REMEDIATION OF HIGH PHENOL CONCENTRATIONS USING CHEMICAL AND BIOLOGICAL TECHNOLOGIES

A Thesis Submitted to the College of Graduate Studies and Research

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

In the Department of Chemical and Biological Engineering

University of Saskatchewan

Saskatoon

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ABSTRACT

This thesis presents the potential of integrating chemical and biological treatment technologies for the removal of high concentrations of phenol in a bioremediation medium. High concentrations of phenol in wastewater are difficult to remove by purely biological methods. Chemical oxidation is one way to treat high concentrations of phenol but complete oxidation is not always possible or will make the treatment process uneconomical. An experimental design approach, based on central composite rotatable design (CCRD) was used to evaluate the effects of process parameters on phenol oxidation by Fenton's reagent and chlorine dioxide. Performance of the chemical oxidation was evaluated by determining the percentage of phenol oxidized at equilibrium. The reaction mechanism for the oxidation of phenol by Fenton's reagent was proposed based on identification of the intermediate compounds.

The effects of H_2O_2 concentration (2000 to 5000 mg L^{-1}) and $FeSO_4.7H_2O$ concentration (500 to 2000 mg L^{-1}) were investigated on phenol oxidation and optimal concentrations of H_2O_2 and $FeSO_4.7H_2O$ for complete oxidation of 2000 mg L^{-1} phenol in medium were found to be 4340 mg L^{-1} and 1616 mg L^{-1}, respectively, at 25°C and pH 3. The main oxidation products were identified as catechol, hydroquinone and maleic acid.

In the case of phenol oxidation by chlorine dioxide, the effects of chlorine dioxide concentration (500 to 2000 mg L^{-1}), temperature (10 to 40°C) and pH (3 to 7) on the oxidation of 2000 mg L^{-1} of phenol were determined. The optimal concentration of chlorine dioxide to completely oxidize 2000 mg L^{-1} of phenol was 2000 mg L^{-1}. The

other parameters did not significantly affect the oxidation over the ranges studied. The main oxidation products were identified as 1,4-benzoquinone and 2-chloro-1,4-benzoquinone.

Finally, the biodegradation of 1,4-benzoquinone, the main oxidation product of phenol oxidation by chlorine dioxide, was studied in batch and continuous systems using *Pseudomonas putida* 17484 in two dose McKinney's medium. The effects of 1,4-benzoquinone concentration and temperature were studied on biodegradation of 1,4-benzoquinone in batch reactors. Under optimal conditions, it was found that 150 mg L^{-1} 1,4-benzoquinone could be successfully biodegraded at 15°C. In a continuous reactor operating at 15°C the highest removal rate with 500 mg L^{-1} of 1,4-benzoquinone was found to be 246 mg L^{-1} h^{-1}. The values of μmax, Ks and yield were also determined as 0.74±0.03 h^{-1} and 14.17±3.21 mg L^{-1} and $2x10^{13}$ cell mg^{-1}, respectively.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt gratitude and thanks to my supervisors Dr. Gordon Hill and Dr. Mehdi Nemati for providing both a wonderful research environment and constant guidance in every aspect of this project. Secondly, I would like to thank the members of my advisory committee Dr. Ajay Dalai, Dr. Aaron Phoenix, Dr. Catherine Niu and Dr. Oon Doo Baik for their critical input and encouragement to work hard during the course of my Ph.D. degree.

I wish to acknowledge Dr. Hossein Nikakhtari for his invaluable support and practical advice on the theoretical and experimental work of this project. I would also like to thank the entire faculty and staff in the department of Chemical and Biological Engineering. Richard Blondin and Kerry Peru (Water Science and Technology Directorate, Environment Canada) deserve special mention for providing their technical expertise and advice on analytical methods.

My gratitude goes to the University of Saskatchewan for providing the infrastructure and financial support from the Natural Sciences and Engineering Research Council of Canada, Environment Canada and a local petroleum processing company.

Finally, I would to thank my wife Apeksha Singla and my family for their support and love.

Dedicated in loving memory of my father

TABLE OF CONTENTS

LIST OF TABLES

Table page

LIST OF FIGURES

NOMENCLATURES

C_{H2O2} — Initial hydrogen peroxide concentration, mg L^{-1}

$C_{FeSO4.7H2O}$ — Initial ferrous sulfate concentration, mg L^{-1}

C_{PH} — Initial phenol concentration, mg L^{-1}

T — Reaction temperature, °C

X_1 — Coded value of hydrogen peroxide concentration

X_2 — Coded value of ferrous sulfate concentration

Y — Response factor, % phenol oxidized

$C_{Fe(II)}$ — Ferrous ions concentration, mM

C_{ClO2} — Initial concentration of chlorine dioxide, mg L^{-1}

μ — Specific growth rate, h^{-1}

μ_{max} — Maximum specific growth rate, h^{-1}

S — Substrate concentration, mg L^{-1}

e_{cb}^- Electron in the conduction band

h_{vb}^+ Hole in the Valance band

CHAPTER 1

1. INTRODUCTION

1.1 Problem of water contamination

Water covers 70% of the earth's surface and undoubtedly is the most valuable resource on earth. Recently, the need for fresh, clean and uncontaminated water has become a problem of great importance since many sources have been exhausted and others are likely to be contaminated because of rapid industrialization and increasing world population. Toxic wastes are being released into the environment, causing extensive environmental contamination such that many of our natural water reserves are damaged beyond repair (Ollis and Al-Ekabi, 1993). Increasing environmental concerns and strict guidelines to control waste from industries has led scientists and engineers to search for improved technologies, such as advanced oxidation treatments for the removal of toxic chemicals from wastes.

To meet increasing demands for petroleum as a source of energy and as primary raw material for the chemical industry, increased recovery activities are vigorous with world oil production reaching 4229 million metric tons per year in 2005 (Energy Information Administration, 2006). Petroleum may pollute water bodies due to oil spills resulting from the rupture of the oil carrying pipes, sudden releases from oil wells and leakage from storage tanks. The recent BP and Enbridge oil spills are examples of these types of petroleum pollution. When this occurs, many water soluble petroleum compounds are discharged into the aquatic environment. Some of these compounds are not only toxic but only partly, or barely, biodegradable. When natural organisms break down these

compounds, they begin to use up the dissolved oxygen in water. When the dissolved oxygen level in the water drops below some critical value, it kills aquatic life such as fish and microorganisms.

1.2 Phenol

During the last two decades, phenolic compounds have become the subject of intense research in the preservation of our environment. Phenol is the common name of hydroxybenzene, C_6H_5OH, an aromatic compound having one hydroxyl group attached to the benzene ring. Phenol has also been called carbolic acid, phenic acid, phenylic acid, phenyl hydroxide or oxybenzene. Phenol is produced both naturally and synthetically by chemical processes. Naturally, phenol has been extracted from coal tar distillation. Synthetically, cumene oxidation accounts for 95% of phenol production worldwide at a rate of 6.4 million metric tons produced in 2001 (Jordan et al. 2002). Despite being toxic, phenols are widely used compounds as raw materials to synthesize other industrially important chemicals (Schmidt 2005 and Busca 2007). Below are listed some of main uses of phenol:

- Bisphenol A is produced by the condensation reaction of two moles of phenol and one mole of acetone. It is widely used in the production of polycarbonates, ophthalmic lens and automotive components.
- Phenolic resins are produced by condensation of phenol and formaldehyde and used as adhesives in the plywood industry and as plasticizers. They are also used as disinfectants and in germicidal paints. Aminophenol, used in the manufacture of dyes and photographic applications, is obtained from phenol.

- Acetylsalicylic acid, a derivative of phenol, is used in the manufacture of aspirin.

Phenol has been selected as a model compound in this study because it is a common pollutant found in the effluent of various industrial wastes including petroleum operations, and phenol like compounds are produced in the degradation pathways of high molecular weight polycyclic aromatic hydrocarbons (PAHs).

1.2.1 Chemical and physical properties of phenol

Phenol was first isolated from coal tar in 1834 and named carbolic acid. Since then its usage has been growing and 6.4 million tons were produced in 2001. Table 1.1 shows some of the physical and chemical properties of phenol. Phenol is a colorless, hygroscopic crystalline solid at room temperature. Phenol is very soluble in water and in many organic solvents (such as alcohols, ethers, chloroform and several other polar solvents). It has low solubility in paraffinic hydrocarbons.

Table 1.1 Chemical and physical properties of phenol (Kirk-Othmer, 1999)

Property	Phenol
Formula	C_6H_5OH
Molecular weight (g/mol)	94.11
Water solubility (g/L at 25 ºC)	87
Melting point (ºC)	43
Boiling point (ºC)	181.8
Auto ignition temperature	715 ºC
Flash point (open cup)	87 ºC
pK_a	9.89 X 10^{-10}

1.3 Environmental pollution caused by phenolic waste

Phenol is the most ubiquitous contaminant originating from a variety of industries (Huang et al., 2007). The effluent from industries such as oil refineries, paper mills, olive oil mills, wood processing, coal gasification, textiles, resins and agro-industrial wastes discharge phenols much higher than the toxic levels set for this compound. Table 1.2 shows the concentration of phenol in the effluents generated in different industrial operations.

Table 1.2 Phenol concentrations in industrial effluents (Busca et al. 2008)

Industry	Phenol concentration, mg L^{-1}
Coking operations	28 – 3900
Coal processing	9 – 6800
Petrochemicals	2.8 – 1220
Pulp and paper	0.1 - 1600
Gas production	4000
Refineries	6 – 500
Pharmaceuticals	1000
Benzene manufacturing	50

Such high concentrations of phenol pose severe health hazards to aquatic and human health. Phenol concentrations when present at ppb level give disagreeable taste and odours when treated with chlorine. Chlorination of drinking water containing phenols also results in the formation of chlorophenols, which are more toxic than phenol and difficult to remove. Phenol is a very corrosive poison and is readily absorbed by contact with skin, by inhalation or by ingestion. Deichmann and Klepinger (1981) reported that ingestion of substances containing as little as 1 mg L^{-1} of phenol can have fatal consequences in humans. Phenol concentrations higher than 2 mg L^{-1} is considered toxic to fish and concentrations between 10 to 100 mg L^{-1} results in the death of most aquatic life (Huang et al. 2007). Drinking phenol contaminated water causes elevated cases of diarrhea, nausea, mouth sores, dark urine, paralysis of the central nervous system and kidney damage (Senturk et al. 2009). Because of its harmful effects at low

concentrations, the US environmental protection agency (EPA) has listed phenol as a priority pollutant and requires lowering the phenol concentration below 1 mg L^{-1} before a contaminated stream can be released (Ayranci and Duman, 2005).

1.4 Treatment methods for the removal of phenolic wastes

A variety of treatment methods, such as incineration, adsorption, wet oxidation, biological treatments and chemical oxidation have been used for removal of phenols from aqueous solutions. The applied treatment, which could be a single treatment or a combination of these treatments, must guarantee the removal of contaminants to allowable discharge limits. The choice of treatment depends upon the type of organic contaminants, their concentration, and volume of the effluent treated and cost of the treatment. The following treatment methods have been widely reported for the removal of phenolic wastes from the aqueous solution.

1.4.1 Incineration

Incineration of organic waste is particularly useful for treating small quantities of wastes with high pollutant concentrations. The incinerators normally used could be horizontal, vertical or fluidized bed vessels. Incineration of organic waste presents the disadvantage of high investment costs for equipment and high operating costs for energy because of additional fuel requirements. Another drawback of this treatment is the production of CO_2 and NO_X resulting from the oxidation of organic compounds at elevated temperatures.

1.4.2 Adsorption onto activated carbon

Adsorption is a separation method in which organic pollutants are removed from the wastewater by adsorption onto the surface of solid particles where it is accumulated for further extraction or destruction. Adsorption of phenol onto activated carbon is a widely studied treatment method because of the affinity of phenols for the active surface of carbon (Garcia-Araya et al., 2003). The high costs associated with recovering activated carbon particles from the treated wastewater is a disadvantage of this treatment (Banat et al., 2000). Kyuya et al. (2004) reported pore size distribution and surface area of the activated carbon are two important factors that affect the adsorption of phenol. Because of the high costs associated with activated carbon, the use of naturally occurring adsorbents such as spent oil shale, natural clay minerals like montmorillonite, kaolinite, and illite have also been reported for the removal of organic contaminants from wastewater (Darwish et al., 1996; Gutierrez and Fuents, 1996; Lo et al., 1997).

1.4.3 Wet and supercritical water oxidation

Wet oxidation, also called wet air oxidation, utilizes the oxidizing properties of air or oxygen to remove organic and inorganic compounds at elevated temperature and pressure. The wet oxidation process was first used and patented by Zimmerman in 1950, to completely mineralize organic compounds to carbon dioxide and water using air or oxygen (Bhargava et al. 2006). The temperature and pressure used for wet oxidation depends upon the type of pollutant removed. However wet oxidation is usually operated at temperatures in the range 180 to 315ºC and pressures ranging from 2 to 20 MPa. Wet air oxidation could achieve 70-90% chemical oxygen demand (COD) removal without

producing NO_X, SO_2, HCl or furans, but the high capital and operating costs are major drawbacks for the large scale adoption of wet air oxidation (Mantzavinos et al. 1999). Devlin and Harris (1984) studied the oxidation of phenol using oxygen and proposed a reaction mechanism. They also observed that complete oxidation of phenol to CO_2 and water entails high treatment costs because of the refractory nature of organic acids to further oxidation.

When the operating conditions (temperature and pressure) of wet oxidation are above the critical point of pure water (22.06 MPa and 647.13 K), the oxidation is called supercritical water oxidation. Several authors have studied the supercritical water oxidation for removal of phenol from wastewater. Like wet oxidation, supercritical water oxidation's high operation costs, complicated reactor design and high energy input are main drawbacks that prevent its wide application.

1.4.4 Photochemical Oxidation

Photochemical oxidation is a widely studied treatment for the destruction of organic pollutants from wastewater. This treatment is based on supplying energy to organic contaminants by UV irradiation and photolysis and decomposition of the organic contaminant by bond cleavage and free radical generation (Esplugas et al., 2002). The high energy of UV radiation can also break the chemical bonds of water to generate free radicals as shown in the following reaction:

$$H_2O \xrightarrow{h\nu} HO^{\bullet} + H^{\bullet} \qquad (1.1)$$

Numerous studies have been reported in the literature on the removal of phenolic waste using photochemical oxidation. UV radiation alone suffers from the drawback of high energy costs due to the requirement of excess UV radiation. Furthermore, not all the emitted radiation is used, only a part of absorbed radiation by the organic contaminant produces chemical changes. Bali et al. (2003) compared UV, UV/H_2O_2 and UV/H_2O_2/Fe^{2+} (photo-Fenton's oxidation) for the removal of phenol and observed that UV radiation alone is the slowest of these and cannot bring complete mineralization of phenol. Combining UV radiation with chemical oxidants such as H_2O_2, ozone and semiconductors like TiO_2 is a very promising technique for the destruction of organic pollutants from wastewater.

1.4.5 Biodegradation of phenolic wastes

Biological treatment of wastewater containing organic pollutants is the natural and economic alternative compared to other treatment options. The cost of biodegradation of organic contaminants is reported to be 5 to 20 times less than chemical treatments such as ozonation and hydrogen peroxide (Mantzavinos et al., 1999). Bioremediation involves the use of specific microorganisms to degrade the organic pollutants in the wastewater and tailings. The microorganisms need carbon (organic contaminants) as a source of energy and inorganic salts or nutrients to reproduce and carry out their metabolic activities. The organic contaminants in wastewater serve as carbon and energy sources for the microorganisms. The main nutrients used to maintain the microbial activity are nitrogen (N), sulphur (S), potassium (K), magnesium (Mg) and calcium (Ca). Besides the organic contaminant and nutrients, biodegradation of an

organic pollutant is dependent on several other factors such as pH, temperature, availability of oxygen in case of aerobic processes and concentrations and chemical structure of the target pollutant. The microorganisms may completely destroy or convert these contaminants to harmless or simple inorganic compounds such as water and CO_2. The clean-up of the Alaskan shoreline of Prince Williams sound after the oil spill of the Exxon Valdez in 1998 is an example of a large scale application of bioremediation that got much public attention (Boopathy, 2000).

Bioremediation can be classified as *in-situ* or *ex-situ*. The *ex-situ* approach involves the physical removal of the contaminated waste from the original location and conducting the treatment in a bioreactor. *In-situ* involves treatment of the contaminated waste in place. Although the in-situ approach is less expensive, the ex-situ approach may provide higher efficiencies as a result of carefully controlled conditions in the bioreactor. The *ex-situ* approach has been found to be more predictable and to be controlled easier than *in-situ* bioremediation (Carberry and Wik, 2001).

Numerous studies have been reported in the literature on the biodegradation of phenol using different microorganisms. Several microorganisms such as *Pseudomonas resinovorans* strain P-1, *Brevibacillus* sp. strain P-6, *Pseudomonas aeruginosa* and *Pseudomonas pseudomallei* have been reported which use phenol as the carbon and energy source but at low concentrations only (Yang and Lee, 2007). Biodegradation of phenol in wastewater is effective in the range of 5-500 mg L^{-1} and higher concentrations slow the growth rate of microorganisms. Concentrations higher than 1450 mg L^{-1} are

toxic and kill the entire population of microorganisms in the wastewater (Sevillano et al., 2008). Feitkenhauer et al. (2001) studied the biodegradation of phenol using the thermophile *Bacillus themoleovorans* sp. A2 and reported specific growth rates as high as 2.8 h^{-1} at an initial phenol concentration of 15 mg L^{-1}. Kotresha and Vidyasagar (2007) used *Pseudomonas aeruginosa* (MTCC 4996) for phenol biodegradation and reported this strain could remove phenol concentration up to 1300 mg L^{-1} at pH 7 and the optimal temperature of 37ºC. As reported, phenol concentration higher than 1450 mg L^{-1} could not be removed by biological treatment alone. Biological treatments need to be combined with other treatments, such as chemical oxidation, for removing high concentrations of the contaminants effectively and economically from the wastewater.

1.4.6 Chemical oxidation

Chemical treatment involves the use of chemical agents to completely destroy or convert the contaminants to harmless or less toxic compounds, or intermediates that can be further degraded by microorganisms (Hamby D. M., 1996). The chemical agents used are normally strong oxidants and the oxidation of the contaminants takes place in a short time. With sufficient contact time, contaminants may be completely mineralized to simpler compounds such as water and carbon dioxide. Chemical oxidation of organic pollutants is a promising alternative when wastewater contains non-biodegradable and/or toxic contaminants and also when the contaminant concentration is high. The most commonly used oxidants that initiate the oxidation reactions include ozone, Fenton's reagent (a mixture of hydrogen peroxide and ferrous ion), sub- or supercritical water, and

permanganate or persulfate (Rivas, 2006). Table 1.3 shows the oxidation potential of commonly used chemical oxidants.

Table 1.3 Standard oxidation potential (against the standard hydrogen electrode) of commonly used chemical oxidants (Legrini et al. 1993)

Compound	Oxidation potential (volts)
Fluorine	3.03
Hydroxyl radical ($HO^{\bullet}$)	2.80
Ozone (O_3)	2.10
Hydrogen peroxide (H_2O_2)	1.78
Potassium permanganate ($KMnO_4$)	1.67
Chlorine dioxide (ClO2)	1.50
Chlorine (Cl)	1.30

Chemical oxidation can be divided into either classical chemical treatments or advanced oxidation processes (AOPs). Classical chemical oxidation is the direct addition of the oxidant to the wastewater containing pollutants. The following are the most common chemical oxidants used for this purpose:

1.4.7 Chlorine

Chlorine is a low cost disinfectant and has been used for a long time for drinking water disinfection. It is a strong oxidant and very simple to use in water treatment

systems. The main disadvantages of chlorine are low selectivity and the reaction of chlorine with organic compounds to form chlorinated organics.

1.4.8 Potassium permanganate

Potassium permanganate is a strong oxidizer and has been used for a long time in wastewater systems. Potassium permanganate, when used in combination with ferrous iron as catalyst, releases sulfate ions (SO_4^{2-}) which have oxidation potentials slightly less than Fenton's reagent. It works in a wide range of pH but is expensive. One of the disadvantages of potassium permanganate is the formation of magnesium dioxide, through oxidation, which precipitates and needs to be removed by clarification or filtration.

1.4.9 Ozone

Ozone is a strong oxidant and has been used since the beginning of the 20^{th} century (Gottschalk et al., 2000). Ozone can oxidize organic compounds either by direct oxidation at low pH (<4) or indirectly through the generation of hydroxyl radicals at high pH (>9), or both at mid pH (4-9) (Nam et al., 2000). Although ozonation for destruction of organic pollutants from wastewater is a well established technology, it suffers from the drawback of high operation costs due to its requirement for electrical energy input. Because it is a gas, it must be produced onsite, and special equipment is needed for its generation.

1.4.10 Hydrogen peroxide

Hydrogen peroxide is a strong oxidant which could be used alone or with a catalyst. The catalyst usually used with hydrogen peroxide is ferrous sulfate and this combination is called Fenton's reagent. Other metal catalysts such as Fe^{3+}, Cu^{2+} and Al^{3+}could also be used. Hydrogen peroxide has numerous advantages such as low cost, high oxidizing power, and ease of handling compared to other oxidizing agents. Hydrogen peroxide when used alone has low reactivity and causes incomplete oxidation of many organic contaminants (Kamenev et al., 1995, Ikehata and Gamal El-Din, 2006). Its reactivity is enhanced by the use of metal catalysts which generate hydroxyl radicals. This combination falls under the category of AOPs.

1.5 Advanced oxidation processes (AOPs)

AOPs are based on the generation of reactive species such as hydroxyl radicals (HO·), very strong and non selective oxidizing agents, which have oxidation potential higher than ozone and hydrogen peroxide (Neyens and Baeyens, 2003). AOPs exploit the high oxidation potential of hydroxyl radicals in achieving complete oxidation of organic contaminants. Based on the generation of hydroxyl radicals, AOPs can be divided into two categories:

- UV based processes (UV/O_3, $UV/O_3/H_2O_2$, UV/TiO_2)

- Hydrogen peroxide based processes (O_3/H_2O_2, H_2O_2/Fe^{2+}, UV/H_2O_2)

AOPs are promising in eliminating non-biodegradable and toxic organic contaminants from wastewater and alternative treatment methods for meeting legislated discharge limits of these contaminants. (Zazo et al., 2005).

1.5.1 UV based processes

The degradation rates of organic contaminants can be enhanced by combining photochemical oxidation with chemical oxidants such as hydrogen peroxide and/or ozone or semiconductors like TiO_2 (Naffrechoux et al., 2000). The most common UV based AOPs used for the degradation of organic contaminants are UV/O_3, $UV/O_3/H_2O_2$, and photocatalytic oxidation (UV/TiO_2).

1.5.2 UV/O_3

The Combined UV/O_3 process is an advanced oxidation treatment for effective oxidation and removal of organic contaminants from wastewater. This process has been used at large scale for the destruction of toxic and refractory contaminants and also for the disinfection of drinking water by destroying bacteria and viruses. The efficiency of the combined UV/O_3 process in removing organic contaminants from wastewater has been proven to be higher than those where UV or ozone has been used individually (Wu et al., 2004). Ozone absorbs UV radiation at a 254 nm wavelength and produces H_2O_2 as an intermediate which then decomposes to HO· as follows:

$$O_3 + 3H_2O \xrightarrow{h\nu} 3H_2O_2 \xrightarrow{h\nu} 6HO^{\bullet} \qquad (1.2)$$

Several authors have studied the removal of toxic and refractory organic contaminants from wastewater using combined UV/O_3. Beltran et al. (1998) investigated the oxidation of nitrobenzene and 2,6-dinitrotoluene using ozonation combined with hydrogen peroxide

and UV radiation and compared the results with UV radiation and ozonation alone. They reported that the combination of UV and ozone achieved the highest removal rate.

1.5.3 Photocatalytic oxidation (UV/TiO_2)

Photocatalytic oxidation has been an efficient and promising alternative for the destruction of pollutants from wastewater. When TiO_2 is exposed to UV irradiation at sufficient energy, electrons (e_{cb}^-) and positive holes (h_{vb}^+) are produce. These holes are strong oxidizing agents and react with water molecules to produce highly reactive hydroxyl radicals as shown in the following reaction:

$$h^+ + H_2O \rightarrow HO^\bullet + h^+ \quad (1.3)$$

Oxygen when present at sufficient concentration in the system scavenges the electrons to produce superoxide ions. Hydrogen peroxide is formed and converted to hydroxyl radicals through a series of reactions:

$$O_2 + e^- \rightarrow O_2^- \quad (1.4)$$

$$O_2^- + h^+ \rightarrow HO_2^\bullet \quad (1.5)$$

$$h^+ + HO_2^\bullet + e^- \rightarrow H_2O_2 \quad (1.6)$$

$$H_2O_2 \xrightarrow{h\nu} 2HO^\bullet \quad (1.7)$$

Numerous studies have been conducted on the removal of phenolic waste using the UV/TiO_2 method. The Low efficiency of the UV/TiO_2 process is its major drawback preventing large-scale applications (Lv and Lu, 2008). Barakat et al. (2005) investigated

the oxidation of phenol and monochlorophenols using $UV/TiO_2/H_2O_2$, UV/TiO_2 and UV/H_2O_2 and found that addition of hydrogen peroxide to the photocatalytic system improved the phenol degradation efficiency from 30 to 97%. Badawy et al. (2009) studied the degradation of olive oil mill waste, a major source of phenolic waste, using photo-Fenton and UV/TiO_2 and reported that phenol was decreased by 93.44% using photo-Fenton but only by 34.11% using UV/TiO_2.

1.5.4 Hydrogen peroxide based AOPs

Hydrogen peroxide is a safe and easy to handle chemical oxidant for removing organic pollutants from wastewater. As mentioned before, the reactivity of hydrogen peroxide alone as a chemical oxidant is very low and sometimes does not react at all with organic compounds (Neyens and Baeyens, 2003). Its reactivity can be enhanced by combining it with other chemical oxidants such as ozone, by using metal catalysts as in Fenton's reagent, or by combining with UV radiation.

1.5.5 H_2O_2/O_3 process

Addition of hydrogen peroxide to ozone initiates a series of radical reactions, generating highly reactive hydroxyl radicals by the decomposition of ozone. The overall reaction between hydrogen peroxide and ozone for generation of hydroxyl radicals is as follows

$$2O_3 + H_2O_2 \rightarrow 2HO^{\bullet} + 3O_2 \qquad (1.8)$$

Numerous studies have been reported on the use of combined O_3/H_2O_2 for the destruction of different types of organic contaminants from wastewater. Mokrini et al.

(1997) investigated the oxidation of phenol and benzoic acid using UV, ozone, hydrogen peroxide and their combination. They found a marked increase in oxidation rate when ozone is combined with hydrogen peroxide and/or UV at low pH (3-7). Tizaoui et al. (2007) studied the removal of landfill leachate using ozone and O_3/H_2O_2 and observed that the efficacy of ozone was doubled when combined with hydrogen peroxide with an optimal hydrogen peroxide concentration of 2 g L^{-1}.

1.5.6 H_2O_2/UV

As mentioned, ultraviolet (UV) radiation alone might not be effective in removing bio-recalcitrant contaminants from wastewater, but combining it with a chemical oxidant such as hydrogen peroxide generates high efficiency hydroxyl radicals. Photolysis of hydrogen peroxide generates hydroxyl radicals when water containing H_2O_2 is exposed to UV radiation (λ=200 to 250 nm) as shown by:

$$H_2O_2 \xrightarrow{h\nu} 2HO^{\bullet} \qquad (1.9)$$

De et al. (1998) investigated the photo oxidation of phenol, 2 and 4-chlorophenols using the UV/H_2O_2 process and observed a marked increase in degradation rate when combined UV/H_2O_2 was used instead of either UV or hydrogen peroxide alone. Vilhunen and Sillanpaa (2009) studied the effect of wavelength (255 to 280 nm), viewing angle (15 and 120º), and phenol and hydrogen peroxide concentrations on phenol degradation using a UV Light Emitting Diode (LED) and H_2O_2. They found that phenol degradation is most efficient at 280 nm. The disadvantage of UV/H_2O_2 is the absorption of UV by the wastewater which would compete with hydrogen peroxide for radiation.

