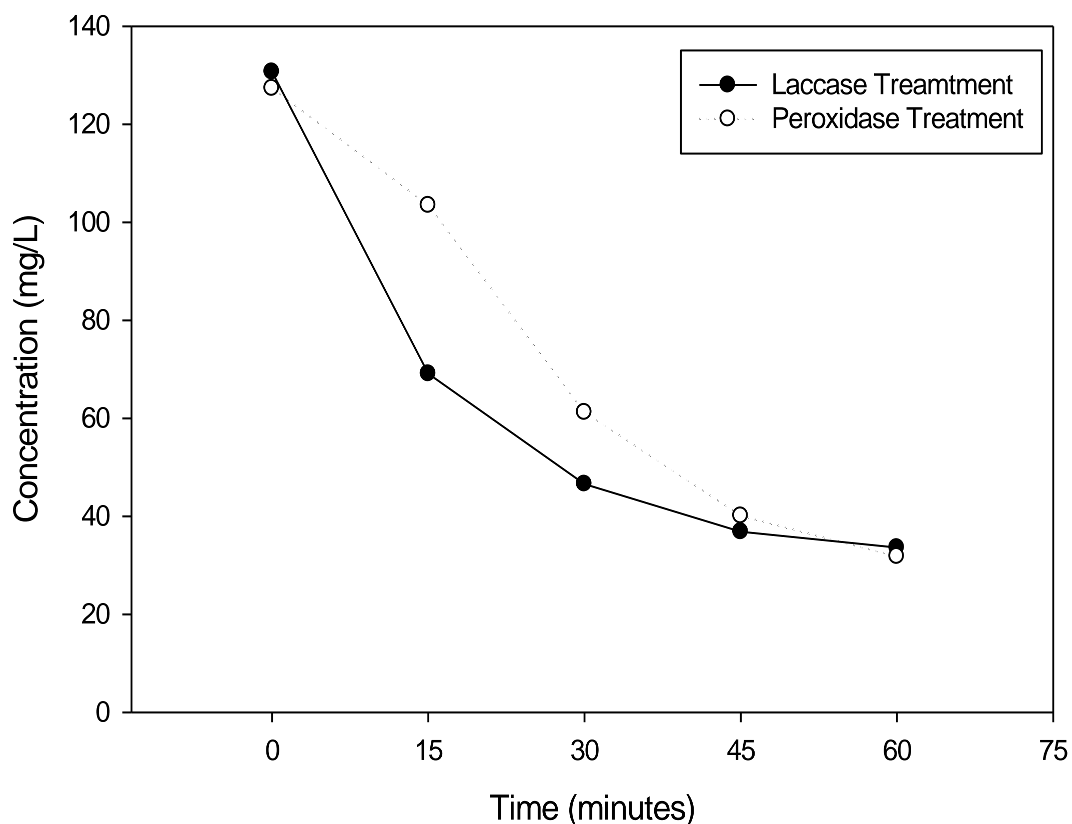

Figure 4.1: Laccase/Peroxidase Treatment of Phenol in De-ionized Water

Since the reaction is believed follow first order kinetics, the rate constant for each reaction was determined. The equation describing first order kinetics is: ln [A] =

$-kt + \ln [A_0]$, where A_0 equals the initial concentration, A equals the concentration at time t, and k is equal to the first order rate constant. The first order rate constant is equal to the slope of the line given by ln [A], or natural log of the concentration, verses time. An example of the plot used to determine the first order rate constants (k) for phenol is shown in Figure 4.2. Table 4.1 summarizes the first order rate constants and R^2 values for both enzymes for each compound. The first order rate constant for laccase reduction of phenol was found to be -0.0223 min^{-1} with an R^2 value of 0.90. The first order rate constant for peroxidase was determined to be -0.0248 min^{-1} with an R^2 of 0.91, which is very similar to the rate obtained by using laccase. Thus both enzymes appeared to be equally effective in a simple matrix. The high values of R^2 (>0.9) indicate that first order fit is acceptable.

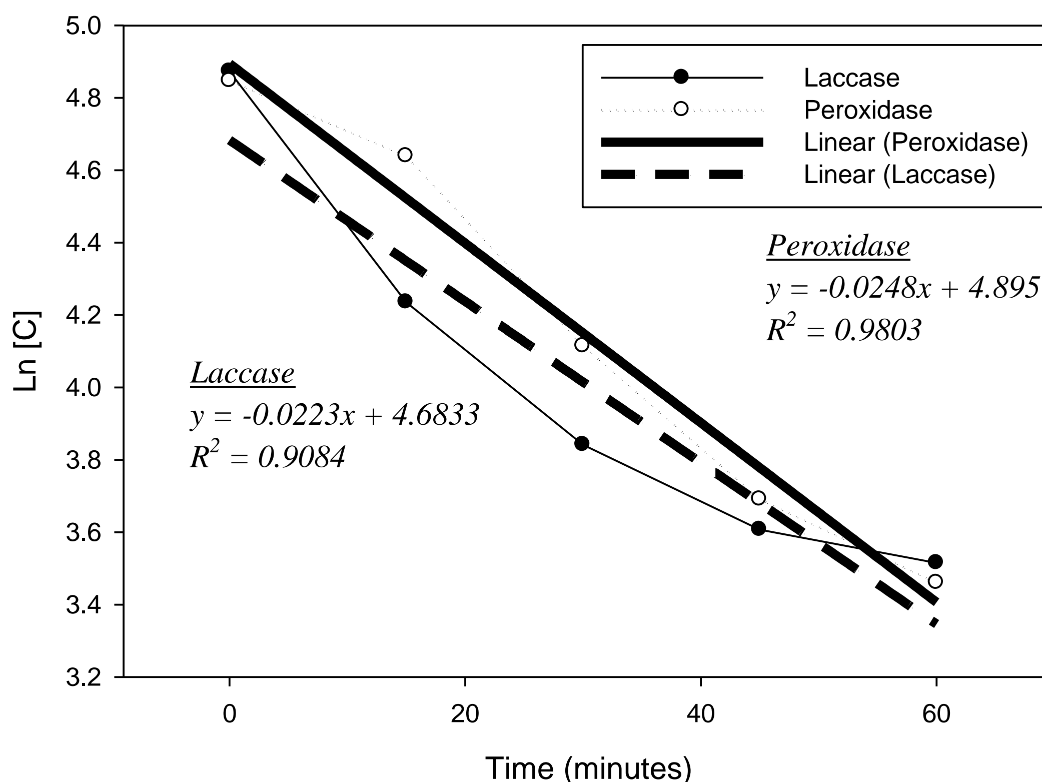

Figure 4.2: Example Plot for Determining Rate Constant, K

Table 4.1: Individual Testing First Order Rate Constants

Compound	**Laccase k (min^{-1})**	**Peroxidase k (min^{-1})**
Phenol	-0.0223 (R^2 = 0.91)	-0.0248 (R^2 = 0.98)
O-cresol	-0.0114 (R^2 = 0.97)	-0.0134 (R^2 = 0.99)
P-cresol	-0.0107 (R^2 = 0.95)	-0.0157 (R^2 = 0.98)
2,4-Dimethyl Phenol	-0.0201 (R^2 = 0.92)	-0.0243 (R^2 = 0.90)

The reduction of o-cresol by laccase and peroxidase catalyzed per oxidation is shown in Figure 4.3. The concentration of o-cresol was decreased by laccase from 67.6 mg/L to 32.9 mg/L in 60 minutes. This corresponds to a reduction in o-cresol by

laccase of 51.3 percent. Similarly, using the peroxidase enzyme, the concentration of o-cresol decreased from 61.2 mg/L to 27.2 mg/L. Peroxidase reduced the concentration of o-cresol by 55.6 percent over the 60 minutes of reaction time. The first order rate constants for laccase and peroxidase for the removal of o-cresol are shown in Table 4.1. Peroxidase/hydrogen peroxide treatment had a slightly higher rate of o-cresol removal than the laccase system. The first order rate constant for laccase was determined to be -0.0107 min^{-1} with an R^2 value of 0.97. Peroxidase had a first order rate constant of -0.0134 min^{-1} and an R^2 value of 0.99. The rate of removal for o-cresol was approximately one half than that of phenol removal for both enzymes.

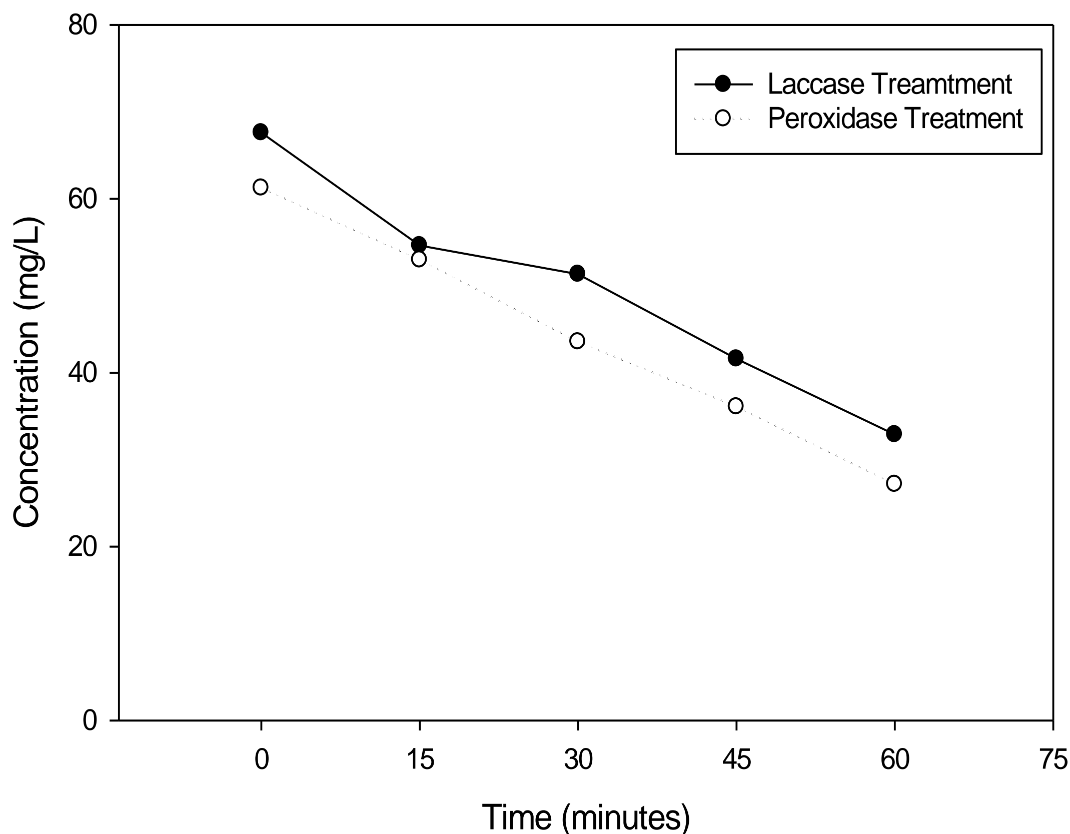

Figure 4.3: Laccase/Peroxidase Treatment of O-cresol in De-ionized Water

Changes in p-cresol concentration, catalyzed by laccase or peroxidase, are presented in Figure 4.4. Laccase reduced the initial concentration of p-cresol from 56.3 mg/L to 27.9 mg/L. Laccase treatment reduced the concentration in p-cresol by 50.4 percent in 60 minutes. The first order rate constant was determined to be -0.107 min^{-1} for laccase with an R^2 value of 0.95 (Table 4.1). The peroxidase enzyme lowered the concentration of p-cresol from 53.2 mg/L to 21.7 mg/L. The reduction in the concentration of p-cresol by peroxidase was 59.3 percent over the 60 minutes of reaction time. The first order rate constant for peroxidase was found to be -0.0157

min^{-1} with an R^2 value of 0.98 (Table 4.1). These rate constants are somewhat similar, although peroxidase is a little higher rate. The first order reduction rate constant for p-cresol was comparable to that of o-cresol, but was nearly half that of phenol and 2,4-dimethyl phenol.

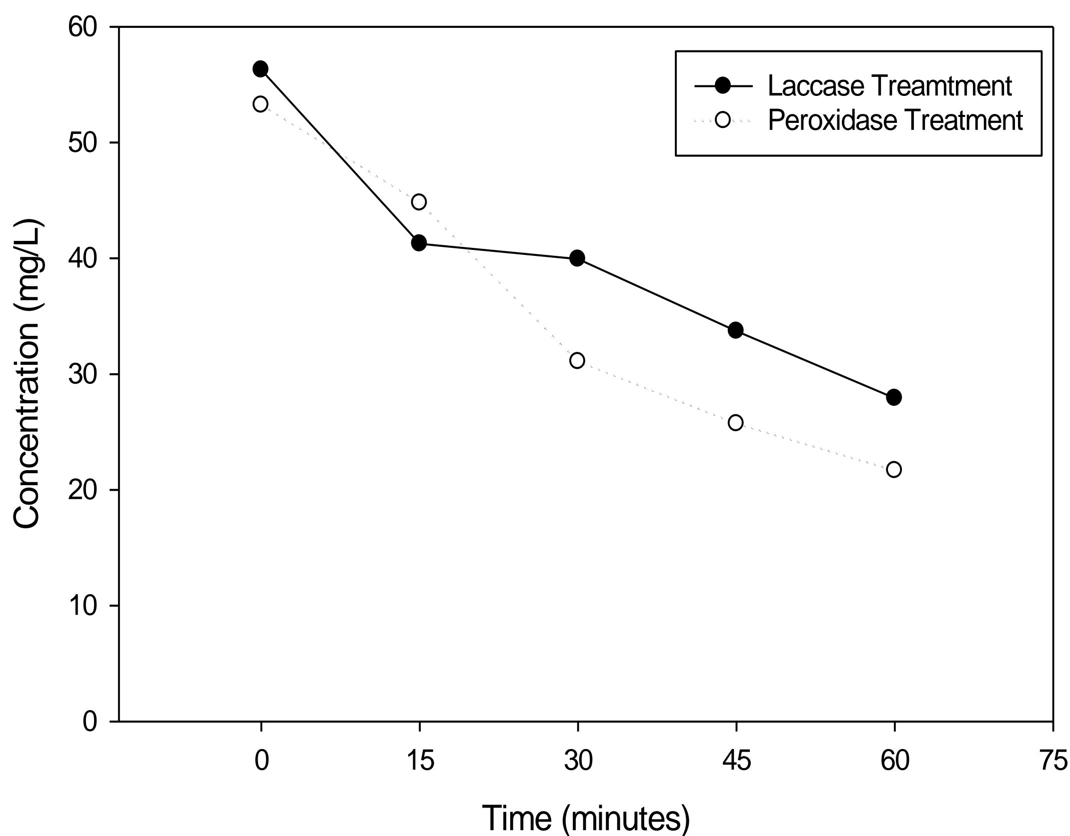

Figure 4.4: Laccase/Peroxidase Treatment of P-cresol in De-ionized Water

The effects of laccase and peroxidase on 2,4-dimethl phenol in a synthetic solution is shown in Figure 4.5. Laccase decreased the concentration of 2,4-dimethyl phenol from 31.3 mg/L to 10.2 mg/L. This reduction in 2,4-dimethyl phenol by laccase treatment was 67.4 percent over 60 minutes. The first order rate constant,

shown in Table 4.1, for 2,4-dmp treated with laccase was found to be -0.0201 min^{-1} with an R^2 value of 0.92. The concentration of 2,4-dimethyl phenol decreased from 34.2 mg/L to 7.9 mg/L using the peroxidase enzyme with 60 minutes reaction time. The reduction in 2,4-dimethyl phenol by peroxidase was 76.9 percent. The first order rate constant for peroxidase was found to be -0.0243 min^{-1} with an R^2 of 0.90 (Table 4.1). These rates appear to be similar. However, the peroxidase/peroxide removed the 2,4-dmp at a rate that was almost 20 percent faster than laccase/peroxide. The rates are comparable to that of phenol, and almost double the rates of o-cresol and p-cresol.

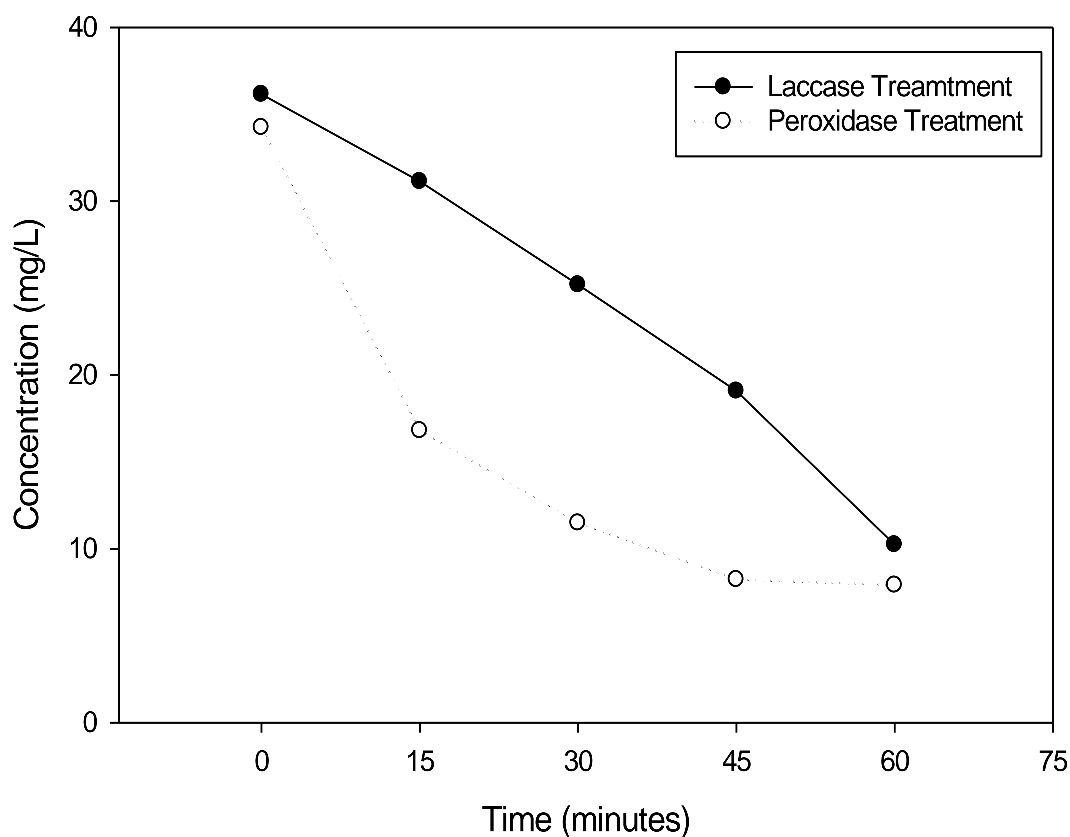

Figure 4.5: Laccase/Peroxidase Treatment of 2,4-Dimethyl Phenol in De-ionized Water

To facilitate comparison of the effectiveness of laccase/peroxide and peroxidase/peroxide treatment on the removal of the target phenolic compounds, the concentrations for each compound were normalized to the initial concentration and presented in the same figure to show the fraction remaining. Figure 4.6 shows the fraction remaining of each compound removed by the laccase/peroxide treatment over the 60 minutes. The figure shows that laccase/peroxide treatment initially removed phenol at the fastest rate. 2,4-dmp was removed to almost the same degree as phenol after 60 minutes. However, there was an initial lay. O-cresol and p-cresol on the hand were removed at a much lower rate and to a lesser degree than phenol and 2,4-dmp.

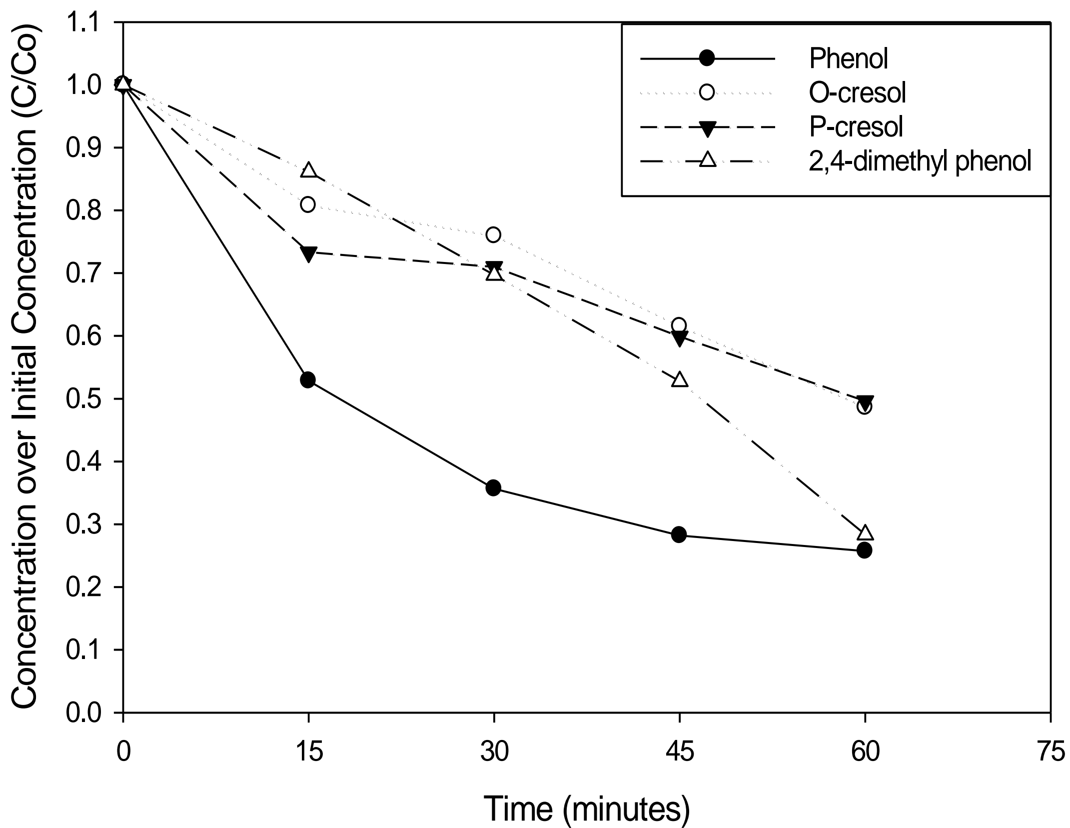

Figure 4.6: Fraction Remaining using Laccase/Peroxide Treatment

The fraction remaining of each compound reduced by peroxidase/peroxide treatment is shown in Figure 4.7. Peroxidase/peroxide treatment initial removed 2,4-dmp at a faster rate than the other compounds. Although phenol was initially reduced at a lower rate than 2,4-dmp, they were removed to almost the same degree after the 60 minutes. P-cresol and o-cresol were removed to a lesser degree and a slower rate than the phenol and 2,4-dmp. The p-cresol was initially removed at a slightly faster rate than o-cresol, but over the 60 minutes, both were removed to a similar degree.

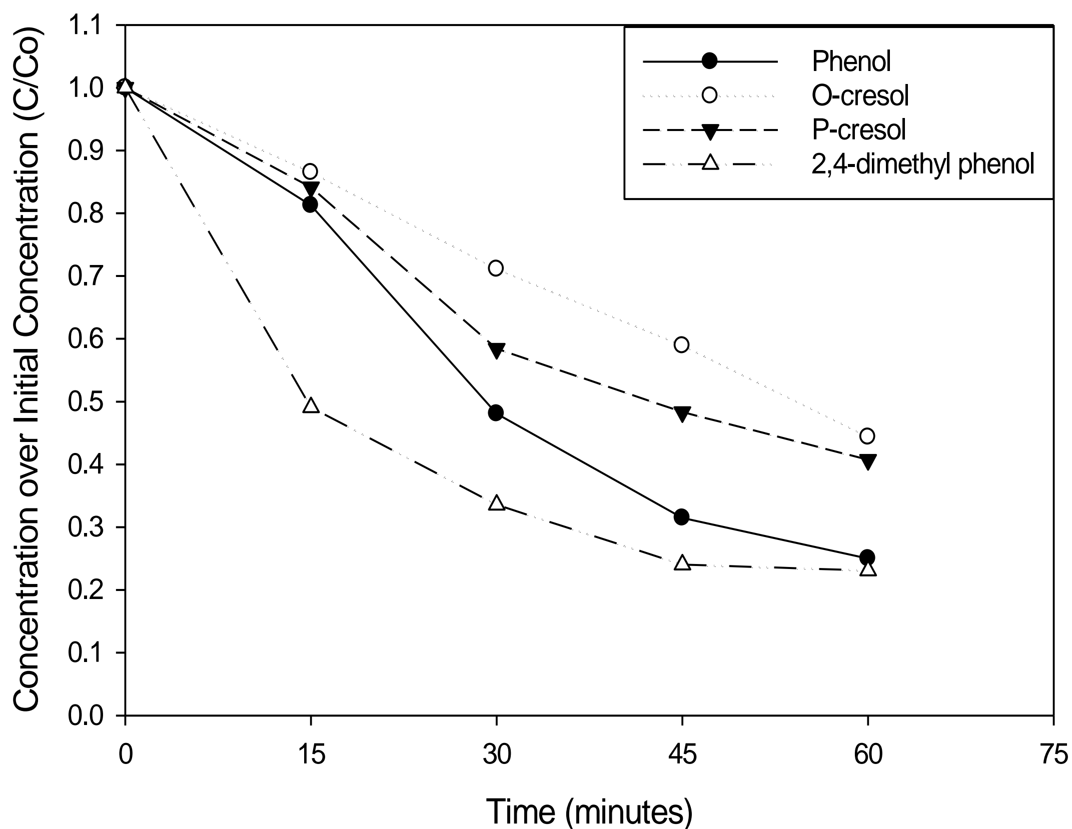

Figure 4.7: Fraction Remaining using Peroxidase/Peroxide Treatment

Defined Mixture of Phenolic Compounds in a Simple Matrix (Synthetic Wastewater)

The second part of phase one was to determine the enzyme's capacity for reducing a mixture of phenolic compounds in a synthetic wastewater. This testing was preformed to check for synergism or antagonism between compounds. The synthetic wastewater was created by adding 100 ppm phenol, 100 ppm o-cresol, 100 ppm p-cresol, and 100 ppm 2,4-dimethyl phenol to de-ionized water. The samples were then tested using gas chromatography to determine the initial concentrations of each compound. An initial concentration of 300 mg/L of hydrogen peroxide and either 200 units of laccase or 2600 units of peroxidase was used to initiate the testing. Concentrations were determined at 15 minute intervals after enzyme addition. The results of this experiment are discussed in this section.

The efficiency of laccase at reducing the concentration of phenol over time in the mixed wastewater is shown in Figure 4.8. The initial phenol concentration in the synthetic wastewater was 111.2 mg/L. The laccase treatment lowered the concentration of phenol to 43.7 mg/L (60.7 percent) over 60 minutes.

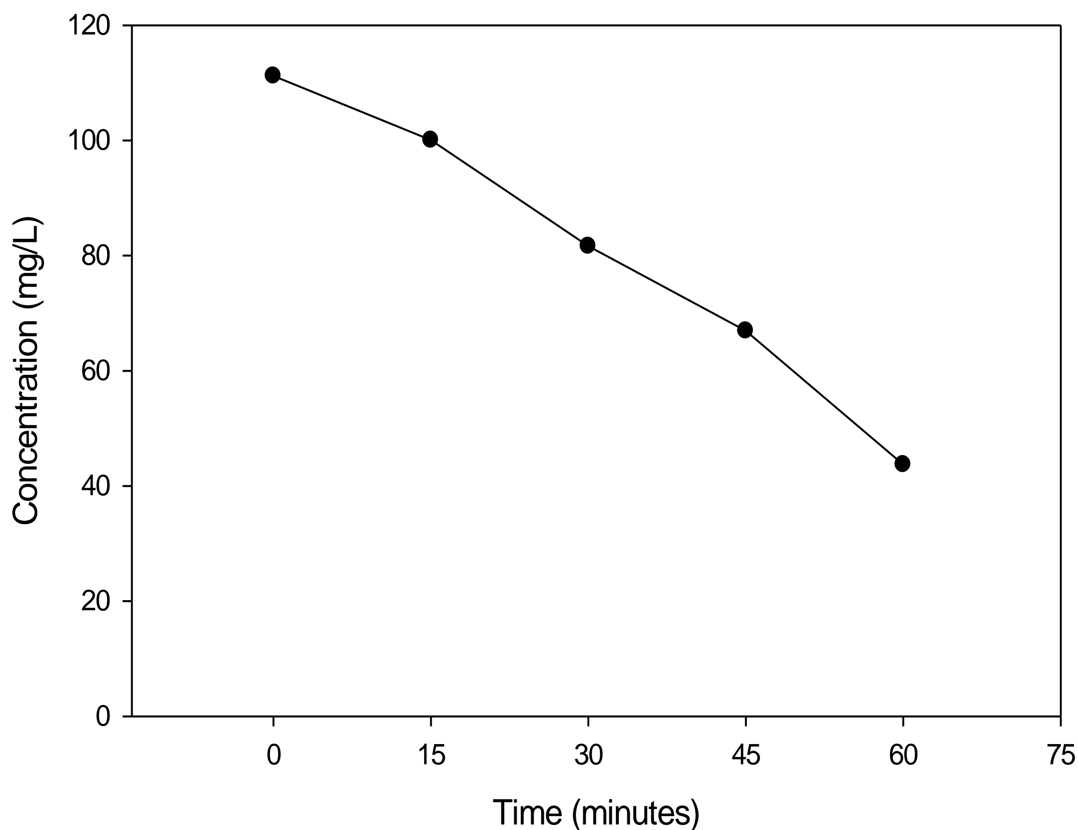

Figure 4.8: Laccase Treatment of Phenol in the Defined Mixture

Table 4.2 contains the first order rate constants and R^2 values for both enzymes for each compound. Again due to the reaction following first order kinetics, the rate constant for each reaction could be determined. In first order kinetics ln [A] = -kt + ln [A_0], where A_0 equals the initial concentration, A equals the concentration at time t, and k is equal to the first order rate constant. Thus, the first order rate constant is equal to the slope of the line given by ln [A], or natural log of the concentration, verses time. The first order rate constant was found to be -0.0151 min^{-1} and had an R^2

value of 0.94. The rate constant for phenol was similar to that of 2,4-dimethyl phenol, but somewhat higher than the first order rate constant for o-cresol and p-cresol.

Table 4.2: First Order Rate Constants for Each Compound in the Defined Mixture

Compound	**Laccase k (min^{-1})**	**Peroxidase k (min^{-1})**
Phenol	-0.0151 (R^2 = 0.94)	-0.0157 (R^2 = 0.99)
O-cresol	-0.0103 (R^2 = 0.95)	-0.0067 (R^2 = 0.95)
P-cresol	-0.0087 (R^2 = 0.99)	-0.0093 (R^2 = 0.98)
2,4-Dimethyl Phenol	-0.0162 (R^2 = 0.97)	-0.0172 (R^2 = 0.999)

Figure 4.9 shows the decrease in o-cresol concentration over time. O-cresol concentration in the synthetic wastewater was reduced by laccase 43.6 percent. The concentration dropped from 46.8 mg/L initially to 26.4 mg/L after 60 minutes. The decrease in o-cresol is similar to that of p-cresol, but lower than that of phenol and 2,4-dimethyl phenol. Laccase reduction of o-cresol has a first order rate constant of -0.0103 min^{-1} and a R^2 value of 0.95 (Table 4.2). The reduction rate constant is slightly high than the rate constant for p-cresol, but significantly lower than that of phenol and 2,4-dimethyl phenol.

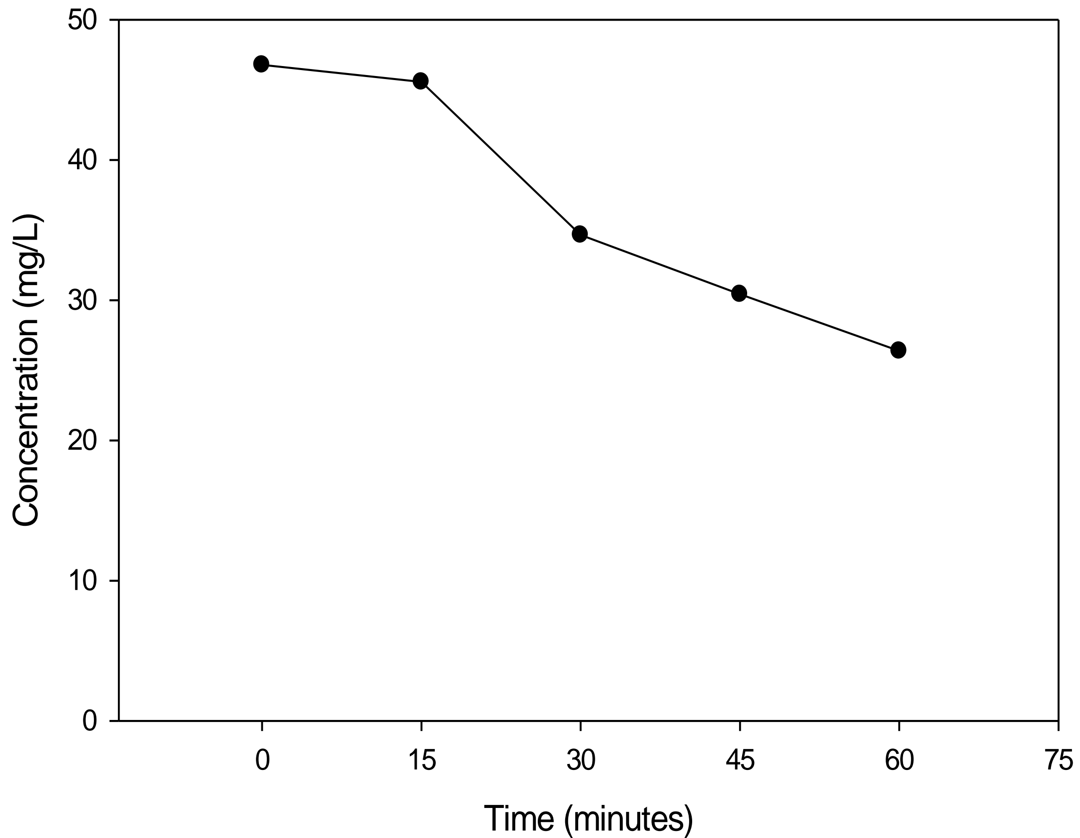

Figure 4.9: Laccase Treatment of O-cresol in the Defined Mixture

The reduction of p-cresol by laccase over time is shown in Figure 4.10. The concentration of p-cresol decreased from 31.6 mg/L to 18.9 mg/L in 60 minutes. The reduction in p-cresol by laccase was 40.2 percent. Again this drop in concentration resembles that of o-cresol, but is much less than the phenol and 2,4-dimethyl phenol. Table 4.2 shows how the first order rate constant of laccase for decreasing p-cresol concentration compares to the other compounds tested in the synthetic wastewater. The first order rate constant was found to be -0.0087 min^{-1} with an R^2 value of 0.99. This is the lowest first order rate constant for laccase in this part of phase one testing,

and the first order rate constant for phenol is nearly double that of p-cresol. Another note is that the rate constant for peroxidase reducing p-cresol is very similar to the laccase rate constant, thus both enzymes appear to be equally effective at reducing p-cresol in concentration with other compounds.

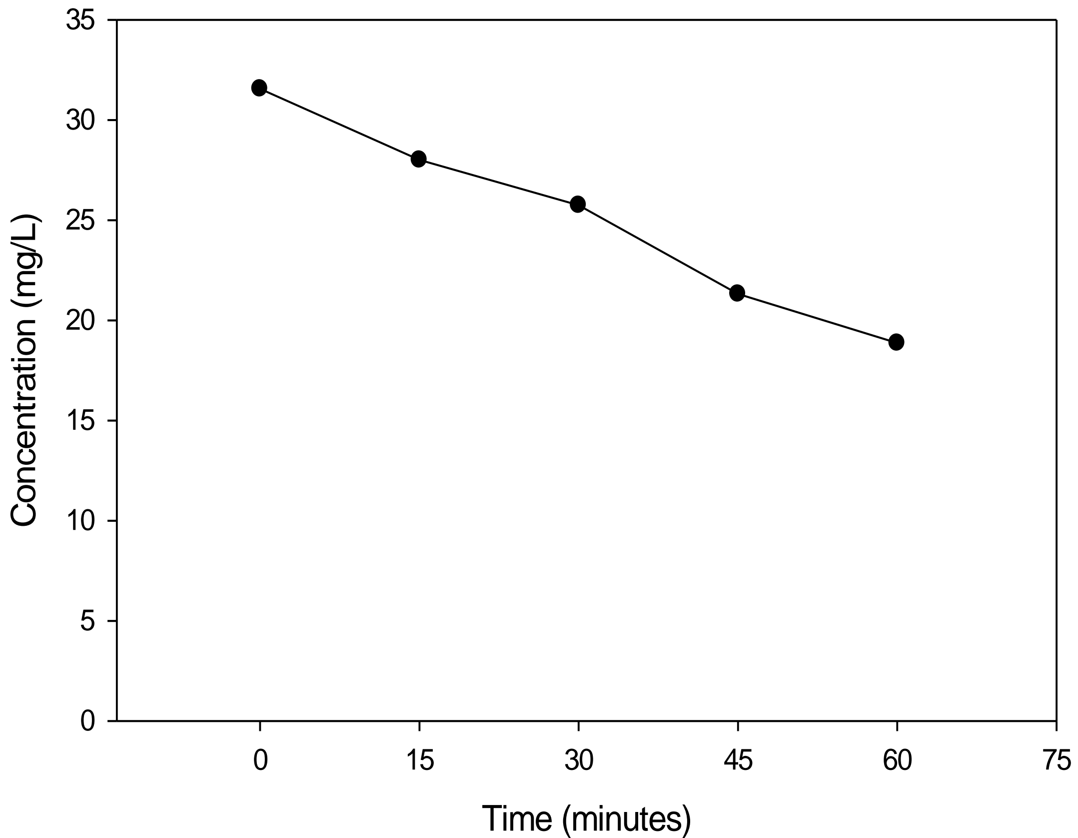

Figure 4.10: Laccase Treatment of P-cresol in the Defined Mixture

The first order rate constants for each compound in the synthetic wastewater are shown in Table 4.2. The first order rate constant for 2,4-dimethyl phenol was

found to be -.0162 min^{-1} with a R^2 value equal to 0.97. This is close to the rate constant for phenol, but is higher than the rate constant for o-cresol and nearly double the rate constant for p-cresol. Figure 4.11 shows the decrease in 2,4-dimethyl phenol concentration over time. 2,4-dimethyl phenol was lowered by laccase treatment from a concentration of 23.4 mg/L to 8.9 mg/L after 60 minutes. The reduction of 2,4-dimethyl phenol by laccase was 62 percent. The percent reduction is very close to that of phenol.