1.5.7 Fenton's reagent (H_2O_2/Fe^{2+})

In 1894, Fenton found that a combination of ferrous salts and hydrogen peroxide was able to rapidly oxidize maleic acid (De et al., 2005). Since then, the addition of ferrous salts to hydrogen peroxide has been used to promote the oxidation of a variety of organic compounds. In the presence of Fe^{2+} and an acidic medium, hydrogen peroxide breaks down to HO· and OH^- according to the following reaction:

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + HO^* + Fe^{3+} \qquad k = 76 \text{ (mol L}^{-1}\text{S}^{-1}\text{)} \qquad (1.10)$$

The reaction between hydrogen peroxide and Fe^{2+} is a fast reaction. If the rate of degradation of the organic compound is slow, there is build up of hydroxyl radicals. And if sufficient Fe^{2+} is not present in the solution, unreacted hydrogen peroxide will also present in the solution. In such a case, excess hydrogen peroxide and hydroxyl radical may react as follows:

$$HO^* + H_2O_2 \rightarrow H_2O + HO_2^* \qquad (1.11)$$

$$2HO^* \rightarrow H_2O_2 \qquad (1.12)$$

$$HO^* + HO_2^* \rightarrow H_2O_2 + \frac{1}{2}O_2 \qquad (1.13)$$

$$HO_2^* + HO_2^* \rightarrow H_2O_2 + O_2 \qquad (1.14)$$

Hydroxyl radicals are very reactive and unstable, so they attack the organic contaminants instantaneously. Numerous studies have been reported on the use of Fenton's reagent to remove organic pollutants from wastes originating from different industries. Yalfani et al. (2009) studied the degradation of phenol using hydrogen peroxide generated from formic acid and oxygen by alumina supported palladium

catalyst. They observed complete oxidation of 100 mg L^{-1} phenol in 6 h, with 60% mineralization, compared to only 48% mineralization achieved in the conventional Fenton's process with 300 mg L^{-1} hydrogen peroxide added. Azbar et al. (2004) found the Fenton's process (Fe^{2+}/H_2O_2) to be the most economical process compared to other AOPs such as O_3, O_3/UV, H_2O_2/UV, O_3/H_2O_2/UV for the removal of chemical oxygen demand (COD) and color from a polyester and acetate fiber dyeing effluent.

Many parameters such as pH, temperature, Fe^{2+} and H_2O_2 concentrations and the presence of inorganic salts might affect the efficiency or degradation rate of Fenton's reagent. Several authors have reported the effect of pH, temperature and concentration of Fe^{2+} ions and H_2O_2 on Fenton's reaction. Fewer studies have been published on the effect of inorganic salts, especially those needed as nutrients for biodegradation, on Fenton's reaction.

1.5.8 Effect of inorganic salts on Fenton's reaction

Industrial or contaminated waters usually contain not only organic pollutants but also several inorganic salts. Ground water also contains chloride and sulfate ions at concentrations ranging from 0.1 to 100 mM (Siedlecka et al., 2007). Han and Wang (2009) reported that the negative effect of inorganic ions on Fenton's oxidation of MTBE was in the following order $PO^{3-}_4 > HPO^{2-}_4 > H_2PO^-_4 > Cl^- > SO^{2-}_4$. These inorganic ions basically have the following effects:

- formation of complexes with Fe^{2+} and Fe^{3+}, whose reactivity is less compared to free iron species (De Laat, 2004),

- precipitation reactions which decrease the activity of dissolved Fe^{3+} species,
- scavenging of hydroxyl radicals by inorganic ions and formation of less reactive inorganic radicals, and
- oxidation reactions with these inorganic radicals.

Lu et al. (1997) also studied the effect of different inorganic ions on the oxidation of dichlorvos (2,2-dichlorovinyl dimethyl phosphate) and reported that phosphate ions mainly suppress the Fenton's reaction due to the formation of a complex with iron species.

Fenton's reagent was reported as one of the most efficient and effective methods for the generation of hydroxyl radicals. It is a widely used oxidation method because of ease of operation i.e. no need to purchase special equipment, low cost of chemicals and mild operating conditions (ambient temperature and pressure). The possibility of recycling the iron makes this process more economical and feasible. For iron recycling, the following procedures are used: raising the pH, separating the iron floc, and re-acidifying the iron sludge.

1.6 Integrated AOPs with a biological process

Combining AOPs with biological treatment methods is a cost effective way to remove the toxic and recalcitrant organic compounds found in industrial wastewaters. Biodegradation is 5 to 20 times cheaper than the advanced oxidation processes; unfortunately it is not applicable when the wastewater contains non biodegradable or high concentrations of toxic pollutants (Mantzavinos et al., 1999). Partial oxidation using

AOPs to improve the biodegradability of toxic waste is an economic alternative for the removal of contaminants from the wastewater. The sequence in combined AOPs with biological treatment depends on the types and concentrations of pollutants present in the wastewater. Nam et al. (2001) studied the degradation of a mixture of polycyclic aromatic hydrocarbons using combined biodegradation and modified Fenton's reagent and found that biodegradation followed by chemical oxidation was the most efficient for PAH removal. Moussavi et al. (2009) compared catalytic ozonation alone with combined catalytic ozonation followed by biodegradation for phenol removal and observed that combined catalytic ozonation with biological process is an effective and economic alternative for phenol removal from saline wastewater compared to ozonation alone. Complete mineralization of organic compounds with chemical oxidant is possible, but short-chain carboxylic acids (maleic, oxalic, acetic and malonic) are often formed and reported to be resistant to chemical oxidation (Chamarro et al., 2001). So, complete mineralization with chemical agents will make the process uneconomical. Chamarro et al. (2001) reported the stoichiometric coefficient for oxidation of phenol, 2,4-dichlorophenol and nitrobenzene to be 0.5 mol of organic compound /mol of hydrogen peroxide compared to 1 for formic acid. They have also proposed the ratio of BOD_5/COD as a test for measuring the biodegradability of treated water.

1.7 Research Objectives

A review of the literature reveals that the majority of earlier works have focused on advanced oxidation of various contaminants in water and information on the oxidation of organic contaminants in a bioremediation medium does not exist. In the present work,

therefore, advanced oxidation of phenol in a bioremediation medium was investigated using Fenton's reagent and chlorine dioxide. Potential for the combination of advanced oxidation and biodegradation for the removal of phenol and intermediates resulting from chemical oxidation was then investigated.

The specific objectives of this Ph.D. research are:

1. Study of phenol oxidation in a bioremediation medium by Fenton's reagent using the central composite rotatable design method (CCRD) and verification of optimal conditions by the response surface methodology. Numerous studies have been published on oxidation of phenol using Fenton's reagent in water and optimal conditions have been reported. The effect of individual inorganic ions have also been reported, but no study has been reported on the oxidation of phenol in a complex bioremediation medium, which involves the combination of several inorganic salts suitable as nutrients for growing microorganisms.
2. Identification and quantification of major intermediates of phenol oxidation in a bioremediation medium by Fenton's reagent using HPLC and proposal of reaction mechanism based on the identification of these intermediate oxidation compounds. Determine the kinetic parameters of the proposed model by fitting to the experimental data into the model.
3. Study of phenol oxidation in a bioremediation medium by chlorine dioxide using the central composite rotatable design method (CCRD) and verification of optimal conditions by the response surface methodology.

4. Identification and quantification of intermediates of phenol oxidation in a bioremediation medium by chlorine dioxide and developing a kinetic model for the reaction.
5. Biodegradation studies of 1,4-benzoquinone (one of the main oxidation product of phenol oxidation by chlorine dioxide) under different operating conditions using *Pseudomonas putida* 17484 in batch and continuous systems and evaluation of its biokinetics.

1.8 Layout of thesis

This thesis has been prepared in accordance with the guidelines set by the University of Saskatchewan for a manuscript based thesis. This thesis is comprised of five manuscripts that have been written and submitted for publications as each research objective was achieved during the last four years of study. Any relevant information, not present in the manuscript has been added in this thesis. Chapter 2 describes the experimental design strategy using central composite rotatable design (CCRD) method to obtain adequate information in the design space with a minimum number of experiments. The optimal experimental conditions were then obtained using response surface methodology and Design Expert software 6®. Oxidation of the phenol does not necessary mean that all of the toxicity is gone; in fact the oxidation of phenol may result in intermediate compounds, especially aromatic compounds, which are more toxic than phenol. So identification and quantification of these intermediates were carried out. A reaction mechanism was proposed based on the intermediate compounds identified in this study and kinetic coefficients were determined, details of which are described in Chapter

3. Chlorine dioxide was another oxidant selected for the oxidation of phenol in a bioremediation medium. Optimal experimental conditions such as pH, temperature and concentration were obtained using CCRD. Results of this study are presented in Chapter 4. The identification of intermediate compounds and kinetics of phenol oxidation by chlorine dioxide were carried out and the results are presented in Chapter 5. The major intermediate compounds of phenol oxidation by chlorine dioxide were 1,4-benzoquinone and 2-chloro-1,4-benzoquinone. There is no information found on toxicity of these two compounds in the literature. Biodegradation of 1,4-benzoquinone, the main intermediate of phenol oxidation, was investigated in batch and continuous system and biokinetic parameters were obtained. The details of this study are presented in Chapter 6. Chapter 7 presents the major conclusions drawn and recommendations for future work. References used in all chapters are compiled at the end of this thesis.

CHAPTER 2

2. OXIDATION OF PHENOL IN A BIOREMEDIATION MEDIUM USING FENTON'S REAGENT

A similar version of this chapter has been published in Environmental Technology:

Kumar P., H. Nikakhtari*, M. Nemati, G. A. Hill "Oxidation of phenol in a bioremediation medium using Fenton's Reagent. Environ. Technol**.** 31 (1), 47-52, (2010).

Contribution of the Ph.D. Candidate

The design of phenol oxidation experiments using Fenton's reagent were done by Pardeep Kumar using commercial software: Design Expert 6.0® with technical input and guidance from Dr. Hill and Dr. Nemati. The written text for this manuscript has been prepared by Pardeep Kumar, while Dr. Hill and Dr. Nemati provided the editorial input.

Contribution of this Chapter to Overall Study

With the primary objective of this Ph.D. work exploring the possibility of integrating chemical and biological processes for removal of high concentrations of phenol, this chapter presents the determination of optimal experimental conditions for partial oxidation of phenol in a bioremediation medium using Fenton's reagent. It also discusses the intermediates of phenol oxidation and their identification. The performance of Fenton's reagent in terms of phenol oxidation at steady state was compared with

* Hossein Nikakhtari (PDF) assisted in the analysis of the experimental data for the manuscript.

chlorine dioxide in Chapter 3 and 4. The identification of major phenol oxidation intermediates would be helpful in assessing the toxicity of these intermediates and potential of biodegradation in a subsequent step.

2.1 Abstract

The oxidation of phenol by Fenton's reagent was investigated in a medium suitable for bioremediation in batch system. An experimental design approach, based on central composite rotatable design (CCRD) was used to quantify the effects of H_2O_2 concentration (2000 to 5000 mg L^{-1}) and $FeSO_4.7H_2O$ concentration (500 to 2000 mg L^{-1}). Performance of the chemical oxidation by Fenton's reagent was evaluated by determining the percentage of phenol oxidized at steady state. The Analysis Of Variance (ANOVA) test indicated both H_2O_2 and $FeSO_4.7H_2O$ concentrations had a positive effect on phenol oxidation. Hydrogen peroxide concentration was the dominating parameter for the removal of phenol by Fenton's reagent. The optimal concentrations of H_2O_2 and $FeSO_4.7H_2O$ for complete oxidation of 2000 mg L^{-1} phenol in medium were found to be 4340 mg L^{-1} and 1616 mg L^{-1}, respectively, at 25°C and pH 3. Oxidation of phenol in culture medium was found to be significantly different than in pure water.

Keywords: Chemical oxidation; Fenton's reagent; CCRD; Bioremediation medium; Phenol

2.2 Introduction

Phenol and other cyclic or polycyclic aromatic compounds are commonly found in the effluents produced in chemical and petrochemical industries. Because of their

toxicity and severe risks to aquatic life and humans, government agencies demand the removal of these compounds from wastewater prior to discharge to receiving waters. In Canada, the Canadian Water Quality Guidelines (Canadian Council of Ministers of the Environment, 1999) require low levels such as 4 μg L^{-1} for the discharge of phenols (mono- and dihydric) and 0.5 μg L^{-1} for pentachlorophenols (Environment-Govt. of Saskatchewan, 2006). A phenol concentration of 2 mg L^{-1} is considered toxic to the fish and concentrations between 10 and 100 mg L^{-1} result in the death of aquatic life (Korbathi and Tanyolac 2003).

Biological removal of phenol in water is effective for concentrations up to 50 mg L^{-1} and other approaches are required for the removal of higher concentrations of phenol (Yavuz et al., 2007). Advanced oxidation processes (AOPs) can be used effectively to treat high levels of organic compounds and has become popular for treatment of phenolic wastes (Villota et al., 2007). Advanced oxidation processes are based on the generation of reactive species such as hydroxyl radicals (HO·) which are strong and non-specific oxidation agents (Zazo et al., 2005).

Chemical oxidation of organics with Fenton's reagent is one such AOP, used for the treatment of different industrial wastewaters (Lin and Peng 1995; Zak 2005; Benatti et al., 2006). Fenton's reaction is a homogeneous reaction in which hydrogen peroxide and ferrous sulfate are used (Peres et al., 2004). Fe^{2+} acts a catalyst and initiates the decomposition of hydrogen peroxide to generate hydroxyl radicals according to the following reaction:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^- + HO\cdot \quad (2.1)$$

The produced hydroxyl radicals react instantly with the organic compound and initiate its decomposition by H-abstraction and addition to C-C double bonds, resulting in the generation of oxidation products as shown in reaction 2.2 (Peres et al., 2004). Hydroxyl radicals have higher oxidation potentials than ozone and hydrogen peroxide.

$$RH + HO\cdot \rightarrow \text{Oxidized products} \quad (2.2)$$

Hydrogen peroxide may also directly oxidize organic substrates:

$$RH + H_2O_2 \rightarrow \text{Oxidized products} \quad (2.3)$$

Generated hydroxyl radicals may be scavenged by the reactions with hydrogen peroxide and Fe^{2+}:

$$HO\cdot + Fe^{2+} \rightarrow HO^- + Fe^{3+} \quad (2.4)$$

$$HO\cdot + H_2O_2 \rightarrow HO_2^{\cdot} + H_2O \quad (2.5)$$

Fe^{2+} is regenerated through the reaction of Fe^{3+} with hydrogen peroxide and hydroperoxyl radicals as shown in reactions 2.6 and 2.7 (Heredia et al., 2001).

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ + HO_2^{\cdot} \quad (2.6)$$

$$Fe^{3} + HO_2^{\cdot} \rightarrow Fe^{2+} + H^+ + O_2 \quad (2.7)$$

Although several authors have studied phenol and chlorophenol oxidation by Fenton's reagent in water (Tang and Huang 1996; Chedeville et al., 2005; Mahvi et al., 2007; Villota et al., 2007; Yavuz et al. 2007; Zazo et al., 2007), there is no prior report on the use of Fenton's reagent in a medium required for the purpose of microbial growth and bioremediation. Complete mineralization of organic substrates to CO_2 requires high quantities of H_2O_2 making the process uneconomical (Zazo et al., 2005). In order to reduce the quantity of Fenton's reagent, its application as a part of an integrated chemical and biological treatment process is of interest (Villota et al., 2007). Low molecular weight polycyclic aromatic hydrocarbons (PAHs) may be effectively removed by biotreatment compared to high molecular weight PAHs and the remaining high molecular weight PAHs could subsequently be removed by chemical oxidation. So for an integrated biological-chemical treatment, the feed for the chemical treatment may come from the bioremediation step which would necessarily contain mineral nutrients.

In the present study, the oxidation of phenol by Fenton's reagent in nutrient rich culture medium is investigated. The optimal H_2O_2 and $FeSO_4.7H_2O$ concentrations required for the oxidation of a high phenol concentration (2000 mg L^{-1}) in a batch system were determined using the central composite rotatable method.

2.3 Materials and Methods

2.3.1 Chemicals

All chemicals were analytical grade. Phenol (99.5%) was obtained from Merck (Darmstadt, Germany). Hydrogen peroxide solution 30% (w %) was obtained from Sigma Aldrich (Oakville, Canada). Ferrous ion was supplied in the form of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) and obtained from Fisher Scientific (New Jersey, U.S.A). The growth medium (two dose McKinney's modified media, (Nikakhtari and Hill, 2006)) used in all the experiments consisted of (mg in 1 L reverse osmosis water): K_2HPO_4, 750; KH_2PO_4, 849; $(NH_4)_2SO_4$, 474; NaCl, 60; $CaCl_2$, 60; $MgSO_4$, 60; $Fe(NH_4)SO_4$, 20; and 1 mL of trace mineral solution. The trace mineral solution consisted of (mg in 1 l reverse osmosis water): $ZnSO_4.7H_2O$, 200; $MnCl_2$, 60; H_3BO_3, 600; $CoCl_2$, 400; $CuCl_2$, 20; $NiCl_2$, 40; Na_2MoO_4, 60. The natural pH of the medium was 7.0 and was adjusted to the reaction pH condition using concentrated H_2SO_4 as described below.

2.3.2 Experimental Design

The experimental design approach used was based on the central composite rotatable design method (CCRD). The surface response methodology was used to investigate the effects of two independent variables on the response function and to determine the optimal conditions maximizing the percentage removal of phenol. The CCRD method helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interactions between the parameters. Hydrogen peroxide (X_1) and $FeSO_4.7H_2O$ (X_2) concentrations were the two parameters investigated.

The range of tested concentrations for hydrogen peroxide and $FeSO_4.7H_2O$ were 2000-5000 mg L^{-1} and 500 – 1500 mg L^{-1}, respectively. The range of tested variables was chosen based on preliminary experiments. From these preliminary experiments, it was found that by using 2000 mg L^{-1} hydrogen peroxide and 400 mg L^{-1} $FeSO_4.7H_2O$, 30% phenol oxidation was achieved. Complete phenol oxidation was achieved using 5000 mg L^{-1} hydrogen peroxide and 1500 mg L^{-1} $FeSO_4.7H_2O$. The coded levels and actual values of hydrogen peroxide and $FeSO_4.7H_2O$ concentrations are shown in Table 2.1.

Table 2.1 Coded and actual values of the independent variables

Design Parameter	-1.414	-1	0	+1	+1.414
X_1 C_{H2O2}, mg L^{-1}	2000	2439	3500	4561	5000
X_2 $C_{FeSO4.7H2O,}$ mg L^{-1}	500	719.9	1250	1780	2000

The response factor (Y) was the percentage of phenol oxidized. Based on the factorial design, thirteen experiments as outlined in Table 2.2 were required in this CCRD study, including five replications at the centre point (coded 0, 0).

2.3.3 Experimental procedure

All oxidation experiments were carried out in batch mode. The reactors consisted of 500 ml Erlenmeyer flasks, shaken at 200 rpm (New Brunswick Scientific Co., Inc, Edison, N.J. U.S.A). In each experiment the reactor was loaded with 200 mL of two dose McKinney's medium containing 2000 mg L^{-1} phenol. The pH of the reaction medium was adjusted to 3 using 1 M H_2SO_4, to enhance hydrogen peroxide stability and to

prevent precipitation of iron at higher pH (Park et al., 2006). Due to this requirement for acidic pH, effect of pH was not investigated. The reaction temperature was maintained at 25°C by conducting all experiments in environmental chambers. The effect of temperature was investigated during the kinetic study which is presented in Chapter 3 and not as part of CCRD experiments. The required amount of $FeSO_4.7H_2O$ was then added and sufficient time was given for the dissolution of $FeSO_4.7H_2O$. The reaction was initiated by the addition of the designated amount of hydrogen peroxide. 4 mL samples were taken from the reactor after six hours to determine the concentration of the residual phenol and intermediates. This length of time was sufficient to ensure the final equilibrium conversions had been achieved (i.e. no further change was observed in concentration of phenol after this period). Samples were immediately transferred into vials which contained sodium sulfite at molar concentrations equivalent to the initial hydrogen peroxide concentration to stop the reaction (Peres et al., 2004). The collected samples were then analyzed for phenol, hydroquinone and catechol concentrations.

2.3.4 Analytical Methods

The concentrations of phenol and aromatic intermediates (hydroquinone and catechol) were determined using an HPLC (Agilent HPLC 1100 with a Diode Array Detector) equipped with a C_{18} column (Nova pack: 4.6 x 150 mm: 4μm). The mobile phase was a mixture of acetonitrile and water (12.5/87.5 v/v) with a flow rate of 2.1 mL min^{-1}. Detection was carried out using an ultraviolet detector at a wavelength of 254 nm. The maleic acid was identified using Dionex Ion chromatograph (ICS-2500) with a thermal conductivity detector (CD25A), an Ionpac CG5A guard column and an Ionpac

CS5A analytical column. The eluent was 1.0 mM KOH at a flow rate of 1.5 mL h^{-1}. The TSI was used to analyze CO_2 production but not quantified because it was off scale.

The concentration of hydrogen peroxide in the original solution was determined by the standard method of titration with standard (0.1 N) potassium permanganate (Solvay Chemical Inc., 2004). 5 g of the hydrogen peroxide sample is taken in a 500 mL volumetric flask containing 250 mL of water and 2 mL of H_2SO_4 (1:3). Dilute to volume with water and mix well.20 mL of this solution is taken in a 500 mL Erlenmeyer flask containing 15 mL of H_2SO_4 (1:3) and 60 mL of water. Standardized potassium permanganate solution is added from a 50 mL burette until the first appearance of pink color that persists for 30 seconds.

2.4 Results and Discussion

Table 2.2 includes actual values of the two variables and the oxidation performance achieved in each experimental run. Over the entire range of concentrations used in this study, high phenol oxidation was achieved. As can be seen from Table 2.2, an almost 86% conversion (18.12 x 10^3 mM) of a 2000 mg L^{-1} of phenol concentration was achieved using a combination of 2000 mg L^{-1} of hydrogen peroxide and 1250 mg L^{-1} of $FeSO_4.7H_2O$ in the bioremediation medium. When the hydrogen peroxide concentration was increased, greater phenol oxidation was observed due to the generation of more hydroxyl radicals. At a hydrogen peroxide concentration of 3500 mg L^{-1}, keeping the $FeSO_4.7H_2O$ concentration constant at 1250 mg L^{-1}, 96% (20.40 x 10^3 mM) of 2000 mg L^{-1} of phenol was oxidized.

Table 2.2 Non-codified values of variables and performance achieved in Fenton's oxidation of phenol in growth media (C_{PH} = 2000 mg L^{-1}*, pH 3 and T = 25°C).

Run no.	X_1, (C_{H2O2}, mg L^{-1})	X_2, ($C_{FeSO4.7H2O}$, mg L^{-1})	Y_{Phenol} Conversion
1	3500	1250	95.90
2	3500	2000	99.90
3	3500	1250	97.80
4	3500	1250	98.10
5	5000	1250	99.50
6	3500	1250	95.70
7	2439	1780	94.70
8	3500	1250	93.40
9	4561	719.7	97.80
10	2439	719.7	90.40
11	3500	5000	95.13
12	4561	1780	100.0
13	2000	1250	85.25

*C_{PH} was the initial phenol concentration

The experiments at the centre point (C_{H2O2} = 3500 mg L^{-1} and $C_{FeSO4.7H2O}$ = 1250 mg L^{-1}) were repeated five times. The values of % phenol oxidation in these repeated tests were 95.90%, 97.80%, 98.10%, 95.70% and 93.40% and the average value and standard deviation were 96.18% and 1.89, respectively. The observed variation in % phenol

oxidation is mainly due to the experimental errors associated with sampling and analytical methods.

Figure 2.1 shows the comparison of phenol oxidation by Fenton's reagent in both water and medium under similar conditions. Five percent more phenol was found oxidized in water (90%, 19.12 x 10^3 mM) than in medium (85%, 18.06 x 10^3 mM).

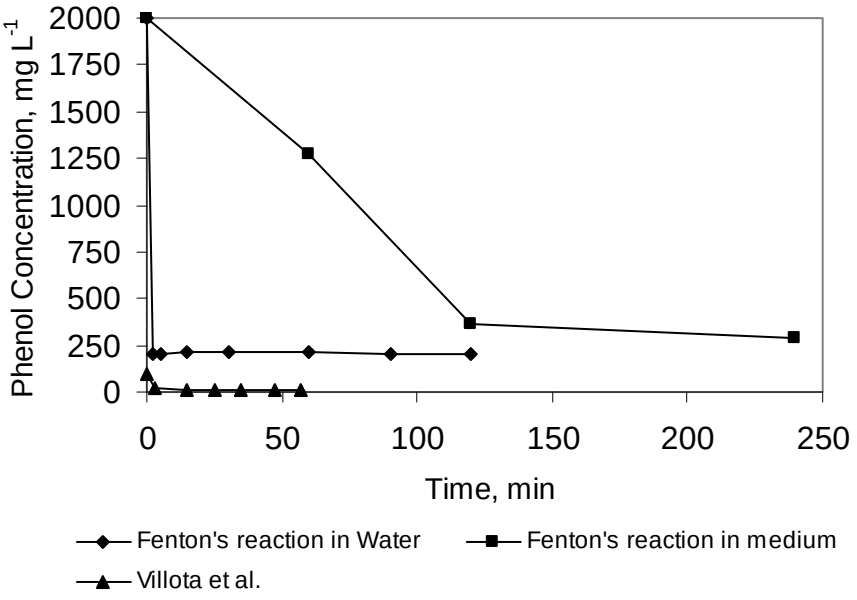


Figure 2.1 Comparison of Phenol oxidation by Fenton's reagent in water and medium under same experimental conditions (C_{PH} = 2000 mg L^{-1}, C_{H2O2} = 2000 mg L^{-1}, $C_{FeSO4.7H2O}$ = 1250 mg L^{-1}, pH 3 and T = 25°C).

Figure 2.1 also shows the phenol oxidation by Fenton's reagent in water by Villota et al. (2007). Comparing phenol oxidation by Fenton's reagent in water with medium, it was found that phenol oxidation was very fast and reaches to equilibrium in about 15 minutes. Similar results have been obtained by Villota et al. (2007) of phenol oxidation by

Fenton's reagent in water. These tests indicate that the extent of conversion and the oxidation rate of phenol in the growth medium are quite different from that reported for the same reaction in water.

The model coefficients were found by fitting CCRD data in Table 2.2 with a second order polynomial Eq. (2.7).

$$Y = \alpha_0 + \sum_{i=1}^{k} \alpha X_i + \sum_{i=1}^{k} \alpha_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \alpha_{ij} X_i X_j + e_{ij} \qquad (2.7)$$

The 13 experiments were conducted to calculate five coefficients of the second order polynomial regression model. The results of the ANOVA test and estimated values of the variable coefficients and their interaction effects are shown in Table 2.3. The p value of the model is less than 0.05, which means at the 95% confidence limit the model is a good representation of the data.

Table 2.3 Test of significance of factors and interactions after ANOVA for the full model (all factors included).

Parameter Concentration	Sum of square	Df	Mean square	F ratio	P value	Estimated coefficient
Model	186.6	5	37.32	11.60	0.0028	60.8
X_1 C_{H2O2}	134.9	1	134.9	41.94	0.0003	$1.57x10^{-2}$
X_2 $C_{FeSO4.7H2O}$	21.94	1	21.94	6.82	0.0349	$-1.28x10^{-3}$
X_1^2	20.29	1	20.29	6.31	0.0403	$-1.52x10^{-6}$
X_2^2	5.17	1	5.17	1.61	0.2454	$3.07x10^{-6}$
X_1X_2	1.10	1	1.10	0.34	0.5767	$9.33x10^{-7}$

*Df: Degree of freedom

The p values associated with X_2^2 and X_1X_2 terms were greater than 0.05, which means these terms are insignificant at 95% confidence interval and were excluded from the model. The final equation of % phenol oxidation after excluding the insignificant parameters is as follows:

$$Y = 59.5 + 0.0152\ X_1 + 0.00312\ X_2 - 0.00000162\ X_1^2 \qquad (2.8)$$

Where,

X_1 : Hydrogen peroxide concentration, mg L^{-1}

X_2 : Ferrous sulfate concentration, mg L^{-1}

Table 2.4 shows that after removing the insignificant terms, the p values for all the remaining terms were less than 0.05. The coefficient of determination (R^2) for the simplified model was calculated to be 0.86, indicating that model could explain 86% of the variability.

Table 2.4 Test of significance of factors and interactions after ANOVA for the simplified model (excluding insignificant terms).

Parameter Concentration	Sum of square	Df	Mean square	F ratio	P value	Estimated coefficient
Model	180.3	3	60.10	18.78	0.0003	59.5
X_1 C_{H2O2}	134.9	1	134.9	42.17	0.0001	$1.57x10^{-2}$
X_2 $C_{FeSO4.7H2O}$	21.94	1	21.94	6.82	0.0279	$-3.12x10^{-3}$
X_1^2	23.45	1	23.45	7.33	0.0241	$-1.62x10^{-6}$

*Df: Degree of freedom

As demonstrated in Figure 2.2, the values predicted by the second-order model agree reasonably well with the experimental data, indicating an acceptable agreement between the model prediction and the experiments. The mean absolute relative error calculated for the model was 1.33%.

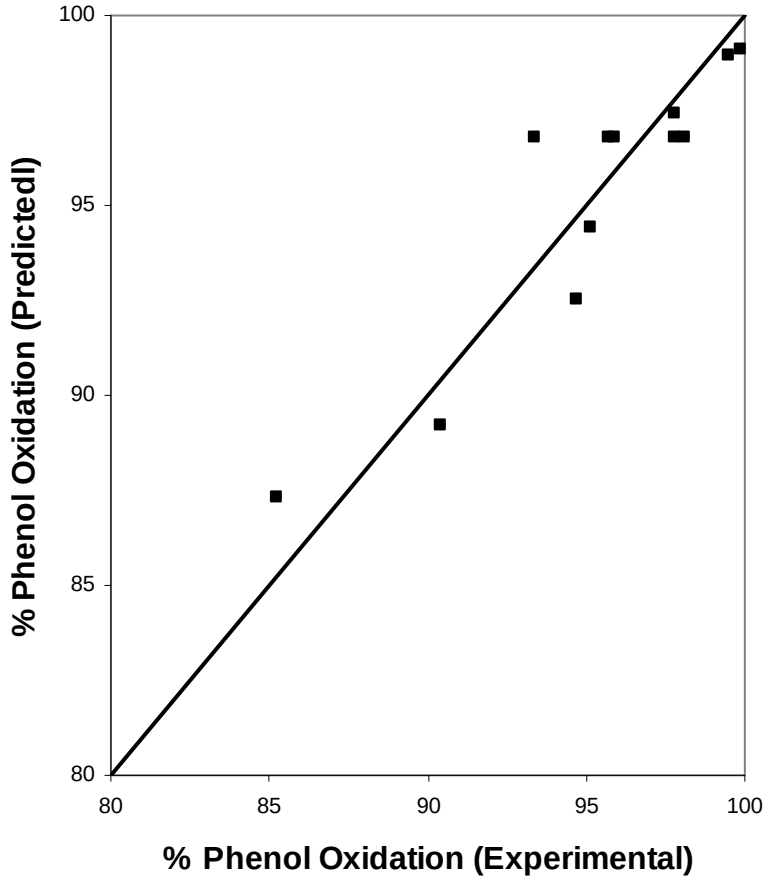


Figure 2.2 Parity chart for the experimental data against the values predicted by the modified second order response model.

Analysis of Equation (2.8) shows that both the first and second order effects associated with hydrogen peroxide concentration (X_1) and only the first order effect associated with ferrous sulfate concentration (X_2) are significant. Figure 2.3 is a 3-dimensional plot of percentage phenol removal against hydrogen peroxide and ferrous sulfate concentrations. The hydrogen peroxide and ferrous sulfate concentrations have positive effects on the response factor.

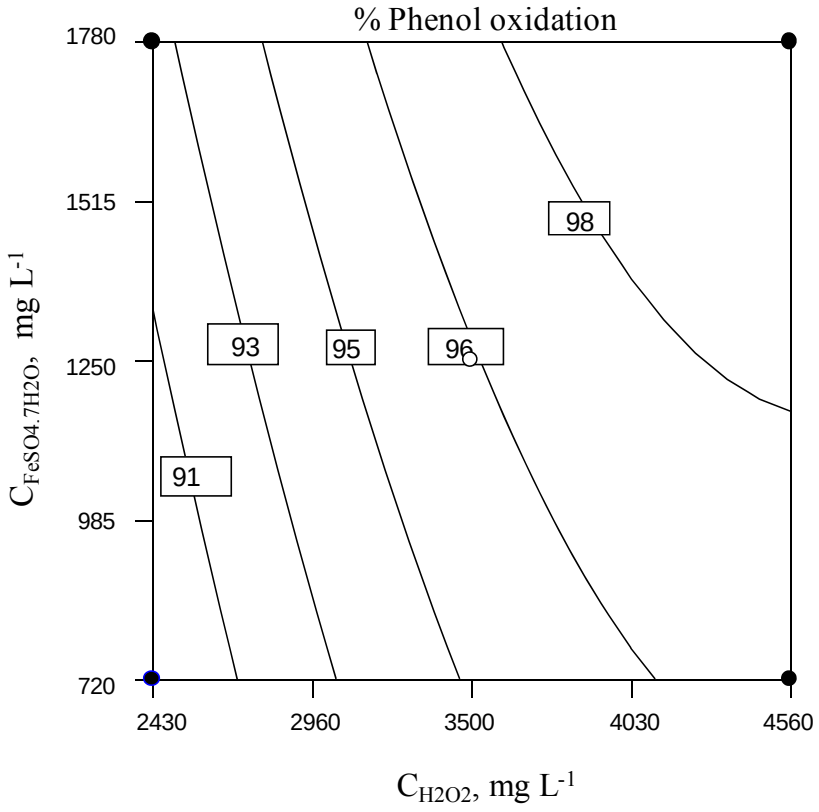


Figure 2.3 Contour plot for percentage of phenol removal as a function of hydrogen peroxide and ferrous sulfate concentrations (C_{PH} = 2000 mg L^{-1}, pH 3 and T = 25°C).

As can be seen in Figure 2.3, at any given ferrous sulfate concentration, there was a dramatic increase in the percentage phenol removal with increase in hydrogen peroxide concentration. It is also observed that at a given hydrogen peroxide concentration, increasing the ferrous sulfate concentration causes a significant increase in the percentage phenol removal but to a lesser degree when compared with hydrogen peroxide. Using 2439 mg L^{-1} (X_1 = -1) as the initial concentration of hydrogen peroxide and 719 mg L^{-1} (X_2 = 0) as the ferrous sulfate concentration, approximately 90% (19.13 x10^3 mM) of the phenol was oxidized. By almost doubling the initial concentration of hydrogen peroxide

the phenol removal increased to approximately 99% (21.04 x 10^3 mM). The same was not true for the effect of ferrous sulfate. By doubling the initial concentration of ferrous sulfate there was only a 5% increase in phenol oxidation.

Villota et al. (2007) studied phenol oxidation by Fenton's reagent in water and found that increasing the molar ratio of H_2O_2/C_6H_5OH from 3.5 to 33.1, caused a 5.7 % increase in the conversion of phenol. The molar ratio of H_2O_2/C_6H_5OH of 8 was found to be the best value for a 100 mg L^{-1} of initial phenol concentration with 2 mg L^{-1} of ferrous sulfate concentration. In the present study, which deals with oxidation of phenol in bioremediation medium, the best value of the molar ratio of H_2O_2/C_6H_5OH was found to be 6 for 2000 mg L^{-1} of initial phenol concentration with a concentration of $FeSO_4.7H_2O$ of 1616 mg L^{-1}.

There have been reports on optimization of phenol removal in the water using the traditional one factor at a time statistical approach (Yavuz et al., 2007). But no study has been done so far to find the optimal operating conditions of Fenton's reagents in a bioremediation medium. Using the optimization method developed by Derringer and Suich (described by Myer and Montgomery, 2002 (Myer and Montgomery, 2002), the best hydrogen peroxide and ferrous sulfate concentrations for complete oxidation of 2000 mg L^{-1} phenol in culture medium were determined to be 4340 and 1616 mg L^{-1}, respectively. A final verification experiment was performed at these optimal operating conditions (C_{PH} = 2000 mg L^{-1} (21.25 x 10^3 mM), C_{H2O2} = 4340 mg L^{-1}, $C_{FeSO4.7H2O}$ =1616 mg L^{-1}, pH 3 and T = 25°C) and the percentage phenol removal was measured to be

99.9% (21.23 x 10^3 mM), indicating that the developed model and optimization strategy were correct.

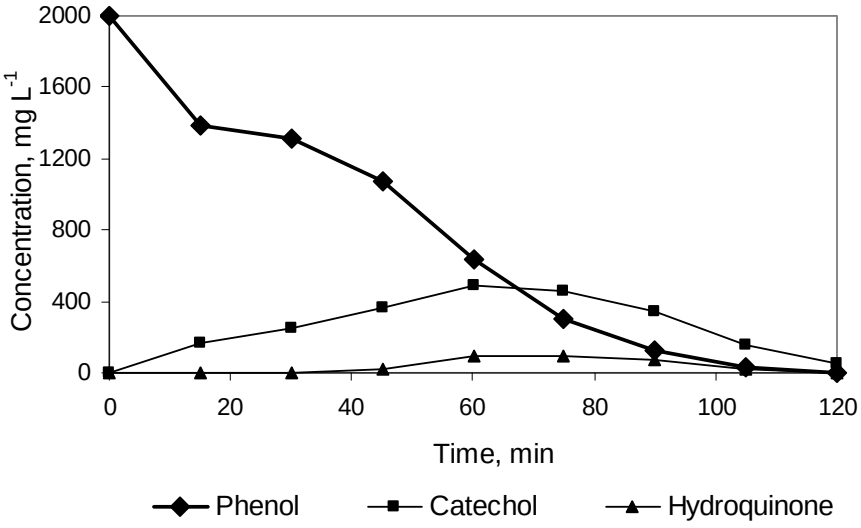


Figure 2.4 Transient oxidation of phenol and formation and oxidation of intermediates at optimal conditions of hydrogen peroxide and ferrous sulfate (C_{PH} = 2000 mg L^{-1}, pH 3, T = 25°C, C_{H2O2} = 4340mg L^{-1} and $C_{FeSO4.7H2O}$ = 1616 mg L^{-1}).

The oxidation of the phenol as a function of time for this run is shown in Figure 2.4. Included in this Figure are also the measured concentration profiles for catechol and hydroquinone which are the intermediate products of phenol oxidation. The two other oxidation products identified were maleic acid and carbon dioxide. The oxidation of phenol results in formation of dihydroxylated compounds, mainly catechol and hydroquinone. The concentrations of these intermediates increased with time and reached to maximum in 60 min. These dihydroxylated compounds were oxidized further and complete oxidation was achieved in 2 hrs.

2.5 Conclusions

A CCRD experimental design was used to determine the ability of Fenton's reagent to oxidize phenol in a medium suitable for bioremediation of organic contaminants. It was found that increases in H_2O_2 and $FeSO_4.7H_2O$ concentrations had a positive effect on phenol oxidation. Hydrogen peroxide concentration, however, was found to be the dominating parameter for the removal of phenol by Fenton's reagent. Using a second order polynomial model, the optimal H_2O_2 and $FeSO_4.7H_2O$ concentrations were evaluated with a focus on the complete removal of 2000 mg L^{-1} of phenol. The optimal conditions for the Fenton's reaction in the bioremediation medium for complete 2000 mg L^{-1} phenol conversion were determined to be C_{H2O2} = 4340 mg L^{-1} and $C_{FeSO4.7H2O}$ = 1616 mg L^{-1}. Almost all phenol was found to be oxidized under these optimal conditions at equilibrium in two hours. The main oxidation products were identified as catechol, hydroquinone, maleic acid and carbon dioxide.

CHAPTER 3

3. KINETIC MODELLING OF PHENOL IN A BIOREMEDIATION MEDIUM USING FENTON'S REAGENT

This chapter will be submitted for publication in International Journal of Chemical Reactor Engineering:

Kumar P., H. Nikakhtari*, M. Nemati, G. A. Hill "Kinetic Modelling of Phenol Oxidation in a Bioremediation Medium Using Fenton's Reagent ", to be submitted.

Contribution of the Ph.D. Candidate

The batch oxidation experiments and kinetic modelling developed for the purpose of this study were planned and executed by Pardeep Kumar with technical input and guidance from Dr. Hill and Dr. Nemati. The manuscript was written by Pardeep Kumar, with editorial input from Dr. Hill and Dr. Nemati and is almost ready for submission.

Contribution of this Chapter to Overall Study

After obtaining the optimal experimental conditions and identification of major intermediate compounds of phenol oxidation in a bioremediation medium by Fenton's reagent as presented in Chapter 2, dynamic batch phenol oxidation experiments were conducted to quantify phenol and intermediate oxidation compounds at different hydrogen peroxide concentrations and temperatures. The reaction mechanism and kinetic model involved determination of kinetic parameters, namely rate constants and activation energies, which were determined for all the reactions involved. The determined rate

* Hossein Nikakhtari (PDF) assisted in the analysis of the experimental data for the manuscript.

constants and amount of hydrogen peroxide required for complete phenol oxidation was compared to those for chlorine dioxide, the other tested oxidant, in Chapters 4 and 5.

3.1 Abstract

The present study is aimed at kinetic modeling of phenol oxidation using Fenton's reagent in a medium suitable for bioremediation of organic pollutants. Batch experiments were conducted to study the effect of H_2O_2 concentration (29.26 to 146.31 mM), temperature (5 to 35 °C) and to compare the oxidation of phenol in a bioremediation medium to that in pure water. The reaction mechanism used for kinetic modeling is based on the intermediate oxidation products identified in this study using HPLC and ion chromatography. Progress of the chemical oxidation by Fenton's reagent was monitored by determining the residual phenol concentration and concentrations of evolved intermediate compounds at regular time intervals. The rate of phenol oxidation and ultimate conversion of phenol were found to increase with increase in hydrogen peroxide concentration and temperatures over the ranges studied. Kinetic parameters, namely rate constants and activation energies, were determined by best-fitting the experimental data to a proposed reaction model.

3.2 Introduction

Increasing environmental awareness, health hazards and more stringent environmental regulations necessitate the incorporation of improved technologies to control industrial waste waters. Phenols and phenol like compounds commonly occur in industrial wastewaters because of their wide use in the chemical industries and other

manufacturing operations (Arana et al., 2007). Phenolic compounds also form in wastewater because of the oxidation of higher molecular weight polycyclic aromatic hydrocarbons (PAHs). Phenols, even at low levels (ppb), give objectionable taste and odor to drinking water and pose toxicity problems at concentrations higher than 50 mg L^{-1} (Yavuz et al., 2007). High concentrations of phenols in wastewater exert high oxygen demand and concentrations between 10 and 100 mg L^{-1} result in the death of aquatic life (Korbathi and Tanyolac, 2003).

It has been observed that high phenol concentrations in industrial wastewaters kill the indigenous bacteriological population in wastewater treatments plants, so biological processes alone can not be used to treat high concentration of phenols in wastewater (Yavuz et al., 2007). Chemical oxidation processes have attracted the attention of researchers during the last two decades and could be used as a pre-treatment to oxidize high concentrations of phenols to short chain organic acids which are more easily biodegraded or they can be used as a final treatment to remove the non-biodegradable organics and meet the discharge limits of toxic compounds (Nam et al., 2001; Luis et al., 2009). Advanced oxidation processes (AOPs), based on the generation of reactive species such as hydroxyl radicals ($OH^{\bullet}$), have become popular for degradation of phenolic wastes (Villota et al., 2007).

Fenton's reagent is one such AOP, consisting of a mixture of H_2O_2 and iron salts (ferrous ions). Fenton's reaction is a homogeneous reaction in which Fe^{2+} acts as a catalyst and

hydroxyl radicals are generated by transfer of an electron between Fe^{2+} and H_2O_2 as shown in the following reaction (Han and Wang 2009):

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^- + HO^{\bullet} \quad (3.1)$$

Hydroxyl radicals are powerful oxidants having oxidation potentials (E_0= 2.80 V) higher than more commonly used oxidants such as ozone (E_0=2.07 V) and hydrogen peroxide (E_0= 1.78 V) (Heredia et al., 2001). The hydroxyl radicals are extremely unstable and attack the organic substrate either by hydrogen atom abstraction to form organic free radicals and water, or add to the aromatic ring forming compounds such as dihydroxybenzenes (Peres et al., 2004).

$$RH + HO^{\bullet} \rightarrow \text{Oxidized products} \quad (3.2)$$

Hydrogen peroxide may also directly oxidize organic substrates:

$$RH + H_2O_2 \rightarrow \text{Oxidized products} \quad (3.3)$$

Hydroxyl radicals generated may be scavenged by reactions with hydrogen peroxide and Fe^{2+}:

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \quad (3.4)$$

$$HO^{\bullet} + Fe^{2+} \rightarrow HO^{-} + Fe^{3+} \quad (3.5)$$

Fe^{2+} is regenerated through the reaction of Fe^{3+} with hydrogen peroxide and hydroperoxyl radicals as shown in reactions (3.6) and (3.7) (Esplugas et al., 2002).

$$Fe^{3+} + H_2O_2 \rightarrow Fe\text{-}OOH^{2+} + H^{+} \quad (3.6)$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + H^{+} + O_2 \quad (3.7)$$

The Fenton's oxidation process has been successfully used to treat different industrial wastewaters. Esplugus et al. (2002) compared different advanced oxidation treatments using O_3, O_3/H_2O_2, UV, UV/O_3, UV/H_2O_2, O_3/UV/H_2O_2, Fe^{2+}/H_2O_2 and photocatalysis for phenol removal in an aqueous solution and observed that Fenton's reagent achieved the fastest removal rate among the applied oxidants. Chemical treatments or advanced oxidation could be specifically applied as a pre-treatment to lower the concentration of toxic pollutants, oxidize recalcitrant pollutants to more biodegradable compounds or as a final treatment to remove the residual recalcitrant compounds and to meet the discharge limits set for the toxic compounds.

Although chemical oxidation could be applied to completely mineralize the organic pollutants to CO_2 and H_2O, high doses of oxidant required for this purpose would make the oxidation process uneconomical. Thus chemical oxidation should be combined with other less expensive treatment methods, such as biodegradation, to remove these

pollutants economically from the wastewater. Nam et al. (2001) studied the degradation of polycyclic aromatic hydrocarbons using combined biodegradation and Fenton's reagent and found that biodegradation followed by chemical oxidation was more efficient for PAH removal. The sequencing used in integrated chemical oxidation and biodegradation depends upon the type of organic pollutants which are present in the wastewater.

Industrial wastewaters usually contain inorganic salts along with organic pollutants and these inorganic compounds were found to lower the efficiency of the Fenton's reaction (Truong et al., 2004; Bacardit et al., 2007; Siedlecka et al., 2007). Siedlecka and Stepnowski (2005) studied phenol oxidation using Fenton's reagent in the presence of chloride and sulfate ions and found that presence of these inorganic anions have significant negative effect on the oxidation process. The presence of inorganic ions has two negative effects: (1) the scavenging of hydroxyl radicals by chloride and sulfate ions to form anion radicals such as $SO_4^{\bullet-}$, $Cl^{\bullet-}$ and $Cl_2^{\bullet-}$ (2) the reaction of inorganic anions with $Fe3^+$ to form iron complexes which affects the regeneration of Fe^{2+} species. Han and Wang (2009) studied the effect of inorganic anions on oxidation of MTBE by Fenton's reagent and observed that the oxidation process is sensitive to the presence of anions. They found that the presence of anions decreased the oxidation rate of MTBE in the following order PO^{3-}_4 >HPO^{2-}_4 >$H_2PO^-_4$ >Cl^- >SO^{2-}_4. Kumar et al. (2010a) compared the steady state phenol oxidation in a bioremediation medium, which contained a mixture of anions, and pure water and found that the oxidation of phenol in a bioremediation medium was much slower than in pure water.

Although a number of researchers have studied phenol oxidation by Fenton's reagent in water and identified the oxidation products, no study has been reported on oxidation of phenol by Fenton's reagent in a bioremediation medium. Chemical oxidation can be used as a pre-treatment to oxidize recalcitrant compounds to more biodegradable compounds. Alternatively, biodegradable compounds of the wastewater can be removed by bioremediation first. This is then followed by chemical oxidation of the non-biodegradable components of the wastewater. During this sequence of bioremediation followed by chemical oxidation, the wastewater could contain inorganic salts present in the bioremediation medium. This underscores the importance of studying chemical oxidation in a bioremediation medium. A suitable bioremediation medium, such as McKinney's medium, is a mixture of several inorganic salts which are required for optimal growth and activity of microorganisms used for bioremediation purposes. We have reported the equilibrium oxidation of phenol in a bioremediation medium in the preceding chapter and determined the optimal concentrations of H_2O_2 and $FeSO_4 \bullet 7H_2O$ using the CCRD (central composite rotatable design) approach (Kumar et al., 2010a).

The objective of the present study was to investigate the dynamic oxidation of phenol by Fenton's reagent in the bioremediation medium and to develop a kinetic model based on a reaction mechanism involving the oxidation products identified in this study and which are in agreement with those reported in the literature.

3.3 Materials and Methods

3.3.1 Chemicals

All the chemicals used were of analytical grade quality. Phenol stock solution was prepared in two-dose McKinney's medium using 99.5% pure phenol in solid form, manufactured by Merck (Darmstadt, Germany). Hydrogen peroxide (30% by weight) was obtained from Sigma Aldrich (Oakville, Canada). The ferrous ions, which act as catalyst in the Fenton's reaction, were supplied in the form of ferrous sulfate heptahydrate ($FeSO_4 \bullet 7H_2O$) obtained from Fisher Scientific. Reverse osmosis water was used for preparing all solutions. The two dose McKinney's medium used in all experiments consisted of (mg in 1 L reverse osmosis water): K_2HPO_4, 750; KH_2PO_4, 849; $(NH_4)_2SO_4$, 474; NaCl, 60; $CaCl_2$, 60; $MgSO_4$, 60; $Fe(NH_4)SO_4$, 20; and 1 mL of trace mineral solution. The trace mineral solution consisted of (mg in 1 L reverse osmosis water): $ZnSO_4 \bullet 7H_2O$, 200; $MnCl_2$, 60; H_3BO_3, 600; $CoCl_2$, 400; $CuCl_2$, 20; $NiCl_2$, 40; Na_2MoO_4, 60. The natural pH of the medium was 7.0 and was adjusted to the designated reaction pH condition using 1 M H_2SO_4.

3.3.2 Experimental procedure

The experimental procedure for phenol oxidation by Fenton's reagent has been described in the preceding chapter. In brief, all the oxidation experiments were conducted in batch mode using 500 mL Erlenmeyer flasks which represented the reactors. Each reactor was charged with 200 mL of two-dose McKinney's medium with an initial phenol concentration of 21.27 mM. The required amount of $FeSO_4 \bullet 7H_2O$ was then added and the reactors were placed on a rotary shaker operated at 200 rpm. The pH of the reaction

medium was adjusted to 3 by using 1 M H_2SO_4. The reaction temperature was maintained at 25°C by conducting all the experiments in temperature controlled chambers. The reaction was started by adding the designated amount of hydrogen peroxide in each reactor. During all the experiments, samples were withdrawn from the reactors at regular intervals to determine the concentrations of remaining phenol and intermediates compounds (catechol and hydroquinone). Samples were transferred into 4 mL vials which contained a sufficient amount of sodium sulfite to stop the reaction between hydroxyl radicals and phenol (Chedeville et al., 2005). Fenton's reaction was performed in the bioremediation medium to study the kinetics of phenol oxidation by changing the concentration of hydrogen peroxide (29.26 mM to 146.31 mM) and temperature (5 to 35°C). Some of the oxidation intermediates were identified and quantified using ESI/LC/MS, HPLC and ion chromatography. The concentration of hydrogen peroxide was varied in the range 29.26 to 146.31 mM. The concentrations of Fe(II) and phenol were fixed at 5.39 mM and 21.27 Mm, respectively.

3.3.3 Analytical Methods

The intermediates of phenol oxidation were identified in the solution using a high resolution liquid chromatograph (Waters 2695, Milford USA) coupled to an inline diode array detector (Waters 996 PDA) connected in series to a Quattro Ultima triple quadrupole mass spectrometer equipped with an electrospray interface (ESI, Micromass, UK) operating in the negative ion mode. After identification of oxidation intermediates (catechol and hydroquinone), phenol and the intermediates were quantified by means of high performance liquid chromatography (HPLC, Agilent 1100; California, USA) with a

dioide array detector equipped with a Nova pack C_{18} column (4.5 x 150 mm; 4μm). The mobile phase was a mixture of acetonitrile and water (12.5/87.5 v/v) at a flow rate of 2.1 mL min^{-1}. Detection was carried out at a wavelength of 254 nm. The maleic acid was identified using a Dionex Ion chromatograph (ICS-2500) with a thermal conductivity detector (CD25A), an Ionpac CG5A guard column and an Ionpac CS5A analytical column (Bannockburn, USA). The eluent was 1.0 mM KOH at a flow rate of 1.5 mL h^{-1}. Concentration (30% wt) of the hydrogen peroxide solution was determined by titration with standard (0.1 N) potassium permanganate, details of which are described in Chapter 2.

3.4 Results and Discussion

The effects of different parameters on oxidation of phenol by Fenton's reagent were studied by varying the concentration of hydrogen peroxide (29.26 mM to 146.31 mM) and temperature (5 to 35°C). The pH of the reaction medium was kept constant at 3.

3.4.1 Effect of medium dose

To study the effect of medium dose, experiments of phenol oxidation by Fenton's reagent were conducted with different doses of bioremediation medium. Figure 3.1 demonstrates the results and also compares published phenol oxidation in water data (Villota et al., 2007).

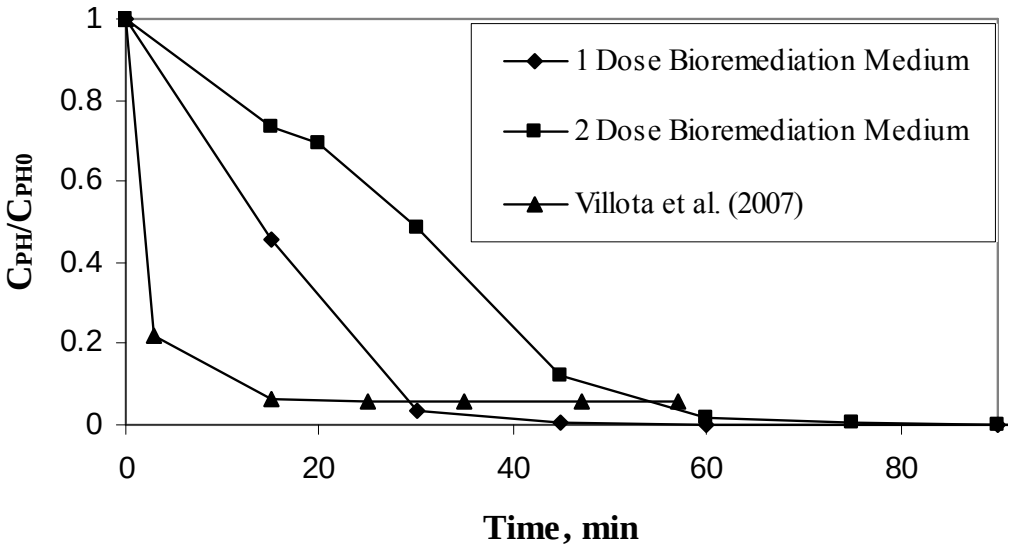


Figure 3.1 Comparison of phenol oxidation by Fenton's reagent in bioremediation medium at different doses (C_{PH} = 21.27 mM, molar ratio of H_2O_2 to phenol = 6.8, Fe(II) = 5.39 mM, pH 3 and T = 25°C). Data for oxidation of phenol from pure water was obtained at C_{PH} = 1.06 mM, molar ratio of H_2O_2 to phenol = 6.3, Fe(II) = 0.065 mM, pH 3 and T = 25°C (Villota et al., 2007).

As can be seen from Figure 3.1, the presence of inorganic salts had a significant effect on phenol oxidation by Fenton's reagent and the rate of phenol oxidation decreased when bioremediation medium is used instead of water and when the medium dose is increased. This is most likely due to scavenging of hydroxyl radicals by the inorganic anions, Fe^{3+} complexation with inorganic anions and generation of inorganic radicals which react with phenol at much slower rates than hydroxyl radicals.

3.4.2 Effect of hydrogen peroxide concentration

The steady state and dynamic conversion of phenol as a function of initial H_2O_2 concentration in bioremediation medium are shown in Figure 3.2. To maintain the clarity in the figure, only the data for phenol oxidation at different hydrogen peroxide concentrations is shown in here and concentration profiles for intermediates are not included. As can be seen in this figure, final conversion of phenol and the rate of phenol oxidation increase with the increase in hydrogen peroxide concentration owing to the generation of more hydroxyl radicals. The main oxidation products of phenol by Fenton's reagent were identified as catechol, hydroquinone, maleic acid and carbon dioxide.