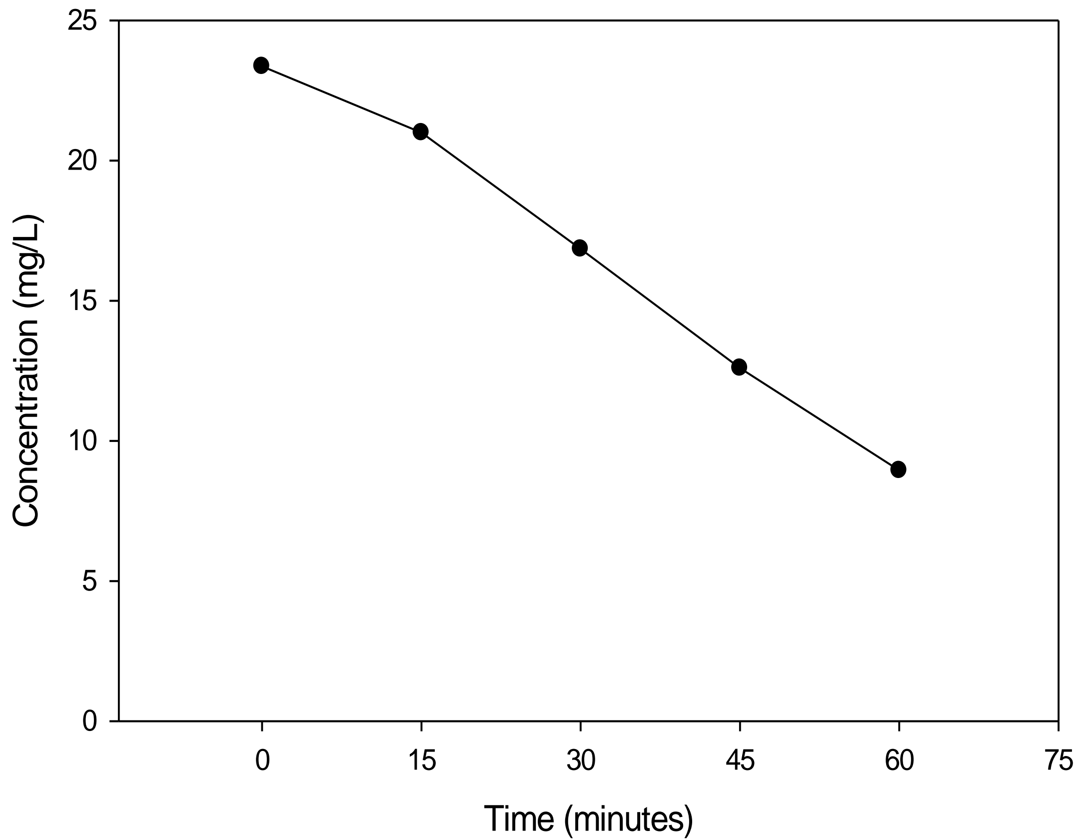

Figure 4.11: Laccase Treatment of 2,4-Dimethyl Phenol in the Defined Mixture

The reduction of phenol by the peroxidase enzyme can be seen in Figure 4.12. The concentration of phenol was reduced from an initial concentration of 110 mg/L to 42.9 mg/L by peroxidase treatment. Over the 60 minutes reaction time phenol was reduced by 61 percent. The first order rate constant for the peroxidase enzyme's ability to remove phenol can be seen in Table 4.2. The reduction rate constant was found to be -0.0157 min-1 with a R^2 value of 0.99. The rate constant for peroxidase is almost identical to that of laccase, thus they seem to be equally efficient. However, the rate for reducing phenol is similar to that of 2,4-dimethyl phenol, and much higher than the first order rate constant for reducing p-cresol and well over two times that of o-cresol.

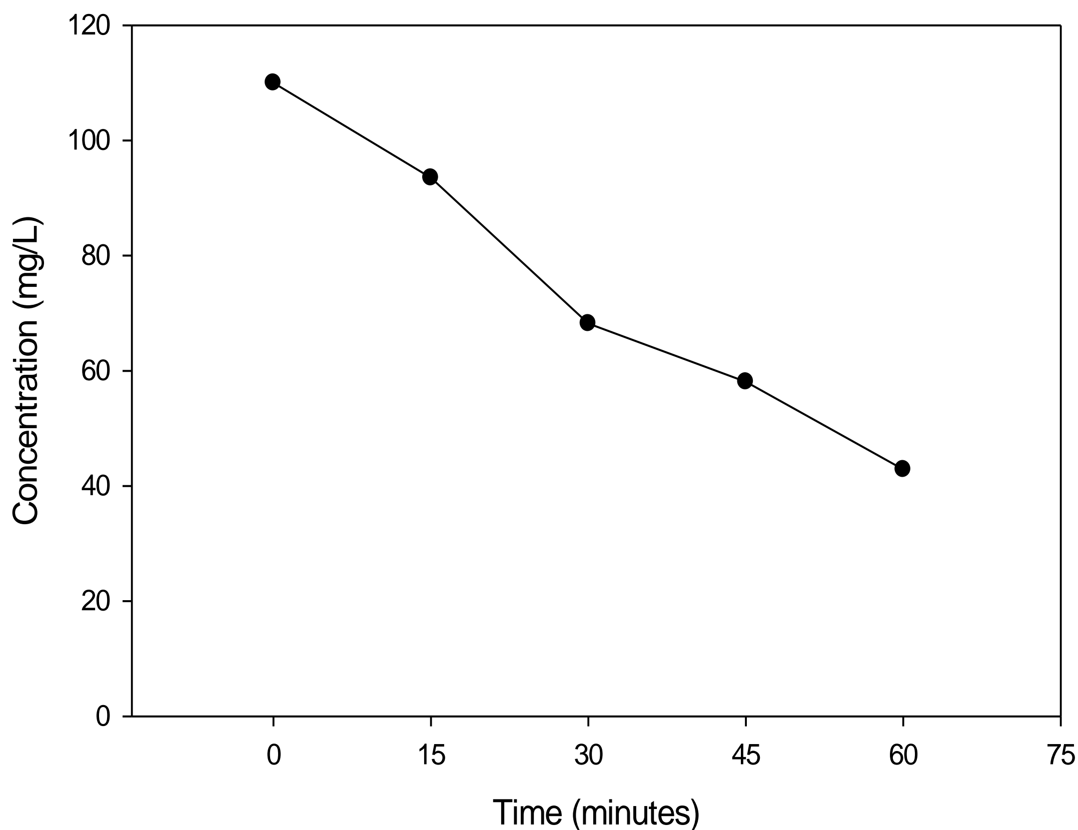

Figure 4.12: Peroxidase Treatment of Phenol in the Defined Mixture

The concentrations of o-cresol over time are shown in Figure 4.13. O-cresol was lowered only 34.3 percent by peroxidase treatment. The concentration dropped from 41.1 mg/L initially to 27 mg/L after 60 minutes time. O-cresol had the lowest percent reduction by the peroxidase enzyme. The percent reduction was close to half of that observed for phenol and 2,4-dimethyl phenol. The first order rate constant for peroxidase reduction of o-cresol was found to be -0.0067 min^{-1} with R^2 equal to 0.95 (Table 4.2). This rate constant was the lowest first order rate constant for peroxidase

and the lowest for this part of this phase one. The reduction rate constant is one and a half times lower that that of phenol and one third that of 2,4-dimethyl phenol.

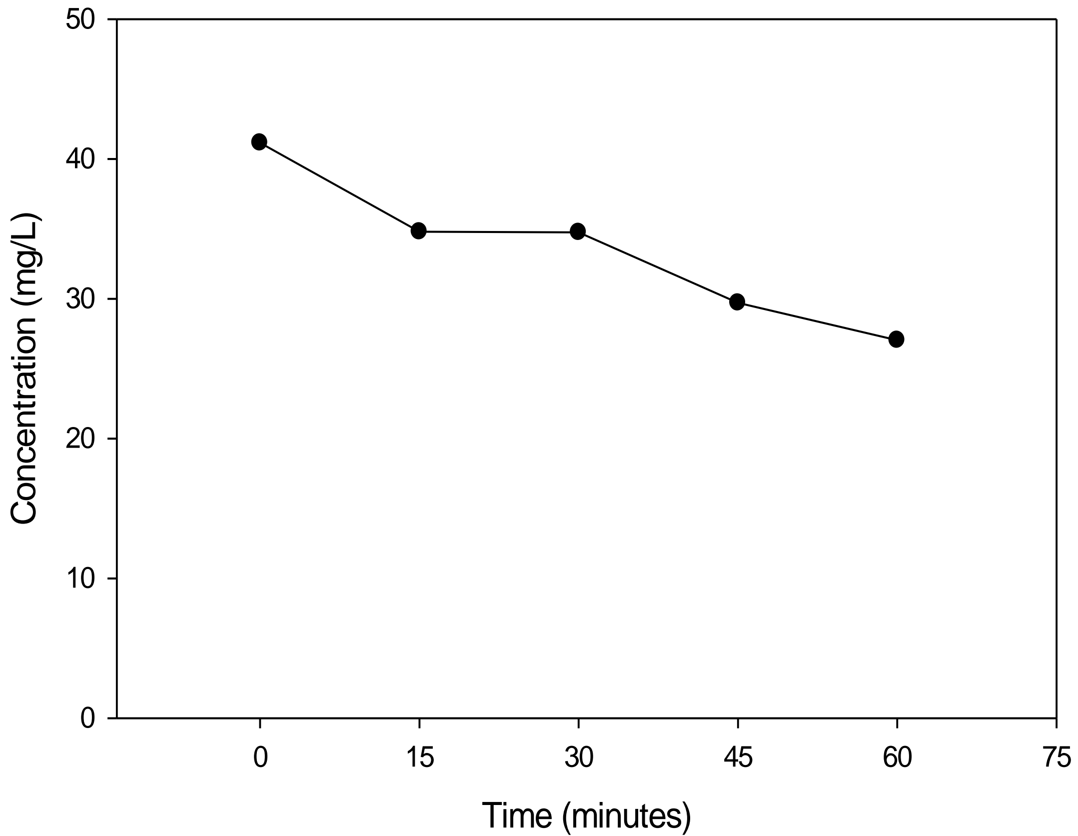

Figure 4.13: Peroxidase Treatment of O-cresol in the Defined Mixture

P-cresol concentration over time can be seen in Figure 4.14. P-cresol concentration dropped 42.3 percent over the 60 minutes. The synthetic wastewater was found to have an initial concentration of 30.7 mg/L and that was reduced by peroxidase to 17.7 mg/L. The percent reduced was very similar to that of o-cresol, and lower than that of phenol or 2,4-dimethyl phenol. Table 4.2 shows the first order rate constant of peroxidase for decreasing the concentration of each compound in the

synthetic wastewater. The first order rate constant for reducing p-cresol was found to be -0.0093 min^{-1} with an R^2 value of 0.98. The first order rate constant for p-cresol is nearly half that of 2,4-dimethyl phenol.

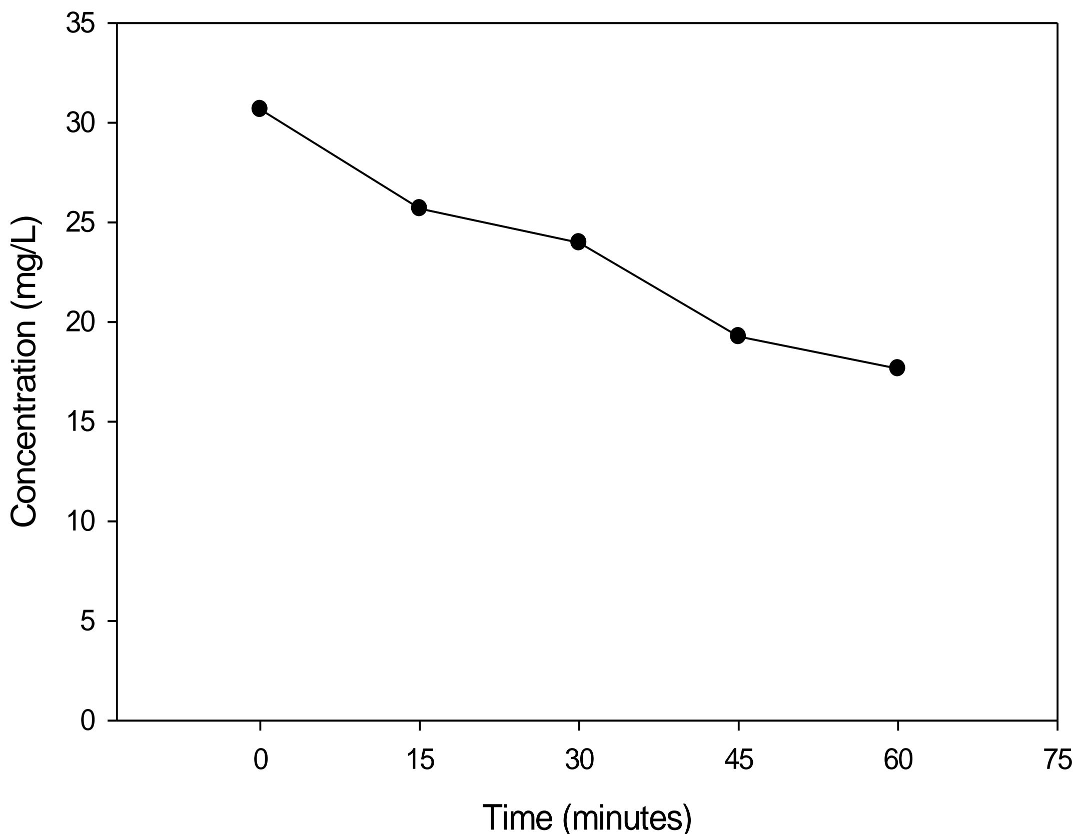

Figure 4.14: Peroxidase Treatment of P-cresol in the Defined Mixture

The reduction in 2,4-dimethyl phenol concentration by peroxidase can be seen in Figure 4.15.Peroxidase reduced 2,4-dimethyl phenol from a concentration of 21.8 mg/L to 7.8 mg/L after 60 minutes. The reduction of 2,4-dimethyl phenol by peroxidase was 64.2 percent. The percent reduction is similar to that of phenol, and nearly doubles that of o-cresol. The first order rate constant for the peroxidase

enzyme's ability to remove phenolic compounds can be seen in Table 4.2. The reduction rate constant for reducing 2,4-dimethyl phenol was found to be -0.0172 min-1 with a R^2 value of 0.999. The rate constant for peroxidase is similar to that of laccase, thus they seem to be equally efficient. However, the rate for reducing 2,4-dimethyl phenol is similar to that of phenol, and is almost double than the first order rate constant for reducing p-cresol and almost three times that of o-cresol.

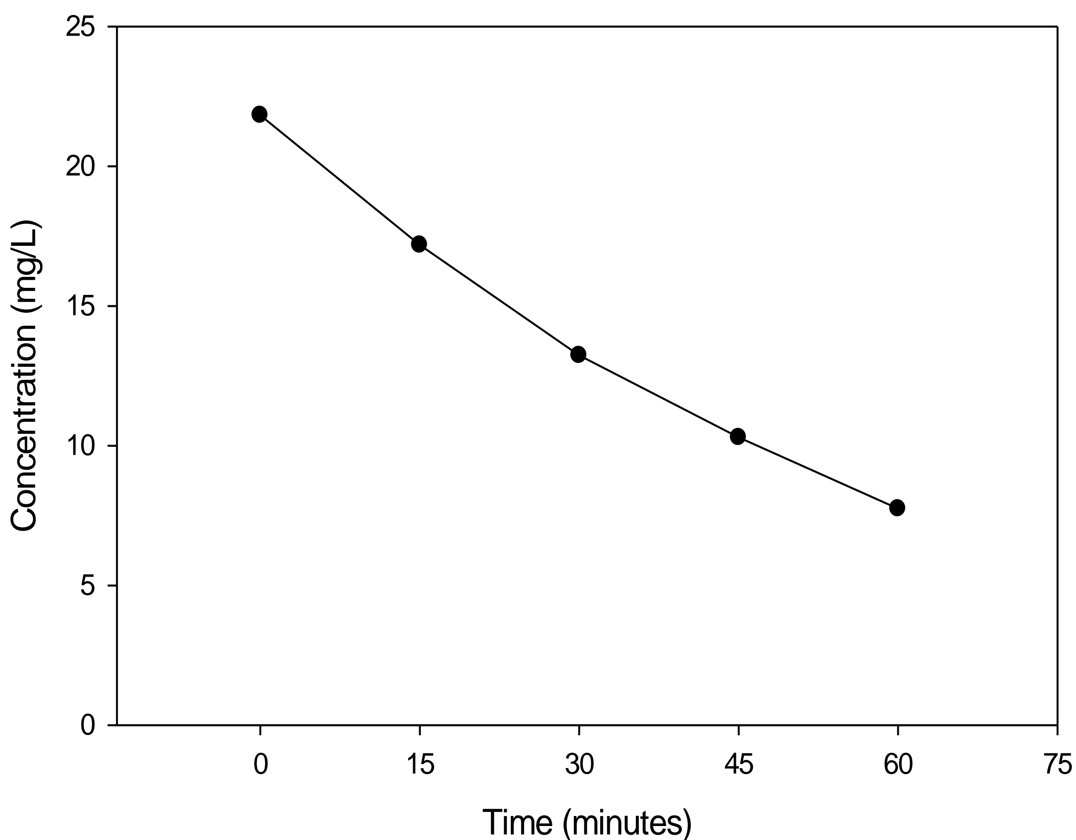

Figure 4.15: Peroxidase Treatment of 2,4-Dimethyl Phenol in the Defined Mixture

Upon initial comparison it appears that in this particular case the two enzymes are to some extent equally efficient, albeit peroxidase does have a slightly higher rate

constant for all compounds except o-cresol. The first order reduction rate constant is however lower when all compounds are in the synthetic wastewater verses the enzymatic treatment of just the single compound in de-ionized water, which is shown in Table 4.3. This difference in rate could be due to antagonism between the different compounds, meaning the compounds compete for the enzyme, making the rate of reduction lower for the mixture. It is hypothesized that in real refinery wastewater the competition for the enzyme will be even greater than in the de-ionized mixture due to the other compounds in the wastewater besides the target phenols, thus the rates for laccase and peroxidase may be smaller.

Table 4.3: Laccase and Peroxidase Rate Constants from Individual Testing and Synthetic Testing for Comparison

Compound	**Laccase Single k (min^{-1})**	**Laccase Mixture k (min^{-1})**	**Peroxidase Single k (min^{-1})**	**Peroxidase Mixture k (min^{-1})**
Phenol	-0.0223	-0.0151	-0.0248	-0.0157
O-cresol	-0.0114	-0.0103	-0.0134	-0.0067
P-cresol	-0.0107	-0.0087	-0.0157	-0.0093
2,4-Dimethyl Phenol	-0.0201	-0.0162	-0.0243	-0.0172

Hydrogen Peroxide Testing in a Simple Matrix

In part three of phase one, the effect of hydrogen peroxide on the enzymes was examined. Phenol, 2,4-dimethyl phenol, o-cresol, and p-cresol were added to de-ionized water to create a synthetic wastewater. To determine if the concentration of

hydrogen peroxide produced any variances in the reaction rate of laccase and peroxidase, tests were done using doses of: 600 mg/L, 300 mg/L, 150 mg/L, and 75 mg/L of hydrogen peroxide. The selected hydrogen peroxide dosage was combined with either 2600 units of peroxidase or 200 units of laccase and was added to synthetic wastewater. The samples were then tested for individual phenolic compound concentrations on the gas chromatograph in fifteen minute intervals for thirty minutes.

The first order rate constants for each compound and each hydrogen peroxidase dose were examined using laccase/peroxide treatment (Table 4.4). To determine the first order rate constant, the natural log of the concentration at time t, is plotted verses time t. The slope of the line created is equal to the first order rate constant. The governing equation is $\ln [A] = -kt + \ln [A_0]$, where A_0 equals the initial concentration, A equals the concentration at time t, and k is equal to the first order rate constant.

As seen in Table 4.4 the hydrogen peroxide concentration can affect the rate at which the phenol concentration is reduced. The R^2 values show how well the data fits the first order model. In this part of the study the fit was moderate (0.80) to very good (> 0.95). Laccase/peroxide treatment had the highest reduction rate constant when using 600 mg/L hydrogen peroxide to remove the phenolic compounds. The rate for 600 mg/L rate was on average 44 percent better than the rate for 300 mg/L of hydrogen peroxide, over two times higher than the rate when 150 mg/L of hydrogen peroxide was used, and over five times higher than the rate when 75 mg/L of hydrogen peroxide was used. Although 600 mg/L causes the laccase enzyme to

reduce the total phenolic compound concentrations the most, 300 mg/L is still an excess amount of hydrogen peroxide for the reduction to occur at a satisfying rate.

The effect of varying hydrogen peroxide concentration on the destruction of each of the phenolic compounds by laccase is shown in Figure 4.16. The first order rate constants, derived from the data presented in Figure 4.16, are summarized in Table 4.4. The first order rate constant increased as the initial hydrogen peroxide dose increased. The highest rate constant was determined for hydrogen peroxide concentration of 600 mg/L. This demonstrates the dependence of the degradation rate on peroxide dose. It should be noted that hydrogen peroxide doses above 100 ppm are considered impractical at the temperatures and pressures typically encountered in refineries due to safety concerns.

Table 4.4: First Order Rate Constants for Laccase Treatment of Phenol with Varied H_2O_2 Concentrations

Hydrogen Peroxide Concentration	**Phenol**	**O-cresol**	**P-cresol**	**2,4-Dimethyl Phenol**
600 mg/L	-0.0330 (R^2 = 0.93)	-0.0356 (R^2 = 0.95)	-0.0421 (R^2 = 0.91)	-0.0385 (R^2 = 0.88)
300 mg/L	-0.0268 (R^2 = 0.97)	-0.0209 (R^2 = 0.99)	-0.0307 (R^2 = 0.92)	-0.0269 (R^2 = 0.99)
150 mg/L	-0.0180 (R^2 = 0.80)	-0.0169 (R^2 = 0.92)	-0.0114 (R^2 = 0.97)	-0.0130 (R^2 = 0.97)
75 mg/L	-0.0092 (R^2 = 0.99)	-0.0067 (R^2 = 0.95)	-0.0054 (R^2 = 0.98)	-0.0097 (R^2 = 0.87)

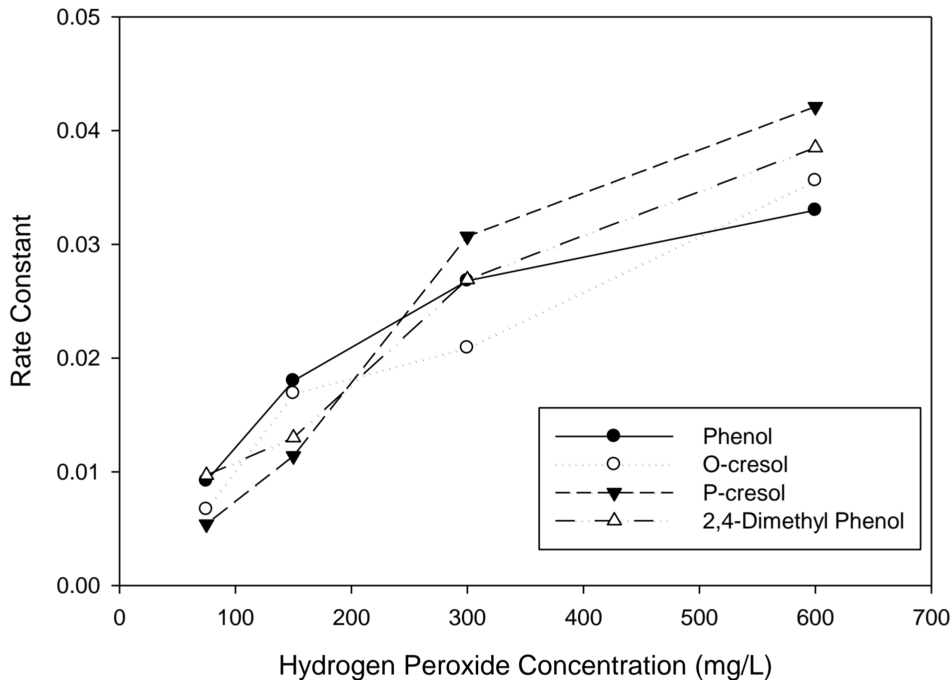

Figure 4.16: Rate Constants for Laccase/Peroxide with
Varied Hydrogen Peroxide Concentrations

The efficiency of the peroxidase enzyme to reduce the selected phenolic compounds over thirty minutes using varied hydrogen peroxide concentrations was also examined. The concentrations of hydrogen peroxide tested were again 600 mg/L, 300 mg/L, 150 mg/L, and 75 mg/L. The selected hydrogen peroxide dosage was combined with either 2600 units of peroxidase. The samples were then tested for individual phenolic compound concentrations on the gas chromatograph in fifteen minute intervals for thirty minutes.

The first order reduction rate constants for the peroxidase/peroxide treatment were examined. The rate constant for each individual phenolic compound for each hydrogen peroxide concentration is presented in Table 4.5. As before it is very clear that the hydrogen peroxide concentration can affect the rate at which the phenolic

compounds in the solution are oxidized or reduced. The R^2 values show how well the data fits the first order model. For the peroxidase/peroxide testing the fit to the first order model was moderate (0.84) to very good (> 0.95).

When using 600 mg/L of hydrogen peroxide the highest reduction rate for the Peroxidase/peroxide treatment removing the phenolic compounds was achieved. The rate for 600 mg/L rate was again on average 40 percent better than the rate for 300 mg/L of hydrogen peroxide. The rate for 600 mg/L hydrogen peroxide was also higher than that when 150 mg/L of hydrogen peroxide was used and significantly higher than the rate when 75 mg/L of hydrogen peroxide was used, approximately three times and just under four times respectively. Even though 600 mg/L causes the peroxidase enzyme to reduce the total phenolic compound concentration the most, 300 mg/L is still an excess amount of hydrogen peroxide for the reduction to occur at a satisfying rate.

The effect of varying hydrogen peroxide concentration on the destruction of each of the phenolic compounds by peroxidase is shown in Figure 4.17. The first order rate constants, derived from the data, are summarized in Table 4.5. The first order rate constant again increased as the initial hydrogen peroxide dose increased. The highest rate constant was determined for hydrogen peroxide concentration of 600 mg/L. This demonstrates the dependence of the degradation rate on peroxide dose.

Table 4.5: First Order Rate Constants for Peroxidase Treatment of Phenol with Varied H_2O_2 Concentrations

Hydrogen Peroxide Concentration	Phenol	O-cresol	P-cresol	2,4-Dimethyl Phenol
600 mg/L	-0.0399 ($R^2 = 0.99$)	-0.0370 ($R^2 = 0.99$)	-0.0314 ($R^2 = 0.91$)	-0.0224 ($R^2 = 0.84$)
300 mg/L	-0.0319 ($R^2 = 0.99$)	-0.0322 ($R^2 = 0.91$)	-0.0225 ($R^2 = 0.99$)	-0.0123 ($R^2 = 0.93$)
150 mg/L	-0.0214 ($R^2 = 0.99$)	-0.0072 ($R^2 = 0.99$)	-0.0105 ($R^2 = 0.95$)	-0.0079 ($R^2 = 0.94$)
75 mg/L	-0.0150 ($R^2 = 0.98$)	-0.0066 ($R^2 = 0.95$)	-0.0089 ($R^2 = 0.98$)	-0.0056 ($R^2 = 0.89$)

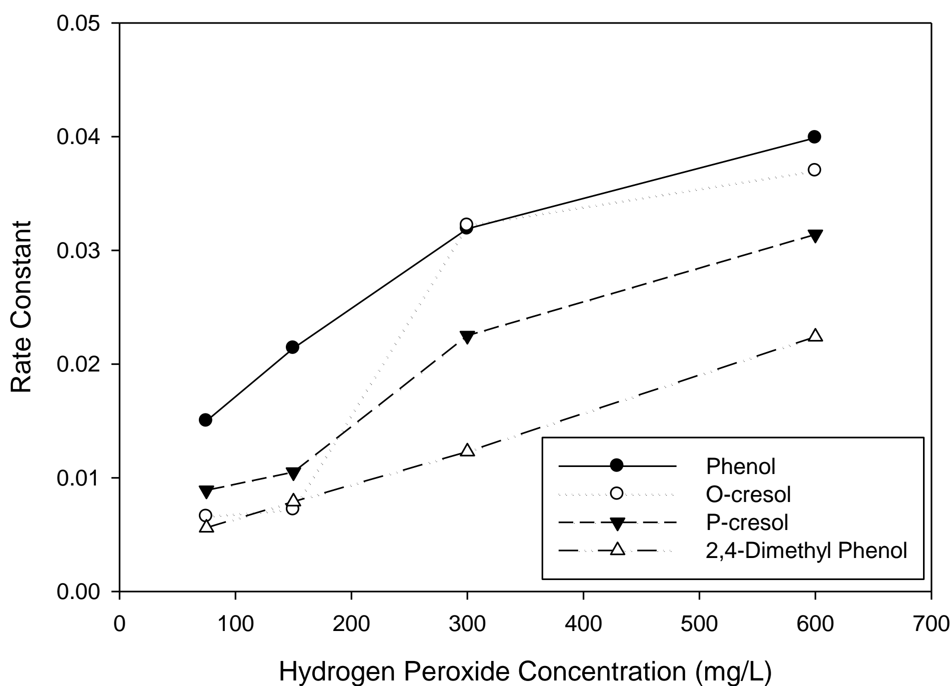

Figure 4.17: Rate Constants for Peroxidase/Peroxide with Varied Hydrogen Peroxide Concentrations

Phase Two: Refinery Wastewater

In the second phase of testing, the research was conducted to determine how well laccase and peroxidase removed phenols from refinery wastewaters, and if there were any effects from the other contaminants in the wastewater. The results of this phase of the study are presented in this section for each sample tested, as then summarized for all samples.

Frontier Stripped Sour Water

The efficacy of laccase enzyme for reducing phenolic compounds in stripped sour water from the Frontier refinery was examined. The concentration of major phenolic constituents was determined by gas chromatography. A dose of 200 units of laccase and 300 mg/L of hydrogen peroxide were added to the Frontier sour water sample and the concentration of the phenolic compounds were determined at fifteen minute intervals over a one hour test period. Six replicates of the frontier sour water were tested using the laccase enzyme. These six replicates were averaged and it was determined that the sour water initially contained 56.6 mg/L of phenol, 22.3 mg/L of o-cresol, 32.7 mg/L of p-cresol, and 1.6 mg/L of 2, 4-dimethy phenol. Thus, the total amount of phenols in the sample was 113.2 mg/L.

Table 4.6 contains the first order rate constants and R^2 values for both enzymes for each compound. Since the reaction follows first order kinetics, the rate constant for each reaction could be determined by plotting the natural log of the concentration verses time. In first order kinetics $\ln [A] = -kt + \ln [A_0]$, where A_0 equals the initial concentration, A equals the concentration at time t, and k is equal to

the first order rate constant. The first order rate constant is equal to the slope of the line given by ln [A], or natural log of the concentration, verses time.

Table 4.6: First Order Rate Constants for Laccase and Peroxidase in Frontier Sour Water

Compound	**Laccase k (min^{-1})**	**Peroxidase k (min^{-1})**
Phenol	-0.0122 (R^2 = 0.83)	-0.0135 (R^2 = 0.96)
O-cresol	-0.0085 (R^2 = 0.84)	-0.0083 (R^2 = 0.97)
P-cresol	-0.0060 (R^2 = 0.94)	-0.0048 (R^2 = 0.81)
2,4-Dimethyl Phenol	-0.0183 (R^2 = 0.82)	-0.0164 (R^2 = 0.87)

Figure 4.18 shows the reduction of phenol concentrations over time. The concentration of phenol decreased from 56.6 mg/L to 24.0 mg/L in 60 minutes. The overall reduction in phenol by laccase was 57.6 percent. It is important to note that only a small reduction in phenol was observed over the first 45 minutes of the reaction, and the majority of phenol oxidation occurred between 45 and 60 minutes. This differs from the testing with phenol in a "clean matrix" where phenol degradation occurred quickly (see Figure 4. __). As shown in Table 4.6, the first order reduction rate constant was found to be -0.0122 min^{-1} with R^2 equal to 0.83. The R^2 value indicates a moderate fit using first order kinetics.

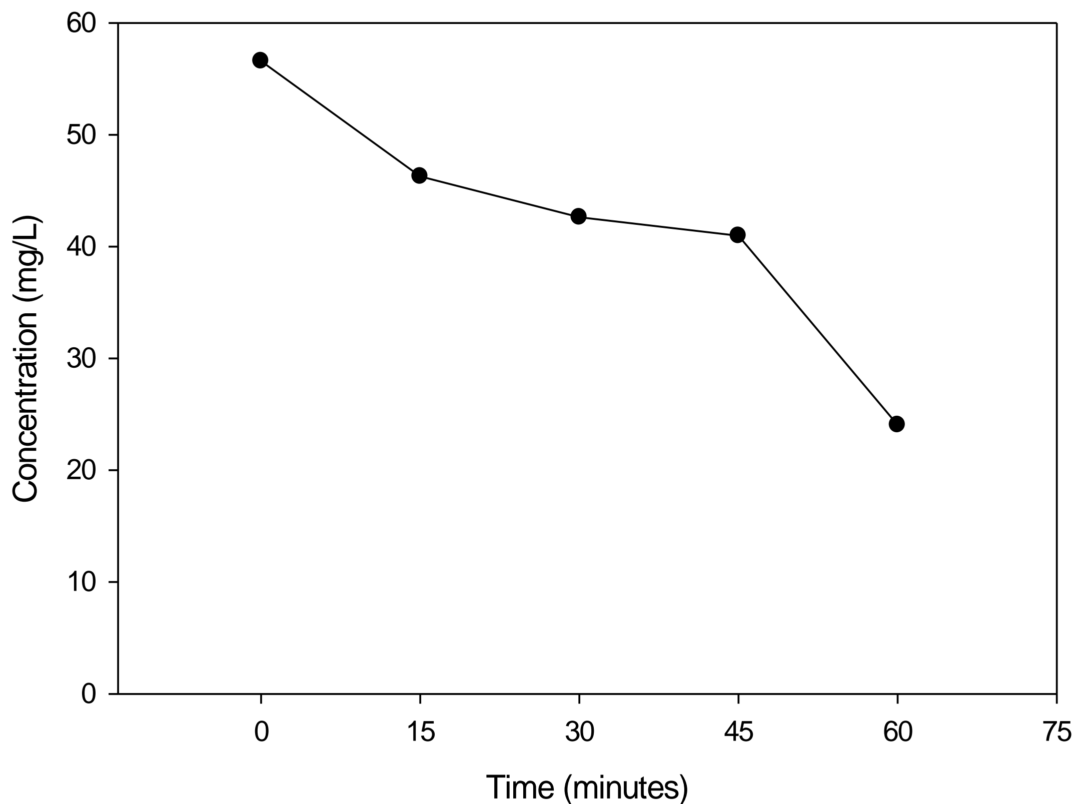

Figure 4.18: Laccase Treatment of Phenol in Frontier Stripped Sour Water

The reduction of o-cresol by laccase is presented in Figure 4.19. O-cresol was reduced by laccase from an initial concentration of 19.5 mg/L to 12.1 mg/L after 60 minutes of reaction time. The reduction of o-cresol by laccase treatment was 37.9 percent. Like for phenol, a majority of the reduction occurred after 30 minutes and was only a little over half of that observed for phenol. The first order rate constant for o-cresol was found to be -0.0085 min-1 with a R2 value of 0.84. The rate is lower than that of phenol.

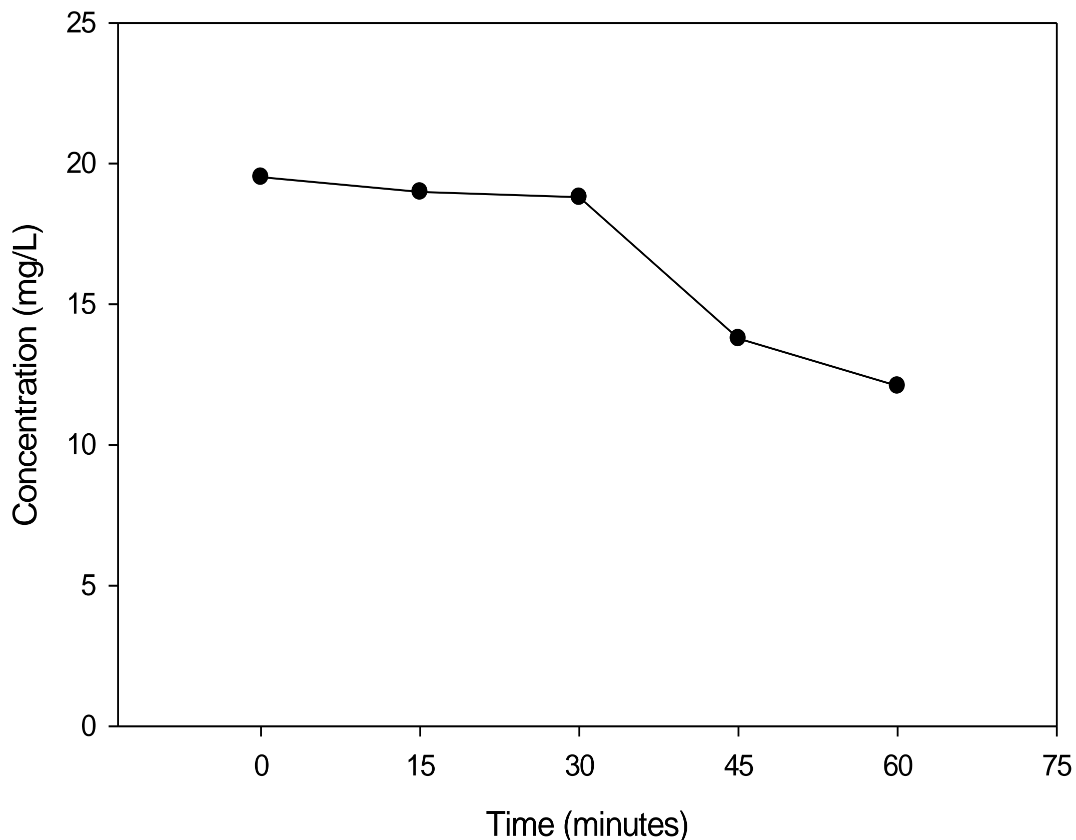

Figure 4.19: Laccase Treatment of O-cresol in Frontier Stripped Sour Water

For p-cresol, a 33 percent reduction in concentration was observed (Figure 4.20). The concentration decreased from an initial concentration of 32.7 mg/L to 21.9 mg/L after 60 minutes of reaction. The percent reduction was similar to that of o-cresol, and again close to half of the observed reduction of phenol. P-cresol had the lowest first order rate constants shown in Table 4.6. The observed rate constant for laccase enzyme's reduction of p-cresol was -0.0060 min^{-1} and the R^2 value was 0.94. The first order rate constant for laccase enzyme's reduction of p-cresol was the lowest observed for any individual phenolic compound.