The proposed reaction mechanism of phenol oxidation by Fenton's reagent is shown in Figure 3.3 and is based on the available literature and identification of oxidation products in this work. The identification of these intermediates and their oxidation kinetics are important because some of these intermediates have higher toxicity than phenol itself (Villota et al., 2007). Based on the mechanisms proposed, the hydroxyl radical attacks the phenol molecule at the ortho- and para- positions to give dihydroxybenzenes, mainly catechol and hydroquinone (Zazo et al., 2005).

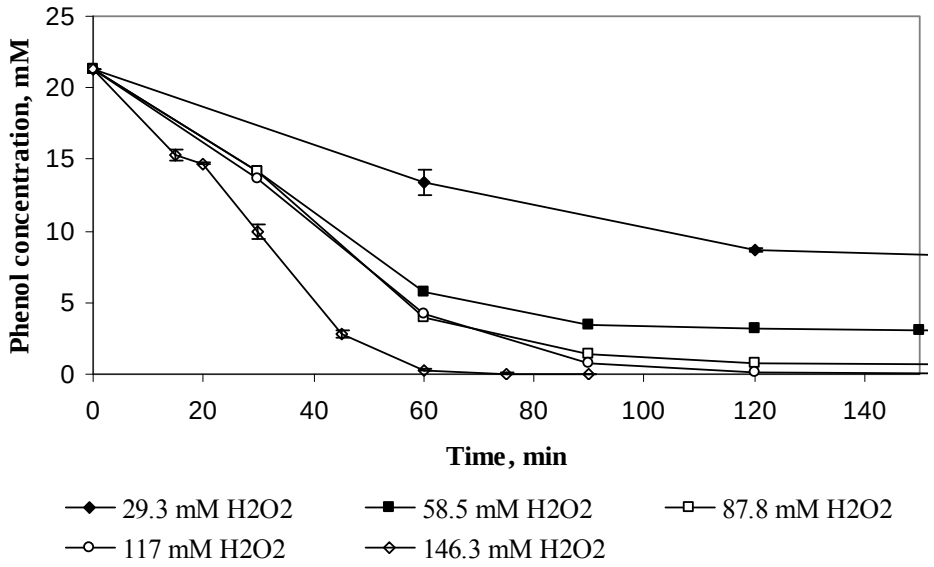


Figure 3.2 Effect of H_2O_2 concentration on phenol oxidation by Fenton's reagent (C_{PH} = 21.27 mM, pH 3, T = 25°C and $C_{Fe(II)}$ = 5.39 mM).

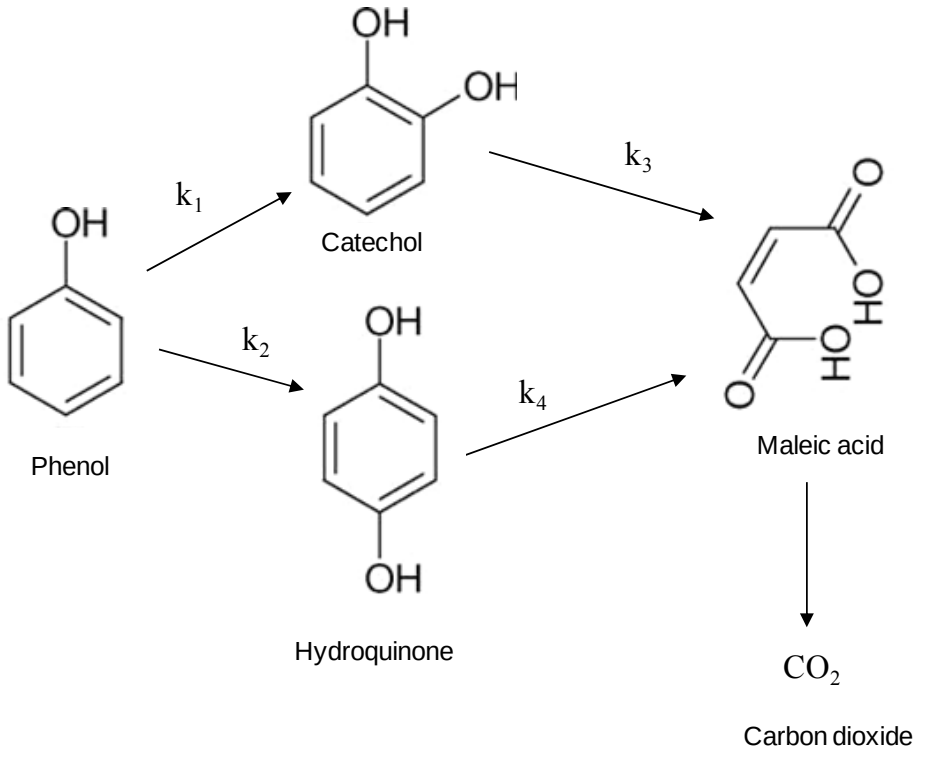


Figure 3.3 Proposed reaction mechanism of phenol oxidation by Fenton's reagent

The oxidation of phenol to catechol occurred instantaneously, but there was a delay observed in the formation of hydroquinone, indicating that the hydroxyl radical attack at the ortho position of the aromatic ring was the predominant mechanism (Villota et al, 2007). The formation of hydroquinone started when the catechol concentration reached to its maximum (Figures 3.4 and 3.5).

One could speculate the formation of hydroquinone by another mechanism, namely the formation of hydroquinone from the oxidation of catechol. To study this, catechol was oxidized with various concentrations of Fenton's reagent in bioremediation and the products of oxidation were identified. No hydroquinone peak was observed on the HPLC graph under any operating conditions.

This delay time in the formation of hydroquinone was found to be a function of reaction temperature and independent of initial hydrogen peroxide concentration at fixed temperature. Values of experimentally measured delay times at different temperatures are shown in Table 3.2. The break down of the aromatic ring takes place by further reaction of hydroquinone and catechol with hydroxyl radicals to give maleic acid and carbon dioxide.

Based on the proposed mechanism shown in Figure 3.3, the main oxidation reactions of phenol by Fenton's reagent can be summarized as shown below.

The oxidation of phenol to catechol:

$$C_6H_5OH + H_2O_2 \xrightarrow{k_1} C_6H_4(OH)_2 + H_2O \quad (3.8)$$

The oxidation of phenol to hydroquinone

$$C_6H_5OH + H_2O_2 \xrightarrow{k_2} C_6H_4(OH)_2 + H_2O \quad (3.9)$$

The oxidation of catechol to maleic acid

$$C_6H_4(OH)_2 + 7H_2O_2 \xrightarrow{k_3} C_4H_4O_4 + 2CO_2 + 8H_2O \quad (3.10)$$

The oxidation of hydroquinone to maleic acid

$$C_6H_4(OH)_2 + 7H_2O_2 \xrightarrow{k_4} C_4H_4O_4 + 2CO_2 + 8H_2O \quad (3.11)$$

Assuming all reactions are first order with respect to each reactant concentration (Ana et al., 2009) and second order overall, the rate equation for each compound can be written as:

$$\frac{dC_{Ph}}{dt} = -k_1 C_{Ph} C_{H_2O_2} - k_2 C_{ph} C_{H_2O_2} \quad (3.12)$$

$$\frac{dC_C}{dt} = k_1 C_{Ph} C_{H_2O_2} - k_3 C_C C_{H_2O_2} \quad (3.13)$$

$$\frac{dC_H}{dt} = k_2 C_{Ph} C_{H_2O_2} - k_4 C_H C_{H_2O_2} \quad (3.14)$$

Where,

C_{PH} is the phenol concentration, mM

C_{H2O2} is hydrogen peroxide concentration, mM

C_C is catechol concentration, mM

C_H is hydroquinone concentration, mM

Using the observed delay times for the formation of hydroquinone shown in Table 3.2, Equations (3.12) to (3.14) were solved simultaneously (using Runge-Kutta 4th order method on Microsoft Excel 2007) and rate constants k_1, k_2, k_3, k_4 were determined by minimizing the sum of squares of error between the predicted and the experimental concentration values. The model predictions fit reasonably well to experimental data as shown in Figures 3.4 and 3.5.

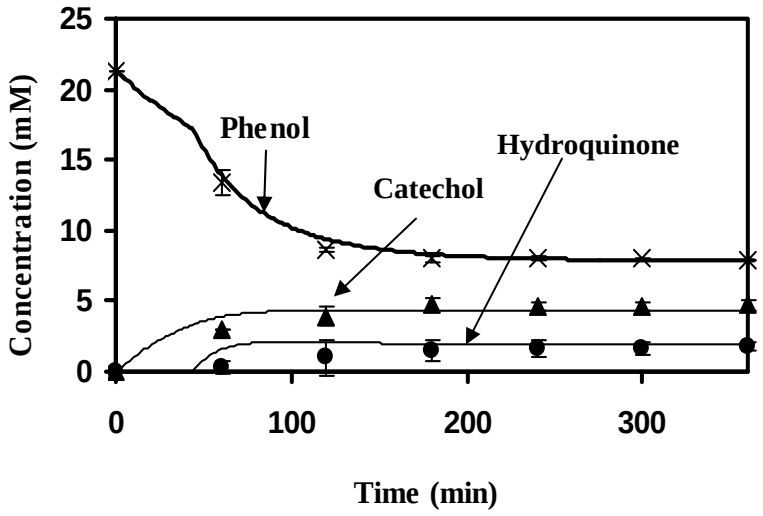


Figure 3.4 Fitting of the overall kinetic model to the phenol oxidation experimental data at low hydrogen peroxide concentration (C_{H2O2} =29.26 mM, C_{PH} = 21.27 mM, $C_{Fe(II)}$ = 5.39 mM, pH 3 and T = 25°C). Error bars represent one standard deviation.

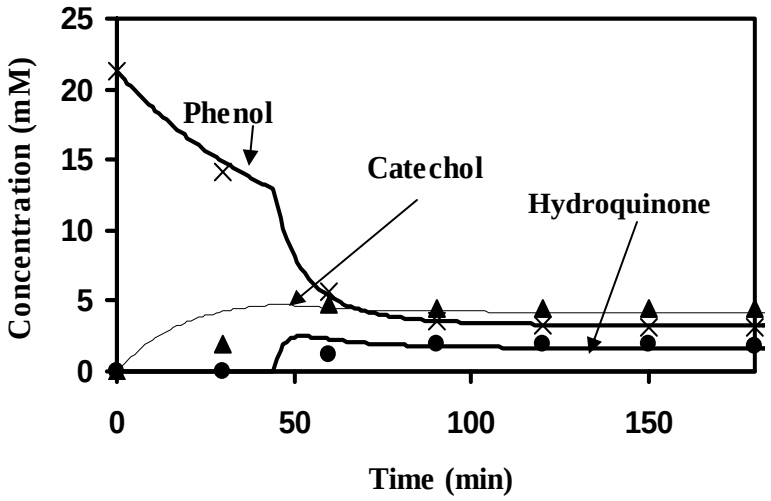


Figure 3.5 Fitting of the overall kinetic model to the phenol oxidation experimental data at high hydrogen peroxide concentration (C_{H2O2} =58.52 mM, C_{PH} = 21.27 mM, $C_{Fe(II)}$ = 5.39 mM, pH 3 and T = 25°C).

The kinetic rate constants determined using different concentrations of hydrogen peroxide are listed in Table 3.1. Table 3.1 includes also average value and standard deviations for each constant. The large values of standard deviations associated with the rate constants are due to several factors: sampling, stopping the reactions, analytical analysis, as well as other complex reactions taking place in the presence of inorganic salts in the bioremediation medium. The inorganic anions react with hydroxyl radicals and generate inorganic radicals, which compete with hydroxyl radicals for organic compounds (Siedlecka and Stepnowski, 2005). Since we have several inorganic salts in

the aqueous phase, it makes the reaction medium very difficult to analyze. But the mechanism that we have considered fits well to the experimental data.

Table 3.1 Values of rate constants for oxidation of phenol by Fenton's reagent at different H_2O_2 concentrations (C_{PH} = 21.27 mM, $C_{Fe(II)}$ = 5.39 mM, pH 3 and T = 25°C).

Concentration of H_2O_2, mM	**k_1 (L/mM min)**	**k_2 (L/mM min)**	**k_3 (L/mM min)**	**k_4, (L/mM min)**
29.26	$8.17x10^{-5}$	$1.74x10^{-4}$	$1.56x10^{-4}$	$8.57x10^{-4}$
58.52	$1.32x10^{-4}$	$1.242x10^{-3}$	$3.02x10^{-4}$	$3.44x10^{-3}$
87.79	$9.50x10^{-5}$	$7.31x10^{-4}$	$2.93x10^{-4}$	$2.93x10^{-3}$
117.05	$2.11x10^{-5}$	$7.99x10^{-5}$	$6.83x10^{-5}$	$3.74x10^{-4}$
146.31	$2.49x10^{-5}$	$1.50x10^{-3}$	$9.46x10^{-5}$	$1.03x10^{-3}$
Average	$7.02x10^{-5}$	$7.22x10^{-4}$	$1.82x10^{-4}$	$1.68x10^{-3}$
Std. dev.	$4.63x10^{-5}$	$6.09x10^{-4}$	$1.08x10^{-4}$	$1.29x10^{-3}$

To evaluate the kinetic model, a validation experiments was conducted at a different hydrogen peroxide concentration than that used to determine the kinetic rate constants. The average values of the rate constants, shown in Table 3.1, were then used to predict the phenol oxidation data as shown in Figure 3.6. As can be seen the model predictions fit the experimental results with reasonable accuracy.

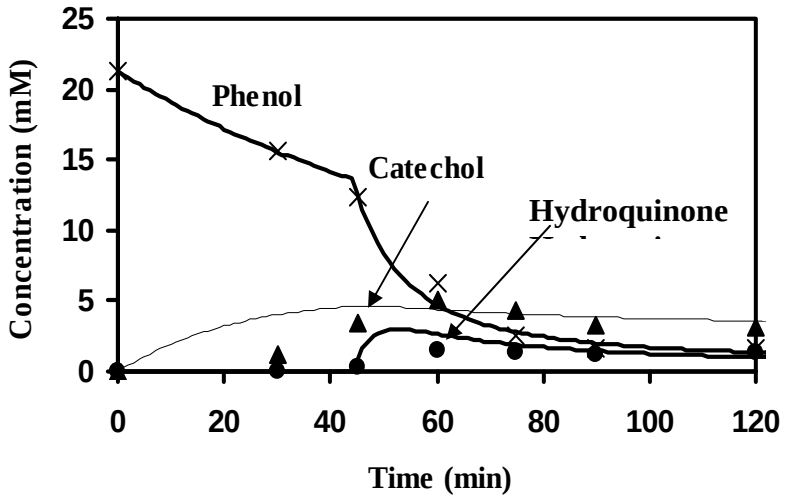


Figure 3.6 Verification of the overall kinetic model to the phenol oxidation experimental data using average values of rate constants (C_{H2O2} =73.16 mM, C_{PH} = 21.27 mM, $C_{Fe(II)}$ = 5.39 mM, pH 3 and T = 25°C).

3.4.3 Effect of temperature

To study the effect of temperature on phenol oxidation by Fenton's reagent, the reaction temperature was varied from 5°C to 35°C keeping the initial concentrations of phenol and hydrogen peroxide constant at 21.27 and 58.52 mM, respectively. The delay time in the formation of hydroquinone was a function of temperature, and decreased with increase in the reaction temperature as shown in Table 3.2.

Table 3.2 Values of rate constants for oxidation of phenol by Fenton's reagent at different temperatures (C_{PH} = 21.27 mM, C_{H2O2} =58.52 mM, $C_{Fe(II)}$ = 5.39 mM and pH 3).

Temperature °C	**k_1 (L/mM min)**	**k_2 (L/mM min)**	**k_3 (L/mM min)**	**k_4 (L/mM min)**	**Delay time (min)**
5	$4.71x10^{-5}$	$2.74x10^{-4}$	$1.41x10^{-4}$	$1.43x10^{-3}$	170
15	$9.20x10^{-5}$	$5.99x10^{-4}$	$2.18x10^{-4}$	$1.72x10^{-3}$	90
25	$1.32x10^{-4}$	$1.242x10^{-3}$	$3.02x10^{-4}$	$3.44x10^{-3}$	45
35	$3.54x10^{-4}$	$4.35x10^{-3}$	$8.55x10^{-4}$	$1.00x10^{-2}$	38
Activation energy (E_a), KJ/mol	45.5 (R^2= 0.96)	63.9 (R^2= 0.97)	40.5 (R^2= 0.91)	45.9 (R^2= 0.89)	

Figures 3.7 and 3.8 show the fitting of the experimental data obtained at 5 and 35°C to the kinetic model. As can be seen in these figures, the proposed model fits very well to the experimental data.

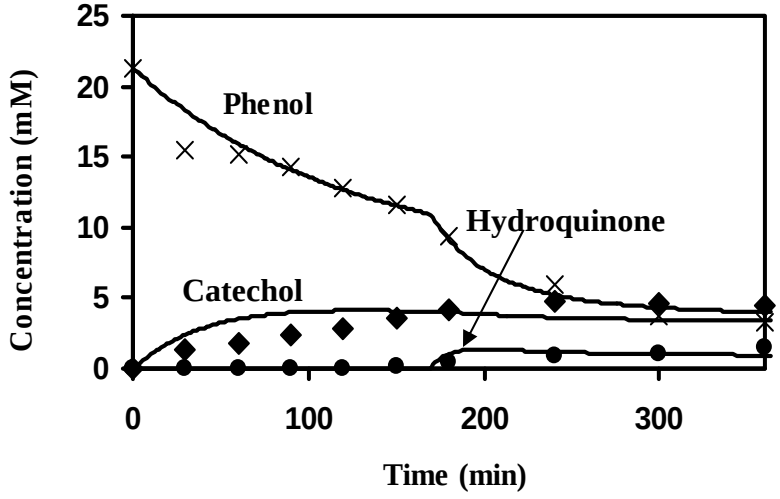


Figure 3.7 Fitting of the overall kinetic model to the phenol oxidation experimental data at 5 °C (C_{H2O2} =58.52 mM, C_{PH} = 21.27 mM, $C_{Fe(II)}$ = 5.39 mM, pH 3).

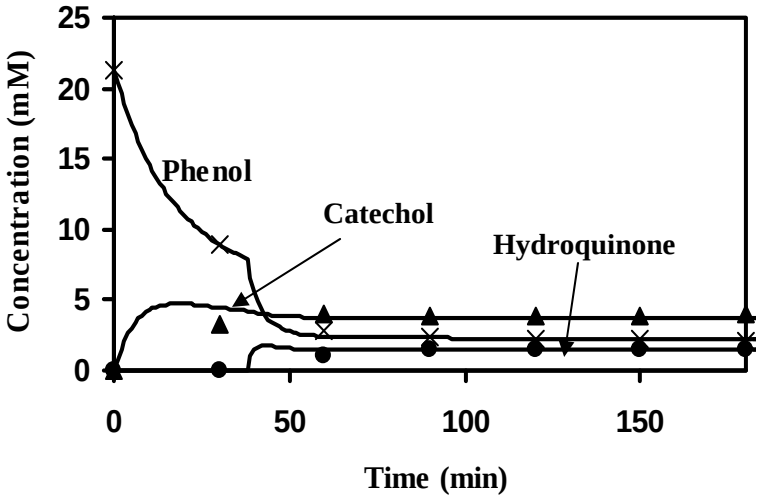


Figure 3.8 Fitting of the overall kinetic model to the phenol oxidation experimental data at 35 °C (C_{H2O2} =58.52 mM, C_{PH} = 21.27 mM, $C_{Fe(II)}$ = 5.39 mM, pH 3).

The calculated rate constants were then used to determine the activation energy for all the reactions involved and are shown in Table 3.2. Figure 3.9 shows the Arrhenius plot for the rate constant k_1. Similar Arrhenius plots have been obtained for the other rate constants.

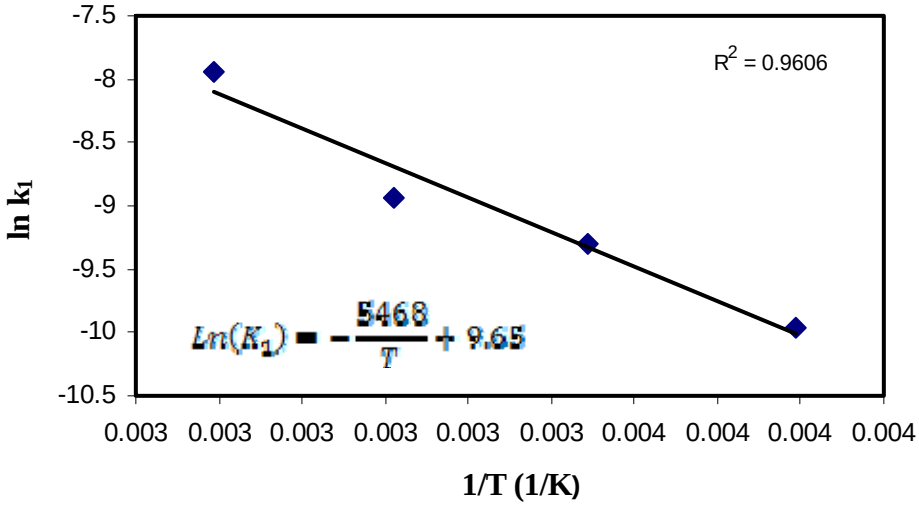


Figure 3.9 Arrhenius plot for rate constant k_1

3.5 Sensitivity analysis

Sensitivity analysis was performed to assess the effect of various coefficients on theoretical predictions by the kinetic model. In order to do this the value of one coefficient was changed by 10% increments from (-50% to +50%), while the others were kept constant. The sum of square deviations based on concentrations of phenol, catechol and hydroquinone was calculated for each tested conditions. Normalized sum of the squared deviations was defined as the ratio of sum of the squared deviation calculated with varied parameter to the sum of the squared deviations calculated with the best fit parameters.

The representative results of such analysis for some of the experimental runs are shown in Figure 3.10. The other runs showed similar trends. As can been seen in Figure 3.10 (A to D), the model is more sensitive to k_1 and k_3 and to a much lesser extent to k_2 and k_4. For the same percentage of change, in best fit values of k_1 and k_3 resulted in higher residual errors than k_2 and k_4.

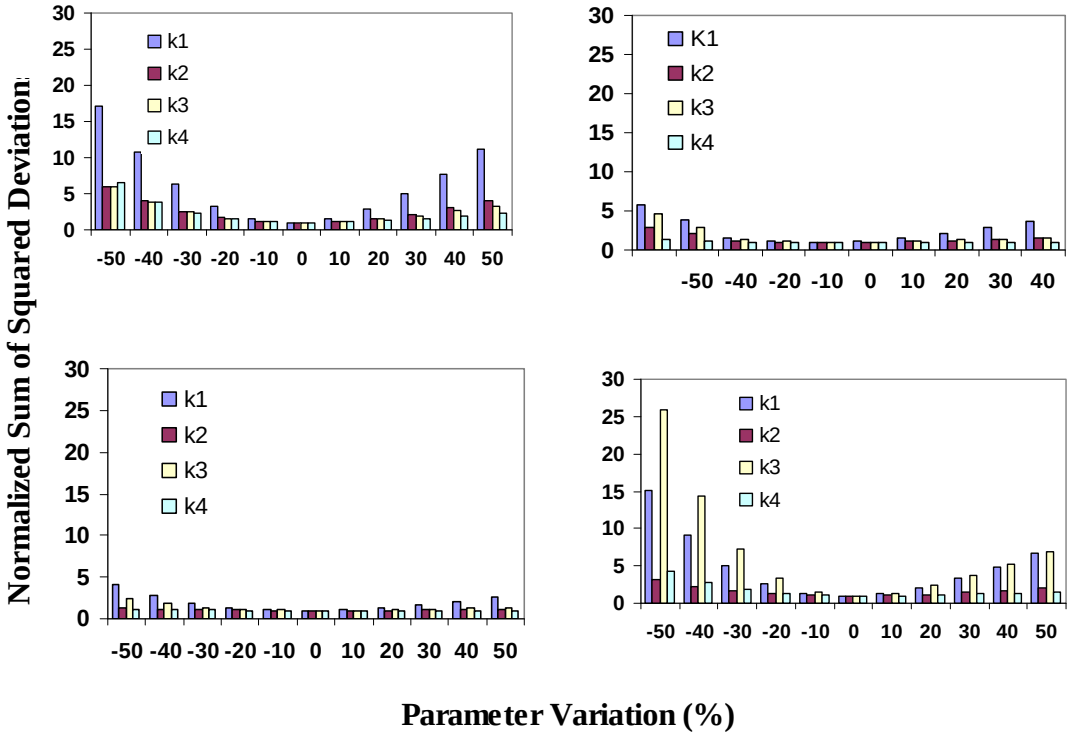


Figure 3.10 Results of sensitivity analysis for oxidation of phenol by Fenton's reagent at different hydrogen peroxide concentrations (top) and temperatures (bottom) A: 29.26 mM hydrogen peroxide and 25ºC; B: 117.05 mM hydrogen peroxide and 25ºC; C: 5ºC and 29.26 mM hydrogen peroxide D: 35ºC and 29.26 mM hydrogen peroxide.

3.6 Conclusions

The kinetics of Fenton's reaction has been determined in a bioremediation medium. The rate of phenol oxidation and overall phenol conversion were found to increase with the increase in hydrogen peroxide concentration. The average values of the second order rate constants k_1, k_2, k_3, k_4 for oxidation of phenol and intermediates by Fenton's reagent at 25°C were found to be 7.02×10^{-5}, 7.22×10^{-4}, 1.82×10^{-4}, 1.68×10^{-3} L/ mM min respectively. The rate of phenol oxidation was found to increase with increase in

temperature of the reaction (Table 3.2). The oxidation of phenol by Fenton's reagent in water was much faster than in the bioremediation medium.

CHAPTER 4

4. OXIDATION OF PHENOL IN A BIOREMEDIATION MEDIUM USING CHLORINE DIOXIDE

A similar version of this chapter has been published in the Journal of Chemical Technology and Biotechnology:

Kumar P., H. Nikakhtari*, M. Nemati, G. A. Hill "Oxidation of phenol in a bioremediation medium using Chlorine Dioxide", J. Chem. Technol. Biotechnol. 85, 720-725, (2010).

Contribution of the Ph.D. Candidate

The design of phenol oxidation experiments using chlorine dioxide were done by Pardeep Kumar using commercial software Design Expert 6.0® with technical input and guidance from Dr. Hill and Dr. Nemati. The written text for this manuscript has been prepared by Pardeep Kumar, while Dr. Hill and Dr. Nemati provided the editorial input.

Contribution of this Chapter to Overall Study

The primary objective of this Ph.D. project was to explore the possibility of combining the chemical and biological processes for removal of high phenol concentrations. This chapter presents the oxidation of phenol in a bioremediation medium using chlorine dioxide and determination of the optimal experimental conditions for maximizing phenol removal. The optimal experimental conditions were compared with the optimal conditions obtained for oxidation of phenol by Fenton's reagent in Chapter 2.

* Hossein Nikakhtari (PDF) assisted in the analysis of the experimental data for the manuscript.

The identification of oxidation products in this study and the fact that removal of these intermediates, even by applying high levels of chlorine dioxide, was not possible was the basis of the biodegradation study of these intermediates in Chapter 6.

4.1 Abstract

High concentrations of phenol in wastewater are difficult to remove by purely biological methods. Chemical oxidation is one way to treat high concentrations of phenol but complete oxidation will make the treatment process uneconomical. For the purpose of integrating chemical and biological treatment, the oxidation of phenol using chlorine dioxide was investigated in a medium suitable for bioremediation. The effects of chlorine dioxide concentration (500 to 2000 mg L^{-1}), temperature (10 to 40°C) and pH (3 to 7) on the oxidation of 2000 mg L^{-1} of phenol were determined. Chlorine dioxide concentration was found to be the dominating parameter for the removal of phenol in the nutrient rich medium. The optimal concentration of chlorine dioxide to completely oxidize 2000 mg L^{-1} of phenol was 2000 mg L^{-1}. Compared to Fenton's reagent, half as much chlorine dioxide is needed to oxidize 2000 mg L^{-1} phenol. The reaction of chlorine dioxide with phenol was very fast and reached to equilibrium within 10 minutes. The main oxidation products were identified as 1,4-benzoquinone and 2-chloro-1,4-benzoquinone. Compared to Fenton's reagent, chlorine dioxide is a superior oxidant for removal of phenol from both pure water and bioremediation medium.