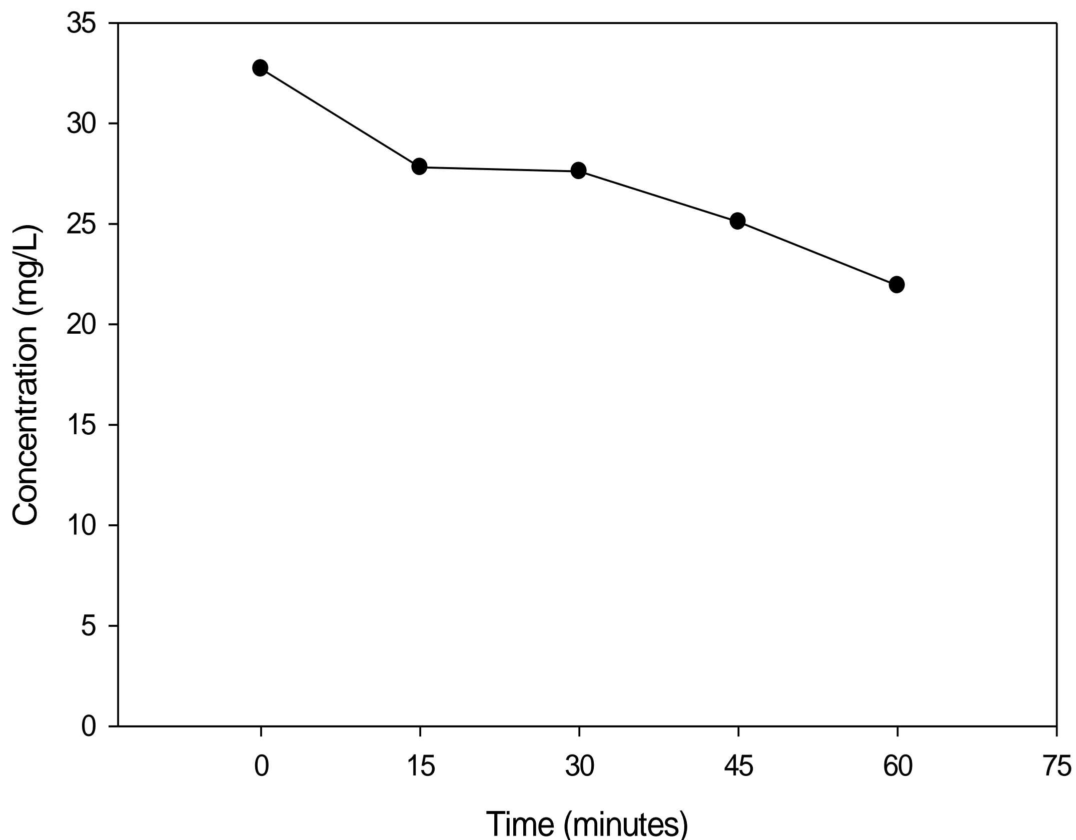

Figure 4.20: Laccase Treatment of P-cresol in Frontier Stripped Sour Water

Figure 4.21 shows the concentration over time for 2,4-dimethyl phenol. The concentration of 2, 4-dimethyl phenol was reduced from 1.6 mg/L to 0.54 mg/L over a period of 60 minutes. Laccase decreased the amount of 2, 4-dimthyl phenol by 66.3 percent. The percent reduction of 2, 4-dimethyl phenol closely matched that of phenol and was almost double that of o-cresol and p-cresol. The first order rate constant for the laccase enzyme's ability to remove phenolic compounds can be seen in Table 4.6. The reduction rate constant for reducing 2,4-dimethyl phenol was found to be -0.0183 min^{-1} with a R^2 value of 0.82. The rate for reducing 2,4-dimethyl phenol is the highest

for the monitored phenolic compounds, and more than double than the first order rate constant for reducing o-cresol and over three times that of p-cresol. However, this may be skewed by the lower initial concentration.

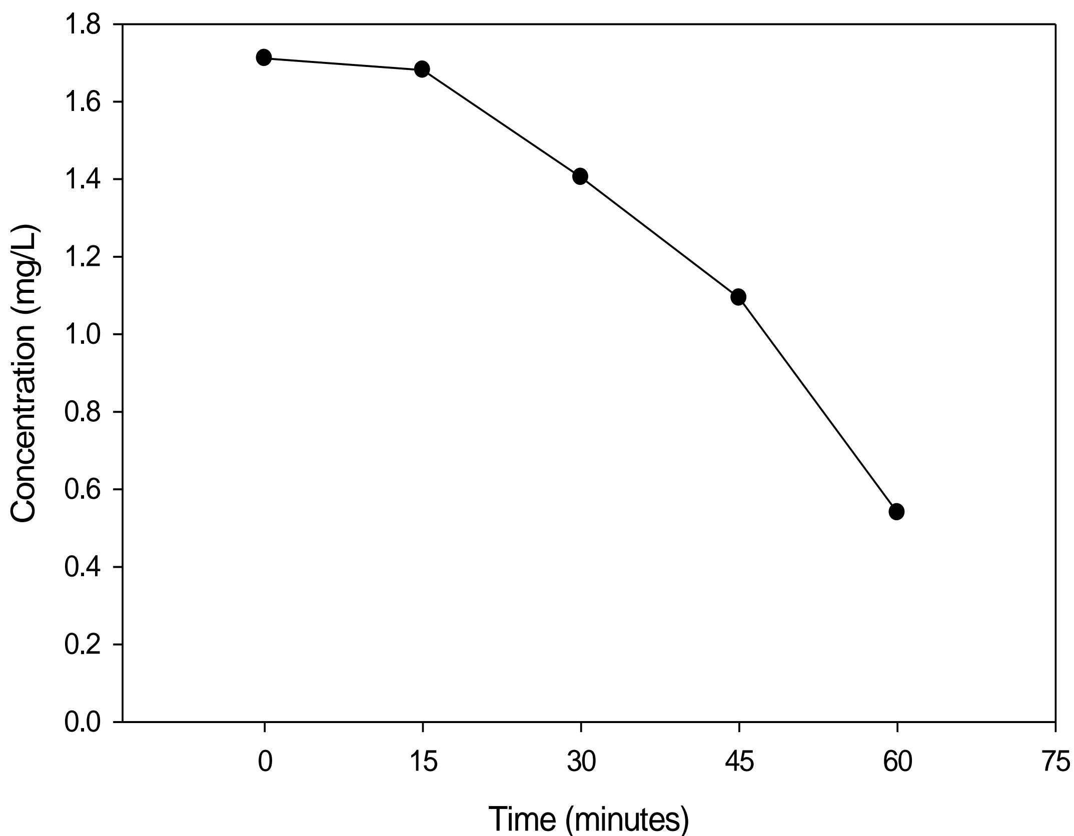

Figure 4.21: Laccase Treatment of 2,4-Dimethyl Phenol in Frontier Stripped Sour Water

The concentrations of each compound tested were normalized to show the percent remaining of each compound tested with the laccase enzyme (Figure 4.22). Phenol and 2,4-dimethyl phenol have the lowest remaining concentrations with 42.4 and 31.8 percent respectively. O-cresol and p-cresol were significantly higher

fractions remaining and nearly double that of phenol and 2,4-dimethyl phenol. The percent remaining for o-cresol and p-cresol were 62.1 and 67 percent respectively. One important thing to note from these tests using laccase is that the majority of the phenolic reduction occurs between the reaction time of 30 and 60 minutes. This is different than for the clean system, where phenolic reduction occurred during the first 15 minutes. One likely explanation is that laccase/peroxide was initially expended oxidizing other wastewater constituents.

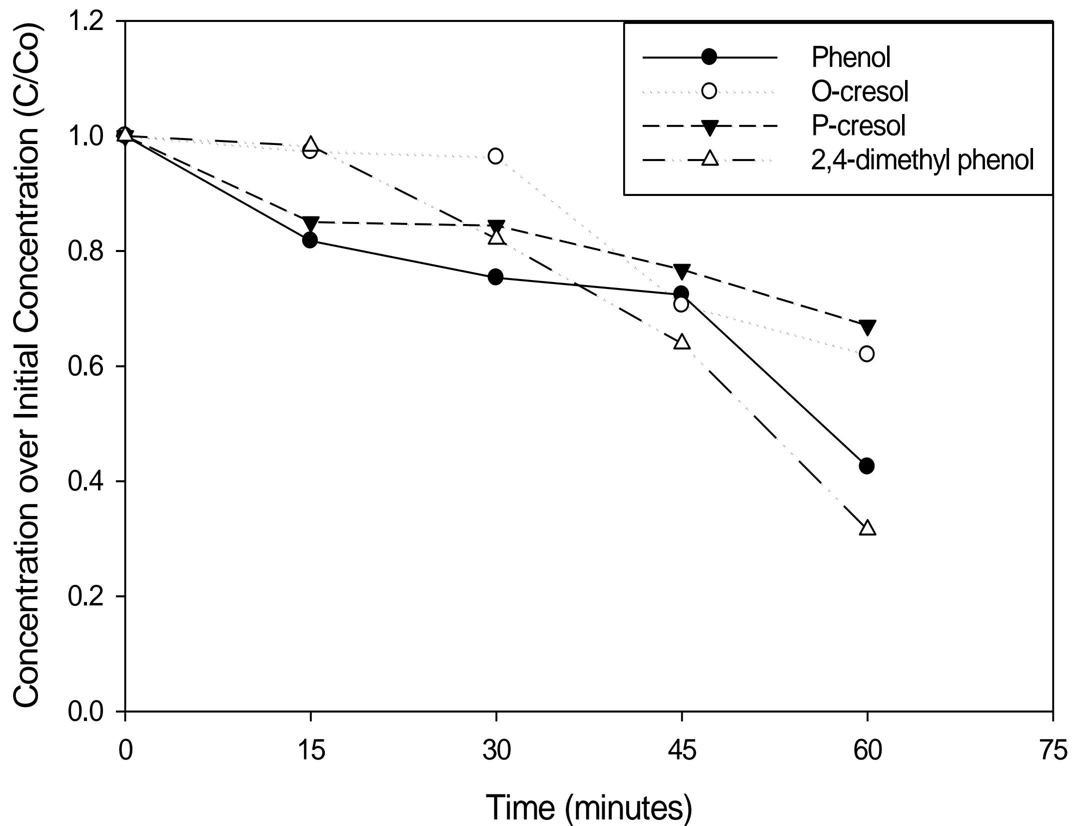

Figure 4.22: Fraction Remaining of Phenolic Compounds using Laccase Treatment

The Frontier sour water was treated with the peroxide enzyme to determine the efficiency for reducing phenolic compounds from the wastewater. An initial dose of 2600 units of peroxidase where added along with 300 mg/L of hydrogen peroxide to the sour water and the concentrations where measured with the gas chromatograph over fifteen minute intervals. As before, six replicate samples were tested using the peroxidase enzyme and the average initial concentrations contained in the sour water were: 35.8 mg/L of phenol, 18.2 mg/L of o-cresol, 24.1 mg/L of p-cresol, 1.9 mg/L of 2, 4-dimethyl phenol, and 77.1 mg/L total phenol. The first order rate constants were also determined for peroxidase and are shown in Table 4.6 as well.

The average concentration of phenol in the Frontier sour water was lowered from 35.8 mg/L to 15.6 mg/L over one hour (Figure 4.23). Peroxidase reduced the amount of phenol in the sour water by 56.4 percent. The first order rate constant for reducing phenol was found to be -0.0135 min-1 with a value of 0.96 for R^2 (Table 4.6). The R^2 value indicates a very good fit with the first order model. Unlike, the laccase enzyme, peroxidase catalyzed a rapid oxidation of phenol with significant reduction in concentration occurring over the first 30 minutes.

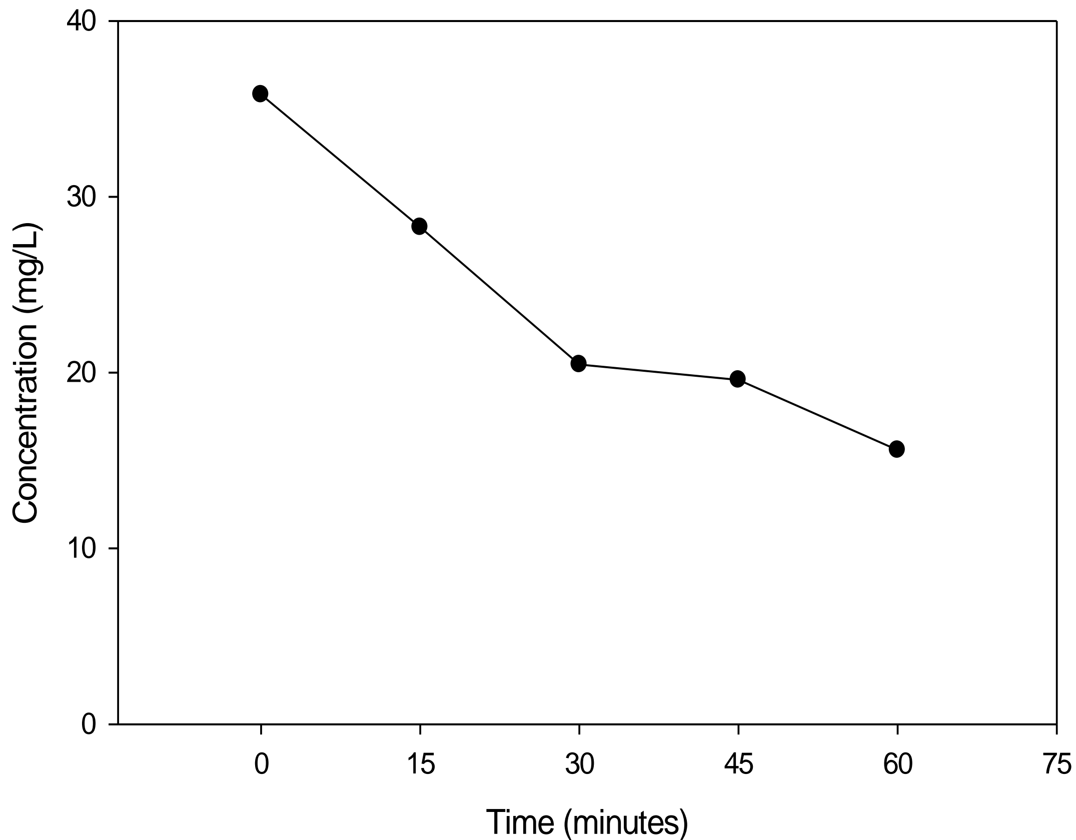

Figure 4.23: Peroxidase Treatment of Phenol in Frontier Stripped Sour Water

The reduction of o-cresol in Frontier sour water by peroxidase is shown in Figure 4.24. O-cresol was found to have an initial concentration of 18.2 mg/L. Over a reaction time of 60 minutes, peroxidase reduced the amount of o-cresol from 18.2 mg/L to 11.2 mg/L. Corresponding to a 38.5 percent decrease in concentration. The first order rate constant for peroxidase reduction of o-cresol was found to be -0.0083 min^{-1} with R^2 equal to 0.97 (Table 4.6). The reduction rate constant is over one and a half times lower than that of phenol. Again the first order model yields a good fit for the data.

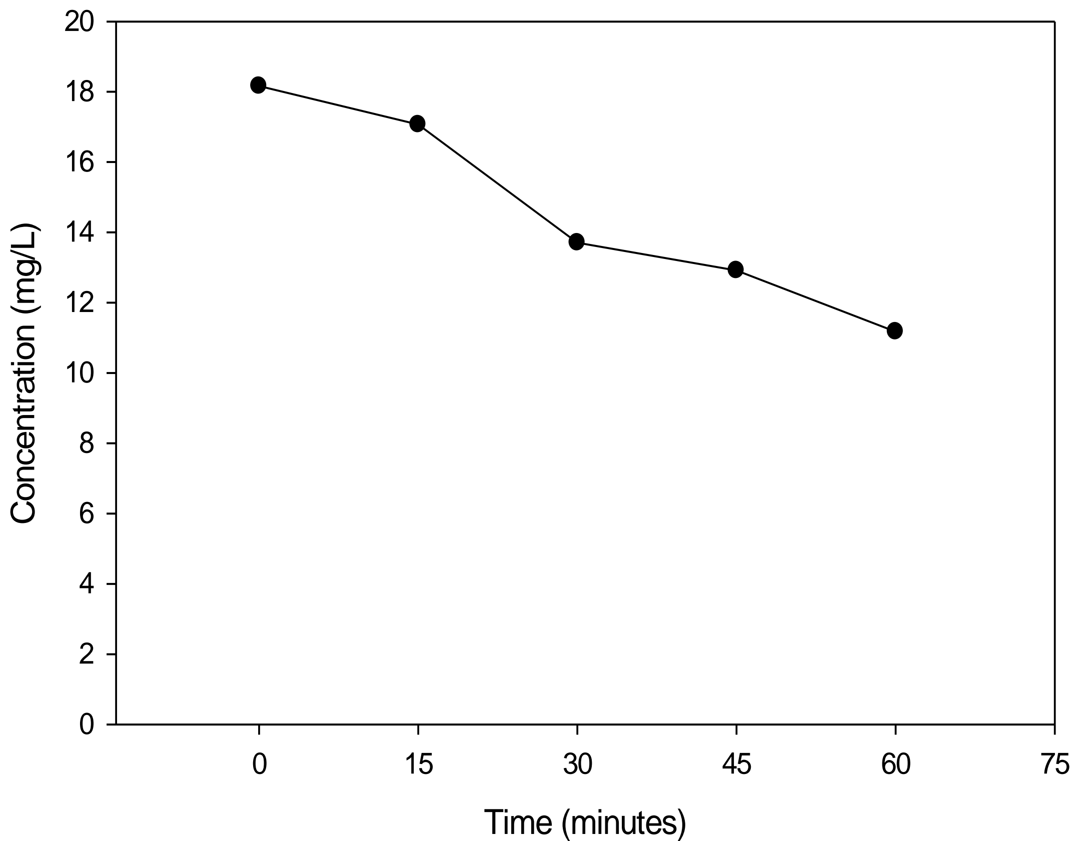

Figure 4.24: Peroxidase Treatment of O-cresol in Frontier Stripped Sour Water

The decrease in p-cresol in Frontier sour water by peroxidase over time can be seen in Figure 4.25. P-cresol concentration was reduced from an initial concentration of 24.1 mg/L to a concentration of 17.1 mg/L (29 percent) over the one hour reaction period. Once again, the peroxidase enzyme produced significant reduction in p-cresol over the first 30 minutes of reaction. This is different from the results determined using laccase, where the majority of the reduction occurred during the last 15 to 30 minutes of the reaction. The first order rate constant for peroxidase reduction of p-

cresol was found in Table 4.6 was -0.0048 min^{-1} with R^2 equal to 0.81. The reduction rate constant for p-cresol is almost three times lower than that of phenol.

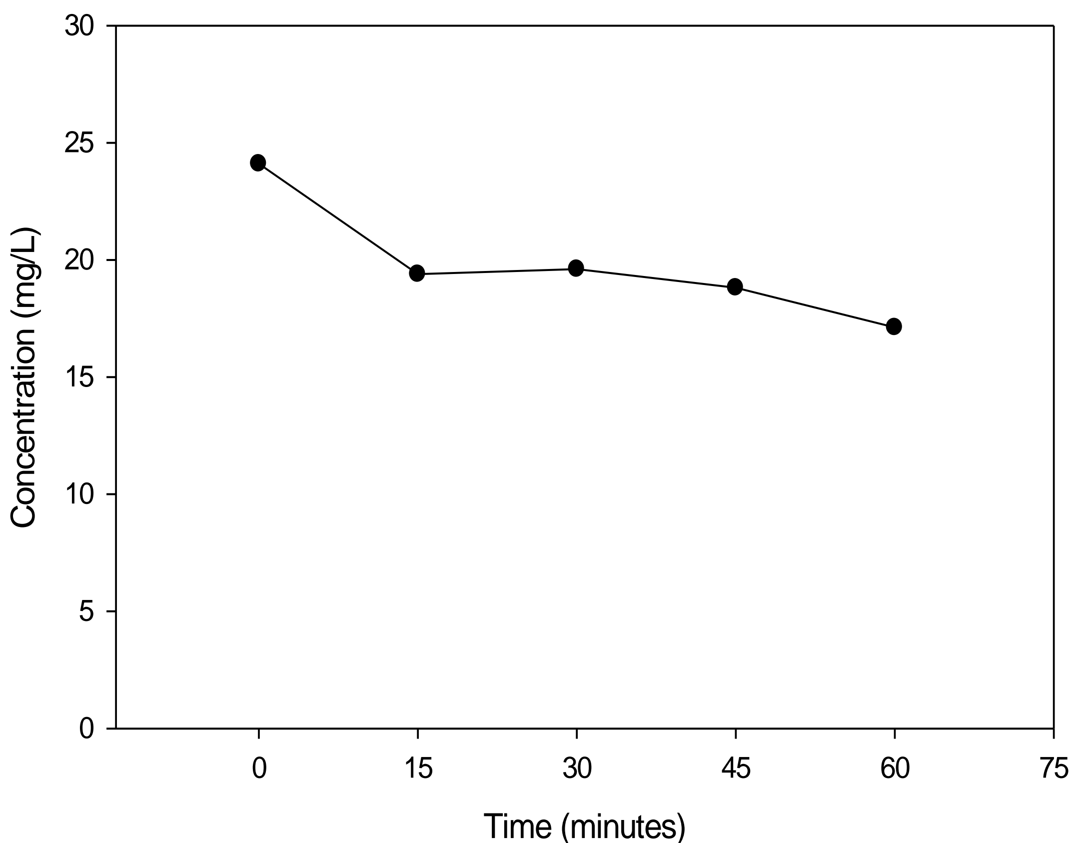

Figure 4.25: Peroxidase Treatment of P-cresol in Frontier Stripped Sour Water

The peroxidase enzyme was able to remove 66.3 percent of the 2,4-dimethyl phenol in the Frontier stripped sour water (Figure 4.26). The concentration of 2,4-dimethyl phenol was reduced from 1.9 mg/L to .64 mg/L over a 60 minute reaction time. The decrease in concentration for 2,4-dimethyl phenol was the highest of the four compounds tested at 67 percent, and almost double that of p-cresol and o-cresol. However, it is important to note that 2,4-dimethyl phenol made up only a small

fraction of the total phenolics in the sample. The first order rate constant found in Table 4.6 for peroxidase enzyme's reduction of 2,4-dimethyl phenol is -0.0164 min-1 with a R2 value of 0.87. The peroxidase reduction rate for 2,4-dimethyl phenol is the highest rate for peroxidase in this particular refinery matrix.

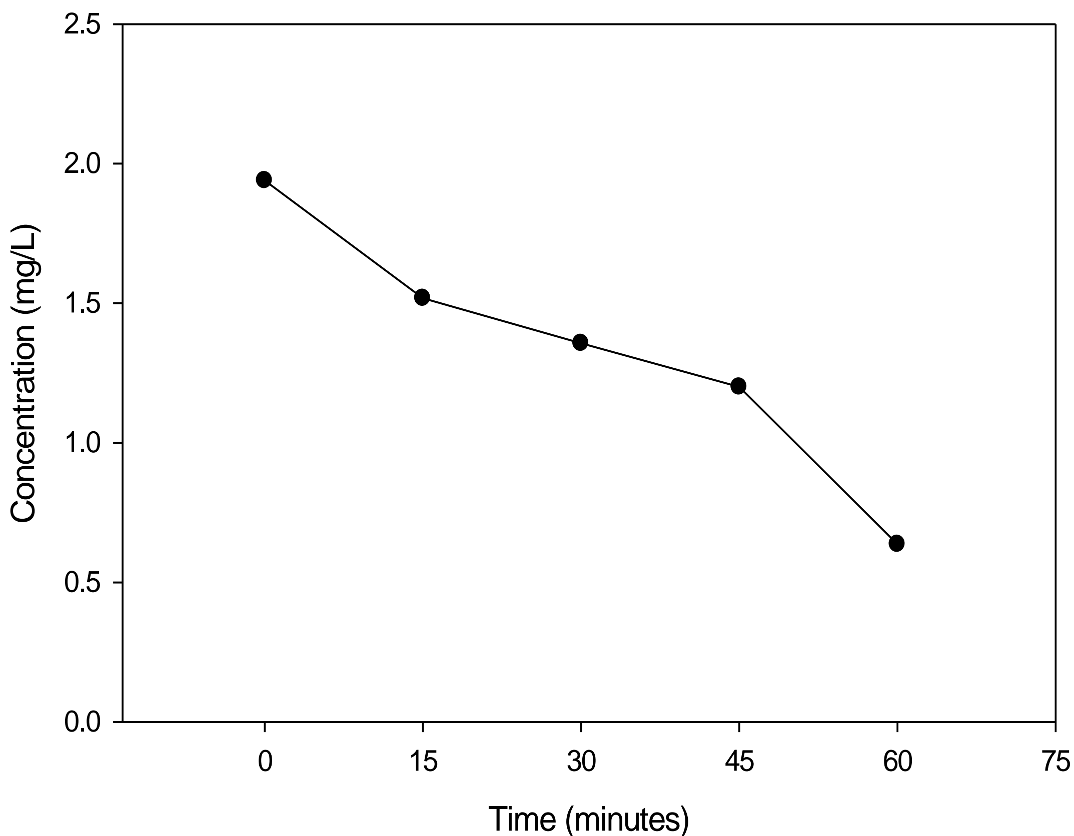

Figure 4.26: Peroxidase Treatment of 2, 4-Dimethyl Phenol in Frontier Stripped Sour Water

In Figure 4.27 the concentrations of each compound are normalized to show the percent remaining of each compound. Phenol and 2,4-dimethyl phenol had the lowest remaining concentrations with 43.6 and 33 percent respectively. Since phenol is the largest contributor to the total phenolics in the sample, the high fraction

removed is much more important than that observed for 2,4-dimethyl phenol. One important observation from the graph is that with the peroxidase enzyme the majority of the reduction occurs during the first 30 minutes of the reaction. This is quite different than the trend observed for the laccase/peroxide treatment system.

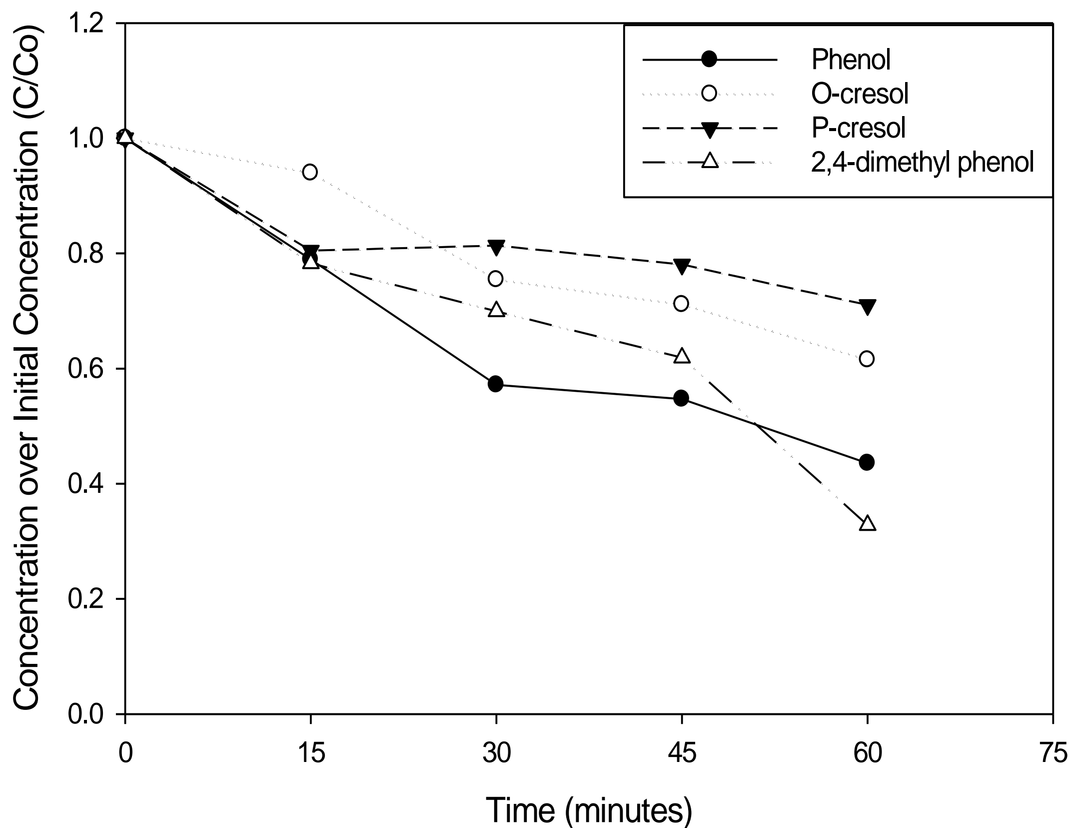

Figure 4.27: Fraction Remaining of Phenolic Compounds using Peroxidase Treatment

CITGO Stripped Sour Water

Stripped sour water from CITGO's Lake Charles Refinery in Louisiana was used to test the laccase enzyme's removal efficiency of phenolic compounds. An initial dose of 200 units of laccase and 300 mg/L of hydrogen peroxide were added to

the sample and the concentrations of phenol, o-cresol, p-cresol, and 2,4-dimethyl phenol were determined at fifteen minute intervals. Six replicate sour water tests were run using the laccase enzymatic treatment. The CITGO sour water was found to contain an average of 63.7 mg/L of phenol, 14.6 mg/L of o-cresol, 36.8 mg/L of p-cresol, and 2.1 mg/L of 2,4-dimethyl phenol. The total amount of phenols in the CITGO stripped sour water was averaged 117.2 mg/L.

To better compare the reduction of laccase and peroxidase the first order rate constants for each was examined. These first order rate constants are found in Table 4.7. In first order kinetics $\ln [A] = -kt + \ln [A_0]$, where A_0 equals the initial concentration, A equals the concentration at time t, and k is equal to the first order rate constant. The first order rate constant is equal to the slope of the line given by ln [A], or natural log of the concentration, verses time.

Table 4.7: First Order Rate Constants for Laccase and Peroxidase in CITGO Stripped Sour Water

Compound	**Laccase k (min^{-1})**	**Peroxidase k (min^{-1})**
Phenol	-0.0100 (R^2 = 0.94)	-0.0125 (R^2 = 0.95)
O-cresol	-0.0058 (R^2 = 0.96)	-0.0043 (R^2 = 0.96)
P-cresol	-0.0065 (R^2 = 0.92)	-0.0063 (R^2 = 0.95)
2,4-Dimethyl Phenol	-0.0122 (R^2 = 0.94)	-0.0122 (R^2 = 0.95)

The change over time in phenol concentration cause by laccase/peroxide treatment can be seen in Figure 4.28. The initial phenol concentration was 63.7 mg/L for the CITGO stripped sour water, and was reduced to 36.6 mg/L over a 60 minute reaction time. Thus the phenol concentration was lowered by 42.5 percent. Little

reduction in phenol occurred over the first 15 minutes. The first order rate constant for this 42.5 percent reduction in phenol by laccase was found to be -0.0101 with a R^2 equal to 0.94.

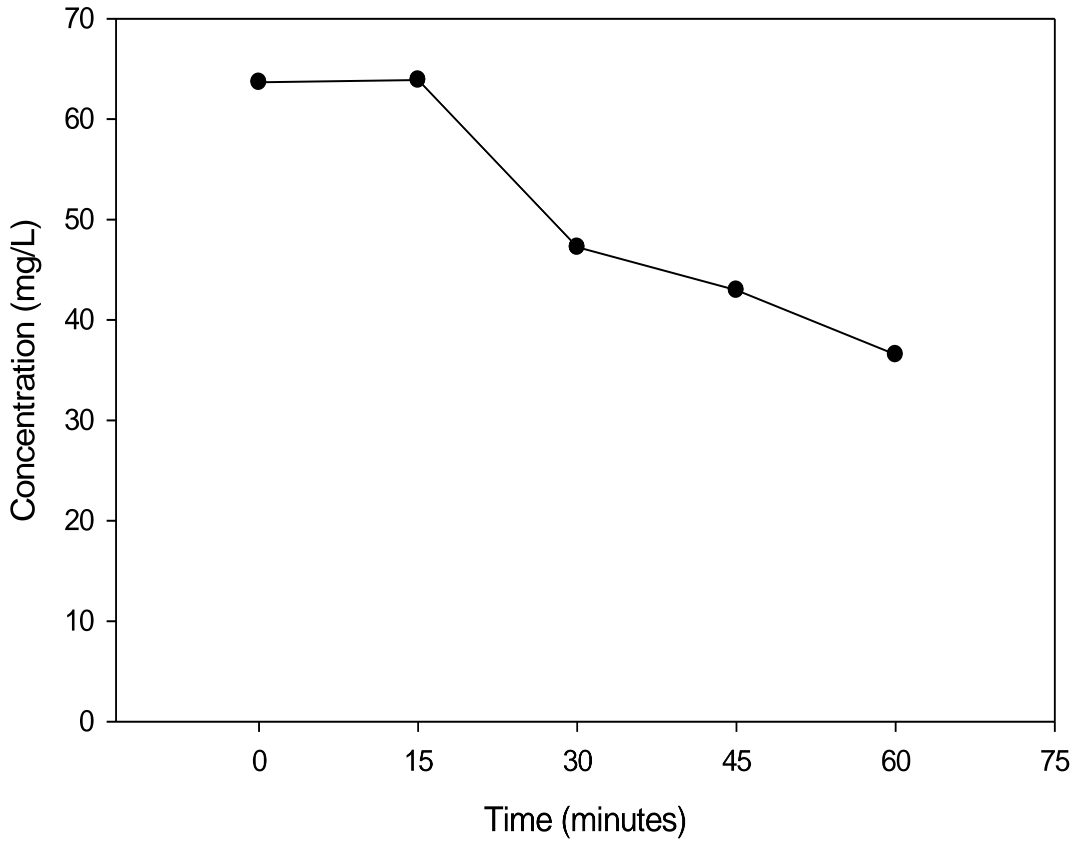

Figure 4.28: Laccase Treatment of Phenol in CITGO Stripped Sour Water

Shown in Figure 4.29 is the concentration of o-cresol over the 60 minute reaction time. The concentration of o-cresol decreased from 14.6 mg/L to 10.6 mg/L in 60 minutes. The reduction in o-cresol caused by laccase/peroxide treatment was 27.4 percent. As for phenol, little reduction of o-cresol occurred during the first 15 minutes of the reaction. The first order rate constant for laccase reducing o-cresol was

found to be -0.0058 min^{-1} with an R^2 value of 0.96 (Table 4.7). The laccase enzyme's first order reduction rate constant for reducing o-cresol was nearly half that of phenol.

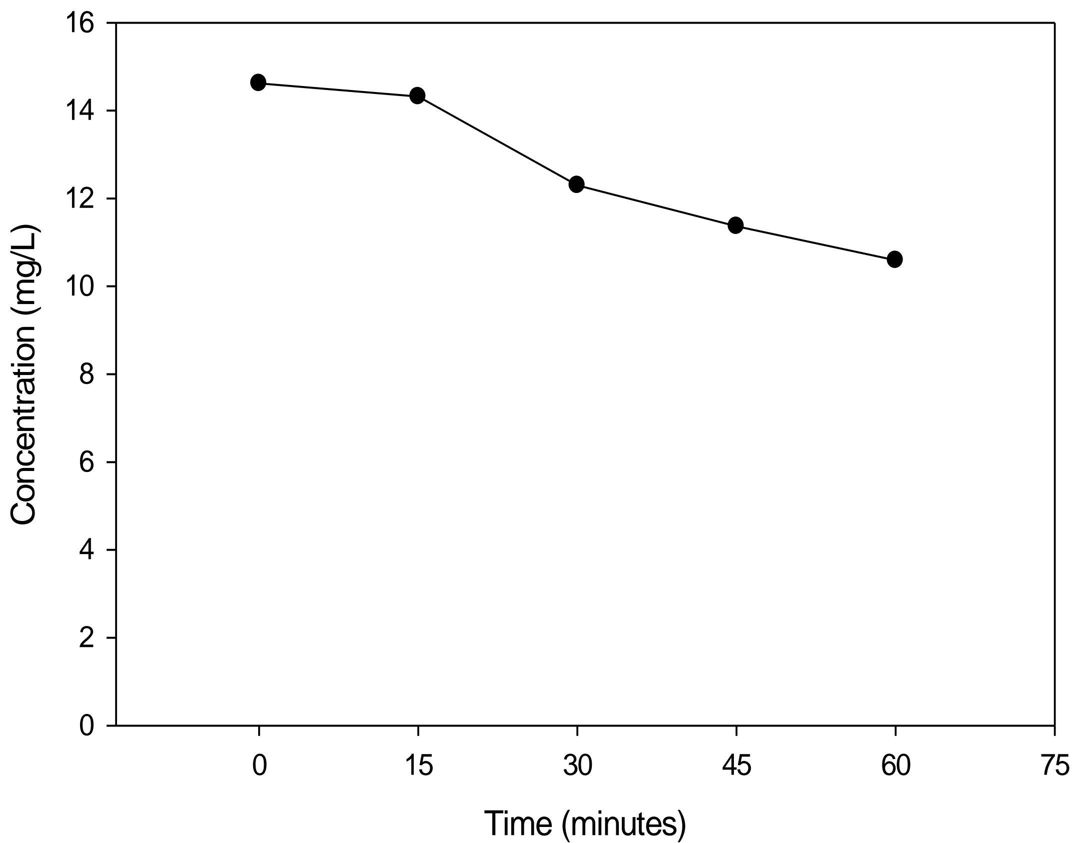

Figure 4.29: Laccase Treatment of O-cresol in CITGO Stripped Sour Water

The p-cresol concentration over time is shown in Figure 4.30. P-cresol concentration decreased 32.9 percent over the 60 minutes when using laccase/peroxide treatment. The stripped sour water was found to have an initial concentration of 36.8 mg/L and that was reduced by laccase to 24.7 mg/L. The percent reduced was very similar to that of o-cresol, and somewhat lower than that of

phenol. The first order rate constant for p-cresol reduced by laccase was found to be -0.0065 min^{-1} with an R^2 value of 0.92 (Table 4.7). The rate constants for laccase and peroxidase for reducing p-cresol were essentially equal; therefore the enzymes are equally effective. Although the first order reduction rate constant for p-cresol was equivalent to that of o-cresol, the rate was nearly half that of phenol.

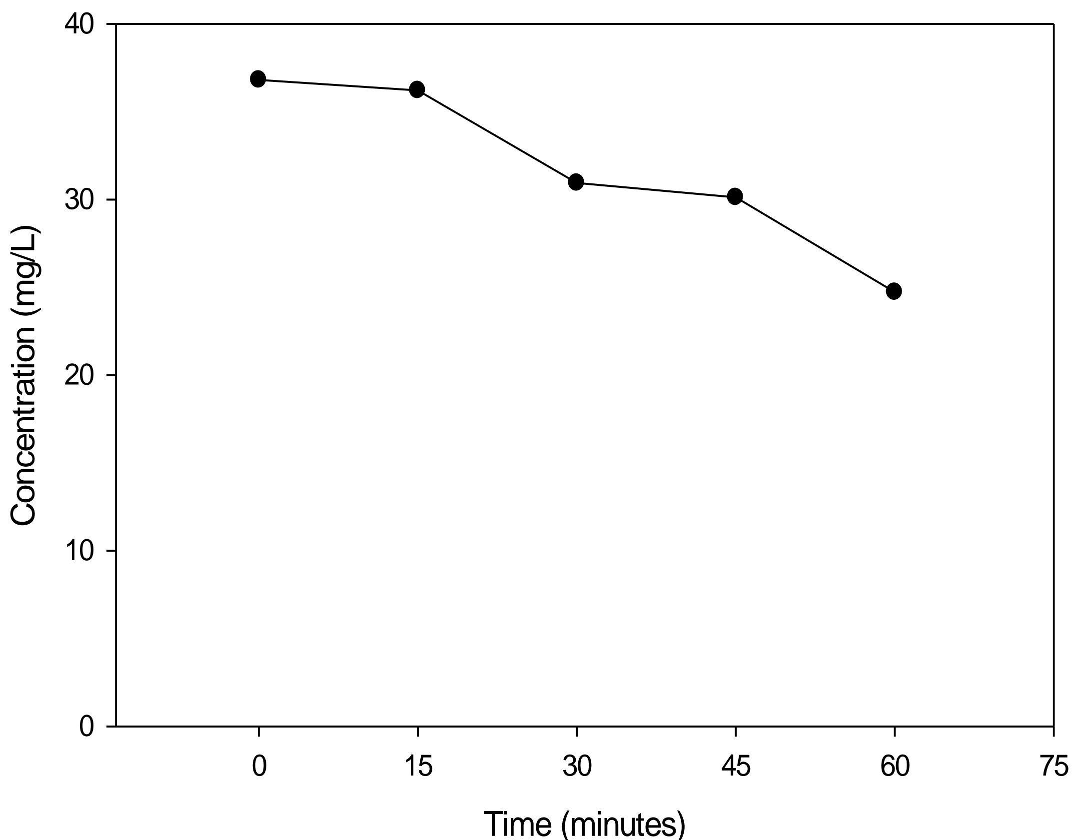

Figure 4.30: Laccase Treatment of P-cresol in CITGO Stripped Sour Water

The reduction of 2,4-dimethyl phenol by laccase is shown in Figure 4.31. 2,4-dimethyl phenol was lowered by laccase treatment from a concentration of 2.11 mg/L to 1.02 mg/L after 60 minutes of reaction time. The reduction of 2,4-dimethyl phenol

was 51.7 percent. Although 2,4-dimethyl phenol was had the highest percent reduction by the laccase enzyme, since phenol is a much more significant component of the wastewater, its reduction is much more important. The first order rate constant for the oxidation of 2,4-dimethyl phenol by laccase and hydrogen peroxide is -0.0122 min-1 with R2 equal to .94 and can be found in Table 4.7. The laccase reduction rate for 2,4-dimethyl phenol was almost double the rate for o-cresol and p-cresol, and about 25 percent higher than the oxidation rate of phenol.