Keywords: Chlorine dioxide; Chemical oxidation; Bioremediation medium; Phenol; 1,4-benzoquinone, 2-chloro-1,4-benzoquinone.

4.2 Introduction

Phenol and other phenolic compounds often appear in the wastewater effluents of a variety of chemical industries (Benitez et al., 1997). Because of their high toxicity and poor biodegradability these compounds must be removed from the wastewater prior to discharge to receiving waters. Low concentrations of phenol give objectionable taste and odor to drinking water. Higher concentrations of phenol not only pose health hazards to humans, but also destroy all forms of aquatic life (Korbathi and Tanyolac, 2003). The Canadian Water Quality Guidelines set very low discharge limits of phenolic compounds e.g. 4 μg L^{-1} for phenols (mono- and dihydric) (Canadian Council of Ministers of the Environment, 1999). Thus phenols should be specifically treated to meet the very low discharge limits of these compounds in Canada.

Phenol at concentrations higher than 50 mg L^{-1} is resistant to biological degradation, so chemical oxidation as a pre-treatment step has attracted the attention of researchers in the last two decades and is becoming popular for treatment of higher levels of phenolic wastes (Villota et al., 2007; Yavuz et al., 2007). Oxidation of phenol by Fenton's reagent in water has been studied extensively and results are reported in literature (Benitez et al., 1997; Villota et al., 2007; Yavuz et al., 2007). Chlorine has been used for the disinfection of drinking water for a long time. But chlorination of water containing phenols results in the formation of chlorinated phenolic compounds (Chalm and Choshen, 1987).

Chlorine dioxide (ClO_2) is considered as an alternative to chlorine for disinfection of drinking water and wastewaters because it does not form chlorinated phenolic

compounds (Chen et al., 1982). Chlorine dioxide is not only a disinfectant but a strong oxidant as well; it reacts with phenol without formation of chlorophenols (Stevens, 1982; Wajon, 1982). Therefore, it could be used as an oxidizing agent for pre-treatment of high concentrations of phenol. Figure 4.1 shows the proposed mechanism of phenol oxidation by chlorine dioxide based on intermediates identified. Phenol dissociates to form phenoxide and hydronium ions, depending on the polarity of the solvent. Phenoxyl radicals were generated by transfer of one electron from phenoxide to chlorine dioxide. Chlorine dioxide reacts with phenoxyl radicals to form 1,4-benzoquinone and hypochlorous acid (Ganiev et al., 2003).

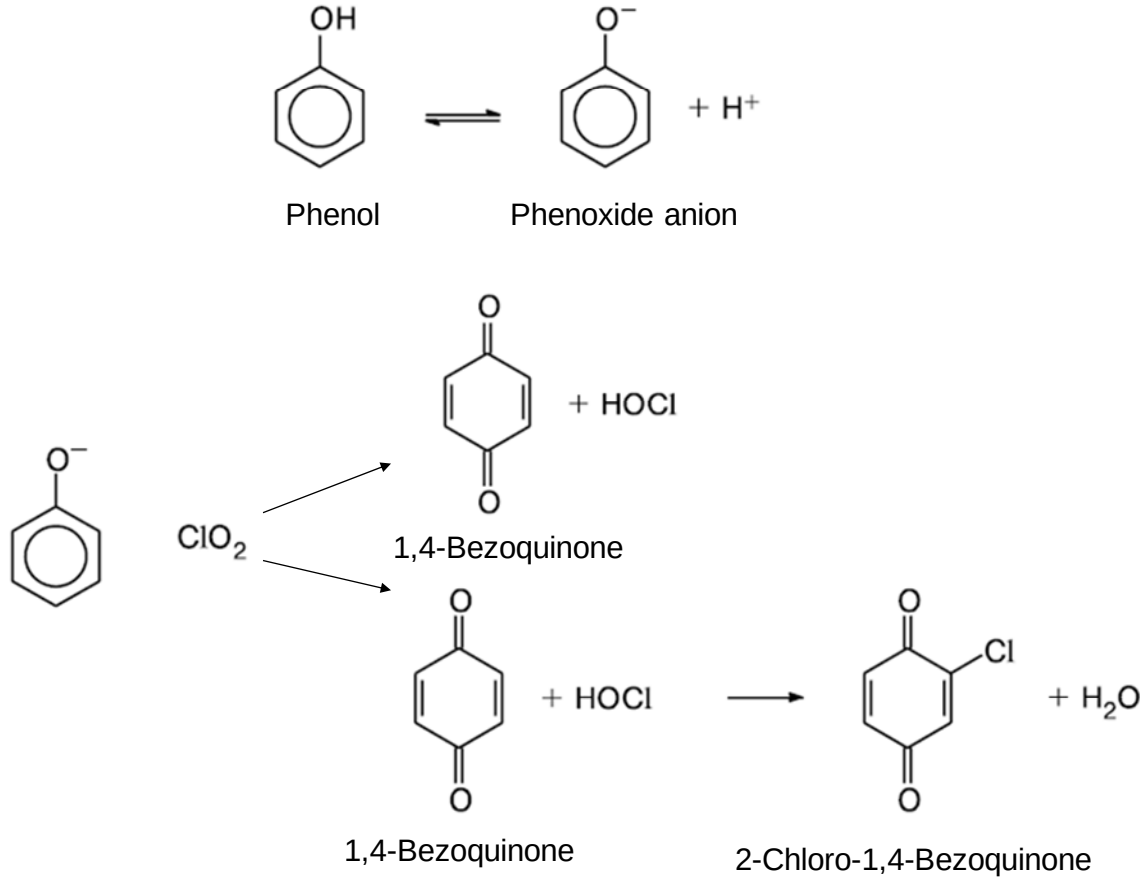


Figure 4.1 Proposed mechanism of phenol oxidation by chlorine dioxide based on the intermediates identified (Ganiev et al., 2003).

Although several authors have studied oxidation of phenol and other phenolic compounds by chlorine dioxide in water, there is no study found in the literature on the use of chlorine dioxide for phenol oxidation in a medium required for the purpose of bioremediation (Jackson et al., 1987; Ganiev et al., 2003; Zhi-yun et al., 2004; Jinquan et al., 2006; Xiao-yi et al., 2007). Xiao-yi et al. (2007) compared the oxidation of phenol by microwave induced ClO_2 – CuO_x /Al_2O3 catalytic oxidation with traditional ClO_2 oxidation in water and reported that the microwave induced catalytic oxidation process is

superior and resulted in lower oxidant dosage compared to traditional ClO_2 oxidation of phenol. Ganiev et al. (2003) studied phenol oxidation by chlorine dioxide in different solvents such as 2-methylpropan-1-ol, ethanol, 1,4-dioxane, acetone, acetonitrile, ethyl acetate, dichloromethane, heptane, tetrachloromethane and water and found that the rate of phenol oxidation increases with an increase of solvent polarity. Ganiev et al. (2004) studied the oxidation of different phenolic compounds using chlorine dioxide in acetonitrile. They identified and quantified the yield of oxidation products of phenol which were 1,4-benzoquinone (50%), 2-chloro-1,4-benzoquinone (20%) and diphenoquinone (10%). Amor et al. (1984) studied the oxidation of various phenolic compounds with chlorine dioxide and found that paraquinone was the major phenol oxidation product with only a small amount of chloroquinone (Amor et al., 1984).

Complete oxidation of organic pollutants to CO_2 requires a high quantity of oxidants making the oxidation process uneconomical (Zazo et al., 2005). The practical interest of chemical oxidation stems from the interest in integration of chemical and biological processes, wherein the partially oxidized wastewater is further treated by biological processes (Villota et al., 2007). In some cases low molecular weight polycyclic aromatic hydrocarbons (PAHs) may be effectively removed from wastewater by biological processes and the remaining heavier components may be completely removed by chemical oxidation to meet the low discharge limits. So for an integrated chemical biological treatment in which chemical oxidation serves as the final step, the original wastewater or the feed from an earlier biotreatment step would normally contain mineral nutrients. In the present study, the oxidation of phenol by chlorine dioxide is investigated

in a medium suitable for bioremediation. The optimal concentration of chlorine dioxide, pH and temperature required for the oxidation of high phenol concentration (2000 mg L^{-1}) are investigated using the central composite rotatable method. The oxidation of phenol by chlorine dioxide and Fenton's reagent in growth medium are also compared (Kumar et al., 2010).

4.3 Materials and Methods

4.3.1 Reagent

All chemicals were analytical grade. Phenol solutions were prepared using reagent grade phenol (99.5%) obtained from Merck (Darmstadt, Germany). Fresh chlorine dioxide was prepared in batches before the start of each experiment by acidification of sodium chlorite (38.4%; w/v) with hydrochloric acid (37%; w/v). The nutrient rich medium (two dose McKinney's modified media) consisted of (mg in 1 L reverse osmosis water): K_2HPO_4, 750; KH_2PO_4, 849; $(NH_4)_2SO_4$, 474; NaCl, 60; $CaCl_2$, 60; $MgSO_4$, 60; $Fe(NH_4)SO_4$, 20; and 1 mL of trace mineral solution. The trace mineral solution consisted of (mg in 1 L reverse osmosis water): $ZnSO_4.7H_2O$, 200; $MnCl_2$, 60; H_3BO_3, 600; $CoCl_2$, 400; $CuCl_2$, 20; $NiCl_2$, 40; Na_2MoO_4, 60 (Nikakhtari et al., 2009a). The natural pH of the medium was 7.0 and was adjusted to the desired reaction pH using 1.0 M HCl.

4.3.2 Preparation of chlorine dioxide (ClO_2):

Chlorine dioxide is produced from acidification of sodium chlorite solution (38.4% $NaClO_2$) and hydrochloric acid solution (37% HCl). The following procedure is used to prepare ClO_2:

$$5\ NaClO_2 + 4HCl \rightarrow 4\ ClO_2 + 5\ NaCl + 2\ H_2O \qquad (3.1)$$

1. For generation of 300 ml chlorine dioxide of 3000 mg L^{-1} concentration i.e. 0.9 g ClO_2, the amount of sodium chlorite and hydrochloric acid determined is as follows:

Mass of $NaClO_2$ required:

$$NaClO_2 = \frac{5 \times 90.5}{4 \times 67.5} \times 0.9\,g = 1.508\ g \qquad (3.2)$$

Required volume of sodium chlorite:

$$NaClO_2\ needed = \frac{1.508}{1.34 \times 0.384} = 2.93\,mL \qquad (3.3)$$

Mass of HCl required:

$$HCl = \frac{4 \times 36.5}{4 \times 67.5} \times 0.9\,g = 0.487\ g \qquad (3.4)$$

Required volume of Hydrochloric acid:

$$HCl\ needed = \frac{0.487}{1.2 \times 0.37} = 1.1\ mL \qquad (3.5)$$

2. The required amount of RO water (296 mL) was transferred in a brown colored open top Pyrex glass bottle.
3. Add 2.93 mL of sodium chlorite (38.4% concentrated).

4. Then slowly add the 1.1 mL hydrochloric acid (37% concentrated).With continuous stirring the solution with Teflon stirrer.

5. Keep the reaction solution in the fume hood for 24 hours for reaction to complete.

4.3.3 Experimental Design

Central composite rotatable design (CCRD) was used to design the experiments and study the effect of three variables on the phenol oxidation. The surface response methodology was used to optimize the response surface and to determine the optimal conditions maximizing the percentage of phenol removal. CCRD gives as much information as three- level factorial deign but with a minimum number of experiments and also allows the user to analyze the interaction between the parameters. The chlorine dioxide concentration (X_1), temperature (X_2) and pH (X_3) were the three parameters investigated in this work.

The range of selected variables was chosen based on preliminary experiments. The range of chlorine dioxide, temperature and pH were 500-2000 mg L^{-1}, 10-30 °C and 3-7, respectively. The coded levels and the actual values of three parameters are shown in Table 4.1.

Table 4.1 Coded and actual values of the independent variables

Design Parameter	-1.68	-1	0	+1	+1.68
X_1 C_{ClO2}, mg L^{-1}	500	804	1250	1696	2000
X_2 Temperature, °C	10	16	25	34	40
X_3 pH	3	3.8	5	6.2	7

The response factor (Y) was the percentage of phenol oxidized at steady state (i.e. no further change observed in concentration of phenol after this time period). The number of experiments required for CCRD was 20 with 2^k factorial points, 2k axial points and 2k replications at the centre, where k is the number of factors (Box and Draper, 1987). Once optimal conditions were determined two additional experiments with higher concentrations of chlorine dioxide (2500 and 3000 mg L^{-1}) were conducted to verify whether oxidation of intermediates could be achieved as shown in Figure 4.8.

4.3.4 Experimental procedure

All phenol oxidation experiments were carried out in batch mode. 500 mL Erlenmeyer (shake) flasks were used as batch reactors with each flask loaded with 200 mL of two dose McKinney's medium containing 2000 mg L^{-1} phenol. The gyratory shaker (New Brunswick Scientific Co., Inc, Edison, N.J. U.S.A) was operated at 200 rpm. The pH of the reaction medium was adjusted to desired values using 1.0 M HCl. The desired reaction temperature was achieved by placing the gyratory shaker in temperature controlled environmental chambers. The reaction was initiated by the addition of the designated amount of chlorine dioxide. No change in residual phenol concentration was

found for even the slowest reaction after two hours, so two hour reaction duration was selected for all experiments. Four mL samples were taken from the reactor after two hours to determine the concentrations of the residual phenol, 1,4-benzoquinone and 2-chloro-1,4-benzoquinone which were the intermediate reaction products. These were the only compounds that were observed using the HPLC analysis technique discussed below.

4.3.5 Analytical methods

The initial concentration of chlorine dioxide was determined using a chlorine dioxide pocket colorimeter II test kit (Hach, 5870051). For identification of phenol oxidation products, the samples of phenol oxidation were analyzed on an HPLC (Agilent HPLC 1100 with a Diode Array Detector) equipped with a C_{18} column (Nova pack: 4.6 x 150 mm: 4μm) operated at 27°C. The mobile phase was a mixture of acetonitrile and water (10/90 v/v) with a flow rate of 2.1 mL min^{-1}. Detection was carried out using an ultraviolet detector at a wavelength of 254 nm. Then based on the reaction mechanism described in Figure 4.1, the three peaks were matched with pure 1,4-benzoquinone, 2-chloro-1,4-benzoquinone and phenol, each with a retention time of 2.06, 4.56 and 5.52 minutes, respectively. No other peaks were observed indicating that diphenoquinone was not a product of these reactions.

4.3.6 Chlorine dioxide measurement

Chlorine dioxide concentration is measured by using HACH pocket colorimeter (with a Silicon Photodiode Detector). Detection is done at 528 nm. 10 ml sample of chlorine dioxide is taken in the sample cell. Immediately four drops of glycine are added to the sample cell and swirl to mix. Then one DPD (N, N-diethyl-p-phenylenediamine) free powder pillow is added and pink color develops immediately. Chlorine dioxide concentration is measured placing the cell in the instrument.

4.4 Results and Discussion

Table 4.2 shows the experimental values of the three variables and percentage phenol oxidized in each run. As can be seen, 27% of 2000 mg L^{-1} of phenol was oxidized with 500 mg L^{-1} of chlorine dioxide at pH 5 and 25°C in growth medium. The percentage removal of phenol increased with increase in chlorine dioxide concentration (Figure 4.3 and 4.4). Complete oxidation of 2000 mg L^{-1} of phenol was obtained using 2000 mg L^{-1} of chlorine dioxide at pH 5 and 25°C.

The model coefficients were found by fitting the CCRD second order polynomial to the experimental data in Table 4.2 by the least square method using Design expert 6.0®. A total of 20 experiments, including 6 replications at the centre point, were conducted to calculate 10 coefficient of the complete second order polynomial regression model. Only the "p values" for X_1, X_1^2 terms were less than 0.05, so the final overall relationship for % phenol oxidization (Y) was obtained in coded values as follows:

$$Y = 54.99 + 19.05\ X_1 + 3.17\ X_1^2 \quad (4.1)$$

Where,

X_1 Chlorine dioxide concentration, mg L^{-1}

The p value of this model is less than 0.05, which means that the model is statistically significant at the 95% confidence level.

Table 4.2 Oxidation using chlorine dioxide in nutrient rich media (C_{PH} = 2000 mg L^{-1*}).

Run no.	X_1, ClO_2 (mg L^{-1})	X_2,temperature (°C)	X_3, pH	Y, % phenol removed
1	804	16	6.2	42.5
2	1250	40	5	53.7
3	1250	10	5	44.9
4	1250	25	5	58.8
5	1250	25	7	50.9
6	1250	25	5	56.1
7	1250	25	3	50.3
8	2000	25	5	100
9	1250	25	5	55.9
10	804	16	3.8	39.3
11	804	34	3.8	41.2
12	804	34	6.2	34.7
13	1696	34	6.2	73.0
14	1250	25	5	55.8
15	1696	16	6.2	73.9
16	1250	25	5	55.8
17	1696	34	3.8	79.9
18	1696	16	3.8	68.4
19	500	25	5	27.1
20	1250	25	5	56.1

*C_{PH} was the initial phenol concentration

The coefficient of determination (R^2) (measure of how well a model approximate experimental data points) was calculated to be 0.97, indicating that 97% of the variability was explained by the model. A parity plot comparing the experimental and predicted values for phenol oxidation by chlorine dioxide obtained by Equation (4.1) is shown in Figure 4.2. There is obviously a good agreement between the predicted and experimental values. The mean absolute relative error calculated for the model was 4.98%.

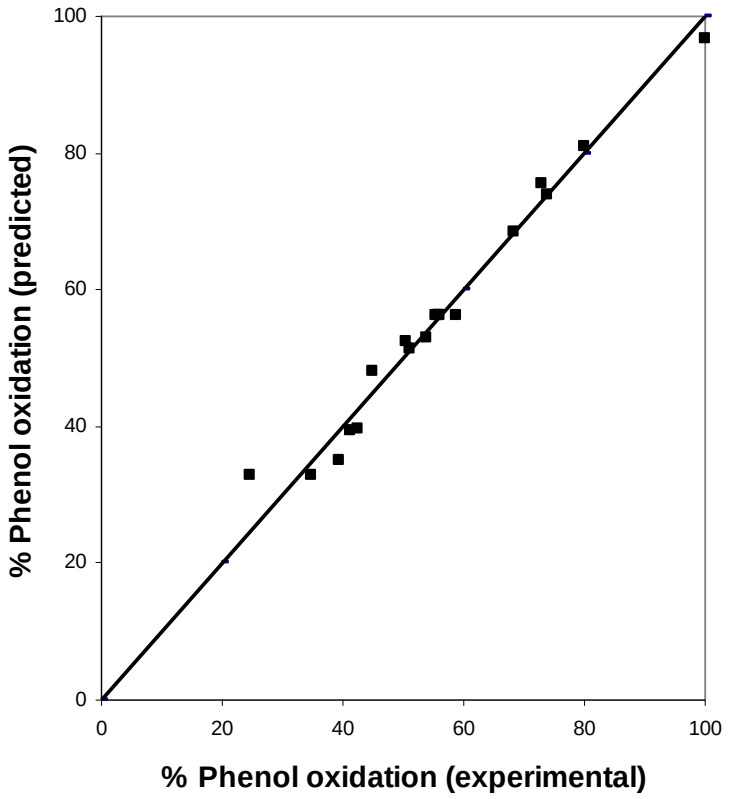


Figure 4.2 Comparison between the experimental and predicted % phenol oxidized values using the second order response model (Equation 4.1).

The effects of different operating variables on phenol oxidation by chlorine dioxide are shown in 3-dimensional plots (Figure 4.3 and 4.4). Figure 4.3 shows the

effect of chlorine dioxide concentration and temperature on percentage of phenol oxidation at a pH of 5.0.

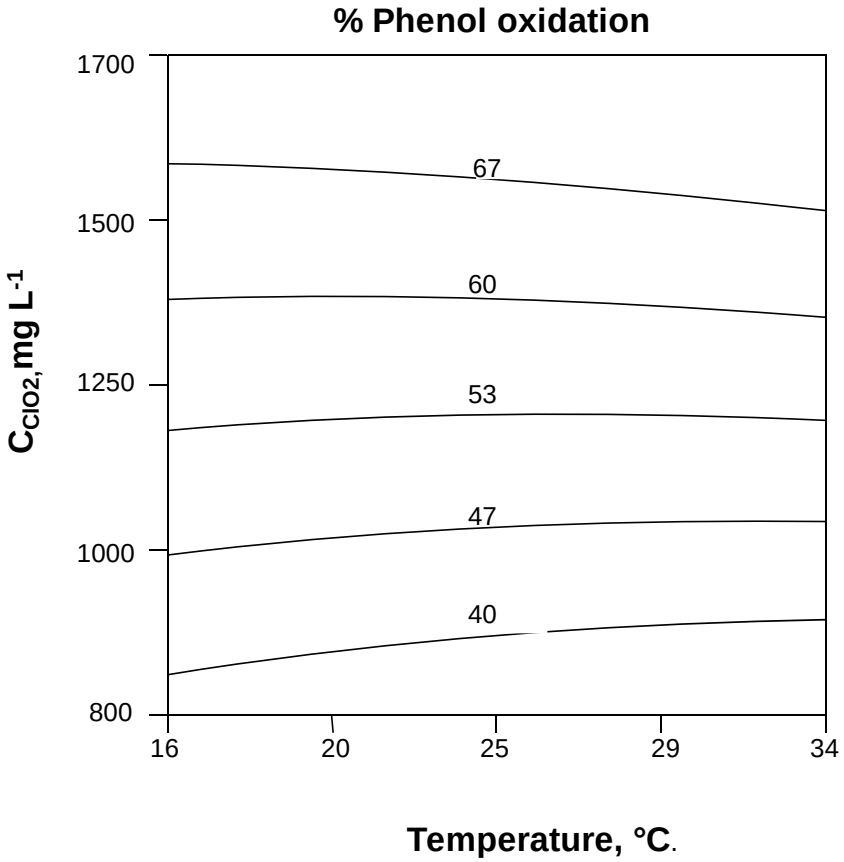


Figure 4.3 Response surface predictions of % phenol oxidized by chlorine dioxide: effect of chlorine dioxide concentration and temperature.

The percentage of phenol oxidized increases quadratically with an increase in chlorine dioxide concentration for a fixed value of temperature. It is also observed that for a given value of chlorine dioxide concentration, an increase in temperature does not have a significant effect on phenol removal.

Figure 4.4 shows the effect of chlorine dioxide concentration and pH on phenol oxidation (%) at a temperature of 25 °C.

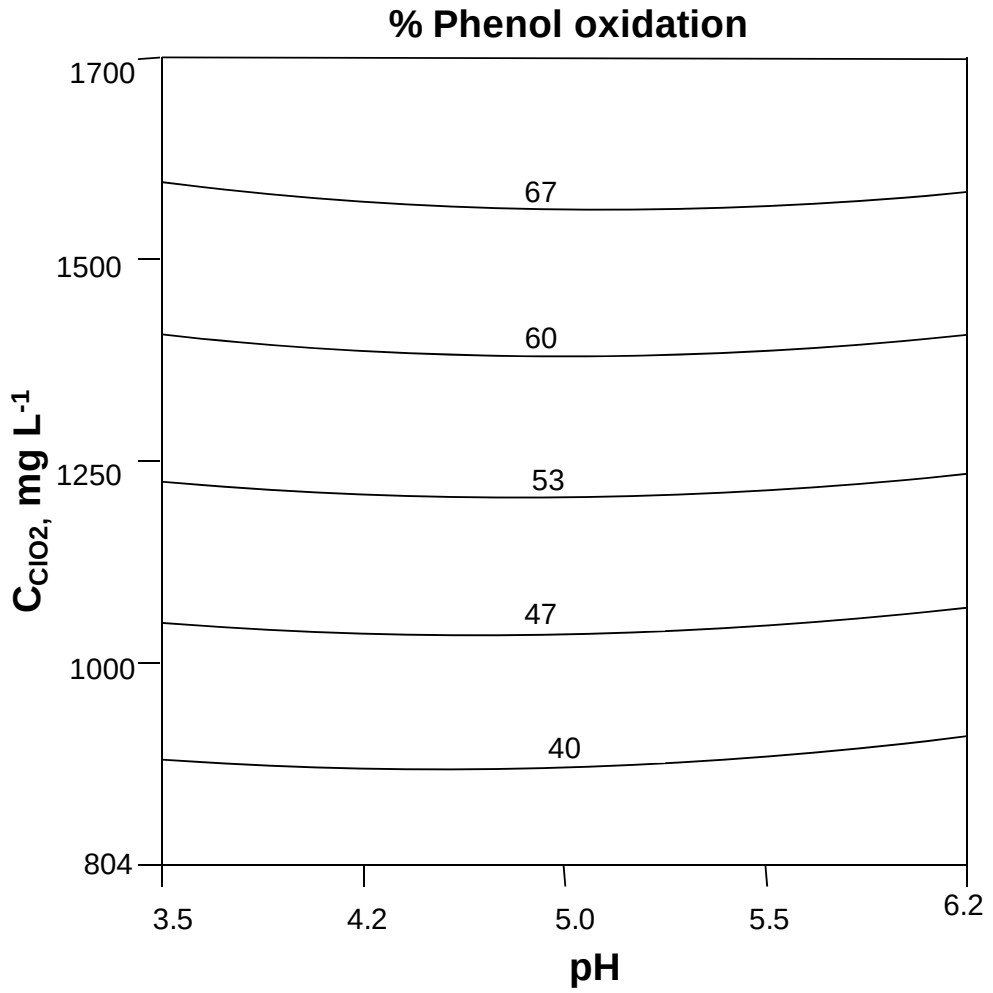
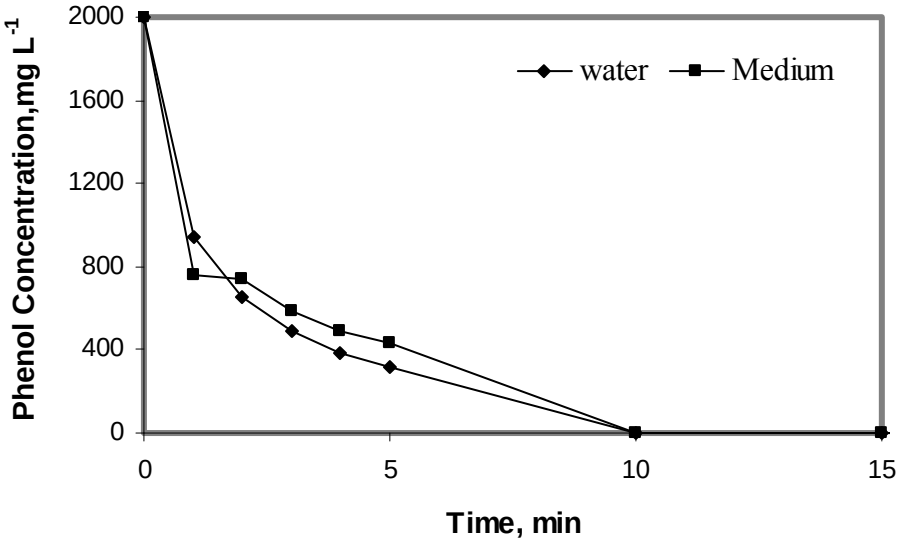


Figure 4.4 Response surface predictions of % phenol oxidized by chlorine dioxide using second order polynomial: effect of chlorine dioxide concentration and pH.

It can be seen again that an increase in chlorine dioxide concentration has the same positive effect on phenol oxidation (%), but an increase in pH has no effect on phenol removal. Although not part of the above CCRD study, one further run was performed at 25 °C using 1250 mg L^{-1} of chlorine dioxide at the very alkaline pH of 11. 50% of the phenol was oxidized which agreed within experimental error with the mean value observed (55±5%) at all other pH conditions using that same temperature and chlorine

dioxide concentration, indicating that increasing the pH to alkaline values did not affect the oxidation of phenol.



F**igure 4.5** Comparison of phenol oxidation by chlorine dioxide in water and medium under identical experimental conditions (C_{PH} = 2000 mg L^{-1}, pH 7, T = 25°C and C_{ClO2} = 2000 mg L^{-1}).