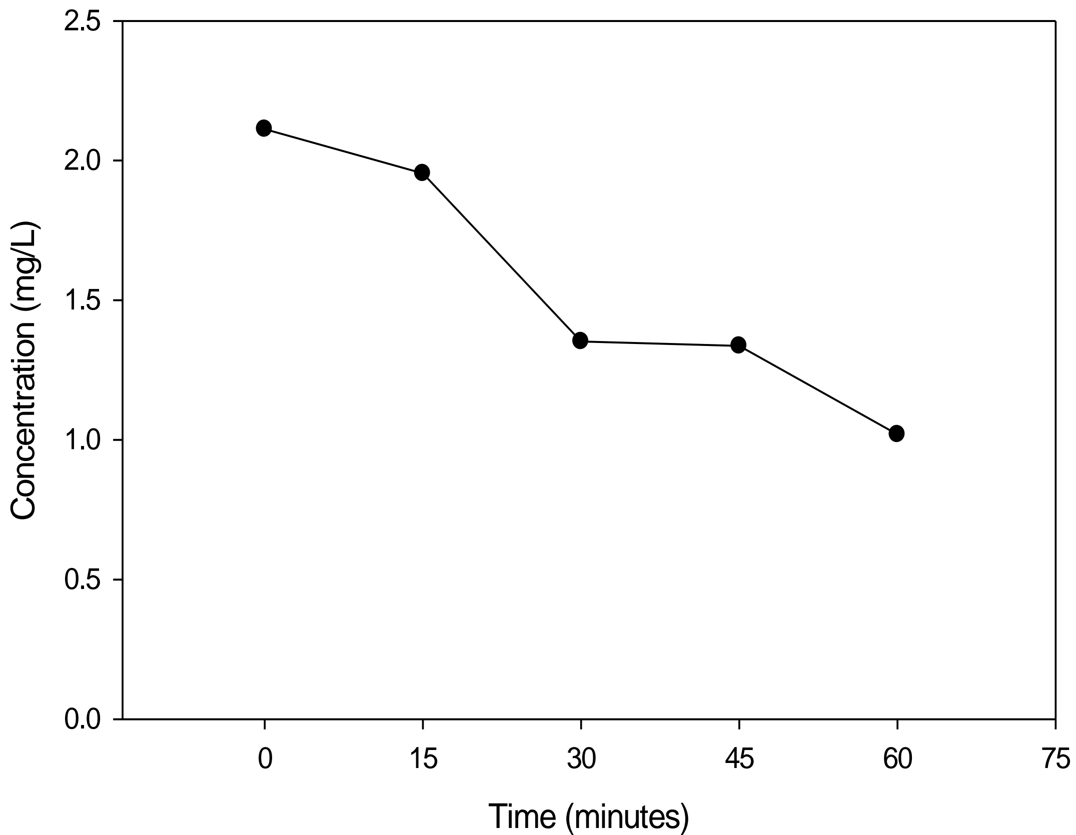

Figure 4.31: Laccase Treatment of 2,4-Dimethyl Phenol in CITGO Stripped Sour Water

In Figure 4.32 the concentrations of each compound are normalized to show the percent remaining of each compound. The compounds with the highest percent remaining were o-cresol and p-cresol as they were significantly higher than that of phenol and 2,4-dimethyl phenol. Phenol and 2,4-dimethyl phenol had the lowest remaining concentrations with 57.5 and 47.6 percent respectively. One important point seen in the graph is that the major drop in concentrations occurred after 15 minutes, and this lay in total phenols removed was not observed in the clean system.

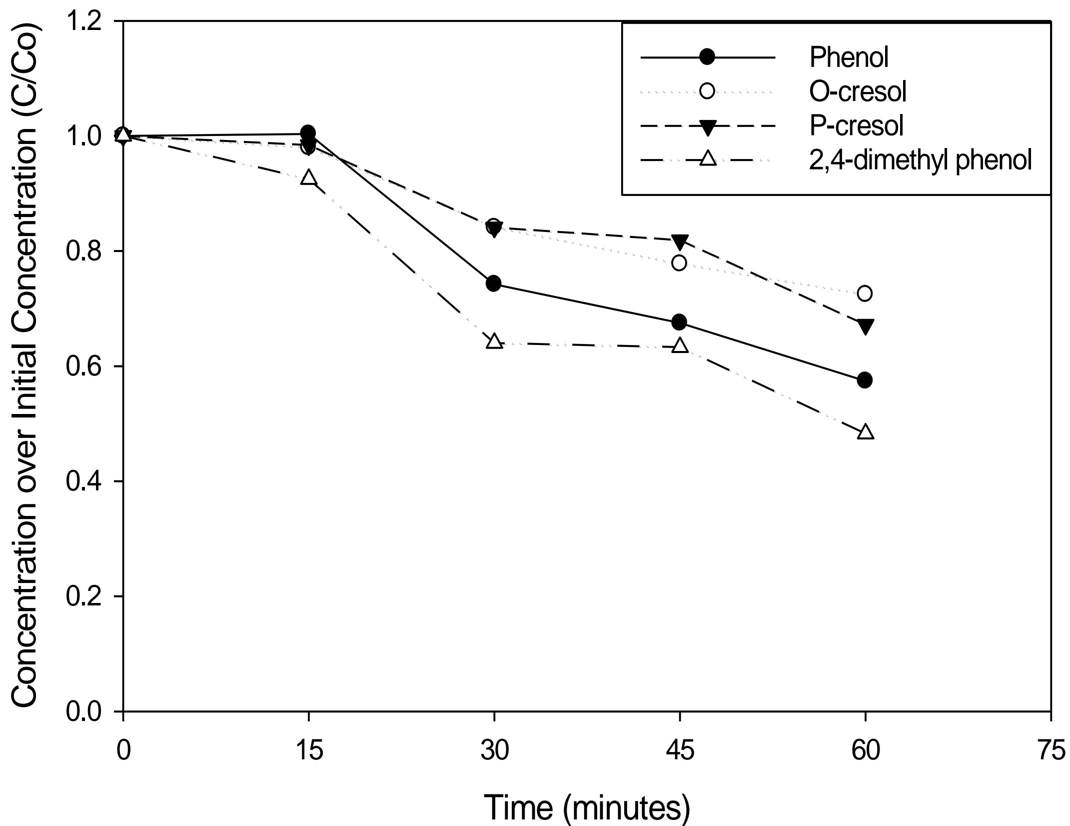

Figure 4.32: Fraction Remaining of Phenolic Compounds using Laccase Treatment on CITGO Stripped Sour Water

The effectiveness of peroxidase enzyme for decreasing concentrations of phenolic compounds in stripped sour water from CITGO's Lake Charles Refinery in Louisiana was studied. An enzyme dose of 2600 units of peroxidase and 300 mg/L of hydrogen peroxide were added to the sample and the concentrations of individual phenolic compounds were determined at fifteen minute intervals. Six replicate sour water tests were conducted using the peroxidase for enzymatic treatment. The CITGO sour water was found to initially contain an average of 51.9 mg/L phenol, 10.1 mg/L o-cresol, 24.5 mg/L p-cresol, and 1.9 mg/L 2,4-dimethyl phenol. The total average amount of phenolic compounds in the stripped sour water was 88.3 mg/L.

The reduction of phenol by peroxidase over time is shown in Figure 4.33. Phenol was decreased, by peroxidase/peroxide treatment, from a concentration of 51.9 mg/L to 24.9 mg/L after 60 minutes. The one hour reduction of phenol was 52 percent. The first order rate constant for peroxidase treatment of phenol was determined to be -0.0125 min^{-1} with a R^2 value of 0.95 (Table 4.7). There was very little reduction in phenol over the first 15 minutes of the reaction. Linear reduction in phenol was observed after 15 minutes.

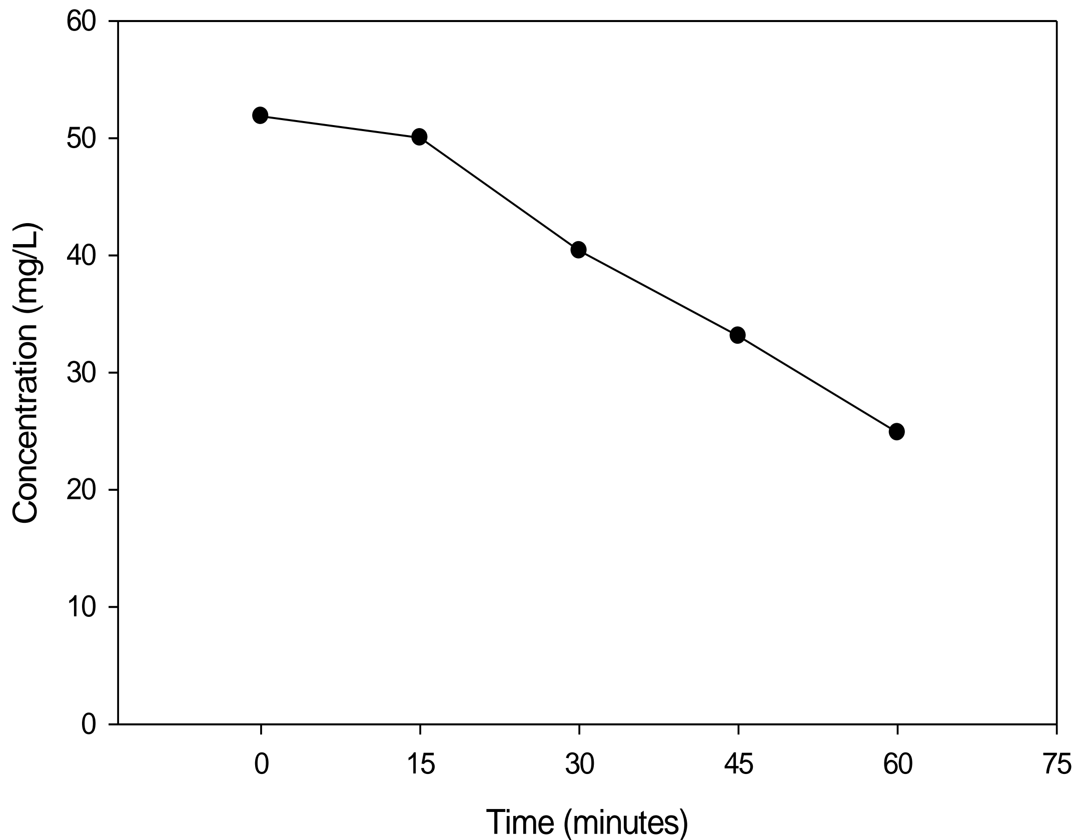

Figure 4.33: Peroxidase Treatment of Phenol in CITGO Stripped Sour Water

The concentrations over time of o-cresol in CITGO stripped sour water by peroxidase/peroxide treatment are shown in Figure 4.34. The concentration decreased from 10.1 mg/L initially to 7.8 mg/L (22.8 percent) after 60 minutes time. The rate of o-cresol change was consistent over the 60 minute test period. The percent reduction was less than half of that observed for phenol. The first order rate constant for peroxidase reduction of o-cresol was -0.0043 min^{-1} with R^2 equal to 0.96 (Table 4.7). The reduction rate constant for o-cresol is almost three times lower than that of phenol.

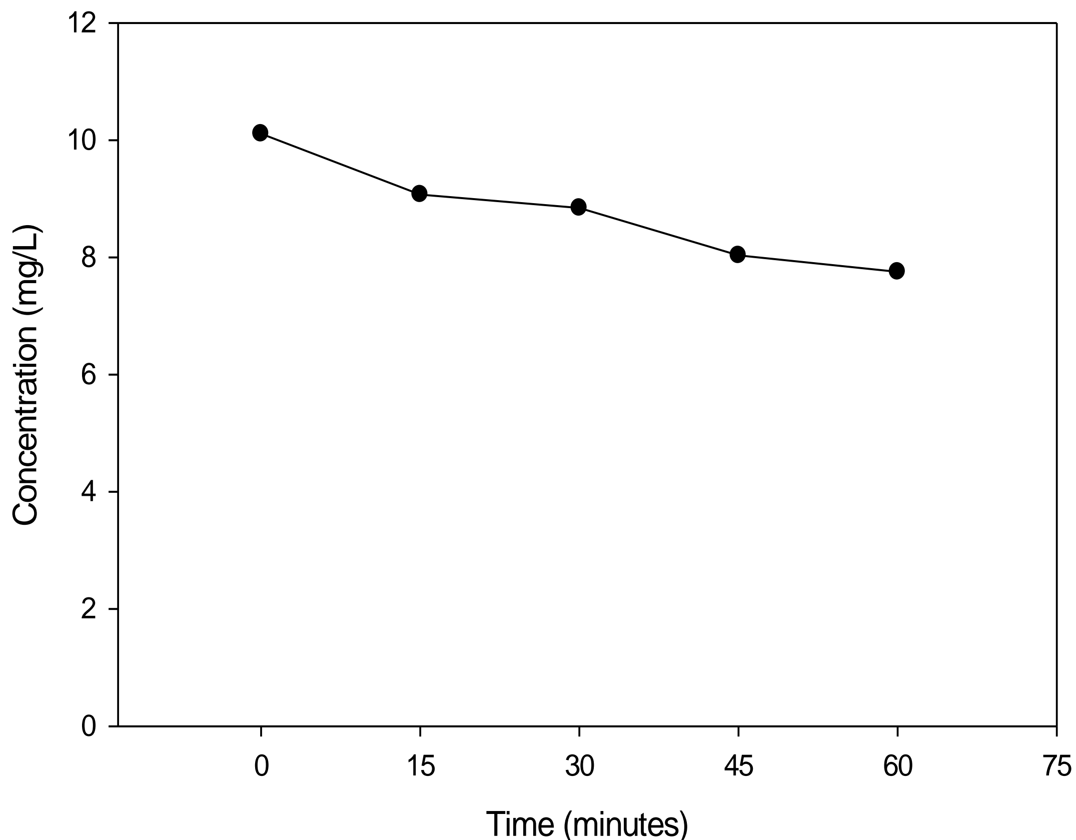

Figure 4.34: Peroxidase Treatment of O-cresol in CITGO Stripped Sour Water

The decrease in p-cresol concentration over time by peroxidase oxidation is shown in Figure 4.35. The peroxidase enzyme lowered the concentration of p-cresol from 24.5 mg/L to 16.8 mg/L. This corresponds to a reduction in p-cresol by peroxidase of 31.4 percent. The percent reduction of p-cresol is comparable to o-cresol, and is almost half that of phenol.. The first order rate constant for peroxidase reduction of p-cresol was found to be -0.0063 min^{-1} with R^2 equal to 0.95 (Table 4.7). This rate constant was slightly higher than the first order rate constant for peroxidase reduction of o-cresol. The reduction rate constant for p-cresol is about two times

lower though that that of phenol. P-cresol degradation began during the first 15 minutes of the reaction.

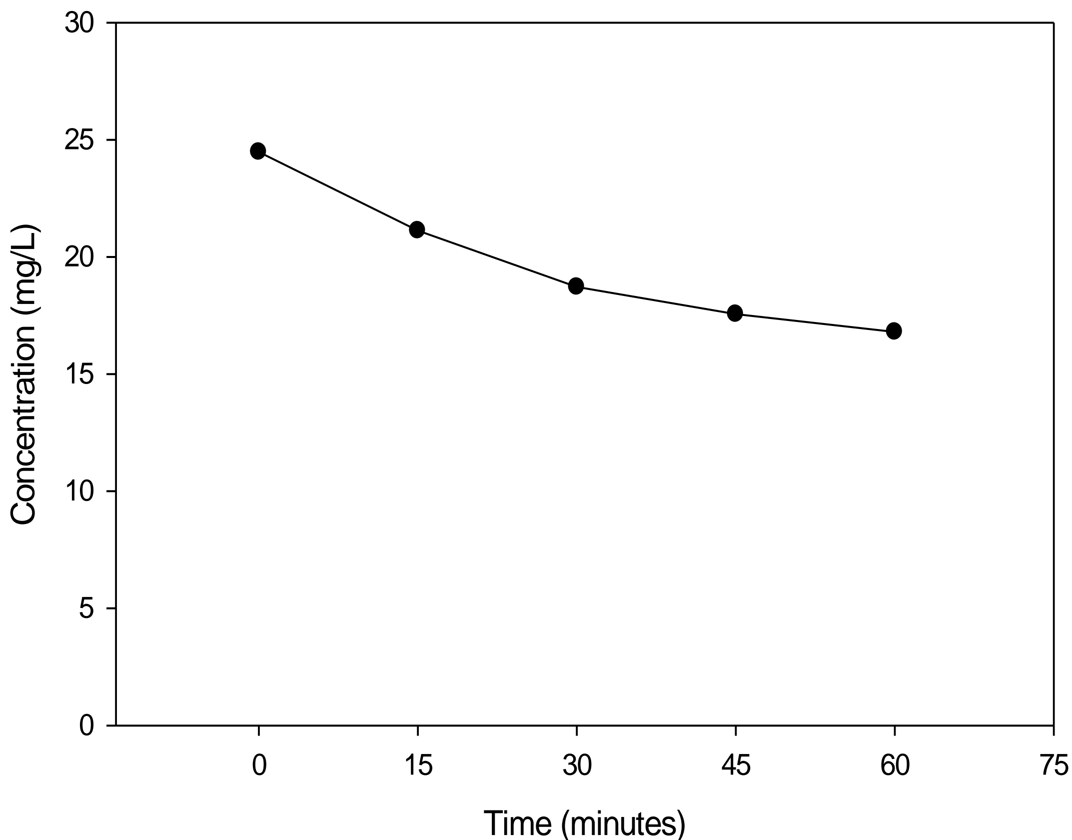

Figure 4.35: Peroxidase Treatment P-cresol in CITGO Stripped Sour Water

2,4-dimethyl phenol was lowered by peroxide treatment from a concentration of 1.87 mg/L to 0.92 mg/L after 60 minutes (Figure 4.36). The reduction of 2,4-dimethyl phenol was 50.8 percent. Most of the 2,4-dmp degradation occurred during the first 30 minutes of reaction. The percent reduction is close to that of phenol. However, there was much more phenol in the waste than 2,4-dmp. The first order rate constants for each compound in the synthetic wastewater are shown in Table 4.7. The

first order rate constant for 2,4-dimethyl phenol was found to be -.0162 min^{-1} with a R^2 value equal to 0.97 using peroxidase enzymatic treatment. This is very close to the rate constant for phenol, but is about two times higher than the rate constant for o-cresol and p-cresol.

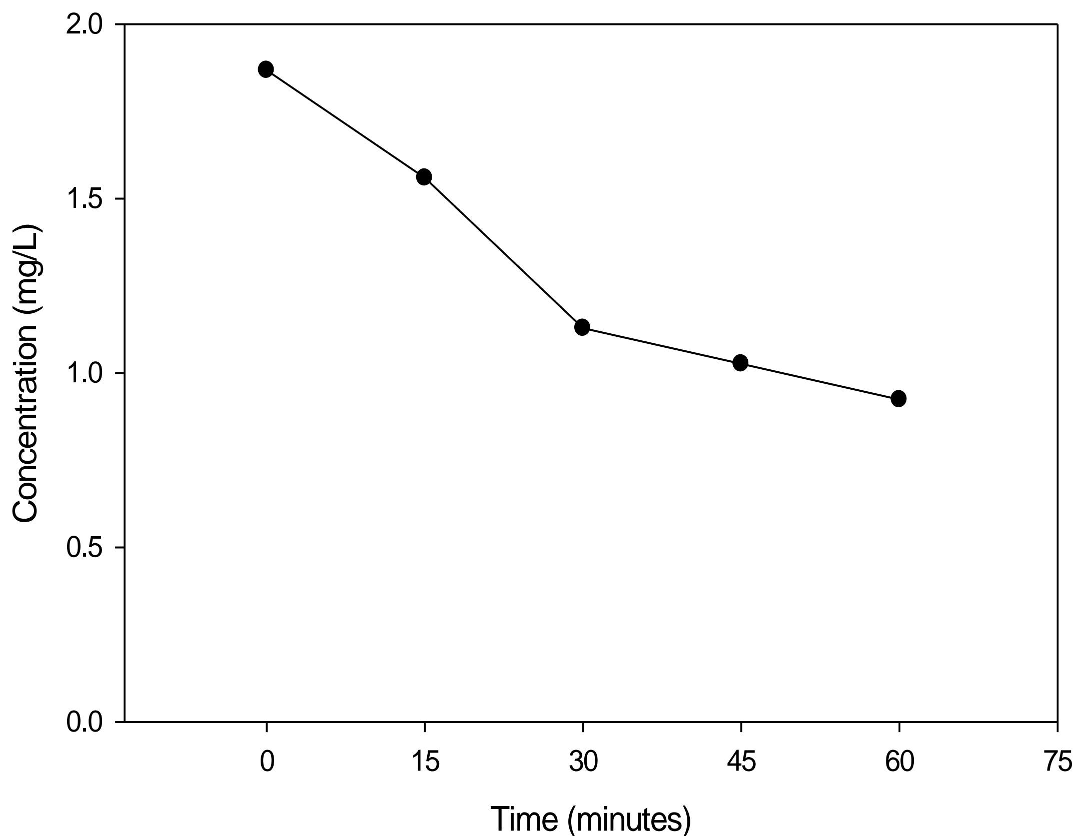

Figure 4.36: Peroxidase Treatment of 2,4-Dimethyl Phenol in CITGO Stripped Sour Water

For each individual phenolic compound the concentrations were normalized to the initial concentration so that the fraction remaining is shown (Figure 4.37). Phenol and 2,4-dimethyl phenol had the lowest remaining concentrations with 57.5 and 47.6

percent respectively. O-cresol and p-cresol were the phenolic compounds with the highest fraction remaining as they were notably higher than that of phenol and 2,4-dimethyl phenol. Unlike laccase/peroxide treatment, there was no lay with peroxidase/peroxide treatment.

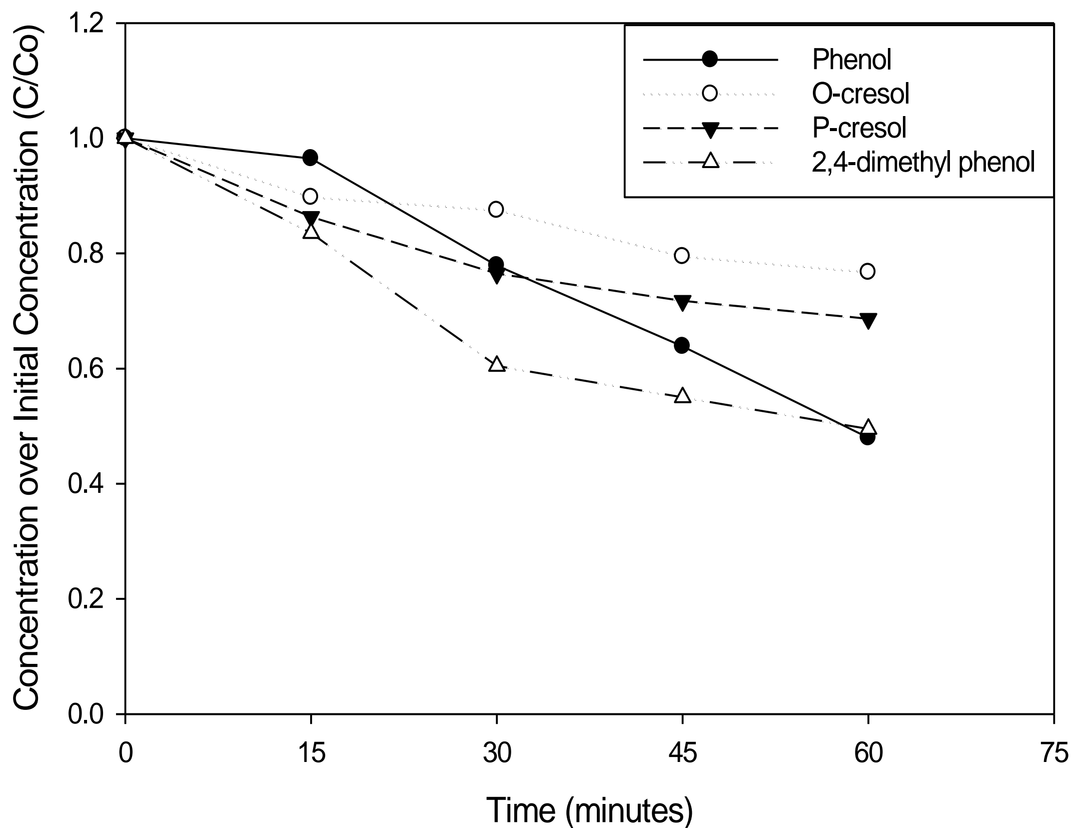

Figure 4.37: Fraction Remaining of Phenolic Compounds using Peroxidase Treatment on CITGO Stripped Sour Water

The first order rate constants for the oxidation of each selected compound reduced by peroxidase are found in Table 4.7. In this particular refinery wastewater matrix the reduction rates were significantly lower than the rates for each compound tested in de-ionized water (Table 4.1 and Table 4.2). Again an important thing to note

is that in this particular case the rate constants for laccase and peroxidase are essentially the same, thus the enzymes are more or less equally efficient.

Frontier Desalter Effluent

Desalter effluent from Frontier's Cheyenne Refinery in Wyoming was used to test the laccase enzyme's removal efficacy of phenolic compounds. An initial dose of 200 units of laccase and 300 mg/L of hydrogen peroxide were added to the sample and the concentrations of phenol, o-cresol, p-cresol, and 2,4-dimethyl phenol were determined at fifteen minute intervals. There were three replicate tests run using the laccase enzymatic treatment. The desalter effluent initially contained none of the target compounds. Therefore, 100ppm of phenol, o-cresol, and p-cresol were added to the sample. The sample was then tested using the gas chromatograph and was found to contain 103.8 mg/L of phenol, 98.6 mg/L of o-cresol, and 76.9 mg/L of p-cresol. The total amount of phenols in the Frontier desalter water was initially 279.3 mg/L.

Table 4.8: First Order Rate Constants for Laccase and Peroxidase in Frontier Desalter

Compound	**Laccase k (min^{-1})**	**Peroxidase k (min^{-1})**
Phenol	-0.0118 ($R^2 = 0.95$)	-0.0122 ($R^2 = 0.95$)
O-cresol	-0.0131 ($R^2 = 0.94$)	-0.0135 ($R^2 = 0.96$)
P-cresol	-0.0116 ($R^2 = 0.95$)	-0.0095 ($R^2 = 0.97$)

The change in phenol concentration over time by laccase/peroxide treatment in the Frontier desalter effluent is shown in Figure 4.38. The initial phenol concentration was 103.8 mg/L and was reduced by the laccase enzymatic treatment to 50.4 mg/L over a 60 minute reaction time. Thus the phenol concentration was lowered by 51.4 percent. One observation is that more phenol reduction occurred in the first 30 minutes of the reaction. The first order rate constant for laccase reduction of phenol was found to be -0.0118 min^{-1} with R^2 equal to 0.95 (Table 4.8).

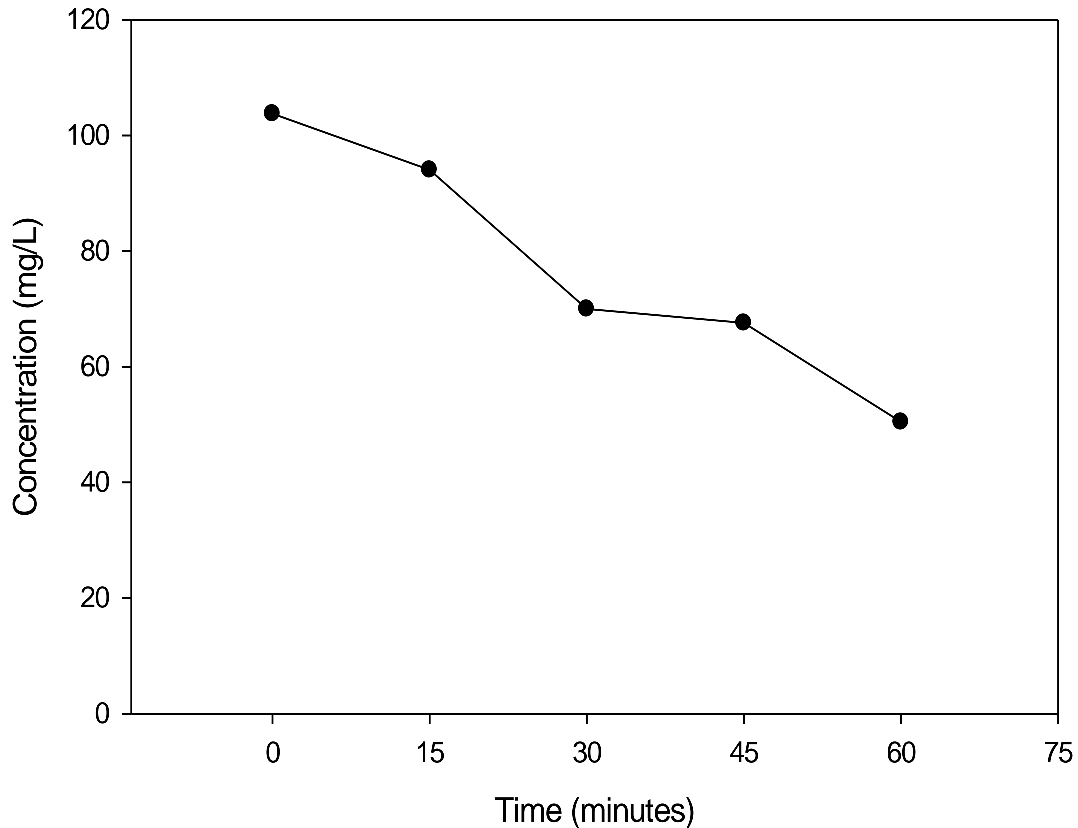

Figure 4.38: Laccase Treatment of Phenol in Frontier Desalter Effluent

The decrease in o-cresol concentration by laccase can is shown in Figure 4.39. The laccase enzymatic treatment reduced the concentration of o-cresol from 98.6

mg/L to 42.5 mg/L over a 60 minute reaction time. The reduction in o-cresol concentration was lowered by 56.9 percent. Like phenol, the reduction of o-cresol occurred in the first 30 minutes of the reaction. The first order rate constants for o-cresol are found in Table 4.8. The first order rate constant for laccase reduction of o-cresol was found to be -0.031 min^{-1} with R^2 value of 0.95. The reduction rate of o-cresol was the highest rate for laccase in the Frontier desalter effluent. The rate for laccase oxidation of o-cresol was slightly higher than that of phenol.

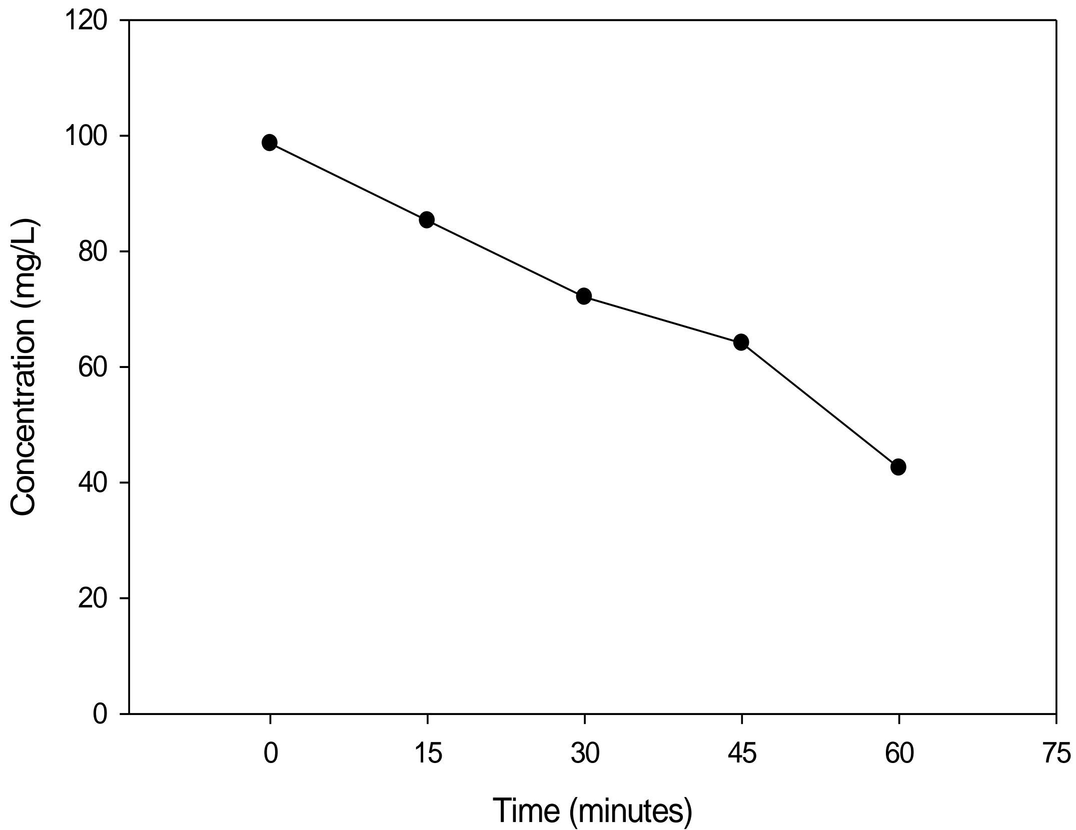

Figure 4.39: Laccase Treatment of O-cresol in Frontier Desalter Effluent

Figure 4.40 shows the concentration over time for p-cresol using the laccase enzymatic treatment. P-cresol concentration decreased from an initial concentration

of 76.9 mg/L to 39 mg/L over the 60 minutes, a reduction of 49.3 percent. The first order reduction rate constant for laccase/peroxide reduction of p-cresol found in Table 4.8 is -0.0116 min^{-1} with R^2 equal to 0.95. The rate constant for p-cresol is essentially the same as that of phenol, but slightly lower than the rate constant for o-cresol.

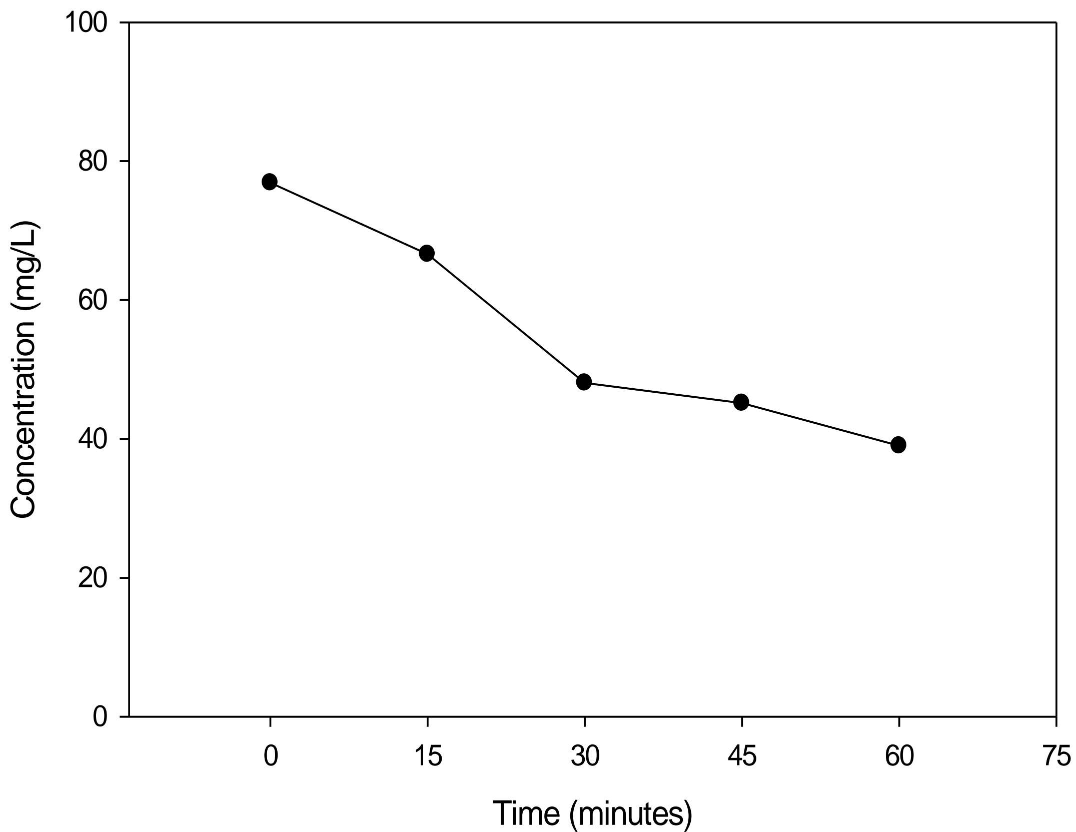

Figure 4.40: Laccase Treatment of P-cresol in Frontier Desalter Effluent

In Figure 4.41 the concentrations for each compound added are normalized to show the percent remaining for each phenolic compound in the Frontier desalter effluent. O-cresol had the lowest percent remaining with 43.1 percent, while phenol and p-cresol had similar percent remaining with 49.6 and 50.7 percent respectively.

The majority of the reduction in these compounds occurred during the first thirty minutes of the reaction time, which is closer to what was observed in the "clean matrix" system of phase one.