To study the effect of mineral nutrients in the growth medium, oxidation of phenol by chlorine dioxide in a nutrient rich medium has been compared with oxidation of phenol in water. With chlorine dioxide the components of nutrient medium did not have any significant effect on phenol oxidation (Figure 4.5).

This is in contrast to phenol oxidation by Fenton's reagent, in which nutrients present in water reduced the efficiency and slowed down the oxidation of 2000 mg L^{-1} phenol by 5% and 100 minutes, respectively (Kumar et al., 2010a). Figure 4.6 compares the 2000

mg L^{-1} phenol oxidation in nutrient rich medium by Fenton's reagent (2000 mg L^{-1} hydrogen peroxide and 1500 mg L^{-1} ferrous sulfate) obtained in our earlier work and that with 2000 mg L^{-1} chlorine dioxide.

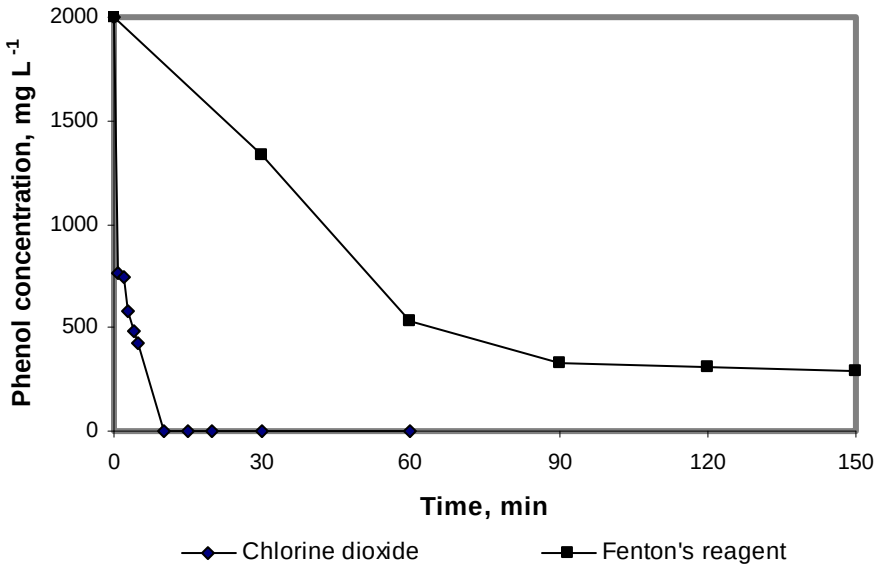


Figure 4.6 Comparison of phenol oxidation in growth medium by Fenton's reagent (C_{PH} = 2000 mg L^{-1}, C_{H2O2} = 2000 mg L^{-1}, $C_{FeSO4.7H2O}$ = 1500 mg L^{-1}, pH 3 and T = 25°C) and chlorine dioxide (C_{ClO2} = 2000 mg L^{-1}, pH 7, T = 25°C).

It can be seen that the reaction of phenol with chlorine dioxide is very fast and complete oxidation of phenol occurs in 10 minutes. Only after 100 minutes (an order of magnitude longer time duration) did Fenton's reagent reach steady state and at that point only 85% of the original phenol was oxidized. Complete oxidation of 2000 mg L^{-1} of phenol was found only using 4000 mg L^{-1} H_2O_2 and 1500 mg L^{-1} of $FeSO_4.7H_2O$ (Kumar et al., 2010a). So for complete oxidation of 2000 mg L^{-1} of phenol, the quantity of required chlorine dioxide was half of the Fenton's reagent, with a much shorter reaction time.

The use of chlorine dioxide for disinfection of potable water and as an oxidant for the removal of organic compounds in pure water has been well documented in the literature and optimized concentrations reported. But no study has been found in the literature reporting the optimal operating conditions for oxidation of phenol by chlorine dioxide in a nutrient rich medium. Using the optimization method developed by Derringer and Suich (described by Myer and Montgomery, 2002) (Myer and Montgomery, 2002), the optimal chlorine dioxide concentration for complete oxidation of 2000 mg L^{-1} of phenol is 2000 mg L^{-1} (i.e. 1 part ClO_2 for 1 part phenol). The percentage oxidation of phenol was found to be 100% at these optimal conditions.

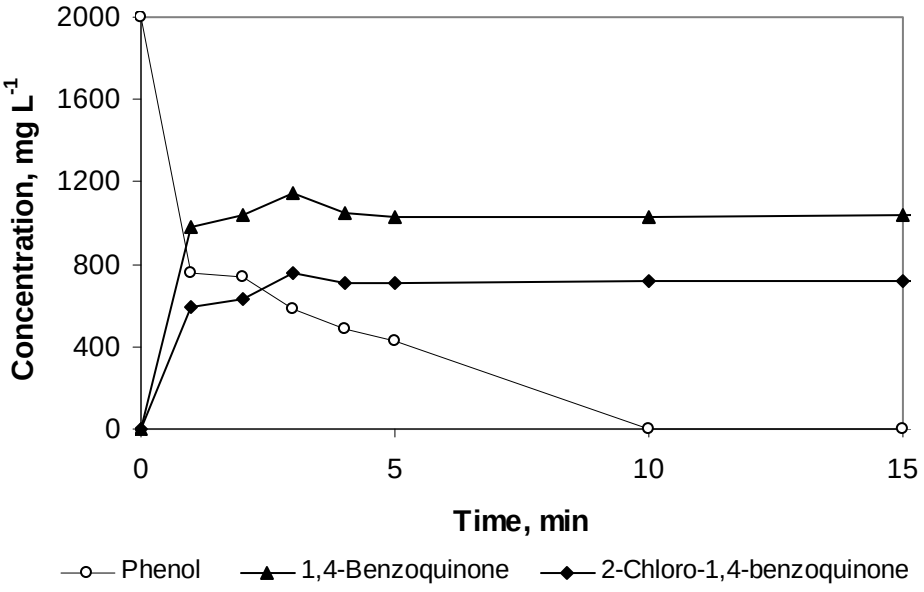


Figure 4.7 Transient oxidation of phenol and evolution of intermediates (C_{PH} = 2000 mg L^{-1}, pH 7, T = 25°C, C_{ClO2} = 2000 mg L^{-1}).

Although complete oxidation of phenol can be efficiently and quickly achieved using chlorine dioxide, the oxidation of phenol produces intermediate organic compounds (1,4-

benzoquinone and 2-chloro-1,4-benzoquinone) which may be more toxic than phenol itself. Figure 4.7 shows the transient formation and steady state concentrations of both 1,4-benzoquinone and 2-chloro-1,4-benzoquinone during the complete oxidation of phenol.

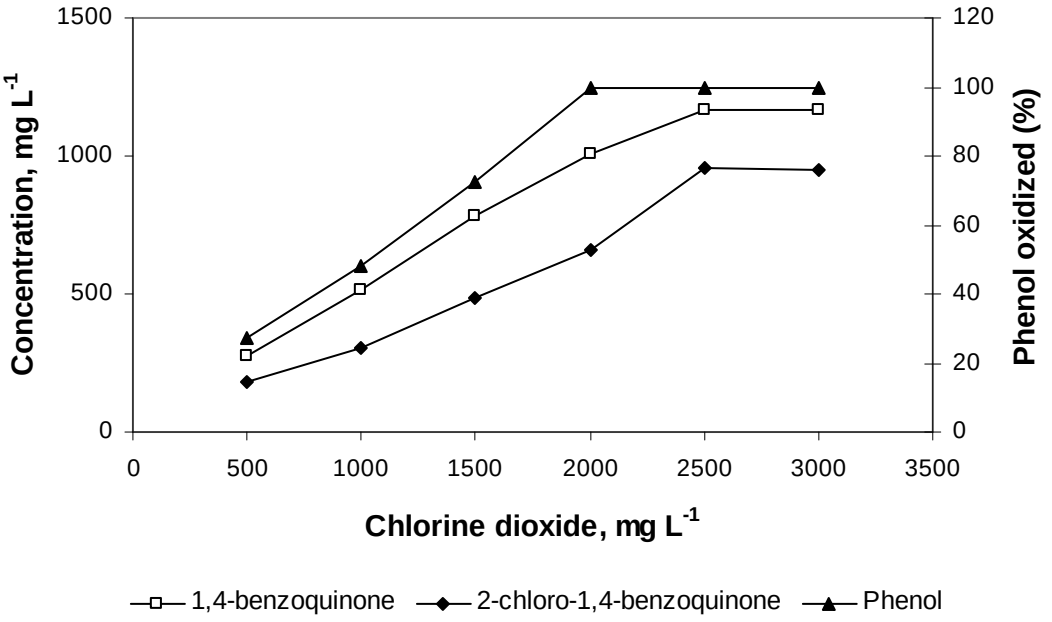


Figure 4.8 Effect of chlorine dioxide concentration on phenol oxidation (C_{PH} = 2000 mg L^{-1}, pH 7, T = 25°C).

Two additional runs, using higher chlorine dioxide concentrations, failed to oxidize the intermediate compounds as shown in Figure 4.8. As more and more of the phenol were oxidized, more and more of the intermediates were produced. Partial oxidation of phenol will make bioremediation of these compounds more feasible.

4.5 Conclusions

The oxidation of phenol by chlorine dioxide has been studied using CCRD experimental design method in a medium suitable for bioremediation of organic contaminants. Chlorine dioxide concentration was found to be the dominating parameter for the oxidation of phenol in the growth media. Mineral nutrients present in growth media were found to have no effect on phenol oxidation compared phenol oxidation in pure water. Comparing the phenol by chlorine dioxide with Fenton's reagent, it was found that almost half of chlorine dioxide amount was needed to oxidize 2000 mg L^{-1} phenol than Fenton's reagent. The pH of the reaction medium was found to have no effect on phenol oxidation by chlorine dioxide, so phenol oxidation by chlorine dioxide could be applied over a wide range of pH. The reaction of chlorine dioxide with phenol was found to be very fast and reaches to equilibrium within 10 minutes. All of 2000 mg $L^{-1}$of phenol was found to be oxidized within 10 minutes. The main oxidation products were identified as 1,4-Benzoquinone and 2-chloro-1,4-benzoquinone.

5. KINETIC MODELLING OF PHENOL OXIDATION IN A BIOREMEDIATION MEDIUM USING CHLORINE DIOXIDE

Contribution of the Ph.D. Candidate

The kinetic modelling and experimental work for phenol oxidation by chlorine dioxide has been done by Pardeep Kumar with technical input and guidance from Dr. Hill and Dr. Nemati. All the written text for this chapter has been prepared by Pardeep Kumar, with Dr. Hill and Dr. Nemati provided the editorial input.

Contribution of this Chapter to Overall Study

This chapter presents the determination of kinetic rate constants for oxidation of phenol in a bioremediation medium using chlorine dioxide. A comparison is made with the kinetic parameters of phenol oxidation by Fenton's reagent as found in Chapter 3. After identification of major oxidation products described in Chapter 4, dynamic experiments were conducted to study the oxidation of phenol and evolution of the intermediate compounds. The quantification of phenol and intermediate products helped us to devise a successful biodegradation strategy presented in Chapter 6.

5.1 Abstract

Integrated chemical and biological treatment is a cost effective treatment for removing high concentrations of phenolic compounds in wastewater which are difficult to remove by purely biological methods. Batch experiments were conducted to study the

effect of chlorine dioxide concentration (7.41 to 29.63 mM) on the oxidation of 21.27 mM of phenol in a bioremediation medium and compared with oxidation of phenol in water. Samples were taken at regular time intervals to keep track of the chemical oxidation reaction and to measure the residual phenol concentration and evolution of oxidation intermediates. The rate of phenol oxidation and final conversion at equilibrium increases with increase in chlorine dioxide concentration over the range studied. Kinetic rate constant was determined by fitting the experimental data to a second order rate expression and compared with the rate constants obtained with phenol oxidation by Fenton's reagent. Half as much chlorine dioxide (by mass) compared to Fenton's reagent and a much shorter reaction time are needed to oxidize 21.27 mM phenol.

Keywords: Chlorine dioxide; 1,4-benzoquinone; Bioremediation medium; Phenol; chlorine dioxide, Kinetic modeling.

5.2 Introduction

Phenols are widely used compounds in various chemical manufacturing industries today (Prpich et al., 2005; Ko et al., 2007 and Prasannakumar et al., 2009). The effluents released from these chemical industries are one of the major sources of phenolic waste in the environment and high, toxic levels are frequently discharged to the environment. Because of high phenol toxicity, and stringent environmental regulations the development of improved technologies to control these wastewaters are necessary (Zazo et al., 2009). Phenolic compounds even at ppb concentrations give disagreeable taste and smell to chlorinated water (Cham et al., 1987). Phenol concentrations, more than 50 ppb, exhibit toxicity problems to aquatic and human lives. In addition, they impose high

oxygen demand and concentrations more than 100 mg L^{-1} result in the death of aquatic life (Korbathi and Tanyolac 2003).

Biological treatment of toxic wastes is often the most economical alternative, but it is inefficient at high phenol concentrations (Benitez et al., 1997). Phenol concentrations higher than 1450 mg L^{-1} are extremely toxic and kill all bacteria used to treat wastewater, so biological treatments alone can not be used to remove high phenol concentrations from wastewater (Sevillano et al., 2008). Chemical oxidation is an alternative treatment technology and popular for the oxidation of high phenol concentrations (Suryaman et al., 2006). Complete oxidation of phenol to CO_2 can be achieved using chemical agents, but high amount of chemical oxidants make the treatment uneconomical (Zazo et al., 2005). Therefore, integrated chemical and biological treatments are more cost effective for the removal of high concentrations of phenolic compounds from wastewater (Edalatmanesh et al., 2008). The chemical oxidation can be used as a pre-treatment to increase the biodegradability of wastewater or after biodegradation to meet the discharge limits set for these toxic compounds.

The use of chlorine as a disinfectant in drinking water has been undertaken for a long time. But chlorination of drinking water containing phenols results in the formation of chlorinated phenols, which are more toxic than phenol (Cham et al., 1987). The formation of chlorophenols during the chlorine treatment of water was one of the main reasons to consider chlorine dioxide, which is a strong oxidant, as an alternative to chlorine for water disinfection. In our previous study of phenol oxidation by chlorine

dioxide in a bioremediation medium, we found the optimal concentration of chlorine dioxide, pH and temperature for oxidation of 21.27 mM of phenol using CCRD (central composite rotatable design) method (Kumar et al., 2010b). We also identified the oxidation intermediates of phenol oxidation by chlorine dioxide and proposed the reaction mechanism based on the available literature. The main identified intermediates of phenol oxidation by chlorine dioxide were 1,4-benzoquinone and 2-chloro-1,4-benzoquinone. The proposed reaction mechanism based on identification of these intermediate oxidation compounds is shown in Figure 4.1. Amor et al. (1984) found that chlorine dioxide treatment of phenols results in the formation of paraquinones as major oxidation products in agreement with out investigation.

Numerous studies have been conducted on the use of chlorine dioxide for oxidation of phenols and polycyclic aromatic hydrocarbons (PAHs) in pure water (Stevens et al., 1982 and Wajon et al., 1982). Prior to our earlier study, there was no information in the literature on the oxidation of phenol by chlorine dioxide in a bioremediation medium. It is important to study the kinetics of phenol oxidation in a bioremediation medium because minerals nutrients, which could affect the oxidation, would be present when chemical treatment is used after bioremediation. Ganiev et al. (2003) studied oxidation of phenolic compounds using chlorine dioxide in pure water mixed with different organic solvents and found that solvent polarity affects the rate of reaction. They also identified and quantified the major intermediate compounds in their final solutions which were 1,4-benzoquinone (50%), 2-chloro-1,4-benzoquinone (20%) and diphenoquinone (10%) (Ganiev et al., 2004). Xiao-yi et al. (2007) studied the oxidation of phenol by microwave

induced ClO_2 – CuO_x /Al_2O3 catalytic oxidation and found that microwave induced catalytic oxidation lowers the required level of oxidant and increases the reaction rate compared with traditional ClO_2 oxidation in water.

In the present study, the dynamics of phenol oxidation by chlorine dioxide were studied in a bioremediation medium and rate constant was determined by fitting a second order kinetic rate expression to the experimental data based on the proposed reaction mechanism. Due to time limitation, only concentration of ClO_2 was included in the model. The data for concentration of intermediate compounds is available and model will be expanded in the near future. The rate constant of phenol oxidation by chlorine dioxide was compared with rate constants of phenol oxidation by Fenton's reagent.

5.3 Materials and Methods

5.3.1 Reagent

All reagents used were of analytical grade quality. Phenol solutions prepared in two dose bioremediation medium using analytical grade phenol (99.5%) obtained from Merck (Darmstadt, Germany). Fresh chlorine dioxide was prepared before the start of each experiment by mixing sodium chlorite (38.4%) with hydrochloric acid (37%). Reverse osmosis water was used to prepare all solutions. The two dose bioremediation medium used in all the experiments consisted of (mg in 1 L reverse osmosis water): K_2HPO_4, 750; KH_2PO_4, 849; $(NH_4)_2SO_4$, 474; NaCl, 60; $CaCl_2$, 60; $MgSO_4$, 60; $Fe(NH_4)SO_4$, 20; and 1 mL of trace mineral solution. The trace mineral solution consisted

of (mg in 1 L reverse osmosis water): $ZnSO_4.7H_2O$, 200; $MnCl_2$, 60; H_3BO_3, 600; $CoCl_2$, 400; $CuCl_2$, 20; $NiCl_2$, 40; Na_2MoO_4, 60.

5.3.2 Experimental Procedure

The experimental procedure for phenol oxidation by chlorine dioxide has been described in detail in Chapter 4. In brief, all phenol dynamic oxidation experiments were carried out in batch mode using 500 mL Erlenmeyer (shake) flasks as batch reactors. Initially 200 mL of two dose bioremediation medium was charged into each flask containing 21.27 mM phenol and placed on the gyratory shaker (New Brunswick Scientific Co., Inc, Edison, N.J. U.S.A) which was operated at 200 rpm. All the phenol oxidation experiments by chlorine dioxide were conducted at 25ºC in temperature controlled environmental chambers. The reaction was initiated by the addition of the designated amount of chlorine dioxide. The reaction of chlorine dioxide with phenol was very fast and reached equilibrium in 10 minutes. Samples were taken every minute for first five minutes. When equilibrium was reached, samples were taken up to one hour to ensure no further change in phenol concentration. One mL samples were taken from the reactor at the designated time and reactions were stopped in these samples using sodium thiosulfate (Na_2SO_3) solution. The concentrations of the residual phenol, 1,4-benzoquinone and 2-chloro-1,4-benzoquinone were determined using the HPLC analysis technique discussed below.

5.3.3 Analytical Methods

Fresh chlorine dioxide was prepared before the start of each experiment and the initial concentration of chlorine dioxide was determined using a chlorine dioxide pocket colorimeter II test kit (Hach, 5870051). Phenol and the intermediates of phenol oxidation by chlorine dioxide were identified using HPLC (Agilent HPLC 1100 with a Diode Array Detector) equipped with a C_{18} column (Nova pack: 4.6 x 150 mm: 4μm) operated at 27°C. The mobile phase was a mixture of acetonitrile and water (10/90 v/v) with a flow rate of 2.1 mL min^{-1}. Quantification of oxidation intermediates were carried out using a Diode array detector at a wavelength of 254 nm. The retention times for 1,4-benzoquinone, 2-chloro-1,4-benzoquinone and phenol were 2.06, 4.56 and 5.52 minutes, respectively.

5.4 Results and Discussion

We have previously studied the effects of pH between 3 and 7, and temperature in the range 10-30ºC, on the equilibrium oxidation of phenol by chlorine dioxide in a bioremediation medium using CCRD and found no noticeable effects. In the present study, dynamic experiments of phenol oxidation were conducted using different chlorine dioxide concentrations ranging from 7.41 to 29.63 mM. The pH and reaction temperature were fixed at 7 and 25°C (the optimal value of chlorine dioxide determined in our earlier work was 2000 mg L^{-1} for 2000 mg L^{-1} of phenol oxidation).

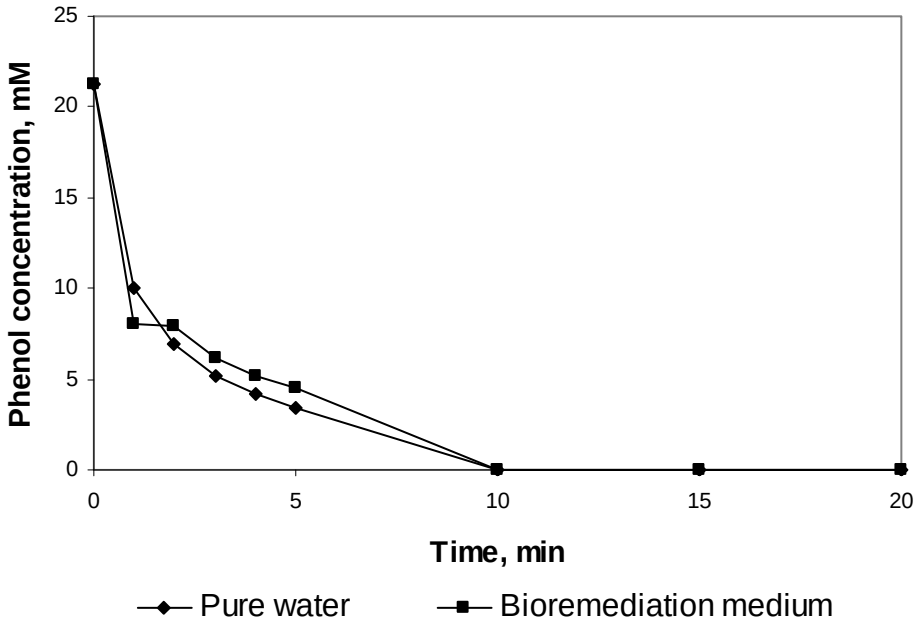


Figure 5.1 Comparison of phenol oxidation in pure water and bioremediation medium at same experimental conditions (C_{PH} = 21.27 mM, C_{ClO2} = 29.63mM, pH 7 and 25°C).

Figure 5.1 compares the oxidation of phenol by chlorine dioxide in water and bioremediation medium and as can be seen, inorganic salts present in the bioremediation medium did not have any effect on phenol oxidation. This is in contrast to Fenton's oxidation of phenol in which these inorganic salts slowed down the oxidation reaction (3 h to reach equilibrium compared to 10 min in case of chlorine dioxide) and effected the final conversion of phenol (85% conversion at equilibrium compared to complete oxidation by chlorine dioxide) (Kumar et al., 2010b). The dynamic phenol conversion as a function of initial chlorine dioxide concentration is shown in Figure 5.2. As can be seen from this figure, the rate of phenol oxidation and final phenol conversion increases with increase in chlorine dioxide concentration.

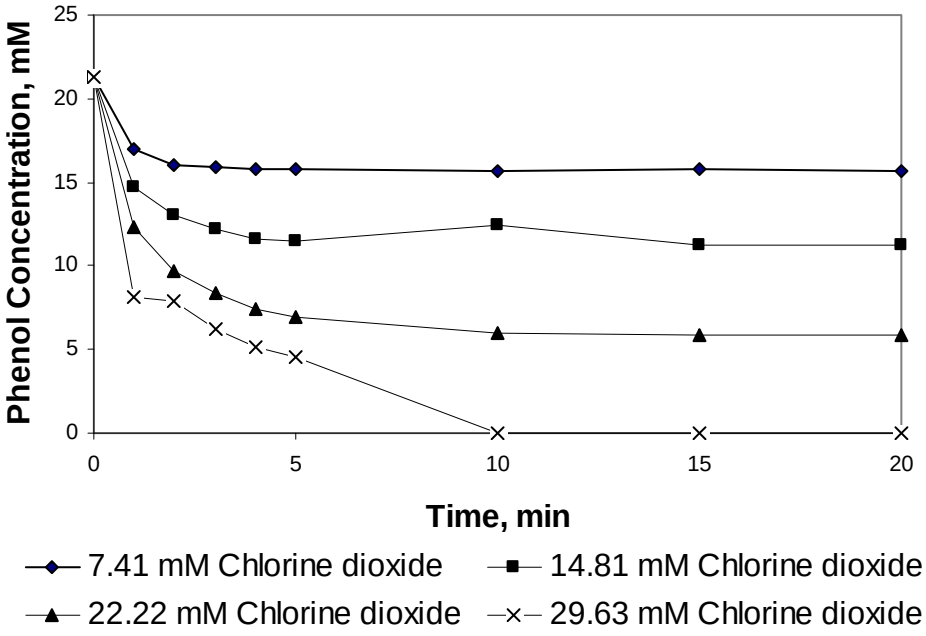


Figure 5.2 Effect of chlorine dioxide concentration on phenol oxidation (C_{PH} = 21.27 mM, pH 7, T = 25°C).

Kumar et al. (2010b) identified 1,4-benzoquinone and 2-chloro-1,4-benzoquinone as major oxidation products of phenol oxidation by chlorine dioxide and a reaction mechanism was proposed based on the identification of these intermediate compounds and available literature. It was also found that these two intermediates persist even after applying higher chlorine dioxide concentrations (Kumar et al., 2010b). Compared to phenol oxidation by Fenton's reagent, the oxidation of phenol by chlorine dioxide is a very fast reaction and reaches to equilibrium within 10 min.

The oxidation of phenol by chlorine dioxide can be represented by the following reaction:

$$C_6H_5OH + ClO_2 \xrightarrow{k_1} \text{Oxidation products} \qquad (5.1)$$

Assuming phenol oxidation by chlorine dioxide is first order with respect to each reactant concentration (Ganiev et al., 2004) and overall second order rate equation can then be written as:

$$\frac{dC_{Ph}}{dt} = -k_1 C_{Ph} C_{ClO_2} \qquad (5.2)$$

Where,

C_{Ph} is the phenol concentration, mM

C_{ClO2} is chlorine dioxide concentration, mM

Equation (2) is solved using the Runge-Kutta 4th order method and the rate constant k_1 is determined by least square minimization on Microsoft Excel 2007©. The model predictions fit very well to the experimental data as shown in Figure 5.3.

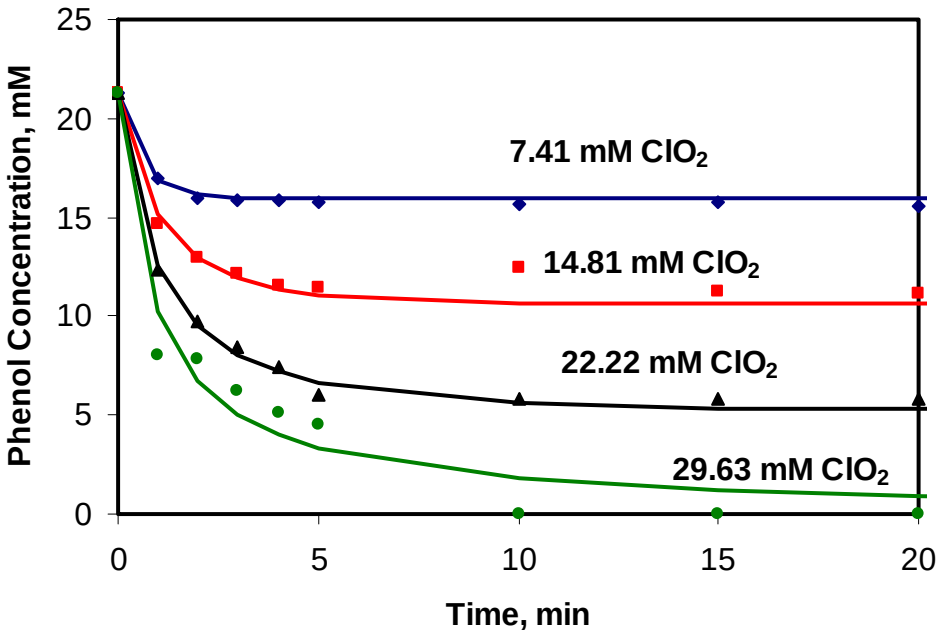


Figure 5.3 Fitting of kinetic equation (solid curves) to the phenol oxidation experimental data (symbols) at various initial chlorine dioxide concentrations (C_{PH} = 21.27 mM, pH 7 and T = 25°C).

The kinetic rate constant determined using different chlorine dioxide concentrations for phenol oxidation are summarized in Table 5.1. The average value of rate constant of phenol oxidation by chlorine dioxide is 0.039±0.008 L/mM min.

Table 5.1 Values of rate constants for oxidation of phenol in a bioremediation medium by chlorine dioxide at different ClO_2 concentrations (C_{PH} = 21.27 mM, pH 7 and T = 25°C).