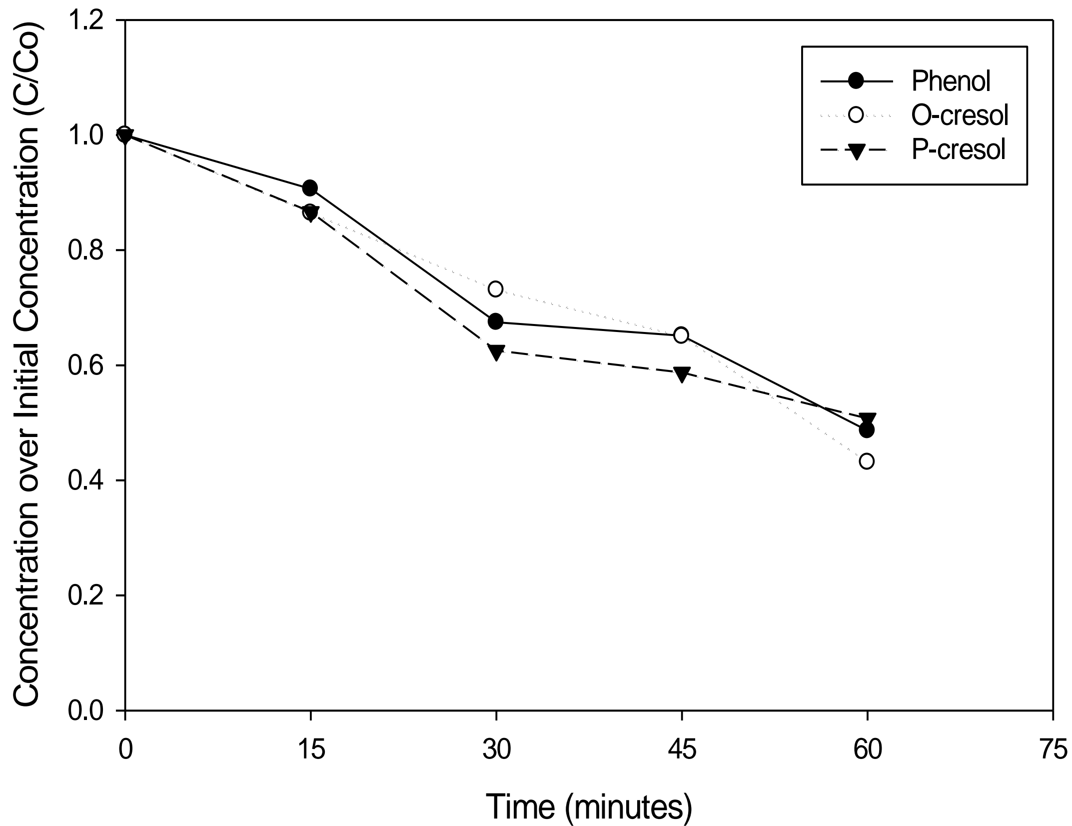

Figure 4.41: Fraction Remaining of Phenolic Compounds using Laccase Treatment on Frontier Desalter Effluent

The efficiency of the peroxidase enzyme for reducing phenolic compounds from the wastewater was tested in the same Frontier Desalter Effluent. An initial amount of 2600 units of peroxidase where added along with 300 mg/L of hydrogen peroxide to the effluent. As before, three replicates were tested using the peroxidase enzyme and the average initial concentrations contained in the desalter effluent were:

105.6 mg/L of phenol, 56.0 mg/L of o-cresol, 59.2 mg/L of p-cresol, and 220.8 mg/L total phenol. The first order rate constants for the oxidation of each selected compound reduced by peroxidase are also shown in Table 4.8.

Figure 4.42 shows the decrease in concentration of phenol by peroxidase/peroxide treatment over the 60 minutes of reaction. The concentration of phenol was reduced from 105.6 mg/L to 46.8 mg/L in 60 minutes, a reduction of 55.7 percent. The first order rate constant for peroxidase reduction of phenol was found to be -0.0122 min^{-1} with R^2 value of 0.95 (Table 4.8). One observation is that the reduction of phenol in this particular matrix occurred abruptly in the first 15 minutes, leveled off, and then decreased again in the last 15 minutes.

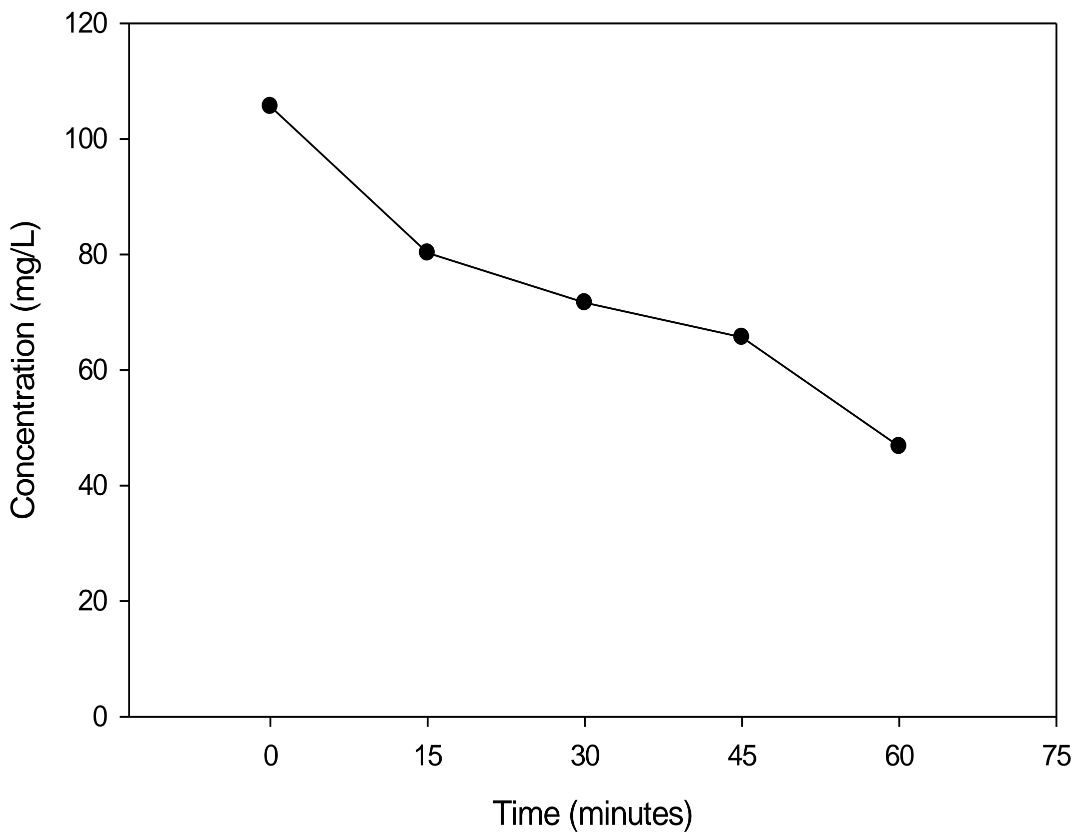

Figure 4.42: Peroxidase Treatment of Phenol in Frontier Desalter Effluent

Figure 4.43 shows the concentration of o-cresol in the Frontier deslater effluent over time reduced by peroxidase treatment. O-cresol concentration decreased 58.8 percent from 56.0 mg/L initially to 23.1 mg/L in 60 minutes. This reduction resembles that of phenol. The first order rate constant for o-cresol reduced by peroxidase was found to be -0.0135 min^{-1} with an R^2 value of 0.96 (Table 4.8). The first order reduction rate constant for o-cresol was higher than that of phenol by approximately 10 percent.

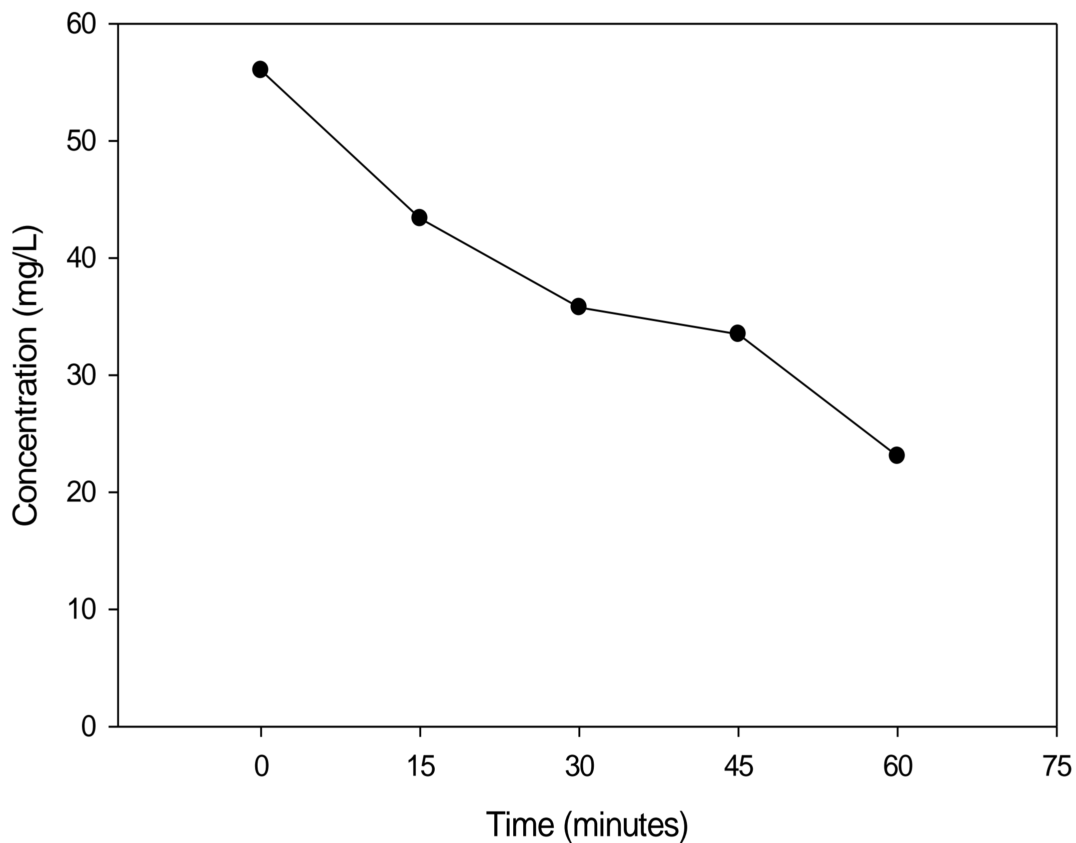

Figure 4.43: Peroxidase Treatment of O-cresol in Frontier Desalter Effluent

The reduction of p-cresol concentration in the Frontier desalter effluent over time by peroxidase is shown in Figure 4.44. Peroxidase treatment lowered the concentration from 59.2 mg/L to 33.9 mg/L in 60 minutes. This percent reduction corresponds to a 42.7 percent decrease and is lower than that of phenol and o-cresol. The first order rate constant for p-cresol reduced by peroxidase was found to be -0.0095 min^{-1} with an R^2 value of 0.97 (Table 4.8). The first order reduction rate constant for p-cresol was lower than that of phenol and o-cresol, which were approximately 30 percent and 45 percent higher respectively.

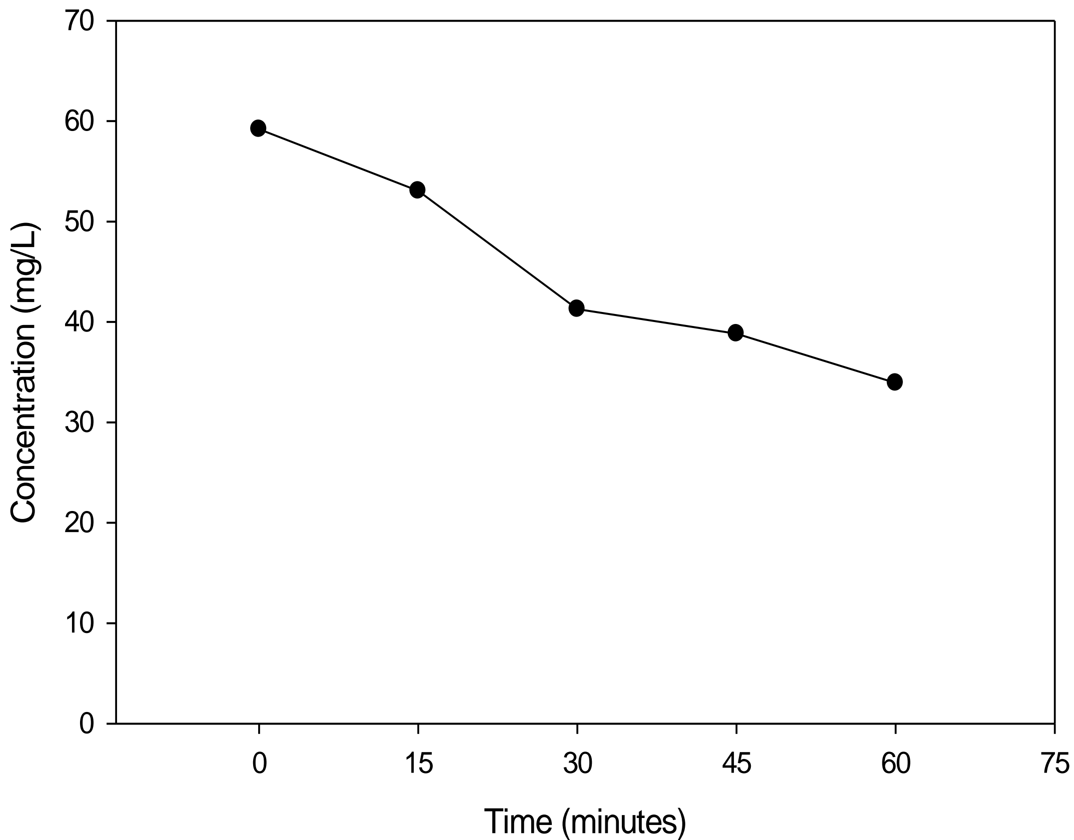

Figure 4.44: Peroxidase Treatment of P-cresol in Frontier Desalter Effluent

In Figure 4.45 the concentrations of phenol, o-cresol, and p-cresol were normalized to show the fraction remaining of each compound tested with the peroxidase enzyme. Phenol and o-cresol have the lowest remaining concentrations with 44.3 and 41.2 percent respectively. P-cresol was slightly higher with 57.3 percent remaining. One important thing to note from this graph is that a big reduction occurs between the reaction time 0 and 15 minutes and then again between 45 and 60 minutes.

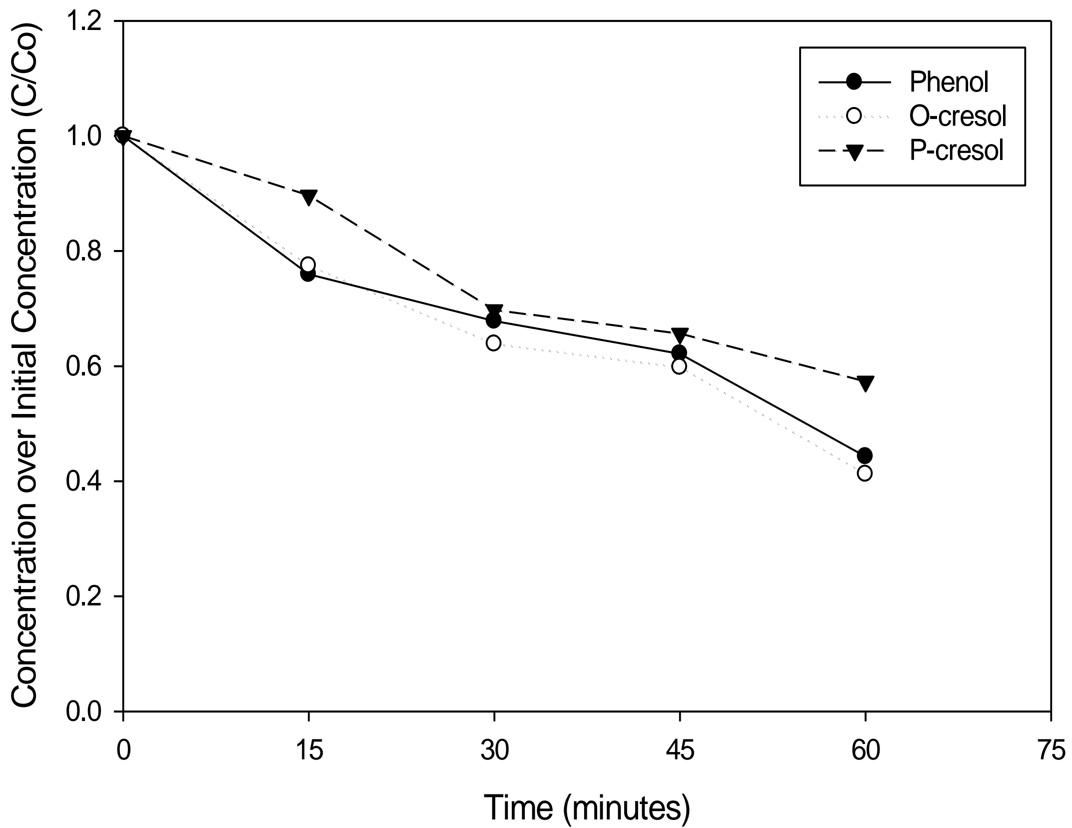

Figure 4.45: Fraction Remaining of Phenolic Compounds using Peroxidase Treatment on Frontier Desalter Effluent

CITGO Desalter Effluent

The efficiency of laccase enzyme to remove selected phenolic compounds was examined using desalter effluent from CITGO's Lake Charles Refinery in Louisiana. A dose of 200 units of laccase and 300 mg/L of hydrogen peroxide were added to the sample and then tested at fifteen minute intervals on the gas chromatograph to determine the concentrations of phenol, o-cresol, p-cresol, and 2,4-dimethyl phenol. Three different tests were run using the laccase enzymatic treatment. The desalter effluent initially contained 41.6 mg/L of phenol, 20.3 mg/L of o-cresol, 37.1 mg/L of p-cresol, and 2.6 mg/L of 2,4-dimethyl phenol. This data was used to determine the first order rate constants. These first order rate constants and R^2 values for both enzymes for each compound are shown in Table 4.6. An R^2 value greater than 0.90 insures the data fits the first order kinetic model well.

Table 4.9: First Order Rate Constants for Laccase and Peroxidase in CITGO Desalter

Compound	**Laccase k (min^{-1})**	**Peroxidase k (min^{-1})**
Phenol	-0.0141 (R^2 = 0.97)	-0.0173 (R^2 = 0.89)
O-cresol	-0.0079 (R^2 = 0.83)	-0.0119 (R^2 = 0.99)
P-cresol	-0.0092 (R^2 = 0.90)	-0.0111 (R^2 = 0.79)
2,4-Dimethyl Phenol	-0.0147 (R^2 = 0.97)	-0.0214 (R^2 = 0.97)

The decrease in concentration of phenol over the 60 minute reaction time is shown in Figure 4.46. The CITGO desalter effluent contained an initial phenol concentration of 41.6 mg/L. Phenol concentration was reduced 58.4 percent by the laccase enzymatic treatment to a concentration of 17.3 mg/L. The first order rate

constant for laccase reduction of phenol was found to be -0.0141 min^{-1} with R^2 value of 0.97 (Table 4.9).

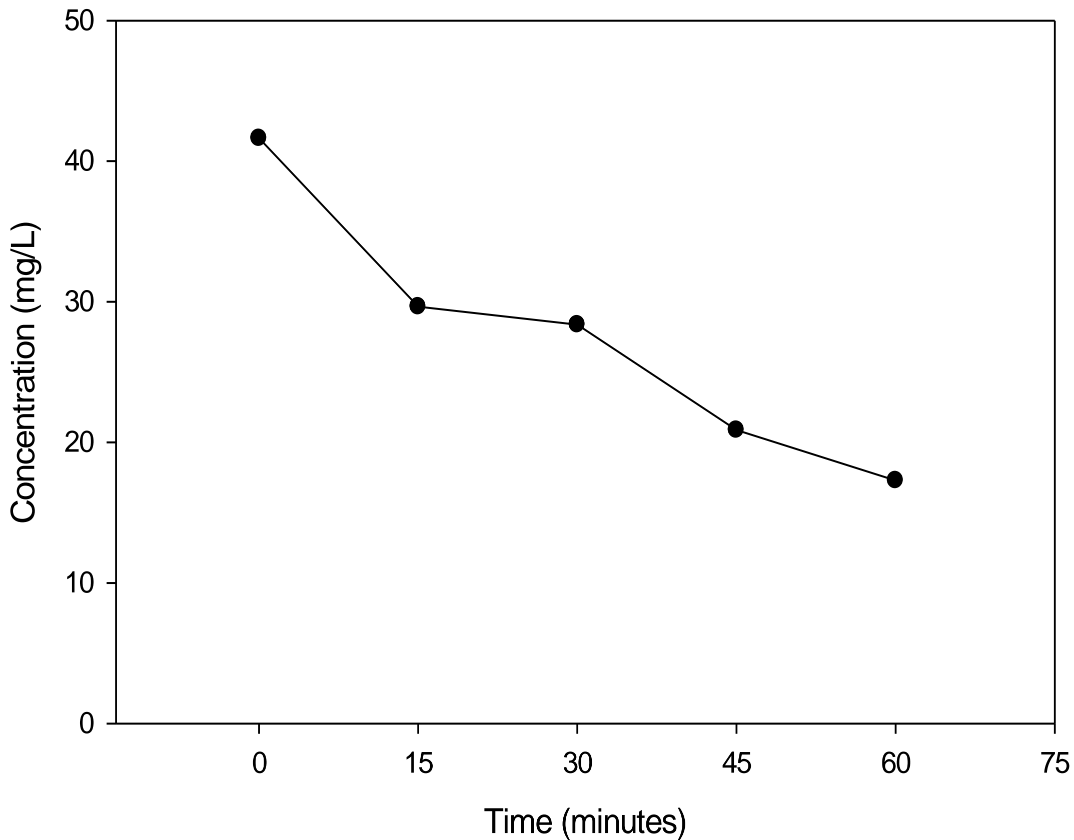

Figure 4.46: Laccase Treatment of Phenol in CITGO Desalter Effluent

The reduction in o-cresol concentration by laccase treatment is shown in Figure 4.47. O-cresol was lowered by laccase treatment from a concentration of 20.3 mg/L to 13.1 mg/L after 60 minutes. This corresponds to a 35.5 percent reduction of o-cresol. Most of the o-cresol degradation occurred during 15 and 30 minutes of reaction. The percent reduction is significantly less than phenol. The first order rate constant for laccase reduction of o-cresol found in Table 4.9 was -0.0079 min^{-1} with

R^2 equal to 0.83. This rate constant is almost two times lower than that of phenol.

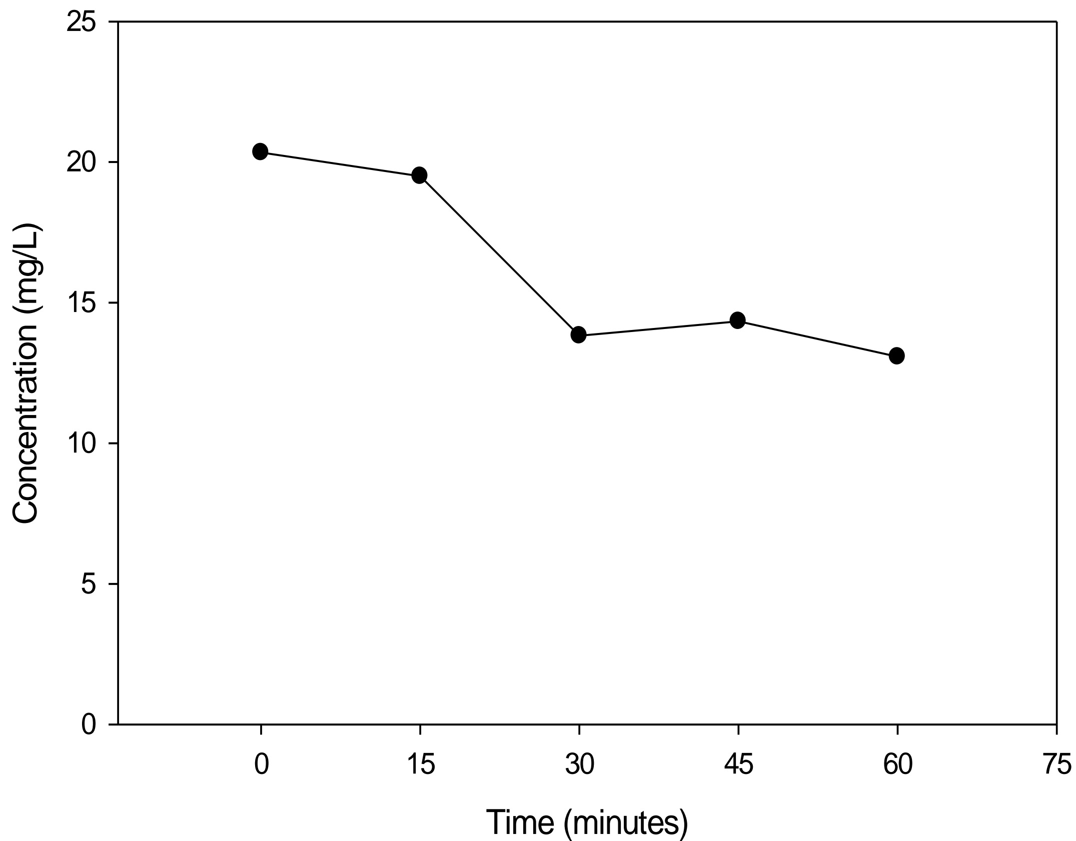

Figure 4.47: Laccase Treatment of O-cresol in CITGO Desalter Effluent

The concentration of p-cresol over time reduced by laccase is shown in Figure 4.48. Laccase treatment reduced the P-cresol from a concentration of 37.1 mg/L to 22.5 mg/L over the span of 60 minutes, a reduction of 39.4 percent. This percent resembles that of o-cresol, and is lower than phenol. Similar to o-cresol, the majority of p-cresol degradation occurred during the first 30 minutes. The first order rate constant for laccase reduction of p-cresol was found to be was -0.007992 min^{-1} with R^2 value of 0.90. This rate constant was lower than the first order rate constants for

laccase reduction of phenol, but slightly higher than the removal rate of o-cresol.

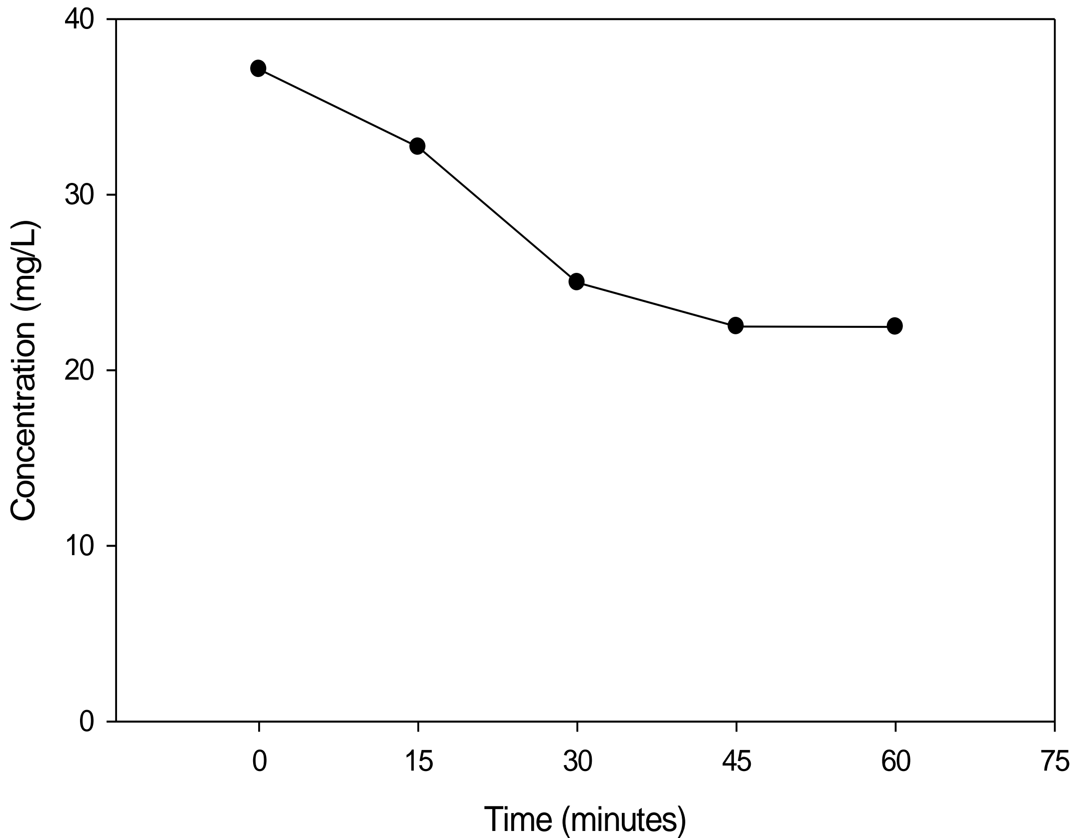

Figure 4.48: Laccase Treatment of P-cresol in CITGO Desalter Effluent

Figure 4.49 shows the concentration of 2,4-dimethyl phenol in the desalter effluent over the 60 minutes. 2,4-dimethyl phenol decreased from a concentration of 2.6 mg/L to 1.1 mg/L due to laccase/peroxide treatment. This corresponds to a reduction of 57.7 percent. The percent reduction is very close to that of phenol, and is much higher than p-cresol and o-cresol. However, there was much more phenol in the CITGO desalter effluent than 2,4-dimethyl phenol. The first order rate constant for laccase reduction of 2,4-dimethyl phenol was determined to be -0.0147 min^{-1} with a

great R^2 value of 0.97. This rate constant for 2,4-dimethyl phenol was the highest first order rate constant for the laccase/peroxide treatment. The reduction rate constant for 2,4-dimethyl phenol is almost two times high that that of o-cresol and p-cresol, and is slightly higher than the rate constant for phenol.

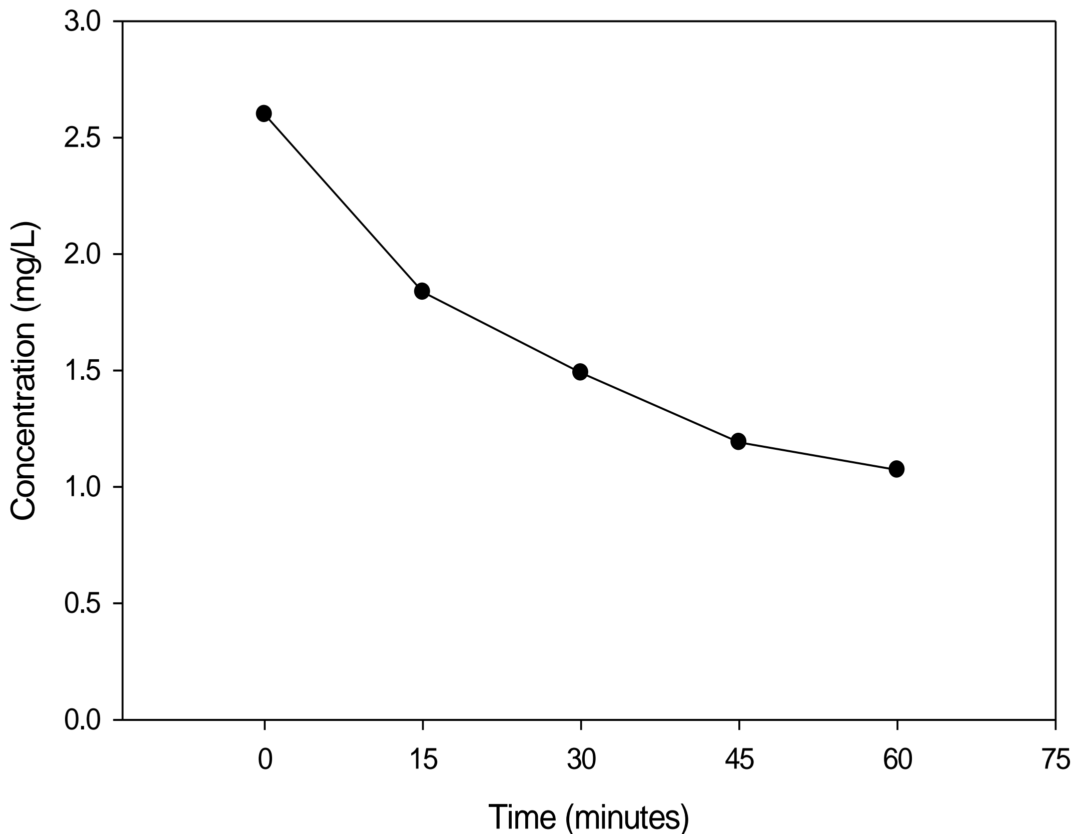

Figure 4.49: Laccase Treatment of 2,4-Dimethyl Phenol in CITGO Desalter Effluent

In Figure 4.50 the individual concentrations for each compound were normalized to the initial concentration to show the fraction remaining. Phenol and 2,4-dimethyl phenol had the smallest percent remaining with 41.6 and 42.3 percent respectively. This percent was slightly higher than the percent remaining for o-cresol

and p-cresol, which were 64.5 and 60.6 percent respectively. Like the clean system, the most degradation occurred during the first 30 minutes of the reaction, however, with o-cresol and p-cresol there was a lay of 15 minutes.

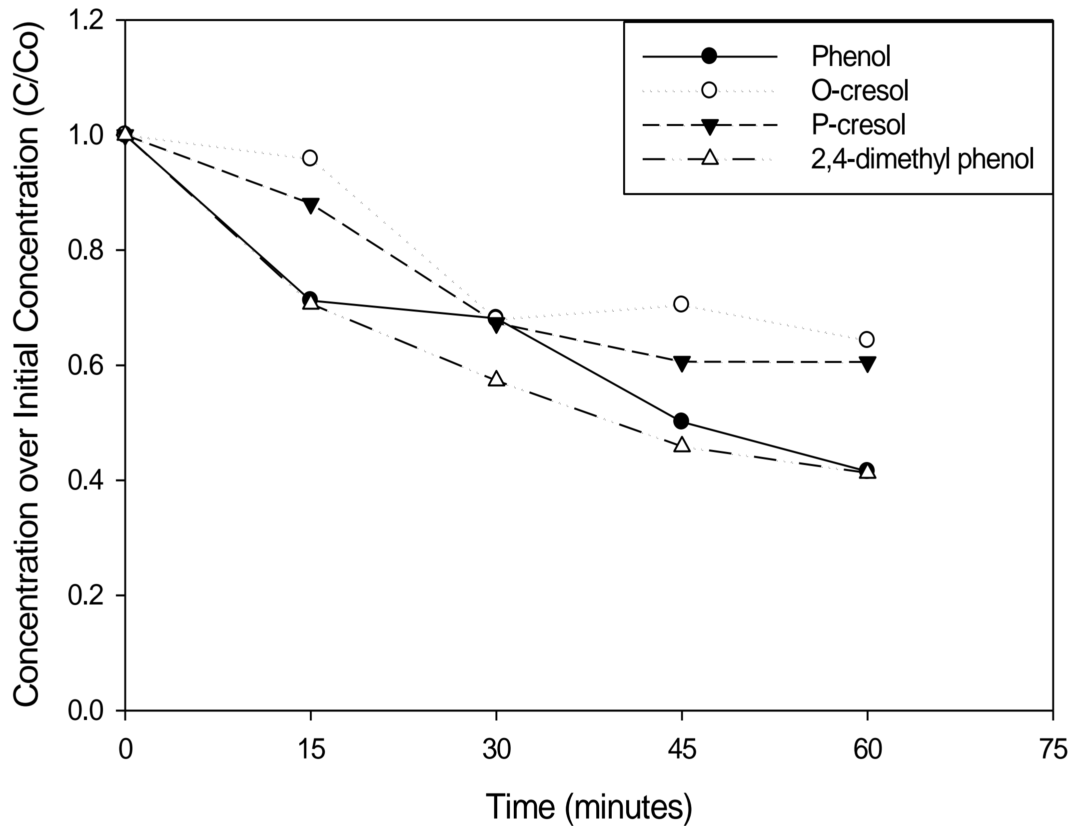

Figure 4.50: Fraction Remaining of Phenolic Compounds using Laccase Treatment on CITGO Desalter Effluent

The efficacy for reducing phenolic compounds from the wastewater was tested in the same Frontier Desalter Effluent using the peroxidase enzyme. An initial amount of 2600 units of peroxidase where added along with 300 mg/L of hydrogen peroxide to the desalter effluent. As before, three duplicate samples were tested using the peroxidase enzyme and the average initial concentrations contained in the water

were: 62.4 mg/L of phenol, 29.5 mg/L of o-cresol, 58.2 mg/L of p-cresol, 4.4 mg/L of 2,4-dimethyl phenol, and 220.8 mg/L total phenol. The first order rate constants for peroxidase were also examined and are shown in Table 4.9.

Figure 4.51 shows the decrease in phenol concentration over time by peroxidase/peroxide treatment. The CITGO desalter effluent contained an initial phenol concentration of 62.4 mg/L, and this decreased to 22.1 over 60 minutes. This corresponds to a reduction in phenol of 64.6 percent. The decrease in phenol over time followed an exponential reduction, and a majority of the reduction occurred in the first 30 minutes of the reaction. The first order rate constant for peroxidase reducing phenol was found to be -0.0173 min^{-1} with an R^2 value of 0.89 (Table 4.9).

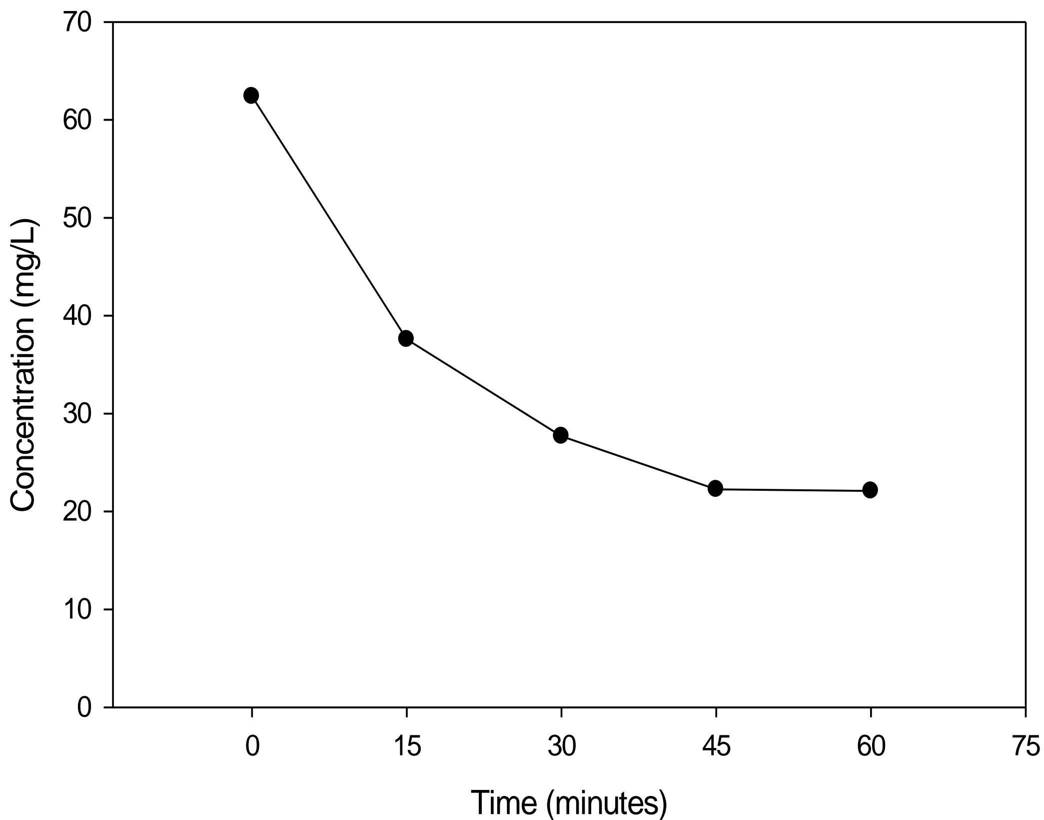

Figure 4.51: Peroxidase Treatment of Phenol in CITGO Desalter Effluent

The reduction in concentration of o-cresol by the peroxidase enzyme is shown in Figure 4.52. The desalter effluent contained an initial o-cresol concentration of 29.5 mg/L and was lowered to 14.1 mg/L after 60 minutes by peroxidase/peroxide treatment. This corresponds to reduction of o-cresol by peroxidase of 52.2 percent. The percent reduction is somewhat less than that of phenol. Although like phenol, the majority of the reduction occurred in the first 30 minutes of the reaction. The first order rate constant for peroxidase/peroxide reducing o-cresol was determined to be -0.0119 min^{-1} with an R^2 value of 0.99 (Table 4.9). Again, the high R^2 value shows that the first order model yields a very good fit for the data. The peroxidase enzyme's first order reduction rate constant for o-cresol was nearly half that of phenol.

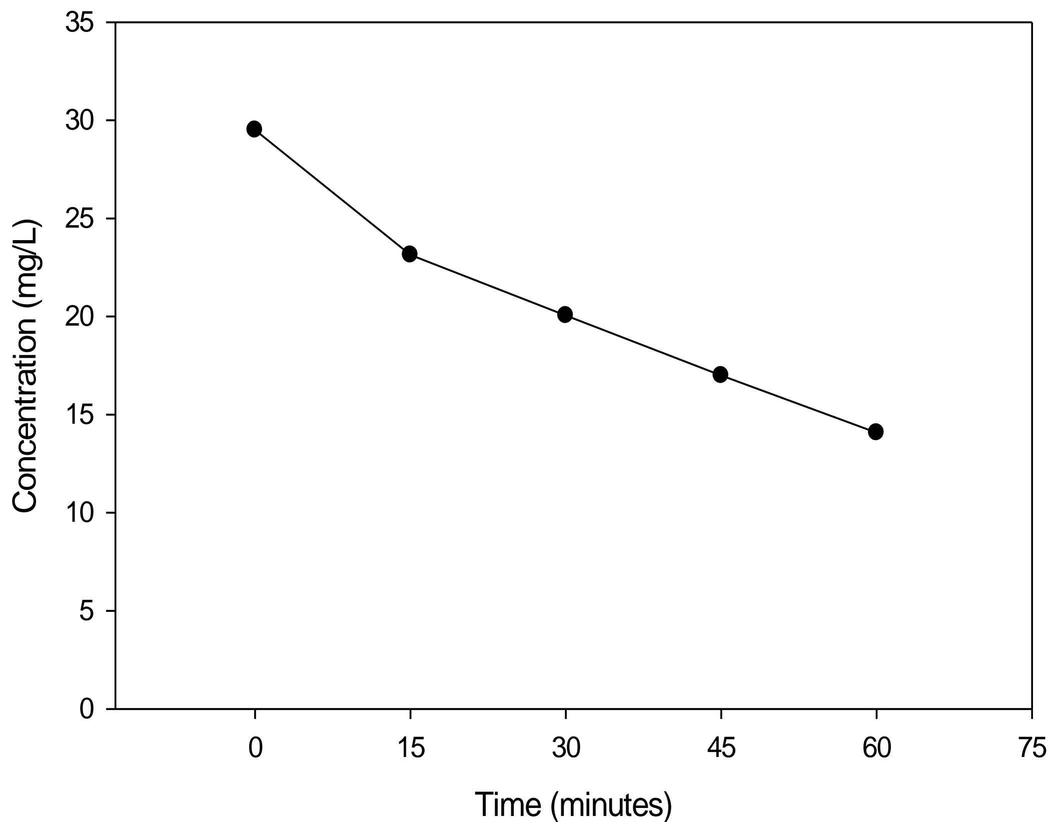

Figure 4.52: Peroxidase Treatment of O-cresol in CITGO Desalter Effluent

The concentration over time for peroxidase/peroxide treatment of p-cresol over time is shown in Figure 4.53. Peroxidase/peroxide treatment reduced the p-cresol in the desalter effluent from a concentration of 58.2 mg/L to 26.2 mg/L over the span of 60 minutes, a 55 percent reduction. This percent resembles that of o-cresol., but again is less that the percent removal for phenol. Similar to phenol and o-cresol, most of the degradation of p-cresol occurred in the first 15 minutes of the reaction. The lay after the 15 minutes could be due to low hydrogen peroxide levels in the sample, or more realistically, that the peroxidase/peroxide was oxidizing other compounds in the matrix other than p-cresol. The first order rate constant for peroxidase was found to be -0.0111 min^{-1} with an R^2 value of 0.79 (Table 4.9). The first order reduction rate constant for p-cresol was comparable to that of o-cresol, but was nearly half that phenol. The lower R^2 value indicates a moderate fit with the first order kinetic model.

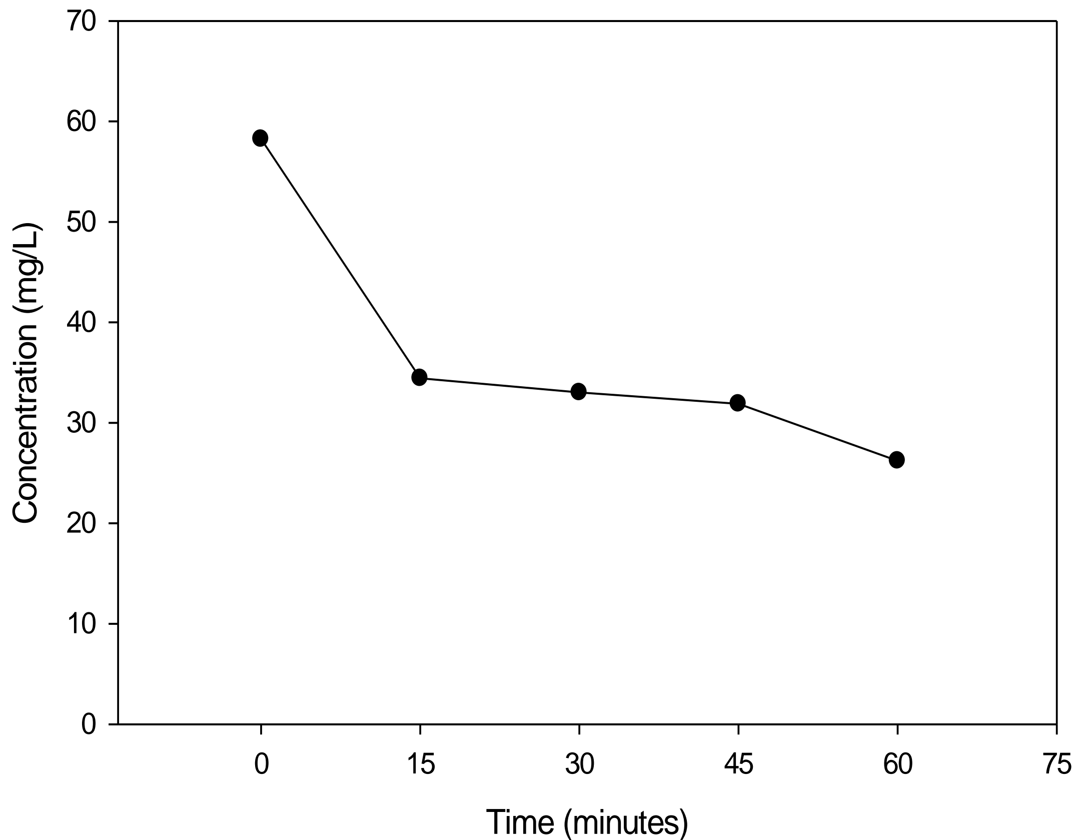

Figure 4.53: Peroxidase Treatment of P-cresol in CITGO Desalter Effluent

The concentration of 2,4-dimethyl phenol over time reduced by peroxidase/peroxide is shown in Figure 4.54. 2,4-dimethyl phenol was reduced by the treatment from an initial concentration of 4.4 mg/L to 1.2 mg/L. The peroxidase lowered the 2,4-dimethyl phenol concentration by 72.7 percent for the 60 minutes. This decrease in 2,4-dimethyl phenol concentration is more than any of the other three compounds, however it is important to note that 2,4-dimethyl phenol made up only a small fraction of the total phenolics in the sample. The first order rate constant for peroxidase reducing 2,4-dimethyl phenol was found to be -0.0214 min^{-1} with an

R^2 value of 0.97, shown in Table 4.9. The rate constant is approximately 25 percent higher than that of phenol, and close to two times that of p-cresol and o-cresol.

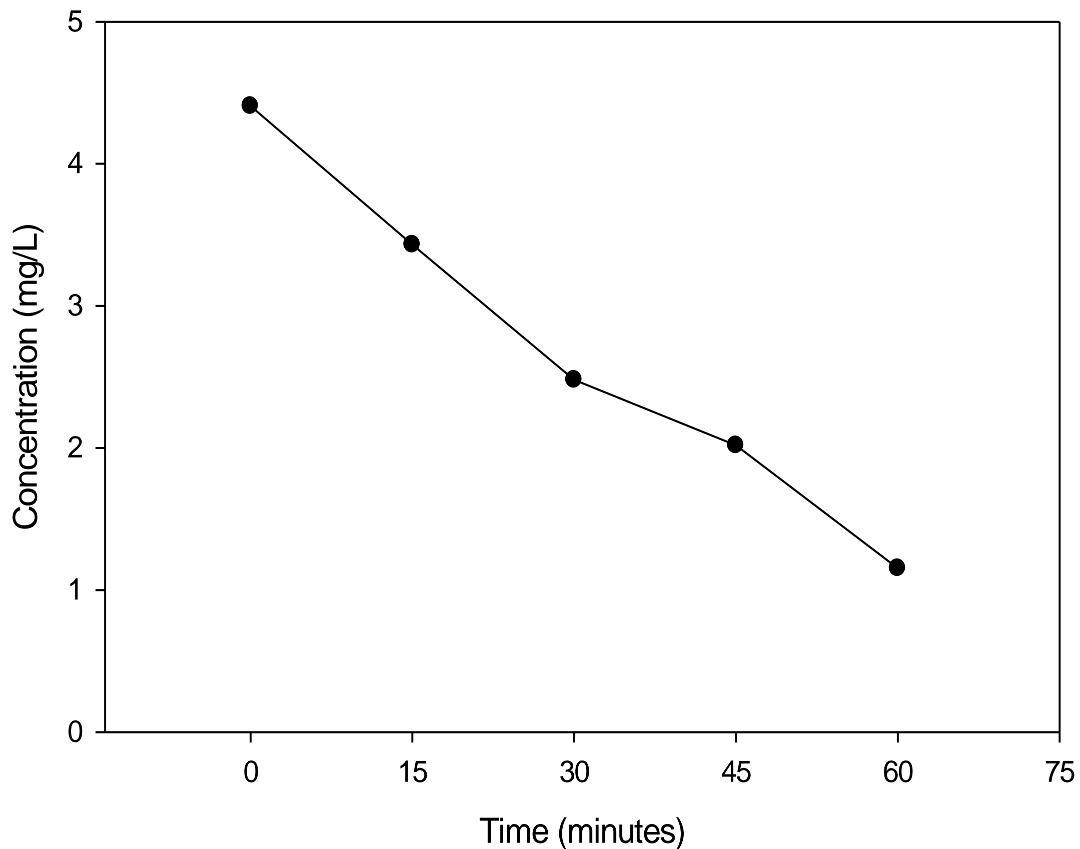

Figure 4.54: Peroxidase Treatment of 2,4-Dimethyl Phenol in CITGO Desalter Effluent

The concentrations of each individual compound tested were normalized to the initial concentration to show the fraction remaining using the peroxidase enzyme (Figure 4.55). The percent remaining for o-cresol and p-cresol was 47.8 and 45 percent respectively. O-cresol and p-cresol fraction remaining was nearly double that of phenol and 2,4-dimethyl phenol. Phenol and 2,4-dimethyl phenol had the lowest remaining concentrations with 35.4 and 27.3 percent respectively. Similar to

laccase/peroxide treatment, the majority of the degradation occurred during the first 30 minutes of the reaction, however with peroxidase/peroxide treatment there was no lay in the reduction.

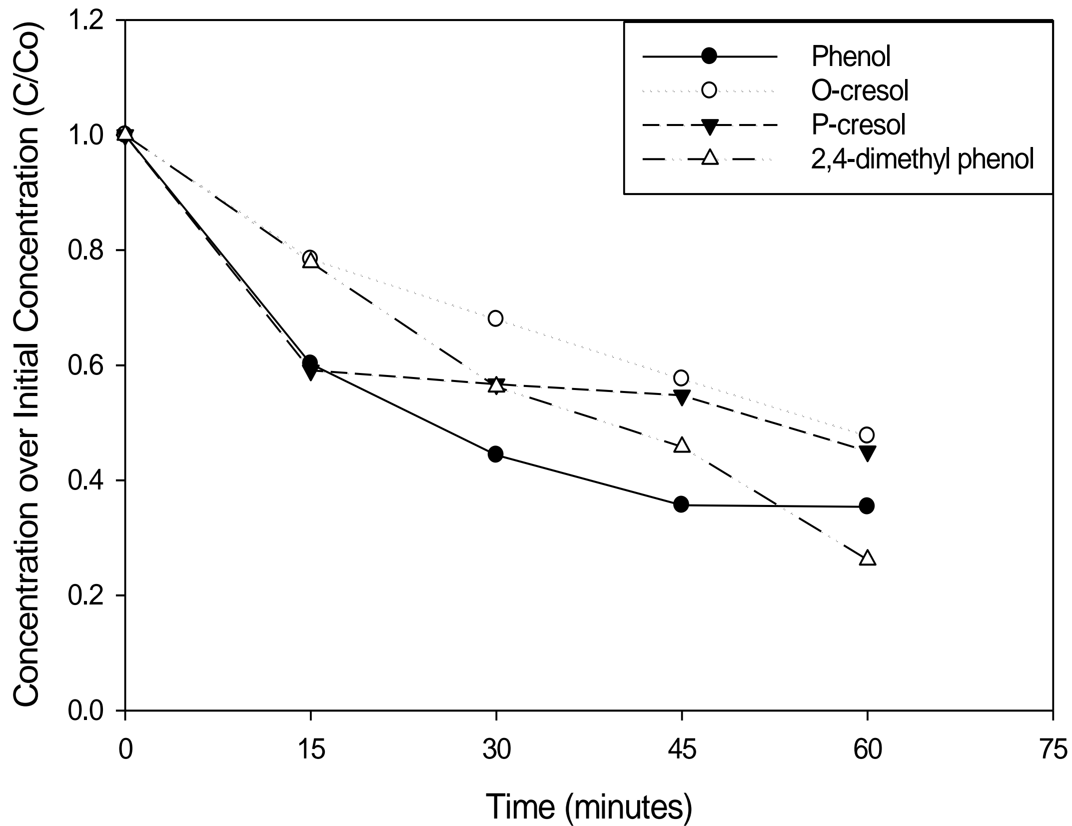

Figure 4.55: Fraction Remaining of Phenolic Compounds using Peroxidase Treatment on CITGO Desalter Effluent

Dartmouth Biox Influent

The laccase enzyme's ability to reduce the concentration of major phenolic constituents in biox influent from Imperial Oil's Dartmouth Refinery in Nova Scotia was examined. An initial dose of 200 units of laccase and 300 mg/L of hydrogen peroxide were added to the biox influent. Gas chromatography was used to determine

the concentrations of the phenol, o-cresol, p-cresol, and 2,4-dimethyl phenol at fifteen minute intervals. Four replicate tests were run using the laccase/peroxide enzymatic treatment. The biox influent was found to contain a non-detectable amount of the target phenolic compounds; therefore, 100ppm of phenol, 100ppm of o-cresol, 100ppm of p-cresol, and 50ppm of 2,4-dimethyl phenol were added to the biox influent. The biox influent was then test and initially contained 69.9 mg/L of phenol, 63.8 mg/L of o-cresol, 34.6 mg/L of p-cresol, and 2.1 mg/L of 2,4-dimethyl phenol, a total amount of 172.8 mg/L phenolics. This data was used to determine the first order rate constants and R^2 values for laccase/peroxide reduction of each individual compound; these are shown in Table 4.10.

Table 4.10: First Order Rate Constants for Laccase and Peroxidase in the Dartmouth Biox Influent

Compound	**Laccase k (min^{-1})**	**Peroxidase k (min^{-1})**
Phenol	-0.0113 (R^2 = 0.83)	-0.0141 (R^2 = 0.95)
O-cresol	-0.0117 (R^2 = 0.80)	-0.0154 (R^2 = 0.98)
P-cresol	-0.0104 (R^2 = 0.92)	-0.0149 (R^2 = 0.91)
2,4-Dimethyl Phenol	-0.0119 (R^2 = 0.97)	-0.0148 (R^2 = 0.91)

The decrease in phenol concentration by the laccase enzyme over time is shown in Figure 4.56. The initial 69.9 mg/L of phenol in the water was lowered to 36.0 mg/L over the 60 minutes. The concentration decrease of phenol in the biox influent corresponds to a 48.5 percent reduction by laccase/peroxide treatment. It is important to note that the majority of the degradation of phenol occurred during the first 30 minutes of the reaction, very little occurred after that time. For reducing

phenol laccase/peroxide had a first order rate constant of -0.0113 min^{-1} and an R^2 value of 0.83 (Table 4.10). The low R^2 value indicates that the data had a moderate fit with the first order model.

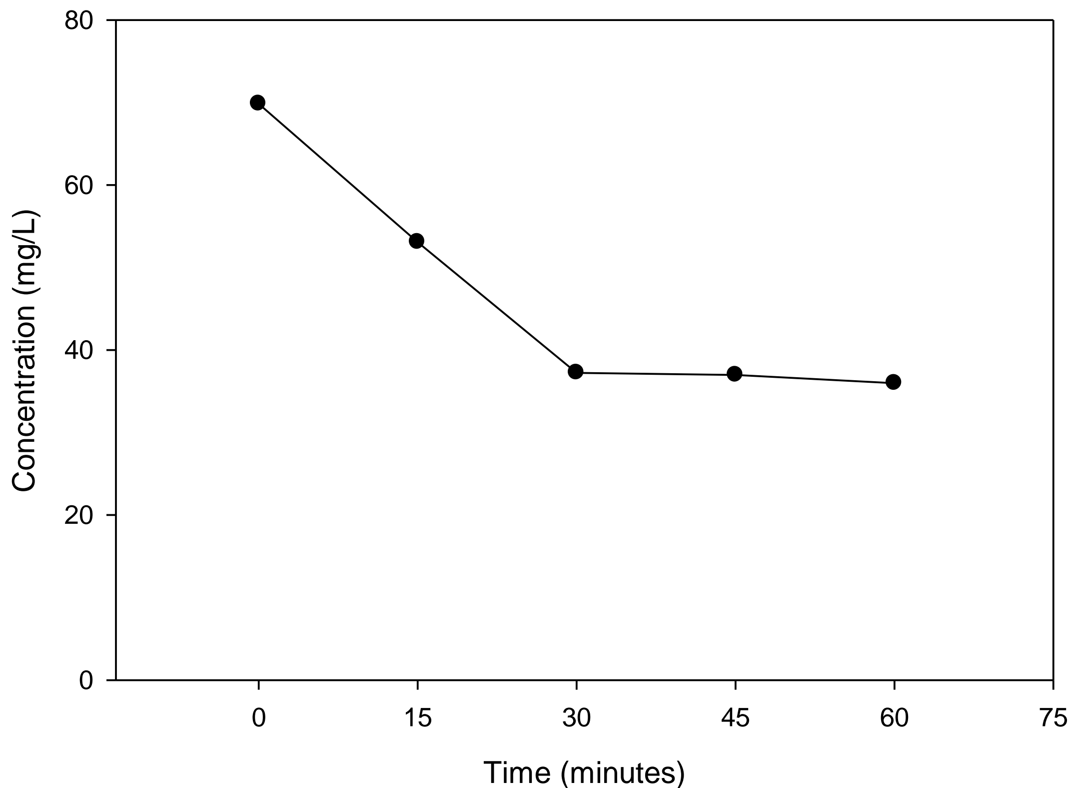

Figure 4.57: Laccase Treatment of Phenol in Dartmouth Biox Influent

Figure 4.58 shows the reduction of o-cresol over time caused by the laccase/peroxide addition. The Dartmouth biox influent contained an average initial o-cresol concentration of 63.8 mg/L, which was reduced to 32.5 mg/L over 60 minutes reaction time. This corresponds to reduction of 49.1 percent. Like the decrease in phenol, a majority of the reduction of o-cresol also occurred during the first 30 minutes of the reaction. Laccase had a first order rate constant of -0.0117 min^{-}

^{1} and an R^2 value of 0.80 (Table 4.10). Also like phenol, the first order rate constant was very similar (less than 5 percent better) to phenol and the data also yield a moderate fit with the first order model.

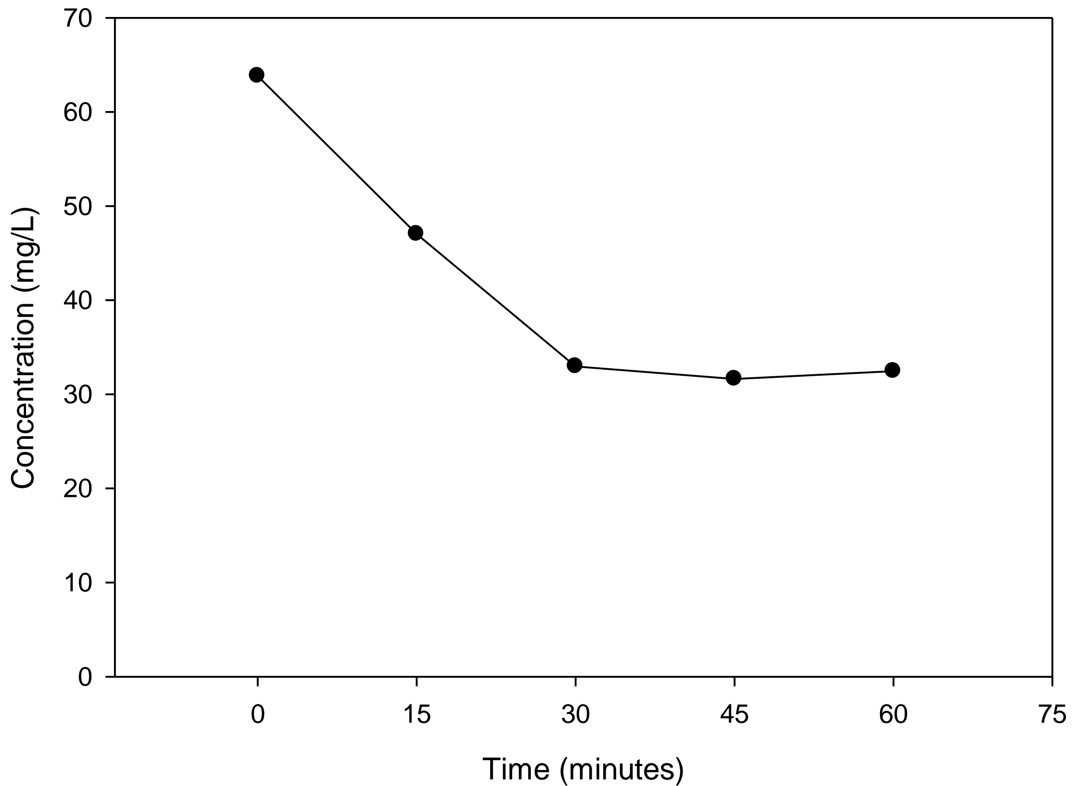

Figure 4.58: Laccase Treatment of O-cresol in Dartmouth Biox Influent

Figure 4.59 shows the decrease in p-cresol concentration over time due to laccase/peroxide treatment. The p-cresol concentration in the biox influent decreased from 34.6 mg/L to 17.7 mg/L after 60 minutes, a 48.8 percent reduction. This reduction is very similar to that of phenol and o-cresol. The first order rate constant for p-cresol was determined to be -.0104 min^{-1} with a R^2 value of 0.92 (Table 4.10).

The rate constant for p-cresol was lower than the rate constant of o-cresol and phenol by approximately 10 percent.

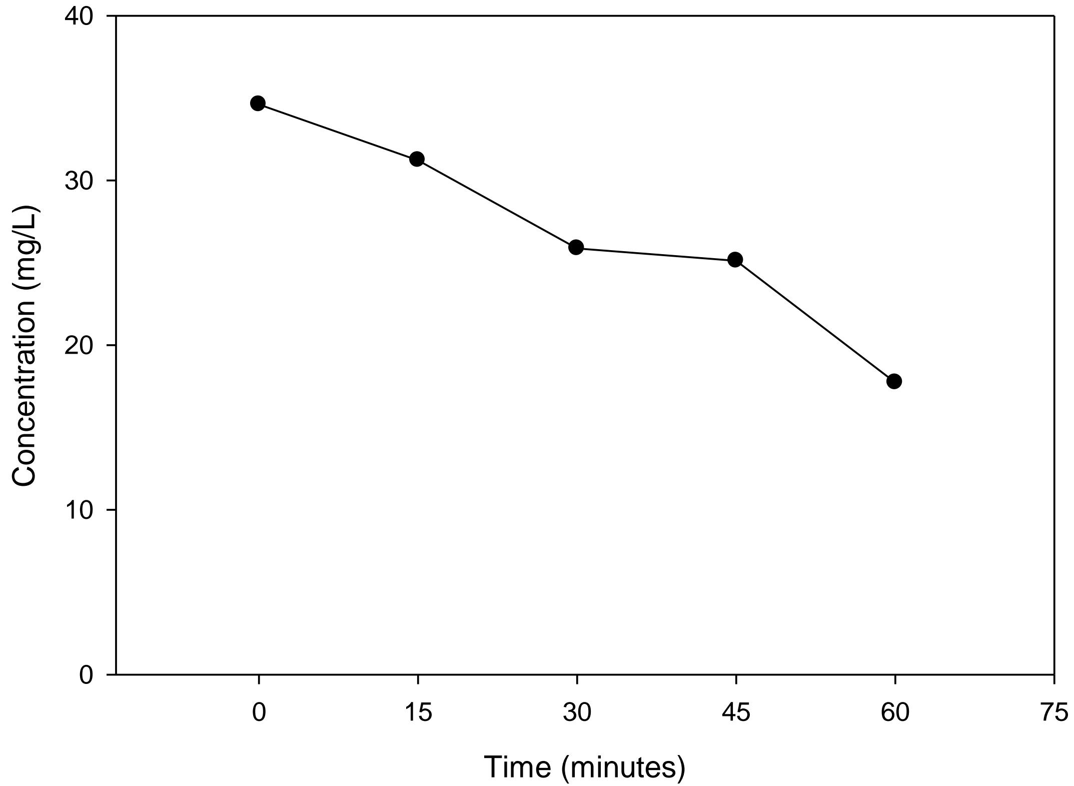

Figure 4.59: Laccase Treatment of P-cresol in Dartmouth Biox Influent

The change in concentration of 2,4-dimethyl phenol over time caused by laccase/peroxide is shown in Figure 4.60. The concentration of 2,4-dimethyl phenol decreased from an initial concentration of 2.1 mg/L to 0.99 mg/L after 60 minutes (52.4 percent). Although 2,4-dimthyl had the highest percent reduction using the laccase/peroxide treatment, however, the 48.5 percent removal for phenol is much more important because phenol is the largest contributor to the total phenolics. The first order rate constant for laccase reducing 2,4-dimethyl phenol was found to be -

.0119 min^{-1} with a R^2 equal to 0.97 (Table 4.10). Although this is the highest rate for laccase/peroxide treatment, it is less than 5 percent better than the rate constants of o-cresol and phenol.

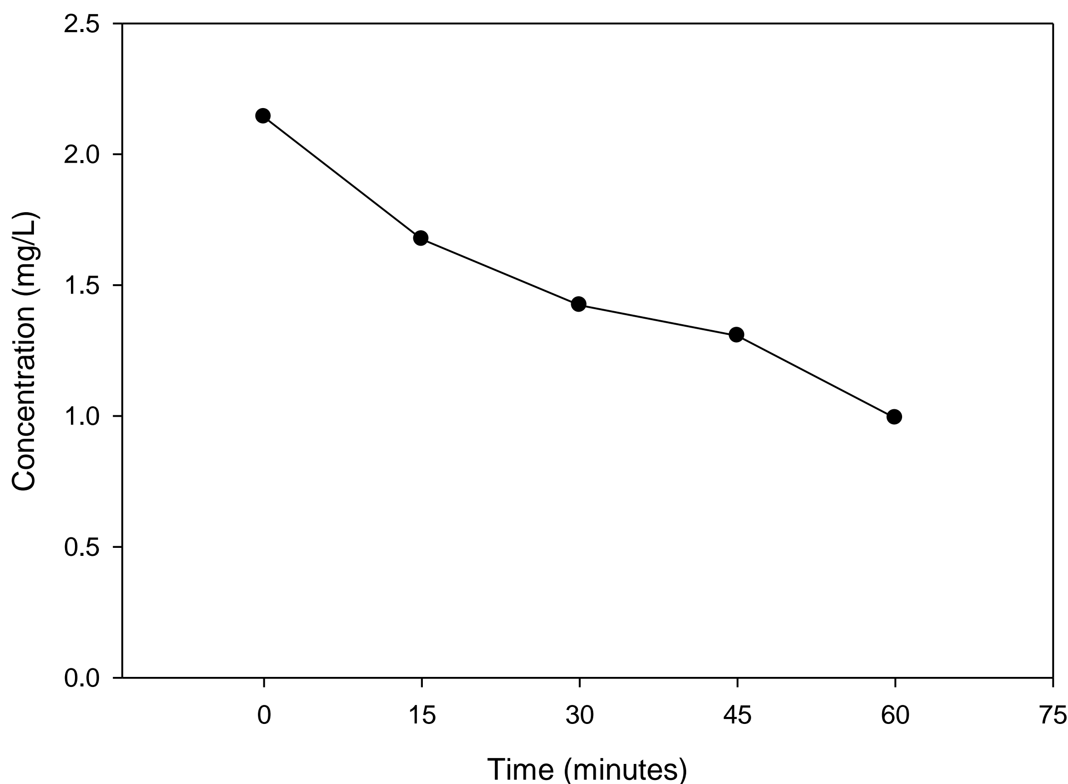

Figure 4.60: Laccase Treatment of 2,4-Dimethyl Phenol in Dartmouth Biox Influent

To compare the decrease in the individual phenolic compounds, the concentrations of each enzyme were normalized to the initial concentration and are shown in Figure 4.61. This then shows the percent remaining for each compound over the 60 minutes. In this case, phenol had the highest percent remaining with 51.5, while 2,4-dimethyl phenol had the lowest with 47.6 percent. O-cresol and p-cresol had 50.9 and 51.2 percent remaining, respectively. An important observation from the

graph is that with the laccase/peroxide treatment the majority of the reduction occurred during the first 30 minutes of the reaction, similar to that observed in a clen system.

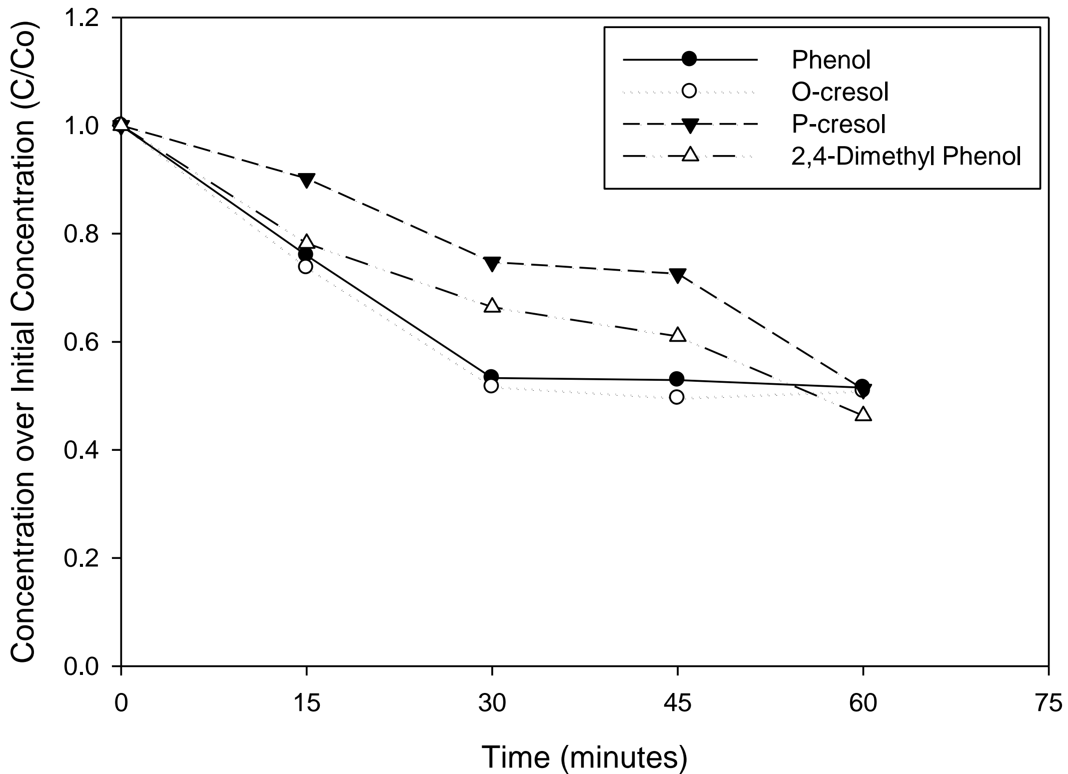

Figure 4.61: Fraction Remaining of Phenolic Compounds using Laccase Treatment on Dartmouth Biox Influent

The peroxidase enzyme was tested on the same biox influent from Dartmouth for its efficiency to remove the same phenolic compounds. An initial dose of 2600 units of peroxidase and 300 mg/L of hydrogen peroxide were added to the biox influent sample. Like the laccase testing 100ppm of phenol, 100ppm of o-cresol, 100ppm of p-cresol, and 50ppm of 2,4-dimethyl phenol were added to the sample since the original concentrations were non-detectable. Once added the concentrations

of phenol, o-cresol, p-cresol, and 2,4dimethyl phenol where measured with the gas chromatograph over fifteen minute intervals. There were three replicate test run using the peroxidase enzyme; and the average initial concentrations contained in the biox influent after the addition of phenols were: 101.3 mg/L of phenol, 38.4 mg/L of o-cresol, 39.8 mg/L of p-cresol, 2.2 mg/L of 2,4-dimethyl phenol, and 177.9 mg/L total phenol. The first order rate constants were also determined for peroxidase and are shown in Table 4.10.

Figure 4.62 shows the decrease in phenol concentration over time using the peroxidase enzyme coupled with hydrogen peroxide. The phenol concentration in the Dartmouth biox influent was reduced by peroxidase 61 percent from an initial phenol concentration of 101.3 mg/L to 22.1 mg/L after 60 minutes. The majority of the reduction occurred during the first and last 15 minutes of the reaction, with a lay in the middle. The first order rate constant for peroxidase for decreasing phenol was found to be -.0141 min^{-1} with a R^2 value of 0.95 (Table 4.10).

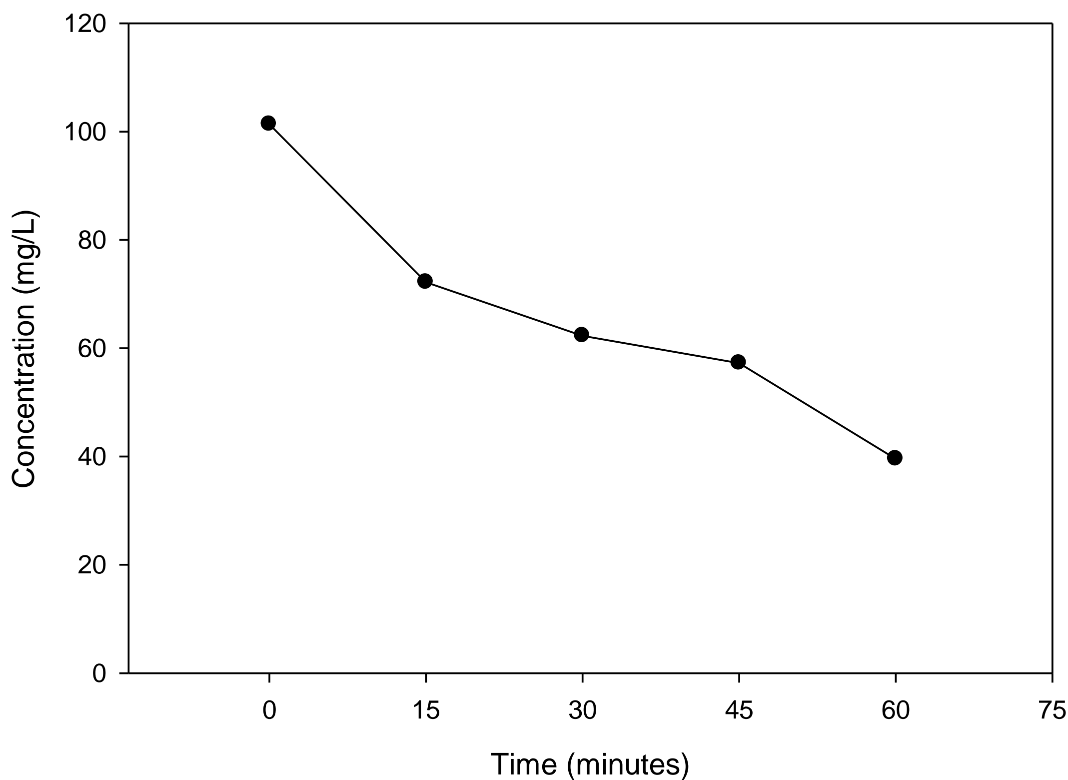

Figure 4.62: Peroxidase Treatment of Phenol in Dartmouth Biox Influent

The initial concentration of o-cresol contained in the biox influent was 38.4 mg/L (Figure 4.63). Peroxidase/peroxide decreased the concentration to 15.6 mg/L after 60 minutes. This corresponds to a reduction of o-cresol of 59.4 percent. The percent reduction is very close to that of phenol. The first order rate constant for peroxidase reducing o-cresol was found to be -.0154 min^{-1} with a R^2 equal to 0.98 (Table 4.10). The first order rate constant for o-cresol is close to 10 percent better than the rate for phenol.

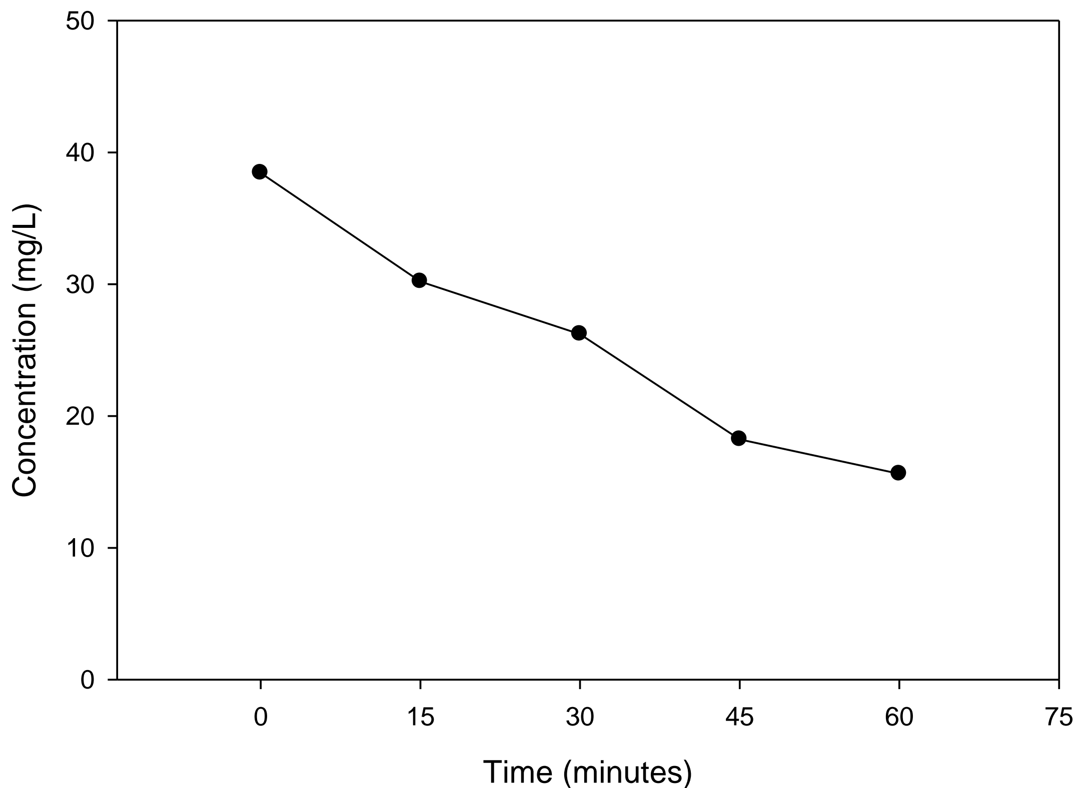

Figure 4.63: Peroxidase Treatment of O-cresol in Dartmouth Biox Influent

The change in concentration over time for p-cresol is shown in Figure 4.64. Peroxidase treatment reduced the P-cresol in the biox influent from a concentration of 39.8 mg/L to 15.9 mg/L over the 60 minutes, a 60.1 percent reduction. This percent resembles that of o-cresol and phenol. However, unlike phenol and o-cresol the major degradation of p-cresol occurred after 30 minutes reaction. The first order rate constant for p-cresol was found to be -.0149 min^{-1} with a R^2 equal to 0.91 (Table 4.10). The rate constant of p-cresol was about 5 percent higher than that of phenol and approximately 5 percent lower than that of o-cresol.