Concentration of ClO_2, mM	**k_1 (L/mM min)**
7.41	0.049
14.8	0.034
22.2	0.035
29.2	0.036
Average	0.039

Ganiev et al. (2003) reported the value of second order rate constant for phenol oxidation by chlorine dioxide in water as 0.014 L/mM min (24±0.016 L mol^{-1} S^{-1}). The value of rate constant obtained for phenol oxidation by chlorine dioxide in bioremediation medium in this work is much higher than that reported by Ganiev et al. (2003). In our previous study, we reported that phenol oxidation by Fenton's reagent yielded rate constants ranging from $1.32x10^{-4}$ to $1.242x10^{-3}$ L/mM min for 21.27 mM phenol oxidation by 58.52 mM hydrogen peroxide concentration. This clearly indicates a much slower oxidation process when Fenton's reagent is used compared to chlorine dioxide. For 21.27 mM of phenol oxidation, Fenton's reagent oxidation (C_{H2O2} =58.52 mM, $C_{Fe(II)}$ = 5.39 mM and pH 3) could only achieve 85% phenol oxidation in 3 h, while chlorine dioxide achieved complete conversion in 10 min. Thus chlorine dioxide is clearly superior for removal of 21.27 mM of phenol in a bioremediation medium.

5.5 Conclusions

The kinetics of phenol oxidation by chlorine dioxide has been determined in a bioremediation medium. The rate of phenol oxidation and overall phenol conversion were found to increase with the increase in chlorine dioxide concentration. The average value of the second order rate constant k_1 for oxidation of phenol by chlorine dioxide at 25°C was found to be 0.039±0.008 L/mM min. Compared to Fenton's reagent, chlorine dioxide oxidation of phenol was found more efficient and much faster in bioremediation medium. Chlorine dioxide achieved 100 % of 21.27 mM phenol conversion in 10 min compared to 85% in 3 h with Fenton's reagent (58.52 mM H2O2).

6. BIODEGRADATION KINETICS OF 1,4-BENZOQUINONE IN BATCH AND CONTINUOUS SYSTEMS

A similar version of this chapter has been submitted for publication in Biodegradation Journal:

Kumar P., M. Nemati, G. A. Hill "Biodegradation kinetics of 1,4-benzoquinone in batch and continuous systems", Biodegra. J. (2010).

Contribution of the Ph.D. Candidate

The acclimatisation of *Pseudomonas putida* (ATCC 17484) to utilize 1,4-benzoquinone, experimental design and determination of biokinetics for biodegradation of 1,4-benzoquinone was done by Pardeep Kumar with technical input and guidance from Dr. Hill and Dr. Nemati. The written text for this manuscript has been prepared by Pardeep Kumar, while Dr. Hill and Dr. Nemati provided the editorial input.

Contribution of this Chapter to Overall Study

After identification and quantification of intermediate products of phenol oxidation by chlorine dioxide in Chapter 4 and 5, the next objective was to study the biodegradation of these intermediate compounds**.** This was important as the results of kinetic study showed that chemical oxidation of intermediates were not possible even with high levels of chlorine dioxide. With the same objective the biodegradation of 1,4-benzoquinone, the major intermediate of phenol oxidation by chlorine dioxide, was conducted in batch and

continuous systems. This chapter presents the results of evaluation of biokinetics for the *Pseudomonas putida* biodegradation of 1,4-benzoquinone.

6.1 Abstract

Combining chemical and biological treatments are a potentially economic approach to remove high concentration of recalcitrant compounds from wastewaters. In the present study, the biodegradation of 1,4-benzoquinone, an intermediate compound formed during phenol oxidation by chlorine dioxide, was investigated using *Pseudomonas putida* (ATCC 17484) in batch and continuous bioreactors. Batch experiments were conducted to determine the effects of 1,4-benzoquinone concentration and temperature on the microbial activity and biodegradation kinetics. Using the generated data, the maximum specific growth rate, biodegradation rate and apparent activation energy were determined as 0.94 h^{-1}, 6.71 mg of 1,4-benzoquinone L^{-1} h^{-1} and 14.8 Kcal mol^{-1}, respectively.

Biodegradation in a continuous bioreactor indicated a linear relationship between substrate loading and biodegradation rates prior to wash out of the cells, with a maximum biodegradation rate of 246 mg L^{-1} h^{-1} observed at a loading rate of 275 mg L^{-1} h^{-1} (residence time : 1.82 h). Biokinetic parameters were also determined using the steady state substrate and biomass concentrations at various dilution rates and compared to those obtained in batch cultures.

Keywords: Phenol oxidation, 1,4-benzoquinone, *Pseudomonas putida* 17484, batch and continuous bioreactors, biodegradation, kinetics.

6.2 Introduction

Phenols and phenol like compounds are widely occurring compounds in wastewaters produced from all the major chemical industries (Benitez et al., 1997 and Prpich et al., 2005). Biological treatments alone are not effective for the removal of phenolic compounds because of their bactericidal nature at high concentrations (Cao et al., 2009). Therefore, advanced oxidation treatments have been applied for the removal of high concentrations of phenolic and other toxic compounds from various industrial wastewaters (Herrmann et al., 1993, Kowalska et al., 2004 and Pignatello et al., 2006). The high amounts of chemical oxidants required to completely mineralize the organic contaminants make the chemical treatment processes uneconomical (Zazo et al., 2005). Integrating the chemical and biological treatments is an attractive alternative to minimize the treatment costs for the removal of high concentrations of toxic compounds from wastewater (Edalatmanesh et al., 2008).

The results of steady state oxidation of phenol in a bioremediation medium using chlorine dioxide have presented and discussed in chapter 4. These include the optimal concentration of ClO_2, temperature and pH. Based on the result of that study, 2000 mg L^{-1} of phenol were completely oxidized by 2000 mg L^{-1} chlorine dioxide. This quantity of chlorine dioxide was half the amount of Fenton's reagent (4000 mg L^{-1} H_2O_2 and 1500 mg L^{-1} of $FeSO_4.7H_2O$) that was needed to accomplish the same task (Kumar et al.,

2010b). With chlorine dioxide, the main oxidation products were identified as 1,4-benzoquinone and 2-chloro-1,4- benzoquinone. The reaction mechanism of phenol oxidation by chlorine dioxide is shown in Figure 4.1. Figure 4.7 shows the dynamic profile of oxidation of 2000 mg L^{-1} phenol in the presence of 2000 mg L^{-1} chlorine dioxide and the evolution of the oxidation products. It was also observed that these two intermediates persist even when much higher concentrations of chlorine dioxide were applied (Figure 4.8).

The Inability of chlorine dioxide to oxidize 1,4-benzoquinone and 2-chloro-1,4-benzoquinone, and the result of a preliminary experiment which confirmed the biodegradability of 1,4-benzoquinone indicated that an integrated chemical and biological treatment may be a suitable alternative to exclusive chemical oxidation, and highlighted the necessity to study the biodegradation of these intermediate compounds. The present work focuses on biodegradation of 1,4-benzoquinone, one of the major intermediate compounds of phenol oxidation by chlorine dioxide, using *Pseudomonas putida* (ATCC 17484).

1,4-benzoquinone is one of the most toxic xenobiotics and is generated during the oxidation of benzene and a wide variety of its derivatives (Nan et al., 2007). A few studies have been reported on photochemical degradation (Shevchuk and Kirso 1981), chemical oxidation by hydrogen peroxide (Medici 1986) and electrochemical detoxification (Pulgarin et al., 1994) of 1,4-benzoquinone. Pulgarin et al. (1994) studied the electrochemical detoxification of 1,4-benzoquinone using Ti/IrO_2 and Ti/SnO_2

electrodes and found that nature of the electrode is the most important parameter. No prior study has been published on the biodegradation of 1,4-benzoquinone in the literature. The objective of this study was to investigate the biodegradation kinetics of 1,4-benzoquinone in both batch and continuous stirred tank bioreactors, with the focus being on the effects of 1,4-benzoquinone concentration and loading rate, as well as temperature. The batch experiments were conducted to assess the effect of initial concentration of 1,4-benzoquinone, pH and temperature. Because CSTR is preferred for large scale treatment, biokinetic study of 1,4-benzoquinone was also carried out in the CSTR. Generated data were used to determine the biokinetic coefficients.

6.3 Materials and Methods

6.3.1 Culture and medium

Pseudomonas putida (ATCC 17484) was used in biodegradation experiments in both batch and CSTR systems. *Pseudomonas putida* was initially grown in 500 mL Erlenmeyer (shake) flasks containing 200 mL sterilized McKinney's medium with 200 mg L^{-1} of phenol. When the optical density (OD) reached a maximum value (0.422), 20 mL (10% v/v) of the culture was transferred to a second shake flask containing McKinney's medium and a mixture of 100 mg L^{-1} of phenol and 50 mg L^{-1} of 1,4-benzoquinone. Following the complete biodegradation of phenol and 1,4-benzoquinone, 20 mL (10% v/v) of this second culture was used to inoculate two dose McKinney's medium containing only 100 mg L^{-1} 1,4-benzoquinone. This procedure ensured *Pseudomonas putida* was acclimatised to utilize 1,4-benzoquinone. Reverse osmosis water was used for preparing all solutions. The growth medium (two dose McKinney's

medium) used in all experiments consisted of (mg in 1 L reverse osmosis water): K_2HPO_4, 750; KH_2PO_4, 849; $(NH_4)_2SO_4$, 474; NaCl, 60; $CaCl_2$, 60; $MgSO_4$, 60; $Fe(NH_4)SO_4$, 20; and 1 mL of trace mineral solution. The trace mineral solution was consisted of (mg in 1 L reverse osmosis water): $ZnSO_4 \cdot 7H_2O$, 200; $MnCl_2$, 60; H_3BO_3, 600; $CoCl_2$, 400; $CuCl_2$, 20; $NiCl_2$, 40; Na_2MoO_4, 60. Subculturing were carried out every 2 days using the acclimatised culture (10% v/v inoculum) and two dose McKinney's medium containing 100 mg L^{-1}1,4-benzoquinone. The cultures were maintained at room temperature (23°C).

6.3.2 Experimental procedure

Batch experiments were conducted to study the kinetics of microbial growth and biodegradation of 1,4-benzoquinone at a pH of 7. The effects of initial concentration of 1,4-benzoquinone and temperature were investigated using 500 mL Erlenmeyer (shake) flasks as batch reactors. Each flask was loaded with 200 mL sterile two dose McKinney's medium containing either 25, 50, 100, 150 or 200 mg L^{-1} 1,4-benzoquinone. These were then inoculated with freshly grown *Pseudomonas putida* (10% v/v) on 100 mg L^{-1} 1,4-benzoquinone. Samples were taken at regular intervals using stainless steel needles and hypodermic syringes. Samples used for 1,4-benzoquinone concentration measurement were filtered through 0.22 μm nylon microfilters to remove biomass prior to analysis by HPLC. For the measurement of biomass concentration, a two mL sample was taken for cell counting. All the shake flasks were completely covered with aluminum foil and placed on the gyratory shaker (New Brunswick Scientific Co., Inc, Edison, N.J. U.S.A) operated at 100 rpm. The effect of temperature was determined by conducting

biodegradation of 1,4-benzoquinone experiments at three different temperatures (10, 15 and 25°C). All the biodegradation experiments were performed in the temperature controlled chambers to maintain the desired temperatures. Control experiments were conducted under similar conditions without inoculation.

A BIOFLOW III reactor (New Brunswick Scientific, Inc., Edison, N.J. U.S.A) was used as the continuous flow reactor (CSTR). The CSTR was vigorously stirred with a mechanical agitator equipped with Rushton turbine impeller. Filtered air, required for bacterial activity, was supplied through a ring sparger. The working volume of the reactor was 750 mL.

Figure 6.1 Continuous stirred tank reactor for biodegradation of 1,4-benzoquinone.

Initially the CSTR was operated batchwise for two days using 100 mg L^{-1} of 1,4-benzoquinone dissolved in 2 dose McKinney's medium. The reactor was inoculated with 10% (v/v) freshly grown *Pseudomonas putida* culture. The bioreactor was switched to continuous mode when 1,4-benzoquinone was completely degraded. The feed consisted of 2 dose McKinney's medium with 500 mg L^{-1} of 1,4-benzoquinone that was pumped continuously into the bioreactor, at an initial flow rate of 45 mL h^{-1}, using a peristaltic pump. The bioreactor was covered with aluminum foil to prevent exposure to light. The bioreactor temperature was maintained at 15°C by circulating cold water through the jacket. This temperature was selected based on batch experiments. In the batch experiments, it was found that 1,4-benzoquinone starts to naturally degrade at 25°C and slows down the biodegradation of 1,4-benzoquinone. The flow rate of the feed was increased stepwise. At each flow rate sufficient time was given for establishment of steady state which was verified by a relatively constant residual substrate concentration. Once steady state was confirmed, a sample was taken from the bioreactor for measuring cell number by the plate counting technique. The flow rate of the feed was increased until cells wash out occurred. The applied dilution rates were in the range 0.06 to 0.75 h^{-1}.

6.3.3 Analytical methods

Optical density can be used as an indication of biomass concentration, when *Pseudomonas putida* is grown on phenol. However, with 1,4-benzoquinone, progress of biodegradation results in a change of colour (yellowish to brown) and thus optical density could not be used. Thus biomass concentration was measured using the plate counting technique. Agar plates were prepared by pouring the agar mixture, 3 g Difco Bacto agar

and 3 g tryptose phosphate in 100 mL sterilized R.O. water, on Petri dishes. Following the serial dilutions, 100 μL of diluted sample was used to inoculate the agar plates. The number of developed colonies were then determined and converted to cell concentration.

The substrate concentration (1,4-benzoquinone) was determined using an HPLC (Agilent HPLC 1100, California, USA) equipped with a C_{18} column (Nova pack: 4.6 x 150 mm: 4μm) and a Diode Array Detector. The mobile phase was a mixture of acetonitrile and water (10/90 v/v) with a flow rate of 2.1 mL min^{-1}. Detection was carried out using the Diode Array Detector at a wavelength of 254 nm. The retention time for 1,4-benzoquinone was 2.06 min.

6.4 Results and Discussion

6.4.1 Effect of initial concentration of 1,4-benzoquinone

Figure 6.1 (A-D) shows the results of microbial growth and substrate removal as a function of time in the batch reactors containing different initial concentrations of 1,4-benzoquinone at 15°C. Control experiments, using sterilized 1,4-benzoquinone solutions without inoculation, were run at each tested concentration to show that removal of 1,4-benzoquinone was due to bacterial activity and not by natural degradation. In all biodegradation experiments a direct relationship between microbial growth and substrate utilization were observed and the specific growth rates and biodegradation rates were determined using the exponential growth phase data.

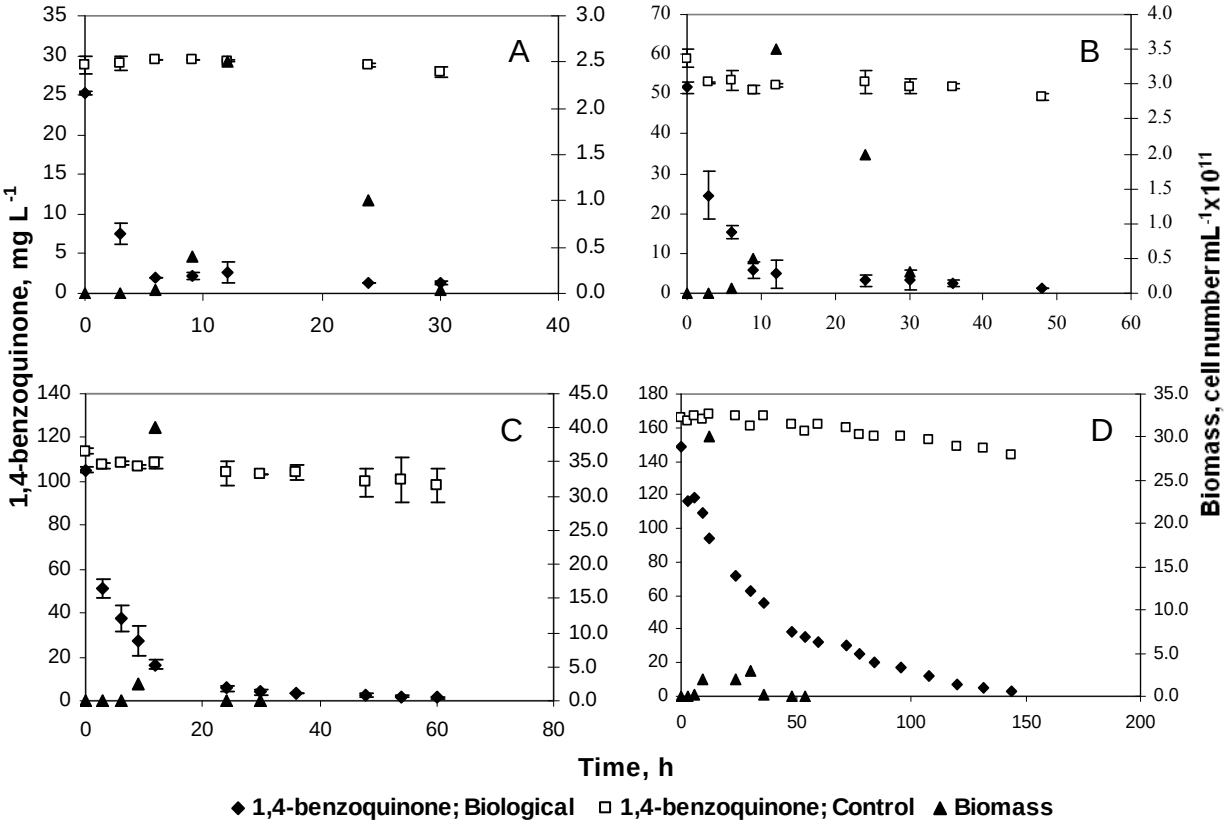


Figure 6.1 Substrate and biomass concentrations as a function of time in batch reactors at various initial substrate concentrations at a fixed temperature of 15°C. A: initial substrate concentration of: 25 mg L^{-1}; B: 50 mg L^{-1}; C: 100 mg L^{-1}; D: 150 mg L^{-1}. Error bars represent one standard deviation and in some case not visible due to the small magnitude.

Table 6.1 shows the values of specific growth and biodegradation rates observed at different initial concentrations of 1,4-benzoquinone. Both specific growth rate and biodegradation rate increase with increase in initial substrate concentration up to a maximum value at a 1,4-benzoquinone concentration of 100 mg L^{-1} . With further increase in initial substrate concentration, the specific growth rate and biodegradation rate decline, indicating substrate inhibition.

Table 6.1 Summary of specific growth and biodegradation rates determined at various initial substrate concentrations at15°C. (batch reactors)

Initial substrate concentration mg L^{-1}	Specific growth rate h^{-1}	Biodegradation rate mg L^{-1} h^{-1}
25	0.77±0.03	2.48±0.04
50	0.83±0.06	3.74±0.21
100	0.91±0.04	6.71±0.21
150	0.85	1.13
200	0.39	0.91

The maximum values of specific growth rate and biodegradation rate were 0.91±0.04 h^{-1} and 6.71±0.27 mg L^{-1} h^{-1}, observed at an initial substrate concentration of 100 mg L^{-1}. A similar trend was observed with the overall yield of biomass; increasing with increase in initial substrate concentration, reaching a maximum at a substrate concentration of 50 mg L^{-1} and decreasing with further increase in initial substrate concentration. The maximum value of overall yield obtained was $6x10^{11}$ cell mg^{-1} substrate, and was observed with 50 mg L^{-1} substrate.

6.4.2 Effect of temperature on biodegradation of 1,4-benzoquinone

Figure 6.2 shows the results of microbial growth and substrate removal as a function of time at different temperatures of 10, 15 and 25°C.

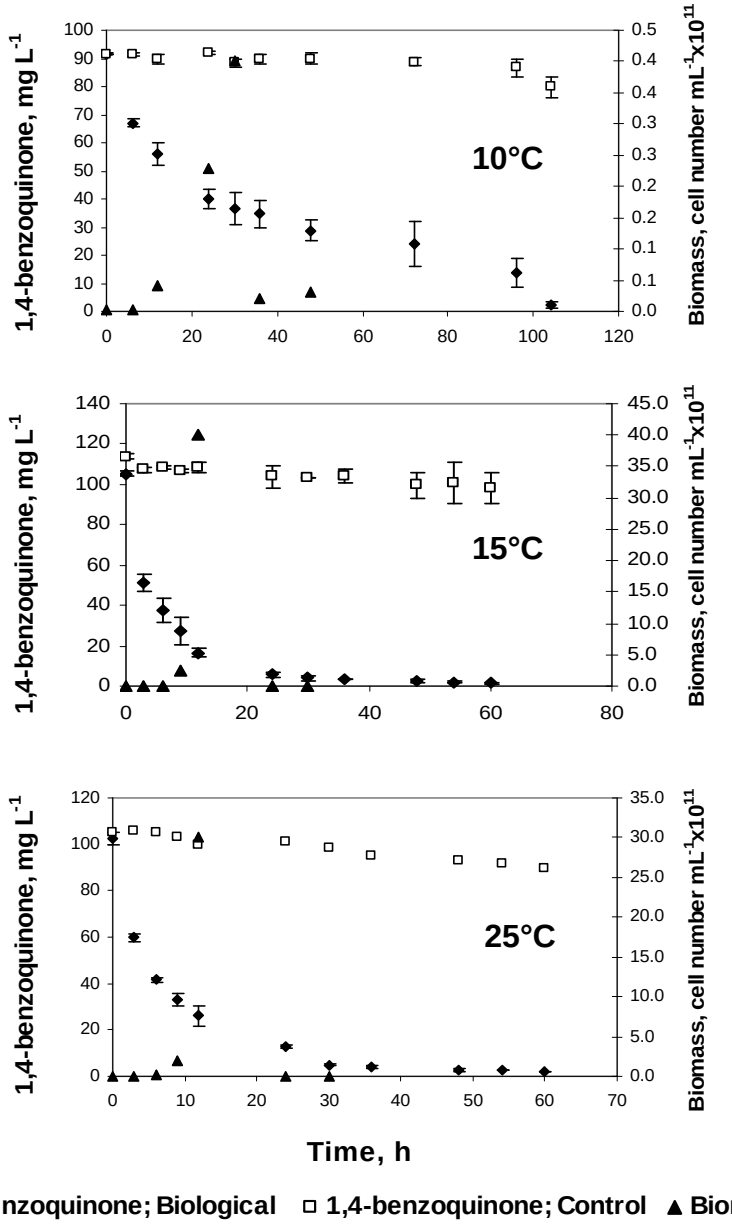


Figure 6.2 Substrate and biomass concentration profiles as a function of time in a batch reactor at different temperatures and 100 mg L^{-1} of 1,4-benzoquinone concentration A: 10°C; B: 25°C. Error bars represent one standard deviation.

The values of specific growth rate and biodegradation rate at various temperatures for 1,4-benzoquinone at initial concentration of 100 mg L^{-1} and pH 7 are shown in Table 6.2.

Table 6.2 Summary of specific growth rate and biodegradation rate at different temperatures and 100 mg L^{-1} of substrate (batch reactors)

Temperature, °C	Specific growth rate h^{-1}	Biodegradation rate mg L^{-1} h^{-1}
10	0.19	0.65
15	0.91±0.04	6.71±0.27
25	0.90	3.15

The value of specific growth rate was found to increase with increase of temperature in the range 10 to 15°C. But did not change significantly when temperature was increased from 15 to 25°C. Natural degradation of 1,4-benzoquinone was observed at 25°C that might have generated some unidentified toxic intermediates which inhibit the growth of *Pseudomonas putida*. Fitting the experimental data into the Arrhenius equation, the value of activation energy for microbial growth was determined to be 14.8 Kcal/mol.

6.4.3 Biodegradation of 1,4-benzoquinone in a continuous stirred tank reactor

Figure 5 shows the steady state profiles of biomass and substrate concentrations obtained at different dilution rates ranging from 0.06 to 0.75 h^{-1}. It can be seen from this figure that the biomass concentration increases with increasing dilution rate from 0.06 to 0.25 h^{-1}, while 1-4-benzoquinone was completely consumed.

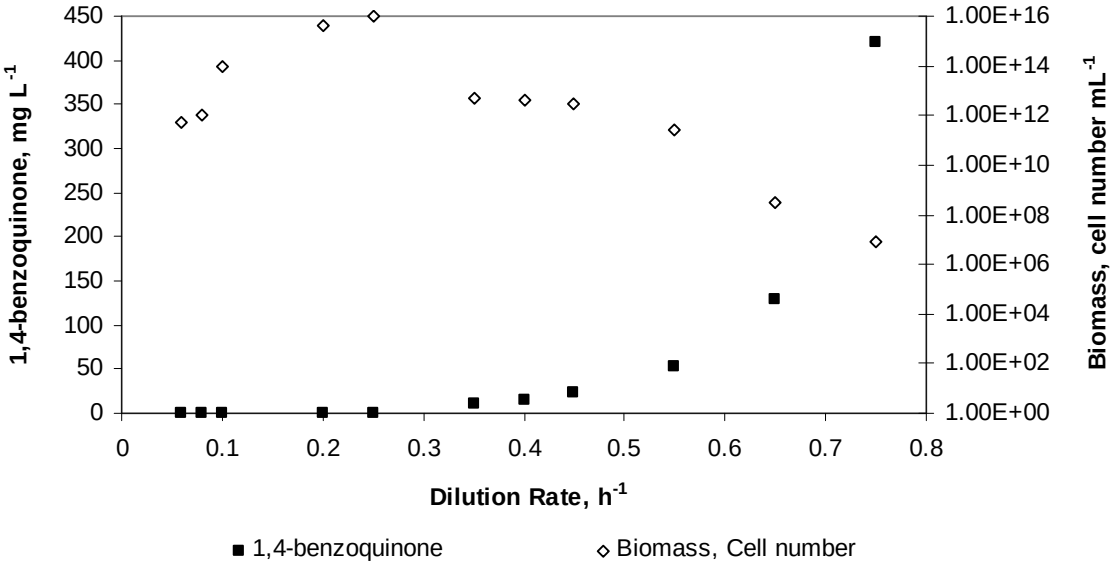


Figure 6.3 Steady state profiles of biomass and substrate concentrations as a function of dilution rate at 500 mg L^{-1} 1,4-benzoquinone.

Further increase in dilution rate resulted in a decrease in biomass concentration and increase in residual 1-4-benzoquinone concentration. The maximum biomass concentration ($1x10^{16}$ cell numbers mL^{-1}) was found at a dilution rate of 0.25 h^{-1} at which complete removal of substrate was also achieved. The residual 1,4-benzoquinone

concentrations approached to the feed concentration at a dilution rate of 0.75 h^{-1} and wash out in form of significant decrease in biomass concentration was observed.

To determine the kinetic parameters, Monod's model was used and the maximum specific growth rate and saturation constant were determined by fitting the steady state experimental data to equation 6.1:

$$\mu = \frac{\mu_{max} S}{K_S + S} \tag{6.1}$$

The Linewaver-Burk, Eadle-Hofstee and Hanes-Woolf* plots were used to analyze the data (Shuler and Kargi, 2002). The values of maximum specific growth rate and saturation constant were determined by these three plots. The average values of maximum specific growth rate and saturation constant were 0.74±0.03 h^{-1} and 14.17±3.21 mg L^{-1}, respectively.

The rate of biodegradation of 1,4-benzoquinone as a function of its loading rate obtained in the CSTR operated at an initial substrate concentration of 500 mg L^{-1} is shown in Figure 6.4.

* The mathematical equations and plots for Linewaver-Burk, Eadle-Hofstee and Hanes-Woolf plots are shown in Appendix B

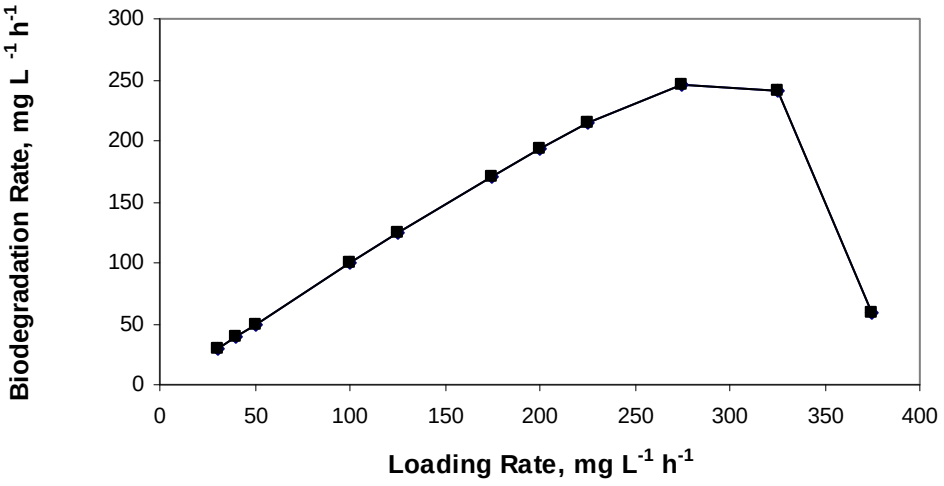


Figure 6.4 Biodegradation rate as a function of loading rate in CTSR operated at an initial substrate concentration of 500 mg L^{-1} and 15°C.