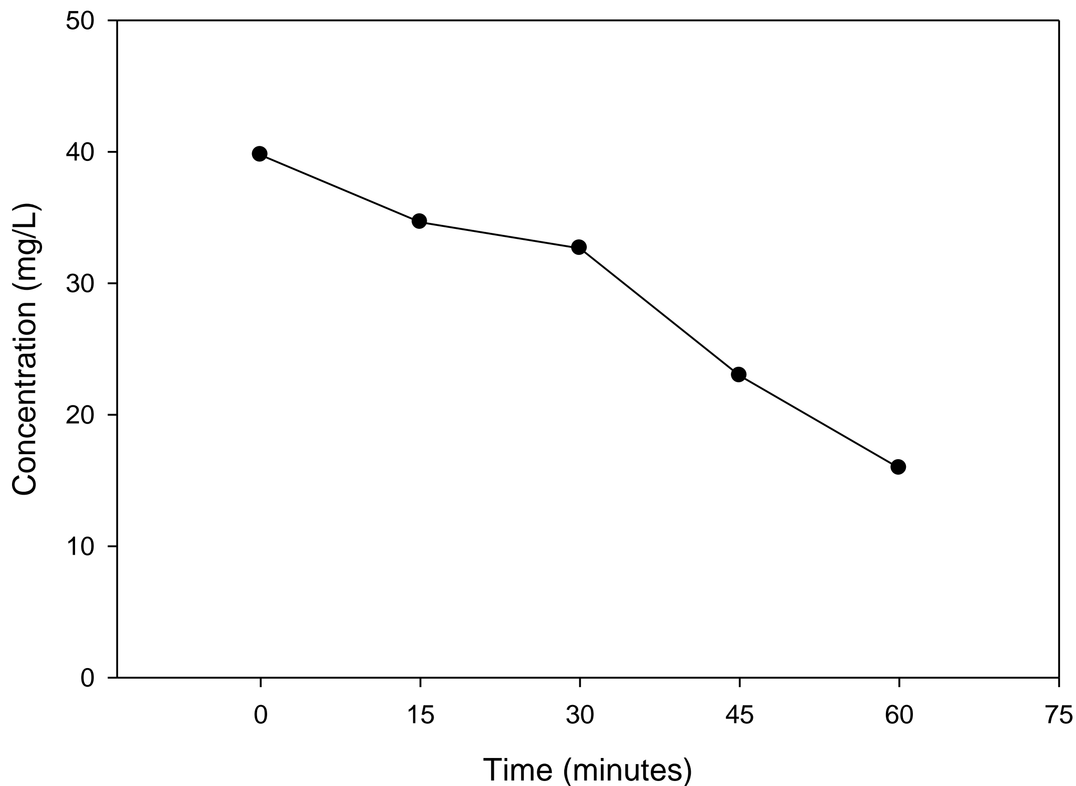

Figure 4.64: Peroxidase Treatment of P-cresol in Dartmouth Biox Influent

The change in concentration of 2,4-dimethyl phenol over time caused by peroxidase/peroxide is shown in Figure 4.65. The 2,4-dimethyl phenol was reduced from an initial concentration of 2.2 mg/L to .94 mg/L. The peroxidase lowered the 2,4-dimethyl phenol concentration by 59.1 percent for the 60 minutes. Similar to o-cresol the majority of the reduction occurred during the first 30 minutes of the reaction. Although the percent reduced is similar to that of the other phenolic compounds, since the other phenolic compounds make up 99 percent of the total phenolics their reduction is much more important. The first order rate constant for phenol reducing 2,4-dimethyl phenol was found to be -.0148 min^{-1} with a R^2 equal to

0.91 (Table 4.10). This rate constant is very similar to the rate for reducing p cresol, slightly lower than that of o-cresol, and slightly higher than the phenol first order rate constant.

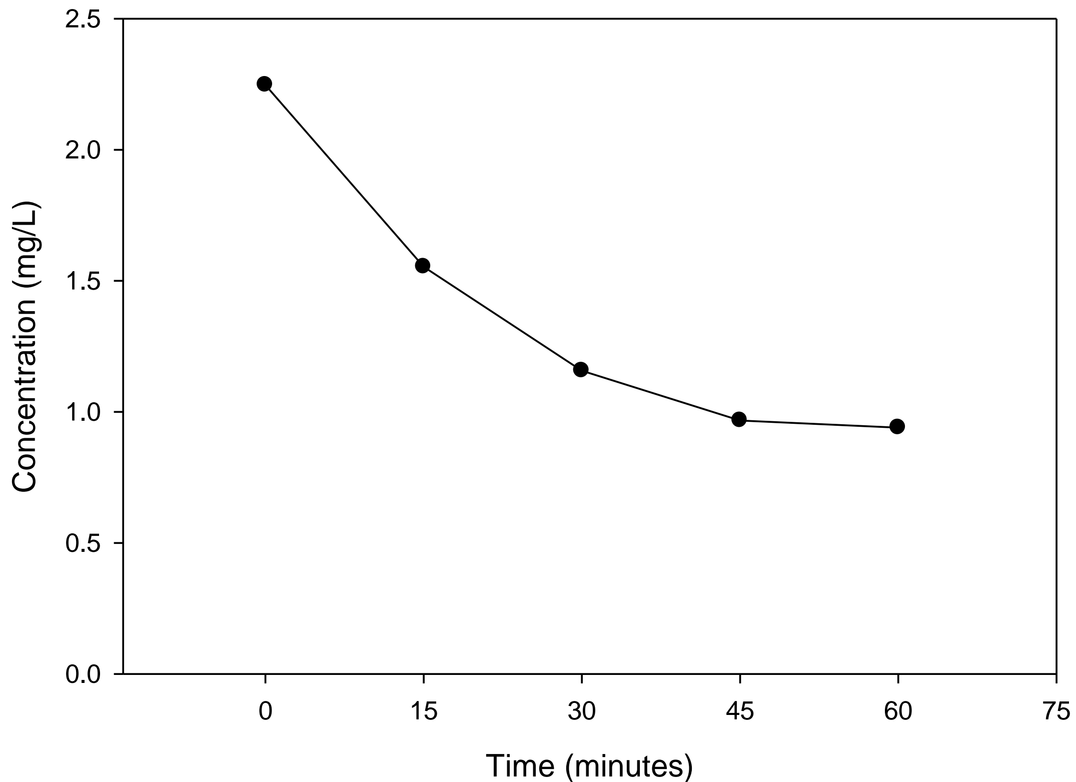

Figure 4.65: Peroxidase Treatment of 2,4-Dimethyl Phenol in Dartmouth Biox Influent

The concentrations for each individual compound tested were normalized to the initial concentration in order to compare the fraction remaining for each compound reduced by the peroxidase enzyme (Figure 4. 66). Phenol and p-cresol had the lowest remaining concentrations with 39 and 39.9 percent respectively. The percent remaining for o-cresol and 2,4-dimethyl phenol was 40.6 and 40.9 percent

respectively. The peroxidase enzyme essentially reduced all of the compounds the same amount.

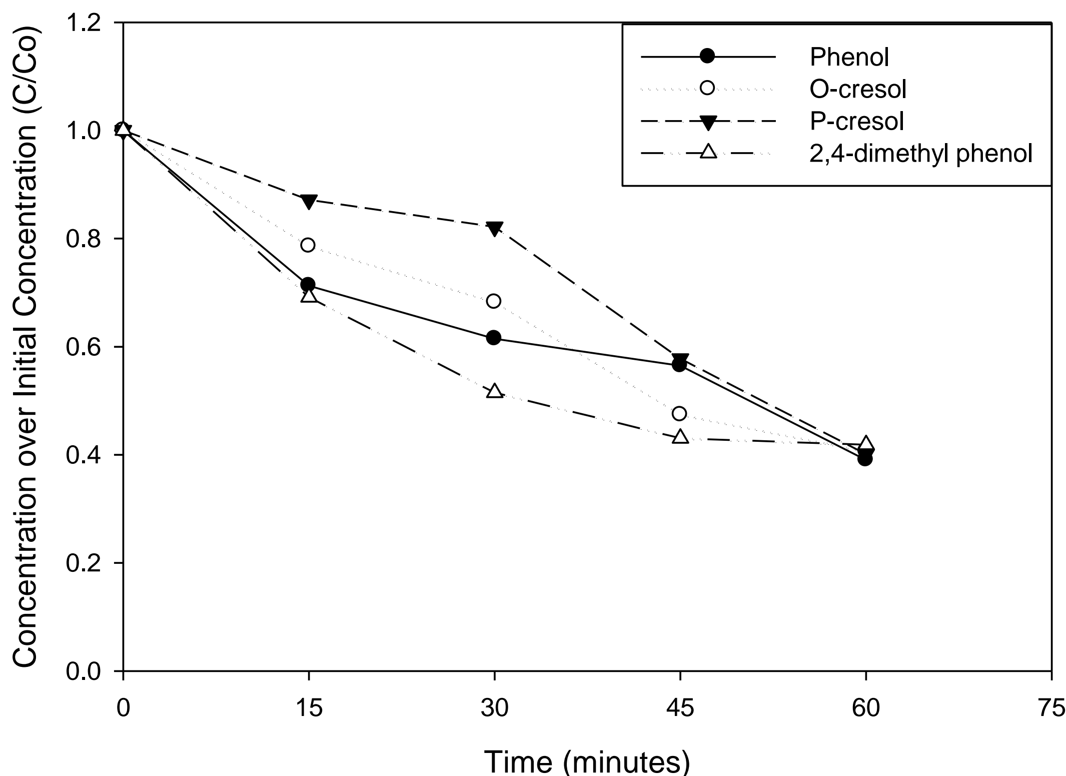

Figure 4.66: Fraction Remaining of Phenolic Compounds using Peroxidase Treatment on Dartmouth Biox Influent

Sarnia Biox Effluent

The efficacy of the laccase enzyme to remove selected phenolic compounds was studied using biox effluent from Imperial Oil's Sarnia Refinery in Southwestern Ontario. The initial testing of the biox effluent revealed a non-detectable amount of the target phenolic compounds were present in the biox effluent, therefore 100ppm of phenol, 100ppm of o-cresol, and 100ppm of p-cresol were added for testing. An initial dose of 200 units of laccase and 300 mg/L of hydrogen peroxide were added to

the sample and then tested at fifteen minute intervals to determine the concentrations. Three replicate tests were run using the laccase/peroxide enzymatic treatment and the biox effluent was then found to contain an average of 56.2 mg/L phenol, 62.3 mg/L o-cresol, 59.8 mg/L p-cresol, and a total amount of phenols of 178.3 mg/L. The data above was used to determine the first order rate constants for each phenolic compound for each enzyme; these are shown in Table 4.11.

Table 4.11: First Order Rate Constants for Each Compound in the Sarnia Biox Effluent

Compound	**Laccase** **k (min^{-1})**	**Peroxidase** **k (min^{-1})**
Phenol	-0.0153 (R^2 = 0.96)	-0.0152 (R^2 = 0.98)
O-cresol	-0.0119 (R^2 = 0.99)	-0.0128 (R^2 = 0.97)
P-cresol	-0.0060 (R^2 = 0.99)	-0.0108 (R^2 = 0.90)

Phenol concentration in the biox effluent was reduced by laccase/peroxide treatment from 56.2 mg/L to 21.1 mg/L over 60 minutes (Figure 4.67). This reduction in phenol by the laccase enzymatic treatment corresponds to 62.5 percent. Similar to that observed in the clean system, laccase catalyzed a rapid oxidation of phenol with significant reduction in concentration occurring over the first 15 minutes. The first order rate constant for phenol reduced by laccase/peroxide was found to be -0.0153 min^{-1} with a R^2 equal to 0.96 (Table 4.11). The high R^2 value indicates a very good fit with the first order model.

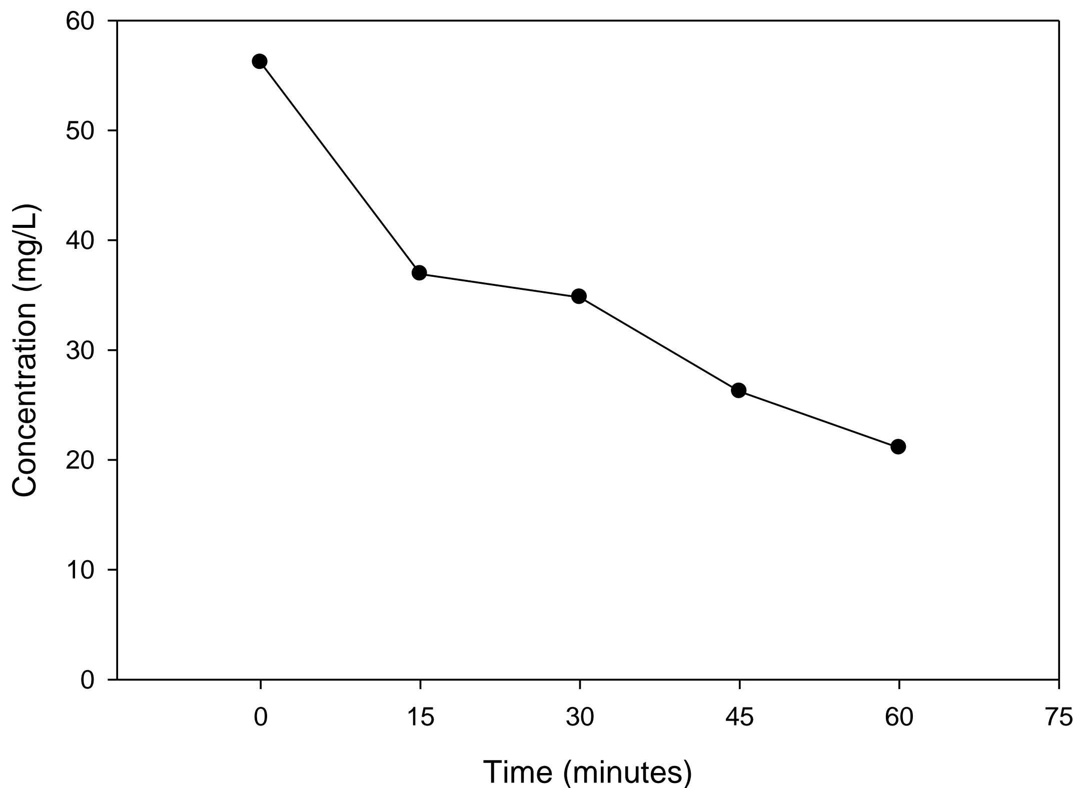

Figure 4.67: Laccase Treatment of Phenol in Sarnia Biox Effluent

The decrease in o-cresol concentration over the one hour reaction time is shown in Figure 4.68. The Sarnia biox effluent was found to contain an initial o-cresol concentration of 62.3 mg/L; laccase/peroxide reduced the concentration to 30.5 over the 60 minute reaction time (51 percent). This reduction was slightly lower than phenol, however similar to phenol, the majority of the reduction occurred in the first 30 minutes. The first order rate constant, found in Table 4.11, for o-cresol reduced by laccase/peroxide was -0.0119 min^{-1} with a R^2 equal to 0.99. The rate constant of o-cresol was lower than the rate for the reduction of phenol by over 25 percent.

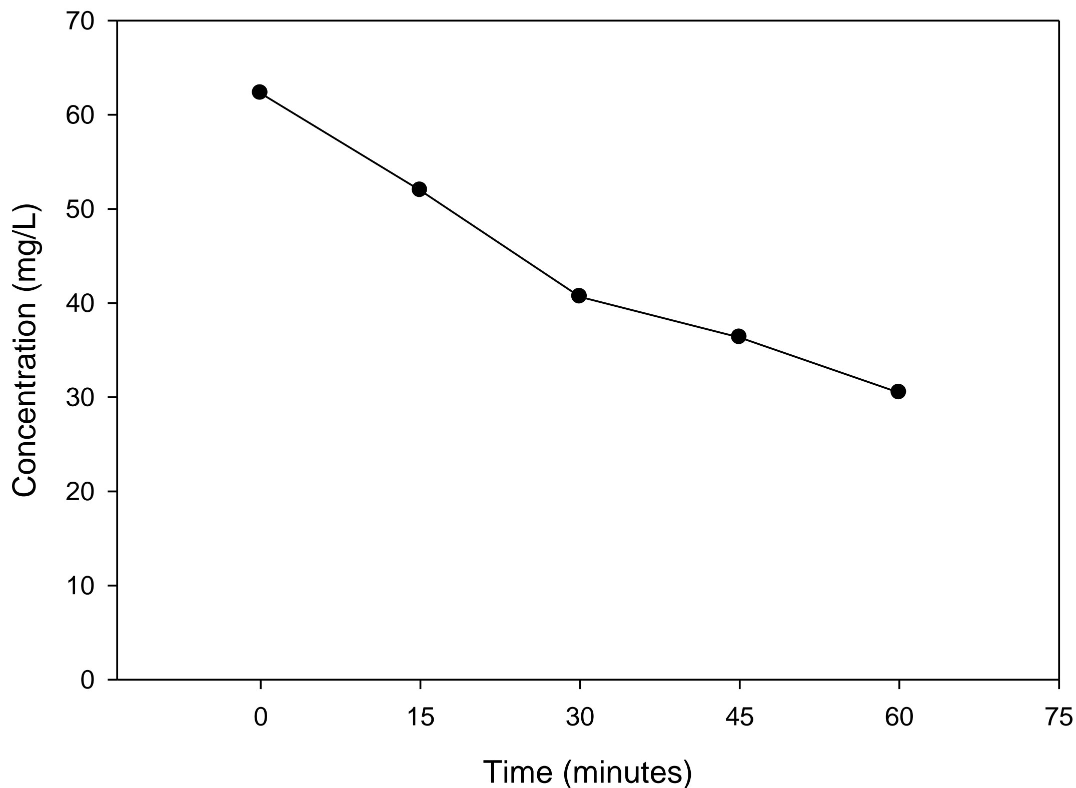

Figure 4.68: Laccase Treatment of O-cresol in Sarnia Biox Effluent

Figure 4.69 shows the change in concentration of p-cresol over time caused by laccase/peroxide treatment. The p-cresol decreased from 59.8 mg/L initially to 41.4 mg/L after 60 minutes. This reduction by laccase/peroxide of p-cresol corresponds to 31.3 percent; nearly half of the reduction seen for phenol, and significantly smaller than that of o-cresol. The first order rate constant for p-cresol reduced by laccase was found to be -0.0060 min^{-1} with a R^2 value of 0.99 (Table 4.11). The rate constant of p-cresol was significantly lower than the rate for phenol and approximately half the rate determined for o-cresol.

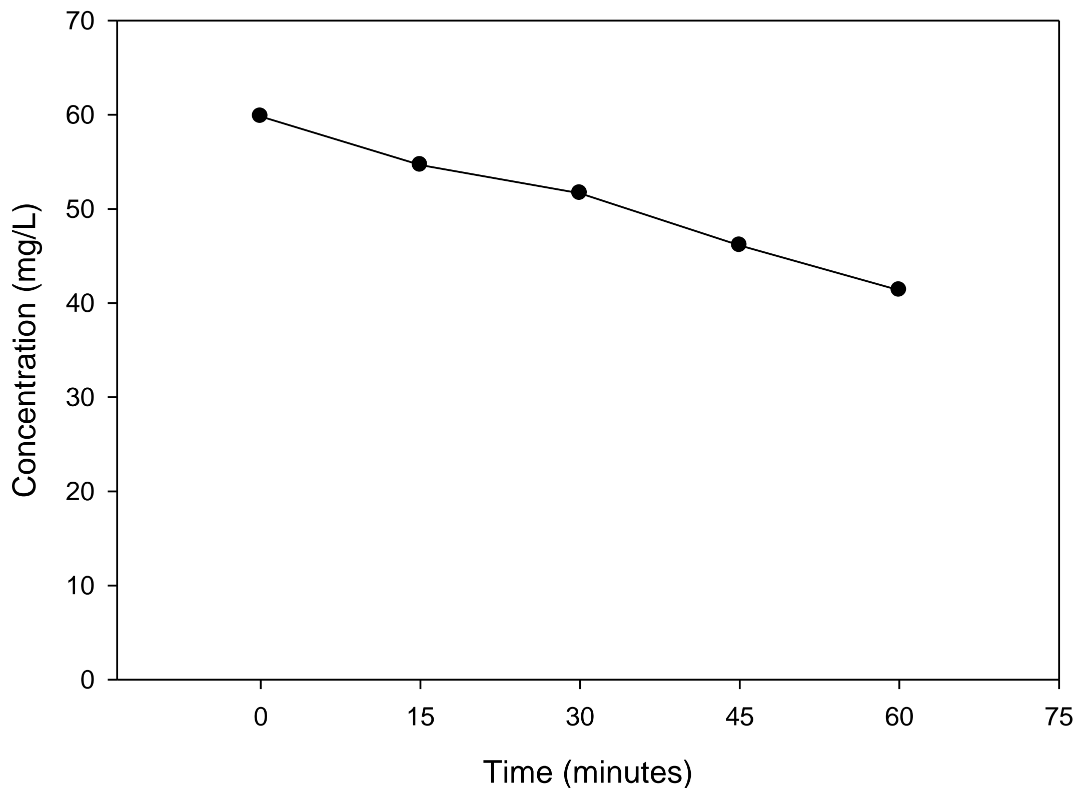

Figure 4.69: Laccase Treatment of P-cresol in Sarnia Biox Effluent

The concentrations for each individual phenolic compound were normalized to the initial concentration to show the fractions remaining over the 60 minute reaction; these are shown in Figure 4.70. Phenol had the smallest fraction remaining with 37.5 percent. This percent was about 25 percent lower than the fraction remaining for o-cresol and nearly half that of p-cresol, which were 49 and 68.7 percent respectively. The decrease in phenol occurred in the first 15 minutes of the reaction; one explanation for this similarity to the clean system could be that the biox effluent has little to no other contaminants.

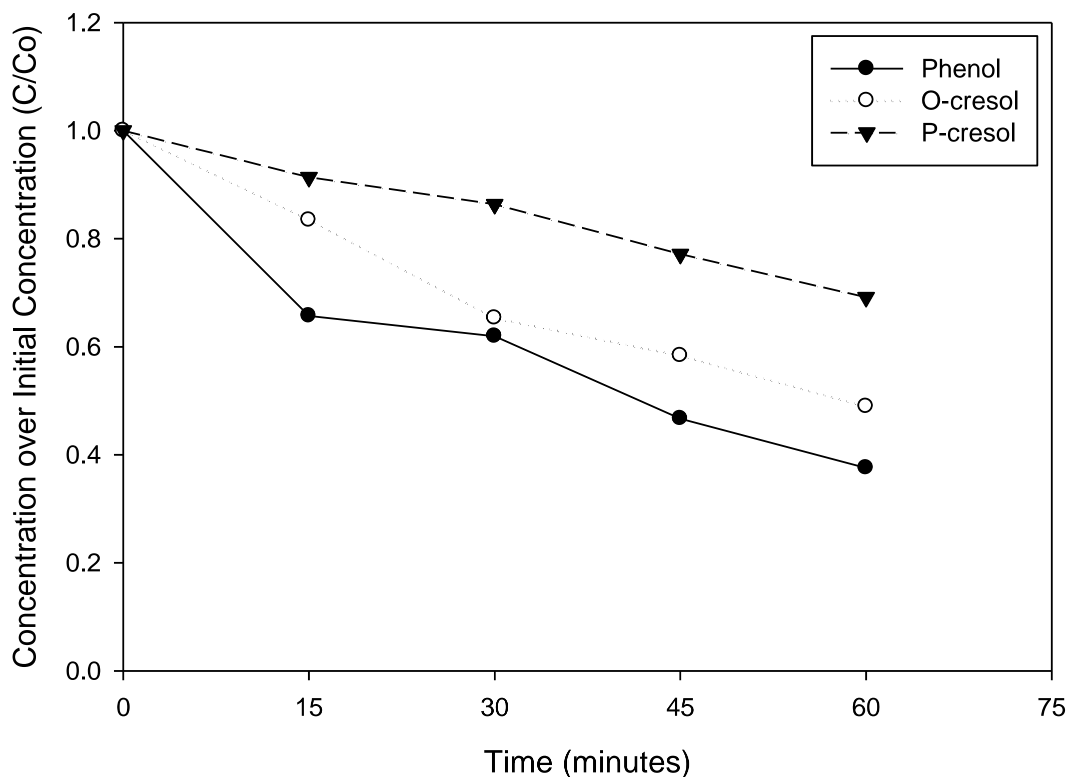

Figure 4.70: Fraction Remaining of Phenolic Compounds using Laccase Treatment on Sarnia Biox Influent

The biox effluent from the Sarnia Refinery was also tested using the peroxidase enzyme. Once again 100ppm phenol, 100ppm o-cresol, and 100ppm p-cresol were mixed into the sample since the original concentrations were non-detectable. An initial dose of 2600 units of peroxidase and 300 mg/L hydrogen peroxide were added to the biox effluent sample. There were three replicate test run using the peroxidase enzyme and the average initial concentrations contained in the biox effluent after the addition of phenols were: 47.8 mg/L phenol, 66.2 mg/L o-cresol, 61.4 mg/L p-cresol and 175.4 mg/L total phenol. The first order rate constants for peroxidase/peroxide treatment were also examined (Table 4.11).

Figure 4.71 shows the decrease in phenol concentration over time in Sarnia biox effluent due to peroxidase/peroxide treatment. The phenol concentration in the biox effluent was reduced from 62.4 mg/L to 22.1 mg/L after 60 minutes, a 58.8 percent reduction. The first order rate constant for phenol reduced by peroxidase was found to be -0.0152 min^{-1} with a R^2 value of 0.98 (Table 4.11). The high R^2 value indicates a very good fit with the first order model.

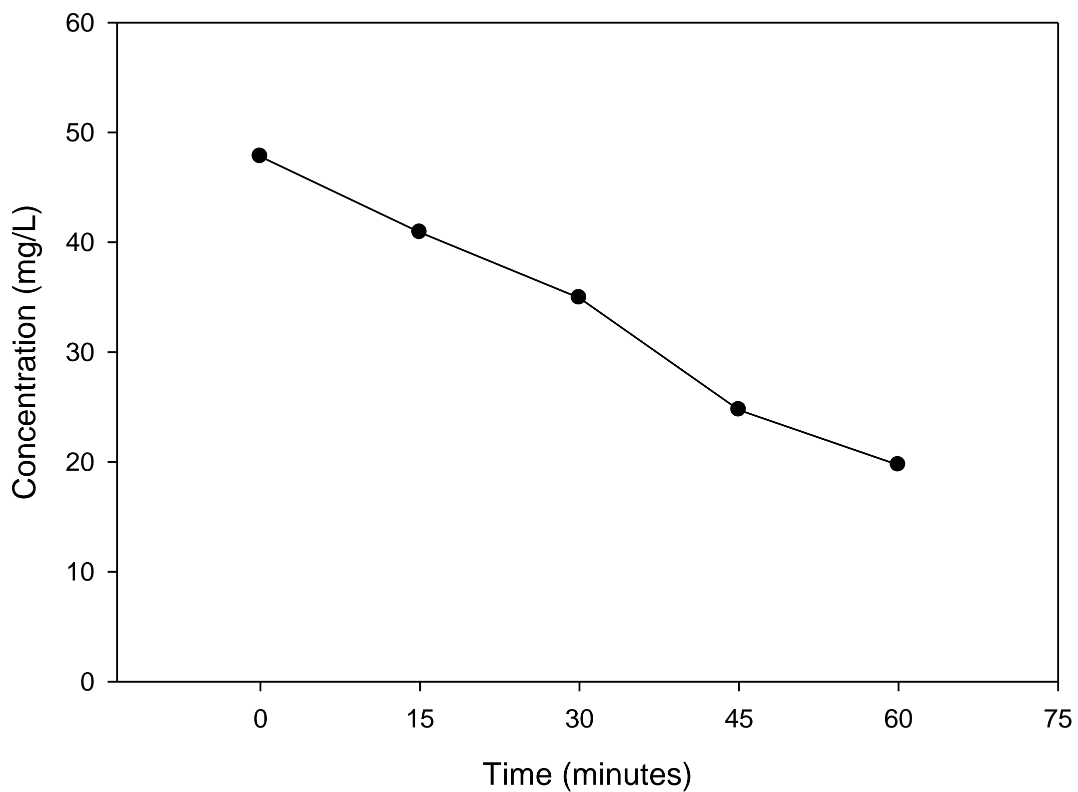

Figure 4.71: Peroxidase Treatment of Phenol in Sarnia Biox Effluent

The o-cresol concentration in the biox effluent decreased from 66.2 mg/L initially to a concentration of 31.7 mg/L after 60 minutes time (Figure 4.72). This corresponds to a reduction in o-cresol concentration of 52.1 percent. The percent

reduction is slightly less than phenol. The majority of the degradation occurred during the first 45 minutes of the reaction. The first order rate constant for o-cresol was examined and was determined to be -0.0128 min^{-1} with a R^2 value equal to 0.97 (Table 4.11). The rate constant of o-cresol was approximately 20 percent lower than the rate constant for phenol.

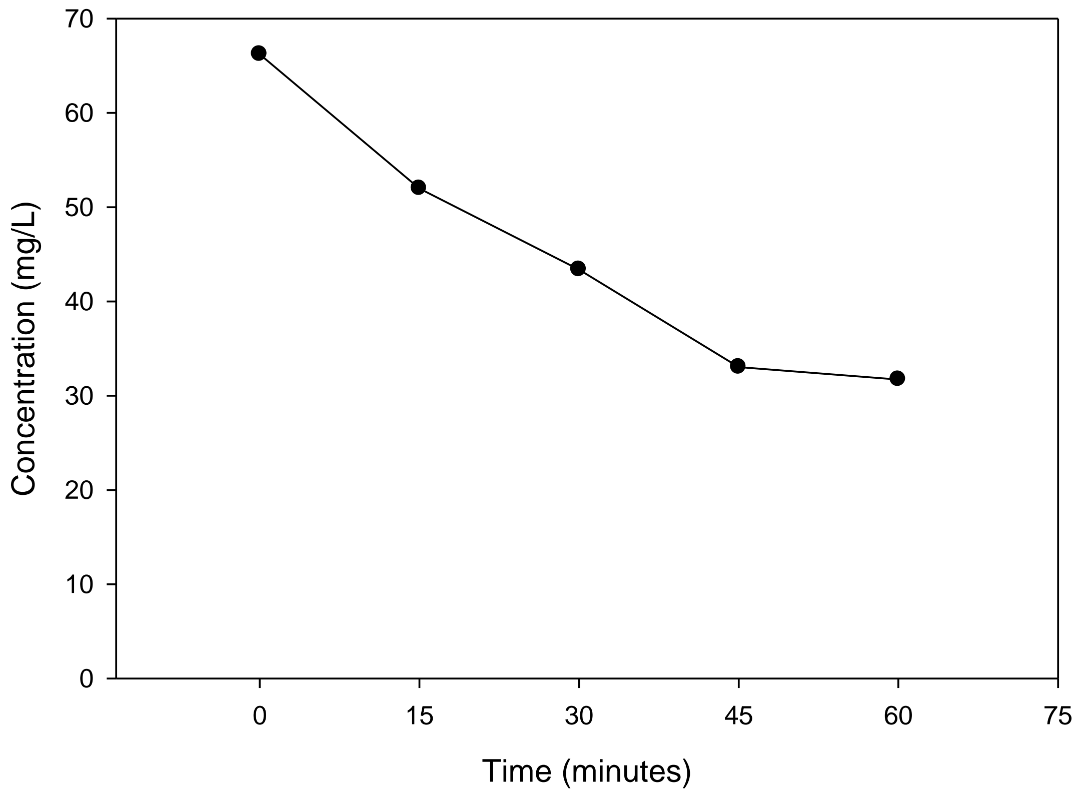

Figure 4.72: Peroxidase Treatment of O-cresol in Sarnia Biox Effluent

The change in p-cresol concentration over time caused by peroxidase/peroxide treatment is shown in Figure 4.73. Peroxidase reduced the P-cresol in the Sarnia biox effluent from a concentration of 61.4 mg/L to 30.8 mg/L over the 60 minutes, a reduction of 49.8 percent. This percent resembles that of o-cresol., but again is less

that the percent removal for phenol. The first order rate constant for peroxidase reducing p-cresol was found to be -0.0108 min^{-1} with a R^2 value of 0.90 (Table 4.11). The rate constant of p-cresol was the lowest rate for peroxidase/peroxide, 40 percent lower than the rate for phenol.

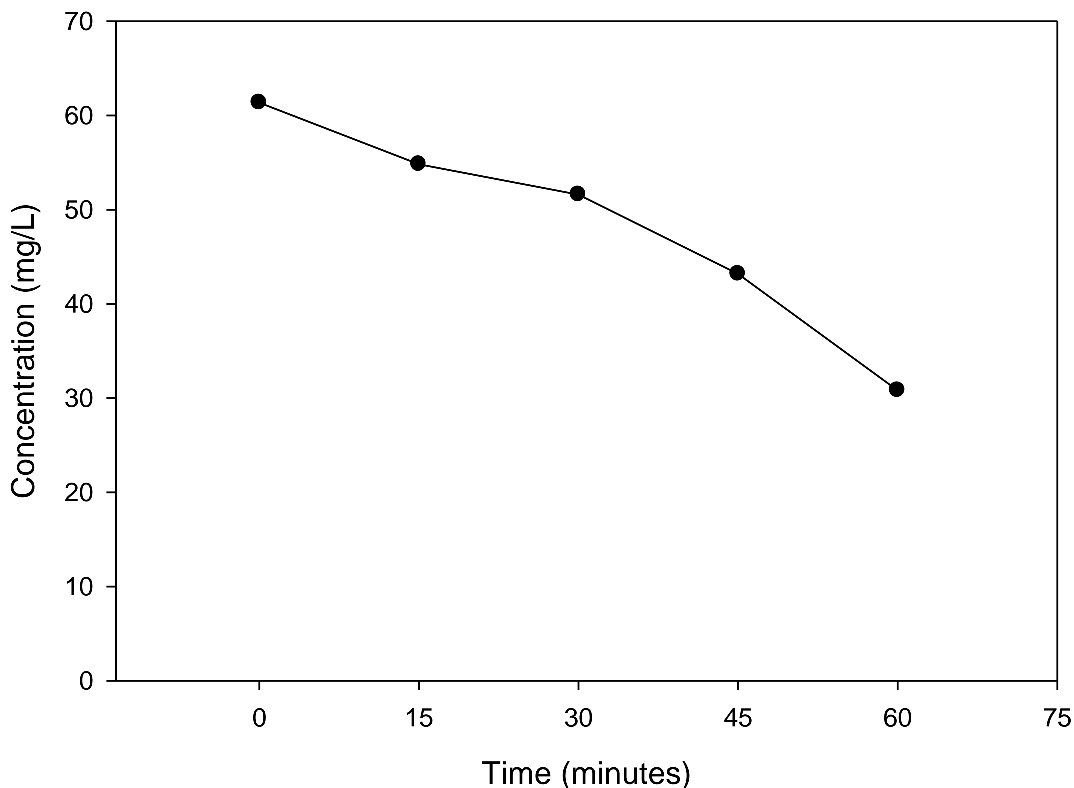

Figure 4.73: Peroxidase Treatment of P-cresol in Sarnia Biox Effluent

The concentrations of each compound added to the biox effluent were normalized to the initial concentration to show the fraction remaining after 60 minutes using the peroxidase enzyme (Figure 4.74). The percent remaining for o-cresol and p-cresol was 47.9 and 50.2 percent respectively. O-cresol and p-cresol percent remaining was a little higher that of phenol. Phenol had the lowest remaining

concentration with 41.2 percent. The decrease in the phenol due to peroxidase/peroxide followed a more linear pattern, whereas o-cresol reduction occurred during the first 45 minutes, and p-cresol degradation occurred after 30 minutes of reaction.

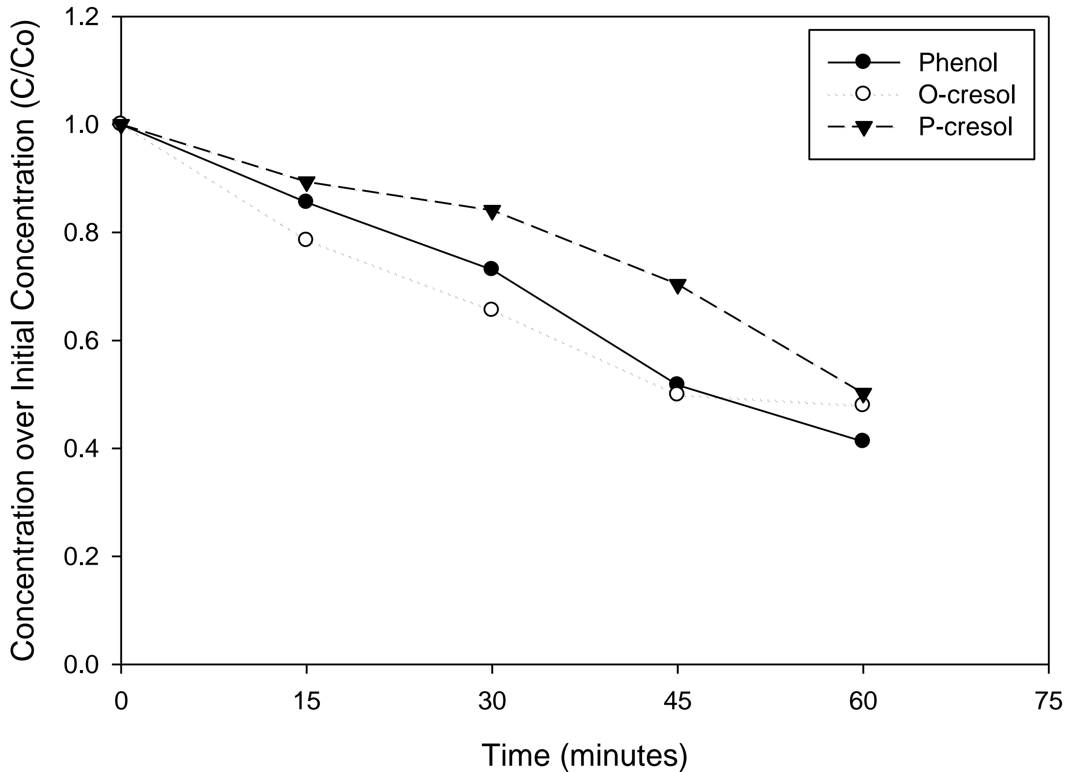

Figure 4.74: Fraction Remaining of Phenolic Compounds using Peroxidase Treatment on Sarnia Biox Influent

Phase Three: Effects of Enzyme/Peroxide Treatment on Nitrification.

The use of laccase and peroxidase was shown to reduce the concentration of phenols in Phase I and II. To confirm that the enzymatic treatment reduced the toxicity of various wastewaters to the nitrification process, Phase III testing was conducted using a commercially available culture of Nitrosomonas and Nitrobacter

known as Nitrotox. Wastewater samples were treated with 500 units of laccase or peroxidase enzyme, 100 mg/L peroxide, and allowed to react for 30 minutes. Samples included in this phase of testing were: CITGO stripped sour water, Frontier stripped sour water, Dartmouth biox influent, Nanticoke biox influent, and Sarnia biox influent. Dilutions of the wastewater were made over the range expected in the wastewater treatment process. The rate of nitrification in these dilutions was measured as the reduction in ammonia over time.

The rate of nitrification as a function of the percent of CITGO Sour Stripper wastewater added to the culture is presented in Figure 4.75. The control was wastewater that was untreated by enzyme and peroxide. With no process wastewater added, the rate of nitrification was 29.4 mg NH_4-N/L/hr, which is a relatively high rate compared to those observed in the field. The rate of nitrification in the control sample decreased as the percentage of wastewater increased, indicating that the CITGO Sour Stripper water was inhibitory to nitrification. A fifty percent reduction in nitrification occurred at a wastewater concentration of just slightly more than 5 percent. The nitrification rate continued to decrease with increasing Sour Stripper Water percentage. When the wastewater concentration approached 40 percent of the total reactor volume, nitrification was almost completely inhibited. This shows that the untreated wastewater was very inhibitory to nitrification, even when present as a relatively small percentage of the total influent volume.

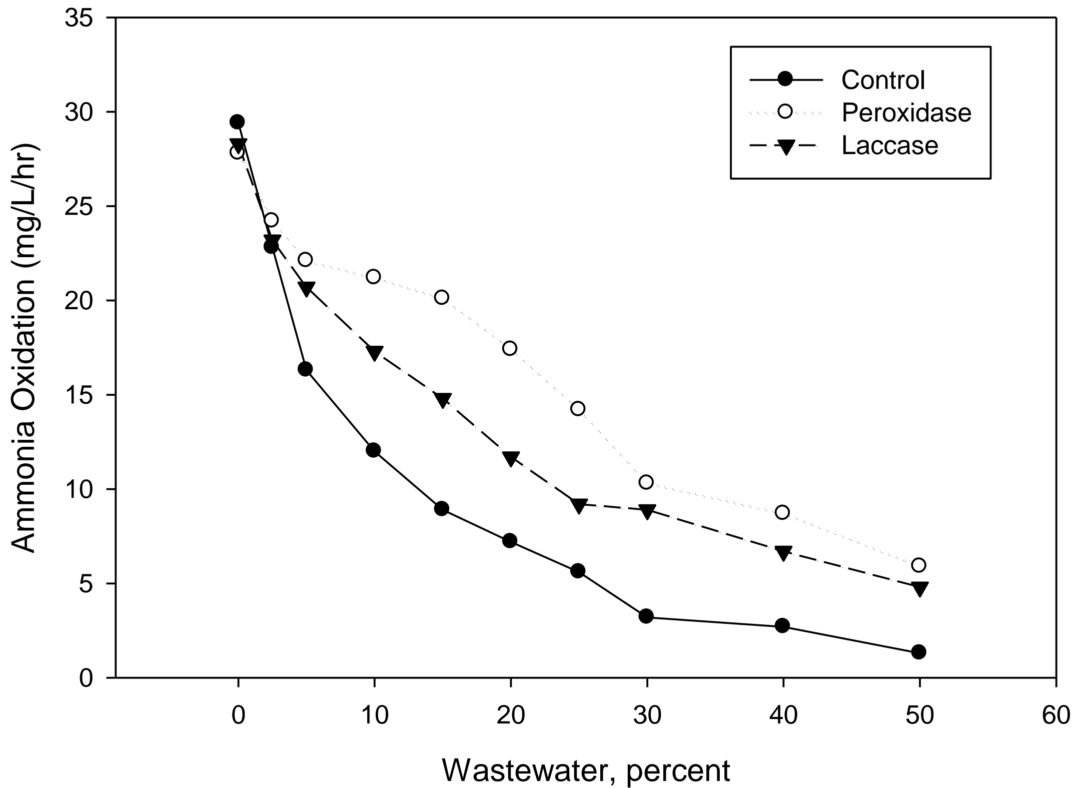

Figure 4.75: Ammonia Oxidation Using CITGO Stripped Sour Water

The wastewater treated with laccase was also inhibitory to nitrification as indicated by the decrease in ammonia oxidation when the percent of wastewater was increased. However, the degree of inhibition was significantly less than observed for the control. Fifty percent reduction in nitrification occurred when the percent wastewater was higher than 15 percent. This was three times higher than observed for the control with no enzyme/peroxide treatment. Almost 20 percent of the non-inhibited nitrification activity remained in the reactor with the highest (40 %) wastewater concentration. Although significantly inhibited, the activity of 6.7 mg NH4-N/L/hr is sufficiently high enough to attain a high degree of ammonia removal

from a process with 12 to 24 hours of hydraulic residence time. This demonstrates that the use of laccase enzyme can decrease the inhibition of nitrification in the CITGO sour stripper water.

The CITGO sour stripper wastewater was also treated with peroxidase and peroxide. As shown in Figure 4.75, the peroxidase wastewater was still inhibitory to nitrification as indicated by the decrease in ammonia oxidation when the percent of wastewater was increased. The degree of inhibition was significantly less than observed for the control and lower than that for the laccase treated samples. Fifty percent reduction in nitrification occurred when the percent wastewater was higher than 25 percent. This concentration was five times higher than observed for the control with no enzyme/peroxide treatment and almost twice as high as the laccase treated wastewater. Almost 30 percent of the non-inhibited nitrification activity remained in the reactor with the highest expected (40 %) wastewater concentration. Although significantly inhibited, the activity of 8.7 mg NH_4-N/L/hr is sufficiently high enough to attain a high degree of ammonia removal from a process with 12 to 24 hours of hydraulic residence time. This demonstrates that the use of peroxidase enzyme can decrease the inhibition of nitrification in the CITGO sour stripper water. Furthermore, the peroxidase enzyme was more effective in reducing the inhibition of process toxicity than the laccase enzyme. This mirrors the findings of Phase II, where the peroxidase enzyme was slightly more effective in removing the targeted phenolic compounds.

Figure 4.76 shows the rate of nitrification as a function of the percent of Frontier sour stripper wastewater added to the culture. The control wastewater was

untreated by enzyme and peroxide. With the control, the rate of nitrification was 25.4 mg NH_4-N/L/hr, which is a relatively high rate compared to those observed in the field. The rate of nitrification in the control sample decreased as the percentage of wastewater increased, indicating that the Frontier stripped sour water was inhibitory to nitrification. A fifty percent reduction in nitrification occurred at a wastewater concentration of just slightly less than 10 percent. The nitrification rate continued to decrease with more Frontier sour water percentage. When the wastewater concentration approached 50 percent of the total reactor volume, nitrification was almost completely inhibited (less than 2 mg NH_4-N/L/hr). This shows that the untreated wastewater was very inhibitory to nitrification, even when present as a relatively small percentage of the total influent volume.

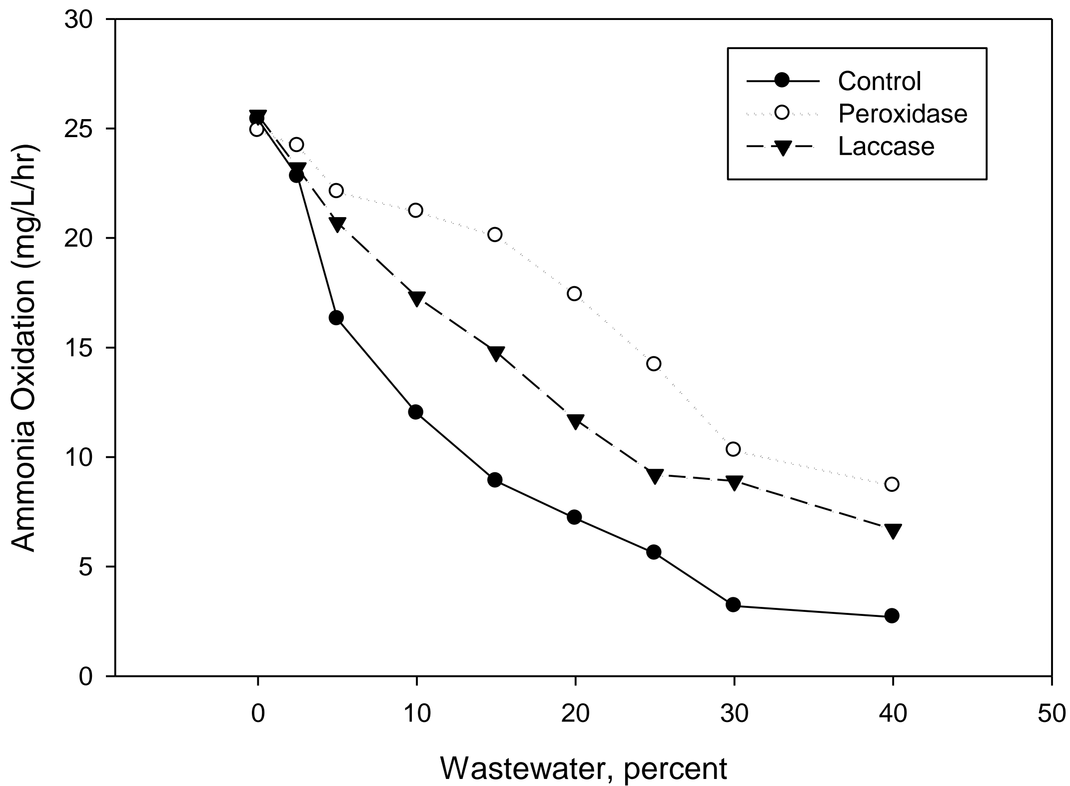

Figure 4.76: Ammonia Oxidation Using Frontier Stripped Sour Water

The wastewater treated with laccase was also inhibitory to nitrification as indicated by the decrease in ammonia oxidation when the percent of wastewater was increased. However, the degree of inhibition was significantly less than observed for the control. Fifty percent reduction in nitrification occurred when the percent wastewater was higher than 20 percent. This was about two times higher than that observed for the control with no enzyme/peroxide treatment. Close to 20 percent of the non-inhibited nitrification activity remained in the reactor with the 40 percent wastewater concentration. Although significantly inhibited, the activity of 5.9 mg NH_4-N/L/hr is sufficiently high enough to attain a high degree of ammonia removal from a process with 12 to 24 hours of hydraulic residence time. This demonstrates

that the use of laccase enzyme can lower the amount of inhibition to nitrification in the Frontier sour water.

The Frontier stripped sour wastewater was also treated with peroxidase and peroxide. This enzymatic treatment using peroxidase and peroxide was still inhibitory to nitrification as indicated in Figure 4.76 by the decrease in ammonia oxidation when the percent of wastewater was increased. Nonetheless, the degree of inhibition was still significantly less than observed for the control and slightly lower than that for the laccase treated samples. Fifty percent reduction in nitrification occurred when the percent wastewater was higher close to 30 percent. This concentration was three times higher than that observed for the control and almost one and half times higher than the laccase treated wastewater. Almost 30 percent of the non-inhibited nitrification activity remained in the reactor with the highest expected wastewater concentration of 40 percent. Although significantly inhibited, the activity of 8.7 mg NH_4-N/L/hr is sufficiently high enough to attain a high degree of ammonia removal from a process with a hydraulic residence time of 12 to 24 hours. This demonstrates that the use of peroxidase enzyme can decrease the inhibition of nitrification in the Frontier sour water. As before, the peroxidase enzyme was slightly more effective in reducing the inhibition of process toxicity than the laccase enzyme; but this was expected as the peroxidase enzyme was slightly more effective in removing the targeted phenolic compounds in Phase II.

In Figure 4.77 the rate of nitrification as a function of the percent of Dartmouth biox influent wastewater added to the culture is shown. With the control, wastewater untreated by enzyme and peroxide, the rate of nitrification was 23.5 mg

NH_4-N/L/hr. The rate of nitrification in the control sample decreased as the percentage of wastewater increased, indicating that the Dartmouth biox influent water was inhibitory to nitrification.   A wastewater concentration of around 15 percent caused a decrease in nitrification of fifty percent. The higher the Dartmouth biox influent percentage the more the nitrification rate continued to decrease. When the wastewater concentration approached 40 percent of the total reactor volume, nitrification was completely inhibited (non-detectable amount). This shows that the untreated biox influent was very inhibitory to nitrification, even when present as a relatively small percentage of the total influent volume.

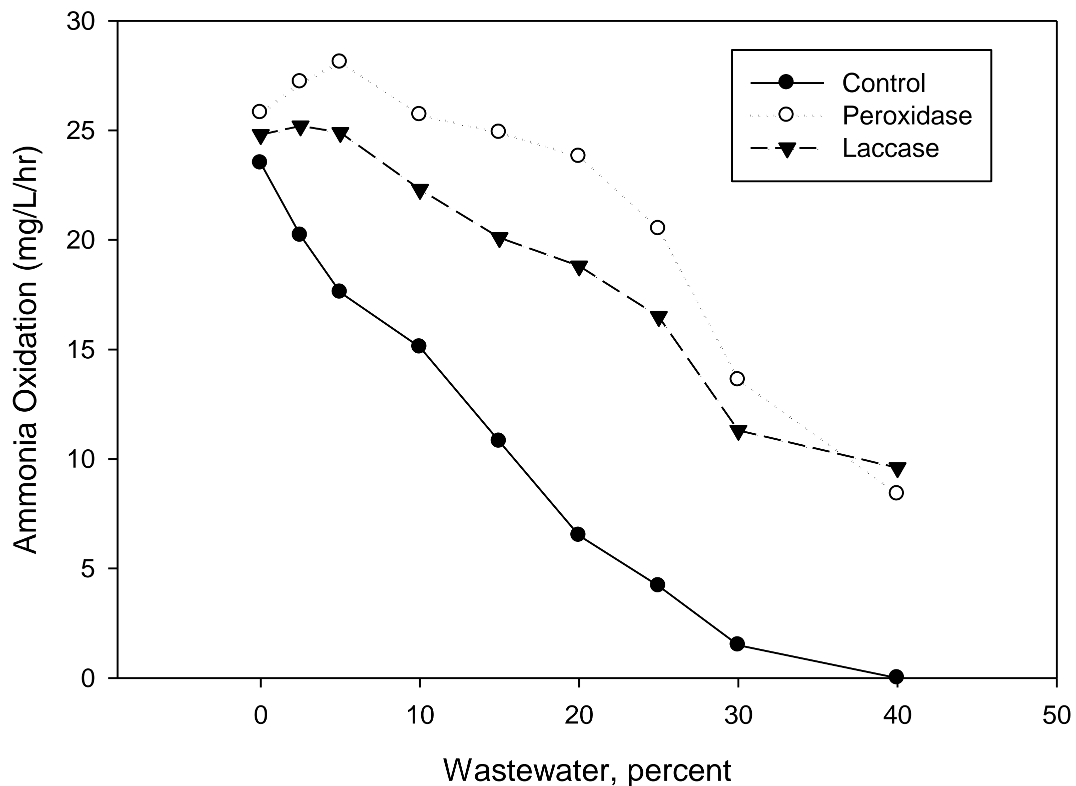

Figure 4.77: Ammonia Oxidation using Dartmouth Biox Influent

The biox influent was treated using the enzymatic approach above, when using the laccase/peroxide treatment the wastewater was also inhibitory to nitrification as indicated by the decrease in ammonia oxidation when the percent of wastewater was increased (Figure 4.77). Yet, the degree of inhibition was significantly less than observed for the control. Fifty percent reduction in nitrification occurred when the percent wastewater was almost 30 percent. This was about two times higher than that observed for the control. Also, when the reactor contained a wastewater concentration of 40 percent of the total reactor volume (complete inhibition in the control) the non-inhibited nitrification activity remained over 35 percent. Although significantly inhibited, the activity of 9.6 mg NH_4-N/L/hr is sufficiently high enough to attain a high degree of ammonia removal from a process with 12 to 24 hours of hydraulic residence time. This demonstrates that the use of laccase enzymatic treatment can lower the amount of inhibition to nitrification in the Dartmouth biox influent.

The Dartmouth biox influent was also treated using the peroxidase/peroxide treatment method. This enzymatic treatment using peroxidase and peroxide was still inhibitory to nitrification, as indicated in Figure 4.77 by the decrease in ammonia oxidation when the percent of wastewater was increased. Nevertheless, the degree of inhibition was still significantly lower than that observed for the control and that for the laccase/peroxide treated samples. Fifty percent reduction in nitrification occurred when the percent wastewater was higher close to 30 percent. This concentration was over two times that observed for the control and about the same as the laccase treated wastewater. Almost 43 percent of the non-inhibited nitrification activity remained

when the wastewater concentration of 35 percent of the total reactor volume was present. Even though the activity is significantly inhibited at 8.4 mg NH_4-N/L/hr, this is sufficiently high enough to attain a high degree of ammonia removal from a process with a 12 to 24 hour hydraulic residence time. This demonstrates that the use of peroxidase/peroxide enzymatic treatment can decrease the inhibition of nitrification in the Dartmouth biox influent. Again, as expected from the results in Phase II, the peroxidase enzyme was slightly more effective in reducing the inhibition of process toxicity than the laccase enzyme.

The rate of nitrification as a function of the percent of Nanticoke desalter wastewater added to the culture is shown in Figure 4.78. The control wastewater was wastewater untreated by enzyme and peroxide. With no process wastewater added, the rate of nitrification was 27.8 mg NH_4-N/L/hr. The rate of nitrification in the control sample decreased as the percentage of wastewater increased, indicating that the Nanticoke desalter water did inhibit nitrification. A wastewater concentration of around 5 percent caused a decrease in nitrification of fifty percent. The higher the Nanticoke desalter percentage the more the nitrification rate continued to decrease, by 25 percent the rate of nitrification had decreased to a non-detectable amount. This shows that the untreated wastewater was very inhibitory to nitrification, even when present as a small percentage of the total influent volume.

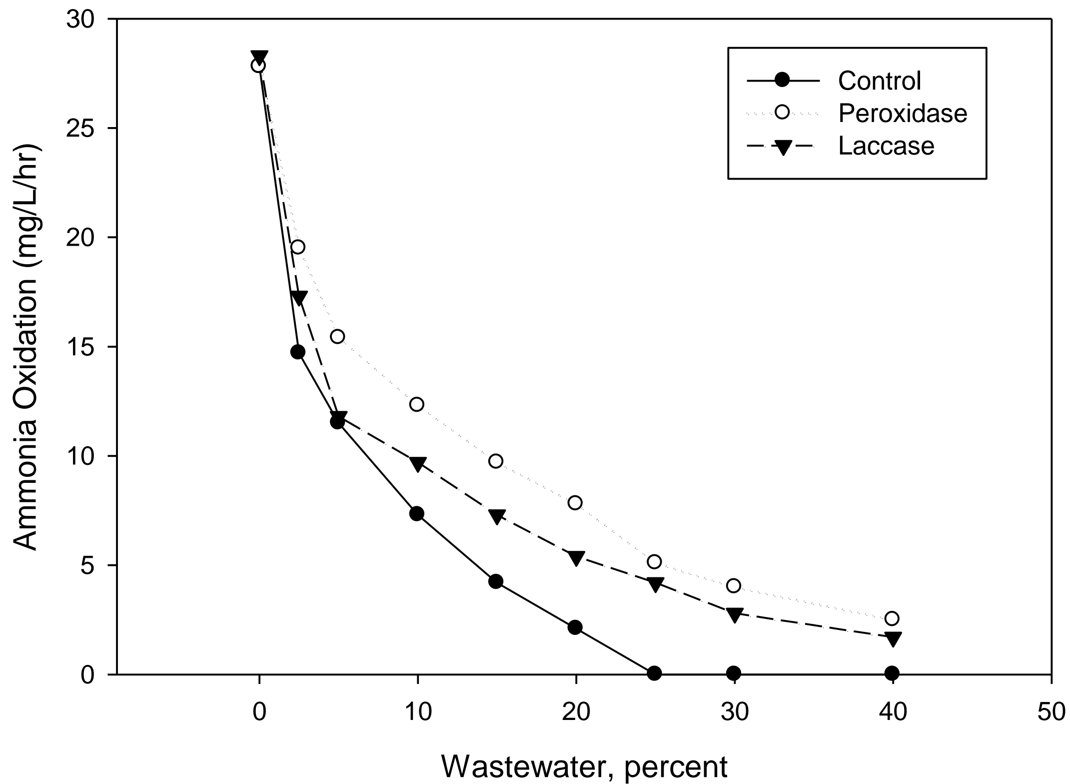

Figure 4.78: Ammonia Oxidation using Nanticoke Desalter

The wastewater was treated using the laccase/peroxide treatment, this was also inhibitory to nitrification as indicated by the decrease in ammonia oxidation when the percent of wastewater was increased (Figure 4.78). However, the inhibition was to a significantly lesser degree than that observed for the control. Fifty percent reduction in nitrification occurred when the percent wastewater was almost 10 percent. This was about two times higher than that observed for the control. Also, when the reactor contained a wastewater concentration of 25 percent of the total reactor volume (complete inhibition in the control) the non-inhibited nitrification activity remained close to 15 percent. Although significantly inhibited, the activity of

4.2 mg NH_4-N/L/hr is enough to achieve a sufficient degree of ammonia removal from a process with 12 to 24 hours of hydraulic residence time. Thus, by using laccase enzymatic treatment, the amount of inhibition to nitrification in the Nanticoke desalter can be lowered.

The Nanticoke desalter was also treated using the peroxidase/peroxide treatment method. This enzymatic treatment using peroxidase and peroxide was still inhibitory to nitrification, as indicated by the decrease in ammonia oxidation when the percent of wastewater was increased as shown in Figure 4.78. The degree of inhibition, however, was still significantly lower than that observed for the control and that for the laccase/peroxide treated samples. Fifty percent reduction in nitrification occurred when the percent wastewater was higher close to 15 percent. This concentration was over three times that observed for the control. Almost 20 percent of the non-inhibited nitrification activity remained when the wastewater concentration of 25 percent of the total reactor volume. Even though the activity is significantly inhibited at 5.1 mg NH_4-N/L/hr, this is sufficiently high enough to attain a high degree of ammonia removal from a process with a 12 to 24 hour hydraulic residence time. This demonstrates that the use of peroxidase/peroxide enzymatic treatment can decrease the inhibition of nitrification in the Nanticoke desalter. Although this particular wastewater was not tested in Phase II, the results continue to follow the trend shown in that phase of the study.

The rate of nitrification as a function of the percent of Sarnia biox effluent wastewater added to the culture is shown in Figure 4.79. The rate of nitrification in the control, wastewater untreated by enzyme and peroxide, was 26.8 mg NH_4-N/L/hr.

This decreased as the percentage of wastewater increased, indicating that the Sarnia biox effluent water did inhibit nitrification. A fifty percent reduction in nitrification was seen at a wastewater concentration around 5 percent. The higher the Sarnia biox effluent percentage the more the nitrification rate continued to decrease. When the wastewater concentration approached 40 percent of the total reactor volume, nitrification had decreased to a non-detectable amount. This shows that the untreated wastewater was very inhibitory to nitrification, even when present as a relatively small percentage of the total influent volume.

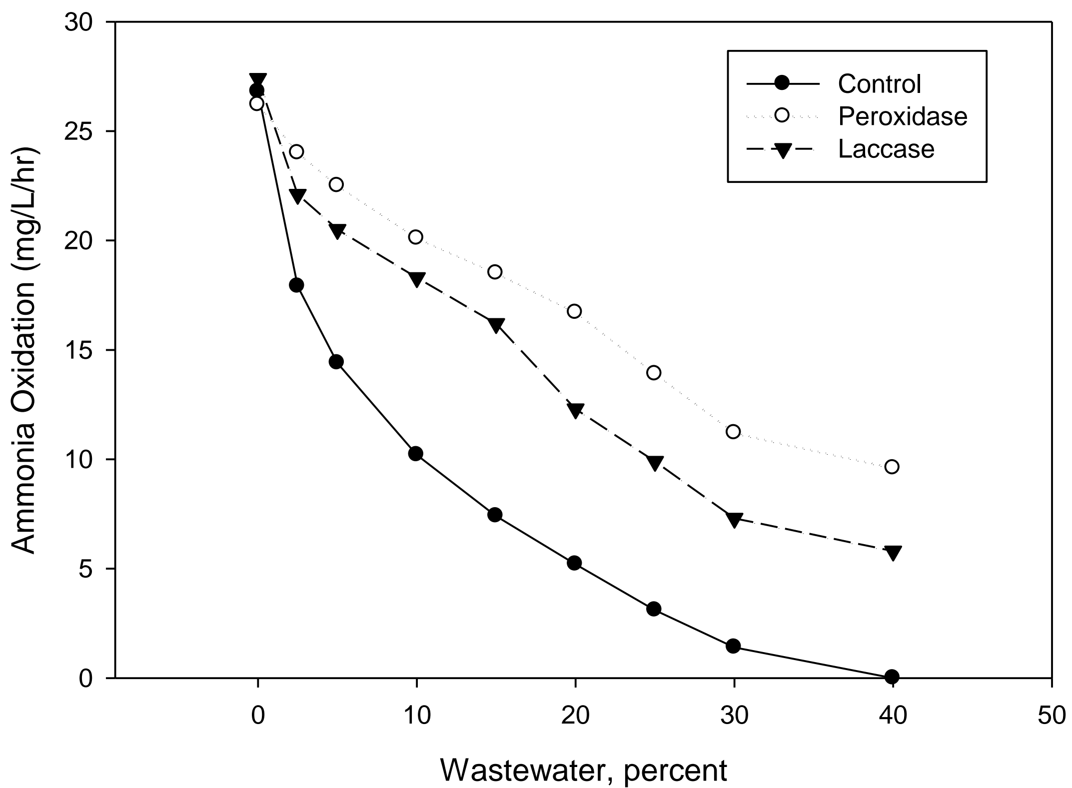

Figure 4.79: Ammonia Oxidation using Sarnia Biox Effluent

The wastewater treated with the laccase/peroxide treatment was also inhibitory to nitrification as indicated in Figure 4.79 by the decrease in ammonia oxidation when the percent of wastewater was increased. However, the degree of inhibition was significantly less than that observed for the control. When the percent wastewater was almost 20 percent, a fifty percent reduction in nitrification occurred. This was about four times higher than that observed for the control. Also, when the reactor contained a wastewater concentration of 40 percent of the total reactor volume the non-inhibited nitrification activity remained close to 20 percent. Although significantly inhibited, the activity of 5.8 mg NH_4-N/L/hr is high enough to achieve a sufficient degree of ammonia removal from a process with an hydraulic residence time of 12 plus hours. As a result, the amount of inhibition to nitrification in the Sarnia biox effluent can be lowered by using laccase enzymatic treatment.

The Sarnia biox effluent was also treated using the peroxidase/peroxide treatment method. This enzymatic treatment was still inhibitory to nitrification, as indicated by the decrease in ammonia oxidation when the percent of wastewater was increased (Figure 4.79). The degree of inhibition, however, was still significantly lower than that observed for the control and that for the laccase/peroxide treated samples. Fifty percent reduction in nitrification occurred when the percent wastewater was higher close to 25 percent. This concentration was over five times that observed for the control. When the wastewater concentration reached 25 percent of the total reactor volume, almost 35 percent of the non-inhibited nitrification activity remained. Even though the activity is inhibited, 9.6 mg NH_4-N/L/hr is sufficiently high enough to attain a high degree of ammonia removal from a process with a 12 to 24 hour

hydraulic residence time. This demonstrates that the use of peroxidase/peroxide enzymatic treatment can also decrease the inhibition of nitrification in the Sarnia biox effluent. Furthermore, the peroxidase enzyme was more effective in reducing the inhibition of process toxicity than the laccase enzyme. This mirrors the results of Phase II, where the peroxidase/peroxide treatment was slightly more effective in removing the target phenolic compounds from the wastewater.

Chapter 5

Summary and Recommendations

The research presented in this thesis supports the hypothesis in that laccase and peroxidase, coupled with hydrogen peroxide, do in fact reduce the concentrations of the chosen phenolic compounds (phenol, o-cresol, p-cresol, and 2,4-dimethyl phenol) in refinery wastewater. Although this enzymatic approach to phenol remediation may appear to be a common subject for research, the matrix in which previous research has been done was buffer solutions or de-ionized water. Whereas the matrix researched in this thesis was not only DI water, but actual refinery wastewater from five different refineries across the US and Canada, each with different wastewater matrices. Therefore the results of this thesis will contribute to the understanding of how this enzymatic approach can be applied to refinery wastewaters. In this chapter, a summary of the findings along with recommendations for further work are presented.

Summary

This study was conducted in three phases. The first phase involved the enzymatic treatment of phenolic compounds in a simple matrix (de-ionized water) to determine both the efficiency of the enzymes and also the effect of hydrogen peroxide on the enzymes. The second phase involved the enzymatic treatment of various

refinery wastewaters (CITGO, Frontier, and Imperial Oil). Lastly, the third phase investigated the effect of enzymatic treatment on nitrification.

Phase one considered the efficiency of laccase and peroxidase treatment in both a simple matrix with only one individual compound present and a simple matrix with a defined mixture of phenolic compounds present. The testing of the individual compounds showed that the peroxidase/peroxide treatment had a slightly higher rate of removing the compounds from the simple matrix than the laccase/peroxide treatment (refer to Table 4.1). However, the overall percent remaining for all compounds were similar for both treatment options. The phenol and 2,4-dmp were reduced to about 25 to 30 percent remaining after one hour, whereas o-cresol and p-cresol were reduced to a remaining percent of about 45 to 50 percent (see Figures 4.5 and 4.6)

In the simple matrix with defined concentrations of phenolic compounds it was determined that the peroxidase/peroxide treatment had a faster rate of removal and was more efficient. Phenol and 2,4-dmp had higher reduction rates than the o-cresol and p-cresol. Also noticed in this part of phase one is that the rates for removing the phenolic compounds in the defined mixture were lower than the rate for removing the compounds individually.

In Phase Two the results for each individual wastewater differed slightly from each other. One important observation is that the rates for reducing the target phenols were generally lower than the rates determined in Phase One. Another difference is with most of the wastewater samples, the majority of the reduction did not occur until the last 15 to 30 minutes of the reaction; whereas, the reaction occurred in the first 15

to 30 minutes in Phase One with the clean system. Table 4.12 shows the average first order reduction rate constants for all compounds in the defined mixture.

Table 4.12: Average First Order Rate Constants for Phase Two

Compound	**Laccase k (min^{-1})**	**Peroxidase k (min^{-1})**
Phenol	-0.0125 (R^2 = 0.91)	-0.0141 (R^2 = 0.95)
O-cresol	-0.0098 (R^2 = 0.89)	-0.0110 (R^2 = 0.97)
P-cresol	-0.0083 (R^2 = 0.94)	-0.0096 (R^2 = 0.89)
2,4-Dimethyl Phenol	-0.0114 (R^2 = 0.93)	-0.0130 (R^2 = 0.93)

To confirm the finding in Phases One and Two, Phase Three testing was conducted using a commercially available culture of Nitrosomonas and Nitrobacter known as Nitrotox. Dilutions of treated wastewater were made over the range expected in the wastewater treatment process. The rate of nitrification in these dilutions was measured as the reduction in ammonia over time. Compared to the control (no enzymatic treatment), the ammonia reduction in the peroxidase/peroxide and laccase/peroxide treated samples was significantly higher.

Recommendations

In order to reduce the toxicity of phenolic compounds to the nitrification process, the amount of phenols in the wastewater being treated needs to be reduced. Enzymatic treatment using peroxidase/peroxide and laccase/peroxide is a viable option for the degradation of phenols. Throughout the course of this thesis, better optimization of parameters was recognized as a potential area for improvement and therefore, should be obtained in further studies.

For instance, it should be determined how pH affects the enzymatic treatment process. Moreover, further studies on pH could determine if a constant pH would yield a better reduction rate.

More studies using other enzymes should be included in this research topic in order to determine the most effective enzyme. Additionally, relationships involving other contaminants should also be explored. Furthermore, a cost analysis of enzyme production for all viable treatment options could provide more insight into real-world practical application.

Bibliography

Alberti, B. N., and Klibanov, A. M. (1981). "Enzymatic Removal Of Dissolved Aromatics From Industrial Aqueous Effluents." *Biotechnology And Bioengineering*, 373-379.

Alemzadeh, I., and Nejati, S. (2009). "Phenols removal by immobilized horseradish peroxidase." *Journal of Hazardous Materials*, 166(2-3), 1082-1086.

Armstrong, T., Scott, B., Taylor, K., and Gardner, A. (1996). "Refining Details Notebook Sour Water Stripper." *Today's Refinery*(June).

Baaziz, M. "The Activity and Preliminary Characterization of Peroxidases in Leaves of Cultivars of Date Palm, Phoenix Dactylifera L." *New Phytologist* 111 (1989): 403-11.

Balci, S., and Dincel, Y. (2002). "Ammonium ion adsorption with sepiolite: use of transient uptake method." *Chemical Engineering and Processing*, 41(1), 79-85.

Ben-Youssef, C., Zepeda, A., Texier, A. C., and Gomez, J. (2009). "A two-step nitrification model of ammonia and nitrite oxidation under benzene inhibitory and toxic effects in nitrifying batch cultures." *Chemical Engineering Journal*, 152(1), 264-270.

Beychok, M. R. (1967). *Aqueous Wastes from Petroleum and Petrochemical Plants*, Wiley, London, New York [etc.].

CASTion. (2008). "Sour Water."

Cooper, V. A., and Nicell, J. A. (1996). "Removal of phenols from a foundry wastewater using horseradish peroxidase." *Water Research*, 30(4), 954-964.

Dow Chemical Company. (1958). *Dowex ion exchange*, [Dow Chemical Company].

Elston, J., and Karmarkar, D. (2007). "Aqueous Ammonia Stripping Technology for SCR Applications." Foster Wheeler Power Group, Clinton, NJ.

Fang, H.-Y., Chou, M.-S., and Huang, C.-W. (1993). "Nitrification of ammonia-nitrogen in refinery wastewater." *Water Research*, 27(12), 1761-1765.

Gary, J. H., and Handwerk, G. E. (1984). *Petroleum refining: technology and economics*, M. Dekker, New York.

Hengstebeck, R. J. (1959). *Petroleum processing; principles and applications*, McGraw-Hill, New York.

Huiyi, J. X. "Distillation Tower." www.p3planningengineer.com, Singapore.

Hwang, Y. L., Keller, G. E., and Olson, J. D. (1992). "Steam Stripping for Removal of Organic Pollutants from Water.1. Stripping Effectiveness and Stripper Design." *Industrial & Engineering Chemistry Research*, 31(7), 1753-1759.

Jorgensen, T. C., and Weatherley, L. R. (2003). "Ammonia removal from wastewater by ion exchange in the presence of organic contaminants." *Water Research*, 37(8), 1723-1728.

Kim, Y. M., Park, D., Lee, D. S., and Park, J. M. (2008). "Inhibitory effects of toxic compounds on nitrification process for cokes wastewater treatment." *Journal of Hazardous Materials*, 152(3), 915-921.

Klibanov, A. M. (1982). "Enzymatic Removal Of Hazardous Pollutants From Industrial Aqueous Effluents." *Enzyme Engineering*, 6, 319-324.

Klibanov, A. M., and Alberti, B. N. (1981). "The Enzyme Peroxidase for the Removal of Phenols, Aromatic-Amines and Other Toxic-Chemicals from Industrial Aqueous Effluents." *Abstracts of Papers of the American Chemical Society*, 182(AUG), 42-ENVR.

Klibanov, A. M., and Morris, E. D. (1981). "Horseradish-Peroxidase For The Removal Of Carcinogenic Aromatic-Amines From Water." *Enzyme And Microbial Technology*, 3(2), 119-122.

Lante, A., Crapisi, A., Krastanov, A., and Spettoli, P. (2000). "Biodegradation of phenols by laccase immobilised in a membrane reactor." *Process Biochemistry*, 36(1-2), 51-58.

Lee, D., Lee, J. M., Lee, S. Y., and Lee, I. B. (2002). "Dynamic simulation of the sour water stripping process and modified structure for effective pressure control." *Chemical Engineering Research & Design*, 80(A2), 167-177.

Leffler, W. L. (1979). *Petroleum refining for the non-technical person*, Petroleum Pub. Co., Tulsa, Okla.

Li, M., Feng, C. P., Zhang, Z. Y., Lei, X. H., Chen, N., and Sugiura, N. (2010). "Simultaneous regeneration of zeolites and removal of ammonia using an

electrochemical method." *Microporous and Mesoporous Materials*, 127(3), 161-166.

Liu, Y. Q., Tay, J. H., Ivanov, V., Moy, B. Y. P., Yu, L., and Tay, S. T. L. (2005). "Influence of phenol on nitrification by microbial granules." *Process Biochemistry*, 40(10), 3285-3289.

Melin, G. A., Niedzwiecki, J. L., and Goldstein, A. M. (1975). "Optimum Design of Sour Water Strippers." *Chemical Engineering Progress*, 71(6), 78-82.

Meyers, R. A. (1997). *Handbook of petroleum refining processes*, McGraw-Hill, New York.

Miland, E., Smyth, M. R., and Fagain, C. O. (1996). "Phenol removal by modified peroxidases." *Journal of Chemical Technology and Biotechnology*, 67(3), 227-236.

Moeder, M., Martin, C., and Koeller, G. (2004). "Degradation of hydroxylated compounds using laccase and horseradish peroxidase immobilized on microporous polypropylene hollow fiber membranes." *Journal of Membrane Science*, 245(1-2), 183-190.

Nakamoto, S., and Machida, N. (1992). "Phenol Removal from Aqueous-Solutions by Peroxidase-Catalyzed Reaction Using Additives." *Water Research*, 26(1), 49-54.

Nicell, J. A., Bewtra, J. K., Biswas, N., Stpierre, C. C., and Taylor, K. E. (1993). "Enzyme-Catalyzed Polymerization and Precipitation of Aromatic-Compounds from Aqueous-Solution." *Canadian Journal of Civil Engineering*, 20(5), 725-735.

Park, J. B., Lee, S. H., Lee, J. W., and Lee, C. Y. (2002). "Lab scale experiments for permeable reactive barriers against contaminated groundwater with ammonium and heavy metals using clinoptilolite (01-29B)." *Journal of Hazardous Materials*, 95(1-2), 65-79.

Singh, N., and Singh, J. (2002). "An enzymatic method for removal of phenol from industrial effluent." *Preparative Biochemistry & Biotechnology*, 32(2), 127-133.

Sintobrator. (2002). "Waste Water Nitrogen Removal System." Japanese Advanced Environment Equipment, Japan.

Tong, Z., Zhao, Q. X., Hui, H., Qin, L., and Yi, Z. (1997). "Removal of toxic phenol and 4-chlorophenol from waste water by horseradish peroxidase." *Chemosphere*, 34(4), 893-903.

U.S., E. I. A. (2008). "Number and Capacity of Petroleum Refineries."

U.S., E. I. A. (2009). "Oil (petroleum)."

Waddams, A. L. (1980). *Chemicals from petroleum: an introductory survey*, Gulf Pub. Co., Book Division, Houston.

Wang, S. B., and Peng, Y. L. (2010). "Natural zeolites as effective adsorbents in water and wastewater treatment." *Chemical Engineering Journal*, 156(1), 11-24.

Wang, Y., Kmiya, Y., and Okuhara, T. (2007). "Removal of low-concentration ammonia in water by ion-exchange using Na-mordenite." *Water Research*, 41(2), 269-276.

Watson, S. M., Valos, F. W., and Waterbury, J. B. (1981). "The Family Nitrobacteraceae." *The Prokaryotes*.