The loading and biodegradation rates were calculated using the concentration of 1,4-benzoquinone in the feed, steady state residual concentration of 1,4-benzoquinone and dilution rate. It could be seen from this figure that biodegradation rate increases with increase in loading rate up to 300 mg L^{-1} hr^{-1} after that biodegradation starts to decline with increase in loading rate. The maximum biodegradation rate obtained in the CSTR was 246 mg L^{-1} h^{-1} at a loading rate of 275 mg L^{-1} h^{-1} (residence time: 1.82 h). In the CSTR, biomass yield for was found to vary with change in dilution rate; it first increased with increase in dilution rate (0.06 to 0.25 h^{-1}) and then declined with further increase in dilution rate (0.35 to 0.75 h^{-1}). The maximum biomass yield obtained was $2x10^{13}$ cell mg^{-1} substrate. Comparing the data obtained in batch and CSTR, the value of maximum specific growth rate was found to be higher in batch reactors but at a lower substrate concentration. However, the CSTR is very efficient in terms of biodegradation rate of

1,4-benzoquinone, with the rate being 36 times higher than the biodegradation rate obtained in batch reactor.

As mentioned earlier, 1,4-benzoquinone is one of the main intermediate compounds of phenol oxidation by chlorine dioxide and it is not oxidized even when high concentrations of chlorine dioxide are used. So in this work, the biodegradation of 1,4-benzoquinone was successfully demonstrated in both batch and continuous stirred tank reactors. No study has been reported in the past on the biodegradation of 1,4-benzoquinone in the literature, so biokinetic parameters of 1,4-benzoquinone could not be compared with literature data. However, for comparison purposes, the biokinetic parameters of 1,4-benzoquinone was compared with phenol and are presented in Table 6.3.

Table 6.3 Comparison of biokinetic parameters of 1,4-benzoquinone and phenol

Reference	Microbial culture	Substrate	Maximum specific growth Rate (h^{-1})	Saturation constant (mg L^{-1})	Biodegradation rate (mg L^{-1} h^{-1})
Hill and Robinson, 1975	*Pseudomonas putida* ATCC*17484*	Phenol	0.534	0.015	
Kumar et al. 2005	*Pseudomonas putida MTCC* 1194	Phenol	0.216	20.59	
Nikakhtari and Hill, 2006	*Pseudomonas putida* ATCC*17484*	Phenol and naphthalene	0.75	<1	
Present work (batch)	*Pseudomonas putida* ATCC*17484*	1,4-benzoquinone	0.91±0.04		6.71±0.2
Present work (continuous)	*Pseudomonas putida* ATCC*17484*	1,4-benzoquinone	0.74±0.03	14.23±3.21	

Data shown in Table 6.3 indicates that the value of maximum specific growth rate ($0.91\pm0.04\ h^{-1}$) obtained in this study is significantly higher than the maximum specific growth rate of *Pseudomonas putida* on phenol. The value of saturation constant obtained is comparable to those reported in the literature but using phenol as the substrate.

6.5 Conclusions

The biodegradation of 1,4-benzoquinone, one of the intermediate of phenol oxidation by chlorine dioxide, was studied in batch and continuous reactors. Complete biodegradation of up to 150 mg L^{-1} of 1,4-benzoquinone was achieved in batch reactors. The values of maximum specific growth rate and biodegradation rate obtained in the batch reactor were $0.91\pm0.04\ h^{-1}$ and 6.71 ± 0.27 mg $L^{-1}\ h^{-1}$, respectively. Over the tested temperature range (10-25°C) the optimal temperature for microbial growth and biodegradation of 1,4-benzoquinone was 15°C. In the continuous bioreactor, increase of loading rate up to a value of 275 mg $L^{-1}\ h^{-1}$ (residence time: 1.82 h) caused a linear increase in biodegradation rate with a maximum value of 246 mg $L^{-1}\ h^{-1}$ obtained at the higher loading rate . Using the experimental data generated in the CSTR, the specific growth rate and saturation constant were determined as $0.74\pm0.03\ h^{-1}$ and 14.23 ± 3.21 mg L^{-1}, respectively. Finally an integrated chemical and biological approach has been shown to be a successful strategy for the removal of phenol.

Chapter 7

7. Conclusions and Recommendations

7.1 Conclusions

The possibility to integrating chemical oxidation with biodegradation to remove high concentrations of phenol, a ubiquitous environmental contaminant, from bioremediation medium was explored in this work. The two chemical oxidants chosen for this study were Fenton's reagent (mixture of hydrogen peroxide and ferrous sulfate) and chlorine dioxide. The optimal conditions for the removal of phenol were determined for each oxidants and their performance has been compared in term of percentage of phenol removed. Identification of the oxidation intermediates, which is important for using biodegradation as a further step, was carried out in each case. Kinetic modeling of advanced oxidation with each oxidant was performed and the kinetic coefficients were determined. Finally, biodegradation of the main intermediate resulting from oxidation of phenol by chlorine dioxide was investigated.

Our preliminary experiments showed that the presence of inorganic salts in bioremediation medium have a significant effect on phenol oxidation by Fenton's reagent. The analysis of variance (ANOVA) test indicated that both H_2O_2 and $FeSO_4.7H_2O$ were statistically significant and increases in their concentrations had a positive effect on phenol oxidation. However, the dominating parameter affecting the percentage of phenol oxidized was the hydrogen peroxide concentration. The quadratic polynomial model developed predicted the optimal conditions for the Fenton's reaction in

the bioremediation medium as C_{H2O2} = 4340 mg L^{-1} and $C_{FeSO4.7H2O}$ = 1616 mg L^{-1}. The main oxidation products were identified as catechol, hydroquinone, maleic acid and carbon dioxide.

The increase of initial hydrogen peroxide concentration (1000 to 5000 mg L^{-1}) increased the rate of phenol oxidation and overall phenol conversion at a constant pH of 3 and $C_{FeSO4.7H2O}$ = 1500 mg L^{-1} due to the increase in hydroxyl radicals generation. A kinetic model was proposed based on the identification of oxidation intermediates and kinetic parameters, namely rate constants and activation energies were determined by fitting the experimental data into the proposed model. The average values of the second order rate constants k_1 (oxidation of phenol to catechol), k_2 (oxidation of phenol to hydroquinone), k_3 (oxidation of catechol to maleic acid), k_4 (oxidation of hydroquinone to maleic acid) for oxidation of phenol and intermediates by Fenton's reagent at 25°C were found to be $7.02x10^{-5}\pm4.63x10^{-5}$, $7.22x10^{-4}\pm6.09x10^{-4}$, $1.82x10^{-4}\pm1.08x10^{-4}$, $1.68x10^{-3}\pm1.29x10^{-3}$ L mM min^{-1}, respectively. Increase of temperature from 5 to 35°C enhanced the phenol oxidation rate. The activation energy determined for each reaction E_{a1} (phenol to catechol), E_{a2} (phenol to hydroquinone), E_{a3} (catechol to maleic acid), E_{a4} (hydroquinone to maleic acid) was 45.5, 63.9, 40.5 and 45.9 KJ mol^{-1}, respectively. The oxidation of phenol by Fenton's reagent in water was found to be much faster than in the bioremediation medium.

The experimental design method based on CCRD was used to determine the optimal experimental conditions for oxidation of 2000 mg L^{-1} phenol by chlorine dioxide in a

bioremediation medium. The three parameters investigated were chlorine dioxide concentration (500 to 2000 mg L^{-1}), temperature (10 to 40°C) and pH (3 to 7). Chlorine dioxide concentration was found to be the dominate parameter for the removal of phenol in the bioremediation medium. The optimal concentration of chlorine dioxide to completely oxidize 2000 mg L^{-1} of phenol was 2000 mg L^{-1}. The pH and temperature did not have significant impact phenol oxidation by chlorine dioxide. Comparison of the data obtained for chlorine dioxide and Fenton's reagent demonstrated that the amount of chlorine dioxide required for complete oxidation of 2000 mg L^{-1} phenol was half of the Fenton's reagent (2000 mg L^{-1} ClO_2 vs. 4000 mg L^{-1} H_2O_2). Unlike Fenton's reagent, chlorine dioxide oxidized phenol at the same rates in water and bioremediation medium. The main oxidation products were identified as 1,4-benzoquinone and 2-chloro-1,4-benzoquinone. Compared to Fenton's reagent, chlorine dioxide is a superior oxidant for removal of phenol from both pure water and bioremediation medium.

The rate of phenol oxidation and overall phenol conversion were found to increase with the increase in chlorine dioxide concentration. The average value of the second order rate constant k_1 for oxidation of phenol by chlorine dioxide was found to be 0.039±0.008 L mM min at 25°C. Compared to Fenton's reagent, chlorine dioxide oxidation of phenol was found more efficient and much faster in bioremediation medium. Chlorine dioxide achieved 100 % conversion of 2000 mg L^{-1} phenol conversion in 10 min compared to 85% in 3 h achieved with Fenton's reagent (2000 mg L^{-1} H_2O_2).

Furthermore, our results showed that complete conversion of the intermediates to CO_2 and water was not possible, even in the presence of a very high concentration of chlorine dioxide. Therefore, biodegradation of 1,4-benzoquinone, the main intermediate of phenol oxidation by chlorine dioxide, was studied in batch and continuous reactors. Batch biodegradation experiments were conducted with different initial 1,4-benzoquinone concentrations ranging from 25 to 150 mg L^{-1} and complete biodegradation of 1,4-benzoquinone was achieved for all tested concentrations. The values of maximum specific growth rate and maximum biodegradation rate obtained in the batch reactor were 0.91±0.04 h^{-1} and 6.71±0.27 mg L^{-1} h^{-1}, respectively. Over the tested temperature range (10-25°C) the optimal temperature for microbial growth and biodegradation of 1,4-benzoquinone was 15°C. In the continuous bioreactor, increase of loading rate up to a value of 275 mg L^{-1} h^{-1} (residence time: 1.82 h) caused a linear increase in biodegradation rate with a maximum value of biodegradation rate of 246 mg L^{-1} h^{-1} obtained at the same loading rate (100% Conversion of 1,4-benzoquinone) . Using the experimental data generated in the CSTR, the maximum specific growth rate (μ_{max}) and saturation constant (Ks) were determined as 0.74±0.03 h^{-1} and 14.17±3.21 mg L^{-1}, respectively. As an overall conclusion, an integrated chemical and biological approach has been shown to be a potentially valuable strategy for the removal of phenol at high concentrations.

7.2 Recommendations

The phenol oxidation in a bioremediation medium using Fenton's reagent indicated that the presence of inorganic salts slowed down the reaction rate and phenol conversion. This work needs to be expanded further to take into account the detailed

reaction mechanism in the presence of these inorganic ions. It was reported in the literature that the inorganic ions slow the Fenton's reaction in the following order PO^{3-}_4 $>HPO^{2-}_4$ $>H_2PO^-_4$ $>Cl^-$ $>SO^{2-}_4$. The bioremediation medium is a mixture of several inorganic salts. Presence of all these salts at once makes the reaction system very complex. To understand the effect of these inorganic salts and reaction mechanism, phenol oxidation should be conducted in the presence of individual and binary combinations of these salts.

The main oxidation products of phenol oxidation by Fenton's reagent in bioremediation medium were catechol, hydroquinone, maleic acid and carbon dioxide. The aromatic intermediates such as catechol and hydroquinone were reported to have higher toxicity than phenol. These intermediates are oxidized by the chemical oxidants, but the short chain organic acids were reported to be resistant to chemical oxidation. So, further biodegradation studies should be conducted on the effluent from the chemical treatment step using *Pseudomonas putida* 17484.

The chlorine dioxide for oxidation of organic compounds is gaining popularity. It is safe, easy to handle and cheaper compared to other AOPs. The use of chlorine dioxide does not result in the formation of sludge as is the case in Fenton's reaction. The main intermediates of phenol oxidation by chlorine dioxide were 1,4-benzoquinone and 2-chloro-1,4-benzoquinone. There is no information reported on the toxicity of these two intermediate compounds, not even in the MSDS literature. The biodegradation studies of 1,4-benzoquinone were successfully conducted in batch and continuous systems. Further

work needs to be done on the toxicity evaluation of these two intermediates. Also, the biodegradation of 2-chloro-1,4-benzoquinone should be investigated in the future. Biodegradation of the actual intermediates formed as a result of phenol oxidation (effluent from chemical treatment step) also needs to be investigated in future for the successful integration of chemical and biological treatments.

Using study was conducted on the oxidation of phenol in a bioremediation medium using two strong oxidizing agents and explored the possibility of combining chemical oxidation with biodegradation. The integrated treatment could be extended to other phenolic compounds and recalcitrant polycyclic aromatic compounds.

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Appendix A

The quantification of phenol and aromatic intermediates (hydroquinone, catechol, 1,4-benzoquinone and 2-chloro-1,4-benzoquinone) were determined using an HPLC. A calibration curve was prepared for each compound using standard solutions in the bioremediation medium. Three injections of each sample were injected into the HPLC and average and standard deviations were used to prepare calibration curves shown in the Figures A.1 to A.5.

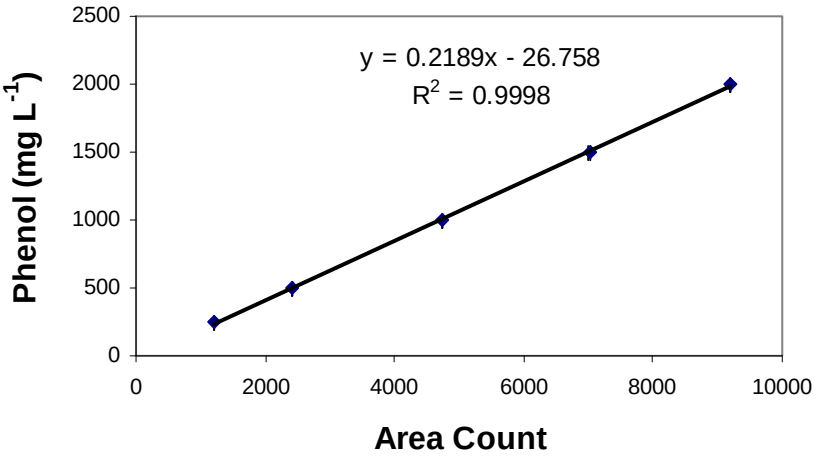

Figure A.1 Calibration of phenol (error bars, not visible due to their magnitude, and represent standard deviations in area count).

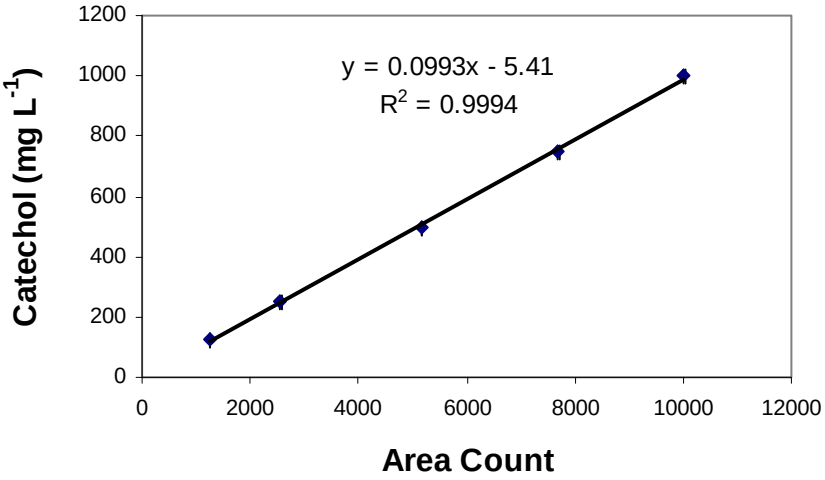

Figure A.2 Calibration of catechol (error bars, not visible due to their magnitude, and represent standard deviations in area count).

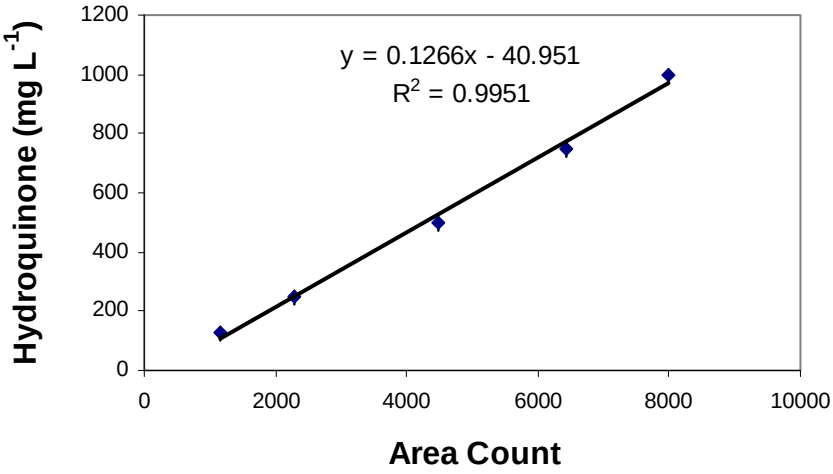

Figure A.3 Calibration of hydroquinone (error bars, not visible due to their magnitude, and represent standard deviations in area count).

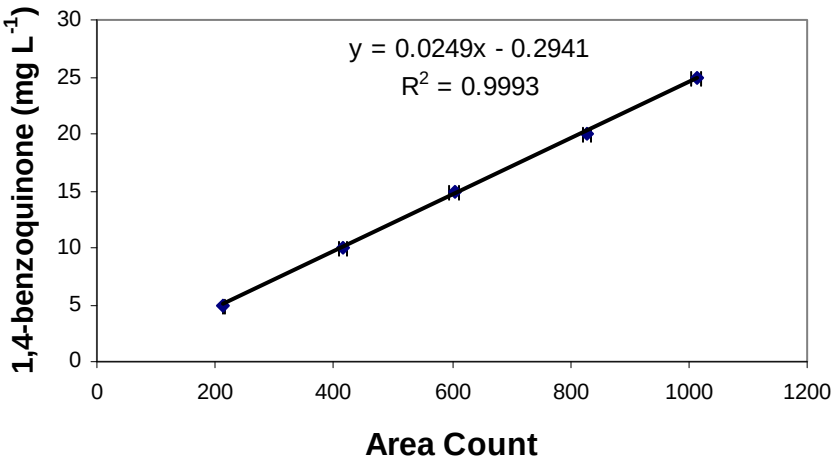

Figure A.4 Calibration of 1,4-benzoquinone (error bars represent standard deviations in area count).

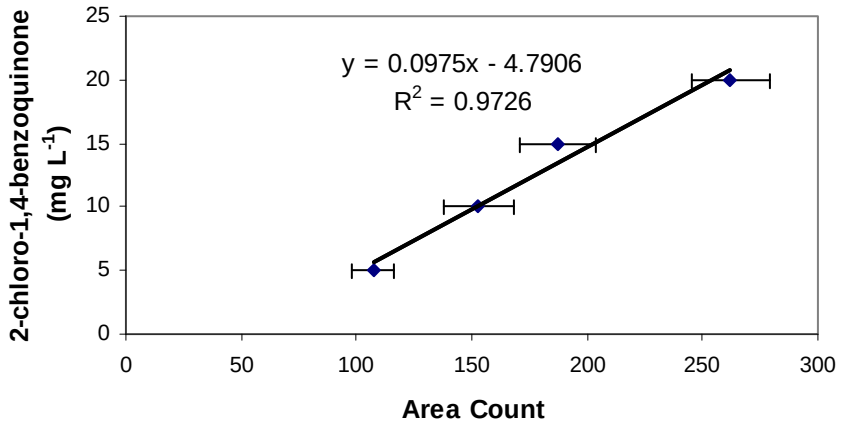

Figure A.5 Calibration of 2-chloro-1,4-benzoquinone (error bars represent standard deviations in area count).

Appendix B

Lineweaver-Burk plot

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_S}{\mu_{max}} \frac{1}{S} \qquad \text{(B-1)}$$

In a continuous reactor operating at steady state, $\mu = D$

Where,

μ = specific growth rate, mg L^{-1}

μ_{max} = maximum specific growth rate, mg L^{-1}

K_S = saturation constant, mg L^{-1}

S = substrate concentration

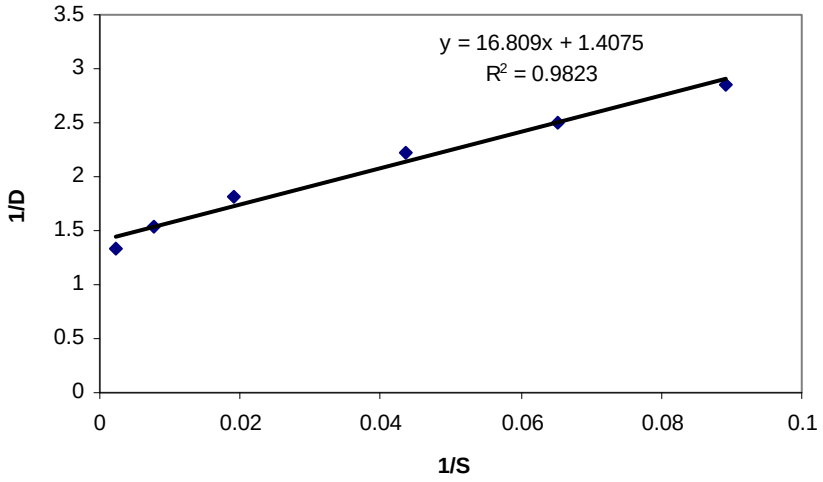

Figure B.1 Lineweaver plot for determination of μ_{max} and K_S.

Eadle-Hosftee plot

$$\mu = \mu_{\max} - K_s \frac{\mu}{S} \qquad \text{(B-2)}$$

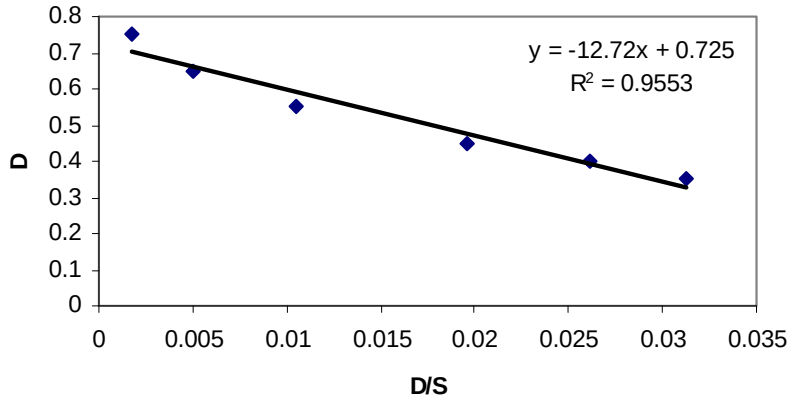

Figure B.2 Eadie-Hofstee Plot.

Hanes-Woolf plot

$$\frac{S}{\mu} = \frac{K_s}{\mu_{\max}} + \frac{1}{\mu_{\max}} S \qquad \text{(B-3)}$$

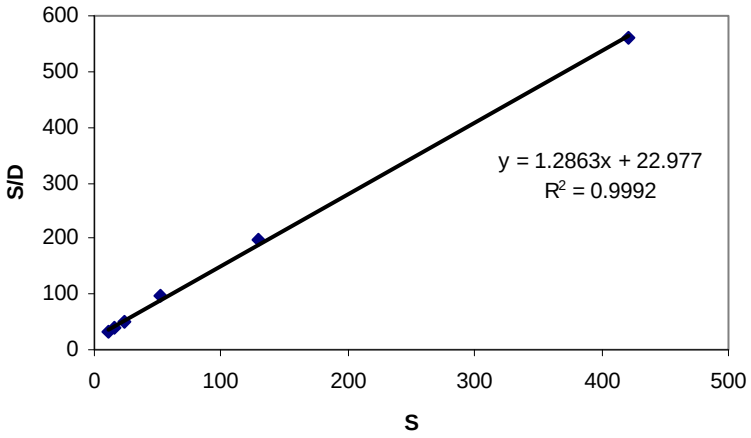

Figure B.3 Hanes-Woolf plot.

Appendix C

Central composite rotatable design method

In this study, optimal experimental conditions required for oxidation of phenol by Fenton's reagent and chlorine dioxide were determined using DOE (Design of Experiment) approach based on central composite rotatable design (CCRD). This method helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interactions among those parameters. The CCRD method is a combination of a factorial design at two levels, star or axial design and repeated experiments at the centre point (Figure C-1).

The central composite design consists of

- 2^K factorial points (also called cube points), where K is the number of factors
- axial points (also called star points)
- centre points

The total number of experiments (N) required is determined by equation C-1.

$$N = 2^K + 2K + n_c \qquad \text{(C-1)}$$

n_c are repeated number of experiments at the centre point to estimate the residual errors. Each of the factors have levels set at five coded levels $-\alpha$, -1, 0, +1, $+\alpha$. Figure C-1 shows the layout of CCRD for three factors.

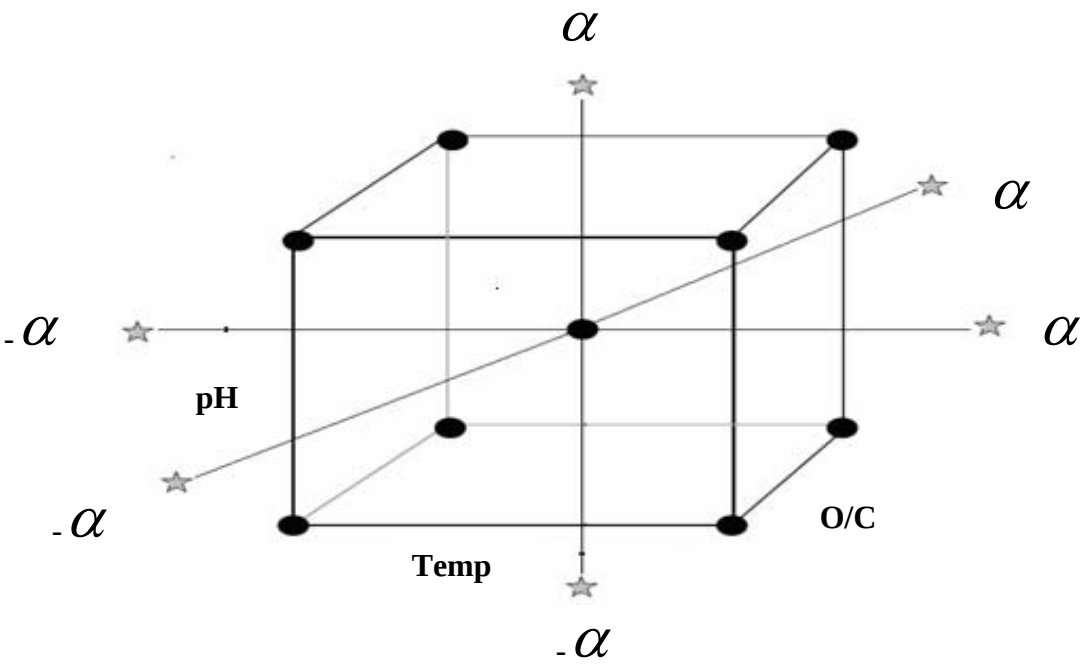

Figure C.1 Layout of central composite design (CCD) for three factors.

The axial points are located at ($\pm\alpha$, 0, 0), (0, $\pm\alpha$, 0), (0, 0, $\pm\alpha$) where α is the distance of the axial point from centre and makes the design rotatable. The value of α depends on the number of experimental runs in the factorial portion of the central composite design:

$$\alpha = [\text{number of factorial runs}]^{1/4} \qquad \text{(C-2)}$$

For full factorial design

$$\alpha = [2^K]^{1/4} \qquad \text{(C-3)}$$